

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*  
**NDA 22-249**

**PHARMACOLOGY REVIEW(S)**

Tertiary Pharmacology Review

**By:** Paul C. Brown, Ph.D.  
OND IO

**NDA:** 22-249

**Submission date:** 9/19/07

**Drug:** Bendamustine hydrochloride

**Sponsor:** Cephalon, Inc.

**Indication:** Chronic Lymphocytic Leukemia (CLL)

**Reviewing Division:** Division of Drug Oncology Products

**Comments:**

I concur with the Division pharm/tox recommendation that the non-clinical studies submitted to this NDA provide sufficient information to support the use of Treanda® (bendamustine hydrochloride) for the treatment of patients with chronic lymphocytic leukemia (CLL).

This alkylating agent exhibited the expected toxicity for this class of compound. As expected, bendamustine is mutagenic, carcinogenic, and teratogenic like other nitrogen mustard alkylating drugs.

The sponsor proposed a pregnancy labeling category of D and the Division agreed with this category although some changes in specific wording describing the relevant studies were suggested by the pharm/tox reviewer. I concur with the pregnancy category of D and with the description of the findings in labeling.

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

/s/

-----  
Paul Brown  
3/7/2008 11:17:23 AM  
PHARMACOLOGIST



DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH

## PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-249
SERIAL NUMBER:	002
DATE RECEIVED BY CENTER:	09/19/2007
PRODUCT:	Treanda® (bendamustine hydrochloride)
INTENDED CLINICAL POPULATION:	Chronic Lymphocytic Leukemia (CLL)
SPONSOR:	Cephalon, Inc.
DOCUMENTS REVIEWED:	Electronic submission
REVIEW DIVISION:	Division of Drug Oncology Products (HFD-150)
PHARM/TOX REVIEWER:	M. Anwar Goheer, Ph.D.
PHARM/TOX SUPERVISOR:	John K. Leighton, Ph.D., D.A.B.T.
DIVISION DIRECTOR:	Robert Justice, M.D., M.S.
PROJECT MANAGER:	Dorothy W. Pease /Capt. Frank H. Cross Jr.
Date of review submission to Division File System (DFS):	March 11, 2008

## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

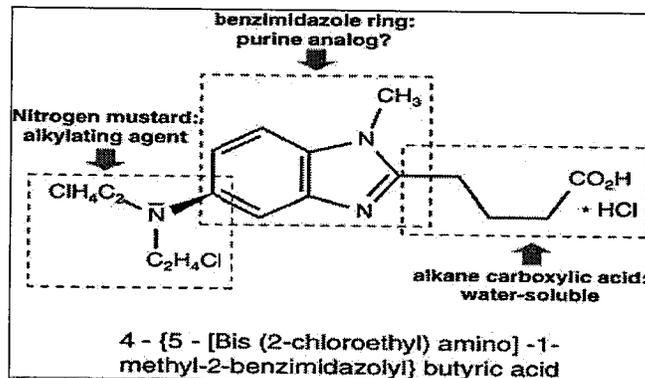
**NDA number:** 22-249  
**Review number:** 2 (labeling review)  
**Sequence number/date/type of submission:** 001 / 09-19-2007 / NDA  
**Information to sponsor:** Yes (X) No ( )  
**Sponsor and/or agent:** Cephalon, Inc.  
 41 Moores Road, Frazer, PA 19355  
**Manufacturer for drug substance:**

**Reviewer name:** M. Anwar Goheer, Ph.D.  
**Division name:** Division of Drug Oncology Products  
**HFD #:** 150  
**Review completion date:** March 11, 2008

#### Drug:

**Trade name:** Treanda (proposed),  
 Cytostasan<sup>®</sup> (Germany) and Ribomustine<sup>®</sup> (Germany)  
**Generic name:** N/A  
**Code name:** BM1, CEP-18083, ID00039, ID00275, ID08736, IMET3393,  
 M000275, M000039, M008736, SDX-105, and ZIMET3393  
**Chemical name:** Bendamustine hydrochloride.  
 1H-Benzimidazole-2-butanoic acid, 5-[bis(2-chloroethyl)amino]-1-methyl- monohydrochloride, or  
 2-Benzimidazole butyric acid, 5-[bis(2-chloroethyl)amino]-1-methyl-, monohydrochloride.  
**CAS registry number:** 3543-75-7  
**Molecular formula/molecular weight:** C<sub>16</sub>H<sub>21</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub>.HCl /  
 and 394.7 (hydrochloride)

#### Structure:



**Relevant INDs/NDAs/DMFs:** IND 67,554  
**Drug class:** Cytotoxic alkylating agent  
**Intended clinical population:** Chronic Lymphocytic Leukemia (CLL)  
**Clinical formulation:** Lyophilized powder for injection (100 mg/vial)

Composition of drug product

Component	Reference to Standard	Function	Amount per Vial
Bendamustine HCl	In house standard	Active Ingredient	100 mg
Mannitol	USP		170 mg
			-
			-

(Excerpted from the sponsor's submission)

**Route of administration:** Intravenous infusion over 30 minutes

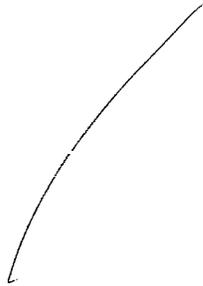
**Proposed Use:** Treanda® is indicated for the treatment of patients with chronic lymphocytic leukemia (CLL). The recommended dose is 100 mg/m<sup>2</sup> administered as an intravenous infusion over 30 minutes on days 1 and 2 of a 28 days cycle, up to 6 cycles.

**Disclaimer:** Tabular and graphical information are constructed by the reviewer unless cited otherwise.

This NDA was submitted pursuant to section 505(b)(2) of the Federal Food, Drug, and Cosmetic Act, for Treanda (bendamustine hydrochloride).

**Data reliance :** Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 22-249 are owned by Cephalon Inc. or are data for which Cephalon has obtained a written right of reference. Any information or data necessary for approval of NDA 22-249 that Cephalon does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Cephalon does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 22-249.

The following recommendations to the sponsor's proposed labeling are given. The Sponsor's proposed wording is followed by the recommendation with the rationale for the recommended changes.



---



---

5 Page(s) Withheld

       Trade Secret / Confidential

✓ Draft Labeling

       Deliberative Process

## 15 REFERECES

1. Clinical reference.
2. Preventing occupational exposures to Antineoplastic and Other Hazardous Drugs in Health Care Settings. NIOSH Alert 2004-165.
- 3 OSHA Technical Manual, TED 1-0.15A, Section VI: Chapter 2. Controlling Occupational Exposure to Hazardous Drugs. OSHA, 1999.  
[http://www.osha.gov/dts/osta/otm/otm\\_vi/otm\\_vi\\_2.html](http://www.osha.gov/dts/osta/otm/otm_vi/otm_vi_2.html)
- 4 American Society of Health-System Pharmacists. ASHP Guidelines on Handling Hazardous Drugs. *Am J Health-Syst Pharm.* 2006; 63:1172-1193.
- 5 Polovich, M., White, J. M., & Kelleher, L.O. (eds.) 2005. Chemotherapy and biotherapy guidelines and recommendations for practice (2nd. ed.) Pittsburgh, PA: Oncology Nursing Society.

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

/s/

-----  
Anwar Goheer  
3/11/2008 03:25:05 PM  
PHARMACOLOGIST

John Leighton  
3/11/2008 03:50:24 PM  
PHARMACOLOGIST



DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH

## PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 22-249  
SERIAL NUMBER: 001  
DATE RECEIVED BY CENTER: 09/19/2007  
PRODUCT: Treanda® (bendamustine hydrochloride)  
INTENDED CLINICAL POPULATION: Chronic Lymphocytic Leukemia (CLL)  
SPONSOR: Cephalon, Inc.  
DOCUMENTS REVIEWED: Electronic submission  
REVIEW DIVISION: Division of Drug Oncology Products  
(HFD-150)  
PHARM/TOX REVIEWER: M. Anwar Goheer, Ph.D.  
PHARM/TOX SUPERVISOR: John K. Leighton, Ph.D., D.A.B.T.  
DIVISION DIRECTOR: Robert Justice, M.D., M.S.  
PROJECT MANAGER: Dorothy W. Pease

Date of review submission to Division File System (DFS): February 27, 2008

## **TABLE OF CONTENTS**

EXECUTIVE SUMMARY	3
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW	7
<b>2.6.1 INTRODUCTION AND DRUG HISTORY.....</b>	<b>7</b>
<b>2.6.2 PHARMACOLOGY.....</b>	<b>13</b>
2.6.2.1 Brief summary.....	13
2.6.2.2 Primary pharmacodynamics.....	14
2.6.2.3 Secondary pharmacodynamics.....	34
2.6.2.4 Safety pharmacology.....	37
2.6.2.5 Pharmacodynamic drug interactions.....	43
<b>2.6.3 PHARMACOLOGY TABULATED SUMMARY.....</b>	<b>44</b>
<b>2.6.4 PHARMACOKINETICS/TOXICOKINETICS.....</b>	<b>44</b>
2.6.4.1 Brief summary.....	45
2.6.4.2 Methods of Analysis.....	46
2.6.4.3 Absorption.....	47
2.6.4.4 Distribution.....	49
2.6.4.5 Metabolism.....	56
2.6.4.6 Excretion.....	67
2.6.4.7 Pharmacokinetic drug interactions.....	64
2.6.4.8 Other Pharmacokinetic Studies.....	69
2.6.4.9 Discussion and Conclusions.....	69
2.6.4.10 Tables and figures to include comparative TK summary.....	70
<b>2.6.5 PHARMACOKINETICS TABULATED SUMMARY.....</b>	<b>70</b>
<b>2.6.6 TOXICOLOGY.....</b>	<b>70</b>
2.6.6.1 Overall toxicology summary.....	71
2.6.6.2 Single-dose toxicity.....	73
2.6.6.3 Repeat-dose toxicity.....	75
2.6.6.4 Genetic toxicology.....	100
2.6.6.5 Carcinogenicity.....	110
2.6.6.6 Reproductive and developmental toxicology.....	113
2.6.6.7 Local tolerance.....	124
2.6.6.8 Special toxicology studies.....	126
2.6.6.9 Discussion and Conclusions.....	128
2.6.6.10 Tables and Figures.....	129
<b>2.6.7 TOXICOLOGY TABULATED SUMMARY.....</b>	<b>129</b>
<b>OVERALL CONCLUSIONS AND RECOMMENDATIONS.....</b>	<b>130</b>
<b>APPENDIX/ATTACHMENTS.....</b>	<b>130</b>

## EXECUTIVE SUMMARY

### I. Recommendations

- A. Recommendation on approvability: The non-clinical studies submitted to this NDA provide sufficient information to support the use of Treanda® (bendamustine hydrochloride) for the treatment of patients with chronic lymphocytic leukemia (CLL).
- B. Recommendation for nonclinical studies: No additional non-clinical studies are required.
- C. Recommendations on labeling: A separate review will be conducted.

### II. Summary of nonclinical findings

A. **Brief overview of nonclinical findings:** Bendamustine hydrochloride [Treanda®, Cytostasan® (Germany), and Ribomustine® (Germany)] belongs to bifunctional nitrogen mustards. Nitrogen mustard and its derivatives are alkylating drugs which dissociate into electrophilic alkyl groups. These groups form covalent bonds with electron-rich nucleophilic moieties. The bifunctional covalent linkage produced can lead to cell death via several pathways. The precise mechanism of action of bendamustine has not been fully characterized.

**Pharmacology:** The cytotoxic activity of bendamustine, bendamustine impurities, and bendamustine degradation products had been studied in human tumor cell lines. The antineoplastic activity of bendamustine was tested *in vivo* in xenograft models. In both *in vivo* and *in vitro* tests, bendamustine showed cell cycle effects analogous to other alkylating drugs including cyclophosphamide and chlorambucil.

**Safety pharmacology:** During safety pharmacology testing, bendamustine at 20 and 25 mg/kg affected the urine output, urinary electrolyte (kaliuretic and natriuretic) and glomerular filtration rate (creatinine clearance) in Sprague Dawley rats, suggesting dysfunction of glomerular filtration. Histopathology lesions in the kidney were also found in the pivotal rat and dog studies described below. Bendamustine hydrochloride perfused at concentrations of 1.5 and 7.5 µg/mL in isolated canine Purkinje fibers had no statistically significant effect on action potential parameters. Bendamustine at 20 µM and 200 µM significantly inhibited the hERG tail current amplitude and caused a significant deceleration of the tail current decay time constant in HEK 293 cells stably expressing the potassium channel. No effects on hERG channel were seen at 2 µM. The clinical significance of these findings are not clear. However, cardiotoxicity was also noted upon histopathological evaluation in the rat and dog pivotal toxicity studies. Heart failure was also noted in the clinical trials. This finding may translate into adverse effects that need to be fully explored in future clinical trials. A QT evaluation in patients is planned as part of post-marketing (phase 4 commitments).

**Pharmacokinetics:** Bendamustine was metabolized by both dog and human microsomes mainly by Phase 1 metabolism to produce an oxidative metabolite and an N-desmethyl metabolite. These processes appeared to be CYP1A2 mediated. Two active

circulating metabolites in human and dog liver preparations were  $\gamma$ -hydroxybendamustine (M3) and N-desmethylbendamustine (M4). The highest tissue levels of bendamustine were in the kidney and liver. The mass balance study in rats showed that significant radioactivity was recovered in both feces (~50%) and urine (~37%). The majority of radioactivity was eliminated rapidly and remained constant from 1 hour to 24 hours.

**Toxicology:** During traditional toxicity assessment, the acute (single dose) toxicity studies were conducted in mice and rats to determine the lethal doses using various routes and are reported in the literature (non-GLP). Bendamustine at 80, or 160 mg/kg/day by stomach tube for 28 consecutive days produced 50% mortalities in rats. Body weight gain and food consumption decreased in a dose dependent manner. Total white blood count and lymphocytes partly recovered in the surviving animals during the recovery phase. There was a dose dependent atrophy of the thymus and inhibition of bone marrow hematopoiesis. Bendamustine at 40 and 60 mg/kg by stomach tube for 90 consecutive days produced 70% and 100% mortalities in Wistar rats, respectively, between week 7 and 14. Prior to death, respiration was impaired and the abdomen of the animals was extremely swollen. White blood cells and lymphocytes were decreased in bendamustine animals. Histopathologically, lymph nodes, spleen and thymus were atrophic.

**Pivotal repeat dose toxicity studies** of 15-weeks intermittent dosing were conducted in Sprague-Dawley rats and beagle dogs (GLP). Male and female  $\pm$  SD (SD) rats (20/sex/group) were dosed bendamustine at 5, 10, or 15 mg/kg/day via 30 minute intravenous infusion once daily for 3 consecutive days in each of five dose cycles. Each dose cycle consisted of 21 days (3 dose days followed by 18 non-dose days). Mortalities (28) occurred in control and test animals throughout the study. Swollen ventral abdomens were observed in control and treated animals. This may be due to infusion apparatus and decreased white blood counts observed in the bendamustine treated groups. Hematological evaluations showed a dose-related decrease in white blood cell and absolute lymphocyte counts. Treatment related microscopic changes were in the kidney (tubular degeneration/ necrosis), heart (cardiomyopathy, focal/multifocal, in male animals only), and bone marrow hyperplasia (femur and sternum). As pointed out in the Pharmacokinetic section, the kidney had high levels of bendamustine and was a significant route of excretion. Bone marrow hyperplasia was not dose-related (4 animals given 5 mg/kg/dose, 1 female given 10 mg/kg/dose, and 1 male given 15 mg/kg/day). All animals with bone marrow hyperplasia died at unscheduled intervals and absence of it in surviving animals suggests that the findings were not test article related or that the timing of the observation was not optimal. The plasma concentrations of bendamustine and both metabolites (M3 & M4) were dose-related over the dose range evaluated. Systemic exposure to bendamustine did not appear to differ consistently with respect to the sex of the animal or the day of dosing. The apparent  $t_{1/2}$  values ranged from 0.14 to 0.36 hr. Concentrations of M3 and M4 were typically below the limit of quantitation by 2 and 1 hour, respectively, after the start of the infusion.

Bendamustine hydrochloride was administered to beagle dogs (3/sex/group) at dosage levels of 1.65, 3.3 or 6.6 mg/kg/day by 30 minute daily intravenous infusion over 4

consecutive days for a total of three treatment cycles. Each cycle was followed by a 31 days recovery period. Three high dose animals (2 males & 1 female) showed deterioration of health and were killed on humane grounds during the recovery phase of the second treatment. Remaining high dose animals were killed on day 29 of this period. Brown/yellow liquid vomitus was noted in treated animals. Body weight loss and reduction in food consumption were noted in both sexes in a dose-related manner. Heart rates were reduced during cycle 2 at 6.6 mg/kg/day (2 males & 1 female, 3/6 animals). Myocardial interstitial inflammation, left atrioventricular valve hemorrhage and leukocytosis were observed in high dose animals. Reduction in WBC counts and lymphocytes were observed in a dose-related manner. Bone marrow suppression (decreased myeloid cells) was observed in animals sacrificed on humane grounds. Lymphoid tissues of high dose animals showed marked or severe changes indicating immunosuppression. Bendamustine also affected testes (seminiferous tubular atrophy), and resulted in mucosal congestion and hemorrhage in the intestines. Systemic exposure was demonstrated at all three dose levels and was dose proportional in cycle 3.

**Genetic toxicology:** Bendamustine induced mutation in Ames test with or without metabolic activation. In the *in vitro* chromosome aberration assay using human lymphocytes, bendamustine produced chromosome aberrations in the presence and absence of metabolic activation. Bendamustine also induced a significant increase in the incidence of micronucleated polychromatic erythrocytes in male and female rats. Hydroxy bendamustine (M3) in the presence and absence of metabolic activation also induced structural chromosomal aberration in human lymphocytes *in vitro*. Therefore, bendamustine is a genotoxic alkylating agent.

**Carcinogenicity:** Although study design in the published paper (Arch Geschwulstforsch 1974; 43(1):16-21) was not adequate to fully assess the carcinogenic potential of bendamustine, intraperitoneal injections of bendamustine for four days produced peritoneal sarcoma in mice. Oral administration for four days induced mammary carcinoma and pulmonary adenomas in mice. Pulmonary adenoma showed signs of malignancy accompanied by pleural rupture dedifferentiation. The evaluations of carcinogenic potentials for oncology drugs are usually not required.

**Developmental and reproductive toxicity:** Embryo-fetal developmental studies were not conducted by the sponsor. Published non-GLP studies referenced by the sponsor have not been adequately conducted to fully evaluate the developmental toxicity of bendamustine. During embryo-fetal developmental toxicity study, intraperitoneal administration of bendamustine produced embryotoxic and teratogenic effects in mice. Malformations observed included exencephaly, cleft palates, and dwarfism (decreased body weights). Bendamustine (ip administration) also caused external (bent/circinate tail) and internal (hydronephrosis and hydrocephalus) malformation in Wistar rats. These developmental findings clearly demonstrate bendamustine as a nitrogen mustard alkylating agent.

**Local tolerance:** Perivenous injection of bendamustine at a concentration of 0.6 or 1.0 mg/ml or intra-arterial injection at a concentration of 0.2 or 0.6 mg/mL produced local irritation at the injection sites in the ear of New Zealand White rabbits.

**B. Pharmacologic activity:** Bendamustine is a bifunctional nitrogen mustard derivative. Nitrogen mustard and its derivatives are alkylating agents which dissociate into electrophilic alkyl groups. These groups form covalent bonds with electron-rich nucleophilic moieties. This bifunctional covalent linkage produced can lead to cell death via several pathways. The exact mechanism of action of bendamustine remains unknown.

**C. Nonclinical safety issues relevant to clinical use:** Reduction in WBC and lymphocytes were observed in a dose related manner during pivotal repeat dose toxicity studies in rats and dogs. Treatment related microscopic changes were seen in kidneys (tubular degeneration/necrosis) in both species. Cardiomyopathy (focal/multifocal) was observed in male rats only. Heart rates of dogs at 6.6 mg/kg/day were reduced during cycle 2 (2 males & 1 female, 3/6 animals). A vigilant monitoring of QT prolongation is warranted until more clinical experience is gained; a clinical study is planned as a part of post-marketing. Bendamustine is mutagenic, carcinogenic, and teratogenic like other nitrogen mustard alkylating drugs.

**APPEARS THIS WAY  
ON ORIGINAL**

## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

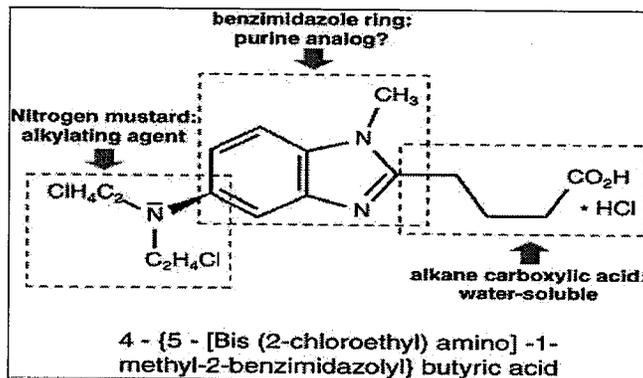
**NDA number:** 22-249  
**Review number:** 1  
**Sequence number/date/type of submission:** 001 / 09-19-2007 / NDA  
**Information to sponsor:** Yes ( ) No (X)  
**Sponsor and/or agent:** Cephalon, Inc.  
 41 Moores Road, Frazer, PA 19355  
**Manufacturer for drug substance:**

**Reviewer name:** M. Anwar Goheer, Ph.D.  
**Division name:** Division of Drug Oncology Products  
**HFD #:** 150  
**Review completion date:** February 27, 2008

#### Drug:

**Trade name:** Treanda (proposed),  
 Cytostasan<sup>®</sup> (Germany) and Ribomustine<sup>®</sup> (Germany)  
**Generic name:** N/A  
**Code name:** BM1, CEP-18083, ID00039, ID00275, ID08736, IMET3393,  
 M000275, M000039, M008736, SDX-105, and ZIMET3393  
**Chemical name:** Bendamustine hydrochloride.  
 1H-Benzimidazole-2-butanoic acid, 5-[bis(2-chloroethyl)amino]-1-methyl- monohydrochloride, or  
 2-Benzimidazole butyric acid, 5-[bis(2-chloroethyl)amino]-1-methyl-, monohydrochloride.  
**CAS registry number:** 3543-75-7  
**Molecular formula/molecular weight:**  $C_{16}H_{21}Cl_2N_3O_2 \cdot HCl$  /  
 \_\_\_\_\_ and 394.7 (hydrochloride)

Structure:



**Relevant INDs/NDAs/DMFs:** IND 67,554  
**Drug class:** Cytotoxic alkylating agent  
**Intended clinical population:** Chronic Lymphocytic Leukemia (CLL)  
**Clinical formulation:** Lyophilized powder for injection (100 mg/vial)

Composition of drug product

Component	Reference to Standard	Function	Amount per Vial
Bendamustine HCl	In house standard	Active Ingredient	100 mg
Mannitol	USP		170 mg
			-
			-

(Excerpted from the sponsor's submission)

**Route of administration:** Intravenous infusion over 30 minutes

**Proposed Use:** Treanda® is indicated for the treatment of patients with chronic lymphocytic leukemia (CLL). The recommended dose is 100 mg/m<sup>2</sup> administered as an intravenous infusion over 30 minutes on days 1 and 2 of a 28 days cycle, up to 6 cycles.

**Disclaimer:** Tabular and graphical information are constructed by the reviewer unless cited otherwise.

This NDA was submitted pursuant to section 505(b)(2) of the Federal Food, Drug, and Cosmetic Act, for Treanda (bendamustine hydrochloride).

**Data reliance :** Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 22-249 are owned by Cephalon Inc. or are data for which Cephalon has obtained a written right of reference. Any information or data necessary for approval of NDA 22-249 that Cephalon does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Cephalon does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 22-249.

**Studies reviewed within this submission:****4.2.1 Pharmacology****4.2.1.1 Primary Pharmacodynamics**

- 4.2.1.1.1 Effect of Bendamustine on Different Human Tumour Cell Lines *In vitro*. Study 0640.00.C7.04.
- 4.2.1.1.5 Cell Cycle Alterations by Bendamustine in Comparison with Other Cytotoxic Agents. Study 0640.00.C7.01.
- 4.2.1.1.6 The Efficacy of Bendamustine is Slightly Decreased by P-glycoprotein and MXR/BCRP Resistance Mechanisms. Study 0640.00.C7.02
- 4.2.1.1.9 Bendamustine Hydrochloride and the Antitumor Drug Screen Program of the National Cancer Institutes (NCI). Study F-DE-NCI-2004.
- 4.2.1.1.7 Analysis of the Cytotoxic Potential of Bendamustine Hydrochloride on Human Lymphocytes as Compared to its Degradation and By-Products. Study 744303.
- 4.2.1.1.8 Analysis of the Cytotoxic Potential of Bendamustine Hydrochloride on Tumor Cell Lines as Compared to its Degradation Products. Study 754900.
- 4.2.1.1.10 Analysis of the Cytotoxic Potential of Bendamustine Hydrochloride on Tumor Cell Lines as Compared to its Metabolite N-Desmethyl Bendamustine. Study 789401
- 4.2.1.1.11 Analysis of the Cytotoxic Potential of Bendamustine Hydrochloride on Tumor cell Lines as Compared to its Metabolite N-Desmethyl Bendamustine. Study 789403.
- 4.2.1.1.2 Effect of Bendamustine Hydrochloride By-Products and  on Tumour Cell Growth. Study 0640.01.C07.06.
- 4.2.1.1.3 Efficacy of Bendamustine Hydrochloride on the Human Mammary Carcinoma MDA-MB 231 in the NMRI nu/nu Mouse after Intravenous Treatment. Study 0640.00.C8.02.
- 4.2.1.1.4 Efficacy of Bendamustine Hydrochloride on the Human Lung Carcinoma LX-1 in the NMRI nu/nu Mouse after Intravenous Treatment. Study 0640.00.C08.01.
- 4.2.1.1.13 Efficacy Dose Response for SDX-105 in a Xenograft Model of SUDHL-1 in SCID Mice. Study T109.
- 4.2.1.1.14 Efficacy of SDX-105 and Rituxan in a Xenograft Model of Daudi in SCID Mice. Study T116.
- 4.2.1.1.15 Efficacy of SDX-105 and Rituxan in a Xenograft Model of Daudi in SCID Mice. Study T124.
- 4.2.1.1.16 Dose Response of SDX-105 and SDX-101 in a Xenograft Model of Daudi in SCID Mice. Study T110.

**4.2.1.2 Secondary Pharmacodynamics**

- 4.2.1.2.1 Effect of Bendamustine Hydrochloride on Different Non-Malignant Cells of Mice and Humans in Comparison to Other Well-Known Cytostatic Drugs. Study 0640.01.C07.07.

- 4.2.1.2.2 Determination of the Cytotoxic Potential of SDX-105 in Cultured Human Hepatocytes after *In vitro* Exposure. Study DM-2005-002.
- 4.2.1.1.12 Cytotoxicity Assay *In vitro* with BALB/C3T3 Cells: Neutral Red (NR) Test with Bendamustine Hydrochloride at Simultaneous Irradiation with Artificial Sunlight. Study 789402.

#### **4.2.1.3 Safety Pharmacology**

- 4.2.1.3.1 Evaluation of Effect on Urine Output, Urinary Electrolyte Balance and Glomerular Filtration Rate in the Rat with a Saline Overload Following Two Successive 30-Minute Intravenous Infusions. Study 20010337 PGR.
- 4.2.1.3.2 Evaluation of Effect on Cardiac Action Potential in Isolated Canine Purkinje Fibers. Study 20010339 PECM.
- 4.2.1.3.3 Bendamustine Hydrochloride: Effects on HERG-1 Tail Currents Recorded from Stably Transfected HEK 293 Cells. Study 853896.

#### **4.2.1.4 Pharmacodynamic Drug Interactions. N/A**

#### **4.2.2 Pharmacokinetics**

##### **4.2.2.1 Analytical Methods and Validation Reports**

- 4.2.2.1.1 Validation of a High Performance Liquid Chromatographic Method for the Measurement of Bendamustine and Two Major Metabolites in Dog Plasma and Urine. Study KLG-09.
- 4.2.2.1.2 Validation Report: Determination of Bendamustine M3 Metabolite, and M4 Metabolite in K2EDTA rat plasma. Study DP-2007-030.

##### **4.2.2.2 Absorption**

- 4.2.2.2.1 Studies on the Pharmacokinetics of Bendamustine [ $^{14}\text{C}$ ] in the Rat. Study DM-2006-012.

##### **4.2.2.3 Distribution**

- 4.2.2.3.1 Disposition of  $^{14}\text{C}$ -Bendamustine in Mice and Rats. Study DM-2007-001.
- 4.2.2.3.2 The Tissue Distribution of Total Radioactivity in the Rat Following Intravenous Administration of [ $^{14}\text{C}$ ]-CEP-18083. Study DM-2005-006.
- 4.2.2.3.3 The Tissue Distribution of Total Radioactivity in the Pigmented Rat Following Intravenous Administration of [ $^{14}\text{C}$ ]-CEP-18083.HCl (Quantitative Whole Body Autoradiography). Study Am 02 DM-2005-007.
- 4.2.2.3.4 Excretion and Distribution Studies of  $^{14}\text{C}$ -Bendamustine in the Dog. Study KLG-05.

#### 4.2.2.4 Metabolism

4.2.2.4.1 *In vitro* Evaluation of CEP-18083 (Bendamustine) as an Inducer of Cytochrome P450 Expression in Cultured Human Hepatocytes. Study DM-2005-004.

4.2.2.4.2 *In vitro* Metabolism studies of <sup>14</sup>C-Bendamustin. Study 99-37-KLG-01.

4.2.2.4.4 *In vitro* Plasma Protein Binding Studies of Bendamustine. Study KLG-06.

4.2.2.4.3 Metabolic Profile of [<sup>14</sup>C]-CEP-18083 (Bendamustine) in Rat Urine and Bile: Preliminary Structural Identification of Metabolites. Study DM-2006-002.

#### 4.2.2.5 Excretion

4.2.2.5.1 The Disposition of [<sup>14</sup>C]-CEP-18083 in the Rat Following Intravenous Administration. Study Am 01 DM-2005-005.pdf

4.2.2.6 Pharmacokinetic Drug Interactions: N/A

4.2.2.7 Other Pharmacokinetic Studies: N/A

#### 4.2.3 Toxicology

##### 4.2.3.1 Single Dose Toxicity Studies

4.2.3.1.1 Bendamustine Single-Dose Toxicity Study in Mice and Rats. Haertl 1989.

##### 4.2.3.2 Repeat Dose Toxicity Studies

4.2.3.2.1 5-Day Intermittent Intravenous Infusion Dose Range Finding Toxicity Study with CEP-18083 (Bendamustine) in Rats with a 16-Day Recovery Period. Study DS-2006-011.

4.2.3.2.2 15-Week Intermittent Intravenous Infusion Toxicity and Toxicokinetic Study with CEO-18083 (Bendamustine) in Rats with a 4-Week Recovery Period. Study DS-2006-010.

4.2.3.2.5 Bendamustine One-Month Oral Toxicity Study in Rats. Horn et al 1984.

4.2.3.2.6 Bendamustine 3-Months Oral Toxicity Study in Rats. Janowski 1985

4.2.3.2.3 Bendamustine Hydrochloride Maximum Tolerated Dose and Five Day Repeated Dose Study in Dogs by Intravenous Infusion. Study 0640.98.C2.01.

4.2.3.2.4 Bendamustine Hydrochloride Toxicity to Dogs by Daily Intravenous Infusion Over a Minimum of Three 4-Day Cycles Each Followed by a Period Without Treatment of up to 31 Days. Study 0640.98.C2.02.

##### 4.2.3.3 Genotoxicity Studies

4.2.3.3.1 Bendamustine Hydrochloride Bacterial Mutation Assay. Study 0640.00.C4.01.

4.2.3.3.2 Bendamustine Hydrochloride *In vitro* Mammalian Chromosome Aberration Test in Human Lymphocytes. Study 0640.00.C4.02.

4.2.3.3.3 CEP-18083: Rat Bone Marrow Erythrocyte Micronucleus Test. Study DS-2007-001.

4.2.3.3.4 Chromosome Aberration Test in Human Lymphocytes *In vitro* with Hydroxy-Bendamustine (HP1). Study 831200.

**4.2.3.4 Carcinogenicity:** N/A

**4.2.3.5 Reproductive and Developmental Toxicity:** N/A

**4.2.3.6 Local Tolerance**

4.2.3.6.1 Perivenous and Intra-Arterial Tolerance Study in the Rabbit. Study 0640.00.C14.01.

**4.2.3.7 Other Toxicity Studies:**

4.2.3.1.2 Estimate the LD<sub>50</sub> Value of \_\_\_\_\_, in Female BDF-1 Mice. Study PT-VIV-108.

**4.3 Literature References**

**Carcinogenicity:**

Oncogenicity of  $\gamma$ -[1-methyl-5-bis-( $\beta$ -chloroethyl)-amino-benzimidazolyl-(2)] -butyric acid hydrochloride in mice. Guttner, Bruns, and Junstand, Arch Geschwulstforsch 43/1: 16-21, 1974.

