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Efficacy of Glucarpidase (Carboxypeptidase G2) in Patients with Acute Kidney Injury After High-Dose Methotrexate Therapy

Brigitte C. Widemann^a, Stefan Schwartz^b, Nalini Jayaprakash^a, Robbin Christensen^c, Ching-Hon Pui^c, Nikhil Chauhan^d, Claire Daugherty^d, Thomas R. King^d, Janet E. Rush^d, and Scott C. Howard^c

^aNational Cancer Institute, Bethesda, MD

^bCharité Universitätsmedizin Berlin, Berlin, Germany

^cSt. Jude Children's Research Hospital, Memphis, TN

^dBTG International Inc., West Conshohocken, PA

Abstract

STUDY OBJECTIVE—As the incidence rate of renal impairment is 2–10% for patients treatment with high-dose methotrexate, and renal impairment develops in 0–12.4% of patients treated for osteosarcoma, we sought to evaluate the efficacy of glucarpidase, a recently approved drug that rapidly hydrolyzes methotrexate to inactive metabolites, which allows for nonrenal clearance in patients with delayed renal methotrexate elimination.

DESIGN—Pooled analysis of efficacy data from four multicenter, single-arm, compassionate-use clinical trials using protocols from 1993–2007.

PATIENTS—Of 476 patients with renal toxicity and delayed methotrexate elimination who were treated with intravenous glucarpidase for rescue after high-dose methotrexate, 169 patients had at least one preglucarpidase (baseline) plasma methotrexate concentration greater than 1 $\mu\text{mol/L}$ and one postglucarpidase methotrexate concentration measurement by high-performance liquid chromatography and were included in the efficacy analysis; renal recovery was assessed in 436 patients who had at least one recorded preglucarpidase and postglucarpidase serum creatinine concentration measurement.

MEASUREMENTS AND MAIN RESULTS—Efficacy was defined as rapid and sustained clinically important reduction (RSCIR) in plasma methotrexate concentration, with a concentration of 1 $\mu\text{mol/L}$ or lower at all postglucarpidase determinations. Median age of efficacy-evaluable patients was 20 years (range 5 wks–84 yrs). Osteosarcoma (36%), non-Hodgkin's lymphoma (27%), and acute lymphoblastic leukemia (20%) were the most frequent underlying

Author for correspondence: Thomas King, BTG International Inc., Five Tower Bridge, Suite 800, 300 Barr Harbor Drive, West Conshohocken, PA 19428-2998, Phone: 610-943-3513, Fax: 610-943-6018, thomas.king@btgplc.com.

Nikhil Chauhan, Claire Daugherty, Thomas King, and Janet Rush all are or were paid employees of BTG International Inc.

DISCLOSURES

Thomas King was responsible for planning, writing, editing, final review, and approval of this manuscript. Claire Daugherty and Nikhil Chauhan provided statistical support, editing, final review, and approval. Janet Rush provided medical review, editing, final review, and approval. All other authors provided content review, editing, final review, and approval.

diagnoses. Median preglucarpidase serum methotrexate was 11.7 $\mu\text{mol/L}$. At the first (median 15 min) through the last (median 40 hrs) postglucarpidase measurement, plasma methotrexate concentrations demonstrated consistent 99% median reduction. RSCIR was achieved by 83 (59%) of 140 patients. Sixty-four percent of patients with renal impairment greater than or equal to Common Terminology Criteria for Adverse Events grade 2 recovered to grade 0 or 1 at a median of 12.5 days after glucarpidase administration.

CONCLUSION—Glucarpidase caused a clinically important 99% or greater sustained reduction of serum methotrexate levels and provided noninvasive rescue from methotrexate toxicity in renally impaired patients.

Keywords

methotrexate; acute kidney injury; carboxypeptidase; glucarpidase

Since the 1950s, methotrexate has been used to treat pediatric and adult cancers¹. Methotrexate inhibits dihydrofolate reductase, a key enzyme in de novo purine biosynthesis². Methotrexate is administered alone or as part of a combined regimen, either in standard or high doses. High-dose methotrexate, usually defined as $>1 \text{ g/m}^2$,² is administered intravenously, followed by leucovorin rescue, and is an integral part of treatment regimens for osteosarcoma, acquired immunodeficiency syndrome–related B-cell lymphoma, primary central nervous system lymphoma, Burkitt's lymphoma, lymphoblastic lymphoma, peripheral T-cell lymphoma, chronic lymphocytic leukemia, adult T-cell leukemia, and leptomeningeal cancer.

