CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125327Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Voraxaze (glucarpidase)

Date: January 9, 2012
To: File for BLA 125327
From: John K. Leighton, PhD, DABT
Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

John K. Leighton 1/9/2012

I have examined pharmacology/toxicology supporting review of Dr. Ricci and secondary memorandum and product labeling provided by Dr. Pilaro. Several issues remain to be finalized, but if addressed as discussed below, would not preclude approval of Voraxaze from a nonclinical perspective.

First, safety of the use of the [redacted] (b) (4) and any carryover into the final drug substance/product, has not been adequately addressed. The issue was identified late in the review cycle and thus is not discussed in the primary pharmacology review or the secondary memorandum. In order to appropriately qualify [redacted] (b) (4) a toxicology study as recommended by the review team will need to be conducted. Alternatively, the review team has also determined that it would be acceptable to approve the BLA if an analytical method with appropriate sensitivity is available by which [redacted] (b) (4) is detected in drug substance or drug product. This has been addressed in teleconferences with the Applicant and is documented in the minutes.

A second topic is the need for a nonclinical study to support the safety of the intrathecal (IT) route of administration. This was discussed in the primary pharmacology review and supervisory memorandum. The primary reviewer indicated that the animal study submitted to the BLA was inadequate to support the safe use of glucarpidase when administered by the intrathecal route. The Division of Hematology Oncology Toxicology defers to the clinical review team as to the need for an adequate nonclinical evaluation to support clinical use of Voraxaze when administered IT, and the study endpoints (safety and/or efficacy).

Finally, the supervisory memorandum discusses the use of glucarpidase treatment together with leucovorin rescue. For clarity, these statements should be understood in the context of leucovorin use described in the package insert for Voraxaze.

MEMORANDUM

TO: Emanuela Lacana, Ph.D. Quality Team Leader
DTP/OBP/OPS/CDER/FDA
THROUGH: Anne M. Pilaro, Ph.D., Supervisory Toxicologist, Jan 6, 2012
DHOT/OHOP/OND/CDER/FDA
FROM: M. Stacey Ricci, Sc.D., Senior Toxicologist, MSR Jan. 6, 2012
DHOT/OHOP/OND/CDER/FDA
BLA #: 125327
SPONSOR: BTG International
PRODUCT: Voraxaze (glucarpidase)
DATE: January 6, 2012

SUBJECT: Nonclinical Consult Request (submitted Dec. 22, 2011)

Background:

The Division of Therapeutic Proteins submitted a *Request for Consultation* on December 22, 2011 regarding the safety of (b) (4) used in the manufacture of Voraxaze. This consult was received from DTP after completion of the primary reviews for the Voraxaze BLA (primary reviews were due December 20, 2011). Following receipt of the Consult Request, an information request was sent to BTG on December 27, 2011 asking that they provide any available animal safety information regarding (b) (4)

(b) (4)[1]

A Drug Master File for (b) (4) was not submitted to FDA, and (b) (4)

According to information provided by a consulting toxicologist hired by BTG[2], the manufacture of (b) (4)

(b) (4)

There are no animal toxicology data available regarding the safety of intravenous administration of (b) (4) There is insufficient information available about the chemical composition of (b) (4) and an assessment regarding potential toxicities of intravenously administered (b) (4), (based on structure-activity relationship) cannot be made.

BTG's proposed lot release specification for residual (b) (4), present in the drug substance is less than or equal to (b) (4) as measured by high performance liquid chromatography with UV detection at 230 nm. Nonclinical studies performed in compliance with 21 CFR part 58 Good Laboratory Practice guidelines were conducted using Lots 2090301, 2090302 and 2090401, which were manufactured from drug substance batches E-CG2-P03, M-CG2-P01 and M-CG2-P02, respectively. The assay used to detect residual (b) (4) was not implemented until after the expiration date of the nonclinical lots, and retrospective analysis of these lots did not detect (b) (4) above the detection limit of (b) (4) Therefore, the animal studies and submitted with the BLA cannot be used to qualify the safety of (b) (4), in Voraxaze at the proposed acceptance limit for lot release.

Nonclinical Recommendation:

There are no data available from animal toxicology studies that can be relied upon to demonstrate the safe use of (b) (4), administered *via* the intravenous route. In order to qualify the safety of (b) (4) present in Voraxaze at the proposed drug substance lot release specification of (b) (4) the nonclinical discipline recommends that BTG conduct a single dose, general toxicology study with a 14 day follow-up observation period including dose groups that receive (b) (4) alone and with Voraxaze. The nonclinical discipline recommends that BTG test multiple dose levels of (b) (4), in order to identify a tolerable level that would qualify the safety of this agent for

intravenous use. Our recommendation is for BTG to test one dose level at the amount of [redacted] (b) (4), that would be expected in a dose of Voraxaze if it contains the upper acceptance limit, plus at least one dose lower and potentially one dose higher than this amount. We also recommend that BTG include dose groups that receive Voraxaze plus additional [redacted] (b) (4) equal to the amounts used in the [redacted] (b) (4) arms alone to evaluate whether the presence of glucarpidase alters the safety of [redacted] (b) (4). Repeat dosing is not necessary to qualify the safety of [redacted] (b) (4) as Voraxaze is indicated for single use. The nonclinical discipline also recommends that the study endpoints include clinical observations, clinical chemistry parameters and histopathology. We recommend that necropsies be performed at the end of the 14 day observation period, and potentially within 24 to 72 hours after dosing if clinical observations indicate that the dosing is not tolerated.

MEMORANDUM

TO: The file
CC: Patricia Keegan, M.D., Director, Division of Oncology Products-2, Office of Hematology and Oncology Products (OHOP), Center for Drug Evaluation and Research (CDER)
John K. Leighton, Ph.D., D.A.B.T., Director, Division of Hematology and Oncology Toxicology (DHOT), OHOP, CDER
FROM: Anne M. Pilaro, Ph.D., Supervisory Toxicologist, DHOT, OHOP, CDER
BLA #: 125327/000
SPONSOR: BTG International, Inc.
PRODUCT: Voraxaze® (glucarpidase; carboxypeptidase-G2 enzyme)
SUBMISSION TYPE: original BLA application
DATE: December 22, 2011

Dec 22, 2011

SYNOPSIS:
BTG International, Inc. (BTG) has submitted an original biologics licensing application (BLA) for their recombinant, carboxypeptidase-G2 enzyme (glucarpidase, Voraxaze®) initially cloned from *Pseudomonas* strain Rs-16, and expressed in *Escherichia coli*. Voraxaze® is indicated "for the (b) (4) reduction of toxic methotrexate concentrations due to impaired renal function."[1]

Glucarpidase is an enzyme that catalyzes the hydrolysis of the carboxyl terminal glutamate residue from folic acid, and its analog methotrexate. Methotrexate (MTX) is an anti-metabolite drug used in the chemotherapy of various adult and pediatric cancers and in adult patients as a treatment for rheumatoid arthritis or severe psoriasis. Methotrexate inhibits dihydrofolate reductase (DHFR), the enzyme responsible for conversion of folic acid and other dihydrofolate derivatives to tetrahydrofolates, which are essential cofactors in the synthesis of purine nucleotides and thymidylate. Inhibition of DHFR activity by MTX results in impaired DNA synthesis, repair and cellular replication, and in neoplastic tissues can in turn lead to decreased tumor growth and survival.

Renal excretion (via glomerular filtration and active tubular secretion) is the primary route of elimination of MTX, and is dependent upon the dosage and route of administration. Within 24 hours following intravenous MTX dosing, approximately 80 to 90% of the MTX dose is excreted unchanged in the urine. However, renal clearance of MTX is nonlinear due to saturation of renal tubular reabsorption, and delayed drug clearance has been identified as a major factor responsible for MTX toxicity. If elimination of MTX is impaired, *e.g.* due to delayed renal clearance, MTX plasma concentrations can remain elevated for prolonged periods of time and result in significant organ toxicity including severe and sometimes fatal skin reactions, bone marrow suppression, aplastic anemia, gastrointestinal toxicity, hepatotoxicity, fibrosis and cirrhosis, acute or chronic interstitial pneumonitis and tumor lysis syndrome. The morbidity associated with impaired MTX clearance is substantial, and these toxicities as well as the effects of delayed renal clearance of MTX are identified as part of the black-

[1] From the indication statement in the current, draft labeling language for Voraxaze®

box warning in the current labeling for methotrexate.[2]

Voraxase® (glucarpidase) is indicated specifically for reduction of toxic plasma MTX levels due to impaired renal clearance. Glucarpidase hydrolyzes MTX and its active metabolite, 7-hydroxymethotrexate into the inactive metabolites, 4-deoxy-4-amino-N10-methylpteroic acid (also known as 2,4-diamino- N10-methylpteroic acid, or DAMPA) and glutamate. Glucarpidase treatment of patients with impaired renal clearance, or following overdose of MTX provides an alternative pathway for clearance of toxic MTX levels, and therefore may function as a rescue agent to ameliorate MTX toxicity. In the current BLA, the Applicant has provided clinical safety and efficacy data that demonstrate rapid and sustained reduction of plasma MTX levels by greater than 90% within 15 minutes of Voraxaze® dosing, resulting in decreased MTX toxicity to patients with impaired renal function. (b) (4)

Nonclinical studies investigating the pharmacology, pharmacokinetics and toxicology of glucarpidase in mice, rats, rabbits, beagle dogs and Rhesus monkeys in support of the safety of Voraxaze® for the proposed indication were reviewed by the primary reviewer, M. Stacey Ricci, Sc.D., and are briefly summarized in the "Executive Summary" and "Integrated Summary and Safety Evaluation" sections of her review. Dr. Ricci's review of BTG's nonclinical information submitted to the BLA consists of her evaluation of completed nonclinical pharmacology, pharmacokinetics, safety pharmacology, and repeat-dose toxicology studies with glucarpidase or the inactive MTX metabolite DAMPA, combined with a review of several published glucarpidase pharmacology studies. Based on the nonclinical safety data submitted in this BLA, Dr. Ricci has recommended approval of Voraxaze® for the specific indication sought by the Applicant. Additionally, based on anticipated clinical use of Voraxase® to treat inadvertent intrathecal MTX overdose, Dr. Ricci has recommended that the Applicant conduct an animal study as a post-marketing requirement (PMR) to establish the safety and efficacy of Voraxaze administered by the intrathecal route, under conditions of the "Animal Rule" (21 CFR 601.90 for biological products), in order to assess the risks and clinical benefits of its use to treat intrathecal MTX overdose in humans. Draft language to convey this PMR to the Applicant is included as Appendix 1 of Dr. Ricci's review.

The pharmacology of glucarpidase was evaluated by *in vivo* testing of its ability to rescue high-dose (HD) MTX-induced toxicity in mice, rabbits and Rhesus monkeys. Because glucarpidase catabolism of MTX produces DAMPA, the safety pharmacology of DAMPA as a single agent was also evaluated. Results from safety pharmacology studies using isolated rabbit hearts or telemeterized dogs exposed to DAMPA did not demonstrate adverse cardiovascular effects, and indicated that rapid elevation of DAMPA levels (an expected effect following glucarpidase treatment of patients with toxic plasma levels of MTX) were well tolerated in the dog. Nonclinical pharmacology studies in mice showed that following toxic doses of MTX, glucarpidase treatment in addition to leucovorin rescue of MTX toxicity did not demonstrate clear, overall improvement in systemic MTX toxicities when compared to MTX treated mice receiving

[2] Summarized from the current labeling for methotrexate, as of December 20, 2011 (available at http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/011719s117lbl.pdf).

LV rescue alone. Glucarpidase treatment of intrathecal HD MTX overdose was also evaluated in both rabbits and monkeys. Rabbits did not respond well to intrathecal glucarpidase treatments, but results in monkeys demonstrated that treatment with glucarpidase alone reduced cerebrospinal fluid MTX concentrations, and support the possible use of glucarpidase treatment of intrathecal MTX overdose.

No treatment-related toxicities were identified in a 3-day, repeat-dose toxicology study conducted in rats treated daily with 50 or 5000 Units/kg glucarpidase. The major toxicity observed in dogs treated every other day for 14 days with 50, 500 or 2500 Units/kg glucarpidase was mortality of undetermined cause, although in some early decedent animals mortality was preceded by adverse clinical signs and changes in hematology and clinical chemistry parameters. Dosing was terminated for the 500 and 2500 Units/kg dose groups after the sixth dose. In the surviving animals, glucarpidase treatment was well-tolerated, with no severe or unanticipated toxicities; however, glucarpidase systemic exposures diminished by Day 13. Anti-glucarpidase antibodies were detected in all surviving glucarpidase-treated animals and potential neutralizing effects or accelerated, antibody-mediated clearance may have contributed to reduced exposures. According to Dr. Ricci's review, the toxicities observed following 6 repeat doses of 500 or 2500 Units/kg of glucarpidase in dogs do not raise safety concerns for the proposed use of Voraxaze® in the clinical setting, as the labeled Voraxaze® dosing regimen will be a single dose of 50 Units/kg (b) (4)

Comment: Comparison of the doses tested in the animal studies to the doses of Voraxaze® tested clinically is not possible. Only limited toxicokinetic data were available from the 14-day repeat-dose toxicology study in dogs; in other toxicity studies serum samples for toxicokinetic evaluation were not collected, or were collected but not evaluated. Additionally, anti-glucarpidase antibodies were detected in the surviving dogs in the repeat-dose toxicology study, suggesting that the exposures that were calculated under-represent the anticipated clinical exposure. Finally, pharmacokinetic data are only available from a limited number of glucarpidase-treated patients (approximate total of 7 subjects), and do not provide sufficient exposure data for comparison with the nonclinical findings.

There were no nonclinical genotoxicity or carcinogenicity studies performed with glucarpidase, as per the guidance provided in ICH S6 *"Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals"* and ICH S9 *"Nonclinical Safety Evaluation for Anticancer Pharmaceuticals."* Nonclinical studies to evaluate the potential effects of glucarpidase on teratogenicity, effects on fertility and reproductive function, and effects on late pregnancy, delivery and juvenile development were not submitted in this BLA. Voraxaze® will be labeled as Pregnancy Category C and the following language (*i.e.* from 21 CFR 201.57.b.9.i.A.3), with concurrence from the Maternal Health Team in CDER's Office of New Drugs, will be used for labeling:

> *"Pregnancy category C.*
> *There are no adequate and well controlled studies with VORAXAZE in pregnant women and animal reproduction studies have not been conducted with VORAXAZE. Therefore, it is not known whether VORAXAZE can cause fetal*

harm when administered to a pregnant woman. VORAXAZE should be given to a pregnant woman only if clearly needed."[3]

The full range of reproductive and developmental toxicity studies, as outlined in the ICH S5(R2) Guidance: *"Detection of Toxicity to Reproduction for Medicinal Products and Toxicity to Male Fertility"* will not be required to support approval of Voraxaze® for the indication sought by the Applicant. Dr. Ricci presents BTG's rationale and her agreement for not conducting these studies in **Section 9: Reproductive and Developmental Toxicity Studies** of her review. The main reasons that the full battery of reproductive and developmental toxicity studies will not be required for Voraxaze® are 1) that the enzyme will be dosed only to patients who are receiving HD MTX for a serious and life-threatening disease, and the clinical benefit will outweigh any potential risk; 2) MTX is genotoxic and teratogenic itself and is contraindicated for use in pregnancy, so the likelihood for the need for glucarpidase treatment is low; 3) glucarpidase is a protein and is cleared from systemic circulation within hours of dosing, so exposures will be relatively low, and 4) the indication for Voraxaze® will be for only a single administration. I concur with Dr. Ricci's assessment and BTG's rationale that nonclinical developmental and reproductive toxicity testing are not necessary to support the approval of Voraxaze® for its indicated use, and that the appropriate language has been added to the labeling to indicate that glucarpidase should only be used in pregnant patients when the expected benefit is likely to outweigh the risk to the fetus.

In summary, the nonclinical studies provided by BTG in support of this BLA showed that glucarpidase was generally well-tolerated in animals. Minor changes in hematology and clinical chemistry parameters, and mortality at relatively high doses were reported. The major clinical toxicities reported with Voraxaze® are well documented and include hypersensitivity reactions, nausea and/or vomiting, paresthesias, flushing, hypotension and headache, which were not predicted by the nonclinical studies. Therefore, together with the known clinical safety record, the nonclinical studies submitted are adequate to conclude that Voraxaze® is reasonably safe for use in patients who have toxic plasma methotrexate concentrations due to impaired renal clearance. BTG will not be required to conduct additional nonclinical genotoxicity, carcinogenicity, fertility, or embryofetal developmental or peri-postnatal reproductive toxicity studies. The Applicant will, however, be required to conduct a nonclinical safety and efficacy study with Voraxaze® administered by intrathecal injection as treatment for intrathecal methotrexate overdose, and provide the results from this study as a post-approval supplement to the BLA.