**Reproductive and Developmental Toxicity:**

**Embryofetal Development**

On the embryotoxic and teratogenic action of the nitrogen mustard derivatives IMET 3393 and IMET 3106 in mice. Heinecke & Klaus, Zbl Pharm 10(10):1067-76, 1971.

The Effect of Nitrogen Mustard Compounds on the Formation of Accessory Ribs in the Fetuses of Mice. Heinecke & Klaus, Arzneimittelforschung 22(1):122-5, 1972.

On the Effect of the "Cytostasan" Mustard Derivative on Murine Pregnancy and Embryonic Development. Wendler, Pabst, and Bertolini, Anat Anz.Bd 139: 100-114, 1976.

**Studies not reviewed within this submission:** None



human small cell lung carcinoma LX-1 in nude mice. In both *in vivo* and *in vitro* tests, bendamustine showed cell cycle effects analogous to other alkylating agents including cyclophosphamide and chlorambucil.

During safety pharmacology testing, bendamustine at 20 and 25 mg/kg affected the urine output, urinary electrolyte (kaliuretic and natriuretic) and glomerular filtration rate (creatinine clearance) in Sprague Dawley rats, suggesting dysfunction of glomerular filtration. A dose of 15 mg/kg had no toxicologically significant effects on renal function.

Effect of bendamustine hydrochloride on action potential was assessed in isolated canine Purkinje fibers. The concentrations of bendamustine hydrochloride (1.5, 4.5, or 7.5  $\mu\text{g}/\text{mL}$ ) were chosen on the basis of maximal plasma drug concentrations ( $C_{\text{max}}$ : 30  $\mu\text{g}/\text{mL}$ ) obtained at an effective pharmacological dose and taking into account a protein binding of 95%, i.e. 1.5  $\mu\text{g}/\text{mL}$  = 5% of 30  $\mu\text{g}/\text{mL}$ . Bendamustine had no statistically significant effect on action potential parameters including amplitude, resting potential, maximal rate of depolarization, and action potential duration under normal (60 ppm) and slow (20 ppm) stimulation rates.

Safety pharmacology study for assessing the potential for delayed ventricular repolarization (QT interval prolongation) by bendamustine was examined by whole cell patch-clamp technique in HEK 293 cells stably expressing potassium channels. Bendamustine at 20 and 200  $\mu\text{M}$  significantly inhibited the HERG-1 tail current amplitude in a dose-dependent manner and caused a significant deceleration of the tail current decay time constant. It was not possible to fit a concentration-response curve and to estimate an  $\text{IC}_{50}$  value for the current inhibition. Bendamustine at two  $\mu\text{M}$  was without effect. Reviewing Medical Officers and Team Leader for this NDA have been informed for these findings.

#### 4.2.1.1 Primary Pharmacodynamics

##### 4.2.1.1.1 Effect of Bendamustine on Different Human Tumour Cell Lines In Vitro. Study 0640.00.C7.04.

#### Key study findings:

- Bendamustine inhibited the cell growth of breast, ovary, and lung cancer cell lines and leukemic tumor cell lines with an IC<sub>50</sub> values between 4 and 500 µM depending on the cell line and the condition of the assay.
- Bendamustine exerted its maximum effect within one hour of incubation.

**Methods:** All cell lines were cultured and maintained in their recommended mediums. The medium containing different concentrations of the test compound were added to the cell culture. In the case of leukemic cell lines, the metabolic activity of the cells was determined by performing the WST-1 assay (Water-soluble stable tetrazolium salt). For adherent cell lines, the total protein content was selected as a sensitivity parameter using the BCA reaction (bicinchoninic acid).

#### Results:

Human tumor cell lines sensitivity towards bendamustine (IC<sub>50</sub>)

CELL LINE	TISSUE	IC <sub>50</sub> VALUE [µM] (on day 5 or 6)
MCF-7 M1	breast	50
MDA-MB-231	breast	80-90
T47D	breast	20
ZR-75-1	breast	100
MDA-MB-435	breast	200
MDA-MB-453	breast	20
LX-1	lung, undifferentiated	80
A549	NSCLC	200
Calu-3	NSCLC	400-500
NCI-H23	NSCLC	50-60
NCI-H358	NSCLC	70-80
NCI-H69	SCLC	4
NCI-H146	SCLC	6
IGROV-1	ovary	200-300
A2780	ovary	100
JAT	ovary	60-70
OVCAR-3	ovary	30
Jurkat	ALL (T-cell)	60
CCRF-CEM	ALL (T-cell)	100-200
CCRF-SB	ALL (B-cell)	20
HL-60	AML	200
THP-1	AML	100-200
KG-1	AML	20
K562	CML	60
Namalwa	non-Hodgkin's	10

(Excerpted from the sponsor's submission)

## Comparison of bendamustine with other alkylating agents

CELL LINE	CYTOSTATIC DRUG	IC <sub>50</sub> VALUE [M]		
		DAY 1	DAY 2	DAY 5 / 6
MDA-MB-435	bendamustine	7 x 10 <sup>-4</sup>	2 x 10 <sup>-4</sup>	1-2 x 10 <sup>-4</sup>
	melphalan	2-3 x 10 <sup>-4</sup>	4 x 10 <sup>-4</sup>	1 x 10 <sup>-5</sup>
	chlorambucil	3 x 10 <sup>-4</sup>	6 x 10 <sup>-5</sup>	5 x 10 <sup>-5</sup>
	doxorubicin	--	7 x 10 <sup>-7</sup>	5 x 10 <sup>-8</sup>
	mitoxantrone	6 x 10 <sup>-7</sup>	2 x 10 <sup>-7</sup>	5-6 x 10 <sup>-9</sup>
	paclitaxel	6 x 10 <sup>-9</sup>	3-4 x 10 <sup>-9</sup>	1-2 x 10 <sup>-9</sup>
	vincristine	3 x 10 <sup>-10</sup>	2-3 x 10 <sup>-10</sup>	1 x 10 <sup>-10</sup>
	5-FU	--	--	5-6 x 10 <sup>-6</sup>
	fludarabine	--	--	4 x 10 <sup>-5</sup>
	methotrexate	--	--	1 x 10 <sup>-7</sup>

(Excerpted from the sponsor's submission)

## Comparison of bendamustine with other alkylating agents

CELL LINE	CYTOSTATIC DRUG	IC <sub>50</sub> VALUE [M]		
		DAY 1	DAY 2	DAY 5 / 6
CCRF-CEM	bendamustine	4 x 10 <sup>-4</sup>	2 x 10 <sup>-4</sup>	2 x 10 <sup>-4</sup>
	melphalan	2 x 10 <sup>-5</sup>	4 x 10 <sup>-6</sup>	3-4 x 10 <sup>-6</sup>
	chlorambucil	1-2 x 10 <sup>-4</sup>	2 x 10 <sup>-5</sup>	3 x 10 <sup>-5</sup>
	doxorubicin	3 x 10 <sup>-7</sup>	5 x 10 <sup>-8</sup>	2-3 x 10 <sup>-8</sup>
	mitoxantrone	2 x 10 <sup>-8</sup>	4 x 10 <sup>-9</sup>	4-5 x 10 <sup>-10</sup>
	paclitaxel	7 x 10 <sup>-9</sup>	3 x 10 <sup>-9</sup>	3 x 10 <sup>-9</sup>
	vincristine	6 x 10 <sup>-10</sup>	3 x 10 <sup>-10</sup>	2-3 x 10 <sup>-10</sup>
	5-FU	--	--	> 10 <sup>-5</sup>
	fludarabine	3 x 10 <sup>-5</sup>	5 x 10 <sup>-6</sup>	8 x 10 <sup>-7</sup>
	methotrexate	--	1 x 10 <sup>-6</sup>	2-3 x 10 <sup>-8</sup>

(Excerpted from the sponsor's submission)

Bendamustine and other alkylating agents exert their growth inhibitory effects depending on the cell lines used and the conditions of the assay.

#### 4.2.1.1.5 Cell Cycle Alterations by Bendamustine in Comparison with Other Cytotoxic Agents. Study 0640.00.C7.01.

##### Key study findings:

- Bendamustine induced cell cycle alterations in THP-1 and CCRF-CEM cells analogous to chlorambucil and melphalan.
- Doxorubicin, methotrexate and vincristine induced cell cycle effects were different to that of bendamustine.

**Methods:** THP-1 (human acute monocytic leukemia) and CCRF-CEM (T-lymphoblastoid leukemia) cells were grown aseptically. Bendamustine or reference compounds were added to the culture medium. One or two days after adding the compounds, cells were stained and analyzed using flow cytometric cell cycle assay.

**Results:** Bendamustine, chlorambucil, and melphalan after one day of treatment in THP-1 and CCRF-CEM cells induced an increase of cells in the early S phase of the cell cycle. This early S phase population may be composed of cells which proceeded from cell cycle arrest to apoptosis. The cell cycle alterations by doxorubicin, methotrexate and vincristine were different than bendamustine.

#### 4.2.1.1.6 **The Efficacy of Bendamustine is Slightly Decreased by P-glycoprotein and MXR/BCRP Resistance Mechanisms.**

Study 0640.00.C7.02.

##### **Key study findings:**

- The efficacy of bendamustine was reduced 7-8 folds in cells over expressing p-glycoprotein and MXR/BCRP (mitoxantrone-resistance protein/breast cancer-resistance protein).

**Methods:** CCRF-CEM, D65, and drug-resistant sublines were grown as suspension cultures. MCF7, 1A9, and their drug-resistant sublines were grown as monolayer cultures. Survival studies were performed in 96-well plates in the presence of different concentrations of bendamustine. ELISA microplate reader was used to quantitate the optical density.

**Results:** The relative resistance was calculated by dividing the IC<sub>50</sub> value of the resistant cells displayed by that of the parental cells.

## Effect of different resistance mechanisms on the efficacy of bendamustine.

Cell line	Resistance mechanism	Relative resistance					
		Bendamustine	Doxorubicin	Mitoxantrone	Etoposide	MTX	Paclitaxel
MCF7	Parent	-	-	-	-	-	-
MCF7 Ad2000	Pgp	7.6	272				
MCF7 AdVp	MXR/BCRP	8.4	74	94			
MCF7/VP	MRP	4.0			32		
MCF7 MTX	DHFR	1.8				455	
D65	Parent	-	-				
D65 Res	Pgp	1.0	524				
CCRF-CEM	Parent	-				-	
CCRF-CEM/T	RFC	1.4				19	
1A9	Parent	-					-
1A9 PTX	Tubulin mut.	2.2					21

Abbreviations: Pgp, P-glycoprotein; MXR/BCRP, mitoxantrone-resistance protein/breast cancer-resistance protein; MRP, multidrug-resistance-associated protein; DHFR, dihydrofolate reductase; RFC, reduced folate carrier; MTX, methotrexate; VP-16, etoposide; PTX, paclitaxel.

\* Statistically significantly different from the parental cells,  $p < 0.05$ .

(Excerpted from the sponsor's submission)

## Sensitivity of parent and resistant cells to bendamustine and various antitumor agents.

Cell line	Resistance mechanism	IC <sub>50</sub> in $\mu$ M	IC <sub>50</sub> in nM				
		Bendamustine	Doxorubicin	Mitoxantrone	Etoposide	MTX	Paclitaxel
MCF7	Parent	33 $\pm$ 12	22 $\pm$ 8	16 $\pm$ 6	300 $\pm$ 173	22 $\pm$ 7	
MCF7 Ad2000	Pgp	250	6000 (6000, 6000)				
MCF7 AdVp	MXR/BCRP	277 $\pm$ 108*	2067 $\pm$ 1071	1500 $\pm$ 866			
MCF7/VP	MRP	133 $\pm$ 65			9667 $\pm$ 577		
MCF7 MTX	DHFR	60				10000	
D65	Parent	510	7 $\pm$ 6				
D65 Res	Pgp	500	3666 $\pm$ 2083				
CCRF-CEM	Parent	54 (28, 80)				14 $\pm$ 6	
CCRF-CEM/T	RFC	78 (26, 130)				269 $\pm$ 69	
1A9	Parent	47 $\pm$ 15					2 $\pm$ 0
1A9 PTX	Tubulin mut.	103 $\pm$ 58					42 $\pm$ 19

Abbreviations: Pgp, P-glycoprotein; MXR/BCRP, mitoxantrone-resistance protein/breast cancer-resistance protein; MRP, multidrug-resistance-associated protein; DHFR, dihydrofolate reductase; RFC, reduced folate carrier; MTX, methotrexate; VP-16, etoposide; PTX, paclitaxel.

\* Statistically significantly different from the parental cells,  $p < 0.05$ .

(Excerpted from the sponsor's submission)

4.2.1.1.9 **Bendamustine Hydrochloride and the Antitumor Drug Screen Program of the National Cancer Institutes (NCI).**  
Study F-DE-NCI-2004.

**Key study findings:**

- Bendamustine did not demonstrate a strong correlation in the NCI screen with any agent.
- Only DTIC (dacarbazine) showed greater than 50% sensitivity agreement with bendamustine.

**Methods:** Bendamustine was tested by the *in vitro* antitumor drug screen program of the NCI and compared with cyclophosphamide and chlorambucil.

**Results:**

NCI 60 Cell line panel results: Average of all cell lines

Compound	Mean GI <sub>50</sub> ( $\mu$ M)	Mean TGI <sub>50</sub> ( $\mu$ M)	Mean LC <sub>50</sub> ( $\mu$ M)
Bendamustine	50	91	100
Cyclophosphamide	70	98	100
Chlorambucil	9.4	34	78

GI<sub>50</sub> = 50% Growth inhibition

TGI = Total growth inhibition

LC<sub>50</sub> = 50% lethality

Comparison of bendamustine sensitivity pattern with other compounds identified by the COMPARE Analysis at NCI

Compound	NSC	Mechanism of action	Correlation (PCC)
DTIC, Dacarbazine	45388	DNA Alkylator, Methylating agent	0.792 (LC <sub>50</sub> )
TOPO1B	376254	Topoisomerase I inhibitor	0.619 (TGI)
N-N-Dibenzyl-Daunomycin	268242	Anthracycline, DNA intercalator (daunomycin analog)	0.574 (TGI)
Melphalan	8806	DNA Alkylator, Nitrogen mustard	0.550 (GI <sub>50</sub> )
YOSHI 864	102627	DNA Alkylator	0.542 (GI <sub>50</sub> )
Ara-AC (Fazarabine)	281272	Antimetabolite and DNA methylation inhibitor	0.524 (TGI)

(Excerpted from the sponsor's submission)

4.2.1.1.7 **Analysis of the Cytotoxic Potential of Bendamustine Hydrochloride on Human Lymphocytes as Compared to its Degradation and By-Products.**  
Study 744303.

**Key study findings:**

- The degradation and by products of bendamustine hydrochloride showed similar or less cytotoxicity than bendamustine hydrochloride with stimulated and non-stimulated human lymphocytes.
- The by-product — with stimulated (phytohemagglutinin treated) and non-stimulated human lymphocytes showed cytotoxic activity about 4 and 10 times higher than bendamustine hydrochloride, respectively.

**Methods:** Human lymphocytes were treated with various concentrations of bendamustine hydrochloride or the degradation and by-products. Cytotoxicity was measured by the analysis of the total metabolic activity of the treated cultures as compared to the controls using the WST-1 test kit.

**Results:**

EC<sub>50</sub>-values of bendamustine hydrochloride and 8 degradations and by-products in human lymphocytes

Compound	EC <sub>50</sub> (µM)	
	Stimulated Lymphocytes	Non-Stimulated Lymphocytes
Bendamustine	40	120
	30	50
	320	80
	>1000	>1000
HP-1	740	>1000
HP-2	>1000	>1000
	ND	20
	10	10
	>1000	210

4.2.1.1.8 **Analysis of the Cytotoxic Potential of Bendamustine Hydrochloride on Tumor Cell Lines as Compared to its Degradation Products.**  
Study 754900.

**Key study findings:** Degradation products of bendamustine did not show higher cytotoxic activity than bendamustine hydrochloride.

**Methods:** Human hepatoma cell line HepG2 and human lymphoblastoid cell line NAMALWA were treated with various concentrations of bendamustine hydrochloride or the degradation products. The cytotoxicity was measured by the WST-1 assay and BCA test kit

**Results:**

EC<sub>50</sub> values of bendamustine hydrochloride and its degradation products

<i>Test item name</i>	<i>EC<sub>50</sub> (mM) with HepG2 cells (WST-1)</i>	<i>EC<sub>50</sub> (mM) with HepG2 cells (BCA)</i>	<i>EC<sub>50</sub> (mM) with NAMALWA cells</i>
Bendamustine hydrochloride	0.38*	0.20*	0.04*
—	0.33	0.24	0.08
—	0.38	0.30	0.37
—	not toxic	not toxic	not toxic

\*= mean of 3 experiments

not toxic = no reduction of viability below 50 % of the negative control up to the highest tested concentration (1 mM).

(Excerpted from the sponsor's submission)

4.2.1.1.10 **Analysis of the Cytotoxic Potential of Bendamustine Hydrochloride on Tumor Cell Lines as Compared to its Metabolite N-Desmethyl Bendamustine.**  
Study 789401.

**Key study findings:** N-desmethyl bendamustine did not show a cytotoxic effect that was higher than the effect of bendamustine hydrochloride.

**Methods:** Human hepatoma cell line HepG2 and human lymphoblastoid cell line NAMALWA were treated with various concentrations of bendamustine hydrochloride or the degradation products. The cytotoxicity was measured by the WST-1 assay and BCA test kit

**Results:**EC<sub>50</sub> values of bendamustine hydrochloride and N-desmethyl bendamustine

Compound	EC <sub>50</sub> (mM)		
	HepG2 cells (WST-1)	HepG2 cells (BCA)	NAMALWA cells
Bendamustine hydrochloride	0.23	0.31	0.01
N-desmethyl bendamustine	0.79	Not toxic	0.06

4.2.1.1.11 **Analysis of the Cytotoxic Potential of Bendamustine Hydrochloride on Tumor cell Lines as Compared to its Metabolite N-Desmethyl Bendamustine.**  
Study 789403.

**Key study findings:** N-desmethyl bendamustine did not show a cytotoxic effect higher than bendamustine hydrochloride.

**Methods:** Primary human lymphocytes and primary human lymphocytes stimulated with phytohemagglutinin (PHA-L) were treated with various concentrations of bendamustine hydrochloride or the metabolite. Cytotoxicity was measured by the analysis of the total metabolic activity of the treated cultures as compared to the controls using the WST-1 test kit

**Results:**EC<sub>50</sub> values of bendamustine hydrochloride and N-desmethyl bendamustine

Compound	EC <sub>50</sub> (mM)	
	Stimulated lymphocytes	Non-stimulated lymphocytes
Bendamustine hydrochloride	0.07	0.33
N-desmethyl bendamustine	0.37	0.75

4.2.1.1.2 **Effect of Bendamustine Hydrochloride By-Products and on Tumour Cell Growth**  
Study 0640.01.C07.06.

**Key study findings:**

- HP-1 and HP-2 (by-products in bendamustine) did not contribute to the antitumor activity of bendamustine in K 562 (human chronic myelogenous leukemia), MDA MB 231 (human breast cancer cells), and HepG2 (human liver carcinoma) cell lines.
- \_\_\_\_\_ was 4-6 more active than bendamustine *in vitro*.

**Methods:** K 562 (human chronic myelogenous leukemia), MDA MB 231 (human breast cancer cells), and HepG2 (human liver carcinoma) cells were cultured according to the suppliers recommendations. Cells were exposed to bendamustine, \_\_\_\_\_, hydroxy-bendamustine (HP-1), \_\_\_\_\_, di-hydroxy-bendamustine (HP-2), \_\_\_\_\_, or \_\_\_\_\_ for different times and their effects on the cell growth were quantitated.

**Results:**

Cytotoxic activity of bendamustine, bendamustine impurities and hydrolysis products in human cancer cell lines

Compound	IC <sub>50</sub> (µM)		
	K562	MDA-MB-231	HepG2
Bendamustine HCl	60-70	55-63	90
HP-1	600	550	>1000
HP-2	>1000	ND	>1000
	190	160	190
	>300	ND*	>1000
	17	8.5	50
	500	600	ND*

ND\* - Not done

4.2.1.1.3 **Efficacy of Bendamustine Hydrochloride on the Human Mammary Carcinoma MDA-MB 231 in the NMRI nu/nu Mouse after Intravenous Treatment.**

**Key study findings:**

- Bendamustine was active against human mammary carcinoma MDA-MB 231 in nude mice.

**Study no.:** 0640.00.C8.02.  
**Volume # and page #:** Module 4.2.1.1.3. (Electronic submission)  
**Conducting laboratory and location:**

/ /

**Date of study initiation:** August 2, 2000  
**QA report:** yes (X) no ( )  
**Drug, lot #, and % purity:** Ribomustine ® (bendamustine),  
batch # 350999, purity not mentioned

### Methods

**Doses:** 25, 37.5, or 50 mg/kg  
**Species/strain:** NMRI nu/nu mouse  
**Number/sex/group or time point (main study):** 4 females per group  
**Route, formulation, volume, and infusion rate:** intravenous  
**Weight:** 18-21 g  
**Unique study design:** MDA-MB 231 mammary carcinoma cells ( $3 \times 10^3$  cells/animal) were inoculated subcutaneously in the left flank of the animals. After the growth of tumor to 24-36 mm<sup>2</sup>, the animals were divided into groups and dosed with bendamustine intravenously once daily on days 1 and 2.

### Observations and times:

Mortality: Daily  
Clinical signs: Daily  
Body weights: Three times weekly  
Tumor size: Three times weekly  
Terminal sacrifice time: Group 1 – Day 11    Group 2 – Day 17  
Groups 3 & 4 – Day 18

### Results

Mortality: 50 mg/kg – 1 animal died on day 5  
Clinical signs: 50 mg/kg – Reduction in activity, moderate cachexia  
Body weights: 50 mg/kg – ↓ 14% on day 7 vs. control

Tumor volume

		Relative Tumor Volume (%)								Activity Rating	
		Day 0	Day 2	Day 4	Day 7	Day 9	Day 11	Day 14	Day 16		Day 18
Group 1 (Control) 0.9% NaCl - Day 1, 2 i.v.	Mean	100	168	280	511	734	1146	-	-	-	
	StdDev	0	35	48	125	234	349	-	-	-	
	n	8	8	8	8	8	8	-	-	-	
	T/C	100.0	94.0	62.8	58.1	52.1	52.7	-	-	-	
Group 2 (Bendamustine) 25 mg/kg - Day 1, 2 i.v.	Mean	100	158	175	197	238	307	866	1091	-	(+)
	StdDev	0	36	77	168	212	355	506	556	-	
	n	8	8	8	8	8	8	8	8	-	
	T/C	100.0	91.6	50.0	32.8	28.3	24.5	-	-	-	
Group 3 (Bendamustine) 37.5 mg/kg - Day 1, 2 i.v.	Mean	100	154	140	168	208	280	492	663	901	+
	StdDev	0	32	48	59	90	104	302	452	550	
	n	7	8	8	8	8	8	8	8	8	
	T/C	100.0	82.1	50.0	24.0	16.2	12.2	-	-	-	
Group 4 (Bendamustine) 50 mg/kg - Day 1, 2 i.v.	Mean	100	138	140	122	119	140	183	210	260	++
	StdDev	0	52	76	89	91	116	212	229	238	
	n	8	8	8	7	7	7	7	7	7	
	T/C	100.0	82.1	50.0	24.0	16.2	12.2	-	-	-	

Statistically significant, Student's *t*-test,  $p \leq 0.05$  when compared with group 1

- = inactive T/C > 75%
- (+) = tumor growth delay T/C > 50% - 75%
- + = tumor inhibition T/C > 25% - 50%
- ++ = tumor stasis T/C ≤ 25% and  $T_x/T_0 > 75\% - 125\%$
- +++ = partial tumor regression  $T_x/T_0 > 10\% - 75\%$
- ++++ = complete remission  $T_x/T_0 \leq 10\%$

T/C = relative tumor volume Test/Control group  
 $T_x/T_0$  = relative tumor volume Test group day<sub>x</sub>/day<sub>0</sub>

(Excerpted from the sponsor's submission)

4.2.1.1.4

**Efficacy of Bendamustine Hydrochloride on the Human Lung Carcinoma LX-1 in the NMRI nu/nu Mouse after Intravenous Treatment.**

**Key study findings**

- Bendamustine showed antitumor activity on human small cell lung carcinoma LX-1 in nude mice.

Study no.:

0640.00.C08.01.

Volume # and page #:

Module 4.2.1.1.4

Conducting laboratory and location:

**Date of study initiation:** August 16, 2000  
**QA report:** yes (X) no ( )  
**Drug, lot #, and % purity:** Ribomustine® (Bendamustine),  
 batch # 350999, purity not mentioned

**Methods**

Doses: 25, 37.5, or 50 mg/kg  
 Species/strain: NMRI nu/nu mouse  
 Number/sex/group or time point (main study): 8 females/group  
 Weight: 17-22 g

Unique study design: Tumor cells were inoculated subcutaneously into the left flank of the animals. After tumor growth between 24 and 42 mm<sup>2</sup>, the animals were assigned to different experimental groups. All animals were treated intravenously once daily on days 1 and 2.

**Observations and times:**

Mortality: Daily  
Clinical signs: Daily  
Body weights: Three times weekly  
Tumor size: Three times weekly  
Terminal kill: Groups 1 and 2 – Day 17 due to large size of tumors  
 Groups 3 and 4 – Day 21

**Results**

Mortality: None  
Clinical signs: Cachexia in high dose animals  
Body weights:

Group	1	2	3	4
Dose (mg/kg)	0	25	37.5	50
Body weight on day 7 vs. initial (%)	100	100	98	91
Tumor volume (T/C) (%)	NA	52	16	8

Tumor volume =  $\{a \times b^2/2\}$  where a is a large diameter and b is a small diameter of the tumor.

N/A – Not applicable

4.2.1.1.13 **Efficacy Dose Response for SDX-105 in a Xenograft Model of SUDHL-1 in SCID Mice.**  
Study T109

**Key study findings:**

- Body weight loss was observed in both cytoxan and SDX-105 (bendamustine) treated mice.
- Tumor volume of bendamustine treated mice reduced in a dose related manner.

**Study no.:** T109  
**Volume # and page #:** Module 4.2.1.1.13.  
**Conducting laboratory and location:** / /  
**Date of study initiation:** August 1, 2003  
**GLP compliance:** No  
**QA report:** yes (X) no ( )  
**Drug, lot #, and % purity:** Cytoxan and SDX-105,  
lot # and purity not provided

**Methods**

**Doses:** Cytoxan (positive control) – 15, 30, 60, or 90 mg/kg/day  
SDX-105 (bendamustine) – 2, 8, 16, or 32 mg/kg/day  
**Species/strain:** SCID mice  
**Number/sex/group or time point (main study):** 5 males/group  
**Route, formulation, volume, and infusion rate:** Intraperitoneal, 0.1 mL  
**Satellite groups used for toxicokinetics or recovery:** No  
**Age:** 8 weeks  
**Weight:** 20-23 g  
**Unique study design or methodology:** SUDHL-1 cells ( $6.4 \times 10^6/0.2$  ml) were inoculated s.c. on each flank of 60 male SCID mice. Tumors were allowed to grow to a size of 80-100 mm<sup>3</sup>. Animals carrying tumors were dosed for 5 consecutive days with cytoxan or SDX-105. The study was terminated when the mean tumor volume of the control group reached 1500 mm<sup>3</sup>.

**Observations and times:**

**Mortality:** Daily  
**Body weights:** Twice weekly

**Results**

**Mortality:** 16 mg/kg/day SDX-105 - 1 ♂ died on day 13  
32 mg/kg/day SDX-105 – 2 ♂ died on days 9 and 13

Body weights: 32 mg/kg/day SDX-105 - ↓ 20%

Tumor volume:

Compound	Dose (mg/kg/day)	Tumor volume (mm <sup>3</sup> )
Control	0	1725
SDX-105	2	1891
	8	1620
	16	509

4.2.1.1.14 **Efficacy of SDX-105 and Rituxan in a Xenograft Model of Daudi in SCID Mice**  
Study T116.

**Key study findings:**

- Rituxan (rituximab) and SDX-105 (bendamustine) inhibited Daudi tumor growth in SCID mice.
- Rituximab and bendamustine combination had a more profound effect than either compound alone.

**Volume # and page #:**

Module 4.2.1.1.14

**Conducting laboratory and location:**

**Date of study initiation:**

November 12, 2003

**GLP compliance:**

No

**QA report:**

yes ( ) no (X)

**Drug, lot #, and % purity:**

Rituximab and SDX-105,  
lot # and purity not mentioned

**Methods:**

Doses:

Group #	Compound	Dose (mg/kg/day)	Route	Treatment (day)
1	Control			Daily for five days
2	Rituximab	75	Iv	Alternative days (0, 2, 4)
3	SDX-105	15	Ip	Daily for five days
4	Rituximab	75	Iv	Alternative days (0, 2, 4)
	SDX-105	15	Ip	Daily for five days
5	Rituximab	75	Iv	Alternative days (0, 2, 4)
	SDX-105	15	Ip	14, 15, 16, 17, & 18

Species/strain: SCID mice  
 Number/sex/group or time point (main study): 3 ♂  
 Route, formulation, volume, and infusion rate: Rituximab – iv SDX-105 – ip  
 Satellite groups used for toxicokinetics or recovery: No  
 Age: 6-8 weeks  
 Weight: ~25 g  
 Unique study design or methodology: Daudi cells ( $1.0 \times 10^7/0.2$  ml) were inoculated s.c. on each flank of 60 male SCID mice. Tumors were allowed to grow to a size of 110-170 mm<sup>3</sup>. Animals carrying tumors were dosed for 5 days with Rituximab, SDX-105, or combination. The study was terminated when the mean tumor volume of control group reached ~1800 mm<sup>3</sup>.

**Observations and times:**

Mortality: Daily  
Body weights: Days 0, 2, 6, 9, 13, 20, 27, and 34

**Results**Mortality:

Group	Treatment	Animal (s) died	Day (s) of death
3	SDX-105	1	23
4	Rituximab & SDX-105	1	23
5	Rituximab followed by SDX-105	1	13

Body weights:

Weight (g)	Days							
	0	2	6	9	13	20	27	34
Control	24.8	26.0	27.4	27.4	29.3	32.2		
SE	1.3	1.6	1.4	1.3	1.5	1.9		
%BW*		105%	110%	110%	118%	130%		
Rituxan (75mg/kg/d)	24.7	24.7	26	25.6	26.4	27.9	29.6	31.7
SE	1.0	1.1	1.5	1.4	1.2	1.4	1.5	1.8
%BW		100%	105%	104%	107%	113%	120%	128%
SDX-105 (15 mg/kg/d)	27.9	24.8	24.3	23.7	23.6	22.0	22.7	24.1
SE	1.4	1.4	1.7	2.1	2.4	1.7	3.1	3.2
%BW		89%	87%	85%	85%	79%	81%	86%
Rituxan & SDX-105	24.8	22.3	22.3	21.5	22.0	22.8	23.1	24.5
SE	1.0	1.6	0.3	0.3	0.6	0.8	0.3	0.8
%BW		90%	90%	87%	88%	92%	93%	99%
Rituxan followed by SDX-105 (Day 14)	24.0	23.3	23.3	23.2	25.3	26.2	26.4	27.4
SE	0.8	0.7	1.3	1.6	1.6	1.6	1.8	1.9
%BW		97%	97%	97%	105%	109%	110%	114%

\* The percent body weight (%BW) of all groups was normalized based on the mean body weight of 100% for each group on Day 0.

(Excerpted from the sponsor's submission)

Body weight loss (up to 15) of groups 3, 4, and 5 were observed during the first two week of dosing.

Tumor volume:

Group #	Compound	Dose (mg/kg/day)	Route	Tumor volume(mm <sup>3</sup> ) on day	
				20	34
1	Control			1913	-
2	Rituximab	75	Iv	645	2224
3	SDX-105	15	Ip	203	743
4	Rituximab	75	Iv	93	370
	SDX-105	15	Ip		
5	Rituximab	75	Iv	231	395
	SDX-105	15	Ip		

Percent tumor volume normalized for each group on day 1

Treatment/Days	0	2	6	9	13	20	27	34	41
control	100	227	289	416	498	1122			
S.E.	0	139	147	218	378	433			
Rituxan (75mg/kg/d)	100	124	131	185	254	430	716	1482	
S.E.	0	37	55	76	111	180	317	628	
SDX-105 (15 mg/kg/d)	100	86	93	89	114	151	352	553	1181
S.E.	0	11	23	31	82	103			
Rituxan & SDX-105	100	97	97	52	62	63	141	251	558
S.E.	0	14	27	33	27	53			
Rituxan followed by SDX-105 (Day 14)	100	88	107	113	182	178	156	305	737
S.E.	0	15	40	69					

(Excerpted from the sponsor's submission)

4.2.1.1.15 **Efficacy of SDX-105 and Rituxan in a Xenograft Model of Daudi in SCID Mice.**  
Study T124.