Methotrexate-induced acute kidney injury is an oncologic emergency that occurs despite best efforts at prevention. Measures that are routinely employed to reduce toxicity, such as administration of pharmacokinetically guided leucovorin, intravenous hydration, and urinary alkalinization, are not uniformly effective. In a recent review of patients with osteosarcoma who received high-dose methotrexate, the overall incidence of renal toxicity ranged from 0–12.4%³. Of the 3887 patients enrolled in 20 clinical trials in which renal toxicity data were available, 68 (1.8%) developed Common Terminology Criteria for Adverse Events (CTCAE, version 2.0) grade 2 or higher nephrotoxicity, with 3 deaths attributed to toxicity³. This series included only patients treated after 1980, when management routinely included intravenous hydration, urinary alkalinization, and leucovorin rescue. Renal impairment occurs more often in adult patients; of 23 patients aged 19–94 years who received high-dose methotrexate for primary central nervous system lymphoma, 11 (48%) experienced a doubling of serum creatinine (sCr) during treatment, and 9 (39%) met the criteria for administration of glucarpidase under the National Cancer Institute protocol with respect to presence of toxic methotrexate concentrations and renal impairment^{4,5}.

High-dose methotrexate administration is accompanied by vigorous intravenous hydration and alkalinization of the urine to avoid intrarenal methotrexate crystal precipitation. Urine methotrexate solubility is directly proportional to pH; the drug and its metabolite (7-hydroxymethotrexate) precipitate in acidic urine (pH <5.5) when their concentrations exceed 2×10^{-3} molar, and their solubilities are 10-fold greater at a pH of 7^{6,7}. When the urine concentration of methotrexate, a bicarboxylic weak acid, increases and urine pH decreases,

the solubility of methotrexate is exceeded⁸. In this milieu, methotrexate precipitates in the renal tubules and crystal nephropathy ensues^{9,10,11}. Renal damage reduces methotrexate clearance, increasing the duration and magnitude of exposure to circulating methotrexate, establishing a cycle of further methotrexate crystal precipitation and renal injury¹².

When methotrexate elimination is delayed, the continued circulation of high concentrations of the drug exposes organ systems to toxicity¹³. The most frequent toxicities of methotrexate are myelosuppression, mucositis, acute hepatitis, and further nephrotoxicity. Fatalities usually result from severe myelosuppression with sepsis, hemorrhage, or renal failure¹⁴. Many of these patients are children, adolescents, and young adults who have received curative resection and were treated with high-dose methotrexate as adjunctive therapy, so the development of a potentially fatal complication to methotrexate may be particularly distressing.

Before 2012, there were no approved pharmaceutical therapies for patients with toxic methotrexate concentrations due to renal impairment. Instead, hemofiltration, high-flux hemodialysis, charcoal hemoperfusion or hemofiltration, peritoneal dialysis, exchange transfusion, or plasma exchange have been employed^{3,15}. High-flux hemodialysis is the most effective method of extracorporeal methotrexate removal but requires 5–6 daily treatments (4–6 hours per session) with a very high blood flow rate (400 mL/min)¹⁶; it often results in transient rebound increases in methotrexate concentrations and has been associated with low blood pressure, fatigue, chest pain, leg cramps, nausea, and headaches^{17,18,19}.

Glucarpidase, previously known as carboxypeptidase G2, is a carboxypeptidase G enzyme isolated from *Pseudomonas* species strain RS-16 that hydrolyzes the terminal glutamate residue from methotrexate, along with other naturally occurring folates and folate analogs²⁰, and was recently approved by the US Food and Drug Administration (Voraxaze; BTG International, Inc., West Conshohocken, PA). The hydrolysis of methotrexate and its active metabolite 7-hydroxymethotrexate by glucarpidase forms the inactive metabolites glutamate, 2,4-diamino-N¹⁰-methylpteroic acid (DAMPA)²¹, and (OH)-DAMPA, which are partially metabolized by the liver^{22,23,24}. The elimination of DAMPA and OH-DAMPA is subsequently achieved extrarenally.

Glucarpidase has been available in the United States and Europe since 1993 under compassionate-use or “named patient” studies. Glucarpidase was approved in January 2012 for the treatment of toxic methotrexate plasma levels in patients with delayed renal clearance of methotrexate due to impaired renal function²⁵. Demonstration of glucarpidase efficacy was based on an analysis of a subset of patients who were treated in a set of multicenter, single-arm, compassionate-use clinical trials conducted by the Cancer Therapy Evaluation Program of the National Cancer Institute (Bethesda, MD)^{26, 27} and 2 European trials conducted primarily in Germany^{28,29}.