Recommendation: In summary, I concur with Dr. Ricci's conclusions regarding the nonclinical findings for Voraxaze® and her current recommendations that the licensing application be approved for marketing for its proposed indication. I also concur with the proposed post-marketing requirement for BTG to conduct an additional nonclinical safety and efficacy study, to obtain data to convey the risks of intrathecal Voraxaze® use as a rescue therapy for intrathecal MTX overdose to patients and prescribing physicians.

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[3] From *Section 8 USE IN SPECIFIC POPULATIONS, Section 8.1 Pregnancy* in the current, draft labeling language for Voraxaze®

A copy of Dr. Ricci's review, with supervisory sign-off, has been conveyed to the regulatory project manager for inclusion in the final action package.

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

PHARMACOLOGY and TOXICOLOGY BLA REVIEW and EVALUATION

Application number:	STN 125327
Supporting document/s:	0005 (DATS# 60013373)
Applicant's letter date:	July 18, 2011
CDER stamp date:	July 18, 2011
Product:	Voraxaze® (glucarpidase)
Indication:	For the (b) (4) reduction of toxic methotrexate concentrations due to impaired renal function
Applicant:	BTG International Inc.
Review Division:	Division of Hematology and Oncology Toxicology (for Division of Oncology Products-2) Office of Hematology and Oncology Products
Reviewer:	M. Stacey Ricci, Sc.D. MSR 12/20/11
Supervisor/Team Leader:	Anne M. Pilaro, Ph.D. Dec 21, 2011
Division Director:	John K. Leighton, Ph.D., D.A.B.T. (DHOT) Patricia Keegan, M.D. (DOP-2)
Project Manager:	Erik Laughner, M.S.

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of STN BLA 125327 are owned by BTG International Inc. or are data for which BTG International Inc. has obtained a written right of reference. Any information or data necessary for approval of STN BLA 125327 that BTG International 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 reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of STN BLA 125327.

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Reviewer:	M. Stacey Ricci, Sc.D.
Supervisor/Team Leader:	Anne M. Pilaro, Ph.D.
Division Director:	John K. Leighton, Ph.D., D.A.B.T. (DHOT) Patricia Keegan, M.D. (DOP-2)
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1 Executive Summary

1.1 Introduction

Voraxaze® (glucarpidase) acts to decrease toxic levels of methotrexate (MTX) in plasma and cerebrospinal fluids, and therefore may function as a rescue agent to ameliorate MTX toxicity resulting from either overdose or impaired MTX clearance. Glucarpidase belongs to the carboxypeptidase class of enzymes that hydrolyze the carboxyl terminal glutamate residue from folic acid, a vitamin required for cellular replication. Glucarpidase also hydrolyzes and inactivates many analogues of folic acid, including MTX (Figure1; from BLA Section 2.4 Nonclinical Overview, p. 4). Glucarpidase hydrolyzes MTX and its active metabolite, 7-hydroxymethotrexate, into the inactive metabolites, 4-deoxy-4-amino-N10-methylpteroic acid (also known as 2,4-diamino- N10-methylpteroic acid, or DAMPA) and glutamate. Glucarpidase is a recombinant form of carboxypeptidase-G2 that was originally cloned from *Pseudomonas* strain Rs-16[1].

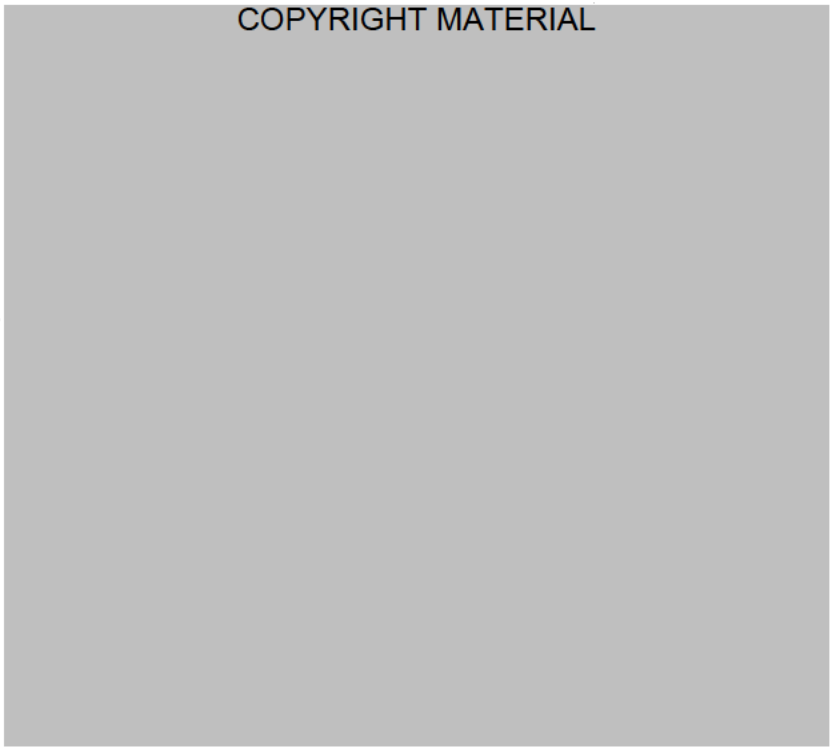


Figure 1 Glucarpidase Enzymatic Activity

Methotrexate is an anti-metabolite that has been used for the treatment of malignancies in pediatric and adult patients since the 1950s. The mechanism of action of MTX is to competitively inhibit dihydrofolate reductase (DHFR), the intracellular enzyme responsible for converting folic acid to reduced folate cofactors, which are necessary for deoxyribonucleic acid (DNA) synthesis. Administration of high dose methotrexate

[1] Sherwood, RF, et al. 1985. *Eur J Biochem*, **148**: 447-453.

(HDMTX; defined as clinical doses greater than 1 g/m^2) is recommended treatment for a number of human malignancies. In patients receiving HDMTX, high concentrations of MTX accumulate in the urine, as the clearance is predominantly renal. To mitigate potential toxicity, HDMTX therapy is always accompanied by vigorous intravenous (IV) hydration and alkalinization of the urine. This can sometimes prove inadequate and results in decreased urinary pH and intra-renal precipitation of MTX and its metabolites, which in turn can lead to renal impairment and delayed MTX elimination. When MTX elimination is delayed, the continued circulation of high concentrations of MTX frequently results in serious toxicities including myelosuppression, severe mucositis, acute hepatitis and further nephrotoxicity.

Measures that are routinely employed to reduce MTX toxicity include leucovorin treatment and additional hydration and urinary alkalinization. Leucovorin is a chemically reduced derivative of folic acid that is used as an antidote to HDMTX toxicity. Leucovorin is readily converted to reduced folic acid derivatives, and has cofactor activity that is equivalent to folic acid. Because it does not require the action of DHFR for its conversion to folic acid derivatives, leucovorin function is unaffected by MTX inhibition of DHFR. Leucovorin does not reduce the amount of circulating MTX, and may not compete effectively with MTX for transport into cells when MTX plasma or other body fluid levels remain high.

The proposed indication for Voraxaze is the (b) (4) reduction of toxic MTX levels resulting from impaired renal function. The recommended dose of Voraxaze is a single intravenous injection of 50 Units/kg of body weight, (b) (4)

1.2 Brief Discussion of Nonclinical Findings

Glucarpidase pharmacology and toxicology was evaluated predominantly using *in vivo* analyses. Animal species used were mice, rats, rabbits, dogs and Rhesus monkeys. Because glucarpidase catabolism of MTX results in elevated DAMPA levels, BTG also evaluated the safety and pharmacokinetics of DAMPA. The applicant provided pharmacology and toxicology data in the form of original research reports from studies conducted by contract research labs or by the National Cancer Institute (National Institute of Health, USA), and published, peer-reviewed journal articles from research conducted at academic institutions.[2]

The use of carboxypeptidase G1 (CPG1) to treat MTX toxicity was first examined in the 1970s. Because clinical data were available for CPG1 that suggested that enzymatic degradation of MTX offered a potential treatment option for HDMTX-related renal toxicity, initial *in vitro* pharmacology studies using glucarpidase (CPG2) were not conducted prior to submission of the original IND. Subsequent *in vitro* analysis of

[2]A tabular listing of the lots of glucarpidase used for the nonclinical and clinical studies is provided as an appendix.

glucarpidase activity in human plasma characterized the ability of glucarpidase to hydrolyze MTX, leucovorin (LV) and the active metabolite of LV, 5-methyltetrahydrofolic acid (5-MeTHF).

Nonclinical proof-of-concept studies involved administration of MTX to animals followed by glucarpidase. Two rescue pharmacology studies in mice evaluated LV rescue of MTX-induced toxicity with or without glucarpidase. The addition of glucarpidase to LV alone improved some MTX-induced tissue damage, but there was not a clear, overall benefit from adding glucarpidase to LV rescue over LV alone. Glucarpidase rescue of intrathecal HDMTX overdose was also evaluated in both rabbits and monkeys. Rabbits did not respond well to intrathecal treatments, but results in monkeys demonstrated that treatment with glucarpidase alone reduced cerebrospinal fluid MTX concentrations[3], and support the possible use of glucarpidase treatment of intrathecal MTX overdose.

Safety pharmacology studies were conducted to evaluate DAMPA toxicity using dogs and isolated rabbit hearts. In telemeterized dogs, treatment with DAMPA elicited minimal effects on blood pressure and heart rate, or on ECG intervals and waveform rhythms. Clinical signs in the dog included changes to the sclera of the eye after the high dose administration, and reddening and/or swelling of the muzzle. In isolated rabbit hearts, DAMPA treatment *in vitro* did not cause any cardiovascular effects that appeared to be treatment-related.

The toxicology program included a 3-day repeat-dose toxicology study in the rat, and a 14-day repeat dose toxicology study in the dog. A dose-escalation study was conducted in the dog preceding conduct of the repeat dose dog study. Glucarpidase was well-tolerated in the rat when administered daily by the IV route for 3 days at doses of 50 or 5000 Units/kg. Single glucarpidase doses of 50, 500, 1000, 2500, 3750 or 5000 Units/kg were evaluated in the dog dose-escalation study. Glucarpidase doses less than or equal to 2500 Units/kg were well-tolerated. Changes in clinical signs, clinical chemistry and hematology parameters that suggested damage to the hepatic and/or renal systems were observed at doses greater than 2500 Units/kg, but tissues were not collected so correlation of the clinical pathology findings with histomorphologic changes was not possible.

In the 14-day repeat-dose study, dogs received 50, 500 or 2500 Units glucarpidase/kg every other day for up to 14 days. Seven early deaths occurred in dogs receiving 500 or 2500 Units/kg. The cause of death in these animals was not determined after microscopic evaluation of the tissues by the study pathologist. Clinical signs in the early decedent animals included central nervous system effects (ataxia, prostration), gastrointestinal system effects (red discharge from anogenital region, soft stool, vomitus, red material in pan/bedding), red discoloration of ears or entire body, labored breathing, and excessive salivation. Liver transaminases were markedly elevated in these animals, although no corresponding lesions in liver were found microscopically. Some of these effects could be the result of prolonged folic acid depletion and might

[3] Adamson *et al, J Clin Oncol.* 1991; 9(4):670-4.

represent an exaggerated pharmacological effect of glucarpidase. Effects could also be consistent with an immunogenic response to glucarpidase in the dog. Anti-glucarpidase antibodies were detected in all glucarpidase-treated animals by Study Day 15. Platelets decreased significantly in a dose-dependent manner on Day 12 in all treated males, but not in females. Toxicokinetic evaluation showed that detectable concentrations of glucarpidase were present in plasma at all doses following the first dose, and that the AUC increased in a supra-proportional manner from the 50 to 500 Units/kg doses and in a dose-proportional manner from 500 to 2500 Units/kg. Lack of data due to early deaths prevented a statistical analysis of the PK data at the 13-day time point, but the few available data points suggested that there was no accumulation of glucarpidase with repeat dosing.

No genetic toxicology, carcinogenicity or reproductive and developmental toxicology studies were conducted using glucarpidase.

1.3 Recommendations

1.3.1 Approvability: Yes, from the nonclinical perspective.

1.3.2 Additional Nonclinical Recommendations

FDA is asking BTG International to conduct an animal study as a post-marketing requirement (PMR) to establish the safety and efficacy of Voraxaze administered by the intrathecal route, based on its expected use to treat accidental intrathecal methotrexate (IT MTX) overdose. The occurrence of accidental IT MTX overdose is infrequent; however, the consequences can be fatal and there are no approved intrathecal rescue agents for IT MTX overdose. The safety and efficacy of intravenous Voraxaze as a rescue agent for toxic plasma MTX concentrations has been established with the present approval of this BLA submission. Intrathecal use of Voraxaze is expected to provide additional therapeutic benefit to the existing standard of care for IT MTX overdose. BTG International provided clinical information from 9 subjects who received investigational glucarpidase to reduce methotrexate levels in cerebrospinal fluid following IT MTX overdose. This animal study is being asked for as a PMR because the number of accidental IT MTX overdose cases constitutes a small subset of patients that experience methotrexate overdose, and because it would be unethical to conduct a placebo-controlled clinical trial to establish the safety and efficacy of intrathecal Voraxaze in this setting. Therefore, BTG International must evaluate intrathecal administration of Voraxaze under conditions of the "Animal Rule" (21 CFR 601.90 for biological products) in order to assess the risks and benefits of its use to treat IT MTX overdose. Safety and efficacy data derived from the evaluation of intrathecal Voraxaze use in cynomolgus monkeys will be included in future product labeling and will inform clinical decisions about the timing and dosage of intrathecal Voraxaze following accidental IT MTX overdose. Draft language that will be sent to BTG International in a possible approval letter is included as Appendix 1 to this review.

1.3.3 Labeling

At present, there are no nonclinical findings to be included in the draft labeling. Following completion of the nonclinical safety and efficacy study evaluating the intrathecal use of Voraxaze and review of the resulting data by the FDA, information regarding intrathecal Voraxaze use will be incorporated into labeling.

2 Drug Information

2.1 Drug

CAS Registry Number:
9074-87-7

Generic Name:
glucarpidase

Code Name(s):
CPG2, CG2, recombinant carboxypeptidase G2, glutamyl carboxypeptidase, N-pteroyl-L-glutamate hydrolase

Chemical Name:
carboxypeptidase G2

Molecular Formula/Molecular Weight:
$C_{1950}H_{3157}N_{543}O_{599}S_7$

Glucarpidase (Voraxaze) has a subunit molecular mass of 41,440 daltons and a dimeric molecular weight of 83 kDa.

Structure or Biochemical Description:
Voraxaze is a zinc-dependent exopeptidase enzyme with co-catalytic zinc ion centers and a conserved aminopeptidase fold. The theoretical amino acid sequence of 390 residues (b) (4)

Pharmacologic Class:
Carboxypeptidase

2.2 Relevant INDs, NDAs, BLAs and DMFs

The clinical development of glucarpidase in the United States (US) began in 1993. It was supplied on a compassionate-use basis by the US National Cancer Institute (NCI, Bethesda, Maryland) under BB-IND 4663. The manufacturer of the initial clinical supply of glucarpidase was the Centre for Applied Microbiology and Research (CAMR; Salisbury, United Kingdom [UK]). In 2002, glucarpidase was acquired by Enact Pharma (Salisbury, UK) and then by Protherics (London, UK & Brentwood, TN) in 2003.

Protherics transferred manufacture of the drug substance to Eurogentec SA (Belgium) and of the drug product to Cangene Corporation (Canada) using a re-derived Master Cell Bank. In June 2004, the NCI began supplying glucarpidase manufactured by Protherics' contractors under BB-IND 11630. BTG International sponsors ongoing investigations of glucarpidase under BB-IND 11557.

2.3 Drug Formulation

Glucarpidase is produced in genetically engineered *Escherichia coli* cells (strain RV308). Voraxaze is supplied as a sterile, freeze-dried preparation in 3 ml USP Type 1 glass vials. Each vial contains a nominal 1000 Units of glucarpidase, intended for injection after reconstitution with 1 ml sterile normal saline. A unit of glucarpidase activity (U) is defined as the quantity of enzyme needed to catalyze the hydrolysis of one micromole of methotrexate in one mL of the reaction mixture at 37°C in one minute.