**Key study findings:**

- SDX-105 (bendamustine) inhibited Daudi tumor growth in SCID mice.
- Bendamustine plus Rituxan (rituximab) significantly enhanced the tumor growth delay.

**Volume # and page #:**

Module 4.2.1.1.15

**Conducting laboratory and location:**

/ /

**Date of study initiation:** April 6, 2004  
**GLP compliance:** No  
**QA report:** yes (X) no ( )  
**Drug, lot #, and % purity:** SDX-105, lot # 02K27,  
 purity not mentioned.  
 Rituximab from ———, lot # L89330 & M02378.

**Methods**

Doses:

Group #	No. of animals	Compound	First treatment cycle			Second treatment cycle		
			Mg/kg/day	Route	Schedule	Mg/kg/day	Route	Schedule
1	4	Saline control	-	Ip	Daily for 5 days	-	-	-
2	4	SDX-105	10	Ip	Daily for 5 days	5	Ip	Days 28, 30
3	4	Rituximab	75	Iv	Days 0, 2 & 4	75	iv	Days 28, 30
4	4	SDX-105	10	Ip	Daily for 5 days	5	Ip	Days 28, 30
		Rituximab	75	Iv	Days 0, 2 & 4	75	Iv	

**Species/strain:** SCID mice  
**Number/sex/group or time point (main study):** 4 ♂/group  
**Route, formulation, volume, and infusion rate:** See table above  
**Satellite groups used for toxicokinetics or recovery:** No  
**Age:** 6-8 weeks  
**Weight:** 20-22 g

**Unique study design or methodology:** Daudi cells ( $1.0 \times 10^7/0.2$  mL) were inoculated s.c. in the flank of each male SCID mouse. Tumors were allowed to grow to a size of 100-140 mm<sup>3</sup>. Mice were randomized and treated with bendamustine and Rituximab as indicated above. The second cycle of treatment was administered on days 28 and 30.

**Results**

Mortality:

Group	Treatment	Day of death	No. of animals died
2	SDX-105	13	1
4	SDX-105 + Rituximab	15	1

Body weights: Group 2 - ↓20% one animal only  
 Group 4 - ↓ 20% one animal only

Group body weight on different days

Groups	0	2	6	10	13	16	20
<b>control</b>	23.7	23.9	23.8	24.9	25.4	26.6	28.2
<b>SE</b>	0.4	0.7	0.7	0.8	0.9	1.0	1.2
<b>% BW</b>		101%	100%	105%	107%	112%	119%
<b>SDX-105</b>	23.2	22.7	21.3	20.9	23.0	23.6	24.1
<b>SE</b>	0.7	0.9	0.9	1.4	1.1	0.8	0.666
<b>% BW</b>		98%	92%	90%	99%	102%	104%
<b>Rituxan</b>	22.7	22.7	22.7	23.2	23.4	24.3	24.3
<b>SE</b>	0.9	0.7	0.7	0.9	0.8	0.9	0.9
<b>% BW</b>		100%	100%	102%	103%	107%	107%
<b>SDX-105 &amp; Rituxan</b>	21.8	21.1	19.8	20.6	19.6	21.0	21.0
<b>SE</b>	0.4	0.3	0.3	0.4	1.1	0.3	0.4
<b>% BW</b>		97%	91%	94%	90%	96%	97%

(Excerpted from the sponsor's submission)

Tumor volume:

Mean time to reach 4X tumor volume and tumor volume on day 20

Group #	Group	4 x time	Tumor volume (mm <sup>3</sup> )
1	Control	6.2 days	2118
2	SDX-105	20.2 days	511
3	Rituximab	14.6 days	847
4	SDX-105 + Rituximab	33.2 days	221

4.2.1.1.16

**Dose Response of SDX-105 and SDX-101 in a Xenograft Model of Daudi in SCID Mice.**  
Study T110

**Key study findings:**

- Tumor volume of SDX-105 (bendamustine) treated mice was reduced in a dose related manner.

**Volume # and page #:**

Module 4.2.1.1.16

**Conducting laboratory and location:**

**Date of study initiation:**

September 12, 2003

**GLP compliance:**

No

**QA report:** yes (X) no ( )  
**Drug, lot #, and % purity:** SDX-105, SDX-101  
lot # and purity not provided

**Methods**

**Doses:** SDX-105 – 10, 15, or 20 mg/kg/day for 5 days  
(Groups 5, 6, & 7)  
SDX-101 – 100, 200, or 300 mg/kg/day  
(Groups 8, 9, & 10)  
Cytoxan – 15, 30, or 60 mg/kg/day as positive control

**Species/strain:** Daudi derived tumors bearing female SCID mice

**Number/sex/group or time point (main study):** 5 ♀/group

**Route, formulation, volume, and infusion rate:**  
SDX-105 & cytoxan by ip, 0.1 mL  
SDX-101 by po, 0.3 mL

**Satellite groups used for toxicokinetics or recovery:** No

**Age:** 6-8 weeks

**Weight:** 21-24 g

**Unique study design or methodology:** Daudi cells ( $1.0 \times 10^7/0.2$  mL) were inoculated s.c. on the flank of each female SCID mouse. Animals were randomized and dosed when the tumor sizes were 80-120 mm<sup>3</sup>. The study was terminated when the tumor volume of the control group reached ~1500 mm<sup>3</sup>.

**Observations and times:**

**Mortality:** Daily  
**Body weights:** Twice weekly

**Results**

**Mortality:** 20 mg/kg/day SDX-105 – 2 died on days 15 & 17  
**Body weights:** 15/20 mg/kg/day SDX-105 – 20% ↓ on day 11  
**Tumor volume**

## Mean tumor volume of ip SDX-105 treated animals

Group	1	5	6	7
Dose (mg/kg/day)	Control	10	15	20
Tumor volume (mm <sup>3</sup> )	1428	707	212	38

## Mean tumor volume of oral SDX-101 treated animals

Group	1	8	9	10
Dose (mg/kg/day)	Control	100	200	300
Tumor volume (mm <sup>3</sup> )	1428	1299	1115	1057

### 2.6.2.3 Secondary pharmacodynamics

#### 4.2.1.2.1 Effect of Bendamustine Hydrochloride on Different Non-Malignant Cells of Mice and Humans in Comparison to Other Well-Known Cytostatic Drugs. Study 0640.01.C07.07.

##### Key study findings:

- Bendamustine hydrochloride affected quiescent lymphocytes isolated from the peripheral blood with an  $IC_{50}$  value of  $7 \times 10^{-5}$  M.
- Chlorambucil and fludarabine inhibited metabolic activity of lymphocytes with  $IC_{50}$  values of  $2 \times 10^{-5}$  M and  $2-3 \times 10^{-5}$  M, respectively.
- Mitoxantrone was most effective in bone marrow stem cells with an  $IC_{50}$  value of  $2 \times 10^{-9}$  M, followed by fludarabine, chlorambucil, and bendamustine, with  $IC_{50}$  values of  $2-3 \times 10^{-5}$ ,  $5 \times 10^{-6}$ , and  $1-2 \times 10^{-5}$ , respectively.

**Methods:** Murine bone marrow cells, murine stroma cells, human bone marrow cells, human stroma cells, and human lymphocytes were obtained, cultured and exposed to test compounds under different conditions. The results obtained with either colony assay (bone marrow stem cells), WST-1 (suspension cultures), or BCA (adherent cell lines) were expressed in relation to untreated controls.

##### Results:

$IC_{50}$  values of different cytostatic compounds on human peripheral lymphocytes

Compound	$IC_{50}$ values (M)		
	Day 1	Day 2	Day 4
Bendamustine	$2 \times 10^{-4}$	$2 \times 10^{-4}$	$7 \times 10^{-5}$
Chlorambucil	$2 \times 10^{-4}$	$2 \times 10^{-4}$	$2 \times 10^{-5}$
Fludarabine	$4 \times 10^{-5}$	$1 \times 10^{-5}$	$2 \times 10^{-5}$
5-Fluorouracil	$7 \times 10^{-4}$	$9 \times 10^{-4}$	$5 \times 10^{-4}$

Cytotoxic activity of bendamustine and other chemotherapeutic agents  
in non-malignant and malignant cells

Cell Type	Mitoxantrone IC <sub>50</sub> (μM)	Fludarabine IC <sub>50</sub> (μM)	Bendamustine IC <sub>50</sub> (μM)	Chlorambucil IC <sub>50</sub> (μM)
BM Stem Cell (human)	0.002-0.003	2-3	10-20	5
BM Stem Cell (murine)	0.003	20-30	50	3
BM Stromal Cell (human)	0.2-0.3	3-4	500-2000	300
BM Stromal Cell (murine)	0.01	60	100-600	ND
Lymphocyte (human)	ND	2	70-100 <sup>s</sup>	20-30
MDA-MB-435	0.005-0.006	30	100-200	40-50
CCRF-CEM	0.0004-0.0005	1	100	30

(Excerpted from the sponsor's submission)

4.2.1.2.2 **Determination of the Cytotoxic Potential of SDX-105 in Cultured Human Hepatocytes after In Vitro Exposure.**  
Study DM-2005-002.

**Key findings:**

- Human hepatocytes treated with chlorpromazine (positive control) showed a marked loss of cell viability and demonstrated that the test system was responsive to cytotoxic agent.
- SDX-105 (bendamustine) at concentrations of up to 100 pg/ml (100 μM) did not show cytotoxicity in cultured human hepatocytes after 2, 24, 48, or 72 hours of exposure.

**Methods:** Cellular ATP content was used to determine the viability of cultured human hepatocytes in this experiment.

**Results:**

Compound	Concentration	Incubation time (hours)	ATP (μM)	Specific activity [ATP (pmol/million cells)]	Percent of VC
Vehicle control (VC)		72	1.1±0.2	633±102	100
Chlorpromazine	500 μM	72	<0.2±0	<114±0	<18
VC		2	2.0±0.3	1157±143	100
SDX-105	100 μM	2	1.9±0.02	1057±11	91
VC		24	1.93±0.2	1103±84	100
SDX-105	11 μM	24	1.85±0.1	1058±74	96

Compound	Concentration	Incubation time (hours)	ATP ( $\mu\text{M}$ )	Specific activity [ATP (pmol/million cells)]	Percent of VC
VC		48	1.8 $\pm$ 0.13	1036 $\pm$ 76	100
SDX-105	100 $\mu\text{M}$	48	1.8 $\pm$ 0.12	1033 $\pm$ 66	99.7
VC		72	1.2 $\pm$ 0.13	671 $\pm$ 73	100
SDX-105	100 $\mu\text{M}$	72	1.4 $\pm$ 0.1	788 $\pm$ 423	117

VC – viable cells

**4.2.1.1.12 Cytotoxicity Assay In Vitro with BALB/C3T3 Cells: Neutral Red (NR) Test with Bendamustine Hydrochloride at Simultaneous Irradiation with Artificial Sunlight**  
Study 789402.

**Key study findings:**

- Bendamustine hydrochloride did not have any phototoxic effects on Balb/c 3T3 cells.

**Methods:** Balb/c3T3 c31 cells were treated with various concentrations of the bendamustine or chlorpromazine (positive control) in the presence and absence of artificial sunlight (wave length >320 nm). The EC<sub>50</sub> values were determined and compared to measure the possible Phototoxicity.

**Results:**

Compound	EC <sub>50</sub> ( $\mu\text{g}/\text{mL}$ )		PIF
	+UV	-UV	
Bendamustine hydrochloride	99.8	92.5	0.9
Chlorpromazine	0.25	17.41	70.3

**2.6.2.4 Safety pharmacology**

**4.2.1.3.1 Bendamustine Hydrochloride (Ribomustoine ®):  
Evaluation of Effect on Urine Output, Urinary Electrolyte Balance  
and Glomerular Filtration Rate in the Rat With a Saline Overload  
Following Two Successive 30-Minute Intravenous Infusions.  
Study 20010337 PGR.**

**Key study findings:**

- Bendamustine hydrochloride affected the urine output, urinary electrolyte balance (Na & K) and glomerular filtration rate (creatinine clearance) in Sprague Dawley rats with a saline overload.

**Study no.:** 20010337 PGR  
**Volume # and page #:** Module 4.2.1.3.1  
**Conducting laboratory and location:** // //

**Date of study initiation:** July 9, 2001  
**GLP compliance:** Yes  
**QA report:** yes (X) no ( )  
**Drug, lot #, and % purity:** Ribomustin (bendamustine), lot # 521000, purity not mentioned

**Methods**

Doses:

Groups	Treatment	Dosage	Time of the Test (a)
1	Negative control group (sterile water/saline, ¼)	Two 30 min infusions on D1 and D2	D2 and D4
2	Furosemide at a dose of 10 mg/kg	30 min infusion on D2 (b)	D2
3	Bendamustine.HCl at 15 mg/kg	Two 30 min infusions on D1 and D2	D2 and D4
4	Bendamustine.HCl at 20 mg/kg	Two 30 min infusions on D1 and D2	D2 and D4
5	Bendamustine.HCl at 25 mg/kg	Two 30 min infusions on D1 and D2	D2 and D4

- (a): . on D2, measurement of diuresis was begun immediately after the start of infusion.  
 . on D4, saline overload was performed 48h ± 1h after the infusion on D2.  
 (b): for group 2, on D1, animals were infused for 30 minutes with saline and only on D2 with furosemide.

(Excerpted from the sponsor's submission)

Species/strain: Sprague-Dawley, SD:  $\sim$  , rats  
 Number/sex/group or time point (main study): 8  $\sigma$ /group  
 Route, formulation, volume, and infusion rate: Intravenous infusion, 30 min.  
 Satellite groups used for toxicokinetics or recovery: No  
 Age: Not mentioned  
 Weight: 229-307 g  
 Unique study design or methodology (if any): On day 4 (48h + 1h after the end of the infusion on day 2) animals were given orally 50 mL/kg of saline and were placed individually in urine collection cages. Urine volumes were determined at 2.5h and 5h after saline overload. Urinary pH, urinary and plasma levels of sodium, potassium, chloride and creatinine as well as osmolarity of urine and plasma were determined on samples collected at 5 hours, on D2 and D4 separately.

**Results**

Mortality: None

Effect of bendamustine on excretion fractions in the rat with saline overloads following two successive 30-minute intravenous infusion.

TREATMENT		EXCRETION FRACTION OF SODIUM (%)	EXCRETION FRACTION OF POTASSIUM (%)	EXCRETION FRACTION OF CHLORIDE (%)	FREE WATER CLEARANCE (mL/h/100g)
VEHICLE	Mean	2.03	27.09	3.32	-0.68
	SD	0.46	6.12	0.67	0.14
	N	8	8	8	8
BENDAMUSTINE.HCl 15 mg/kg	Mean	2.32	31.43	3.70	-0.65
	SD	0.58	6.47	0.88	0.16
	N	8	8	8	8
	%	+14	+16	+11	+5
	P	NS	NS	NS	NS
BENDAMUSTINE.HCl 20 mg/kg	Mean	3.15	46.74	5.05	-0.42
	SD	1.69	25.40	2.59	0.34
	N	8	8	8	8
	%	+55	+73	+52	+39
	P	NS	NS	NS	NS
BENDAMUSTINE.HCl 25 mg/kg	Mean	7.79	113.75	11.57	-0.42
	SD	4.82	60.92	6.70	0.17
	N	8	8	8	8
	%	+284	+320	+248	+38
	P	**	**	**	NS

Vehicle: saline/sterile water (4/1).

Mean: mean value.

SD: Standard Deviation.

N: number of animals.

NS: P > 0.05, \*\*: P ≤ 0.01, when compared with the control group dosed with the vehicle: analysis of variance with NEWMAN-KEULS test if P ≤ 0.05.

%: percentage of variation calculated in relation to the control group dosed with the vehicle.

(Excerpted from the sponsor's submission)

These results showed an immediate (kaliuretic) effect at 25 mg/kg bendamustine. after the second infusion on day 2. Delayed effects were detected on D4, mainly at 25mg/kg.

At this dose, marked effects on electrolyte balance were observed (kaliuretic and natriuretic effects and an increase in elimination of chloride) as well as an increase in glomerular filtration rate, suggesting dysfunction of glomerular filtration. Positive control (furosemide) induced diuretic, kaliuretic, natriuretic, and an increase in elimination of chloride as expected.

4.2.1.3.2 **Evaluation of Effect on Cardiac Action Potential in Isolated Canine Purkinje Fibers.**  
Study 20010339 PECM.

**Key study findings:**

- Bendamustine HCl at concentrations of 1.5, 4.5, and 7.5 µg/mL showed no statistically significant effect on action potential parameters under either normal (60 ppm) or low (20 ppm) stimulation rates.
- Under the same experimental conditions, cisapride ( $3 \times 10^{-7}$ M) induced electrophysiological effect, i.e. an increase in duration of action potential.

**Study no.:** 20010339 PECM  
**Volume # and page #:** Module 4.2.1.3.2  
**Conducting laboratory and location:**   
**Date of study initiation:** July 11, 2001  
**GLP compliance:** Yes  
**QA report:** yes (X) no ( )  
**Drug, lot #, and % purity:** Ribomustin (bendamustine),  
batch # 521000 — purity

**Methods**

**Doses:** Bendamustine - 1.5, 4.5, or 7.5 µg/mL  
Cisapride (positive control) –  $3 \times 10^{-7}$ M

**Species/strain:** Beagle dogs

**Number/sex/group or time point (main study):** 6 Purkinje fiber preparations from 2 male beagle dogs

**Route, formulation, volume, and infusion rate:** Test solutions were dissolved in sterile water/saline solution (1/4, v/v), mixed with Tyrode's solution at a specific concentration (i.e. 1.5, 4.5, or 7.5 µg/mL), then perfused at 2 mL/min. Each solution was perfused for 30 minutes. Values obtained at the end of each period of stimulation at 60 ppm (25<sup>th</sup> minute) and at 20 ppm (30<sup>th</sup> minute) were analyzed.

**Satellite groups used for toxicokinetics or recovery:** None

Age:

8.5 and 12 months

Weight:

12.85 and 13.85 kg

**Results**

Effect of bendamustine on cardiac action potential under normal stimulation rate (60 ppm)

TREATMENT		APA (mV)	RP (mV)	Vmax (V/s)	APD50 (ms)	APD70 (ms)	APD90 (ms)
Pre-dose values (Tyrode)	Mean	124	-92	418	215	256	297
	SEM	3	0	27	11	9	8
	N	6	6	6	6	6	6
Vehicle (*)	Mean	1	0	7	2	1	1
	SEM	1	0	9	2	1	1
	N	6	6	6	6	6	6
Bendamustine HCl 1.5 µg/mL	Mean	-1	0	11	0	4	5
	SEM	2	0	4	4	2	3
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
Bendamustine HCl 4.5 µg/mL	Mean	0	-1	16	-3	3	2
	SEM	3	1	20	3	1	1
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
Bendamustine HCl 7.5 µg/mL	Mean	-1	0	4	1	4	7
	SEM	2	0	25	3	2	1
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS

(\*) Vehicle = 1% sterile water/saline solution (1/4,v/v) in Tyrode.

Pre-dose values: control period with Tyrode.

ppm: pulses per minute.

APA: amplitude of the action potential.

RP: resting potential.

Vmax: maximal rate of depolarisation.

APD50: action potential duration at 50% of repolarisation.

APD70: action potential duration at 70% of repolarisation.

APD90: action potential duration at 90% of repolarisation.

Mean: mean value.

SEM: Standard Error of the Mean.

N: number of preparations.

Results are expressed as variation calculated in relation to values measured during the control period (Tyrode perfusion).

NS: P > 0.05, when compared to the vehicle control period (1% sterile water/saline solution (1/4, v/v) in Tyrode); analysis of variance with NEWMAN KEULS test if P ≤ 0.05.

Note: values of APA, RP, Vmax, APD50, APD70 and APD90 were analysed 25 minutes after starting each infusion period.

(Excerpted from the sponsor's submission)

Effect of bendamustine on cardiac action potential under low stimulation rate (20 ppm)

TREATMENT		APA (mV)	RP (mV)	Vmax (V/s)	APD50 (ms)	APD70 (ms)	APD90 (ms)
Pre-dose values (Tyrode)	Mean	118	-88	417	258	308	354
	SEM	3	0	32	14	13	13
	N	6	6	6	6	6	6
Vehicle (*)	Mean	-1	0	13	2	5	3
	SEM	2	0	7	4	1	2
	N	6	6	6	6	6	6
Bendamustine HCl 1.5 µg/mL	Mean	1	0	5	5	8	5
	SEM	1	1	19	5	3	2
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
Bendamustine HCl 4.5 µg/mL	Mean	-2	0	-16	-2	8	6
	SEM	4	0	34	5	2	3
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
Bendamustine HCl 7.5 µg/mL	Mean	2	0	-27	3	11	11
	SEM	1	1	23	3	1	0
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS

Best Possible Copy

(\*) Vehicle = 1% sterile water/saline solution (1/4,v/v) in Tyrode.

Pre-dose values: control period with Tyrode.

ppm: pulses per minute.

APA: amplitude of the action potential.

RP: resting potential.

Vmax: maximal rate of depolarisation.

APD50: action potential duration at 50% of repolarisation.

APD70: action potential duration at 70% of repolarisation.

APD90: action potential duration at 90% of repolarisation.

Mean: mean value.

SEM: Standard Error of the Mean.

N: number of preparations.

Results are expressed as variation calculated in relation to values measured during the control period (Tyrode perfusion).

NS: P > 0.05, when compared to the vehicle control period (1% sterile water/saline solution (1/4, v/v) in Tyrode); analysis of variance with NEWMAN KEULS test if P ≤ 0.05.

Note: values of APA, RP, Vmax, APD50, APD70 and APD90 were analysed 30 minutes after starting each infusion period.

(Excerpted from the sponsor's submission)

4.2.1.3.3 **Bendamustine Hydrochloride: Effects on HERG-1 Tail Currents Recorded from Stably Transfected HEK 293 Cells**  
Study 853896.

**Key study findings:**

- Bendamustine HCl at the concentrations of 20 and 200 µM inhibited the hERG-1 tail current amplitude and caused a significant deceleration of the tail current decay time constant.
- Bendamustine has no effect on hERG-1 channel at 2 µM.

<b>Study no.:</b>	853896
<b>Volume # and page #:</b>	Module 4.2.1.3.3
<b>Conducting laboratory and location:</b>	
<b>Date of study initiation:</b>	May 25, 2004
<b>GLP compliance:</b>	Yes
<b>QA report:</b>	yes (X) no ( )
<b>Drug, lot #, and % purity:</b>	Bendamustine hydrochloride, batch # AN2542, — purity
<b>Reference item:</b>	E-4031, batch # JSH9677, > — purity
<b>Test system:</b>	HEK 293 cells stably expressing HERG-1 potassium channels.
<b>Test concentrations:</b>	200 µM, 20 µM or 2 µM

**Results**

## Normalized tail current amplitudes

Treatment	Mean relative tail current amplitude	Normalized tail current amplitude <sup>5</sup>	Percent inhibition <sup>6</sup>
Vehicle (Bath solution)	92 %	-	-
Bendamustine HCl (2 µM)	94 %	102 %	-
Bendamustine HCl (20 µM)	74 %	80 %	20 %
Bendamustine HCl (200 µM)	32 %	35 %	65 %

<sup>5</sup> Percent current left at the end of the recording taking into account rundown from exposure to vehicle (0.5% DMSO).

<sup>6</sup> Normalized tail current reduced from 100%. Proportion of the HERG-1 current inhibited by the test item.

(Excerpted from the sponsor's submission)

## Normalized tail current decay time constants

Treatment	Mean relative tail current decay time constant	Normalized tail current decay time constant <sup>7</sup>
Vehicle (Bath solution)	109 %	-
Bendamustine HCl (2 µM)	107 %	94 %
Bendamustine HCl (20 µM)	129 %	118 %
Bendamustine HCl (200 µM)	150 %	138 %

(Excerpted from the sponsor's submission)

Reference item: E-4031 at 100 nM produced an inhibition of ~90% the relative tail current amplitude being 9%.

**Neurological effects:** No studies conducted

**Pulmonary effects:** No studies conducted

**Gastrointestinal effects:** No studies conducted

**Abuse liability:** No studies conducted

**Other:** None

**2.6.2.5 Pharmacodynamic drug interactions:** No studies conducted

**APPEARS THIS WAY  
ON ORIGINAL**

### 2.6.3 PHARMACOLOGY TABULATED SUMMARY

Study type	Test system	Noteworthy findings
Cell cycle alterations by bendamustine in comparison with other cytotoxic agents.	Human leukemic and lymphoma cell lines	Cell cycle alterations induced by bendamustine were similar to those induced by related alkylating agents.
The efficacy of bendamustine is slightly decreased by p-glycoprotein and MXR/BCRP resistance mechanisms.	Murine and human tumor cells and drug resistant sublines	The activity of bendamustine was less affected by these pathways than other drugs tested.
Effect of bendamustine hydrochloride by-products and on tumor cell growth.	Human breast cancer cell line, human leukemic cell line and HepG2 human liver carcinoma cell line	Only showed greater cytotoxicity than bendamustine.
Efficacy of bendamustine on human lung cancer, human mammary carcinoma.	Nu/nu mice	Dose-dependent reduction in tumor growth.
Efficacy of bendamustine and Rituximab in a xenograft model of Daudi in SCID mice.	SCID mice	Rituximab and bendamustine combination showed more profound effect than either compound alone.

### SAFETY PHARMACOLOGY TABULATED SUMMARY

Study type	Test system	Noteworthy findings
Effect on urine output, urinary electrolyte balance and glomerular filtration rate in the rat with a saline overload.	Sprague-Dawley rats, <i>in vivo</i> .	Bendamustine affected urine output, urinary electrolyte balance (kaliuretic, natriuretic) and glomerular filtration rate (creatinine clearance) in rats with a saline overload.
Evaluation of effect on cardiac action potential in isolated canine Purkinje fibers.	Isolated canine Purkinje fibers.	Bendamustine at 1.5, 4.5, and 7.5 µg/mL had no effect on action potential parameters.
Effects on HERG-1 tail currents recorded from stably transfected HEK 293 cells.	HEK 293 cells	Bendamustine at 20 and 200 µM inhibited HERG-1 tail current amplitude and caused deceleration of the tail current decay time constant, no effect at 2 µM.

## 2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary: The pharmacokinetic/toxicokinetic properties of bendamustine have been studied in mice, rats and dogs. Cytostasan (bendamustine) was absorbed quickly into rat blood. Liver, kidney and gastrointestinal tract have higher concentration of bendamustine than blood. The tissue distribution study of bendamustine hydrochloride in rats indicated that the highest tissue levels of bendamustine were in the kidney and liver. The mass balance study showed that significant radioactivity was recovered in both urine and feces.

Bendamustine hydrochloride up to 100  $\mu$ M had no effect on the expression of cytochrome P450 enzymes in primary cultures of human hepatocytes. Treatment of cultured human hepatocytes with prototypical P450 enzymes inducers caused anticipated increases in CYP activities.

Bendamustine was metabolized by both dog and human microsomes mainly by Phase 1 metabolism to produce an oxidative metabolite and an N-desmethyl metabolite. These processes appeared to be CYP1A2 mediated. Metabolism *in vitro*, by dog and human hepatic microsomes produced the same metabolites but the amount of metabolite production was greater in dog than human.

*In vitro* plasma protein binding studies indicated that  $^{14}$ C-bendamustine was relatively highly protein bound to human plasma proteins (~96%) compared to dog plasma proteins (~75%) and that this binding was preferential to human serum albumin. Interaction studies showed that bendamustine was unable to displace warfarin from human serum albumin.

### 2.6.4.2 Methods of Analysis

[see under individual study reviews]

#### Analytical Methods and Validation Reports

##### 4.2.2.1.1 Validation of a High Performance Liquid Chromatographic Method for the Measurement of Bendamustine and Two Major Metabolites in Dog Plasma and Urine. Study KLG-09.

A liquid chromatographic method for the determination of bendamustine, monohydroxy-bendamustine (HP1) and dihydroxy-bendamustine (HP2) in dog plasma and urine has been validated. \_\_\_\_\_ was used as the sample preparation method.

#### Results:

Main results of the validation study are summarized below:

Plasma			
Intra-batch	Bendamustin	HP1	HP2
LLOQ	2 ng/ml	100 ng/ml	500 ng/ml
ULOQ	1200 ng/ml	1100 ng/ml	2500 ng/ml
LLOQ RE (CV)	± 6.7% (≤ 18.7%)	± 11.1% (≤ 1.9%)	± 14.3% (≤ 2.6%)
QC Low RE (CV)	± 5.9% (≤ 6.3%)	± 3.6% (≤ 1.1%)	± 10.4% (≤ 3.7%)
QC Medium RE (CV)	± 3.9% (≤ 2.9%)	± 5.1% (≤ 1.5%)	± 11.6% (≤ 1.4%)
QC High RE (CV)	± 6.8% (≤ 3.4 %)	± 10.5% (≤ 3.7%)	± 14.4% (≤ 2.1%)
Correlation coefficient $r^2$	≥ 0.9990	≥ 0.9976	≥ 0.9965

(Excerpted from the sponsor's submission)

Urine	
Intra-batch	Bendamustin
LLOQ	2 ng/ml
ULOQ	1200 ng/ml
LLOQ RE (CV)	± 17.5% (≤ 23.2%)
QC Low RE (CV)	± 9.6% (≤ 4.2%)
QC Medium RE (CV)	± 8.9% (≤ 2.5%)
QC High RE (CV)	± 15.6% (≤ 1.1%)
Correlation coefficient $r^2$	≥ 0.9928

LLOQ - Lower Limit of Quantification  
RE - Relative error of the mean

ULOQ - Upper Limit of Quantification  
CV - Coefficient of variance

(Excerpted from the sponsor's submission)

4.2.2.1.2 **Validation Report: Determination of Bendamustine M3 Metabolite, and M4 Metabolite in K<sub>2</sub>EDTA rat plasma.**  
Study DP-2007-030

A LC-MS/MS method for the determination of bendamustine, M3 metabolite ( $\gamma$ -hydroxy-bendamustine), and M4 metabolite (N-desmethyl-bendamustine) in K<sub>2</sub>EDTA rat plasma is described and validated by \_\_\_\_\_ project # 1000-06966).

2.6.4.3 **Absorption**

4.2.2.2.1 **Studies on the Pharmacokinetics of Bendamustine [<sup>14</sup>C] in the Rat.**

**Key study findings:**

- Cytostasan was absorbed quickly in rat blood with a long half life.
- Liver, kidney and gastrointestinal tract have higher concentration of bendamustine equivalents than blood.
- Elimination of <sup>14</sup>C-bendamustine was primarily fecal (biliary).

**Study no.:** DM-2006-012.  
**Volume # and page #:** Module 4.2.2.2.1  
**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** May 1985  
**GLP compliance:** No  
**QA report:** yes ( ) no (X)  
**Drug, lot #, and % purity:** Cytostasan (bendamustine)  
lot # and purity not mentioned

**Methods**

Doses: 3.5 mg/kg  
Species/strain: Wistar rats  
Number/sex/group or time point (main study): 3  
Route, formulation, volume, and infusion rate: Intravenous and oral (gavage)  
Satellite groups used for toxicokinetics or recovery: No  
Age: 9 weeks  
Weight: 200 g

**Results**

**Mortality:** None

Elimination of  $^{14}\text{C}$  Cytostasan (% of dose) after oral and intravenous administrations

Time (hours)	Oral				Intravenous			
	Urine		Feces		Urine		Feces	
	♂	♀	♂	♀	♂	♀	♂	♀
6	13.1	14.8			24.3	20.9		
24	17.8	20.2	45.3	31.8	27.1	23.5	45.9	12.6
48			68.3	52.3			49.8	47.9
72	18.2	20.9	74.6	65.0	28.2	25.1	53.7	56.7

Normalized  $^{14}\text{C}$  blood concentration (%) in the rat after oral and intravenous administrations

Time (hours)	Oral administration	Intravenous administration
0.1	4.3±0.5	127.4±6.3
0.2	7.5±1.5	89.5±5.5
0.4	8.5±1.8	59.0±2.7
1.6	8.8±1.1	35.0±1.3
6.4	8.5±0.4	33.2±1.3
24	6.6±0.3	25.8±0.8

Tissue/blood ratio r of the  $^{14}\text{C}$  concentration in the rat 1 hour and 24 hours after the oral administration of  $^{14}\text{C}$  Cytostasan

Organs/Tissue	1 hour p.a.			24 hours p.a.		
	n	mean r	sem	n	mean r	sem
Pylorus	6	56.16	21.38	6	2.38	0.41
Gastric glands	6	7.34	2.71	6	0.37	0.05
Small intestine	6	10.93	4.80	6	0.47	0.03
Liver	6	9.49	1.38	6	1.91	0.30
Pancreas	6	0.35	0.03	6	0.25	0.02
Kidney	3male	18.11	0.77	6	8.06	0.41
	3female	9.30	0.29			
Testes	3	0.11	0.01	3	0.13	0.01
Ovary & fallopian tube	3	0.43	0.03	3	0.47	0.02
Uterus	3	0.56	0.04	3	0.69	0.04
Lungs	6	0.62	0.03	5	0.51	0.02
Heart	6	0.48	0.02	6	0.33	0.01
Spleen	6	0.24	0.01	6	0.37	0.04
Muscle (skeletal)	6	0.18	0.01	6	0.21	0.01
Skin	6	0.20	0.01	6	0.28	0.01
Fat (perirenal)	6	0.08	0.01	6	0.12	0.01
Eyes	6	0.17	0.02	6	0.20	0.01
Adrenal gland	6	0.36	0.02	6	0.30	0.01
Thyroid gland	6	0.47	0.07	6	0.37	0.04
Thymus gland	6	0.20	0.01	6	0.20	0.02
Cerebellum	6	0.05	0.01	6	0.03	0.01
Cerebrum	6	0.04	0.01	6	0.02	0.01

(Excerpted from the sponsor's submission)

#### 2.6.4.4 Distribution

##### 4.2.2.3.1 Disposition of <sup>14</sup>C-Bendamustin in Mice and Rats Study DM-2007-001.

##### Stability studies of <sup>14</sup>C-bendamustine solutions:

The degradation of <sup>14</sup>C-bendamustine during preparation and storage were investigated. The thin layer chromatography and the distribution coefficient of the parent drug were applied to stability studies. It is recommended that <sup>14</sup>C-bendamustine be prepared at 5° C and used in experiments in the shortest possible time, but solutions of the drug can be stored at least 3 and possibly up to 6 months at -15° C without significant deterioration.