Methods

This study was a pooled analysis of efficacy data from four multicenter, single-arm, compassionate-use clinical trials of glucarpidase.^{26–29} Key protocol and inclusion criteria

for the four studies are listed in Table 1. All 4 studies were open-label trials that enrolled consenting patients who had impaired methotrexate clearance due to methotrexate-induced acute kidney injury after high-dose methotrexate therapy. Criteria for leucovorin administration and administration of a second dose of glucarpidase are outlined in Table 1. In order to avoid the known interaction between leucovorin and glucarpidase, leucovorin administration was restricted to a general time frame of 2–4 hours before or after glucarpidase. Consistent with general recommendations for use of high-dose methotrexate, patients were treated with intravenous hydration and bicarbonate to maintain a high urine flow rate and urine pH >7.0. Hemodialysis was instituted if indicated for fluid or electrolyte abnormalities. Allergic signs or symptoms were managed supportively, including the administration of corticosteroids and antihistamines, as needed.

DAMPA and OH-DAMPA are byproducts of the catabolic action of glucarpidase on methotrexate and cross-react with commercial methotrexate immunoassays used in local laboratories causing overestimation of methotrexate concentration after glucarpidase^{30,31}. Therefore, efficacy was assessed in a subset of pooled study patients for whom methotrexate concentrations were assayed at central laboratories using high-performance liquid chromatography (HPLC). Plasma sampling times were similar across the studies: immediately before glucarpidase administration, and after glucarpidase administration at 15 minutes, 1 hour, and 2 to 4 hours. In 3 of the studies, subsequent daily samples were obtained until discharge (up to approximately day 8 after administration of glucarpidase).

Efficacy data collected from the studies were analyzed post hoc in alignment around a primary endpoint—rapid and sustained clinically important reduction (RSCIR) in serum methotrexate concentration—defined as attainment of methotrexate concentration 1 $\mu\text{mol/L}$ in all postglucarpidase determinations. In patients who had at least one postglucarpidase renal function parameter measured, renal recovery was assessed by measuring changes in sCr and the maximum grade of toxicities using CTCAE version 3.

Results

Baseline Clinical and Demographic Characteristics of the Study Patients

The breakdown of patients with data available for the efficacy and renal recovery analyses, by study and for the pooled analysis, is shown in Table 2. Most patients were receiving treatment for osteosarcoma, acute lymphoblastic leukemia, or non-Hodgkin's lymphoma and had received the protocol-recommended dose of glucarpidase 50 U/kg administered intravenously over 5 minutes.

Of the total 476 patients with confirmed glucarpidase dosing, 169 had at least one postglucarpidase methotrexate concentration measurement by HPLC and were therefore included in the efficacy analysis. A total of 156 patients had a confirmed baseline (preglucarpidase) and at least 1 postglucarpidase methotrexate measurement. A total of 447 patients received glucarpidase and had at least 1 postglucarpidase sCr or creatinine clearance measurement. Of these patients, renal recovery was assessed in 436 patients who had recorded sCr measurements pre- and postglucarpidase.

Key baseline (preglucarpidase) demographic and clinical characteristics for the patients evaluable for efficacy are listed by study and for the pooled analysis in Table 3. Study 001 excluded pediatric patients (except for a 10 year old patient who was enrolled), whereas the other 3 studies enrolled patients of all ages. Most patients from Study 001 were diagnosed with non-Hodgkin's lymphoma, primary central nervous system lymphoma, or acute lymphoblastic leukemia, whereas most patients in the other 3 studies were diagnosed with osteosarcoma, non-Hodgkin's lymphoma, or acute lymphoblastic leukemia. Preglucarpidase plasma methotrexate concentrations varied primarily by tumor type. Most patients with a preglucarpidase methotrexate concentration $\geq 50 \mu\text{mol/L}$ had osteosarcoma (31/41, 76%), whereas most patients with a preglucarpidase methotrexate concentration $<50 \mu\text{mol/L}$ had non-Hodgkin's lymphoma (32/99, 32%), or acute lymphoblastic leukemia (27/99, 27%). Similarly, 70% (30/43) of patients with a preglucarpidase methotrexate concentration $\geq 50 \mu\text{mol/L}$ were pediatric compared to 35% (37/106) of patients with a preglucarpidase methotrexate concentration $<50 \mu\text{mol/L}$. The 43 patients who had preglucarpidase methotrexate concentrations of $\geq 50 \mu\text{mol/L}$ had received a higher median methotrexate dose (12.0 g/m^2) than that (4.0 g/m^2) of the 103 patients with lower preglucarpidase methotrexate concentrations ($<50 \mu\text{mol/L}$).