The composition of each vial of Voraxaze is shown in Table 1 (copied from Section 2.3.P, p.1 of the BLA):

Table 1 Voraxaze Drug Formulation Composition (per vial)

Ingredient	Function	Reference	Quantity
Glucarpidase	Active	In-house	1000 Units
Lactose monohydrate	(b) (4)	Ph. Eur. / NF	10 mg (1%)
Tris-HCl (b) (4)		Tris(hydroxymethyl)aminomethane Ph. Eur. / USP	(b) (4)
Zn^{2+}		Zinc Acetate dihydrate (Ph. Eur. / USP)	

2.4 Comments on Novel Excipients

There are no novel excipients.

2.5 Comments on Impurities/Degradants of Concern

There are no impurities or degradants of concern.

2.6 Proposed Clinical Population and Dosing Regimen

Voraxaze is indicated for the treatment of toxic plasma methotrexate concentrations due to impaired renal function. Voraxaze is administered as a single intravenous injection of 50 Units/kg. (b) (4)

2.7 Regulatory Background

Refer to Section 2.2 of this review.

3 Studies Submitted

3.1 Studies Reviewed

The following tables list the original study reports submitted in the BLA.

Table 2 Primary Pharmacodynamic Studies

Title	Study Number	GLP Compliance	Report Date
Methotrexate Toxicity Determination in C57BL/6N Mice	PR001-NCL-TX004	No	June 28, 2005
Amelioration of the Toxic Effects of Methotrexate with Leucovorin or Voraxaze in C57BL/6N mice	PR001-NCL-IN001	No	Sept. 19, 2005
Rescue Pharmacology Study in Mice (*reviewer note: study has no title*)	PR001-NCL-PC002	No	Jan. 12, 2007
An Investigation of the Safety of CPG2 and DAMPA and the Effects of CPG2 on Methotrexate Levels in Rabbits Treated Intrathecally	PR001-NCL-PK003	No	July 1, 2005
Plasma Pharmacokinetics of Methotrexate in Rhesus Monkeys After Varying Doses of Glucarpidase	PR001-NCL-PK002	No	June 2005

Table 3 DAMPA Safety Pharmacology Studies

Title	Study Number	GLP Compliance	Report Date
An Examination of the Cardiovascular Effects of DAMPA and Methotrexate on the Isolated Heart of the Female Rabbit	PR001-NCL-SP001	Yes	Oct. 13, 2006
DAMPA Single Dose Pilot Toxicity/Pharmacokinetics Study by Intravenous Administration to Beagle Dogs Followed by a 7-Day Observation Period	PR001-NCL-PK005	Yes	Sept. 19, 2006
DAMPA Telemetric Evaluation of Cardiovascular Effects After Intravenous Administration in Four Conscious Beagle Dogs	PR001-NCL-PC001	Yes	Oct.18, 2006

Table 4 Pharmacokinetic Studies

Title	Study Number	GLP Compliance	Report Date
Pharmacokinetics of Voraxaze (Glucarpidase) Following a Single Intravenous Administration in the Male Rabbit	PR001-NCL-PK001	Yes	April 12, 2005
Voraxaze Pharmacokinetic Interaction Study in Female Rabbits	PR001-NCL-PK004	Yes	May 3, 2007
DAMPA Single Dose Pilot Toxicity/Pharmacokinetics Study by Intravenous Administration to Beagle Dogs Followed by a 7-Day Observation Period	PR001-NCL-PK005	No	Sept.19, 2006

Table 5 Toxicology Studies

Title	Study Number	GLP Compliance	Report Date
Dose Escalation Tolerance Study of Voraxaze in Beagle Dogs	PR001-NCL-TX001	No	Feb. 7, 2005
Fourteen-Day Repeated Dose Toxicity Study of Voraxaze Administered via Intravenous Injection to Beagle Dogs	PR001-NCL-TX002	Yes	May 20, 2005
Voraxaze (Carboxypeptidase G2): 3 Day Intravenous Administration Toxicity Study in the Rat with a 10 Day Recovery Period	1845-013	Yes	Mar. 9, 2004

3.2 Studies Not Reviewed

The following drug interaction study was not reviewed:

Title	Study Number
The Influence of Leucovorin on the Kinetics of the Degradation of Methotrexate by the Enzyme Voraxaze in Human Plasma	PR001-NCL-PK008

The following analytical methods and validation reports were not reviewed:

Title	Study Number
Qualification of an Analytical Procedure for Determination of Carboxypeptidase G2 Concentration in Rat Plasma by Measurement of Activity	1845-015
Validation of an Analytical Procedure for Determination of Voraxaze (Glucarpidase) in Rabbit Plasma by Measurement of Enzymatic Activity	1845-023
Validation of an Analytical Procedure for Determination of Voraxaze (Glucarpidase) in Dog Serum and Plasma.	1845-025
Validation of an High Performance Liquid Chromatographic Method for Measurement of Methotrexate and 2,4-Diaminio-N10-methylpteroic Acid (DAMPA) in Human Plasma	PTU 018
Validation of an Improved High Performance Liquid Chromatographic Method for Measurement of Methotrexate and 2,4-Diaminio-N10-methylpteroic Acid (DAMPA) in Human Plasma and Serum	PTU 030
Validation of an HPLC Method for Measurement of Methotrexate, DAMPA and 7-Hydroxy Methotrexate in Human Plasma and Serum	PTU 042
Validation of a Chiral HPLC Fluorescence Method for Measurement of (6R)-Leucovorin, (6S)-Leucovorin, (6R)-5-Methyltetrahydrofolic Acid and (6S)-5-Methyltetrahydrofolic Acid in Human Plasma	PTU 045
Validation of an HPLC Method for Measurement of Methotrexate, DAMPA and 7-Hydroxy Methotrexate in Dog Plasma	PTU 048

The following ADME studies were not reviewed:

Title	Study Number
Evaluation of the Potential Induction Effect of DAMPA on Cytochrome P450 (CYP)1A2, CYP2C9 and CYP3A Enzyme Activities in Freshly Isolated Human Hepatocytes	PR001-NCL-PK007
Investigation of the Potential Inhibitory Effect of DAMPA on Human Cytochrome P450 (CYP) Model Substrates	PR001-NCL-PK006

The following pharmacology peer-reviewed journal articles were not reviewed:

Title	Citation	Test System	Testing Facility
Enzymatic Cleavage of Methotrexate Provides A Method for Prevention of Drug Toxicity	Chabner *et al*, *Nature*. 1972; 29:395-7.	BDF1 Male Mice	Lab of Chemical Pharmacology NCI/NIH
Rescue of Experimental Intrathecal MTX Overdose with Glucarpidase	Adamson *et al*, 1991 *J Clin Oncol.*; 9(4):670-4.	Rhesus monkeys	Pediatric Branch NCI/NIH
Methotrexate Pharmacokinetics Following Administration of Recombinant Glucarpidase in Rhesus Monkeys	Adamson *et al*, 1992, *J Clin Oncol.*; 10(8):1359-64.	Rhesus monkeys	Pediatric Branch NCI/NIH
Pharmacokinetics and Metabolism of the MTX metabolite 2,4-Diamino-N^{10}-Methylpteroic Acid	Widemann *et al.*, 2000; *JPET.* 294:894-901	Rhesus monkeys and MOLT-4 *in vitro* cell line	Pediatric Oncology Branch (NCI/NIH)

3.3 Previous Reviews Referenced

No other reviews are referenced.

4 Pharmacology

4.1 Primary Pharmacology

Study title: Methotrexate Toxicity Determination in C57BL/6N Mice

Study no.: PR001-NCL-TX004
Study report location: BLA Section 4.2.1.1.1
Conducting laboratory and location: (b) (4)
Date of study initiation: February 16, 2005
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: MTX was supplied by a commercial source (the CoA was not provided)

Key Study Findings

Daily administration of MTX at doses of 250 or 500 mg/kg demonstrated severe toxicity after 3 days and was evidenced by marked weight loss, myelosuppression and lesions in the small intestine.

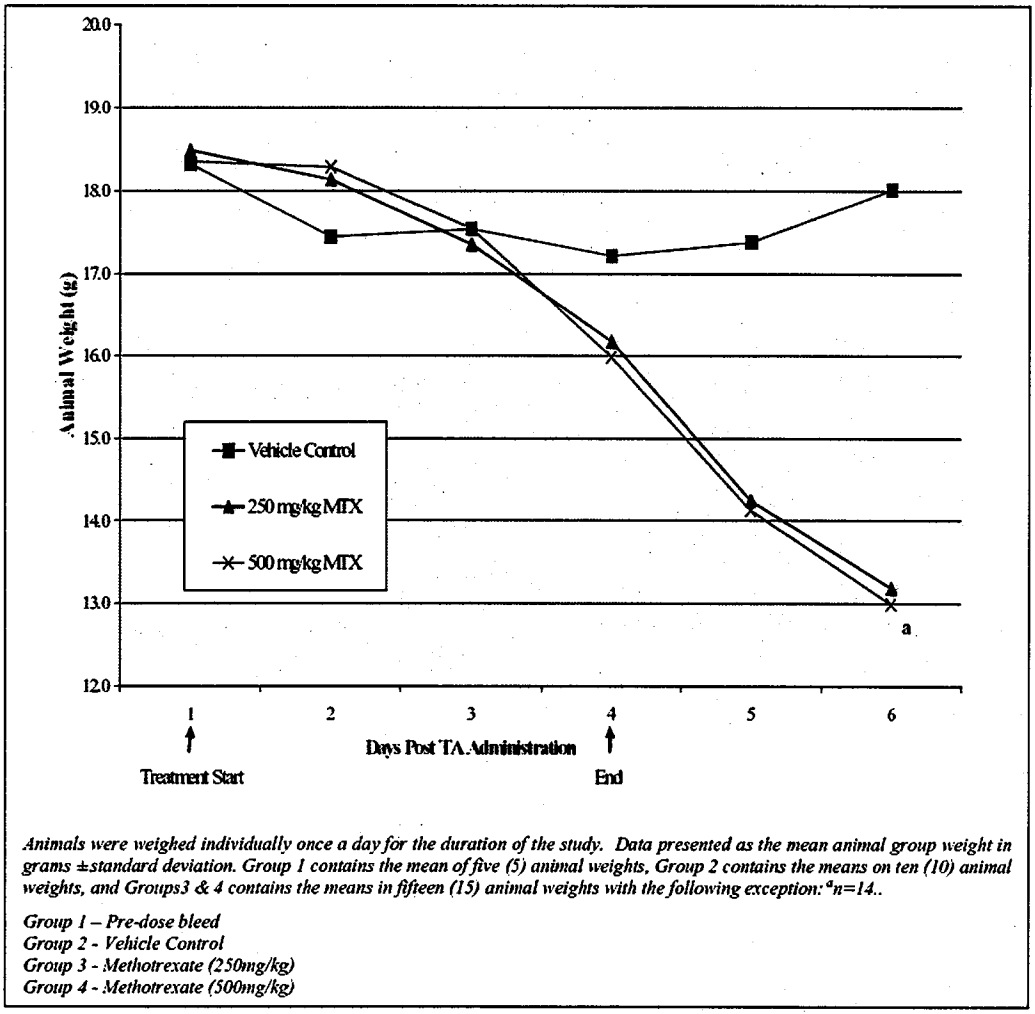
Methods

Doses:	0, 250, 500 mg/kg
Frequency of dosing:	MTX administration planned daily for 4 days
Route of administration:	Intraperitoneal (IP)
Dose volume:	Dose concentrations not provided
Formulation/Vehicle:	PBS
Species/Strain:	*Mus musculus* CRL: C57BL/6NCrlBR
Number/Sex/Group:	Females only used: 10/control; 15/MTX group
Age:	6-8 weeks
Weight:	No weight requirement, but mean body weight was approximately 18.4 in each group
Satellite groups:	5/pre-dose bleed
Deviation from study protocol:	MTX treatments were reduced from once daily for 5 days to once daily for 3 or 4 days, because of body weight losses of 15-20%

Observations and Results

Methotrexate was administered by IP injection to two groups of mice at two separate concentrations to reproduce HDMTX toxicity. There was a marked decline in mean body weight in mice from the MTX-treated groups, shown graphically below (copied from p.15 of the study report):

Figure 2 Body Weight in Mice Following MTX IP Administration (Study # PR001-NCL-TX004)



Animals were weighed individually once a day for the duration of the study. Data presented as the mean animal group weight in grams ±standard deviation. Group 1 contains the mean of five (5) animal weights, Group 2 contains the means on ten (10) animal weights, and Groups3 & 4 contains the means in fifteen (15) animal weights with the following exception: [a]n=14..

Group 1 – Pre-dose bleed
Group 2 - Vehicle Control
Group 3 - Methotrexate (250mg/kg)
Group 4 - Methotrexate (500mg/kg)

Mice in MTX-treatment groups began to show hunched posture, dehydration, rough hair coats and lethargy beginning on Day 5. However, treatments were stopped after the 3rd or 4th dose for all mice on study, because of the degree of weight loss. Mice continued to lose weight and were either found dead or euthanized by Day 7. Necropsies were performed on all mice (both scheduled and unscheduled mortalities).

- Tissues from early decedent mice were damaged by autolysis and not evaluable. Necropsies of surviving MTX-treated animals had gross findings of watery-like fluid in the gastrointestinal (GI) tract (clear, yellow or green in appearance), hepatic discoloration, enlarged gall bladders, reduced unilateral kidney size and bilateral renal pitting.
- Microscopic analysis of acceptable tissues indicated that MTX-treated mice had damage to epithelium of the GI tract (marked loss of villi and crypts, and cellular vacuolation). Lesions were less severe in the distal GI tract. Non-glandular mucosa exhibited minimal to mild hyperkeratosis.

- Hematology results showed decreases in both red and white blood cell parameters in MTX-treated animals when compared to controls.

Study title: Amelioration of the Toxic Effects of Methotrexate with Leucovorin or Voraxaze in C57BL/6N mice

Study no.:	PR001-NCL-IN001
Study report location:	BLA Section 4.2.1.1.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 16, 2005
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	- Glucarpidase, lot #2090302; purity not provided - MTX, from USP catalogue #1414003, Lot # I1D108; purity not provided - Leucovorin, Ben Venue Labs, lot and purity not provided

Key Study Findings

Methotrexate toxicity was evident in all treated groups (Groups 2, 3, 4) and included weight loss, myelosuppression, bone marrow hypocellularity and GI necrosis. Treatment with leucovorin (LV) ameliorated the intestinal necrosis observed, and the addition of glucarpidase added no clear benefit. Leucovorin alone improved MTX-induced myelosuppression, and glucarpidase gave additional benefit. Neither LV nor glucarpidase improved MTX-induced weight loss or bone marrow effects.

Methods

Doses:

Group	MTX	LV	Glucarpidase
1	0 (PBS)	-	-
2	500 mg/kg	-	-
3A	500 mg/kg	6.75 mg/kg	-
3B	500 mg/kg	6.75 mg/kg	-
4A	500 mg/kg	6.75 mg/kg	50 U/kg
4B	500 mg/kg	6.75 mg/kg	50 U/kg

Frequency of dosing: MTX or PBS: daily, Days 1-4
LV: twice daily (6h, 18h post-MTX) on Days 2-4
Glucarpidase: 30 min after final MTX dose on Day 4

Route of administration: MTX, LV and PBS: IP
Glucarpidase: IV

Species/Strain: *Mus musculus* CRL: C57BL/6NCrlBR

Number/Sex/Group: Group 1 & 2: 20 each
Group 3A & 4A: 15 each
Group 3B & 4B: 10 each
Subgroups A were submitted for anatomic and clinical pathology
Subgroups B were submitted for PK analysis

Age: 5-6 weeks

Weight: 15-20 g

Observations and Results

- Mice in all MTX-treated groups experienced weight loss. Treatment was terminated on Day 4 because mice had lost >20% of their initial body weight.
- Seven mice died unexpectedly (5 died in MTX arm only; 1 each in Groups 3 and 4) and four were euthanized moribund (2 each from Groups 3 and 4).
- The GI tract was most susceptible to MTX toxicity, and the most common lesion observed macroscopically was dilation of the small intestine. Incidence and severity of this GI finding was reduced with LV treatment alone and to a greater degree with LV plus glucarpidase treatment. A summary of the gross lesions observed is below (copied from p. 70 of the study report):

Table 6 Summary of Gross Lesions (Study #PR001-NCL-IN001)

Group Number	No gross lesions	GIT; Dilated	Spleen; reduced in size	Kidney; reduced in size	Colon; hemorrhagic	Liver; discoloration	Other*	DOA
1	20/20 (100%)	0/20 (0%)	0/20	0/20	0/20	0/20	0/20	0/20
2	2/16 (12.5%)	9/16 (56.3%)	1/16	1/16	0/16	0/16	1/16[a]	6/16
3A	5/14 (35.7%)	6/14 (42.9%)	5/14	2/14	0/14	1/14	1/14[a, b]	1/14
4A	7/15 (46.7%)	1/15 (6.7%)	1/15	0/15	2/15	2/15	1/15[b]	3/15

Data represented as a ratio of the number of mice with observed lesion over total mice.
DOA = dead on arrival *GIT = gastrointestinal tract*
Autolysis precluded meaningful evaluation. **Other = [a] enlarged gallbladder, [b] distended stomach*

- Histopathology Results:
  - Microscopic examination identified marked to severe necrosis of the epithelium of the small intestine that was most severe in the duodenum, and diminished progressively toward the anus. These findings were present in Group 2 (MTX only); a similar incidence of these findings, but less severe pathology was present in Group 3A and 4A animals.
  - Two mice in Group 2 had minimal to mild focal degeneration of the renal cortex; this was not observed in animals from Groups 3 or 4.
  - Three mice in Group 3A had mild to moderate multifocal hepatocellular lipidosis, and this was not observed in mice from Groups 2 or 4A.
  - Minimal to mild nephrosis, often accompanied by multifocal cortical mineralization, was observed in 7 of 15 Group 4A mice. The nephrosis was characterized by increased cytoplasmic vacuolation in the proximal tubules, with necrosis and mineralization of some affected cells and granular casts in distal tubules. The nephrosis was of a low severity, and considered by the study pathologist that it would not be expected to compromise renal function. Correlation with clinical chemistry results was not possible, as these data were not collected.
- Whole blood collection was incomplete on Day 8 because of early mortalities, particularly from Group 2. In the remaining animals, myelosuppression accompanied MTX treatment. Both RBC and WBC were lower in treated groups when compared to controls. In Group 4 mice there was an increase in basophils, eosinophils, monocytes and polymorphonucleated leukocytes when compared to Group 2 mice. The increase in these leukocytes was also greater than that observed in mice from Group 3.
- There were little to no serum chemistry results provided (i.e. only BUN and creatinine values for Group 1 females were provided in the report).