The distribution of <sup>14</sup>C-bendamustine in mice: Male Swiss mice (20-25 g body weight) were injected with <sup>14</sup>C bendamustine at 5 mg/kg (5 mL/kg). Six animals were killed at 5, 15, 30, 60, 120, and 1440 minutes post dose. Radioactivity determined in the plasma and different tissues is shown below.

Disposition of <sup>14</sup>C bendamustine (5 mg/kg) in mice  
[Values are estimated from the graph, normalized percent of dose]

Tissue/organ	Time (min)	5	15	30	60	120	1440
Plasma		7	1	0.5	0.3	0.1	0.1
Lung		3	0.8	0.5	0.5	0.4	0.3
Heart		3	0.7	0.4	0.6	0.3	0.2
Stomach		0.8	2	0.8	0.4	0.6	0.1
Bone marrow		2	0.7	0.5	0.8	0.4	0.3
Muscle		2	0.6	0.5	0.5	0.6	0.4
Hypophysis		2	0.8	0.6	1	0.5	0.3
Eye		2	0.7	0.7	2	0.6	0.4
Spleen		1	0.7	0.7	0.6	0.5	0.1
Testicle		0.7	0.5	0.8	0.5	0.8	0.1
Brain		0.4	0.09	0.08	0.2	0.06	0.03
Skin		3	2	1	0.8	1	0.7
Lymphatic nodes		2	1	0.9	2	2	0.5
Kidney		20	6	3	1	2	0.8
Liver		20	6	2	0.8	0.9	0.6
Small intestine		8	6	4	1	1	0.08
Large intestine		2	0.8	3	4	6	0.8
Tail		3	2	1	0.5	0.8	0.6
Carcass		30	20	30	30	40	4

4.2.2.3.2 **The Tissue Distribution of Total Radioactivity in the Rat Following Intravenous Administration of [<sup>14</sup>C]-CEP-18083. Study DM-2005-006.**

**Key study findings:**

- Majority of the <sup>14</sup>C-bendamustine administered intravenously was recovered by 24 hours post dose.

**Study no.:** \_\_\_\_\_ **Sponsor:** DM-2005-006.  
183204

**Volume # and page #:** \_\_\_\_\_ **Module** 4.2.2.3.2

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** 07 February 2006

**GLP compliance:** Yes

**QA report:** yes (X) no ( )

**Drug, lot #, and % purity:** <sup>14</sup>C-CEP-18083.HCl (bendamustine)  
batch # RUS 0618, radiochemical purity \_\_\_\_\_

**Methods**

**Doses:** 3 mg salt/kg  
**Species/strain:** Albino (Sprague Dawley) rats  
**Number/sex/group or time point (main study):** Total 5 males  
**Route, formulation, volume, and infusion rate:** Intravenous, 1 mL/kg  
**Satellite groups used for toxicokinetics or recovery:** Total 5 males  
**Age:** 7-8 weeks  
**Weight:** 284-326 g

**Results**

Excretion of total radioactivity (% administered dose)

Sample	Timepoint	001M	002M	003M	004M	005M	Mean	SD
Urine	4 h						28.5	1.0
	8 h						30.9	1.4
	24 h						34.2	3.9
	48 h						35.0	4.1
	72 h						35.4	4.1
	96 h						35.7	4.2
	120 h						36.0	4.2
	144 h						36.3	4.2
Faeces	168 h						36.5	4.2
	24 h						40.8	8.5
	48 h						45.5	7.6
	72 h						46.8	7.7
	96 h						47.5	7.5
	120 h						48.1	7.4
Cage Wash	144 h						48.6	7.2
	168 h						49.0	7.1
	24 h						2.7	1.1
	48 h						3.5	1.9
	72 h						3.9	1.8
	96 h						3.9	1.8
Exp Air 1	120 h						4.0	1.8
	144 h						4.1	1.8
	168 h						4.2	1.8
	24 h						0.0	n.a.
Exp Air 2	24 h						0.0	n.a.
	168 h						0.2	0.0
G.I. Tract	168 h						5.4	2.2
	168 h						5.4	2.2
<b>Total</b>		85.8	95.4	99.7	104.5	91.5	95.4	7.2

\* = Results calculated from data less than 30 d.p.m. above background  
 ° = Mean includes results calculated from data less than 30 d.p.m. above background  
 n.a. = Not appropriate  
 - = no sample collected

(Excerpted from the sponsor's submission)

Plasma concentration as  $\mu\text{g}$  equiv salt/mL

Time (hour)	Concentration
0.08	9.8 $\pm$ 4.1
0.25	5.3 $\pm$ 2.1
0.5	2.8 $\pm$ 0.8
1	1.9 $\pm$ 0.5
6	1.8 $\pm$ 0.7
12	1.6 $\pm$ 0.5
24	1.3 $\pm$ 0.5

4.2.2.3.3

**The Tissue Distribution of Total Radioactivity in the  
Pigmented Rat Following Intravenous Administration of  
[<sup>14</sup>C]-CEP-18083.HCl (Quantitative Whole Body Autoradiography).**

**Key study findings:**

- Highest levels of <sup>14</sup>C-bendamustine at 5 min post dose were in the kidney and liver.

**Study no.:** DM-2005-007  
**Volume # and page #:** Module 4.2.2.3.3  
**Conducting laboratory and location:**  
**Date of study initiation:** February 7, 2006  
**GLP compliance:** Yes  
**QA report:** yes (X) no ( )  
**Drug, lot #, and % purity:** <sup>14</sup>C-CEP-18083.HCl  
batch # RUS 0618, radiochemical purity —,  
specific activity 381 MBq/mmol or 26.1  $\mu\text{Ci}/\text{mg}$

**Methods**

**Doses:** 3 mg salt/kg  
**Species/strain:** Pigmented (Lister Hooded) rats  
**Number/sex/group or time point (main study):** Total 6 males  
**Route, formulation, volume, and infusion rate:** Bolus intravenous via tail vein,  
1 mL/kg  
**Satellite groups used for toxicokinetics or recovery:** No  
**Age:** 7-8 weeks  
**Weight:** 205-235 g

Sampling times: 2 animals were sacrificed at 5 min, 1 and 24 hours post dose.

## Results

Concentration of total activity ( $\mu\text{g}$  equiv salt/g, adjusted)

Tissues	5 min <sup>a</sup>	1 h	24 h
Adrenal cortex	3.81	0.24	0.14
Adrenal medulla	3.95	0.27	0.22
Adrenal (whole)	3.91	0.24	0.15
Aorta	2.23	0.25	0.15
Bladder	0.95	0.30	0.25
Blood	6.43	0.71	0.46
Bone marrow	1.27	0.08	0.06
Brain	0.13	0.02	*0.01
Brown fat	1.40	0.10	0.08
Epididymis	0.56	0.14	0.10
Eye	0.24	0.03	0.05
Harderian gland	2.31	0.11	0.10
Heart	4.66	1.67	0.14
Kidney cortex	35.80	6.72	4.16
Kidney medulla	8.49	2.67	0.32
Kidney (whole)	34.23	7.19	3.07
Lachrymal gland	1.81	0.11	0.08
Large intestine wall	1.60	0.18	0.16
Liver	18.62	1.30	0.46
Lung	5.61	0.54	0.32
Lymph node	1.71	0.15	0.12
Optic nerve	0.98	0.04	0.04
Pancreas	1.95	0.46	0.12
Pineal body	NP	NC	NC
Pituitary gland	3.71	0.15	0.14
Preputial gland	1.94	0.08	0.16
Prostate	1.55	0.19	0.09
Rectum	2.16	0.20	0.20
Salivary gland	3.03	0.16	0.13
Sciatic nerve	NP	NC	NC
Seminal vesicles	0.16	0.02	*0.01
Skeletal muscle	1.33	0.12	0.11

<sup>a</sup> = results from one animal only as one appeared to have been mis-dosed (see Table 1)

\* = value below limit of reliable measurement

NC = not calculable

Results expressed as  $\mu\text{g equiv salt/g}$  (adjusted)

Tissues	5 min <sup>a</sup>	1 h	24 h
Skin - non-pigmented	1.12	0.27	0.30
Skin - pigmented	1.54	0.28	0.33
Small intestine wall	2.44	0.32	0.09
Spinal cord	0.11	0.02	*0.01
Spleen	1.62	0.15	0.13
Stomach wall (non secretory)	1.04	0.17	0.13
Stomach wall (secretory)	1.97	0.14	0.10
Testes	0.18	0.07	0.05
Thymus	0.88	0.10	0.07
Thyroid gland	3.47	0.20	0.16
Uveal Tract	0.73	0.09	0.17
White fat	0.12	*0.01	*0.01
Limit of reliable measurement:	0.02	0.01	0.02
Eye (LSC)	0.56	0.51	0.07

<sup>a</sup> = results from one animal only as one appeared to have been mis-dosed (see Table 1)

\* = value below limit of reliable measurement

LSC = analysed by combustion and liquid scintillation counting

(Excerpted from the sponsor's submission)

The tissue distribution study of bendamustine hydrochloride in rats (Study No 183340, report 26495) indicated that the highest tissue levels of bendamustine were in the kidney and liver. The mass balance study (Study No 183204, report 26775) showed that significant radioactivity was recovered in both urine and feces.

#### 4.2.2.3.4 Excretion and Distribution Studies of <sup>14</sup>C-Bendamustin in the Dog. Study KLG-05.

##### Key study findings:

- Radioactivity excreted in the feces and urine was 66% and 22%, respectively, of the administered dose at 168 hours postdose.
- Maximum radioactivity was found in the liver, kidney, gastrointestinal tract, skin, skeletal muscle, and white fat.

Study no.:

KLG-05

Volume # and page #:

Module 4.2.2.3.4

**Conducting laboratory and location:**

Date of study initiation: July 10, 2000

GLP compliance: Yes

QA report: yes (X) no ( )

**Drug, lot #, and % purity:**

Bendamustine, batch # 21332 (9908005), — purity

<sup>14</sup>C-bendamustine, batch # MKR300500, 36.2 µCi/mg — purity**Methods**

Doses: 3.3 mg/kg  
 Species/strain: Beagle Hsd: DOBE dogs  
 Number/sex/group or time point (main study): Total 2 ♂ & 2 ♀  
 Route, formulation, volume, and infusion rate: Intravenous via a cannulated cephalic vein  
 Satellite groups used for toxicokinetics or recovery: None  
 Age: 9-21 months  
 Weight: 9-11 kg

**Results**

Recovery of radioactivity in organs/tissues following the intravenous administration of <sup>14</sup>C-bendamustine to male and female beagle dogs

Organ	Recovery (% dose administered)			
	1 hour (F)	24 hours (M)	48 hours (F)	168 hours (M)
Adrenal glands	0.02	BLQ	BLQ	BLQ
Bone	0.41	0.15	0.08	0.08
Bone marrow	0.07	0.02	0.03	BLQ
Brain	BLQ	BLQ	BLQ	BLQ
Caecum wall	0.34	0.09	0.68	0.03
Eyes	BLQ	BLQ	BLQ	BLQ
Gall bladder	0.01	0.04	0.03	0.01
Gall bladder contents	2.28	0.29	0.05	0.01
Heart	0.20	0.10	0.14	0.03
Kidney	0.54	0.10	0.29	0.14
Large intestine wall	0.78	0.16	0.75	0.10
Liver	8.52	2.16	2.29	1.32
Lungs	0.40	0.21	0.19	0.07
Lymph node	0.32	0.16	0.28	0.13
Non-pigmented skin	4.34	2.63	3.03	1.97
Ovaries	0.01	N.A.	BLQ	N.A.
Pancreas	0.06	0.03	0.02	BLQ
Pigmented skin	2.29	2.53	2.51	1.45
Pituitary	BLQ	BLQ	BLQ	BLQ
Plasma	1.97	0.83	0.85	0.34
Prostate	N.A.	0.01	N.A.	BLQ
Skeletal muscle	12.89	7.91	5.08	5.85
Small intestine wall	3.98	0.88	1.64	0.39
Spleen	0.25	0.08	0.23	0.05
Stomach wall	7.74	0.24	0.33	0.19
Testes	N.A.	0.01	N.A.	BLQ
Thyroid	BLQ	BLQ	BLQ	BLQ
Urinary bladder	0.06	0.02	0.02	0.02
Uterus	0.12	N.A.	0.02	N.A.
Vena cava	BLQ	BLQ	BLQ	BLQ
White fat	1.27	1.07	1.25	1.61

BLQ Below limits of quantification (<0.005% of dose administered)  
 N.A. – not applicable  
 Blood taken as 4.95% body weight  
 Bone taken as 5.5% body weight  
 Bone marrow taken as 0.35% body weight  
 Eyes, ovaries and testes – one organ lyophilised  
 Gastrointestinal tract tissues were measured without contents  
 Lymph tissue taken as 1.00% body weight  
 Plasma – based on a haematocrit (packed cell volume) value of 45%  
 Skeletal muscle taken as 45.5% body weight  
 Skin taken as 18% body weight (assumed equal distribution of pigmented and non-pigmented skin)  
 Vena cava expressed as % dose/g tissue  
 White fat taken as 7.1% bodyweight

(Excerpted from the sponsor’s submission)

Concentrations of bendamustine and two metabolites (HP1 and HP2) in plasma following intravenous administration of <sup>14</sup>C-bendamustine to male and female dogs

Time-point	Plasma concentration											
	Dog 1 (F)			Dog 2 (M)			Dog 3 (F)			Dog 4 (M)		
	Parent	HP1	HP2	Parent	HP1	HP2	Parent	HP1	HP2	Parent	HP1	HP2
5 min												
20 min												
30 min												
1 hour												
2 hours												
24 hours												
48 hours												
168 ho												

(Excerpted from the sponsor’s submission)

**Conclusions:**

Species	Recovery over 168 hours (%)				
	Feces	Urine	Cage wash	Organs	Total
Dog	66.4	22.2	2.4	2.0	93.0

**2.6.4.5 Metabolism**

**4.2.2.4.1 In Vitro Evaluation of CEP-18083 (Bendamustine) as an Inducer of Cytochrome P450 Expression in Cultured Human Hepatocytes.**  
Study DM-2005-004.

**Key study findings:**

- Bendamustine hydrochloride up to 100 µM had no effect on the expression of cytochrome P450 enzymes in primary cultures of human hepatocytes.
- Treatment of cultured human hepatocytes with prototypical P450 enzymes inducers caused anticipated increases in CYP activities.

**Study no.:** DM-2005-004.  
**Volume # and page #:** Module 4.2.2.4.1  
**Conducting laboratory and location:**

**Date of study initiation:** February 16, 2006  
**GLP compliance:** Yes  
**QA report:** yes (X) no ( )

**Drug, lot #, and % purity:**

Bendamustine hydrochloride (CEP-18083), lot # 00039P0006.  purity.  
Positive tests, omeprazole ( purity), phenobarbital ( purity), isoniazid ( purity), and rifampin ( purity) were obtained from

**Methods**

**Doses:** 1, 10, and 100 µM  
**Species/strain:** 3 separate human livers  
**Unique study design or methodology (if any):** Three preparations of cultured human hepatocytes from 3 human livers were treated with bendamustine or known human CYP enzyme inducers. After treatment, human liver hepatocytes were harvested, microsomes prepared, and microsomal enzymic activities measured.

## Results

Effects of treating cultured human hepatocytes with bendamustine or prototypical inducers on CYP activity (values are expressed as average fold induction)

Treatment	Concentration	Fold induction <sup>a</sup>							
		CYP1A2	CYP2A6	CYP2B6	CYP2C8 §	CYP2C9 §	CYP2C19	CYP2E1	CYP3A4/5
DMSO	0.1% (v/v)	1.00 (n=2)	1.00 ± 0.70	1.00 ± 0.38	1.00 ± 0.61	1.00 ± 0.80	1.00 (n=2)	1.00 ± 0.20	1.00 ± 0.23
Saline	0.1% (v/v)	1.00 (n=1)	1.00 ± 0.43	1.00 (n=2)	1.00 ± 0.66	1.00 ± 0.82	1.00 (n=2)	1.00 ± 0.46	1.00 ± 0.18
Bendamustine	1 µM	0.947 (n=2)	1.25 ± 0.40	1.10 ± 0.13	1.11 ± 0.08	1.10 ± 0.15	1.11 (n=2)	1.09 ± 0.03	1.28 ± 0.35
Bendamustine	10 µM	0.991 (n=2)	0.998 ± 0.349	1.01 ± 0.04	1.08 ± 0.09	1.00 ± 0.24	1.08 (n=2)	1.02 ± 0.05	1.15 ± 0.49
Bendamustine	100 µM	1.06 (n=2)	0.811 ± 0.233	0.982 ± 0.114	0.976 ± 0.170	1.05 ± 0.11	1.10 (n=2)	0.891 ± 0.176	1.12 ± 0.20
Omeprazole	100 µM	32.4 (n=2)	0.455 ± 0.144	7.62 ± 2.47	3.61 ± 2.11	2.43 ± 1.59	1.90 (n=2)	1.07 ± 0.20	1.88 ± 0.67
Phenobarbital	750 µM	1.73 (n=1)	2.20 ± 0.65 ¥	12.9 ± 1.8 ¥	7.99 ± 5.43	3.27 ± 2.85	2.64 (n=2)	1.08 ± 0.43	4.64 ± 1.04 ¥
Isoniazid	100 µM	0.923 (n=1)	0.963 ± 0.573	1.11 (n=2)	0.962 ± 0.380	0.736 (n=2)	0.872 (n=2)	2.92 ± 1.62	0.650 ± 0.419
Rifampin	10 µM	1.38 (n=2)	1.40 ± 0.74	7.75 ± 2.82	7.99 ± 4.75	3.66 ± 3.51	4.74 (n=2)	1.01 ± 0.31	3.54 ± 1.17 ¥

DMSO: Dimethyl sulfoxide

<sup>a</sup> The values shown are the mean ± standard deviation of 3 human hepatocyte preparations: H650, H655 and H656, unless indicated otherwise (eg, n = 2).

All values are rounded to 3 significant figures, and the standard deviation is rounded to the same degree of precision.

§ Significance found among treatment groups (where 0.1% DMSO is the vehicle control) according to Kruskal-Wallis One Way Analysis on Ranks ( $p < 0.05$ ) but unable to specify the groups that statistically differ from the other groups according to Dunnett's test with positive controls.

¥ Statistically significant compared to control (0.1% DMSO) according to Dunnett's Test ( $p < 0.5$ ) with positive controls.

(Excerpted from the sponsor's submission)

**Conclusions:** Bendamustine up to 100 µM did not increase or decrease any CYP-450 activities measured in human cultured hepatocytes.

4.2.2.4.2 **In Vitro Metabolism studies of <sup>14</sup>C-Bendamustine.**  
Study 99-37-KLG-01.

**Key study findings:**

- Bendamustine hydrochloride was metabolized by both dog and human liver microsomes to produce two components, M3 and M4.
- Both M3 and M4 production appear to be mediated by CYP1A2.

**Study no.:** 99-37-KLG-01  
**Volume # and page #:** Module 4.2.2.4.2  
**Conducting laboratory and location:**

**Date of study initiation:** December 2, 1999  
**GLP compliance:** Yes  
**QA report:** yes (X) no ( )

**Drug, lot #, and % purity:**

Bendamustine, batch # 21332 (9908005), — purity.  
<sup>3</sup>H-bendamustine, batch # MKR200400, 44 µCi/mL, ✓ purity.  
 β-Hydroxy bendamustine, batch 00210001, ✓ purity.  
 Monohydroxy-bendamustine, batch # Js 769A, ✓ purity.  
 Human liver microsomes were from 3♂ and 3♀.  
 Dog liver microsomes were from 3 male beagle dogs.

**Results**

Effect of CYP450 specific inhibitors on the microsomal metabolism of bendamustine by human liver microsomes

Inhibitor	Inhibitor Conc (µM)	M3		M4	
		% Activity	Std. dev	% Activity	Std. dev
Furafylline	0.1	56.1	7.2	56.3	7.5
	1	13.6	3.8	14.3	4.2
	50	1.4	0.2	blq	n/a
Tranylcypromine	0.1	92.7	12.5	89.2	15.2
	1	80.0	1.4	77.4	2.4
	50	14.5	0.6	12.4	0.6
Sulfaphenazole	0.1	90.7	7.8	86.0	9.5
	1	96.2	2.0	92.8	3.3
	50	101.6	1.5	98.7	1.5
Quinidine	0.1	99.3	2.7	102.6	4.2
	1	97.2	2.4	99.7	2.7
	50	74.1	5.5	70.3	4.2
4-Methylpyrazole	0.1	96.0	4.3	99.5	7.6
	1	94.8	8.1	94.1	8.8
	50	75.5	6.1	72.4	6.4
Ketoconazole	0.1	82.1	4.9	81.2	4.5
	1	86.7	4.9	86.7	5.4
	50	44.8	4.6	43.7	5.0

Values expressed as percentage of control incubations (no inhibitor present)

Std. dev standard deviation  
 Blq below limits of quantification  
 n.a not applicable

(Excerpted from the sponsor's submission)

## Human CYP1A2 activity compared to control

Compound	Concentration ( $\mu$ M)	% activity
Control	0	100
Bendamustine	20	104 $\pm$ 3
Bendamustine	200	90 $\pm$ 9
Furafylline	1	63 $\pm$ 4

## Human CYP2C9/10 activity compared to control

Compound	Concentration ( $\mu$ M)	% activity
Control	0	100
Bendamustine	20	93 $\pm$ 3
Bendamustine	200	92 $\pm$ 5
Sulfaphenazole	1	56 $\pm$ 5

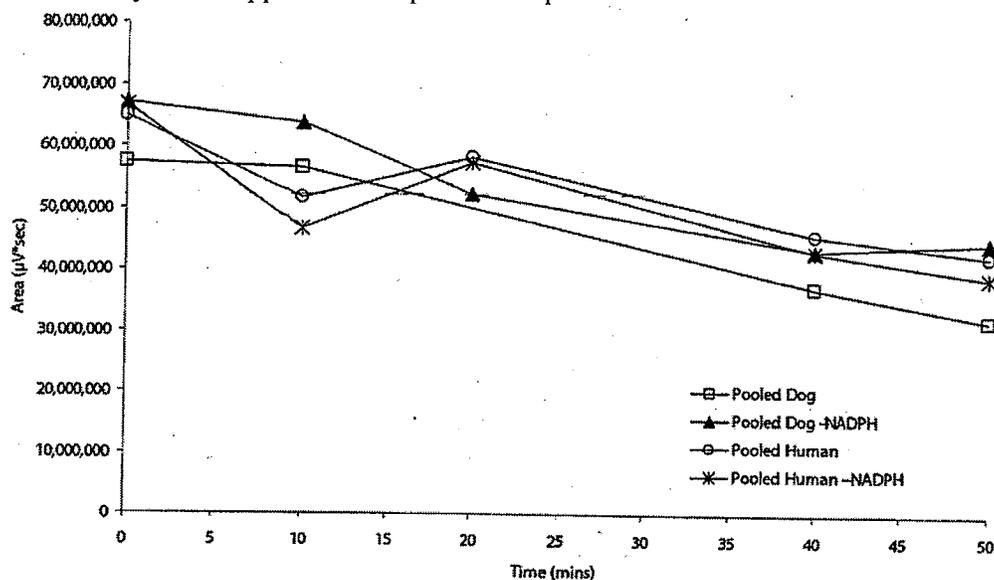
## Human CYP2D6 activity compared to control

Compound	Concentration ( $\mu$ M)	% activity
Control	0	100
Bendamustine	20	100 $\pm$ 8
Bendamustine	200	125 $\pm$ 25
Quinidine	1	7 $\pm$ 1

## Human CYP3A4 activity compared to control

Compound	Concentration ( $\mu$ M)	% activity
Control	0	100
Bendamustine	20	102 $\pm$ 11
Bendamustine	200	96 $\pm$ 8
Ketoconazole	1	4 $\pm$ 7

Metabolism of bendamustine (20  $\mu$ M) by dog and human liver microsomes (0.5 mg/mL) as measured by the disappearance of parent compound



There was no apparent evidence of a species difference in the qualitative nature of the metabolism of bendamustine.

**Conclusions:** Bendamustine was metabolized by both dog and human microsomes mainly by Phase 1 metabolism to produce an oxidative metabolite and an N-desmethyl metabolite. These processes appeared to be CYP1A2 mediated. Metabolism *in vitro*, by dog and human hepatic microsomes produced the same metabolites but the amount of metabolite production was greater in dog than human.

#### 4.2.2.4.3 Metabolic Profile of [ $^{14}$ C]-CEP-18083 (Bendamustine) in Rat Urine and Bile: Preliminary Structural Identification of Metabolites Study DM-2006-002.

##### Key study findings:

- [ $^{14}$ C]-CEP-18083.HCl (bendamustine) is extensively metabolized *in vivo* in rats.
- The major metabolic routes are proposed to be oxidative and/or hydrolytic dehalogenation, oxidation, carboxylic acid formation, N-dealkylation and/or sulfate, glutathione and cysteine conjugation.

**Study no.:** DM-2006-002  
**Volume # and page #:** Module 4.2.2.4.3  
**Conducting laboratory and location:** / /  
**Date of study initiation:** February 7, 2006  
**GLP compliance:** Yes  
**QA report:** yes (X no ( )  
**Drug, lot #, and % purity:** <sup>14</sup>C-CEP-18083 HCl  
 lot # and purity not provided

**Methods**

**Doses:** 3 mg/kg  
**Species/strain:** Sprague Dawley rats  
**Number/sex/group or time point (main study):** 5 males  
**Route, formulation, volume, and infusion rate:** Intravenous  
**Satellite groups used for toxicokinetics or recovery:** No  
**Age:** 7-8 weeks  
**Weight:** 284-326 g  
**Sampling times:** Urine was collected from 0.0 to 4.0 hours, 4 to 8 hours, and 8 to 24 hours post dose.  
 Bile samples were collected from 0.0 to 30 minutes, 30 to 60 minutes, and 60 to 120 minutes after dosing.

**Results**

Concentrations of total radioactivity in rat urine  
 Percent of dose collected during the indicated period

Collection period (hr)	001M	002M	003M	004M	005M
0-4	29.7	28.3	29.3	27.0	28.4
4-8	2.4	1.8	3.4	2.3	2.2
8-24	1.7	0.6	8.1	4.2	1.5



#### 4.2.2.4.4 In Vitro Plasma Protein Binding Studies of Bendamustine. Study KLG-06.

##### Key study findings:

- <sup>14</sup>C-bendamustine was relatively highly protein bound in both human (~94–96%) and dog (~72–78%) plasma.
- Bendamustine did not displace protein bound <sup>14</sup>C-warfarin when co-incubated with human serum albumin at physiological concentration.
- Prednisone, doxorubicin, vincristine, and mitoxantrone have no effect on the binding of bendamustine to human plasma proteins.
- Approximately 14 and 26% of the initial radioactivity of bendamustine hydrochloride (10 and 50 µg/mL) were apparently “covalently” bound to human serum albumin after 6 hours of incubation.

**Study no.:** KLG-06  
**Volume # and page #:** Module 4.2.2.4.4  
**Conducting laboratory and location:**

**Date of study initiation:** 14 July 2000  
**GLP compliance:** Yes  
**QA report:** yes (X) no ( )  
**Drug, lot #, and % purity:** <sup>14</sup>C-bendamustine, batch # MKR300500  
 specific activity 36.2 µM/mg — purity

##### Results

<sup>14</sup>C-bendamustine was incubated in triplicate at 37°C with individual human plasma (n = 3 subjects) and pooled dog plasma (pooled from 3 animals) for approximately 60 minutes. Following the incubation, an aliquot of each concentration was analyzed and results are shown below.

<sup>14</sup>C-bendamustin binding to human and dog plasma

Plasma	Nominal concentration of bendamustin (µg/mL)			
	1	5	10	50
Dog	78.41	78.27	77.72	72.62
Human	96.03 (± 0.04)	96.16 (± 0.11)	96.00 (± 0.13)	94.71 (± 0.17)

Values given are the % radioactivity bound.

Values for human represent the mean values from n = 3 determinations (± standard deviation)

Bendamustine hydrochloride showed more binding with human plasma than dog plasma.

Concentration of radioactivity in red blood cells and plasma following incubation of <sup>14</sup>C-bendamustine (10, 50 and 100 µg/mL) in whole human and dog blood for 30 minutes at approximately 37°C.

Subject number	Concentration (µg/mL)			
	Target	Blood	Cells	Plasma
Human 1	10			
	50			
	100			
Human 2	10			
	50			
	100			
Human 3	10			
	50			
	100			
Dog	10			
	50			
	100			

(Excerpted from the sponsor's submission)

There was no notable difference in the distribution of bendamustine between human and dog blood.

Effect of bendamustine on the extent of binding of <sup>14</sup>C-warfarin to human plasma proteins

Concentration of bendamustine (µg/mL)	Concentration of warfarin (µg/mL)	
	4	10
0	98.53	98.18
10	98.47	98.17
50	98.33	97.96

Values given are the % radioactivity bound

(Excerpted from the sponsor's submission)

Bendamustine has no measurable effect on the binding of <sup>14</sup>C-warfarin to human proteins.

Extent of binding of  $^{14}\text{C}$ -bendamustine to human plasma proteins  
in the presence of four co-administered compounds

Compound	Conc	Nominal bendamustine concentration ( $\mu\text{g/mL}$ )			
		10		50	
		Mean	stdev	Mean	stdev
No drug	0	96.15	0.14	96.32	0.16
Prednisone	0.1 $\mu\text{g/mL}$	96.28	0.13	96.32	0.08
	0.5 $\mu\text{g/mL}$	96.07	0.24	96.29	0.02
Doxorubicin	1 $\mu\text{g/mL}$	96.21	0.11	96.37	0.07
	10 $\mu\text{g/mL}$	96.28	0.11	96.43	0.08
Vincristine	0.1 ng/mL	96.13	0.14	96.35	0.13
	1 ng/mL	96.11	0.15	96.34	0.11
Mitoxantrone	0.1 ng/mL	96.22	0.15	96.31	0.07
	1 ng/mL	95.99	0.06	96.38	0.03

Values given are the % radioactivity bound  
(Excerpted from the sponsor's submission)

Prednisone, doxorubicin, vincristine, and mitoxantrone had no effect on the binding of bendamustine to human plasma proteins.

Incubation of  $^{14}\text{C}$ -bendamustine (10 and 50  $\mu\text{g/mL}$ ) with human serum albumin  
to determine extent of covalent binding

Nominal concentration ( $\mu\text{g/mL}$ )	Experiment	Actual concentration		% remaining	Mean (Stdev)
		t = 0 hours	t = 6 hours		
50	1	48.10	33.71	70.08	69.96 (2.99)
	2	48.69	32.58	66.91	
	3	48.29	35.20	72.89	
10	1	9.36	8.02	85.68	77.09 (7.59)
	2	9.52	7.07	74.26	
	3	9.45	6.74	71.32	

(Excerpted from the sponsor's submission)

This reduction in radioactive concentration after 6 hour of incubation may be due to non-specific absorption to the plastic walls of the incubation vessel or covalent binding to human serum albumin. These samples were dialyzed for 24 hours and further 40 hours (total 64 hours) and results are shown below.