Glucarpidase Treatment Regimen

Of 169 evaluable patients, 118 (69.8%) received a single dose of glucarpidase, 45 (26.6%) received a second dose, and 6 (3.6%) received a third. The criteria for a second dose differed across the 4 studies (Table 1).

Key Efficacy Endpoint

Methotrexate reduction was immediate in most patients, with 136 (87%) of 156 patients experiencing a $\geq 95\%$ reduction in serum methotrexate concentration from the preglucarpidase measurement to the first postglucarpidase assessment, at a median of 15 min postglucarpidase (Figure 1). The median methotrexate concentration at each postglucarpidase assessment time point (15 minutes through day 8) was $<0.54 \mu\text{mol/L}$, corresponding to median reductions from preglucarpidase measurements of $\geq 97\%$.

RSCIR was achieved by 83 (59%) of 140 patients with preglucarpidase methotrexate levels $>1 \mu\text{mol/L}$ (Figure 2). The achievement of RSCIR was clearly related to preglucarpidase methotrexate concentrations. Patients who had a preglucarpidase methotrexate concentrations of $\geq 50 \mu\text{mol/L}$ were less likely to achieve RSCIR than those with concentrations of $<50 \mu\text{mol/L}$. However, an analysis of reduction in methotrexate concentration revealed that patients with higher preglucarpidase methotrexate concentrations had a higher percent reduction in methotrexate concentration following glucarpidase. Ninety-eight percent (44/45) of patients with preglucarpidase methotrexate concentrations of $\geq 50 \mu\text{mol/L}$ and 83% (92/111) of patients with preglucarpidase concentrations of $<50 \mu\text{mol/L}$ had $\geq 95\%$ reductions at the first postglucarpidase measurement.

A further reduction in methotrexate concentration was observed in 8 patients who received a second dose of glucarpidase 48 hours after the first dose and who had a methotrexate

concentration >1 $\mu\text{mol/L}$ before the second dose; however, the extent of this further reduction varied (range 8–97%).

Recovery of Renal Function

In 436 patients with available (premethotrexate and preglucarpidase) sCr measurements, there was a 3.5-fold increase in mean sCr concentration from premethotrexate to the preglucarpidase baseline: 0.79 to 2.79 mg/dL. After administration of glucarpidase, mean sCr continued to rise (an additional increase of 0.24 mg/dL over 3 days), then began to decrease. The mean sCr value at day 22 (148 evaluable patients) was 1.27 mg/dL (Figure 3). Shifts in CTCAE serum creatinine concentration grade from baseline to day 22 for those patients with assessments are listed in Table 4.

Of 410 evaluable patients who developed grade 2 sCr values following high-dose methotrexate that remained at grade 2 at preglucarpidase baseline, 262 (63.9%) recovered to grade 0 or 1, with a median time to recovery of 12.5 days for the 258 patients for whom days to recovery could be calculated.

For patients who had grade 2 sCr values after methotrexate and subsequently demonstrated improvement in grade (to 0 or 1) after glucarpidase, age was a determining factor. In patients aged <18 years old, 75% (150/201 evaluable patients) recovered to grade 0 or 1 at the last assessment, with median times to recovery of 8 days in patients aged <12 years, and 12 days in patients aged 12–18 years. In contrast, only 48% (29/60 evaluable) of patients aged >65 years recovered to grade 0 or 1 sCr values, with a median time to recovery of 16 days.

The adverse event related to glucarpidase that was most commonly assessed was paresthesia, which occurred in 2% of patients. Related events similar to paresthesia that occurred were burning sensation, hypoesthesia, oral paresthesia, skin burning sensation, and formication; all occurred in <1% of patients. The second most common treatment-emergent event was flushing, which occurred in 1.8% of patients. Related events similar to flushing that also occurred were feeling hot, hot flush, and erythema; all occurred in <1% of patients.

Discussion

Differences in protocol design existed among the 4 compassionate-use trials. In this pooled analysis, approximately 76% of patients with osteosarcoma were <18 years of age, and all were younger than 65 years. Among patients <12 years of age, the most frequent tumor was osteosarcoma, followed by acute lymphoblastic leukemia. Conversely, among patients who were ≥ 65 years of age, the most frequent tumor was primary central nervous system lymphoma followed by non-Hodgkin's lymphoma. Osteosarcoma patients received a much higher dose of methotrexate (albeit shorter infusion times) than patients with acute lymphoblastic leukemia, non-Hodgkin's lymphoma, or primary central nervous system lymphoma. As a consequence, patients with osteosarcoma had higher preglucarpidase methotrexate levels than patients with other tumors. Also, a higher percentage of osteosarcoma patients received a second dose of glucarpidase than did patients with other tumors. This was due to design differences between Studies 002 and 006, where patients

received a second dose of glucarpidase if they had high preglucarpidase methotrexate levels. In Studies 001 and 004, patients received additional doses if they had persisting high methotrexate concentrations after the first dose of glucarpidase.