- Decreased bone marrow cellularity was observed in Group 2, 3 and 4 mice when compared to controls.

Study title: Rescue Pharmacology Study in Mice (*study has no title*)

Study no.: PR001-NCL-PC002
Study report location: BLA Section 4.2.1.1
Conducting laboratory and location: (b) (4)
Date of study initiation: September 7, 2006
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: Glucarpidase lot # not provided

Key Study Findings

Methotrexate-induced toxicity was assessed by monitoring body weights. The administration of LV or LV plus glucarpidase reduced MTX-induced weight loss, but the addition of glucarpidase showed no improvement over LV alone. Mortality was increased in MTX+LV (~60%) and MTX+LV+glucarpidase groups (~60%) when compared to MTX alone (~40%).

Methods

Doses:

Group	MTX	LV	Glucarpidase
1	5 mg/kg	-	-
2	5 mg/kg	2.5 mg/kg	-
3	5 mg/kg	2.5 mg/kg	50 U/kg

Frequency of dosing:

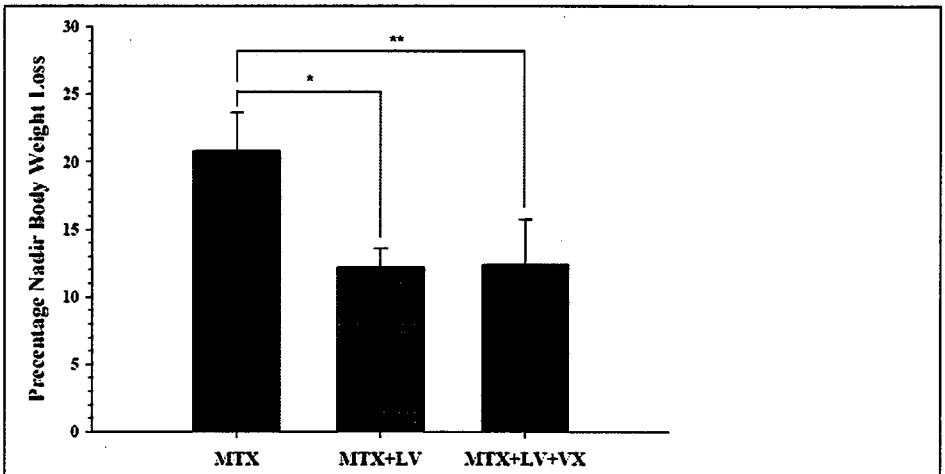
MTX	72 hours constant rate infusion
LV	32, 38, 44, 50, 56, 62, 68, 74, 80 and 86 hours after start of MTX
Glucarpidase	24 and 48 h after start of MTX

Route of administration: MTX: IV
LV: IP
Glucarpidase: IV
Dose volume: MTX: 0.07 mg/kg/hr
Species/Strain: Male Swiss Webster mice
Number/Sex/Group: 10-11/group
Age: Not stated
Weight: 22.2-28.9 g

Observations and Results

Individual body weight data were normalized to pre-treatment body weights, and mean percentage body weight losses were calculated per group. Animal mortality was the secondary measure of MTX-induced toxicity. Statistical analyses demonstrated that the combination of LV plus glucarpidase reduced the extent of MTX-induced weight loss, but there were no significant differences in body weights when comparing mice treated with LV or LV plus glucarpidase (below figure copied from p. 8 of the study report).

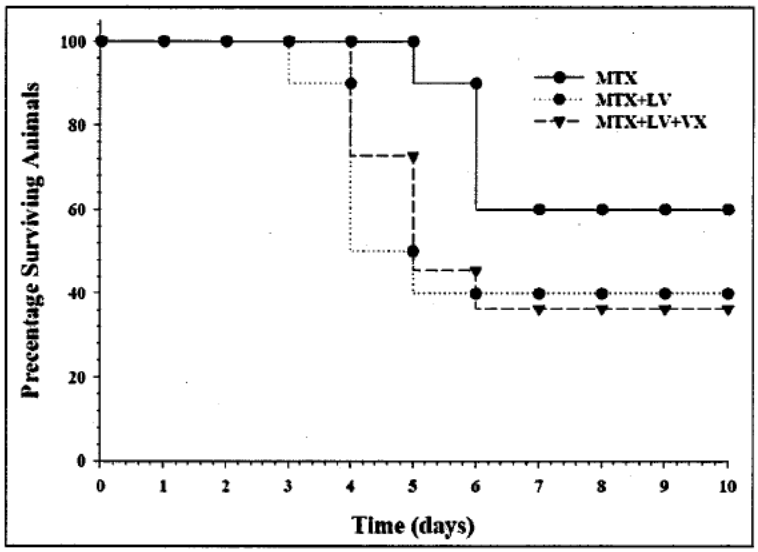
Figure 3 Maximum Weight Loss in Mice Following MTX, LV and Glucarpidase Treatment (Study # PR001-NCL-PC002)



**Note: "MTX" refers to group a (n=6), "MTX+LV" refers to group b (n=4), and "MTX+LV+VX" refers to group c (n=4).

Survival data was presented as the percentage of animals surviving in each group relative to the initiation of MTX infusion (copied from p. 9 of the study report):

Figure 4 Survival in Mice Following MTX, LV and Glucarpidase Treatment (Study # PR001-NCL-PC002)



Study title: An Investigation of the Safety of CPG2 and DAMPA and the Effects of CPG2 on Methotrexate Levels in Rabbits Treated Intrathecally.

Study no.:	PR001-NCL-PK003
Study report location:	BLA Section 4.2.1.1.1
Conducting laboratory and location:	(b) (4)

Background

This report was compiled by (b) (4) on behalf of Protherics. The report states "The study was not fully reported by the investigators but this report has been compiled on behalf of Protherics from the limited documentation that is available." The study was conducted by (b) (4) between January and September 1989. The study investigated the safety of glucarpidase and DAMPA when administered intrathecally to rabbits. This documentation included:

- A brief protocol
- A summary table
- Data sheets related to individual rabbits and their treatment

Methods

The study was conducted in two parts: Part 1 investigated the neurotoxicity of DAMPA and Part 2 examined the potential for glucarpidase to rescue rabbits from MTX toxicity. There was no description of the test articles or how they were formulated. There was no detailed description of the treatment procedure.

- Part 1:
  - Two groups of 4 rabbits received 15 mg DAMPA or 1.0 ml normal saline intrathecally via cisternal puncture. The dose of DAMPA was estimated to be equivalent to the amount of DAMPA produced by cleavage of the highest of MTX dose given to humans.
  - Animals were observed for 72 h for signs of neurotoxicity.
- Part 2:
  - Eight rabbits received 20 mg MTX intrathecally via cisternal puncture.
  - They were then divided into two groups of 4 animals each that received either 1 ml of normal saline or glucarpidase (the amount of glucarpidase is stated in the results table shown in Figure 5 below [copied from p. 3 of the study report] as Not Specified). The summary table and pathology reports indicated that 4 more animals also received glucarpidase alone and an additional 2 animals received MTX and 20 units glucarpidase.
  - Animals were observed for 72 h for signs of neurotoxicity.
  - Samples of brain, lung and heart were examined microscopically by (b) (4)

Observations and Results

Figure 5 Survival of Rabbits Following Intrathecal Dosing (Study # PR001-NCL-PK003)

Treatment Group	Surviving	Dead
Part 1		
Controls (Elliots B)	4	0
DAMPA	3	1
Part 2		
MTX + saline	1	3
MTX + CPG2 (dose NS)	3	1
CPG2	4	0
MTX + CPG2 (20 units)	0	2

NS = not specified

- One of the control animals from Part 1 experienced hypothermia that was treatable. Other changes in the controls were consistent with cardiac failure and with encephalitozoonosis, a protozoan infection. None of the animals died. The report compiler notes that the method of euthanasia was not specified. Therefore it is unknown whether cardiac findings were influenced by the anesthesia used because they were also seen in a control animal.
- One DAMPA-treated animal died approximately 5h after dosing. All the DAMPA treated animals experienced seizures and/or cardiac arrhythmias.
- All animals treated with MTX alone experienced seizures, and 3 died. Two had small foci of acute hemorrhage in the cerebellum, and 2 animals had pulmonary congestion.

- In the group treated with MTX and glucarpidase (dose Not Specified [NS]), 1 animal showed changes compatible with cardiac dysfunction and 1 animal had areas of severe necrosis of the intimal surface of a number of pulmonary arteries that were also associated with severe thrombosis.
- The animals treated with glucarpidase only had no remarkable effects noted.
- The two animals treated with MTX and 20 Units glucarpidase died, possibly as the result of severe cardiac dysfunction. In both animals there was congestion of the meninges of the cerebral cortex and cerebellum.
- Pathology reports indicated that tissue fixation was not completed satisfactorily for all animals' tissues.

This study's results are of questionable value because the supporting documentation is incomplete and the methodology was not properly documented.

Study title: Plasma Pharmacokinetics of MTX in Rhesus Monkeys After Varying Doses of Glucarpidase

Study no.:	PR001-NCL-PK002
Study report location:	BLA Section 4.2.1.1.1
Conducting laboratory and location:	(b) (4)

Background

This research study was conducted to evaluate the effects of varying doses of glucarpidase on plasma MTX concentrations in monkeys, and to determine the lowest dose of glucarpidase that resulted in a >90% decrease in plasma MTX within 15 minutes of enzyme administration. The work was presented at a meeting of the American Association for Cancer Research (AACR) and published as an abstract for the meeting proceedings in July 2003. This study report was compiled on behalf of Protherics from limited documentation that was available. This included:

- A brief protocol and abstract
- A summary table of analytical results
- Data sheets relating to individual monkeys and their treatment

Methods

- Six adult rhesus monkeys (*Macaca mulatta)* between 11 and 16 years old and weighing between 9.4 and 13.8 kg were used for this study.
- Dosing:
  - Animals were administered MTX to achieve steady state plasma levels of 10 microM. An IV loading dose of 300 mg/m^2 was given over 1 hour followed by continuous infusion of 60 mg/m^2/h for 4 hours.
  - Glucarpidase was given IV following completion of the MTX infusion. One animal was treated per dose level. The starting dose was 50 Units/kg; the next animal was dosed with 15 Units/kg, then the third monkey received 5 Units/kg glucarpidase. Three additional animals received 1 Unit/kg.

Glucarpidase was given over a period of 4 minutes (for the 50 Units/kg dose), or over 1 minute for all other dose levels.
  - Leucovorin was given intramuscularly starting 24 hours after the start of the MTX administration, then every 12 hours for four doses.
- Blood samples for PK were collected pre-MTX dosing, 2 and 3 hours after the start of MTX infusion, just prior to glucarpidase administration and then 5, 15, 30, 45, 60 minutes and 2 and 3 hours after injection of glucarpidase.
- Concentrations of MTX and DAMPA were measured using HPLC.

Observations and Results

- All but one animal tolerated the study drugs well without any observed clinical signs. The animal that received the highest dose of glucarpidase (50 Units/kg) became unresponsive 14 minutes after administration of glucarpidase. Supportive care measures were administered, and the animal was considered clinically normal the following morning. There were no differences in MTX or DAMPA levels in this animal compared to the others following glucarpidase treatment.
- Five minutes following glucarpidase treatment, there was an immediate drop in plasma MTX concentrations and a parallel increase in DAMPA concentrations. There did not appear to be a glucarpidase dose-related effect on MTX elimination (the tables below was copied from pp. 6-7 of the study report).

Table 7 Plasma Concentrations of MTX Before and up to 3 Hours After Administration of Glucarpidase (Study # PR001-NCL-PK002)

Time Point	MTX Concentration (uM)					
	Animal ID					
	9S6	88003	829A	15398p	9SL	D28*
	Dose of glucarpidase (units/kg)					
	1	1	1	5	15	50
Predose	ND	ND	ND	NA	ND	ND
2 h	25.35	19.63	17.24	28.09	22.51	27.79
3 h	19.87	19.75	15.70	25.35	24.71	22.48
5 h (EOI)	15.29	16.95	16.20[a]	19.87	14.61	20.70
EOI + 5 min	0.05	ND	0.06	ND	ND	ND
EOI + 15 min	ND	ND	ND	ND	ND	ND
EOI + 30 min	ND	ND	ND	ND	ND	ND
EOI + 45 min	ND	ND	ND	ND	ND	ND
EOI + 1 h	ND	ND	ND	ND	ND	ND
EOI + 2 h	ND	ND	ND	ND	ND	ND
EOI + 3 h	ND	ND	ND	ND	ND	ND

<LOQ indicates that MTX was detected below the LOQ (limit of quantitation 0.05 µM)
- Data missing, EOI – End of Infusion
ND = Not detected, NA = Not analysed, sample lost
a = Infusion ended 30 min early, due to rate 60 mL/h not 50 mL/h
* Adverse reaction 14 min after glucarpidase administration

Table 8 Plasma Concentrations of DAMPA Before and up to 3 Hours After Administration of Glucarpidase (Study # PR001-NCL-PK002)

Time Point	DAMPA Concentration (uM)					
	Animal ID					
	9S6	88003	829A	15398p	9SL	D28*
	Dose of glucarpidase (units/kg)					
	1	1	1	5	15	50
Predose	ND	ND	ND	NA	ND	ND
2 h	ND	ND	ND	ND	ND	ND
3 h	ND	ND	ND	ND	ND	ND
5 h (EOI)	ND	ND	ND[d]	ND	ND	ND
EOI + 5 min	4.9	9.6	4.4	3.0	3.2	10.8
EOI + 15 min	2.8	5.0	2.7	1.3[b]	2.0	5.0[a]
EOI+ 30 min	1.4	3. 7	1.5	1.6[c]	0.9	5.1
EOI + 45 min	0.9	2.7	1.8	1.2	0.9	3.6
EOI+ 1 h	0.7	2.3	0.9	0.8	0.6	3.7
EOI + 2 h	0.2	0.9	0.4	0.4	0.3	1.0
EOI + 3 h	0.1	ND	0.2	ND	0.2	0.8

<LOQ indicates that DAMPA was detected below the LOQ (limit of quantitation for 0.1 uM)
- Data missing, EOI – End of Infusion, ND not detected
a = late sample, EOI + 27 min, b = late sample, EOI + 24 min, c = late sample, EOI + 36 min
d = Infusion ended 30 min early, due to rate 60 mL/h instead of 50 mL/h,
* Adverse reaction 14 min after glucarpidase administration
ND = Not detected, NA = Not analysed, sample lost

4.3 Safety Pharmacology

Study title: An Examination of the Cardiovascular Effects of DAMPA and Methotrexate on the Isolated Heart of the Female Rabbit

Study no.: PR001-NCL-SP001
Study report location: BLA Section 4.2.1.3.1
Conducting laboratory and location: (b) (4)
Date of study initiation: June 15, 2006
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: DAMPA, lot # not provided, 97.8%; Methotrexate, lot # not provided, 99.4% Both test compounds were supplied by Sigma (St. Louis, MO)

Key Study Findings

- There were no adverse effects of DAMPA concentrations of ≤100 microM on hemodynamics, electrocardiography or electrophysiology. The data collected

from 250 microM-treated hearts were not useable because DAMPA precipitated out of the perfusion solution.