Radioactivity associated with human serum albumin after 24 hours dialysis following incubation of  $^{14}\text{C}$ -bendamustine (10 and 50  $\mu\text{g/mL}$ ) with human serum albumin

Nominal concentration ( $\mu\text{g/mL}$ )	Experiment	Actual concentration		% remaining	Mean (Stdev)
		Before dialysis	t = 24 hours		
50	1	33.71	21.05	62.44	59.02 (4.42)
	2	32.58	19.74	60.59	
	3	35.20	19.02	54.03	
10	1	8.02	3.61	45.01	57.92 (11.61)
	2	7.07	4.33	61.24	
	3	6.74	4.55	67.51	

(Excerpted from the sponsor's submission)

Radioactivity associated with human serum albumin after 64 hours dialysis following incubation of  $^{14}\text{C}$ -bendamustine (10 and 50  $\mu\text{g/mL}$ ) with human serum albumin

Nominal concentration ( $\mu\text{g/mL}$ )	Experiment	Actual concentration		% remaining	Mean (Stdev)
		Before dialysis	t = 64 hours		
50	1	33.71	21.80	64.67	56.38 (8.83)
	2	32.58	18.69	57.37	
	3	35.20	16.58	47.10	
10	1	8.02	3.38	42.14	50.34 (7.59)
	2	7.07	3.66	51.77	
	3	6.74	3.85	57.12	

(Excerpted from the sponsor's submission)

Radioactivity "covalently" bound with human serum albumin after 64 hours dialysis following incubation of  $^{14}\text{C}$ -bendamustine (10 and 50  $\mu\text{g/mL}$ ) with human serum albumin

Nominal concentration ( $\mu\text{g/mL}$ )	Experiment	Radioactive concentration (dpm/g)	Incubation volume (mL)	Total radioactivity (dpm)	Total radioactivity in pellet (dpm)	% "Covalently" bound	Mean (Stdev)
50	1	2,708,859	20	54,177,180	6,837,263	12.62	13.53 (8.33)
	2	2,618,352	20	52,367,040	11,665,633	22.28	
	3	2,828,512	20	56,570,240	3,216,989	5.69	
10	1	644,921	20	12,898,420	4,643,400	36.00	25.68 (9.84)
	2	568,396	20	11,367,920	2,798,895	24.62	
	3	541,900	20	10,838,000	1,778,419	16.41	

(Excerpted from the sponsor's submission)

The mean proportions of radioactivity covalently bound were approximately 14 and 26%, respectively.

**2.6.4.6 Excretion****4.2.2.5.1 The Disposition of [<sup>14</sup>C]-CEP-18083 in the Rat Following Intravenous Administration.**  
Study Am 01 DM-2005-005**Key study findings:**

- Major route of excretion of radioactivity was via the feces (~50%).
- Urinary excretion was also substantial (~37%).
- Nearly 78% of the dose was excreted during the first 24 hours after administration.

**Study no.:** DM-2005-005  
**Volume # and page #:** Module 4.2.2.5.1  
**Conducting laboratory and location:** / /

**Date of study initiation:** February 7, 2006  
**GLP compliance:** Yes  
**QA report:** yes (X) no ( )  
**Drug, lot #, and % purity:** <sup>14</sup>C-CEP-18083, batch # RUS 0618,  
 purity, specific activity 381 MBq/mmol or 26.1 µCi/mg.  
 Bendamustine hydrochloride, batch # 05C31 purity

**Methods**

**Doses:** 3 mg/kg  
**Species/strain:** Albino (Sprague Dawley) rats  
**Number/sex/group or time point (main study):** 5 ♂  
**Route, formulation, volume, and infusion rate:** Intravenous via tail vein,  
 1 mL/kg  
**Satellite groups used for toxicokinetics or recovery:** 5 ♂  
**Age:** 7-8 weeks  
**Weight:** 284-326 g

**Observations and times:**

**Urine collected:** 0-4, 4-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 h post dose  
**Feces collected:** 24 hours intervals  
**Expired air collected:** 0-24 h postdose from 2 animals  
**Toxicokinetics:** Blood samples collected at 5 minutes and 0.25, 0.5, 1, 2, 4, 6, 9, 12, and 24 hours post dose.

**Results**

Excretion of total radioactivity (% administered dose)

Sample	Timepoint	001M	002M	003M	004M	005M	Mean	SD
Urine	4 h						28.5	1.0
	8 h						30.9	1.4
	24 h						34.2	3.9
	48 h						35.0	4.1
	72 h						35.4	4.1
	96 h						35.7	4.2
	120 h						36.0	4.2
	144 h						36.3	4.2
Faeces	168 h						36.5	4.2
	24 h						40.8	8.5
	48 h						45.5	7.6
	72 h						46.8	7.7
	96 h						47.5	7.5
	120 h						48.1	7.4
Cage Wash	144 h						48.6	7.2
	168 h						49.0	7.1
	24 h						2.7	1.1
	48 h						3.5	1.9
	72 h						3.9	1.8
	96 h						3.9	1.8
Exp Air 1	120 h						4.0	1.8
	144 h						4.1	1.8
	168 h						4.2	1.8
	24 h						0.0	n.a.
Exp Air 2	24 h						0.0	n.a.
G.I. Tract	168 h						0.2	0.0
Carcass	168 h						5.4	2.2
<b>Total</b>		<b>85.8</b>	<b>95.4</b>	<b>99.7</b>	<b>104.5</b>	<b>91.5</b>	<b>95.4</b>	<b>7.2</b>

\* = Results calculated from data less than 30 d.p.m. above background

° = Mean includes results calculated from data less than 30 d.p.m. above background

n.a. = Not appropriate

- = no sample collected

(Excerpted from the sponsor's submission)

Plasma radioactivity (µg equiv salt/mL)

Sample	Timepoint	006M	007M	008M	009M	010M	Mean	SD
Plasma	0.08 h						9.75	4.12
	0.25 h						5.26	2.12
	0.5 h						2.82	0.80
	1 h						1.94	0.50
	2 h						1.76	0.55
	4 h						1.87	0.67
	6 h						1.80	0.65
	9 h						1.65	0.61
	12 h						1.63	0.54
	24 h						1.34	0.53

(Excerpted from the sponsor's submission)

**Conclusions:** Following single dose intravenous administration of radiolabeled CEP-18083.HCl to Sprague Dawley rats, excretion was rapid, with the majority of the administered dose recovered by 24 h post dose. Relatively high concentrations of total

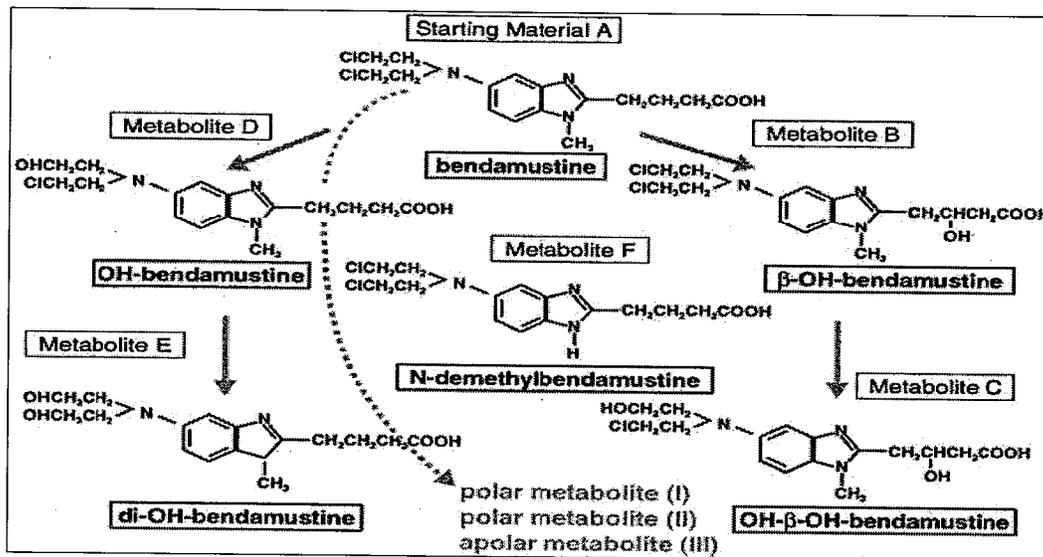
radioactivity excreted were found in feces (~50%) and urine (~37%). The majority of radioactivity eliminated remained constant from 1 h to 24 h.

2.6.4.8 Other Pharmacokinetic Studies:

None conducted

2.6.4.9 Discussion and Conclusions:

The pharmacokinetic studies of bendamustine were carried out in rodents and non-rodents. The drug was rapidly absorbed and broadly distributed after oral and intravenous administration. Elimination was predominately via metabolism, with more excretion in the feces than the urine. Proposed metabolism of bendamustine is shown below [Gandhi-V, Semin Oncol 29 (suppl 13):4-11, 2002].



The intermittent infusion of bendamustine at 5, 10, or 15 mg/kg/day for 15 consecutive weeks demonstrated systemic exposure of male and female rats to bendamustine and its metabolites (M3 and M4). The plasma concentrations of bendamustine and its metabolites were dose-related on days 1 and 3 (cycle 1) and on days 85 and 87 (cycle 5) indicating no accumulation with repeated administration of bendamustine.

**2.6.4.10 Tables and figures to include comparative TK summary***In vitro* microsomal metabolism of <sup>14</sup>C bendamustine (% of total)

Incubation time	50 minutes			
Species	Dog		Human	
Concentration (μM)	20	200	20	200
Compounds				
Parent	18.5	Similar parent/HP1 ratio	22.9	Similar relative amounts as at 20 μM
M2 (HP1)	74.2		70-75	
M3	6.4	<1	<1	
M4	1	<1	<1	

**2.6.5 PHARMACOKINETICS TABULATED SUMMARY**

Study type	Test system	Noteworthy findings
Tissue distribution	Rat	Highest levels of <sup>14</sup> C bendamustine at 5 min postdose were in the kidney and liver.
Excretion and distribution	Dog	Radioactivity excreted in the feces and urine was 66 and 22%, respectively, of the administered dose at 168 hours postdose.
<i>In vitro</i> metabolism	Dog and human liver microsomes	Bendamustine was metabolized by CYP1A2 in both dog and human liver microsomes.
Bendamustine as an inducer of CYP 450 enzymes	Cultured human hepatocytes	Bendamustine up to 100 μM had no effect on the expression of CYP 450 enzymes.
Plasma protein binding	<i>In vitro</i>	<sup>14</sup> C bendamustine was highly protein bound in both human (~95%) and dog (~75%) plasma.

## 2.6.6 TOXICOLOGY

### 2.6.6.1 Overall toxicology summary

General toxicology: A majority of the nonclinical studies were conducted in the former German Democratic Republic before the GLP regulations were in effect. Some of the original reports and published articles were in German and had been translated into English. Subsequent studies including pivotal 15 week intermittent infusion studies in rats and dogs, genotoxicity studies, and safety pharmacology studies were conducted according to GLP regulations.

Single dose intravenous administration of bendamustine in mice induced sedation, tremor, ataxia, convulsion, and respiratory distress. The intravenous LD<sub>50</sub> of bendamustine was 240 mg/m<sup>2</sup> in the mouse and rat. Oral administration of bendamustine in rats for 28 or 90 consecutive days induced weight loss, nasal secretion, writhing, piloerection and colored feces. Prior to death, respiration was impaired and the abdomen of the animals was extremely swollen. White blood cells and lymphocytes were decreased in bendamustine treated animals. Histopathologically, lymph nodes, spleen and thymus were atrophic.

A five day non-GLP intravenous infusion range finding study was conducted in rats to select dose levels for pivotal 15 week intermittent study in rats. In the pivotal study, male and female CD (SD) rats (20/sex/group) were dosed with bendamustine at 5, 10, or 15 mg/kg/day via 30 minute intravenous infusion once daily for 3 consecutive days in each of five dose cycles. Each dose cycle consisted of 21 days (3 dose days followed by 18 non-dose days). Mortalities (28) occurred in control and test animals throughout the study. Swollen ventral abdomens were observed in control and treated animals. This may be due to infusion apparatus and toxicity of the drug. White blood cell and absolute lymphocyte counts were decreased in all treated animals. Treatment related microscopic changes were in the kidney (tubular degeneration/necrosis) and bone marrow hyperplasia (femur and sternum). Bone marrow hyperplasia was not dose related. All animals with bone marrow hyperplasia died at unscheduled intervals. The plasma concentrations of bendamustine and its both active metabolites [ $\gamma$ -hydroxybendamustine (M3) and N-desmethylbendamustine (M4)] were dose-related over the dose range evaluated and did not appear to differ consistently with respect to the sex of the animal or the day of dosing. The apparent t<sub>1/2</sub> values ranged from 0.14 to 0.36 hr.

On the basis of the dose-range finding study in dogs, bendamustine hydrochloride was administered to beagle dogs (3/sex/group) at dosage levels of 1.65, 3.3 or 6.6 mg/kg/day by 30 minute daily intravenous infusion over 4 consecutive days for a total of three treatment cycles. Each cycle was followed by a 31 days recovery period. Three high dose animals (2 ♂ & 1 ♀) showed deterioration of health and were killed on humane grounds during the recovery phase of the second treatment. Remaining high dose animals were killed on day 29 of the second recovery period. Body weight loss and reduction in food consumption were noted in both sexes in a dose-related manner. Heart rates (2 ♂ & 1 ♀, 3/6 animals) were reduced during cycle 2 of high dose animals (6.6

mg/kg/day). Reduction in WBC counts and lymphocytes were observed in a dose-related manner. Bone marrow suppression (decreased myeloid cells) was observed in animals sacrificed on humane grounds. Lymphoid tissues of high dose animals showed marked or severe changes indicating immunosuppression. Bendamustine also affected testes (seminiferous tubular atrophy), and caused mucosal congestion and hemorrhage in the intestines.

Genetic toxicology: A standard battery of genetic toxicity studies was conducted to explore the genotoxic potential of bendamustine. Bendamustine hydrochloride induced mutation in bacterial mutation assay (Ames test) in a dose related manner in the presence and absence of metabolic activation (S9). Bendamustine hydrochloride also induced significant increase in the proportion of cells with chromosomal aberrations in human lymphocytes culture *in vitro*, both in the presence and absence of metabolic activation. The potential of bendamustine hydrochloride to induce micronucleated polychromatic erythrocytes in bone marrow was investigated in male and female Sprague Dawley rats. A single intravenous administration of bendamustine (CEP-18083) at doses up to 25 mg/kg induced a significant increase in the incidence of micronucleated polychromatic erythrocytes in male and female rats. Therefore, bendamustine was positive *in vivo* cytogenetic assay. Hydroxy-bendamustine (main metabolite of bendamustine) also induced structural chromosomal aberrations in human lymphocytes *in vitro* in the absence and presence of S9 mix.

Carcinogenicity: Standard carcinogenicity studies were not performed and are not required for oncology drugs. The design of the published study in 1974 was not adequate to fully assess the carcinogenic potential of bendamustine. Briefly, intraperitoneal injections of bendamustine for four days produced peritoneal sarcoma in female AB/jena mice (non-GLP study). Oral administration for four days induced mammary carcinoma and pulmonary adenomas.

Reproductive toxicology: Studies to assess the embryo-fetal developmental toxicity of bendamustine were not conducted by the sponsor. Embryofetal developmental toxicity studies were conducted in mice and rats and are reported in the literature. In these studies, bendamustine was administered by intraperitoneal injections. The proposed clinical route of administration is intravenous. Intraperitoneal administration of bendamustine produced in both species significant increase in the rate of malformations (dwarfism, exencephaly, and cleft palate). Bendamustine (ip administration) also caused external (bent/circinate tail) and internal (hydronephrosis and hydrocephalus) malformation in Wistar rats. These non-GLP published studies are considered to be consistent for an alkylating agent.

Special toxicology: The perivenous and intra-arterial tolerance of bendamustine hydrochloride was investigated in the New Zealand White rabbit. The histological findings showed a treatment-related effect when the animals were administered the highest two concentrations of bendamustine hydrochloride (0.6 mg/mL and 1.0 mg/mL) by perivenous injection. This effect was characterized by an increase in incidence and degree of perivascular changes indicative of local irritation.

**2.6.6.2 Single-dose toxicity**

**4.2.3.1.1 Bendamustine Single-Dose Toxicity Study in Mice and Rats  
Hartl 1989.**

**Key study findings:**

- Maximum tolerated dose by male mice by iv route was 50 mg/kg.
- Maximum tolerated dose by male mice by oral administration was 100 mg/kg.
- Medium lethal doses (LD<sub>50</sub>) were 80 and 400-500 mg/kg by iv and oral administrations, respectively, for male mice.
- Clinical signs included sedation, tremor, ataxia, convulsions, and respiratory distress.
- Dose dependent atrophies of the thymus, spleen and testes were observed.

**Study no.:** 1  
**Volume # and page #:** Module 4.2.3.1.1.  
**Conducting laboratory and location:** Academy of Sciences of the GDR  
 Central Institute of Microbiology and  
 Experimental Therapy (ZIMET), Jena  
 Germany  
**Date of study initiation:** April 1983  
**GLP compliance:** No  
**QA report:** yes ( ) no (X)  
**Drug, lot #, and % purity:** Bendamustine, lot # 301181, purity not  
 mentioned

**Methods**

Doses:	Mice –	IV – 40-125 mg/kg Oral – 50-700 mg/kg
	Rats	IV – 30-80 mg/kg Oral – 100-600 mg/kg
Species/strain:		Male hybrid mice (ABD2 F1) Male outbred rats (Jelei WIST)
Number/sex/group or time point (main study):		Not mentioned
Route, formulation, volume, and infusion rate:		Oral (gavage) and iv
Satellite groups used for toxicokinetics or recovery:		No
Age:		Not mentioned
Weight:		Mice-18.5-29.0 g Rats 90-410 g
Sampling times:		N/A
Unique study design or methodology (if any):		N/A

**Observations and times:**

Mortality: Daily

Clinical signs: Twice daily

Body weights, Food consumption, Ophthalmoscopy, EKG, Hematology, Clinical chemistry, Urinalysis, Gross pathology, Organ weights, Histopathology, and

Toxicokinetics: Not done

**Results**

Details of the relationship of dosing and clinical signs were not presented.

Mortality:

Species	Route of administration	MTD (mg/kg)	LD <sub>50</sub> (mg/kg)	LD <sub>100</sub> (mg/kg)
Mouse	Iv	50	80	100
	Oral	100	400-500	600
Rat	Iv	30	40	80
	Oral	100	200-300	600

**2.6.6.3 Repeat-dose toxicity****4.2.3.2.1 5-Day Intermittent Intravenous Infusion Dose Range Finding Toxicity Study with CEP-18083 (Bendamustine) in Rats with a 16-Day Recovery Period.****Key study findings:**

- Bendamustine up to 15 mg/kg/day for 5 days did not cause any mortality in rats.
- Food consumption and body weight gain were reduced during the dosing period but recovered at the end of the 16-day recovery period.
- There were no test article related effects on clinical pathology, necropsy findings, or organ weights at the end of the recovery phase.

**Study no.:** DS-2006-011.  
**Study Number:** 6573-176  
**Volume # and page #:** Module 4.2.3.2.1  
**Conducting laboratory and location:**  
**Date of study initiation:** 15 March 2006  
**GLP compliance:** No  
**QA report:** yes ( ) no (X)  
**Drug, lot #, and % purity:** Bendamustine (CEP-18083),  
 batch # A418738, — purity

**Methods**

**Doses:** 5, 10, or 15 mg/kg/day  
**Species/strain:** Male and female CD(SD) rats  
**Number/sex/group or time point (main study):** 5  
**Route, formulation, volume, and infusion rate:** IV infusion by 30 minutes,  
 10 mL/kg  
**Satellite groups used for toxicokinetics or recovery:** No  
**Age:** 65-72 days  
**Weight:** 283 to 378 g for ♂ and 197 to 255 g for ♀

**Observations and times:**

**Mortality:** Twice daily  
**Clinical signs:** Daily  
**Body weights:** Days 1 to 5, 6, 12, and 19  
**Food consumption:** Daily during dosing, from days 6 to 12, and days 12 to 19.  
**Ophthalmoscopy:** Not done  
**EKG:** Not done  
**Hematology:** Schedule sacrifice  
**Clinical chemistry:** Schedule sacrifice

Urinalysis: Schedule sacrifice  
Gross pathology: Necropsy  
Organ weights: Adrenal, brain, heart, kidney, liver, lung, ovary, spleen, , testis, thymus, uterus,  
Histopathology: Not done

**Results**

Mortality: None  
Clinical signs: 15 mg/kg – Few feces  
Body weights: 10 or 15 mg/kg/day – Reduced during the dosing period (maximum 9%), recovered after the recovery phase.  
Food consumption: 10 or 15 mg/kg/day – Reduced food consumption during the dosing period, recovered after 16 day recovery phase.  
Ophthalmoscopy: Not done  
EKG: Not done  
Hematology: No obvious effect at day 22 (necropsy).  
Clinical chemistry: No differences  
Urinalysis: No effect  
Gross pathology: No bendamustine related necropsy findings  
Organ weights: No differences  
Histopathology: Not done  
Toxicokinetics: Not done

4.2.3.2.2 **15-Week Intermittent Intravenous Infusion Toxicity and Toxicokinetic Study with CEO-18083 (Bendamustine) in Rats with a 4-Week Recovery Period.**

**Key study findings:**

- Male and female animals (total 28) died throughout the study.
- No unscheduled mortalities occurred during the recovery phase.
- Swollen ventral abdomens were observed in control and test animals.
- Bendamustine induced dose-related reductions in lymphocytes and WBC.
- Primary target organ of toxicity was the kidney.
- Kidney tubular epithelial karyomegaly at 15 mg/kg did not reverse by the end of the recovery period.
- Cardiomyopathy (focal/multifocal) was observed in male animals only.
- Systemic exposures to bendamustine and two metabolites were demonstrated.

**Study no.:**

— Study Number 6573-175  
 Sponsor Reference Number DS-2006-010

Volume # and page #: Module 4.2.3.2.2.

Conducting laboratory and location:

/ / /

Date of study initiation: May 2, 2006

GLP compliance: Yes

QA report: yes (X) no ( )

Drug, lot #, and % purity: CEP-18083 (bendamustine hydrochloride), lot # A418738, 05C31. — , purity

**Methods**

Doses:

Number	Group	No. of animals		Dose level (mg/kg/day)	Dose concentration (mg/mL)/
		Male	Female		
Toxicity Animals <sup>a</sup>					
1	Control	20	20	0	0
2	Low	20	20	5	0.5
3	Mid	20	20	10	1.0
4	High	20	20	15	1.5
Toxicokinetic Animals <sup>b</sup>					
5	Control	3	3	0	0
6	Low	9	9	5	0.5
7	Mid	9	9	10	1.0
8	High	9	9	15	1.5

- a Toxicity animals designated for recovery phase (5 animals/sex/group) underwent 4 weeks of recovery following dosing before sacrifice
- b Toxicokinetic animals were included only for the purpose of blood sample collections

Species/strain: CD (SD) rats

Number/sex/group or time point (main study): 20

Route, formulation, volume, and infusion rate: 30 minutes intravenous infusion, Mannitol (2.55 mg/mL in 0.9% sodium chloride, 10 mL/kg,

Satellite groups used for toxicokinetics or recovery: Yes

Age: 66-72 days

Weight: 309-388 g for males and 209-286 g for females

Unique study design or methodology: Animals were dosed by 30 minute intravenous infusion once daily for 3 consecutive days in each of five dose cycles. Each dose cycle consisted of 21 days (3 dose days followed by 18 non-dose days).

**Observations and times:**

<u>Mortality:</u>	Twice daily
<u>Clinical signs:</u>	Daily
<u>Body weights:</u>	Weekly
<u>Food consumption:</u>	Weekly
<u>Ophthalmoscopy:</u>	Predose, final week of dosing, and during the recovery phase
<u>EKG:</u>	Not done
<u>Hematology:</u>	Days 4, 25, 46, 67, and 88 of the dosing phase, unscheduled and scheduled sacrifices.
<u>Clinical chemistry:</u>	Days 4, 46, and 88 of the dosing phase, unscheduled and scheduled sacrifices
<u>Urinalysis:</u>	Days 4, 26, and 88
<u>Gross pathology:</u>	Days 106 (dosing phase) and 134 (recovery animals)
<u>Organ weights:</u>	See histopathology inventory for this IND
<u>Histopathology:</u>	Adequate Battery: yes (X), no ( )—explain Peer review: yes ( ), no (X)
<u>Toxicokinetics:</u>	First and last dose days of dose cycles 1 and 5 (Days 1 and 3 and 85 and 87 of the dosing phase) at predose, 5 and 30 minutes, and 1, 2, 4, 6, and 24 hours postdose.

**Results**Mortality:

Fifteen animals were found dead or sacrificed early during the dosing phase

Group	Dose (mg/kg/dose)	Sex	Animal No.	Day of Death	Death Reason	Cause of Death <sup>b</sup>
1	0	Male	B63485	97	Found Dead	Undetermined
2	5	Male	B63495	72	Found Dead	Undetermined
2	5	Male	B63505	32	Found Dead	Undetermined
2	5	Male	B63506	22	General Debilitation	Undetermined
3	10	Male	B63516	76	General Debilitation	Undetermined
3	10	Male	B63525	67	General Debilitation	Undetermined
4	15	Male	B63527	67	General Debilitation	Pyelonephritis
4	15	Male	B63532	50	General Debilitation	Pyelonephritis
4	15	Male	B63544	105	General Debilitation <sup>c</sup>	Hydro nephrosis
1	0	Female	B63586	59	Found Dead	Undetermined
2	5	Female	B63607	65	Found Dead	Lung Thrombosis
2	5	Female	B63613	64	General Debilitation	Glomerulopathy
2	5	Female	B63616	60	General Debilitation	Pyelonephritis
3	10	Female	B63633	92	Found Dead	Pyelonephritis
4	15	Female	B63643	38	Skin Sore	Undetermined

a Entered by inlife technician

b Determined by anatomic pathologist

c Animal died prior to sacrifice

(Excerpted from the sponsor's submission)

## Summary of animals found dead or sacrificed early during the dosing phase

Group	Dose (mg/kg/day)	No. of animals died			Days of death
		♂	♀	Total	
1	0	1	1	2	97 & 59
2	5	3	3	6	22-72
3	10	2	1	3	67-92
4	15	3	1	4	50-105

## Thirteen animals were sacrificed early due to damaged catheters.

Group	Dose (mg/kg/dose)	Sex	Animal No.	Day of Sacrifice
1	0	Male	B63469	80
1	0	Male	B63481	24
2	5	Male	B63496	36
2	5	Male	B63497	36
3	10	Male	B63507	46
3	10	Male	B63508	55
3	10	Male	B63519	25
4	15	Male	B63535	57
4	15	Male	B63536	57
7	10	Male	B63567	23
8	15	Male	B63573	26
1	0	Female	B63588	16
6	5	Female	B63666	80

(Excerpted from the sponsor's submission)

## Summary of animals sacrificed early due to damaged catheters (by group)

Group	Dose (mg/kg/day)	No. of animals sacrificed			Days of sacrificed
		♂	♀	Total	
1	0	2	1	3	16, 24 & 80
2	5	2	0	2	36
3	10	3	0	3	25, 46 & 55
4	15	2	0	2	57
6	5	0	1	1	80
7	10	1	0	1	23
8	15	1	0	1	26

No test article related mortalities were noted during the dosing period.

No unscheduled mortalities occurred during the recovery phase.

Clinical signs: Swollen ventral abdomens were observed in control and treated animals but higher in high dose animals.

Body weights: 5, 10, and 15 mg/kg – Reduced ( $\downarrow < 10\%$ ) during the dosing cycles for males only, no dose response.

Food consumption: 15 mg/kg/day – Reduced ( $\downarrow \sim 10\%$ ) during the dosing cycles

Ophthalmoscopy: No test article related findings

EKG: Not done

Hematology: 15 mg/kg/day on dosing day 4 - white blood cells ( $\downarrow$  70-80%) and lymphocytes ( $\downarrow$  50-75%) vs. control, showed partial recovery during the recovery period.

Clinical chemistry: No effect

Urinalysis: No test article related changes

Gross pathology: No test article related macroscopic changes

Organ weights: No test article related effect

Histopathology: Adequate Battery: yes (X), no ( )—explain  
Peer review: yes ( ), no (X)

#### Summary of microscopic observation – Unscheduled deaths and sacrifices

Animal sex Dosage group Tissue/findings No. in group	Males				Females			
	1	2	3	4	1	2	3	4
Catheter site, abscess, focal	0	0	1	3	0	2	1	1
Inflammation, chronic, focal	0	1	1	1				
Thrombus, focal	0	2	0	1				
Death comment, pyelonephritis	0	0	0	2	0	1	1	0
Hydronephrosis	0	0	0	1				
Heart, cardiomyopathy, multifocal	0	0	0	2				
Focal	0	1	1	0		1		
Kidney, karyomegaly, tubular epithelial, diffuse, unilateral	0	0	0	1	0	1	0	0
bilateral	0	0	0	2				
Hydronephrosis, diffuse, unilateral	0	2	0	2		1	1	
Bilateral	0	0	0	1				
Liver, infiltrate, lympho-histiocytic, multifocal	0	0	3	4	1	0	0	0
Prostate, inflammation, suppurative, diffuse	0	0	0	1				
Infiltrate, lymphocytic, multifocal	0	0	0	1				

#### Summary of microscopic observations – Dosing phase – Final phase sacrifice

Animal sex Dosage group Tissue/findings No. in group	Males				Females			
	1	2	3	4	1	2	3	4
	12	10	10	11	13	12	14	14
Adrenal, cortex, vacuolation, diffuse, bilateral	2	-	-	9				
Heart, cardiomyopathy, multifocal	2	-	-	6	-	0	0	0
Focal	0	-	-	2	0	0	0	0
Kidney, karyomegaly, tubular epithelial, diffuse, unilateral	0	8	2	10				
bilateral	0	8	7	1	0	0	0	10
Regeneration, tubular epithelial, multifocal, bilateral			1	4				
Degeneration/necrosis, tubular, multifocal, bilateral					0	0	0	7
Liver, vacuolation, hepatocytic, multifocal					0	0	0	2

## Summary of microscopic observation – Recovery Phase – Final phase sacrifice

Tissue/findings	Animal sex Dosage group No. in group	Males				Females			
		1	2	3	4	1	2	3	4
		5	5	5	4	5	5	5	5
Heart, cardiomyopathy, focal		0	0	0	2				
Kidney, karyomegaly, tubular epithelial, diffuse, bilateral Multifocal, bilateral Degeneration/necrosis, tubular, multifocal, bilateral		0	0	3	3	0	0	0	1
		0	4	2	1	0	0	3	1
		0	0	0	2				

Toxicokinetics:

Toxicokinetic parameters for bendamustine in male and female rats on the first and last dose-day of cycles 1 (days 1 and 3) and 5 (days 85 and 87).

Sex	Day	Group	Dose (mg/kg/day)	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (hr)	AUC <sub>0-t</sub> (ng•hr/mL)	AUC <sub>0-∞</sub> (ng•hr/mL)	t <sub>1/2</sub> (hr)
Male	1	6	5	3324.6	0.5	1733	1734	0.16
		7	10	7744.4	0.5	4104	NC	NC
		8	15	12882.5	0.5	6835	6837	0.15
	3	6	5	3022.7	0.5	1593	1594	0.15
		7	10	10377.5	0.5	5366	NC	NC
		8	15	15682.0	0.5	8139	NC	NC
	85	6	5	4349.6	0.5	2257	2258	0.14
		7	10	7295.8	0.5	4006	4006	0.36
		8	15	11425.5	0.5	5988	5989	0.14
87	6	5	1727.0	0.5	930	931	0.15	
	7	10	6075.7	0.5	3294	3298	0.18	
	8	15	11343.8	0.5	5915	5918	0.16	
Female	1	6	5	2793.9	0.5	1452	1452	0.14
		7	10	6451.8	0.5	3363	NC	NC
		8	15	11614.3	0.5	6022	NC	NC
	3	6	5	2375.1	0.5	1227	1227	0.14
		7	10	9036.6	0.5	4643	NC	NC
		8	15	11924.7	0.5	6215	NC	NC
	85	6	5	3204.3	0.5	1634	1634	0.15
		7	10	5911.0	0.5	3072	3078	0.20
		8	15	12067.8	0.5	6331	NC	NC
	87	6	5	4037.1	0.5	2031	NC	NC
		7	10	7589.3	0.5	3939	3942	0.17
		8	15	14644.4	0.5	7608	NC	NC

NC: Not Calculable

(Excerpted from the sponsor's submission)

Toxicokinetic parameters for M3 ( $\gamma$ -hydroxybendamustine) in male and female rats

Sex	Day	Group	Dose (mg/kg/day)	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (hr)	AUC <sub>0-t</sub> (ng·hr/mL)
Male	1	6	5	53.9	0.5	31
		7	10	186.0	0.5	121
		8	15	254.4	0.5	145
	3	6	5	59.0	0.5	34
		7	10	204.2	0.5	127
		8	15	288.9	0.5	158
	85	6	5	21.1	0.5	12
		7	10	49.7	0.5	39
		8	15	112.8	0.5	60
87	6	5	28.5	0.5	16	
	7	10	57.6	0.5	33	
	8	15	100.8	0.5	54	
Female	1	6	5	93.1	0.5	48
		7	10	174.8	0.5	91
		8	15	295.9	0.5	155
	3	6	5	103.2	0.5	54
		7	10	239.2	0.5	124
		8	15	382.2	0.5	201
	85	6	5	68.5	0.5	36
		7	10	83.8	0.5	44
		8	15	193.2	0.5	101
	87	6	5	73.9	0.5	39
		7	10	94.5	0.5	50
		8	15	215.9	0.5	113

(Excerpted from the sponsor's submission)

## Toxicokinetics for M4 (N-des-methylbendamustine) in male and female rats

Sex	Day	Group	Dose (mg/kg/day)	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (hr)	AUC <sub>0-t</sub> (ng·hr/mL)
Male	1	6	5	1.8	0.5	0.5
		7	10	4.2	0.5	1.1
		8	15	5.0	0.5	3.0
	3	6	5	2.7	0.5	0.7
		7	10	5.3	0.5	1.3
		8	15	8.8	0.5	4.9
	85	6	5	BLQ<1.0	NC	NC
		7	10	1.5	0.5	0.4
		8	15	3.5	0.5	0.9
87	6	5	BLQ<1.0	NC	NC	
	7	10	2.0	0.5	0.5	
	8	15	3.2	0.5	0.8	
Female	1	6	5	2.8	0.5	0.7
		7	10	6.3	0.5	1.6
		8	15	9.7	0.5	2.4
	3	6	5	3.2	0.5	0.8
		7	10	8.3	0.5	2.1
		8	15	12.2	0.5	3.1
	85	6	5	4.3	0.5	1.1
		7	10	4.5	0.5	1.1
		8	15	9.2	0.5	2.3
	87	6	5	3.8	0.5	1.0
		7	10	4.2	0.5	1.1
		8	15	10.1	0.5	2.5

BLQ&lt;1.0: Below the limit of quantitation of 1.0 ng/mL.

NC: Not Calculable

(Excerpted from the sponsor's submission)

The increases in  $C_{max}$  and AUC on all sampling days [days 1 and 3 (cycle 1) and on days 85 and 87 (cycle 5)] were approximately dose proportional in males and females. No gender differences are noted.

## 4.2.3.2.5

**Bendamustine One-Month Oral Toxicity Study in Rats.  
Horn et al 1984.**

**Key study findings:**

- Bendamustine at 80 and 160 mg/kg caused 50% mortality by week 4.
- Chlorambucil at 10 mg/kg produced 100% mortality by 4<sup>th</sup> week.
- There was a dose dependent decrease in body weight gain of animals treated with bendamustine or chlorambucil.
- AST, ALT, alkaline phosphatase, total cholesterol, urea nitrogen and creatinine did not show treatment related changes.
- Bendamustine at 40, 80, or 160 mg/kg for 4 weeks in rats caused inhibition of bone marrow hematopoiesis and a decrease in lymphocytes in the peripheral blood.

<b>Study no.:</b>	Not mentioned
<b>Volume # and page #:</b>	Module 4.2.3.2.5
<b>Conducting laboratory and location:</b>	Academy of Sciences of the GDR, Research Center for Microbiology and Medicine, Central Institute for Microbiology and Experimental Therapy (ZIMET) Jena, Department of Pharmacology and Toxicology, Germany
<b>Date of study initiation:</b>	Not known
<b>Date of the report:</b>	10-01-1984
<b>GLP compliance:</b>	No
<b>QA report:</b>	yes ( ) no (X)
<b>Drug, lot #, and % purity:</b>	Bendamustine (IMET 3393), batch # 301181, purity not mentioned Chlorambucil as positive control

**Methods**

Doses:	Bendamustine:	5, 10, 20, 40, 80 or 160 mg/kg
	Chlorambucil:	1, 5, or 10 mg/kg
Species/strain:		Male Jelei: Wist rats
Number/sex/group or time point (main study):		10
Route, formulation, volume, and infusion rate:		Oral gavage daily, 10 mL/kg
Satellite groups used for toxicokinetics or recovery:		None
Age:		Not given



Hematology:

## Leucocytes values different from control (↓ %)

Compound	Dose (mg/kg)	Week				
		1	2	3	4	6
Bendamustine	5	17	0	22	31	36
	10	25	28	33	53	30
	20	16	26	42	45	21
	40	50	55	40	37	17
	80	61	44	50	61	-
	160	32	55	65	64	-
Chlorambucil	1	0	40	49	52	33
	5	75	63	73	78	18
	10	59	88	89	-	-

## Lymphocytes values different from control (↓ %)

Compound	Dose (mg/kg)	Week				
		1	2	3	4	6
Bendamustine	5	10	10	6	8	0
	10	12	20	15	12	4
	20	30	33	5	18	3
	40	39	36	17	36	21
	80	65	83	83	86	-
	160	79	90	88	93	-
Chlorambucil	1	10	4	25	29	19
	5	36	71	69	70	41
	10	46	100	48	-	-

Dose-dependent decrease in white blood cell count and lymphocytes were observed.

Clinical chemistry: Changes in leucinarylamidase (LAP) compared to control (↓ %)

Compound	Concentration (mg/kg)	Leucinarylamidase	
		Week 4	Week 6
Bendamustine	10	32	20
	20	42	5
	40	38	25
Chlorambucil	5	53	30

AST, ALT, alkaline phosphatase, total cholesterol, urea nitrogen and creatinine did not show treatment related changes.

Urinalysis:

Protein in the urea at higher concentrations of bendamustine

Gross pathology:

No compound related changes were found.

Organ weights:

## Changes in organ weights compared to control (%)

Compound	Concentration (mg/kg)	Thymus	Spleen	Adrenals	Testes
Bendamustine	40	↓ 54	↓ 20	↑ 77	↑ 5
	80	↓ 94	↓ 14	↑ 105	↑ 91
	160	↓ 91	↑ 21	↑ 227	↑ 90
Chlorambucil	1	↓ 33	↓ 32	↑ 9	↑ 14
	5	↓ 52	↓ 38	↑ 18	↓ 27

Histopathology: Adequate Battery: yes ( ), no (X)  
 Explain: Limited tissues examined  
 Peer review: yes ( ), no (X)

Organ/findings	Bendamustine (mg/kg)			Chlorambucil (mg/kg)	
	40	80	160	1	5
Bone marrow inhibition of hematopoiesis	Slight	Severe	Severe	-	Slight
Intestine, epithelial necrosis	Slight	Slight	Slight	-	-
Lymphatic atrophy	Slight	Moderate	Severe	-	Slight
Inhibition of inflammation	-	Moderate	Severe	-	Slight
Kidney tubules, cariomegaly	Slight	Moderate	Severe	-	Slight
Epithelial necrosis	Slight	Slight	Slight	-	Slight
Enlargement	Slight	-	Moderate	-	-
Lung, bronchopneumonia	-	Slight	Slight	-	-
Lymphatic atrophy	-	Slight	Slight	-	-
Lymph nodes atrophy	Slight	Moderate	Severe	-	-
Spleen, follicular atrophy	Moderate	Severe	severe	-	Slight
Inhibition of hematopoiesis	Slight	Moderate	Severe	-	Slight
Thymus, atrophy	Slight	Severe	Severe	Moderate	Severe

**4.2.3.2.6 Bendamustine 3-Months Oral Toxicity Study in Rats****Key study findings:**

- Bendamustine at 40 and 60 mg/kg produced 70% and 100% mortalities, respectively, between week 7 and 14.
- Bendamustine induced weight loss, nasal secretion, writhing, piloerection and colored feces.
- Prior to death, respiration was impaired and the abdomen of the animals was extremely swollen.
- Histopathologically, lymph nodes, spleen and thymus were atrophic.

Study no.: 43/44  
 Volume # and page #: Module 4.2.3.2.6  
 Conducting laboratory and location:

Date of study initiation: October 6, 1983  
 GLP compliance: No

The present study was conducted in accordance with the \_\_\_\_\_

QA report: yes ( ) no (X)  
 Drug, lot #, and % purity: Bendamustine, batch # 080383,  
 purity not mentioned.

**Methods**

Doses: Bendamustine – 20, 40, and 60 mg/kg/day  
 Chlorambucil – 5 mg/kg/day  
 Species/strain: Wistar rats  
 Number/sex/group or time point (main study): 16  
 Route, formulation, volume, and infusion rate: 0.5 mL/animal  
 Satellite groups used for toxicokinetics or recovery: No  
 Age: Not mentioned  
 Weight: Males mean weight – 284 g  
 Females mean weight – 211 g

**Observations and times:**

Mortality: Daily  
Clinical signs: Daily  
Body weights: Weekly  
Food consumption: Not measured  
Ophthalmoscopy: Not done  
EKG: Not done

Hematology: Before testing, during weeks 3, 11, and 14  
Clinical chemistry: Before testing, during weeks 3, 11, and 14  
Urinalysis: Not done  
Gross pathology: At necropsy  
Organ weights: See histopathology for this IND  
Histopathology: Adequate Battery: yes ( ), no (X)  
 explain: limited tissues examined  
 Peer review: yes ( ), no (X)

**Results**

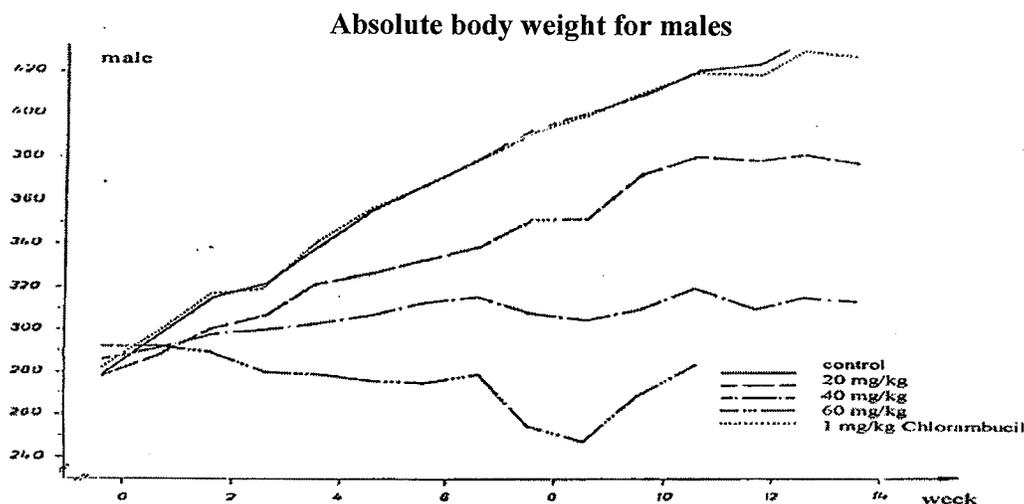
Mortality:

Dose (mg/kg)	Males		Females	
	Animals died (weeks)	Mortality (%)	Animals died (weeks)	Mortality (%)
Control	0	0	0	0
20	2 (11&12)	12.5	2 (4 & 9)	12.5
40	15 (4-14)	94	9 (1-13)	56
60	16 (5-11)	100	16 (2-11)	100

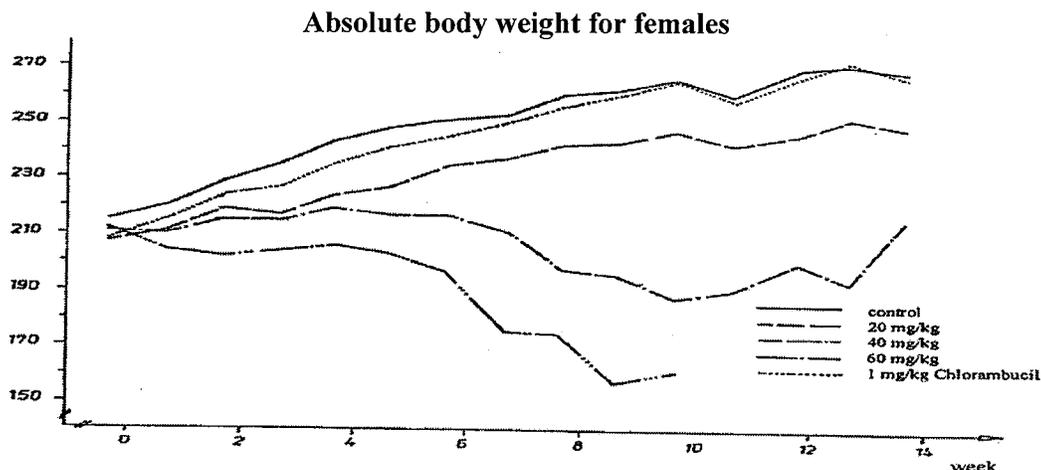
Clinical signs: Bendamustine at 40 & 60 mg/kg – Colored feces, secretion out of nose, piloerection, impaired respiration and swollen abdomen before death.

Chlorambucil – No effect

Body weights: Bendamustine at 20, 40, & 60 mg/kg – Dose-dependent reduction in body weight as compared with control.



(Excerpted from the sponsor's submission)



(Excerpted from the sponsor's submission)

- Food consumption: Not measured
- Ophthalmoscopy: Not done
- EKG: Not done
- Hematology: Bendamustine - ↓ white blood cells, lymphocytes  
Chlorambucil – No effect
- Clinical chemistry: Bendamustine - ↓ AST, ↓ alkaline phosphate, ↑ ALT
- Urinalysis: Protein excretion was increased
- Bone marrow: Bendamustine at 40 & 60 mg/kg – Myelocytes, promyelocytes and myeloblast were increased.
- Gross pathology: Inflammatory changes in the lungs of dead animals
- Organ weights: Changes in organ weight at 40 mg/kg bendamustine as compared to control (%)

Sex	Spleen	Thymus	Brain	lung	kidney	Testes
Male	↓ 39	↓ 38	↓ 5	↑ 77	↓ 14	↓ 15
Female	↓ 30	↓ 39		↑ 97		

- Histopathology: Adequate Battery: yes ( ), no (X)  
explain: limited tissues examined
- Peer review: yes ( ), no (X)

Organ finding	20 mg/kg	40 mg/kg	60 mg/kg
Bone marrow, congestion		Moderate	Severe
Lipid deposition in the tubules		Slight	Moderate
Liver, activation of stern cells	Slight	Moderate	Moderate
Congestion		Slight	Slight
Lymph nodes, number of lymphocytes decreased		Slight	Moderate
Hemosiderosis		Slight	Moderate
Spleen, number of lymphocytes decreased		Moderate	Moderate
White pulp atrophic		Slight	Moderate
Hemosiderosis		Slight	Moderate

Organ finding	20 mg/kg	40 mg/kg	60 mg/kg
Testes, germinative Decreased content of epididymis Juvenile cells			Severe
		Moderate	Severe
		Slight	Moderate
Thymus, number of lymphocytes decreased  atrophy Medulla/cortex border disappeared		Slight/ moderate	Severe
		Moderate	Severe
	Slight	Moderate	Severe

Toxicokinetics: Not done

**4.2.3.2.3 Bendamustine Hydrochloride Maximum Tolerated Dose and Five Day Repeated Dose Study in Dogs by Intravenous Infusion.**

**Key study findings:**

- Maximum tolerated dose was 9.9 mg/kg/day over 3 day treatment cycle to beagle dogs.

**Study no.:** 0640.98.C2.01.  
**Volume # and page #:** Module 4.2.3.2.3.  
**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** January 14, 1997  
**GLP compliance:** Yes  
**QA report:** yes (X) no ( )  
**Drug, lot #, and % purity:** Bendamustine hydrochloride,  
 batch # ChB 101096, — purity

**Methods**

Doses:

Pair	Dosage (mg/kg/day)	No. days dosed	Recovery period (days)
1	3.3	3	-
	6.6	3	16
	9.9	3	23
2	13.2	3	26
3	6.6	5	23

Species/strain: Beagle dogs  
 Number/sex/group or time point (main study): 1 ♂ and 1 ♀/group  
 Route, formulation, volume, and infusion rate: IV by 30 minute infusion  
 Satellite groups used for toxicokinetics or recovery: No  
 Age: 25-33 weeks  
 Weight: 9.1-10.1 kg

**Observations and times:**

Mortality: Regularly during the day  
Clinical signs: Regularly  
Body weights: Days 1, 3 and 5, and twice weekly during the recovery period  
Food consumption: Daily  
Ophthalmoscopy: Not done  
EKG: Before dosing, on second day of dosing and once during the 5-day dosing.  
Hematology: Day 5 and during the recovery phase  
Clinical chemistry: Not done  
Urinalysis: Not done  
Gross pathology: At necropsy  
Organ weights: Adrenals, brain, heart, kidneys, liver, lung, pancreas, pituitary, spleen, thymus, testes (with epididymides) or ovaries, thyroids (with parathyroids), uterus or prostate.  
Histopathology: Not done

**Results**

Mortality: No unscheduled death  
Clinical signs: Pair 1 at 6.6 and 9.9 mg/kg/day – Salivation and vomiting  
 Pair 2 at 13.2 mg/kg/day – Salivation and vomiting  
 Pair 3 at 6.6 mg/kg/day – Salivation, vomiting, and liquid feces  
Body weights: Dose dependent decrease in body weight  
Food consumption: Dose dependent decrease in food consumption  
Ophthalmoscopy: Not done  
EKG: No apparent changes in heart rate (1 pair/reading).

Dog no./sex	Heart rate/min							
	Pre-dose	Day 2 at 3.3 mg/kg	Day 2 at 6.6 mg/kg	After 2 week recovery	Day 2 at 13.2 mg/kg	Day 2 at 9.9 mg/kg	Pre-dose	Day 5 at 6.6 mg/kg
35♂	144	140	169	128			122	
36♀	108	130	112 (R136)	150			156	
37♂	93				106			
38♀	133				167			
39♂	122						135	94
40♀	111						108	102

R Repeat

(Excerpted from the sponsor’s submission)

Hematology: Decreased white blood cell, reticulocyte and platelet counts were decreased.  
Clinical chemistry: Not done  
Urinalysis: Not done  
Gross pathology: No consistent changes related to bendamustine treatment  
Organ weights: No effect  
Histopathology: Not done  
Toxicokinetics: Not done

**4.2.3.2.4 Bendamustine Hydrochloride Toxicity to Dogs by Daily Intravenous Infusion Over a Minimum of Three 4-Day Cycles Each Followed by a Period Without Treatment of up to 31 Days.**

**Key study findings:**

- Dose levels selection was based on the MTD study # RBM001/9712423.
- Bendamustine at high dose (6.6 mg/kg/day) produced vomiting, salivation, body weight loss and reduction in food consumption.
- High dose animals were sacrificed during the second recovery period on humane grounds.
- Heart rates of high dose animals (6.6 mg/kg/day) were reduced during cycle 2 (2♂ & 1 ♀, 3/6 animals)
- Reduction in WBC counts and lymphocytes were observed in a dose-related manner.
- Treatment related changes were seen in lymphoid tissues, heart, testes and intestines, especially in high dose animals.

**Study no.:** RBM 003/974052  
**Volume # and page #:** Report No.: 0640.98.G2.02  
**Conducting laboratory and location:** Module 4.2.3.2.4

**Date of study initiation:** June 16, 1997  
**GLP compliance:** Yes  
**QA report:** yes (X) no ( )  
**Drug, lot #, and % purity:** Bendamustine hydrochloride,  
batch No. ChB 101096, purity.

**Methods**

Doses:

Group	Dosage (mg/kg/day)	Dose volume (mL/kg/day)	Animals/sex/group	No. days dosed	Recovery period (days)	Total cycles
1	0	6.6	3	4	31	3
2	1.65	1.65	3	4	31	3
3	3.3	3.3	3	4	31	3
4	6.6	6.6	3	4	31	3

Species/strain: Beagle dogs  
 Number/sex/group or time point (main study): 3  
 Route, formulation, volume, and infusion rate: IV by 30 minute infusion, 0.9% saline, /  
 Satellite groups used for toxicokinetics or recovery: No  
 Age: 33-40 weeks  
 Weight: 9.3-13.8 Kg

**Observations and times:**

Mortality: Daily  
Clinical signs: Regularly during the day  
Body weights: Weekly  
Food consumption: Daily  
Ophthalmoscopy: Before treatment and during the last treatment  
EKG: Before dosing and after dosing on day 4 of the second and last treatment  
Hematology: Before treatment and at the end of third treatment cycle.  
Clinical chemistry: Before treatment and at the end of third treatment cycle.  
Urinalysis: Before treatment and at the end of third treatment cycle.  
Gross pathology: At necropsy  
Organ weights: See histopathology inventory for this NDA  
Histopathology: See histopathology inventory  
Toxicokinetics: On day 1 of the first treatment and during the last treatment cycle at immediately pre-dose, immediately post dose, 5 minutes, 10 minutes, 20 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, 12 hours, and 24 hours on each of the two occasions.

**Results**

Mortality:

Group	Dosage (mg/kg/day)	Animal died	Days	Animal sacrificed	Days	Cycle
4	6.6	3 (2 ♂ & 1 ♀)	8, 15 and 17	3 (1 ♂ & 2 ♀)	29	2 <sup>nd</sup> cycle recovery phase

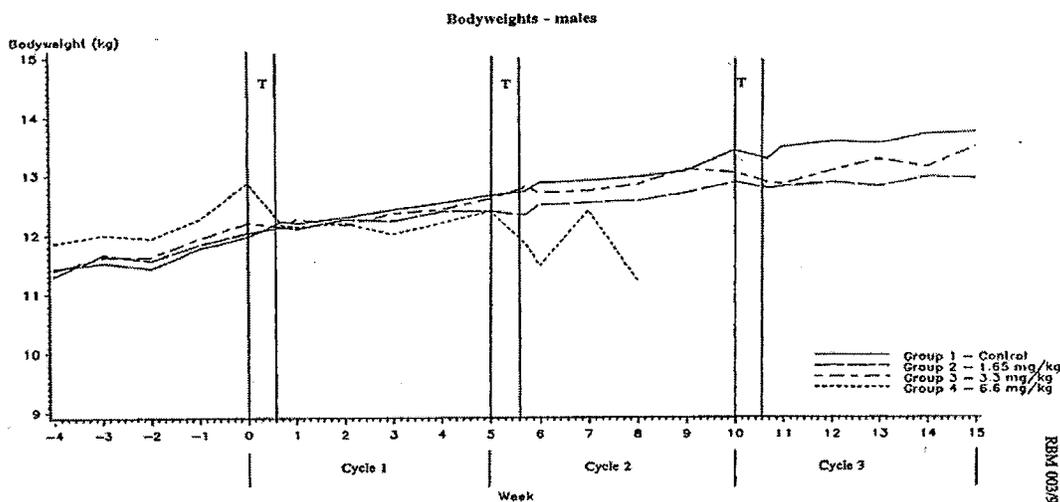
Clinical signs: Group 4 (6.6 mg/kg) – Brown/white frothy vomitus, salivation, red colored feces.

Body weights:

Change in body weight (kg) as compared to initial body weight

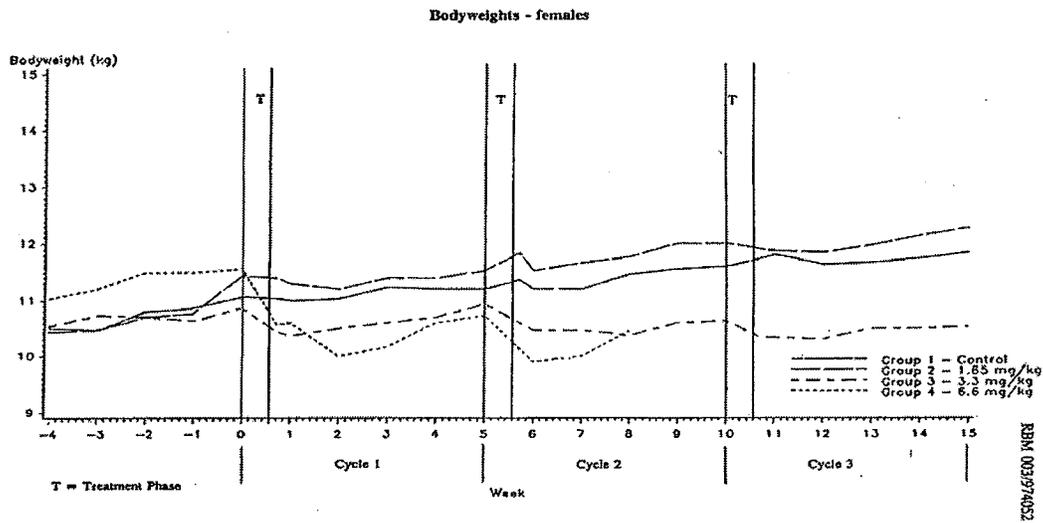
Period	Dosage (mg/kg/day)							
	Control		1.65		3.3		6.6	
	♂	♀	♂	♀	♂	♀	♂	♀
Initial body wt.	12.0	11.1	12.0	11.4	12.2	10.9	12.9	11.6
Cycle 1	0.3	0.0	0.1	0.0	-0.1	-0.4	-0.7**	-1.0**
Recovery cycle 1	0.4	0.2	0.3	0.1	0.5	0.5	0.2	0.2
Cycle 2	0.10.	0.2	-0.1	0.3	0.2	-0.3	-0.6*	-0.6*
Recovery cycle 2	0.7	0.2	0.5	0.2	0.2	0.0	-	-
Cycle 3	-0.1	0.1	-0.1	-0.1	-0.2	-0.3*	-	-
Recovery cycle 3	0.5	0.1	0.2	0.4	0.6	0.2	-	-

\* $p < 0.05$ , \*\* $p < 0.01$  William's test



(Excerpted from the sponsor's submission)

RBM 003/97



(Excerpted from the sponsor's submission)

**Food consumption:**

Change in food consumption (%) compared with control group

Period	1.65 mg/kg/day		3.3 mg/kg/day		6.6 mg/kg/day	
	♂	♀	♂	♀	♂	♀
Cycle 1	↓ 3	↓ 9	↓ 1	↓ 29*	↓ 25*	↓ 51**
Recovery cycle 1	0	0	0	↓ 8	↓ 8*	↓ 21**
Cycle 2	0	0	0	↓ 11	↓ 28	↓ 43**
Recovery cycle 2	0	0	0	↓ 11	-	-
Cycle 3	0	↓ 4	↓ 11*	↓ 23**	--	--
Recovery cycle 3	0	0	0	↓ 8	-	-

\* $p < 0.05$ ,      \*\* $p < 0.01$       William's test

Food consumption was reduced in a dose-related manner. Females were more affected than males. Group 4 (6.6 mg/kg/day) were euthanized for humane grounds during the second recovery period.

**Ophthalmoscopy:** No ophthalmic changes due to treatment.

**EKG:**

Mean (3 animals) heart rates (beats/min)

Period	Dosage (mg/kg/day)							
	Control		1.65		3.3		6.6	
	♂	♀	♂	♀	♂	♀	♂	♀
Pre-dose.	131	172	115	159	121	135	154*	106+
Cycle 2	127	126	130	122	127	113	91*	95
Cycle 3	106	158	114	151	126	122*	-	-

+  $p < 0.05$  Student's t test      \*  $p < 0.05$ ,      \*\*  $p < 0.01$  Williams' test

Reduced heart rates during cycle 2 at 6.6 mg/kg/day (2 ♂ & 1 ♀, 3/6 animals) may be due to treatment with bendamustine hydrochloride.

Hematology:

Changes in hematology values as compared to control (%) at the end of third treatment (week 10.4)

Dosage (mg/kg/day)	WBC total		Lymphocytes	
	♂	♀	♂	♀
1.65	↓ 15	↓ 23	↓ 49	↓ 50
3.3	↓ 42	↓ 51	↓ 71	↓ 72

Clinical chemistry: No clinical chemistry changes attributable to treatment.

Urinalysis: No effect

Bone marrow smears:

Dosage (mg/kg/day)	Animal No./sex	Cellularity	Distribution	Morphology	Bone marrow smear comment
Control	All animals	NAD	NAD	NAD	NAD
1.65	All animals	NAD	NAD	NAD	NAD
3.3	All animals	NAD	NAD	NAD	NAD
6.6	1 ♂	NAD	I	I	Marked increased myeloid cells
	2 ♂ & 2 ♀	NAD	I	NAD	Moderate relative decrease in myeloid cells
	1 ♀	NAD	NAD	NAD	NAD

NAD – No abnormalities detected

Gross pathology: Congestion/hemorrhage in gastrointestinal tract of high dose animals.

Organ weights: Thymus, heart and spleen were slightly decreased.

Histopathology: Adequate Battery: yes (X), no ( )—explain  
Peer review: yes ( ), no (X)

Summary of incidence of treatment related findings

Organ/finding	Sex Animal on study Group Dose (mg/kg/day)	Male			Female		
		1.65	3.3	6.6	1.65	3.3	6.6
		3	3	3	3	3	3
Cecum, lymphoid tissue, marked Severe		0	0	0	0	0	1
		0	0	2	0	0	0
Duodenum, prominent mitotic figures in crypt epithelium		0	0	1	0	0	2
Epididymides, abnormal spermatogenic cell in duct, minimal Moderate		0	0	1			
		0	0	1			
Jejunum, necrosis and inflammation-tips of villi, minimal Slight		0	0	1	0	0	0
		0	0	0	0	0	1
Heart, myocardial interstitial inflammatory, minimal Left Atrioventricular valve, focal hemorrhage, min. Leukocytosis, minimal						1	1
							1
			1				

Organ/finding	Sex Animal on study Group Dose (mg/kg/day)	Male			Female		
		3	3	3	3	3	3
		2	3	4	2	3	4
		1.65	3.3	6.6	1.65	3.3	6.6
Kidneys, glomerulitis, minimal Enlarged nuclei, minimal		0	0	3	0	0	2
		0	0	3	0	1	2
Lymph nodes-mesenteric, reduced lymphoid cellularity, Moderate Marked Absent germinal centers		0	0	1	0	0	1
		0	0	2	0	0	2
		0	0	3	0	0	3
Prostate, acinar epithelial necrosis, slight Moderate		0	0	1			
				1			
Spleen, reduced cellularity of the white pulp, moderate Severe		0	0	0	0	2	0
				3	0	2	3
Testes, focal seminiferous tubular atrophy, minimal slight moderate		1	0	2			
		2	0	1			
		0	3	0			
Thymus, involution/atrophy, minimal Slight Moderate Marked Severe		1	2	0	2	0	0
		2	1	0	1	2	0
		0	0	0	0	0	1
		0		1	0	0	2
		0	0	2	0	0	0

Toxicokinetics:

Mean plasma concentrations ( $\pm$  SD) of bendamustine hydrochloride at the end of the infusion ( $C_{inf}$ ) and mean areas under the plasma concentration-time curves estimated up to 24 hours after the end of the infusion ( $AUC_{24}$ ) are shown below.

Dose level (mg/kg/day)	$C_{inf}$ (ng/mL)				$AUC_{24}$ (ng.h/mL)			
	Cycle 1 (day 1)		Cycle 3 (day 2)		Cycle 1 (day 1)		Cycle 3 (day 2)	
	♂	♀	♂	♀	♂	♀	♂	♀
1.65	1284 $\pm$	1844 $\pm$	1740 $\pm$	2594 $\pm$	492 $\pm$	796 $\pm$	660 $\pm$	1073 $\pm$
	469	534	164	203	199	285	63	172
3.3	3381 $\pm$	4125 $\pm$	3432 $\pm$	3779 $\pm$	1234 $\pm$	1660 $\pm$	1387 $\pm$	1431 $\pm$
	303	609	1120	879	125	314	498	429
6.6	8183 $\pm$	10070 $\pm$	-	-	3113 $\pm$	4197 $\pm$	-	-
	2430	882			885	486		

The plasma concentrations at 24 hours post dose ( $C_{24}$ ) were below the limit of quantification.

The relationships between the mean plasma concentrations of bendamustine at the end of infusion ( $C_{inf}$ ), mean areas under the plasma concentration-time curves, and dose level are presented below.

Dose level (mg/kg/day)	Dose level ratio	$C_{inf}$ (ratio)				AUC <sub>24</sub> (ratio)			
		Cycle 1 (day 1)		Cycle 3 (day 2)		Cycle 1 (day 1)		Cycle 3 (day 2)	
		♂	♀	♂	♀	♂	♀	♂	♀
1.65	1	1	1	1	1	1	1	1	1
3.3	2.0	2.6	2.2	2.0	1.5	2.5	2.1	2.1	1.3
6.6	4.0	6.4	5.5	-	--	6.3	5.3	-	-

The  $C_{inf}$  values and the extent (AUC<sub>24</sub>) of the systemic exposure were generally higher than the proportionate dose increment.