- At concentrations ≥50 microM, MTX was associated with alterations in cardiac electrical conduction and included atrioventricular dissociation, shortening of ventricular repolarization (decreased monophasic action potential duration [$MAPD_{90}$] and QT interval), and sporadic rhythm disturbances.

Methods

Doses:	Two test groups received the following escalating doses of either DAMPA or Methotrexate: 0, 2.5, 50, 100, 250 microM
Frequency of dosing:	Hearts were exposed to approximately 15 minute test periods of increasing test compound concentration
Route of administration:	Perfusion
Dose volume:	Volume added to 600 ml perfusion medium (in microL): 0, 17.2, 326, 344, and 1031 (DAMPA) or 1022 (MTX)
Formulation/Vehicle:	Both test compounds were dissolved in DMSO; final DMSO concentration was ≤0.3%
Species/Strain:	Rabbit/New Zealand White
Number/Sex/Group:	7 hearts (from female rabbits)/group
Age:	13-15 weeks old
Weight:	2.1-2.6 kg
Satellite groups:	n/a
Unique study design:	Isolated hearts were used rather than whole animals.* Rabbits were euthanized via stunning (method not specified), followed by cardiectomy. Hearts were rapidly removed, mounted on a Langendorff apparatus and perfused with buffer. Arteries were secured and hearts were paced with electrodes (for details see Methods section below).
Deviation from study protocol:	Results from the high concentration of 250 microM were removed because DAMPA precipitated out of solution.

* Hearts from female rabbits were used because of their increased susceptibility to develop Torsades de Pointes compared to those from male rabbits (citation provided by BTG)[4].

Methods

Hearts were rapidly removed, mounted on a Langendorff apparatus, and perfused with modified, oxygenated, Krebs-Henseleit buffer. A ventricular drain and fluid-filled latex

[4] Liu X-K, Wang W, Ebert SN, Franz MR, Katchman A, Woosley RL. 1999. Female gender is a risk factor for torsades de pointes in an *in vitro* animal model. J Cardiovasc Pharmacol., **34**:287-294.

balloon were secured in the left ventricle with a purse string suture at the atrial appendage. A pulmonary artery drain was secured. Hearts were paced via pacing electrodes placed into the right atrium. Hearts were deemed acceptable for use in the study if they exhibited acceptable hemodynamic, electrocardiographic, and electrophysiological parameters throughout the equilibration period.

The hearts were maintained at approximately 37°C while suspended in a glass chamber and bathed with Krebs-Henseleit buffer. The glass chamber had multiple ECG electrodes arranged on the wall in a simulated 'Einthoven' configuration. A monophasic action potential probe was placed in contact with the right ventricular endocardium and used to obtain intermittent monophasic action potential (MAP) recordings. For MAP measurements, only stable readings (i.e. with acceptable amplitude and MAP duration [MAPD]) were analyzed. The latex balloon in the left ventricle (LV) was expanded with water to achieve a LV end-diastolic pressure (LVEDP) of approximately 5 mmHg. The balloon was connected with tubing to a pressure transducer to measure LVEDP, LV diastolic pressure (LVDP) and LV systolic pressure (LVSP). Coronary perfusion pressure (CPP) was measured with a pressure transducer connected to a side-arm port of the aortic cannula.

Heart rate (HR), QT, QRS and PR intervals, as well as monophasic action potential duration 30%, 50% and 90% ($MAPD_{30}$, $MAPD_{50}$ and $MAPD_{90}$, respectively) were also determined to assess the effects of the test articles on cardiovascular electrophysiology. Simultaneous MAP and multiple lead volume-conducted ECGs were recorded. The hemodynamic variables of left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), left ventricular developed pressure (LVDP), +dP/dt, -dP/dt, and coronary perfusion pressure (CPP) were monitored throughout the protocol.

Individual hearts were equilibrated for approximately 10-15 minutes after which baseline measurements were collected for approximately 10 minutes. Hearts were then exposed to approximately 15 minute test periods of increasing concentrations of the test compound and measurements were collected continuously.

Table 9 Isolated Rabbit Heart Safety Pharmacology Study Design (Study # PR001-NCL-SP001)

Group	Test Compound	Test Compound Conc. (µM)	Equilibration Time at Each Concentration (minutes)	Stock Solution Concentration (mg/mL)	Volume of Stock Added to 600 mL Perfusion Medium (µL)	Number of Rabbit Hearts
1	DAMPA MW=343.56	0, 2.5, 50, 100, 250	15, 15, 15, 15, 15	30	0, 17.2, 326, 344, 1031	7
2	Methotrexate MW=454.4	0, 2.5, 50, 100, 250	15, 15, 15, 15, 15	40	0, 17.0, 324, 341, 1022	7

Observations and Results

DAMPA Results:

- Actual concentrations of DAMPA taken from perfusate samples were less than intended: i.e. 2.2, 43.6, 91.0 and 73.6 microM. Because the high dose concentration was so far below anticipated, these results from the high dose concentration used are invalid and not described.
- There were no notable effects on cardiac hemodynamics.
- There was a slight increase in the early and mid-phase times to ventricular repolarization.
- No other effects were noted.

Methotrexate Results:

- Actual MTX concentrations measured from perfusate samples were 2.7, 43.4, 92.1 and 236.8 microM.
- One heart experienced severe loss in cardiac function (decreased developed pressure, contractility, relaxation) at nominal MTX concentrations ≥50 microM. Notable effects on cardiac hemodynamics were not reported for any of the other hearts tested.
- A slight, statistically significant decrease in the time to late stage ventricular repolarization was noted at an MTX concentration of 250 microM. In addition, a concomitant, statistically significant decrease in QT interval was also noted (100, 250 microM MTX). There were no notable effects of MTX administration on PR interval or QRS durations at concentrations up to 250 microM.
- Atrioventricular dissociation occurred in 3/7 hearts at MTX concentrations ≥50 microM.

Study title: DAMPA Single Dose Pilot Toxicity/Pharmacokinetics Study by Intravenous Administration to Beagle Dogs Followed by a 7-Day Observation Period

Study no.:	PR001-NCL-PK005
Study report location:	BLA Section 4.2.1.3.1
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	May 25, 3002
GLP compliance:	Yes
QA statement:	No
Drug, lot #, and % purity:	DAMPA, Lot 17402BC (supplied from Sigma-Aldrich), 96.1%

Key Study Findings

- Administration of DAMPA by intravenous infusion at doses up to 60 mg/kg was well tolerated, and no systemic toxicity was observed.
- The plasma elimination half-life of DAMPA was 0.5-1 hour and clearance was between 0.60-0.70 L/h/kg.

Methods

Doses:	15, 30 or 60 mg/kg
Frequency of dosing:	Once, with a 7 day observation period
Route of administration:	Intravenous infusion lasting 15-30 min
Dose volume:	3, 4.5 or 9 ml/kg
Formulation/Vehicle:	0.1N NaOH adjusted to pH 8.5 using HCl
Species/Strain:	Beagle dog
Number/Sex/Group:	1/sex/group
Age:	23-27 weeks
Weight:	10.1-11.0 kg (males) and 7.8-9.2 (females)
Satellite groups:	None
Unique study design:	DAMPA is known to be of limited aqueous solubility and to have the potential to precipitate out of solution in environments of pH less than 7. To prevent kidney damage in the clinic, patients receive a hydration/alkalinization regimen. In this study, each animal received a hydration/alkalinization regimen consisting of IV infusion of 5% dextrose in water for irrigation containing 1.0 mEq $NaHCO_3$ 1 hour before DAMPA, and for at least 5 hours after DAMPA administration.

Animals were observed twice daily. Body weight and food consumption were recorded daily, and samples for TK were collected pre-dose and 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 hours after dosing. Blood gas/pH samples were collected approximately 45 minutes into the predose hydration phase and 1 hour after DAMPA administration. Urine samples were collected pre-treatment, on Day 1 and on Day 6. Necropsy was performed and consisted of macroscopic examination of the thoracic and abdominal cavities and the appearance of the heart, lungs, kidneys and parenteral site. A standard list of tissues and organs were collected and preserved for potential future examination.

Observations and Results

Clinical observations:

- On Day 1, trembling was observed in both the male and female dogs receiving 60 mg/kg approximately 30 min following DAMPA administration. Trembling was no longer observed at 1 hour following dosing.

Toxicokinetics:

- DAMPA concentrations were above the lower limit of quantification (LLOQ) up to 8-12 hours after infusion.
- The AUC_t values increased at greater than dose-proportional levels (see table copied from p. 19 of the study report below).
- The terminal half-life was considered short (less than 1 hour).

Table 10 DAMPA PK Results Using Dogs (Study # PR001-NCL-PK005)

Dose level (mg/kg)	Rate of infusion (mg/kg/min)	Dose level ratio	AUC_t (µmol.h/L)	AUC_t ratio
15	1.0	1	32.0	1
30	1.3	2	122	3.8
60	> 1.8	4	262	8.2

There were no other remarkable findings associated with the single doses of DAMPA tested. The urinalysis and macroscopic examinations of the kidney did not reveal evidence of kidney damage.

Study title: DAMPA Telemetric Evaluation of Cardiovascular Effects After Intravenous Administration in Four Conscious Beagle Dogs

Study no.: PR001-NCL-PC001
Study report location: BLA Section 4.2.1.3.1
Conducting laboratory and location: (b) (4)
Date of study initiation: June 15, 2006
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: DAMPA, Lot #04005DE (supplied by Sigma), 97.8%

Key Study Findings

- Treatment with DAMPA elicited minimal effects on blood pressure and heart rate, and on ECG intervals and waveform rhythms.
- Clinical signs included changes to the sclera of the eye after the high dose treatment, and reddening and/or swelling of the muzzle.

Methods

Doses:	Escalating doses of 0, 15, 30, 60 mg/kg
Frequency of dosing:	Every 7 days (intra-animal dose escalation)
Route of administration:	Intravenous infusion (20 min)
Dose volume:	9.15, 3.1, 4.6, 9.15 ml/kg
Formulation/Vehicle:	0.1 N NaOH adjusted to pH 8.5 with HCl
Species/Strain:	Chronically telemetered non-naïve beagle dogs
Number/Sex/Group:	2/sex each received the escalating doses
Age:	23-34 months
Weight:	11.8 to 16.9 kg
Satellite groups:	No
Unique study design:	Telemetric recordings were obtained for approximately 45 minutes prior to the 1 hour hydration/alkalinization infusion (described in Study PR001-NCL-PK005).
Deviation from study protocol:	None that affected the integrity or results of the study

Observations and Results

Clinical observations were made 30 min prior to and until 5 hours after the hydration/alkalinization infusion. Toxicokinetic samples were collected after completion of the hydration/alkalinization infusion and 2 hours after the DAMPA infusion.

- After the 60 mg/kg dose, all four dogs exhibited reddening of the sclera of both eyes 1-1.5 hours post-dose. One of the dogs exhibited rapid breathing 5 hours post-dose; one other dog experienced reddening and swelling around the eyes and muzzle. These findings cleared that day or within 2 days post-dose.

The following cardiovascular parameters were recorded using the telemetry device: blood pressure, ECG (intervals, morphology and rhythm), heart rate. Baseline values were collected over a 12-hour period prior to the start of any treatment. Dosing phase readings commenced 1 hour prior to the hydration/alkalinization infusion, and continued for up to 12 hours post-DAMPA infusion.

- Statistically significant differences in heart rate were measured following DAMPA treatment, when compared to those obtained after dosing with vehicle:
  - A [mean?] reduction of heart rate by 26 beats per minute (bpm) at 15 minutes following the start of the 20 minute infusion of 60 mg/kg DAMPA ($p<0.001$), and a reduction at 0.83 hour (15 bpm) and 1.83 hour (35 bpm) after completion of dosing ($p<0.05$)
  - A reduction of 30 bpm at 0.83 hour post the 30 mg/kg dose ($p<0.05$)
- Small but statistically significant elevations of QT and QTcR interval (compared with vehicle) were evident after the 60 mg/kg dose:
  - At 7 min and 15 min after starting DAMPA, QT interval was +20 ms, $p<0.01$ and -20 ms, $p<0.01$, respectively, and QTcR was +20 ms, $p<0.01$ and +15 ms, $p<0.05$, respectively.
- Waveform differences were sporadic and not DAMPA-related.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Study title: Pharmacokinetics of Voraxaze (Glucarpidase) Following a Single Intravenous Administration in the Male Rabbit

Study no.:	PR001-NCL-PK001
Study report location:	BLA Section 4.2.2.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Sept. 20, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	• Batch 1 glucarpidase (original preparation), 004, 515 Units/mg (GP-HPLC test result not listed on CoA) • Batch 2 glucarpidase (new preparation), BN 2090302, 437 Units/mg (GP-HPLC: 98.2%)

Key Study Findings

This study evaluated the comparability of pharmacokinetics (PK) between two batches of glucarpidase following IV administration to male rabbits. Lot 004 was the only lot of glucarpidase manufactured at CAMR, the initial location of glucarpidase manufacture. Lot 2090302 was sourced from drug substance batch M-CG2-P01, manufactured at Eurogentec. The following list of manufacturing stages is provided in the Quality CTD (copied from p. 6, Section 2.3.S.2.6 of the BLA):

(b) (4)

Methods

Doses:	500 Units/kg/day
Frequency of dosing:	Single dose
Route of administration:	IV
Dose volume:	0.5 ml/kg
Formulation/Vehicle:	0.9% NaCl
Species/Strain:	Rabbit/Crl:NZW/KbI BR rabbit (males only)
Number/Sex/Group:	10 males/group (5/group treated on Day 1, the other 5/group treated on Day 4)
Age:	Not stated
Weight:	3.18 to 4.07 kg
Satellite groups:	None
Unique study design:	Animals receiving the same treatment were split into groups of 5 and treated on separate days (1 and 4)
Deviation from study protocol:	Dosing solution analysis was compromised following inadequate storage conditions of glucarpidase.

Observations and Results

Reviewer note: The initial dose solution analysis of samples used for Group A indicated that the back-calculated concentrations were not equivalent (samples administered for Batch 1 and 2 were (b) (4) *Units/ml and* (b) (4) *Units/ml, respectively; the expected value was 1000 Units/ml). Reconstituted glucarpidase in physiological saline is not stable when stored for 24 hours at 2-4 °C. These results were repeated using frozen samples of Batch 1, and results were more consistent. Samples analyzed from Day 4 for Batch 1 and 2 were* (b) (4) *and* (b) (4) *Units/ml, respectively. The initial low result of Batch 1 from Group A was considered possibly attributable to its storage temperature. The study director concluded that, based on the concentrations of the frozen samples, the correct dose was given to both Group A and Group B.*

Samples for PK analysis were taken predose, 5, 15, 30, 45 minutes and 1, 2, 4, 8, 12, 24 and 48 hours post-dose. The statistical analysis of the PK parameters using bioequivalence criteria are shown in the table below (copied from p. 23 of the report):

Table 11 Bioequivalence Analysis from Rabbit PK Comparability Study (Study # PR001-NCL-PK001)

Parameter	Geometric Mean		Ratio (Batch 2/Batch 1)	Lower 90% CI	Upper 90% CI	Between Subject CV%
	Batch 1	Batch 2				
AUC(0-30 min)	7.28	8.28	1.14	1.02	1.27	13.2
AUC(0-t_z)	87.0	99.3	1.14	0.998	1.31	16.3
AUC(0-∞)	93.4	103	1.11	0.974	1.26	15.2
C_{max}	17.7	21.1	1.19	1.05	1.36	16.2
$t_{1/2}$	5.14	5.27	1.03	0.854	1.23	21.8
t_{max}*	0.083	0.083	0#	-0.170	0.420	NA

*t_{max} values are median values
#Difference between the median values (Batch 2 – Batch 1)

These results indicate that the average bioavailability of the new batch (Batch 2) was 14% higher than Batch 1. If the bioequivalence criteria were applied strictly, these results would not support a demonstration of bioequivalence between the two batches, as the 90% confidence interval for both AUC and C_{max} exceeds the upper limit of 1.25. The Nonclinical Overview section of the BLA states that "Results of this study (1845-016) showed that the two batches of glucarpidase used during development are bioequivalent if the PK data are normalized for nominal potency" (p.45, Section 2.4)." If the PK data are normalized to the dose according to the glucarpidase concentration specification on the Certificates of Analysis for these two batches (Batch 1 = (b) (4) Units/vial and Batch 2 = (b) (4) Units/vial), then the statistical analysis of the bioequivalence criteria are within regulatory limits (90% confidence interval between 0.8 and 1.25 %) shown on p. 25 of the report.