**APPEARS THIS WAY  
ON ORIGINAL**

**Histopathology inventory.**

Study	43/44	RBM 003/974052	DS-2006-010
Species	Rat	Dog	Rat
Adrenals	*X	*X	*X
Aorta		X	X
Bone Marrow smear	X	X	X
Bone (femur)	X		X
Brain	*X	*X	*X
Cecum	X	X	X
Cervix			X
Colon	X	X	X
Duodenum	X	X	X
Epididymis	X	*X	*X
Esophagus	X	X	X
Eye	X	X	X
Fallopian tube			
Gall bladder		X	
Gross lesions		X	X

Study	43/44	RBM 003/974052	DS-2006-010
Species	Rat	Dog	Rat
Harderian gland			X
Heart	*X	*X	*X
Ileum	X	X	X
Injection site		X	X
Jejunum	X	X	X
Kidneys	*X	*X	*X
Lachrymal gland			
Larynx	X		
Liver	*X	*X	*X
Lungs	*X	*X	*X
Lymph nodes, cervical	X	X	
Lymph nodes mandibular	X	X	X
Lymph nodes, mesenteric	X	X	X
Mammary Gland		X	X
Nasal cavity			
Optic nerves		X	X
Ovaries	*X	*X	*X
Pancreas	X	*X	X
Parathyroid		*X	X
Peripheral nerve			
Pharynx			
Pituitary		*X	*X
Prostate	X	*X	*X
Rectum	X	X	X
Salivary gland	X	X	*X
Sciatic nerve		X	X
Seminal vesicles	X		*X
Skeletal muscle	X	X	X
Skin		X	X
Spinal cord	X	X	X
Spleen	*X	*X	*X
Sternum		X	X
Stomach	X	X	X
Testes	*X	*X	*X
Thymus	*X	*X	*X
Thyroid	X	*X	*X
Tongue		X	X
Trachea		X	X
Urinary bladder	X	X	X
Uterus	X	*X	*X
Vagina	X	X	X
Zymbal gland			

X, histopathology performed

\*, organ weight obtained

**2.6.6.4 Genetic toxicology****4.2.3.3.1 Bendamustine Hydrochloride Bacterial Mutation Assay****Key findings:**

- Bendamustine hydrochloride induced mutation in Ames test with or without metabolic activation (S9).

**Study no.:** KGP 017/002582  
**internal report No.:** 0640.00.C4.01  
**Volume # and page #:** Module 4.2.3.3.1 (Electronic submission)  
**Conducting laboratory and location:**

**Date of study initiation:** 16 March 2000  
**GLP compliance:** Yes  
**QA reports:** yes (X) no ( )  
**Drug, lot #, and % purity:** Bendamustine hydrochloride,  
 batch # 35 09 99, — purity

**Methods**  
**Strains/species/cell line:** Bacterial mutation assay  
 Salmonella typhimurium (TA1535, TA1537, TA98, TA100)  
 Escherichia coli (WP2uvrA/pK101, CM891)  
**Doses used in definitive study:** 50, 150, 500, 1500, 2666, 3833, and 5000 µg/plate  
**Basis of dose selection:** Dose-range finding experiment (mutation test 1)  
**Metabolic activation system:** Aroclor 1254 induced rat liver post-mitochondrial fraction (S9)  
**Negative controls:** Solvent (ethanol)  
**Positive controls:** In the absence of S9 mix – Sodium azide, 9-aminoacridine (9 AC), 2-nitrofluorene (NF), and 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2)  
 In the presence of S9 mix – 2-aminoanthracene (AA) and benzo[a]pyrene (b[a]P)  
**Incubation and sampling times:** All plates were incubated at 37° C for 72 hours.

**Results**

**Study validity:** Three petri dishes were used for each dose level. Revertant colonies were counted using a — automated colony counter. An increase in revertant colony numbers of at least twice the concurrent solvent controls, with some evidence of dose-relationship, in the presence or absence of S9 mix was considered to show evidence of mutagenic activity in this test system.

## Mutation test 1

Compound	Dose level (µg/plate)	S9	TA 98	TA 100	TA 1535	TA1537	CM891
Test	500	-	23±4	121±9	13±1	11±3	165±4
Test	1500	-	30±6	121±1	14±6	8±3	124±8
Test	5000	-	33±7	-	-	-	252±44
Solvent		-	27±3	103±3	14±2	9±2	165±11
NaAz	0.5	-		452±57	167±10		
9AC	30	-				51±7	
NF	1	-	248±3				
AF-2	0.05	-					1895±7
Test	500	+	29±4	116±3	16±2	12±1	206±6
Test	1500	+	36±6	103±4	27±7	18±2	200±11
Test	5000	+	69±1	-	20±2	-	548±47
Solvent	-	+	30±4	99±2	14±3	11±2	176±6
AA	2.0	+			216±20		
AA	10	+					1945±9
B[a]P	5.0	+	439±16	707±1		134±2	

- = Absence

+ = Presence

SD = Standard deviation

NaAz = Sodium azide

9 AC = 9-Aminoacridine

NF = 2-Nitrofluorene

AF-2 = 2-(-2-Furyl)-3-(5-nitro-2-furyl) acrylamide

AA = 2-Aminoanthracene

B[a]P = Benzo[a]pyrene

## Mutation test 2

Compound	Dose level (µg/plate)	S9	TA 98	TA 100	TA 1535	TA1537	CM891
Test	2666	-	34±8	119±7	13±4	-	307±2
Test	3833	-	33±3	91±2	15±6	-	312±3
Test	5000	-	17±4	-	-	-	295±2
Solvent		-	27±3	107±5	14±3	8±2	189±2
NaAz	0.5	-		409±4	150±1		
9AC	30	-				75±2	
NF	1	-	151±4				
AF-2	0.05	-					2002±2
Test	2666	+	48±6	88±2	16±4	-	522±2
Test	3833	+	46±4	-	22±6	-	497±2
Test	5000	+	53±6	-	20±7	-	265±2
Solvent	-	+	31±1	82±1	16±2	11±3	208±2
AA	2.0	+			112±1		
AA	10	+					1842±1
B[a]P	5.0	+	265±4	729±4		51±5	

- = Absence

+ = Presence

SD = Standard deviation

NaAz = Sodium azide

9 AC = 9-Aminoacridine

NF = 2-Nitrofluorene

AF-2 = 2-(-2-Furyl)-3-(5-nitro-2-furyl) acrylamide

AA = 2-Aminoanthracene

B[a]P = Benzo[a]pyrene

Study outcome: Bendamustine in a dose-related manner increased revertant colony numbers as compared to control in the presence and absence of S9 mixture.

**APPEARS THIS WAY  
ON ORIGINAL.**

**4.2.3.3.2 Bendamustine Hydrochloride In Vitro Mammalian Chromosome Aberration Test in Human Lymphocytes.**

**Key findings:**

- Bendamustine hydrochloride showed clastogenic activity, in both the presence and absence of S9 mix, *in vitro* cytogenetic test in human lymphocytes.

**Study no.:** 0640.00.C4.02.  
**Volume # and page #:** Module 4.2.3.3.2 (Electronic submission)  
**Conducting laboratory and location:** / /  
**Date of study initiation:** 14 March 2000  
**GLP compliance:** Yes  
**QA reports:** yes (X) no ( )  
**Drug, lot #, and % purity:** Bendamustine hydrochloride,  
batch # 35 09 99. — purity

**Methods**

Strains/species/cell line: Human lymphocytes  
Doses used in definitive study: 5, 10 and 15 µg/ml  
Basis of dose selection: Assessment of drug toxicity and first test  
Positive controls: Mitomycin (in the absence of S9 mix),  
Cyclophosphamide,(in the presence of S9 mix)  
Incubation and sampling times: 3 hours (exposure period) and 18 hours (recovery period)  
Metabolic activation system: S9 mix  
Number of slides analyzed: Duplicate  
Counting method: Microscopic

**Results****First test**

Exposure time (hours)	S9 mix	Bendamustine (µg/ml)	Cells with aberrations (%)		Relative mitotic index (%)
			excluding gaps	including gaps	
3	-	0	0.5	1.5	100
		2.5	8.0	9.5	70
		5	24.0	24.0	64
		10	38.0	38.0	49
		Mitomycin (0.8 µg/ml)	36.0	36.0	-
3	+	0	0.5	0.5	100
		2.5	6.6	6.5	101
		5	25.0	26.0	71
		10	38.0	38.0	36
		Cyclophosphamide (25)	34.0	34.0	-

**Second test**

Exposure time (hours)	S9 mix	Bendamustine (µg/ml)	Cells with aberrations excluding gaps (%)	Cells with aberrations including gaps (%)	Relative mitotic index (%)
3	-	0	0	0	100
		5	28.0	28.0	82
		10	54.0	54.0	63
		15	37.0	37.0	41
		Mitomycin C (0.8)	21.0	21.0	-
3	+	0	0	0	100
		2.5	13.0	13.0	74
		5	29.0	29.0	67
		10	38.0	38.0	36
		Cyclophosphamide (25)	28.0	28.0	-

**Study validity:** The test substance was considered positive, if statistically significant increase ( $P < 0.01$ ) in the frequency of metaphases with aberrant chromosomes at one or more concentration was observed. The study was accepted as valid.

**Study outcome:** Bendamustine hydrochloride caused clastogenic activity in the presence and absence of S9 mix in this *in vitro* cytogenetic test.

4.2.3.3.3 **CEP-18083: Rat Bone Marrow Erythrocyte Micronucleus Test****Key findings:**

- Maximum tolerated dose by intravenous administration to rats in dose range finding toxicity study was 25 mg/kg.
- A single intravenous administration of bendamustine (CEP-18083) induced significant increase in the incidence of micronucleated polychromatic erythrocytes in male and female rats.

**Study no.:** \_\_\_\_\_ **Study No.:** DS-2007-001  
**Volume # and page #:** AB-42UF.125.BTL  
**Conducting laboratory and location:** Module 4.2.3.3.3 (Electronic submission)

**Date of study initiation:** 23 February 2007  
**GLP compliance:** Yes  
**QA reports:** yes (X) no ( )  
**Drug, lot #, and % purity:** CEP-18083 (bendamustine hydrochloride), lot # 06C10L, batch # A426804, —purity

**Methods**

**Strains/species/cell line:** Sprague Dawley rats  
**Doses used in definitive study:** 6.25, 12.5, and 25 mg/kg  
**Basis of dose selection:** Dose range finding study (toxicity study)

Dose selection toxicity study

Group	1		2		3	
	25		50		100	
Dose (mg/kg)	5♂	5♀	5♂	5♀	5♂	5♀
Number of animals						
Mortality		1	1	5	4	5
Piloerection	5	5	5	5	5	5
Crusty eyes and nose	4	5	4		2	
Lethargy	2	3	5	5	5	5
Lacrimation			5	5	5	5
Ataxia		1		1		
Diarrhea			4		1	
Prostration				1		
Tremors		1				

Maximum tolerated dose was set at 25 mg/kg

Micronucleus assay

Group (mg/kg)	Number of animals/sex for bone marrow collection at		Number of animals per sex for toxicokinetics
	24 hours post-dose	48 hrs post dose	
Low dose (6.25)	5	0	3
Mid dose (12.5)	5	0	3
High dose (25.0)	5	5	3
Positive control	5	0	0
Vehicle control	5	5	0

**Toxicokinetics:** Animals were bled at 5 min, 30 min, 1 hr, and 24 hrs post dose  
**Negative controls:** Saline  
**Positive controls:** Cyclophosphamide monohydrate (40 mg/kg)  
**Incubation and sampling times:** 24 and 48 hours postdose  
**Study validity:** Fluorescent microscope was used for counting cell populations.  
 The test article was considered to induce a positive response if a dose related increase in micronucleated polychromatic erythrocytes was observed and one or more doses were statistically elevated relative to the vehicle control

Summary of Bone Marrow Micronucleus Analysis

Treatment (10 mL/kg)	Sex	Time (hr)	Number of Animals	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Number of MPCE/1000 PCE (Mean +/- SD)	Number of MPCE/PCE Scored
Saline	M	24	5	0.581 ± 0.02	---	0.3 ± 0.27	3 / 10000
	F	24	5	0.603 ± 0.01	---	0.4 ± 0.22	4 / 10000
CEP-18083 6.25 mg/kg	M	24	5	0.604 ± 0.03	4	2.1 ± 0.96	*21 / 10000
	F	24	5	0.575 ± 0.05	-5	2.4 ± 1.92	*24 / 10000
12.5 mg/kg	M	24	5	0.592 ± 0.04	2	8.5 ± 2.98	*85 / 10000
	F	24	5	0.580 ± 0.06	-4	5.0 ± 3.34	*50 / 10000
25 mg/kg	M	24	5	0.535 ± 0.05	-8	12.2 ± 5.39	*122 / 10000
	F	24	5	0.497 ± 0.05	-18	5.4 ± 2.68	*54 / 10000
Cyclophosphamide 40 mg/kg	M	24	5	0.512 ± 0.04	-12	13.8 ± 1.96	*138 / 10000
	F	24	5	0.468 ± 0.03	-22	10.9 ± 1.71	*109 / 10000
Saline	M	48	5	0.584 ± 0.04	---	0.3 ± 0.27	3 / 10000
	F	48	5	0.541 ± 0.06	---	0.4 ± 0.65	4 / 10000
CEP-18083 25 mg/kg	M	48	5	0.434 ± 0.04	-26	6.2 ± 3.47	*62 / 10000
	F	48	5	0.437 ± 0.06	-19	2.6 ± 1.47	*26 / 10000

\*Statistically significant, p ≤ 0.05 (Kastenbaum-Bowman Tables).

PCE = Polychromatic erythrocytes  
 MPCE = Micronucleated polychromatic erythrocytes  
 (Excerpted from the sponsor's submission)

## Toxicokinetic Parameters for Bendamustine, M3 and M4 in Male and Female Rats

Sex	Analyte	Dose (mg/kg)	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (hr)	AUC <sub>0-t</sub> (ng•hr/mL)
Male	Bendamustine	6.25	9942.4	0.083	3741
		12.5	31298.2	0.083	12562
		25	44377.6	0.083	19095
	M3	6.25	95.5	0.083	60
		12.5	130.0	0.5	98
		25	294.0	0.5	217
	M4	6.25	1.3	0.083	NC
		12.5	1.5	0.5	0.6
		25	3.0	0.5	1.4
Female	Bendamustine	6.25	11232.0	0.083	4174
		12.5	33553.2	0.083	19437
		25	58707.2	0.083	25100
	M3	6.25	94.5	0.5	69
		12.5	104.6	0.5	81
		25	172.1	0.5	124
	M4	6.25	BLQ<1.0	NC	NC
		12.5	1.4	0.083	NC
		25	3.7	0.5	1.2

BLQ&lt;1.0: Below the limit of quantitation of 1.0 ng/mL

NC: Not Calculable

(Excerpted from the sponsor's submission)

Study outcome: Bendamustine hydrochloride induced chromosomal breakage and spindle malfunction in *in vivo* cytogenetic assay in Sprague Dawley rats.

#### 4.2.3.3.4 Chromosome Aberration Test in Human Lymphocytes In Vitro with Hydroxy-Bendamustine (HP1).

##### Key findings

- Hydroxy bendamustine in the presence and absence of S9 mix induced structural chromosomal aberration in human lymphocytes *in vitro*.

**Study no.:** 831200.  
**Volume # and page #:** Module 4.2.3.3.4  
**Conducting laboratory and location:**

**Date of study initiation:** April 22, 2004  
**GLP compliance:** Yes

QA reports: yes (X) no ( )  
 Drug, lot #, and % purity: Hydroxy-bendamustine (HP1),  
 batch # AN2607, — purity  
 Donor: 42 year old healthy male

## Methods

Strains/species/cell line: Human lymphocytes  
Doses used in definitive study: 21.8, 38.2, and 66.8 µg/mL with S9 mix  
 38.2, 66.8 and 116.9 µg/mL without S9  
Basis of dose selection: Current OECD Guideline (mitotic indices below  
 50%. 5 mg/mL, 5 µL/mL, or 10 mM which ever is  
 the lowest)  
Negative controls: DMSO  
Positive controls: In the absence of metabolic activation:  
 Ethylmethane sulfonate (EMS)  
 In the presence of metabolic activation:  
 Cyclophosphamide (CPA)  
Incubation and sampling times: 4 and 22 hours  
Metabolic activation system: S9 mix  
Number of slides analyzed: Duplicate  
Counting method: Microscopic

## Results

Preparation interval	Test item concentration in µg/mL	Polyploid cells in %	Mitotic indices in %		Aberrant cells in %	
			of control	incl. gaps*	excl. gaps*	with exchanges
<b>Exposure period 4 hrs without S9 mix</b>						
22 hrs	negative control	0.0	100.0	2.0	1.5	0.0
	solvent control <sup>1</sup>	0.0	100.0	2.5	1.5	0.0
	positive control <sup>2</sup>	0.2	117.0	4.0	2.0 <sup>x</sup>	0.5
	21.8	0.0	111.2	10.0	9.5 <sup>s</sup>	3.0
	38.2	0.0	80.3	14.0	13.0 <sup>s</sup>	3.0
	66.8	0.0	85.4	21.5	18.5 <sup>s</sup>	4.0

\* inclusive cells carrying exchanges

<sup>x</sup> due to the low metaphase quality and the reduced number of evaluable metaphase plates no distinct increase of aberrant cells could be observed

<sup>s</sup> aberration frequency statistically significant higher than corresponding control values

<sup>1</sup> DMSO 0.5 % (v/v)

<sup>2</sup> EMS 660.0 µg/mL

<sup>3</sup> CPA 15.0 µg/mL

(Excerpted from the sponsor's submission)

Preparation interval	Test item concentration in µg/mL	Polyploid cells in %	Mitotic indices in %		Aberrant cells in %	
			of control	incl. gaps*	excl. gaps*	with exchanges
<b>Exposure period 22 hrs without S9 mix</b>						
22 hrs	negative control	0.0	100.0	0.0	0.0	0.0
	solvent control <sup>1</sup>	0.2	100.0	0.0	0.0	0.0
	positive control <sup>2</sup>	0.0	43.3	15.0	14.0 <sup>5</sup>	2.0
	38.2	0.2	77.1	21.5	18.5 <sup>5</sup>	4.5
	66.8	0.2	59.6	28.0	24.5 <sup>5</sup>	6.5
	116.9	0.0	40.7	29.5	27.0 <sup>5</sup>	13.0

- \* inclusive cells carrying exchanges  
 X due to the low metaphase quality and the reduced number of evaluable metaphase plates no distinct increase of aberrant cells could be observed  
<sup>5</sup> aberration frequency statistically significant higher than corresponding control values  
<sup>1</sup> DMSO 0.5 % (v/v)  
<sup>2</sup> EMS 660.0 µg/mL  
<sup>3</sup> CPA 15.0 µg/mL

(Excerpted from the sponsor's submission)

Preparation interval	Test item concentration in µg/mL	Polyploid cells in %	Mitotic indices in %		Aberrant cells in %	
			of control	incl. gaps*	excl. gaps*	with exchanges
<b>Exposure period 4 hrs with S9 mix</b>						
22 hrs	negative control	0.0	100.0	1.5	1.5	0.0
	solvent control <sup>1</sup>	0.0	100.0	4.0	2.5	0.0
	positive control <sup>3</sup>	0.0	29.3	18.0	16.0 <sup>5</sup>	4.0
	21.8	0.4	75.5	5.0	4.0	0.0
	38.2	0.4	78.4	10.0	8.0 <sup>5</sup>	1.0
	66.8	0.4	49.8	9.5	6.0 <sup>5</sup>	1.0

- \* inclusive cells carrying exchanges  
 X due to the low metaphase quality and the reduced number of evaluable metaphase plates no distinct increase of aberrant cells could be observed  
<sup>5</sup> aberration frequency statistically significant higher than corresponding control values  
<sup>1</sup> DMSO 0.5 % (v/v)  
<sup>2</sup> EMS 660.0 µg/mL  
<sup>3</sup> CPA 15.0 µg/mL

(Excerpted from the sponsor's submission)

Study validity: The test substance was considered positive, if statistically significant increase ( $P < 0.01$ ) in the frequency of metaphases with aberrant chromosomes at one or more concentration was observed. The study was accepted as valid.

Study outcome: Hydroxy-bendamustine was clastogenic in chromosome aberration test in the presence and absence of metabolic activation (S9 mix).

**APPEARS THIS WAY  
ON ORIGINAL**

**APPEARS THIS WAY  
ON ORIGINAL**

**2.6.6.5 Carcinogenicity**

Note: Executive CAC minutes are attached.

**Oncogenicity of  $\gamma$ -[1-methyl-5-bis-( $\beta$ -chloroethyl)-amino-benzimidazolyl-(2)]-butyric acid hydrochloride in mice.**

**Key study findings:**

- Intraperitoneal injections of bendamustine for four days produced peritoneal sarcoma in mice.
- Oral administration of bendamustine for four days induced mammary carcinoma and pulmonary adenomas.

<b>Study no.:</b>	Publication (Guttner, Bruns, and Junstand, Arch Geschwulstforsch 43/1: 16-21, 1974)
<b>Volume # and page #:</b>	Module 4.2.3.4
<b>Conducting laboratory and location:</b>	Central Institute of Microbiology and Experimental Therapy, Jena, the German Academy of Science to Berlin, Germany.
<b>Date of study initiation:</b>	Unknown
<b>GLP compliance:</b>	No
<b>QA report:</b>	yes ( ) no (X)
<b>Drug, lot #, and % purity:</b>	Cytostasan (bendamustine hydrochloride) lot # and purity not specified
<b>CAC concurrence:</b>	Not requested

**Methods**

## Doses:

Group No.	Dose (mg/kg/day)	Total dose (mg/kg)	Route of administration	Number of female animals
1	Control	-	-	46
2	12.5	50	Intraperitoneal	86
3	25	100	Intraperitoneal	81
4	62.5	250	Oral	81

Basis of dose selection (MTD, MFD, AUC etc.): Experimental therapy trials

Species/strain: AB/Jena mice

Number/sex/group (main study): See above table

Route, formulation, volume: See above table

Frequency of dosing: Daily for 4 consecutive days

Satellite groups used for toxicokinetics or special groups: No

Age: 9 weeks

Body weight: 18-22 g

Restriction paradigm for dietary restriction studies: None

Drug stability/homogeneity: Not tested

Dual controls employed: No  
 Interim sacrifices: No  
 Deviations from original study protocol: Unknown

Note: Animals were immunized against ectromelia three week before the study initiation by intracutaneous scarification with vaccine serum at the root of the tail. Surviving animals were revaccinated after 34 weeks.

## Results

### Survival time and tumor incidence after treatment with i.p. cytosasan

Number of animals	46			86		81	
Dose (mg/kg/day)	Control			12.5		25	
Total dose *(mg/kg)	Control			50		100	
Application				i.p.		i.p.	
Medium survival time (weeks)	62			14		22	
	RS	PA	sc S	PA	Ip S	PA	Ip S
N1.Tu	37	30	39	81	13	53	38
Rate of death up to 1 <sup>st</sup> tumor (%)	19.6	34.8	15.2	5.8	85.0	34.6	53.0
Number of tumors	2	6	7	1	4	6	8
% Tu/No	4.3	13.0	15.2	1.1	4.7	7.4	22.2
% Tu/N1.Tu	5.4	20.0	18.0	1.2	30.8	11.3	47.4
Significance**)				++	++	-	+++

\*) reference point: beginning of the trial  
 No number of animals at the begin of the trial  
 N1.Tu number of living animals after appearance of the 1<sup>st</sup> tumour  
 RS reticulosarcomas  
 PA pulmonary adenomas  
 sc S subcutaneous sarcomas  
 ip S intraperitoneal sarcomas  
 MC mammary carcinomas  
 \*\*) relate to % Tu/N1.Tu  
 (-: p>0.05; ++: p<0.01; +++: p<0.001)

### Survival time and tumor incidence after treatment with oral cytosasan

Number of animals	46			81			
Dose (mg/kg/day)	Control			62.5			
Total dose (mg/kg)	Control			250			
Application				Oral			
Medium survival time (weeks)	62			58			
	RS	PA	sc S	RS	PA	sc S	MC
N1.Tu	37	30	39	80	78	73	76
Rate of death up to 1 <sup>st</sup> tumor (%)	19.6	34.8	15.2	1.2	3.7	9.9	6.2
Number of tumors	2	6	7	5	77	4	6
% Tu/No	4.3	13.0	15.2	6.2	95.1	4.9	7.4
% Tu/N1.Tu	5.4	20.0	18.0	6.3	98.8	5.5	7.9
Significance**)				-	+++	-	-

\*) reference point: beginning of the trial  
 No number of animals at the begin of the trial  
 N<sub>1.Tu</sub> number of living animals after appearance of the 1<sup>st</sup> tumour  
 RS reticulosarcomas  
 PA pulmonary adenomas  
 sc S subcutaneous sarcomas  
 ip S intraperitoneal sarcomas  
 MC mammary carcinomas  
 \*\*) relate to % Tu/N<sub>1.Tu</sub>  
 (-: p>0.05; ++: p<0.01; +++: p<0.001)

Survival time and tumor incidence after treatment with i.p and oral cytotasan

Number of animals	86		81		81			
Dose (mg/kg/day)	12.5		25		62.5			
Total dose (mg/kg)	50		100		250			
Application	i.p.		i.p.		Oral			
Medium survival time (weeks)	14		22		58			
	PA	ip S	PA	ip S	RS	PA	Sc S	MC
N <sub>1.Tu</sub>	81	13	53	38	80	78	73	76
Rate of death up to 1 <sup>st</sup> tumor (%)	5.8	85.0	34.6	53.0	1.2	3.7	9.9	6.2
Number of tumors	1	4	6	18	5	77	4	6
% Tu/No	1.1	4.7	7.4	22.2	6.2	95.1	4.9	7.4
% Tu/N <sub>1.Tu</sub>	1.2	30.8	11.3	47.4	6.3	98.8	5.5	7.9
Significance**)	++	++	-	+++	-	+++	-	-

\*) reference point: beginning of the trial  
 No number of animals at the begin of the trial  
 N<sub>1.Tu</sub> number of living animals after appearance of the 1<sup>st</sup> tumour  
 RS reticulosarcomas  
 PA pulmonary adenomas  
 sc S subcutaneous sarcomas  
 ip S intraperitoneal sarcomas  
 MC mammary carcinomas  
 \*\*) relate to % Tu/N<sub>1.Tu</sub>  
 (-: p>0.05; ++: p<0.01; +++: p<0.001)

**Conclusions:** Although study design was not adequate to fully assess the carcinogenic potential of bendamustine, intraperitoneal injections of bendamustine for four days produced peritoneal sarcoma. Oral administration for four days induced mammary carcinoma and pulmonary adenomas.

**2.6.6.6 Reproductive and developmental toxicology****Fertility and early embryonic development:** Not conducted**Embryofetal development****On the embryotoxic and teratogenic action of the nitrogen mustard derivatives  
IMET 3393 and IMET 3106 in mice.****Key study findings:**

- Mean body weight of the fetuses decreased with increasing dosage of IMET 3393.
- Malformations observed included exencephaly, cleft palates, and dwarfism.
- IMET 3393 and IMET 3106 caused embryotoxic and teratogenic effect in mice similar to cyclophosphamide.

**Study no.:** Publication (Heinecke & Klaus, Zbl Pharm 1971;110(10):1067-76)  
**Volume # and page #:** Module 4.2.3.5  
**Conducting laboratory and location:** Department of Experimental Therapy & Central Institute of Microbiology and Experimental Therapy, Jena, the German Academy of Science to Berlin.  
**Date of study initiation:** Unknown  
**GLP compliance:** No  
**QA reports:** yes ( ) no (X)  
**Drug, lot #, and % purity:** IMET 3393 (bendamustine) and IMET 3106  
 Lot # and purity not mentioned

**Methods**

**Doses:** IMET 3393 – 35 & 70 mg/kg  
**Species/strain:** AB mice & NMRI mice  
**Number/sex/group:** Variable  
**Route, formulation, volume, and infusion rate:** Intraperitoneal,  
 0.2 ml or 0.4 mg/20 g mouse  
**Satellite groups used for toxicokinetics:** No  
**Study design:** Females dosed on GD 5, 7, 9, or 11 (single dose) and GD 7 to 11 for multi-dose application. Animals were sacrificed on GD 18.  
**Parameters and endpoints evaluated:** Number of living and dead fetuses, their position in the uterine cornu, mean body weight, sex determination, and external abnormalities  
**Dose justification:** Not mentioned

**Results**Mortality (dams): See table below,Clinical signs (dams), Body weight (dams), and Food consumption (dams):

Not mentioned

Toxicokinetics:

Not performed

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

Influence of single-dose application of IMET 3393 in distilled water on resorptions in colony-bred strain AB mice

Dose (mg/kg)	Day P.C.	Pregnant mothers				Implantations		Resorptions	
		Total	Died	Without implant	Evaluable	Total	Mean±SD	Total	%
Control	7-11	16	0	6	10	75	7.5±0.5	2	2.7
Control	11	12	0	0	12	99	8.3±0.3	7	7.1
35	5	9	0	7	2	16	8.0	0	0
70	5	9	5	4	0	0	0	0	0
70	7	16	5	3	8	60	7.5±1.2	26	43.3
70	9	17	8	0	9	65	7.2±0.6	8	12.3
70	11	12	3	0	9	64	7.2±0.5	31	48.3

Influence of single-dose application of IMET 3393 in isotonic sodium chloride solution on resorptions in colony-bred strain AB mice

Dose (mg/kg)	Day P.C.	Pregnant mothers				Implantations		Resorptions	
		Total	Died	Without implant	Evaluable	Total	Mean±SD	Total	%
Control	11	6	0	1	5	35	7.0±0.5	2	5.7
70	7	12	0	4	8	57	7.1±1.0	14	24.6
70	9	2	0	0	2	14	7.0	2	14.3
70	11	10	2	3	5	37	7.3±0.4	14	37.8

The mean number of implantations did not change significantly after single-dose injection on different days of gestation. The rate of resorptions in high dose treated animals increased as compared to control animals. This dose did not appear to be maternally toxic when administered on gestation days 7 or 9.

Influence of single-dose application of IMET 3393 in distilled water on deformations in colony-bred strain AB mice

Dose (mg/kg)	Day P.C.	Fetuses			Abnormalities*	
		Total	Dead	Mean±SD	Total	%
Control	7-11	73	0	7.3±0.3	0	0
Control	11	92	0	7.7±0.3	1	1.1
35	5	16	0	8.0	0	0
70	7	34	1	5.6±1.6	5	14.7
70	9	57	1	6.3±0.7	2	3.5
70	11	33	2	4.7±0.9	3	9.1

\*see table below for details

Influence of single-dose application of IMET 3393 in isotonic sodium chloride solution on deformations in colony-bred strain AB mice

Dose (mg/kg)	Day P.C.	Fetuses			Abnormalities*	
		Total	Dead	Mean±SD	Total	%
Control	11	33	0	6.6±0.6	0	
70	7	43	1	6.1±1.2	3	7.0
70	9	12	0	6.0±1.0	0	
70	11	23	0	5.8±1.3	1	4.4

\*see table below for details

Single dose administration of IMET 3393 at 70 mg/kg caused mortalities, resorptions and deformations of fetuses in pregnant mice.

Influence of a multiple-dose application of nitrogen mustard derivatives on resorptions (solvent: distilled water, ip. at Days 7-11 p.c.)

Dose (mg/kg)	Pregnant mothers				Implantations		Resorptions	
	Total	Died	Without implant	Evaluable	Total	Mean±SD	Total	%
1. IMET 3393 AB/colony bred								
Control	16	0	6	10	75	7.5±0.5	2	2.7
25	21	0	6	15	109	7.3±0.2	16	9.3
37.5	21	0	5	16	108	6.8±0.2	3	2.8
50.0	24	0	7	17	118	7.3±0.3	15	12.7
62.5	32	2	16	14	102	7.2±0.4	2	2.0
2. IMET 3393 NMRI/Han Jena								
Control	25	1	2	22	273	12.4±0.6	35	12.8
37.5	19	0	5	14	156	11.1±0.6	31	19.6
3. Cyclophosphamide NMRI/Han Jena								
Control	25	1	2	22	273	12.4±0.6	35	12.8
10	20	0	8	12	153	12.7±0.9	32	20.9
15	20	0	2	18	218	12.1±0.5	42	19.3
30	19	0	6	13	142	10.9±0.9	68	47.9

Influence of a multiple-dose application of nitrogen mustard derivatives on deformations (solvent: distilled water, ip. at Days 7-11 p.c.)