Table 12 Bioequivalence Analysis Using Dose-Normalized Rabbit PK Data (Study # PR001-NCL-PK001)

Parameter	Geometric Mean		Ratio (Batch 2/Batch 1)	Lower 90% CI	Upper 90% CI	Between Subject CV%
	Batch 1	Batch 2				
AUC(0-30 min) norm	0.0158	0.0156	0.983	0.882	1.10	13.2
AUC(0-t_z) norm	0.189	0.187	0.987	0.863	1.13	16.3
AUC(0-∞) norm	0.203	0.194	0.957	0.842	1.09	15.2
C_{max} norm	0.0384	0.0396	1.03	0.904	1.18	16.2
$t_{1/2}$	5.14	5.27	1.03	0.854	1.23	21.8
t_{max}*	0.083	0.083	0#	-0.170	0.420	NA

Norm = normalised for dose (Batch 1 = 460 Units/kg and Batch 2 = 532 Units/kg)
Where normalisation Units were; ((Units.h/mL)/(Units/kg)) for all AUC values and ((Units/mL)/Units/kg)) for C_{max}
*t_{max} values are median values
#Difference between the median values (Batch 2- Batch 1)

Study title: Voraxaze Pharmacokinetic Interaction Study in Female Rabbits

Study no.:	PR001-NCL-PK004
Study report location:	BLA Section 4.2.2.6.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 26, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Glucarpidase, 2090401, 99.4% (461 Units/mg)

Key Study Findings

The effect of glucarpidase on the PK of leucovorin (LV) and its active metabolite, 5-methyltetrahydrofolic acid (5-MeTHF), in the presence of MTX was investigated in rabbits. Like MTX, LV is a substrate for glucarpidase, though it has a 10-fold lower affinity for LV than for MTX. Systemic exposure to LV generally increased in the presence of glucarpidase and this increase was more marked in animals that also received MTX.

Methods

Doses:	Glucarpidase: 50 Units/kg MTX: 55 mg/kg LV: 13.5 mg/kg (150 mg/m^2)
Frequency of dosing:	Single doses of MTX and glucarpidase were administered; four doses of LV were administered at 2, 5, 26 and 50 hours after glucarpidase or saline
Route of administration:	IV
Dose volume:	Glucarpidase: 0.05 ml/kg MTX: 2.2 ml/kg LV: 1.35 ml/kg
Formulation/Vehicle:	Glucarpidase: saline
Species/Strain:	Rabbits/New Zealand White (females only)
Number/Sex/Group:	

Group	Treatment	Number of females	Animal numbers
1	MTX + Voraxaze + LV	6	1 - 6
2	MTX + saline + LV	6	7 - 12
3	Voraxaze + LV	6	13 - 18
4	Saline + LV	6	19 -24
5	MTX + Voraxaze + LV	6	25 – 30
6	MTX + saline + LV	6	31 - 36

Age:	Not provided
Weight:	3.68-4.95 kg
Satellite groups:	None

Deviation from study protocol: None that would appear to affect the integrity of the study's conclusions.

The dose of MTX was selected to provide a plasma concentration of 100 microM in rabbits at the time of glucarpidase administration, but would not be expected to produce toxicity.

From 4 rabbits per group, blood samples were taken through a surgically implanted cannula in either the jugular or femoral vein according to the following schedule:

Animals	Sample times (time after each LV dose)
13 – 16 (Group 3)	LV Dose 1: Pre-dose, 5, 15 and 30 minutes and 1 hour post-dose
19 – 22 (Group 4)	LV Dose 2 (3 hours after LV Dose 1): Pre-dose, 5, 15 and 30 minutes, 1, 3, 6, and12 hours
25 – 28 (Group 5)	LV Dose 3 (24 hours after LV Dose 1): Pre-dose, 5, 15 and 30 minutes, 1, 3, 6 and 12 hours
31 – 34 (Group 6)	LV Dose 4 (48 hours after LV Dose 1): Pre-dose, 5, 15 and 30 minutes, 1, 3, 6, 12 and 24 hours

From the remaining 2 rabbits per group, blood samples were taken prior to the glucarpidase or saline dose and prior to every LV dose.

Plasma concentrations of (6S)- and (6R)-LV, (6S)- and (6R)-5-MeTHF, MTX and DAMPA, and glucarpidase were measured.

Group 5 and 6 animals underwent complete macroscopic examination upon necropsy. Abnormal tissues or organs were noted and samples were preserved.

Observations and Results

One group 4 animal was euthanized following the third LV dose for what are stated as "animal welfare reasons." Following the first LV dose, glucarpidase had little effect on the PK of LV. On subsequent doses, the C_{max} and AUC values of LV generally increased in the presence of glucarpidase. The increase in systemic exposure to LV in the presence of glucarpidase was more marked in animals receiving MTX. The active metabolite of LV, (6S)-5-MeTHF was generally not quantifiable in plasma but the inactive isomer (6R)-5-MeTHF was present following the first two dose of LV in the groups treated with Voraxaze. Conversion of MTX to DAMPA in the presence of glucarpidase was evident.

Study title: DAMPA Single Dose Pilot Toxicity/Pharmacokinetics Study by Intravenous Administration to Beagle Dogs Followed by a 7-Day Observation Period

Study no.:	PR001-NCL-PK005
Study report location:	BLA Section 4.2.2.7.1
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	May 25, 2006
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	DAMPA (from Sigma-Aldrich #8615531), 11502KC, % purity unknown

Key Study Findings

This study was conducted to assess the maximum tolerated dose of DAMPA to inform dose-selection for a cardiovascular telemetry study in dogs. A diet formulated to maintain urinary pH above 7 and an IV hydration/alkalinization regimen before and after DAMPA administration was used to help prevent kidney damage. Dogs received the following single doses of DAMPA on Day 1 and were euthanized on Day 8:

Group	Treatment	Dose# (mg/kg)	No. of animals Male	Female
1	DAMPA	15	1	1
2	DAMPA	30	1	1
3	DAMPA	60	1	1

#-Expressed in terms of test substance as supplied.

Observations and Results

There were no unscheduled deaths on study. Dogs receiving the high dose experienced trembling approximately 30 minutes following DAMPA administration, but these effects were no longer observed at 1 hour post-dosing. There were no remarkable findings on body weight gain, food consumption, or gross pathology.

Administration of DAMPA at doses up to 60 mg/kg appeared well-tolerated, and no systemic toxicity was observed. A maximum dose of 60 mg/kg was selected for the telemetry safety pharmacology study.

6 General Toxicology

6.1 Single-Dose Toxicity

No single-dose toxicity studies evaluating glucarpidase (Voraxaze) safety were included in the BLA submission.

6.2 Repeat-Dose Toxicity

Study title: Fourteen-Day Repeated Dose Toxicity Study of Voraxaze Administered via Intravenous Injection to Beagle Dogs.

Study no.:	PR001-NCL-TX002
Study report location:	BLA Section 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 15, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Glucarpidase, Lot# 2090302, 98.2% as active form as measured by GP-HPLC

Key Study Findings

Repeated IV injection of glucarpidase resulted in adverse clinical signs, changes in hematology and clinical chemistry, and mortality at doses of 500 and 2500 Units/kg following 6 doses (administered every other day). Possible causes for the clinical signs observed are hypersensitivity to repeated administration of a bacterial enzyme, and effects due to folic acid depletion. The cause of death of the early decedents was not determined microscopically. Repeated doses of 50 Units/kg appeared well-tolerated, but anti-glucarpidase antibodies were detected in all surviving glucarpidase-treated animals at study termination.

Methods

Doses:	Group 1: 0 (vehicle control) Group 2: 50 enzyme Units/kg Group 3: 500 enzyme Units/kg Group 4: 2500 enzyme Units/kg
Frequency of dosing:	Study days 1, 3, 5, 7, 9, 11 (except Groups 3 and 4 females) and 13 (except Groups 3 and 4 males)
Route of administration:	Slow bolus intravenous injection into a peripheral vessel
Dose volume:	2.7, 0.05, 0.53, 2.7 ml/kg (respectively)
Formulation/Vehicle:	0.9% NaCl
Species/Strain:	Canine/Beagle
Number/Sex/Group:	3
Age:	> 4 months
Weight:	Males: 6.4 – 9.4 kg; Females: 6.3 – 8.2 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	After adverse effects and morbidity were seen following the 6th dose to males on Day 11, the females in Groups 3 and 4 were not dosed on Day 11. On day 13, all remaining animals were dosed, except for Group 3 and 4 males. Other protocol deviations are listed in Appendix 21 of the Study Report, and are not considered to have adversely affected the integrity of the study.

Observations and Results

Mortality

Observations for mortality and morbidity were recorded twice daily (a.m. and p.m.).

- There were seven early deaths during the study. All occurred after the sixth dose in animals from either Group 3 or 4 (table below was copied from p. 19 of the study report).

Table 13 Summary of Mortalities During the 14-Day Toxicology Study in Dogs (Study # PR001-NCL-TX002)

Text Table 1 - Summary of Early Deaths Following the Sixth Dose

Group	Sex	Animal #	Study Day	Mode of Death
3	Male	3817	11	Moribund Sacrifice
3	Female	3816	13	Moribund Sacrifice
3	Female	3818	13	Moribund Sacrifice
3	Female	3820	13	Moribund Sacrifice
4	Male	3823	11	Found Dead
4	Male	3825	11	Moribund Sacrifice
4	Female	3824	13	Moribund Sacrifice

Clinical Signs

Clinical observations were recorded pre-test, pre-dose and at 15 minutes, 1, 2, 4, 6, 12 and 24 hours postdose on days of study drug administration. Rectal body temperatures were recorded predose on Study Day 1, at one hour post-dose on Study Days 1 and 7 and one hour postdose or just prior to moribund euthanasia on Study Day 13. A general physical exam of each animal was conducted by a clinical veterinarian pre-test and within two days of scheduled necropsy.

- Clinical signs noted in the early decedents included: red discharge from anogenital region, ataxia, prostration, soft stool, vomitus, red material in pan/bedding, red discoloration of the ears or entire body, labored breathing and increased or excessive salivation. These signs were seen after the 6th dose beginning at 15 minutes postdose on Study Day 11 for the males, and beginning on Study Day 13 for the females.
- Red discoloration of the ears or entire body was recorded across groups (including the control group), but the incidence was higher in the Group 4 animals (dosed with 2,500 U/kg/dose). Soft or watery stool was observed for two females in Group 4 on Study Day 3 or 5. Up until Study Day 9 (after the fifth dose), one male in Group 4 was observed to have ataxia and labored breathing at approximately 15 minutes post-dose.

Body Weights

Body weights were recorded pretest, predose on Study Days 1, 3, 5, 7, 9, 11 (all animals), and 13 (all animals), prior to terminal fasting on Study Day 14 and prior to necropsy.

- There were no differences in body weight that were considered related to dosing with the test article.

Feed Consumption

Feed consumption was measured daily.

- There were no differences in food consumption that were considered related to the test article treatment.

Ophthalmoscopy

An ophthalmological examination was conducted by a board-certified veterinary ophthalmologist pretest and within four days of scheduled necropsy.

- A signed ophthalmologic examination report is included as Appendix 13 of the study report that states the test article treatment did not appear to produce detectable ocular disease.

ECG

Electrocardiogram tracings were recorded from all animals pretest and from surviving animals within one hour postdose on Study Day 13. On Study Day 13, there were no recordings from either Group 3 male or female dogs or from the Group 4 male dogs, and two Group 4 females had no tracings. A signed ECG report is provided in Appendix 14 of the study report.

- There were no statistical differences detected between groups for HR, RR interval, PR interval, QRS duration, QT interval or QTc. Sufficient dogs were not available to evaluate ECG effects in the 500 and 2500 enzyme Units/kg dose groups. The two remaining Group 4 females did not show any treatment-related deviation from pretest.

Hematology

Blood samples for hematological and coagulation analyses were obtained from all animals pretest and on Study Day 11 (moribund animals only), 12 and 14 (remaining females in Group 4 only). Animals were fasted overnight prior to collection (except on Study Day 11). Blood samples were evaluated for:

Total and relative differential leukocyte counts	Mean corpuscular hemoglobin
Erythrocyte counts	Mean corpuscular hemoglobin concentration
Hemoglobin concentration	Platelet counts
Hematocrit value	Prothrombin time
Mean corpuscular volume	Activated partial thromboplastin time

- Samples from moribund male dogs in Groups 3 and 4 that were collected prior to necropsy showed increases in hemoglobin and hematocrit, and decreases in platelet and neutrophils counts compared to pretest values.
- Platelet counts decreased compared to control animals on Study Day 12 in all males receiving glucarpidase. These changes were not seen in female dogs on Study Day 12, but Group 3 and 4 female dogs were not dosed on Study Day 11.
- Samples from surviving Group 4 female animals contained decreased platelet and lymphocyte counts, as compared to pretest values. Samples were not collected from control animals.

Clinical Chemistry

Blood samples for clinical chemistry analysis were obtained from all animals pretest and on Study Days 11 (moribund animals only), 12 and 14 (remaining female dogs in Group 4 only). Animals were fasted overnight prior to collection (except on Study Day 11). Blood samples were evaluated for:

Total protein	Cholesterol	Alanine aminotransferase	Phosphorus
Albumin	Triglycerides	Aspartate aminotransferase	Sodium
Globulin	Total bilirubin	Alkaline phosphatase	Potassium
Albumin/globulin ratio	Urea nitrogen	Gamma-glutamyl transferase	Chloride
Glucose	Creatinine	Calcium	

- Moribund animals (Group 3 and 4 males) had notable elevations in ALT and AST (>10-fold) and approximately 2-fold increases in (BUN, creatinine, ALP, GGT) when compared to baseline.
- When compared to controls, on Day 12, one of two surviving Group 3 males had markedly increased ALT and AST (>100- and >30-fold, respectively). The only surviving Group 4 male had an approximate 10-fold elevation in ALT level, but AST level was similar to control values.
- There were no clear differences from controls in any clinical chemistry parameter from samples collected from female dogs treated with glucarpidase.

Urinalysis

Semi-quantitative urinalysis was conducted via catch pan method (i.e. dogs placed in cages overnight, with pans beneath to catch urine) pretest, and on Study Days 12 and 14 (remaining females in Group 4 only). Feed and water were withheld overnight prior to collection. The urine samples were evaluated for the following:

pH	Urobilinogen	Occult blood
Protein	Leukocytes	Nitrate
Glucose	Specific gravity	Microscopic examination of sediment
Ketone	Color	
Bilirubin	Appearance	

- There were no test article-related differences from samples from controls.

Gross Pathology

Animals euthanized *in extremis* or found dead were necropsied. For moribund animals on Study Day 11, blood samples for hematology, coagulation, clinical chemistry, antibody analysis and bioanalytical samples were collected prior to euthanasia. On Study Day 15, surviving animals were euthanized via anesthesia with sodium pentobarbital to effect followed by exsanguination, and submitted for a complete necropsy examination.

The following organs were examined *in situ*, dissected free and fixed in 10% neutral buffered formalin (except for the testes which were fixed in Modified Davidson's fixative, and the eyes with optic nerve, which were fixed in Davidson's fixative):

Adrenal gland	Gross lesions	Ovary	Testis
Aorta	Heart	Pancreas	Thymus
Bone (femur)	Injection site (last)	Pituitary gland	Thyroid gland
Bone marrow (sternum)	Intestine (cecum, colon, rectum, duodenum, ileum, jejunum)	Prostate gland	Tongue
Brain (brain stem, cerebellum, cerebrum)	Kidney	Salivary gland (mandibular)	Trachea
Cervix	Liver	Skeletal muscle	Urinary bladder
Epididymis	Lung	Skin (abdominal)	Uterus
Esophagus	Lymph node (mandibular, mesenteric)	Spinal cord (cervical, lumbar, thoracic)	Vagina
Eye	Mammary gland	Spleen	
Gall bladder	Nerve (optic, sciatic)	Stomach (cardiac, fundic, pyloric)	

Fixed tissues were shipped to [redacted] (b) (4) for trimming, embedding and sectioning. Slides were stained with hematoxylin and eosin.

- Test-article related findings were seen in animals that died or were euthanized moribund. The most frequent findings included reddened mucosa (red gelatinous material present in the lumen) of the GI tract, and discoloration of the ear, lung, gall bladder and/or injections site.

Organ Weights

Organ weights were recorded prior to fixation for the following organs:

Adrenal gland	Kidney	Pituitary gland
Brain	Liver	Spleen
Heart	Ovary	Testis
Thymus		

- There were no test article-related changes in organ weights when compared to controls animals.

Histopathology

Adequate Battery: Histopathologic examination was performed on all tissues collected from all animals and on all gross lesions (see list under Gross Pathology, above).

Peer Review: The slides were examined by a board-certified (American College of Veterinary Pathologists) pathologist, and were not peer-reviewed.

Histological Findings: The most common microscopic findings in all glucarpidase-treated animals were congestion or hemorrhage of the GI tract, lung and gall bladder. No blood breakdown pigments were noted in the findings of hemorrhage, suggesting that the hemorrhages developed shortly before death.

The cause of death of the early decedent animals was not determined microscopically. All microscopic findings that were possibly related to glucarpidase administration were tabulated and are provided in Appendix 3 to this review (Table 16).

- The most common findings were congestion or hemorrhage at various sites, especially the GI tract, lung, gall bladder and last injection site. None of the hemorrhages were accompanied by blood breakdown pigments.

The Study Pathologist concluded that all findings in early decedent animals were considered related to blood pressure changes and vascular relaxation during the moribund and post-mortem intervals, and also that all findings in terminal animals were considered incidental or related to trauma of injection.

Toxicokinetics

Blood samples were obtained from available animals on Study Days 1 and 13 at the following timepoints: predose and 5, 15, 30, 45 minutes, 1, 2, 4, 8, 12, 24 and 48 hours postdose. The blood samples were collected in tubes containing lithium heparin and placed on ice until centrifuged. The plasma was extracted and stored in a -70°C freezer. The frozen samples were shipped on dry ice to (b) (4) for glucarpidase activity analysis.

Blood samples for antibody analysis were collected from all animals predose on Study Days 1, 8, 11 (moribund animals only), 13 (moribund animals and animal #3826), and 15. The samples were collected in serum separator tubes, centrifuged, and serum was separated into two aliquots (except those collected on Study Day 11). One frozen serum aliquot was shipped to (b) (4) for anti-Voraxaze IgG levels. The remaining aliquots are being retained at (b) (4) An ELISA was used to analyze samples for the presence of anti-Voraxaze antibodies.

- Glucarpidase was not quantifiable in the plasma from the control animals on Study Days 1 and 13. Limited data were available from Study Day 13 samples, so the TK evaluation was predominantly performed using the Day 1 plasma

enzyme activity data. Plasma levels of glucarpidase declined in an essentially monophasic manner. The mean elimination half-life ($t_{1/2}$) was approximately 5 hours, ranging in individual animals from 4.0 to 5.7 hours after dosing with 500 Units/kg and from 3.8 to 7.1 hours after dosing with 2500 Units/kg. Plasma glucarpidase levels decreased to below the limit of quantification (<0.131 Units/ml) in all animals by 8, 24 and 48 hours postdose in the 50, 500 and 2500 Units/kg dose groups, respectively.

Table 14 Toxicokinetic Results from 14-Day Toxicology Study in Dogs (Study# PR001-NCL-TX002)

Text Table 2 - Summary of Toxicokinetic Parameters

Study Day	Dose (units/kg)	Sex	AUC (0-τ) (units/*h/mL)	AUC (0-τ) (norm)	C_{max} (units/mL)	C_{max} (norm)	t_{max} (h)	$t_{1/2}$ (h)
1	50	Male	1.73	0.0346	0.910	0.0182	0.139	NC
		Female	1.09	0.0219	0.855	0.0171	0.0830	NC
	500	Male	39.5	0.0790	8.68	0.0174	0.0830	4.56
		Female	41.5	0.0831	10.2	0.0204	0.0830	4.33
	2500	Male	200	0.0799	42.3	0.0169	0.139	6.01
		Female	224	0.0896	43.2	0.0173	0.0830	5.34
13	50	Male	0.654	0.0131	0.190	0.0114	0.0830	NC
		Female	1.47	0.0294	0.629	0.0126	0.0830	NC
	500	Male	NC	NC	NC	NC	NC	NC
		Female	NC	NC	5.30	0.0106	0.0830	NC
	2500	Male	NC	NC	NC	NC	NC	NC
		Female	310	0.124	42.2	0.0169	0.0830	5.15

C_{max} (norm) = C_{max} [units/mL] / dose [units/kg]
AUC(0-τ) (norm) = AUC [units/mL*h] / dose [units/kg]
NC = Not calculable

The above table was copied from p.21 of the Study report.

Anti-Glucarpidase Antibody Analysis

On Study Day 8, four out of 18 animals developed antibodies to glucarpidase, and on Study Day 13, all remaining animals tested positive for antibody development.

Table 15 Anti-Glucarpidase Antibody Formation in 14-Day Toxicology Study in Dogs (Study # PR001-NCL-TX002)

Text Table 3 - Incidence of Positive Antibody Response to Voraxaze™

	Study Day 1	Study Day 8	Study Day 15*
Group 1 male	0/3	0/3	0/3
Group 1 female	0/3	0/3	0/3
Group 2 male	0/3	1/3	3/3
Group 2 female	0/3	0/3	3/3
Group 3 male	0/3	1/3	3/3
Group 3 female	0/3	0/3	3/3
Group 4 male	0/3	2/3	2/2
Group 4 female	0/3	0/3	3/3

*Some samples were collected on Day 11 or 13 rather than Day 15 due to moribundity.
x/x = # of positive animals/# of animals per sex per group tested.

Dosing Solution Analysis

Three random vials of bulk test article (not reconstituted) were analyzed to confirm enzyme activity prior to the start of the study.

- Results indicated that the enzyme activity ranged from [redacted] (b) (4) and was in the acceptable range.

Two reconstituted vials were analyzed from Study Days 1 and 21.

- The vials from Study Day 1 had enzyme activity [redacted] (b) (4) % of theoretical.
- The vials from Study Day 21 had enzyme activity of [redacted] (b) (4) % of theoretical.

Stability of glucarpidase prepared at a concentration of 0.394 Units/ml and stored at +5°C for 24 hours was also evaluated.

- There was no measurable enzyme activity at this time point.

Statistical Analysis

Means and standard deviations were calculated for all quantitative data. Treated groups of the same sex were compared to Group 1 (vehicle control) at common time points using an ANOVA with a *post hoc* Dunnett's t Test or another appropriate test. A two-sided α of 0.05/0.01 was used to determine if statistically significant differences exist between the control and treated groups. For groups with less than three animals/sex, statistical analyses were not performed.

Study title: Voraxaze (Carboxypeptidase G2): 3 Day Intravenous Administration Toxicity Study in the Rat with a 10 Day Recovery Period

Study no.:	1845-013
Study report location:	BLA Section 4.2.4.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	28 October 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Glucarpidase, #BN 2090301, % purity not provided (purity defined as specific activity on Certificate of Analysis =440 U/mg)

Key Study Findings

Glucarpidase was well tolerated in the rat at both dose levels of 50 and 5000 Units/kg administered daily for 3 days.

Methods

Doses:	0, 50, 5000 Units/kg/day
Frequency of dosing:	Daily for 3 days
Route of administration:	Intravenous
Dose volume:	5 ml/kg
Formulation/Vehicle:	0.9% NaCl
Species/Strain:	Rat/Crl:WI(GLX/BRL/Han)IGSB obtained from (b) (4)
Number/Sex/Group:	10/sex/main study groups 10/sex/satellite study groups 4/sex/recovery groups (control and high dose only)
Age:	Approximate 53 days old
Weight:	Main study animals weighed from 191.9-229.0 g (males) and from 141.6 to 195.4 g (females)
Satellite groups:	Satellite groups represented each of the 3 dose cohorts
Unique study design:	None
Deviation from study protocol:	None that affected the integrity or outcome of the study.

Observations and Results

Mortality

Necropsy procedures were conducted on Day 4 for main study groups and on Day 14 for recovery groups.

- There were no early decedents.

Clinical Signs

Clinical observations were made twice daily (am/pm) for routine health checks and daily for signs of ill health or overt toxicity. Postdose observations were made immediately, 0.5 1, 2 and 4 hours post-dosing. Detailed physical examinations were made weekly.

- There were no remarkable clinical or post-dosing observations.

Body Weights

Body weight was measured on Day 1 before dosing, then twice weekly and before necropsy.

- During the recovery period, mean body weight gain in males in the high dose recovery group was slightly lower than control recovery animals. A similar trend was not seen in females.

Feed Consumption

Food consumption was measured twice weekly.

- No remarkable findings.

Ophthalmoscopy

Ophthalmologic exams were not conducted.

ECG

Electrocardiograms were not conducted.

Hematology

Hematology samples were collected on Day 3 for main study groups and on Day 13 for recovery animals. The following parameters were determined:

Hemoglobin concentration	Red blood cell count
Packed cell volume	Reticulocytes
Mean cell volume	Mean cell hemoglobin
Mean cell hemoglobin concentration	Hemoglobin distribution width
Red cell distribution width	Platelet count
Plateletcrit	Mean platelet volume
Platelet distribution width	Total and differential white cell count

- On Day 3, prothrombin time was slightly decreased in high dose males, and neutrophils (as % total white blood cells) were increased by approximately 25% in high dose females when compared to controls.
- There were no significant differences in hematology parameters between control and glucarpidase-treated animals on Day 13.

Clinical Chemistry

Clinical chemistry samples were collected on Day 3 for main study groups and on Day 14 for recovery animals. The following parameters were determined:

Aspartate aminotransferase	Alanine aminotransferase
Alkaline phosphatase	Sodium
Potassium	Calcium
Inorganic phosphorus	Chloride
Total protein	Albumin
Globulin	Albumin/globulin ratio
Total cholesterol	Glucose
Urea	Total bilirubin
Creatinine	

- No treatment-related changes were observed.

Urinalysis

Urine samples were not collected.

Gross Pathology

Eyes	Spleen	Adrenals	Mandibular lymph nodes	Pituitary
Optic nerves	Pancreas	Kidneys	Thymus	Brain
Harderian glands*	Mesenteric lymph nodes	Testes & epididymides	Lungs	Spinal cord (cervical, thoracic, lumbar)
Skin	Stomach	Ovaries	Heart	Trachea*
Mammary gland	Duodenum	Seminal vesicles	Aorta	Head*
Muscle (quadriceps)	Jejunum	Urinary bladder	Trachea	Dosing sites
Femur with bone marrow and articular surface	Ileum	Prostate	Esophagus	Popliteal lymph nodes
Sternum with bone marrow	Cecum	Uterus	Tongue	Gross lesions

Sciatic nerves	Colon	Vagina	Thyroid/parathyroid	
Liver	Rectum*	Salivary glands	Larynx*	

*Microscopic examination was not performed on these tissues.

No abnormal findings were noted in animals from terminal or recovery groups.

Organ Weights

Organ weights were recorded prior to fixation for the following organs:

Liver	Testes & epididymides	Thyroids & parathyroids
Spleen	Ovaries	Pituitary
Adrenals	Prostate	Brain
Kidney	Heart	

- No meaningful differences were noted in organ weights between control and glucarpidase-treated animals.

Histopathology

Adequate Battery: Yes; see table under Gross Pathology above.

Peer Review: No description of histopathology review procedures was provided.

Histological Findings

- No treatment-related microscopic findings were observed in terminal or recovery animals.

Toxicokinetics

On Day 1 of treatment, blood samples were taken from the lateral caudal vein from non-fasted animals 10 minutes after the end of infusion. Samples collected for antibody analysis were collected pre-dose and on Days 7 and 14.

- Concentrations of plasma glucarpidase 10 minutes after the first dose ranged from 0.96-1.32 mcg/ml (0.4-0.5 U/ml) in the low dose animals and from 249.6-408 mcg/ml (99.8-163.2 U/ml) in high dose animals. Glucarpidase levels were below the limit of quantification in control animals.
- A description of the assay for antibody analysis is not provided, and it appears that samples were not measured for anti-glucarpidase antibodies.

Dosing Solution Analysis

Dosing concentrations were analyzed on Day 1 of main study and Day 1 of satellite study.

- Test article formulations from the main study were within acceptable limits.
- Test article formulations from the satellite study were inconsistent. Two of the samples were below the LLOQ and not quantifiable and the third sample was 2.4 times the expected concentration. Dispensary records indicate that all formulations were prepared correctly.

Study title: Dose Escalation Tolerance Study of Voraxaze in Beagle Dogs

Study no.:	PR001-NCL-TX001
Study report location:	BLA Section 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 20, 2004
	Study completion (February 7, 2005)
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Voraxaze (glucarpidase), Lot #2090302, 98.2%

Key Study Findings

In this dose escalation study using a single male and female animal, doses of up to 2500 Units/kg glucarpidase were well-tolerated. Doses of 5000 Units/kg in the male and 3750 Units/kg in the female were not well-tolerated, and caused severe clinical signs including prostration, labored breathing and vomiting.

Methods

Doses:	Dose 1: 50 Units/kg Dose 2: 500 Units/kg Dose 3: 1000 Units/kg Dose 4: 2500 Units/kg Dose 5: 3750 Units/kg (female only); 5000 Units/kg (male only)
Frequency of dosing:	Single escalated doses with at least a 2-day observation period between each dose (intra-animal dose escalation) Female: Days 1,3,5,7,16 Male: Days 1,3,5,7 and 23
Route of administration:	Intravenous injection
Dose volume:	Dose 1: 0.05 ml/kg Dose 2: 0.53 Dose 3: 1.1 Dose 4: 2.6 Dose 5: 4.0
Formulation/Vehicle:	941 units/ml
Species/Strain:	Beagle dog
Number/Sex/Group:	One female and one male were each administered the escalated doses
Age:	At least 6 months
Weight:	Male: 8.2 kg; Female: 7.4 kg
Satellite groups:	No
Unique study design:	No
Deviation from study protocol:	None

Observations and Results

Mortality

Observations were recorded twice daily (a.m. and p.m.).

- Four hours after receiving a dose of 3750 Units/kg on Day 23, the female dog was euthanized in moribund condition.
- The male dog was euthanized as scheduled on Day 25.

Clinical Signs

Clinical observations were recorded predose on Day 1; at 15 minutes, 1, 2, 4, 6, 12 and 24 hours post-dose on days of dosing, and prior to termination and when a change was noted.

- Both dogs were reported as normal following the 50, 500, 100 and 2500 Units/kg doses.
- Following the 5000 Units/kg dose administered to the male dog on Day 16, the animal experienced the following clinical signs:
    - Within 15 minutes post-dose: prostration, decreased activity, soft stool, discolored ears (red), labored breathing and bloodshot eyes.

  - Vomitus and increased salivation were recorded at approximately one hour post-dose.
  - The animal had no abnormal clinical signs 12 hours post-dose.
- Because of the adverse reaction experienced by the male animal, the female dog was not dosed on Day 16. The female was dosed with 3750 Units/kg on Day 23 and experienced the following:
  - At 15 minutes post-dose, prostration, vomitus, red discoloration of the skin (entire body), labored breathing, excessive salivation.
  - At four hours post-dose, soft stool, red material in the bedding and excessive salivation were recorded.
  - The animal was euthanized approximately 6 hours post-dose.

Body Weights

Body weights were recorded predose on each day of dose administration. The female dog was also weighed on Day 16 but was not dosed that day.

- Body weight increased in the male from Day 1 to 16.
- Body weight decreased in the female by less than 5% from Day 1 to 23.

Feed Consumption

Feed consumption was measured daily for Days 1-9.

- There were no remarkable changes in food consumption during this time period.

Ophthalmoscopy

Ophthalmologic evaluations were not conducted.

ECG

Electrocardiograms were not conducted.

Hematology

Blood samples were collected from both animals predose on Day 16 and from the male only at 24 hours post-dose. Blood samples were evaluated for the following:

Total and relative differential leukocyte counts	Mean corpuscular volume
Erythrocyte counts	Mean corpuscular hemoglobin
Hemoglobin concentration	Mean corpuscular hemoglobin concentration
Hematocrit value	Platelet counts

- Following the 3750 Units/kg dose, the female dog had increased levels of red blood cells, hematocrit and hemoglobin outside of the normal range for the testing facility.

- Neutrophil counts were also increased when compared to the predose value, but were within the historical normal range.

Clinical Chemistry

Blood samples were collected from both animals predose on Day 16 and from only the male dog at 24 hours post-dose. Blood samples were evaluated for the following:

Total protein	Creatinine
Albumin	Alanine aminotransferase
Globulin	Aspartate aminotransferase
Albumin/globulin ratio	Alkaline phosphatase
Glucose	Gamma-glutamyltransferase
Cholesterol	Calcium phosphorus
Triglycerides	Sodium
Total bilirubin	Potassium
Urea nitrogen	Chloride

- Increased ALT was measured in the sample collected from the male dog 24 after the dose of 5000 Units/kg.
- Increases in total protein, ALT, AST, Alk Phos, GGT and triglycerides were measured in the sample collected from the female dog 6 hours after the 3750 Units/kg.