Dose (mg/kg)	Fetuses			Fetuses with abnormalities	
	Total	Dead	Mean ±SD	Total	%
1. IMET 3393 AB/colony bred					
Control	73	0	7.3±0.3	0	
25	93	0	6.6±0.6	0	
37.5	105	2	6.6±0.2	2	1.9
50.0	103	0	6.1±0.3	0	
62.5	109	0	7.1±0.7	0	

Dose (mg/kg)	Fetuses			Fetuses with abnormalities	
	Total	Dead	Mean ±SD	Total	%
<b>2. IMET 3393 NMRI/Han Jena</b>					
Control	238	6	10.6±0.6	4	1.7
37.5	125	3	8.9±0.7	1	0.8
<b>3. Cyclophosphamide NMRI/Han Jena</b>					
Control	238	6	10.8±0.6	4	1.7
10	121	2	10.1±0.8	3	2.5
15	176	1	9.8±0.5	3	1.7
30	74	9	5.7±0.7	7	9.6

The percentage of fetuses resorptions increased with doses.

Offspring (malformations, variations, etc.):

**Influence of single-dose application of IMET 3393 on fetus-development in colony-bred strain AB**

Dose mg/kg	Day p.c.	Abnormalities (foetuses total/deformed)		Body weight of foetuses on day 19 p.c.			Foetuses bearing accessory ribs			
		external	skeletal	n	mean (mg)	SD (mg)	significance I: m C: treated	n foetuses	% foetuses over 13/13*	95% confidence interval
<b>I. IMET 3393 in distilled water</b>										
dest. water	7-11	73/0 -	73/0 -	43 m 30 f	1205.7 ± 14.75 1184.8 ± 18.92	-	-	73	5.5	1.5 - 13.4
dest. water	11	92/1 dwarfism	92/0 -	51 m 41 f	1237.5 ± 25.10 1219.0 ± 14.89	-	-	92	3.3	0.7 - 9.2
35.0	5	16/0 -	not determined	5 m 11 f	1082.0 ± 13.29 1074.5 ± 13.32	-	0.1% 0.1%	-	-	-
70.0	7	34/3 short tail, ectopia	32/1 rib concrecence	18 m 17 f	991.9 ± 44.59 975.9 ± 37.50	-	0.1% 0.1%	32	78.1	60.0 - 90.7
70.0	9	57/2 cleft palate, haematoma	57/0 -	22 m 34 f	943.0 ± 31.74 929.0 ± 37.04	-	0.1% 0.1%	57	29.8	18.4 - 43.4
70.0	11	33/0 -	30/3 rib malformation, rib concrecence, deformity of the spine	21 m 10 f	1040.2 ± 53.61 976.0 ± 79.23	-	0.1% 0.1%	30	6.7	0.8 - 22.1
<b>II. IMET 3393 in isotonic NaCl-solution</b>										
NaCl-solut.	11	33/0 -	33/0 -	17 m 16 f	1253.8 ± 26.98 1252.2 ± 18.63	-	-	33	6.1	0.7 - 20.2
70.0	7	43/0 -	43/3 bended tail, rib concrecence	18 m 18 f	1187.8 ± 38.93 1156.4 ± 30.72	-	- 5%	42	57.1	41.0 - 72.3
70.0	9	12/0 -	12/0	7 m 5 f	1484.3 ± 19.14 1385.0 ± 17.54	1%	0.1% 5.0%	12	8.3	0.2 - 36.5
70.0	11	23/0 -	23/1 deformity of the spine	11 m 12 f	1035.0 ± 28.69 1042.9 ± 34.08	-	0.1% 0.1%	23	8.7	1.1 - 28.0

SD = standard deviation  
C = controls

(Excerpted from the publication)

IMET 3393 at 70 mg/kg on GD 7 and 11 caused more malformations than on GD 9. The mean body weights of fetuses were reduced at higher doses of IMET 3393.

Influence of a multiple-dose application of nitrogen mustard derivatives on fetus development (solvent: distilled water, ip. at days 7-11 p.c.)

Total dose mg/kg	Abnormalities (foetuses total/deformed)		Body weight of foetuses on day 18 p.c.			Foetuses bearing accessory ribs		
	external	skeletal	n	mean SD (mg)	significance f:m C: treated	n foetuses	% foetuses over 13/13*	95% confidence interval
<b>I. IMET 3393 AB/cxlony-bred</b>								
dest. water	73/0 -	73/0 -	43 m 30 f	1205.7 ± 14.75 1184.8 ± 18.92	-	73	5.5	1.5 - 13.4
25.0	93/0 -	93/0 -	47 m 46 f	1181.8 ± 15.41 1139.1 ± 18.48	-	93	24.7	16.4 - 34.8
37.50	105/2 dwarfism	105/0 -	59 m 46 f	1144.0 ± 11.41 1103.5 ± 15.05	- 1% 1%	105	25.7	17.7 - 35.2
50.0	103/0 -	103/0 -	53 m 50 f	1071.0 ± 13.62 1024.5 ± 14.93	5% 0.1% 0.1%	103	25.7	17.7 - 35.2
62.50	100/0 -	100/0 -	54 m 46 f	1033.7 ± 13.05 1005.5 ± 14.09	5% 0.1% 0.1%	100	29.0	20.4 - 38.9
<b>II. IMET 3393 NMR/Han Jena</b>								
dest. water	238/4 exencephali	143/0 -	108 m 125 f	1136.6 ± 14.49 1132.1 ± 11.83	-	143	42.0	33.8 - 50.6
37.50	125/1 haematoma	125/0 -	65 m 58 f	1147.5 ± 16.74 1181.4 ± 20.25	-	125	91.2	84.5 - 95.3
<b>IV. Cyclophosphamide NMR/Han Jena</b>								
dest. water	238/4 exencephali	143/0 -	108 m 125 f	1136.6 ± 14.49 1132.1 ± 11.83	-	143	42.0	33.8 - 50.6
10.0	121/3 haematoma, cleft palate, Hämascos	62/0 -	74 m 45 f	1175.9 ± 16.76 1177.6 ± 18.30	-	62	77.4	65.0 - 87.1
15.0	176/3 dwarfism, haematoma	86/0 -	105 m 70 f	1086.5 ± 13.20 1064.0 ± 14.51	- 0.1% 0.1%	86	95.4	88.5 - 98.7
30.0	74/2 Hämascos	36/5 anomaly of ribs and vertebrae	34 m 40 f	884.6 ± 18.44 888.3 ± 17.89	- 0.1% 0.1%	36	97.2	88.5 - 99.9

\* ) = foetuses of the 2<sup>nd</sup> gestation (mean foetus-weights are not different to those of the 1<sup>st</sup> gestation)

SD = standard deviation

n.r. = not reported

C = controls

(Excerpted from the publication)

**Conclusions:** A significant increase in the rate of malformations (dwarfism, exencephaly, hematoma and cleft palate) was observed at 37.5 mg/kg IMET 3393 treated animals. There was also a significant increase of fetuses bearing accessory ribs. Positive control (cyclophosphamide) at 30 mg/kg produced significant resorptions.

**The Effect of Nitrogen Mustard Compounds on the Formation  
of Accessory Ribs in the Fetuses of Mice**

**Key study findings:**

- Intraperitoneal administration of Endoxan®, IMET 3393, and IMET 3106 from GD 7<sup>th</sup> to 11<sup>th</sup> day caused an increase in the number of fetuses with accessory ribs.

**Study no.:** Publication (Heinecke & Klaus, Arzneimittelforschung 1972;22(1):122-5)

**Volume # and page #:** Module 4.2.3.5

**Conducting laboratory and location:** Central Institute of Microbiology and Experimental Therapy, Jena, the German Academy of Science to Berlin & Department of Experimental Therapy

**Date of study initiation:** Unknown

**GLP compliance:** No

**QA reports:** yes ( ) no (X)

**Drug, lot #, and % purity:** IMET 3106, IMET 3393 (bendamustine) and Endoxan® (cyclophosphamide)

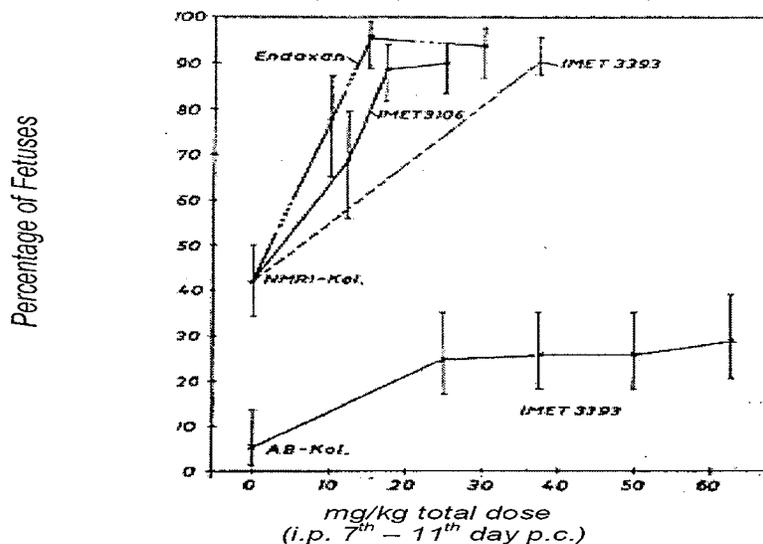
**Methods**

Doses:	Variable
Species/strain:	AB and NMRI (colony bred)
Number/sex/group:	Variable
Route, formulation, volume, and infusion rate:	0.2 ml or 0.4 ml, ip injection
Satellite groups used for toxicokinetics:	No
Study design:	Animals were dosed from GD 7 to 11. Fetuses were collected on GD 18.
Parameters and endpoints evaluated:	Accessory ribs were recorded
Dose justification:	Not mentioned

**Results**Mortality (dams), Clinical signs (dams), Body weight (dams), and Food consumption(dams): Not mentionedToxicokinetics: Not performedTerminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

Five time application of Endoxan, IMET 3393 or IMET 3106 from the 7<sup>th</sup> to 11<sup>th</sup> day p.c. produced an increase in the number of fetuses with accessory ribs as shown below.

The effects of Endoxan, IMET 3393 and IMET 3106 on the formation of accessory ribs (more than 13/13 ribs)



(Excerpted from the sponsor's submission)

The formation of accessory ribs varies from the absence of this phenomenon, being normal, to the increase or decrease of the number of fetuses with accessory ribs depending on the strain of the animals investigated.

Number of ribs in murine fetuses as related to major physical and skeletal deformations

Fetuses	N	Fetuses with		
		Fewer than 13/13 ribs (%)	13/13 ribs (%)	More than 13/13 ribs (%)
Without malformations	1172	3.0	84.7	12.3
With external and skeletal deformations	162	9.3	72.9	17.9
With skeletal deformations	52	19.3	48.2	32.7
With cleft palates	55	3.6	92.8	3.6
With ectopia hepatic or segmented intestines	27	14.8	51.8	33.4
With exencephalus	16	6.3	87.4	6.3

**Conclusions:** Endoxan® (cyclophosphamide), IMET 3393 (bendamustine), and IMET 3106 administrations from the 7<sup>th</sup> to 11<sup>th</sup> day p.c. caused an increase in the number of fetuses with accessory ribs as shown above. Development of accessory ribs was used as parameter in testing teratogenic activities.

### On the Effect of the "Cytostasan" Mustard Derivative on Murine Pregnancy and Embryonic Development

#### Key study findings:

- Intraperitoneal administration of cytostasan at 100 mg/kg killed all dams.
- Cytostasan at 50 mg/kg during dose range findings study killed all embryos.
- Resorption rates at 40 mg/kg cytostasan were higher than at 20 mg/kg.
- Cytostasan produced external (bent/circinate tail, exomphalos, and turricephaly) and internal (hydrocephalus, hydronephrosis, hydroureter) malformations in Wistar rats.

<b>Study no.:</b>	Publication (Wendler, Pabst, and Bertolini, Anat Anz.Bd 139 (S); 100-114, 1976)
<b>Volume # and page #:</b>	4.2.3.5
<b>Conducting laboratory and location:</b>	Anatomical Institute of the Department of Medicine at the Karl Marx University in Leipzig, GDR
<b>Date of study initiation:</b>	Unknown
<b>GLP compliance:</b>	No
<b>QA reports:</b>	yes ( ) no (X)
<b>Drug, lot #, and % purity:</b>	Cytostasan, lot # and purity unknown

#### Methods

Doses:	Dose range study: 20, 25, 35, 40, 50, & 100 mg/kg Finally study: 20 and 40 mg/kg
Species/strain:	Wistar Albino rats
Number/sex/group:	Different
Route and volume:	Intraperitoneally (IP), 10 mL/kg
Satellite groups used for toxicokinetics:	No
Study design:	Rats were dosed on 4, 7, 9, 11 or 13 day p.c., then euthanized on GD 20. Half of the embryo were fixed in SERRA mixture (90% alcohol, 35% formaldehyde: glacial acetic acid 12:6:1 parts) and other half in 96% alcohol.
Parameters and endpoints evaluated:	Mortality, clinical signs, implantation rate, resorptions rates and number of live and dead fetuses, malformation rate and embryo weights.
Dose justification:	Cytostasan at 100 mg/kg killed all animals and at 50 mg/kg killed most of the embryos.

#### Results

<u>Mortality (dams):</u>	50 mg/kg - killed most embryos 100 mg/kg – killed all animals
--------------------------	--

Clinical signs (dams): Not reported  
Body weight (dams): Not reported in the paper  
Food consumption (dams): Not reported  
Toxicokinetics: Not performed

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

Average implantation rates

Dose (mg/kg)	Gestational day	Animals/group	Implantation/group	Average±SD
Control		20	214	10.7±1.9
20	4	9	101	11.2±1.6
	7	7	75	10.7±3.6
	9	3	23	7.7±4.7
	11	5	43	8.6±2.7
	13	6	55	9.2±2.3
40	4	7	68	9.7±3.4
	7	11	108	9.8±3.1
	9	9	100	11.1±2.1
	11	4	49	12.3±2.9
	13	10	95	9.5±1.6

Average resorption rates

Dose (mg/kg)	Gestation day	Implantation/group	Resorption/group	Resorption/group (%)
Control		214	22	10.3
20	4	101	22	21.8
	7	75	26	34.7
	9	23	3	13.0
	11	43	13	30.2
	13	55	8	14.6
40	4	68	28	41.2
	7	108	42	38.9
	9	100	44	44.0
	11	49	18	36.7
	13	95	42	44.2

Number of live fetuses

Dose (mg/kg)	Gestation day dosed	Implantation/group	Live fetuses per group	% of live fetuses
Control		214	192	90
20	4	101	79	78
	7	75	49	65
	9	23	20	87
	11	43	30	70
	13	55	47	85
40	4	68	40	59
	7	108	66	61
	9	100	56	56
	11	49	31	63
	13	95	53	56

Offspring (malformations, variations, etc.):

Malformation rates

Dose (mg/kg)	Gestation day dosed	Implantation/group	# of malformed/group	% of malformed/group
Control		214	3	1.4
20	4	101	12	11.9
	7	75	8	10.7
	9	23	6	26.1
	11	43	1	2.3
	13	55	10	18.2
40	4	68	5	7.4
	7	108	7	6.5
	9	100	15	15.0
	11	49	4	8.2
	13	95	11	11.6

Externally visible malformations

20 mg/kg			40 mg/kg		
Day	%	Findings	Day	%	Findings
4	4.4	Bent tail (1 & 2)	4	2.8	Circinate tail (1 & 2)
7	-	(2 & 3)	7	5.8	Turriccephaly, exomphalos, bent tail, circinate tail (2)
9	-	(1)	9	-	(3)

- 1) "round back
- 2) posterior extremities stretched to extreme extension
- 3) "strangulation marks" Retraction on back at the level of the thoracic spine.

(Copied from the publication)

Malformations of internal organs

	20 mg/kg of BW		40 mg/kg of BW	
4 <sup>th</sup> day	55.6%	Hydrocephalus Hydronephroses	83.3%	Hydrocephalus Hydronephroses
7 <sup>th</sup> day	56.7%	Hydrocephalus Hydronephroses	38.5%	Hydrocephalus Hydronephroses Hydroureter
9 <sup>th</sup> day	100.0%	Hydrocephalus Hydronephroses	57.7%	Hydrocephalus Hydronephroses
11 <sup>th</sup> day	33.3%	Hydrocephalus	44.4%	Hydrocephalus
13 <sup>th</sup> day	66.7%	Hydrocephalus Hydronephroses	84.8%	Hydrocephalus Hydronephroses

(Excerpted from the publication)

Average embryo weight

Dose (mg/kg)	Gestation day	Number of live fetuses	Average weight of the group ± SD (mg)
20	4	61	2.32±0.2
	7	9	2.27±0.3
	9	13	2.21±0.1
	11	17	2.04±0.1
	13	47	2.25±0.3
40	4	40	2.17±0.3
	7	43	2.22±0.3
	9	35	2.01±0.8
	11	17	1.09±0.1
	13	42	2.01±0.2

Intraperitoneal administration of cytotasan at 100 mg/kg killed all the dams and at 50 mg/kg most of the embryos died.

**Prenatal and postnatal development:** Not conducted

**2.6.6.7 Local tolerance****4.2.3.6.1 Perivenous and Intra-Arterial Tolerance Study in the Rabbit.  
Study 0640.00.C14.01.****Key study findings:**

- Perivenous injection of bendamustine at a concentration of 0.6 or 1.0 mg/ml or intra-arterial injection at a concentration of 0.2 or 0.6 mg/mL produced local irritation at the injection sites.

**Study no.:** 0640.00.C14.01  
**Volume # and page #:** Module 4.2.3.6.1  
**Conducting laboratory and location:** / /  
**Date of study initiation:** April 6, 2000  
**GLP compliance:** Yes  
**QA reports:** yes (X) no ( )  
**Drug, lot #, and % purity:** Bendamustine hydrochloride,  
batch # 350999, purity not mentioned  
**Formulation/vehicle:** 0.9% saline solution

**Methods**

## Doses:

Group	Dose route	Dose volume	Dose concentration (right ear)	Total dose (right ear)	Left ear
1	Perivenous	0.2 mL	1.0 mg/mL	0.2 mg	Saline/water
2	Perivenous	0.2 mL	0.6 mg/mL	0.12 mg	Saline/water
3	Perivenous	0.2 mL	0.2 mg/mL	0.04 mg	Saline/water
4	Perivenous	0.2 mL	0.1 mg/mL	0.02 mg	Saline/water
5	Intra-arterial	0.5 mL	0.6 mg/mL	0.3 mg	Saline/water
6	Intra-arterial	0.5 mL	0.2 mg/mL	0.1 mg	Saline/water

**Species/strain:** New Zealand White rabbit  
**Number/sex/group or time point (main study):** 3  
**Route:** Perivenous and intra-arterial  
**Satellite groups used for toxicokinetics or recovery:** none  
**Age:** 12 weeks  
**Weight:** 2.5 to 2.9 Kg

**Observations and times:**

Mortality: Twice a day  
Clinical signs: Daily  
Body weights: Days 1 and 5  
Gross pathology: Study termination (Day 5)  
Histopathology: Auricular only,  
 Peer review: yes ( ), no (X)

**Results**

Mortality: None  
Clinical signs: None  
Local reactions: Slight or moderate bruising at both the test and control sites (more notable in animals injected intra-arterial)

Body weights:

Mean body weight change (g) from day 1 to day 5

Route	Concentration (mg/mL)	Mean body weight changed (g)	
		♂	♀
Perivenous	0.1	32	115
	0.2	28	30
	0.6	-41	-12
	1.0	02	57
Intra-arterial	0.2	71	92
	0.6	142	87

Gross pathology: Congestion in the lungs of two animals

Histopathology:

Microscopic findings (males and females combined)

Group	Vehicle				Bendamustine			
	1	2	3	4	1	2	3	4
Concentration (mg/mL)	0	0	0	0	1.0	0.6	0.2	0.1
<b>Perivenous (perivascular)</b>								
Perivascular eosinophilic fibrillar material, minimal	0	0	1	0	2	2	0	0
Moderate	0	0	0	0	2	2	0	0
Perivascular edema, moderate	1	0	0	0	4	4	0	0
Perivascular inflammation, slight	3	1	1	0	1	4	3	2
Moderate	1	0	0	0	3	0	0	0
Subcutaneous eosinophilic fibrillar material, moderate	0	0	0	0	2	0	0	0
Subcutaneous edema, moderate	0	0	0	0	2	4	0	0
Subcutaneous inflammation, moderate	0	0	0	0	3	0	0	0

## Microscopic findings (males and females combined)

Group	Vehicle		Bendamustine	
	5	6	5	6
Concentration (mg/mL)	0	0	0.6	0.2
Intra-arterial (vascular)				
Perivascular focus of fibrin, minimal	0	0	3	2
Site of needle puncture in arterial wall	0	0	3	1

There were no deaths and no signs of toxicity in any rabbits during the observation period. Local tolerance at the treatment sites was confined to slight to moderate bruising at both test and control injection sites (more notable in animals injected intra-arterially). A slight loss in bodyweight was recorded in high dose four males and six females. The histological findings showed a treatment-related effect when the animals were administered the highest two concentrations of bendamustine hydrochloride (0.6 mg/mL and 1.0 mg/ml) by perivenous injection. This effect was characterized by an increase in incidence and degree of perivascular changes indicative of local irritation, together with an increased extent of these lesions into adjacent subcutaneous tissue.

**Conclusions:** Based on findings in this study, treatment-related findings were seen with bendamustine hydrochloride when administered by perivenous injection at a concentration of 0.6 or 1.0 mg/ml or by intra-arterial injection at a concentration of 0.2 or 0.6 mg/mL.

#### 2.6.6.8 Special toxicology studies:

4.2.3.1.2

Estimate the LD<sub>50</sub> Value of \_\_\_\_\_  
in Female BDF-1 Mice.

#### Key study findings:

- LD<sub>50</sub> was estimated to be 468 mg/kg.

Study no.:

PT-VIV-108

Volume # and page #:

Module 4.2.3.1.2.

Conducting laboratory and location:

Salmedix, Inc.

Date of study initiation:

September 2, 2003

GLP compliance:

No

QA report:

yes ( ) no (X)

Drug, lot #, and % purity:

lot # and purity not provided.

**Methods**

Doses: 63, 200, 630 mg/kg  
 Species/strain: Female BDF-1 mice  
 Number/sex/group or time point (main study): 1 ♀/group  
 Route, formulation, volume, and infusion rate: Intraperitoneal  
 Satellite groups used for toxicokinetics or recovery: None  
 Age: Not provided  
 Weight: Unknown

Unique study design: Single animal is dosed with a minimum volume of 200 uL at 48 hours intervals or until one is confident of the survival of the previous dose animal [Global Harmonised System for the classification of chemicals which cause acute toxicity (OECD Test Guidance 425)]. The testing stops when one of the following stopping criteria is met.

1. 3 consecutive animals survive at the upper bound.
2. 5 reversals occur in any 6 consecutive animals tested;
3. At least 4 animals have followed the first reversal and the specified likelihood-ratios exceed the critical value.

**Results**Mortality:

Test sequence	Animal ID	Dose (mg/kg)	Short term result	Long term result
1	1	20	Survived	Survived
2	3	63	Survived	Survived
3	4	200	Survived	Survived
4	5	630	Died	Died
5	7	200	Survived	Survived
6	8	630	Died	Died
7	11	200	Survived	Survived

No stopping criteria were met.

LD<sub>50</sub>=468 mg/kg

Clinical signs:

630 mg/kg – decreased mobility, labored breathing and deceased

#### 2.6.6.9 Discussion and Conclusions

During traditional toxicity assessment, the acute (single dose, non-GLP) toxicity studies were conducted in mice and rats. Pivotal repeat dose toxicity studies were conducted in Sprague-Dawley rats and beagle dogs (GLP). Microscopic findings in rats that were considered to be related to bendamustine included karyomegaly and tubular degeneration/necrosis of kidney tubular epithelium. Systemic exposure increased in a dose related manner. The  $t_{max}$  value for bendamustine was 0.5 hours and the apparent  $t_{1/2}$  ranged from 0.14 to 0.36 hours in rats. During repeat dose toxicity study in beagle dogs, heart rates were reduced during cycle 2 at 6.6 mg/kg/day (2♂ & 1 ♀, 3/6 animals). This effect had been discussed with reviewing Medical Officers and Team Leader for this NDA. Microscopic findings in high dose dogs were indicative of immunosuppression, and included moderate to severe atrophy in the thymus, reduced lymphoid cellularity and absent germinal centers in the spleen and lymph nodes. The kidney was also identified as a target organ in the dog. The mean maximum plasma concentrations ( $C_{max}$ ) and systemic exposure (AUC) increased with increasing doses. In summary, bendamustine disrupted cellular turnover in gastrointestinal tract, immune system and testes, where rapid cell division occurs.

**Genetic toxicology:** Bendamustine induced mutation in Ames test, produced chromosome aberration in human lymphocytes, and induced micronucleated polychromatic erythrocytes in male and female rats. Therefore, bendamustine is genotoxic alkylating agent.

**Carcinogenicity:** Intraperitoneal injections of bendamustine for four days produced peritoneal sarcoma in mice. Oral administration for four days induced mammary carcinoma and pulmonary adenomas in mice.

**Developmental and reproductive toxicity:** As expected from nitrogen mustard alkylating agent (i.e. chlorambucil and melphalan), bendamustine produced external (bent/circinate tail) and internal (hydrocephalus, hydronephrosis, hydroureter) malformation during embryofetal developmental toxicity study in Wistar rats. Bendamustine (ip administration) also caused embryotoxic and teratogenic effects in mice.

**Local tolerance:** Perivenous injection of bendamustine at a concentration of 0.6 or 1.0 mg/ml or intra-arterial injection at a concentration of 0.2 or 0.6 mg/mL produced local irritation at the injection sites in the ear of New Zealand White rabbits.

**2.6.7 TOXICOLOGY TABULATED SUMMARY**

## General Toxicology;

Summary of multiple dose toxicology studies

Species	Duration/ Route	N/sex/ dose	Doses (mg/m <sup>2</sup> /day)	Significant finding
Rat	1 month/oral	10	240-960	Mortalities, inhibition of bone marrow hematopoiesis and decreased lymphocytes in peripheral blood
Rat	3 months/oral	16	120-360	Mortalities and lymph nodes, spleen, and thymus were atrophic,
Rat	15-week intermittent/iv	20	30-90	Cardiomyopathy (focal/multifocal) in males only, kidney tubular epithelial karyomegaly in treated animals
Dog	15-week intermittent/iv	3	33-132	↓ heart rate of high dose animals, lymphoid tissues, kidney, testes and intestine were affected.

Genetic Toxicology

Study	Concentration or Dose	Results		
		Positive control	No metabolic (-S9)	Plus metabolic (+S9)
Bacterial reverse mutation assay	5000 µg/plate	Yes	Positive	Positive
<i>In vitro</i> human lymphoma cells	15 µg/ml	Yes	Positive	Positive
<i>In vivo</i> rat bone marrow micronucleus assay	6.25, 12.5, and 25 mg/kg	Cyclophospha- mide	Increased PCE/NCE ratio	

Genetic Toxicology of Hydroxy-Bendamustine

Study	Concentration (µg/mL)	Results		
		Positive control	No metabolic (-S9)	Plus metabolic (+S9)
Chromosome aberration test	21.8, 38.2, and 66.8	Yes	Positive	Positive

Reproductive Toxicology

Study	Route	Species	Result
Embryofetal development	Intraperitoneal	Mice	Embryotoxic and teratogenic (cleft palate and costal malformations)
Embryofetal development	Intraperitoneal	Mice	Increased number of fetuses with accessory ribs
Embryofetal development	Intraperitoneal	Rats	Produced external (bent/circinate tail) and internal (hydrocephalus, hydronephrosis, hydroureter) malformations

**OVERALL CONCLUSIONS AND RECOMMENDATIONS**

**Conclusions:** Bendamustine is approvable from pharmacology/toxicology perspective.

**Unresolved toxicology issues (if any):** None

**Recommendations:** This NDA is approvable from a pharmacology/toxicology perspective.

**Suggested labeling:** Separate review will be conducted

Reviewer Signature \_\_\_\_\_

Supervisor Signature \_\_\_\_\_ Concurrence Yes \_\_\_ No \_\_\_

Appendix/attachments:

Attachment A

Executive CAC  
Date of Meeting: November 27, 2007

Committee: Abby Jacobs, Ph.D., OND IO, Acting Chair  
Todd Bourcier, Ph.D., DMEP, Alternate Member  
Chuck Resnick, Ph.D., DCRP, Alternate Member  
John K. Leighton, Ph.D., DDOP, Team Leader  
M. Anwar Goheer, Ph.D., DDOP, Presenting Reviewer

Author of Draft: Anwar Goheer

The following information reflects a brief summary of the Committee discussion recommendations.

NDA # 22-249  
Drug Name: Treanda® (Bendamustine hydrochloride)  
Sponsor: Cephalon, Inc.

Background: Bendamustine hydrochloride is a nitrogen mustard derivative, a agent. It is under review for treatment of chronic lymphocytic leukemia. The recommended dose is 100 mg/m<sup>2</sup> administered as an intravenous infusion over minutes on days 1 and 2 of a 28-day cycle, up to 6 cycles.

The sponsor of this NDA (Cephalon) would like \_\_\_\_\_

Mouse Carcinogenicity Study: In the published paper [Arch Geschwulstforsch 43(1):16-21] female mice were treated orally or intraperitoneally with bendam hydrochloride for four consecutive days and observed until death.

**Intraperitoneal injections** of bendamustine for four days produced peritoneal **Oral administration** for four days induced mammary carcinomas and pulmon adenomas.

Executive CAC Recommendations and Conclusions:

- The Committee noted that the full spectrum of potential carcinogenicity v evaluated in the study. However, the Committee concurred that the follo neoplasms were drug related: peritoneal sarcomas after intraperitoneal administration; pulmonary adenomas and mammary carcinomas after ora administration.

APPEARS THIS WAY  
ON ORIGINAL

Reviewer: Anwar Goheer, Ph.D.

NDA No. 22-249

- The Committee did not concur that the reticulosarcomas seen after oral administration were clearly drug related.

Abigail Jacobs, Ph.D.  
Acting Chair, Executive CAC

cc: \  
/Division File, DDOP  
/JKLeighton, DDOP  
/MAGoheer, DDOP  
/DWPease, DDOP  
/ASeifried, OND IO

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

/s/

-----  
Anwar Goheer  
2/27/2008 02:13:37 PM  
PHARMACOLOGIST

John Leighton  
2/27/2008 02:58:22 PM  
PHARMACOLOGIST

# PHARMACOLOGY FILING CHECKLIST

**NDA Number:** 22-249

**Applicant:** Cephalon

**Stamp Date:** 9/19/2007

**Drug Name:** Bendamustine

**NDA Type:** NME

On initial overview of the NDA application for RTF:

	Content Parameter	Yes	No	Comment
1	On its face, is the pharmacology/toxicology section of the NDA organized (in accord with 21 CFR 314 and current guidelines for format and content) in a manner to allow substantive review to begin?	√		CTD format
2	Is the pharmacology/toxicology section of the NDA indexed and paginated in a manner allowing substantive review to begin?	√		Electronic submission
3	On its face, is the pharmacology/toxicology section of the NDA legible so that substantive review can begin?	√		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted in this NDA (carcinogenicity, mutagenicity*, teratogenicity*, effects on fertility, juvenile studies, acute and repeat dose adult animal studies*, animal ADME studies, safety pharmacology, etc)?			Carcinogenicity – not done/not required Mutagenicity – done Teratogenicity – not done, publications are submitted, 505(b)(1) or (2)? Fertility – not done/not required Juvenile studies – not done/not required Acute and repeat dose toxicity – done (3 cycles in dogs, 5 cycles in rats) ADME – done Safety pharmacology – done(cardio, local tolerance)
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	√		Similar formulations were used in pivotal preclinical and clinical studies
6	On its face, does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the sponsor <u>submitted</u> a rationale to justify the alternative route?	√		Same route of administration

	Content Parameter	Yes	No	Comment
7	Has the sponsor <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	√		
8	Has the sponsor submitted all special studies/data requested by the Division during pre-submission discussions with the sponsor?		√	Raw data for teratology studies is not submitted. Published papers in 1971 and 1976 are submitted
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		√	Published studies were conducted by ip route, direct comparison to maximum recommended human dose (iv) cannot be made.
10	If there are any impurity – etc. issues, have these been addressed? (New toxicity studies may not be needed.)	√		
11	Has the sponsor addressed any abuse potential issues in the submission?		√	
12	If this NDA is to support a Rx to OTC switch, have all relevant studies been submitted?		√	Not applicable. This NDA is NME
13	From a pharmacology/toxicology perspective, is the NDA fileable? If "no" please state below why it is not.	√		

Any Additional Comments:

Exec. CAC meeting is on Nov. 27, 2007

Reviewing Chemist (Ravindra Kasliwal) has been contacted regarding CMC assessment of impurities.

\_\_\_\_\_  
Reviewing Pharmacologist

\_\_\_\_\_  
Date

\_\_\_\_\_  
Team Leader/Supervisor

\_\_\_\_\_  
Date

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

/s/

-----  
Anwar Goheer  
10/29/2007 08:11:17 AM  
PHARMACOLOGIST

John Leighton  
11/5/2007 09:26:25 AM  
PHARMACOLOGIST