Urinalysis

Not conducted.

Gross Pathology/Histopathology

Organs and tissues were not examined or collected.

Special Evaluation

Toxicokinetics

Toxicokinetic samples were not collected.

A separate report from [redacted] (b) (4) titled "Analysis of Dog Serum Samples for the Detection of anti-Voraxaze (Glucarpidase) Antibodies by ELISA" was provided in the Appendices. A method was developed for the qualitative determination of dog anti-glucarpidase antibodies in serum. Serum samples were collected for anti-glucarpidase antibody formation on the following days: 9, 17 (male only, 24 hours post-dose), 23 (female only, approximately 6 hours post-dose).

- Four samples were analyzed (male: Days 9, 17 and female: Days 9, 23), and the samples from Day 17 and Day 23 gave absorbance values above the method

detection limit and were considered positive. Samples from Day 9 were negative.

Dosing Solution Analysis

Not provided or discussed.

7 Genetic Toxicology

No experiments to evaluate potential genetic toxicity of glucarpidase were submitted to the BLA. The Applicant BTG references the ICH S6 Guidance Document to support their position that genetic toxicology studies were not required to support marketing.

8 Carcinogenicity

No experiments to assess the carcinogenic potential of glucarpidase were submitted to the BLA. No carcinogenicity studies were completed as glucarpidase is indicated for single use only.

9 Reproductive and Developmental Toxicology

No experiments to evaluate reproductive and developmental toxicity were submitted to the BLA. BTG gave the following rationale for why reproductive toxicology studies were not completed:

- Glucarpidase will generally be administered only once to patients who have already been exposed to HDMTX, a known teratogen.
- Glucarpidase will be used in a patient population with a serious and life-threatening illness with poor prognosis.
- Appropriate labeling will indicate that glucarpidase should only be used when the expected benefit is likely to outweigh the risk.
- As indicated in the labeling, MTX is contraindicated in pregnancy, and therefore glucarpidase administration to pregnant women should seldom be an issue.
- Glucarpidase is a protein, and therefore it is unlikely that it will pose any more of a reproductive toxicology risk than MTX.

10 Special Toxicology Studies

All toxicology studies conducted were reviewed in the above sections.

11 Integrated Summary and Safety Evaluation

The pharmacology and toxicology of glucarpidase was evaluated primarily through *in vivo* testing. Glucarpidase toxicity was evaluated using dogs and rats, and its ability to rescue HDMTX-induced toxicity was evaluated in mice, rabbits and Rhesus monkeys. Because glucarpidase catabolism of MTX produces DAMPA, the safety pharmacology of DAMPA as a single agent was also evaluated using dogs and isolated rabbit hearts.

The toxicology of glucarpidase as a single agent was evaluated in a 3-day repeat dose study in rats with a 10-day recovery period, and a 14-day repeat dose study in dogs without recovery. The rats received daily doses of either 50 or 5000 Units/kg glucarpidase for 3 days, and no treatment-related findings were identified. Dogs received IV injection of glucarpidase (0, 50, 500 or 2500 Units/kg) every other day for up to 14 days. After receiving the 6th dose, dogs receiving 500 or 2500 Units/kg experienced adverse clinical sings, changes in hematology and clinical chemistry parameters, and 7 of these animals died or were euthanized moribund. Repeat doses of 50 Units/kg for 14 days appeared well-tolerated, but systemic exposures diminished by Day 13 based on TK sample analysis for glucarpidase. Anti-glucarpidase antibodies were detected in all surviving glucarpidase-treated animals and potential neutralizing effects or accelerated, antibody-mediated clearance may have contributed to reduced exposures. The toxicities observed following 6 repeat doses of 500 or 2500 Units/kg of glucarpidase in dogs do not raise safety concerns for the proposed use of Voraxaze in the clinical setting, as the labeled Voraxaze dosing regimen will be a single dose of 50 Units/kg (b) (4)

Results from safety pharmacology studies using isolated rabbit hearts or telemeterized dogs exposed to DAMPA did not demonstrate adverse cardiovascular effects, and indicated that rapid elevation of DAMPA levels (as expected following glucarpidase treatment of HDMTX plasma levels) were well tolerated in the dog.

In addition to conducting pharmacodynamic studies examining IV glucarpidase rescue of HDMTX plasma concentrations in animals, BTG conducted glucarpidase rescue studies following IT MTX overdose using rabbits and Rhesus monkeys. The rabbit study (PR001-NCL-PK003) was compiled by a contractor using an incomplete data set from the conducting laboratory (NCI), and the monkey study was provided as a published journal article.[5] In both studies, LV was not administered. In the rabbit study, the high number of mortalities and damage to brain and heart demonstrate that the rabbit is not a useful model for glucarpidase rescue of IT MTX overdose. In the monkey study, Rhesus monkeys received toxic IT MTX doses that were immediately followed by IT glucarpidase. All monkeys treated with both glucarpidase and MTX survived without neurologic sequelae. Mean lumbar MTX CSF concentrations 15 minutes after glucarpidase administration decreased by an estimated magnitude of 3 logarithms. The only treatment related finding was asymptomatic cerebrospinal fluid pleocytosis, which developed in all animals. This study supports the use of the NHP as a potentially useful model for conducting safety and efficacy studies to support the clinical use of glucarpidase by the IT route under the Animal Rule (21 CFR 601.90).

FDA is asking BTG International to conduct an animal study as a post-marketing requirement (PMR) to establish the safety and efficacy of Voraxaze administered by the intrathecal route, based on its expected use to treat accidental intrathecal methotrexate (IT MTX) overdose. BTG International provided clinical information from less than10 human subjects who received investigational glucarpidase to reduce methotrexate

[5] Adamson *et al*, *J Clin Oncol.* 1991; 9(4):670-4..

levels in cerebrospinal fluid following IT MTX overdose. Following consultation with the reviewing Medical Officer, these additional clinical data are considered not sufficient to obviate the need for animal data, because there were no human control data (i.e., IT MTX overdose alone) available for comparison of safety or efficacy of glucarpidase treatment. See Appendix 1 for the draft language for a Post-Marketing Requirement for an animal study using monkeys to model Voraxaze rescue of IT MTX overdose.

12 Appendix/Attachments

Appendix 1

Postmarketing Requirements Under 505(o)

Section 505(o)(3) of the Federal Food, Drug and Cosmetic Act (FDCA) authorizes FDA to require holders of approved drug and biological product applications to conduct postmarketing studies and clinical trials for certain purposes, if FDA makes certain findings required by the statute.

We have determined that analysis of existing clinical data describing all known cases of intrathecal administration glucarpidase following intrathecal methotrexate overdose does not constitute substantial evidence of safety or efficacy to support the use of glucarpidase to treat inadvertent intrathecal methotrexate overdose.

(b) (4) FDA has determined that you are required to complete the following:

1. To conduct an animal safety and efficacy study in monkeys to evaluate Voraxaze treatment of intrathecal methotrexate overdose under the conditions of the "Animal Rule" (21 CFR 601.90 for biological products). The animal safety and efficacy study conducted under 21 CFR 601.90 is expected to provide data that will establish a dosing regimen of Voraxaze that will provide clinically meaningful benefit to patients with intrathecal methotrexate overdose.

   The timetable you submitted on XXX XX, XXXX, states that you will conduct this study according to the following schedule:

   Draft Protocol Submission: XXX XXXX
   Final Protocol Submission: XXX XXXX
   Final Report Submission: XXX XXXX

Appendix 2

Lots of Glucarpidase Used in Completed Nonclinical and Clinical Studies

This table was copied from p. 1 of Quality Section 3.2.P.2 Drug Product, from the BLA.

Table 1 Lots of glucarpidase used in completed non-clinical and clinical studies

Study Title	Non-clinical or Clinical	Drug product lot used
Study PR001-NCL-PK003: An investigation of the safety of CPG2 and DAMPA and the effects of CPG2 on methotrexate levels in rabbits treated intrathecally	Non-clinical	Development lot
Rescue of Experimental Intrathecal Methotrexate Overdose with Carboxypeptidase-G2 [Adamson *et al*, 1991]	Non-clinical	Development lot
Methotrexate Pharmacokinetics Following Administration of Recombinant Carboxypeptidase-G2 in Rhesus Monkeys [Adamson *et al*, 1992]	Non-clinical	Development lot
Study PR001-NCL-PK002: Plasma Pharmacokinetics (PK) of Methotrexate (MTX) in Rhesus Monkeys After Varying Doses of Carboxypeptidase-G2 (CPG2)	Non-clinical	CAMR Lot 004

Table 1 Lots of glucarpidase used in completed non-clinical and clinical studies (continued)

Study Title	Non-clinical or Clinical	Drug substance lot used (Drug Product Lot No., if different)
NCI Study (PR001-CLN-002): Special Exception Protocol for the Use of Carboxypeptidase-G2 ± Thymidine for MTX Toxicity and Renal Failure; conducted November 1993 – June 2004	Clinical	CAMR Lot 004
Berlin Study (PR001-CLN-001): Study of Recombinant Carboxypeptidase G2 (CPG2) for the Management of Patients With Delayed Methotrexate (MTX) Clearance of Intrathecal MTX Overdosage. Rescue Protocol for Patients Treated at Centers of the GMALL Study Group; conducted January 2000 – June 2003	Clinical	CAMR Lot 004
Bonn Study (PR001-CLN-003): A Trial of Carboxypeptidase-G2 (CPG2) for the Management of Patients With Methotrexate Toxicity and Renal Dysfunction; conducted March 1997 – March 2002	Clinical	CAMR Lot 004
Study 1845/013: Voraxaze™ (Carboxypeptidase G2): 3 Day IV Administration Toxicity Study in the Rat with a 10 Day Recovery Period	Non-clinical	Cangene Corporation Lot 2090301
Study 1845/016 (PR001-NCL-PK001): Pharmacokinetics of Voraxaze following a Single IV Administration in the Male Rabbit	Non-clinical	CAMR Lot 004 and Cangene Corporation Lot 2090302
Study PR001-NCL-TX001 (GTL00001): Dose Escalation Tolerance Study of Voraxaze™ in Beagle Dogs	Non-clinical	Cangene Corporation Lot 2090302
Study PR001-NCL-TX002 (GTL00003): 14-Day Repeat-Dose Toxicity Study of Voraxaze™ Administered IV to Beagle Dogs	Non-clinical	Cangene Corporation Lot 2090302
PK Study (PR001-CLN-005): A Trial to Determine the Pharmacokinetics of Glucarpidase (Voraxaze™) in Subjects With Normal and Impaired Renal Function; conducted July 2004 – October 2004	Clinical	Cangene Corporation Lot 2090302
Study PR001-NCL-IN001 (GTL00005): Amelioration of the Toxic Effects of Methotrexate with Leucovorin or Voraxaze in C57BL/6N mice	Non-clinical	Cangene Corporation Lot 2090302
PD Study (PR001-CLN-006): Special Exception Protocol for the Use of Carboxypeptidase-G2 for MTX Toxicity; conducted July 2004 – November 2005	Clinical	Cangene Corporation Lot 2090302
Study PR001-NCL-PK004: Pharmacokinetic interaction study in female rabbits	Non-clinical	Cangene Corporation Lot 2090401
Study PR001-CLN-017: An open-label study to assess the pharmacokinetics in subjects receiving high dose methotrexate, with or without Voraxaze	Clinical	Cangene Corporation Lot 2090601

Appendix 3

Histopathology Results from the Repeat Dose Toxicology Study in Dogs (Study # PR001-NCL-TX002)

The following microscopic findings were tabulated from Study # PR001-NCL-TX002. All findings that appeared to be test article-related are listed. Numbers of animals with findings from the early decedent group are in red.

Table 16 Histopathology Results from the 14-Day Repeat Dose Toxicology Study in Dogs Using Voraxaze

		Males				Females			
		0	50	500	2500	0	50	500	2500
Terminal euthanasia, N		3	3	2	1	3	3	0	2
Early death, N		0	0	1	2	0	0	3	1
FINDINGS	**GRADE**								
Adrenal gland; degeneration	minimal				1				
Brain; hemorrhage	minimal	1		1					
Brain; inflammation, chronic	minimal			1					
Ear; inflammation	minimal				1[g]				
	mild				1				1
Ear; congestion or hemorrhage	minimal				1[g]			2	
Eye; inflammation, chronic	mild								1
Gall bladder; hemorrhage	minimal							1	
	mild		1	1	2		1	1	1+1
	moderate							1	
Heart; hemorrhage	mild				1				
Injection site, last; hemorrhage	minimal	2		1		1	1		
	mild				1		1		1
	moderate				1			3	
Intestine, cecum; congestion or hemorrhage	minimal	1						1[e]	1[h]
	moderate			1[d]	1[i]				
Intestine, cecum; inflammation, chronic	minimal						1[c]		
Intestine, colon; congestion or hemorrhage	minimal							1[e]	
	mild			1[d]	1[i]				

		Males				Females			
		0	50	500	2500	0	50	500	2500
Terminal euthanasia, N		**3**	**3**	**2**	**1**	**3**	**3**	**0**	**2**
Early death, N		**0**	**0**	**1**	**2**	**0**	**0**	**3**	**1**
FINDINGS	**GRADE**								
Intestine, duodenum; congestion or hemorrhage	mild				1[i]				
	minimal							1[e]	
	moderate			1					
Intestine, duodenum; inflammation, chronic	minimal			1[b]	1[g]		1[c]		
Intestine, ileum; congestion or hemorrhage	minimal				1[g]				
	mild				1[i]				
Intestine, ileum; inflammation, chronic	minimal			1[b]					
Intestine, jejunum; congestion or hemorrhage	minimal				1[g]			1	1[h]
	moderate			1[d]	1[i]				
Intestine, rectum; congestion or hemorrhage	minimal		1		1[g]		1[c]		1
	mild				1[i]				1[h]
	moderate			1[d]					
Liver; hemorrhage	mild							1	
Liver; inflammation, chronic	mild			1					
Lung; congestion or hemorrhage	minimal				1				
	mild				1				1
	marked			1					
Lung; inflammation, chronic	minimal	1	2	1			3		1
	mild					1			
Lymph node, mesenteric; hemorrhage	minimal			1	1[i]				
Lymph node, mesenteric; necrosis, lymphoid	minimal			1	1[i]				
Lymph node, popliteal; hemorrhage	minimal				1[i]			1	
Lymph node, mandibular;	minimal							1	

		Males				Females			
		0	50	500	2500	0	50	500	2500
Terminal euthanasia, N		3	3	2	1	3	3	0	2
Early death, N		0	0	1	2	0	0	3	1
FINDINGS	**GRADE**								
hemorrhage									
Lymph node, mandibular; necrosis, lymphoid	minimal				1[i]				
Pancreas; hemorrhage	minimal				1[g]				
Pancreas, single cell necrosis	minimal			1					
Pituitary gland	cyst		1			1	2	2	
Spleen; necrosis, lymphoid	minimal				1[i]				
Spleen; hemorrhage	Minimal				1[i]				
Stomach, cardiac; mineralization,	minimal						1[a]		
	mild			1[f]					
Stomach, fundic; mineralization	minimal				1[i]				
	mild			1[f]					
Stomach, fundic; hemorrhage	minimal				1[i]				
Stomach, pyloric; congestion	minimal			1	1[g]				
Stomach, pyloric; inflammation, chronic	minimal						1[a]	1	
Thymus; hemorrhage	minimal				1	1			
	mild							2	

[a-i] *Superscripts are used to denote the same animal when they had multiple findings within the same organ system.*

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

BLA Number: 125327 **Applicant: BTG International** **Stamp Date: 18 July 2011**

Drug Name: Voraxase **BLA Type: NME; rolling submission**

On **initial** overview of the NDA/BLA application for filing:

Note: The nonclinical portions of the rolling BLA submission (Modules 2.4, 2.6 and 4) were received December 16, 2010 and replaces sequence 003 received May 10, 2010.

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
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).	-	-	Not applicable. The final formulation has remained unchanged since the first (lot 001) and most recent batch (M-CG2-P11) of glucarpidase manufactured.
6	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 applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted 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?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
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?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?		X	This is also not applicable for a protein therapeutic.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	-	-	Not applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? YES

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

M. Stavros — Sept. 8, 2011
Reviewing Pharmacologist — Date

Team Leader/Supervisor — Sept 8, 2011
Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908