In these studies, regardless of design or inclusion or exclusion criteria, administration of glucarpidase consistently caused a rapid and sustained reduction in methotrexate concentration (median reductions 97%) at each postglucarpidase assessment (15 minutes through 8 days after administration). A single dose of glucarpidase lowered methotrexate concentrations to $<1 \mu\text{mol/L}$ in all postglucarpidase assessments, which can be regarded as a clinically important reduction, in 59% of patients. Patients with higher preglucarpidase methotrexate concentrations had higher percent reductions in methotrexate concentrations. Regardless of starting methotrexate concentration, nearly all patients experienced a reduction in methotrexate concentration after glucarpidase of at least 85%. These data demonstrate that a RSCIR was achieved in slightly more than half of all treated patients and occurred mostly in patients with methotrexate concentrations of $<50 \mu\text{mol/L}$. However, almost all patients responded to glucarpidase with a marked reduction in methotrexate concentration, and this occurred without regard to the preglucarpidase methotrexate concentration.

The HPLC assay methodology employed in these studies for the measurement of serum methotrexate had a lower limit of quantitation of $0.05 \mu\text{mol/L}$. Therefore, a patient with a preglucarpidase methotrexate level of $1\text{--}4 \mu\text{mol/L}$ would not be expected to show a reduction of 99%. Glucarpidase only hydrolyzes methotrexate in the vascular space. Intracellular methotrexate will not be hydrolyzed, and release of intracellular methotrexate into the vascular space accounts for later increases in methotrexate level, or “rebound”. Rebound was assessed in 3 of the 4 studies and was defined as a postglucarpidase methotrexate concentration that was >2 times the nadir methotrexate concentration, where the difference in methotrexate concentration between the two values was $>1 \mu\text{mol/L}$. In Studies 001, 002, and 006, between 14% and 23% of patients met the criteria for rebound (14%, 23%, and 15%, respectively). This underscores the need to accompany glucarpidase with leucovorin in order to provide intracellular rescue.

The early recognition of toxic methotrexate concentrations and intervention to decrease them are cornerstones of patient management. Prolonged exposure to high systemic methotrexate concentrations appears to influence the extent of tissue damage and is associated with poorer clinical outcomes and higher incidence of death³². The threshold systemic methotrexate concentrations above which serious toxicities are likely to develop are reported to be $5\text{--}10 \mu\text{mol/L}$ at 24 hours, $1 \mu\text{mol/L}$ at 48 hours, and $0.01 \mu\text{mol/L}$ at 72 hours after methotrexate administration as a 3- or 4-hour infusion³³. After a 24-hour infusion, methotrexate levels are usually measured at 42 hours (target concentration <0.5 to $1 \mu\text{mol/L}$) and 66 hours (target concentration $<0.1 \mu\text{mol/L}$). In an analysis of 1,141 patients treated with 4,909 courses of high-dose methotrexate, 20 patients (1.8% of patients, 0.4% of courses) received glucarpidase approximately 46 hours after high-dose methotrexate, with no patient deaths³⁴. This frequency is similar to that noted in a recent meta-analysis of data from almost 4,000 patients with osteogenic sarcoma who received high-dose methotrexate: grade 2 nephrotoxicity occurred in 1.8% of patients. However, most of these patients were

treated with dialysis-based methods or supportive care. Additionally, Widemann et al reported 12 deaths in an analysis of patients treated with high-dose methotrexate, 6 of which were directly attributable to irreversible methotrexate toxicity.²⁶ Risk factors for development of grade 4 or 5 renal toxicity were grade 4 toxicity prior to administration of glucarpidase, inadequate initial increase in leucovorin dose, and administration of glucarpidase more than 96 hours after the start of methotrexate³. Renal impairment occurs more frequently in older patients; in a series of 23 patients receiving high-dose methotrexate for primary central nervous system lymphoma, 48% of patients (aged 19–94 yrs) experienced a doubling of sCr during treatment, and 9 patients met criteria for administration of glucarpidase under a National Cancer Institute treatment protocol⁵.

Currently available methods of methotrexate removal, including extracorporeal methods, are suboptimal. These methods are slow and expose potentially neutropenic and thrombocytopenic patients to increased risk of infection and hemorrhage. Thus, there is a need for an effective treatment that rapidly decreases methotrexate concentrations in patients with delayed methotrexate elimination due to renal impairment. Glucarpidase meets this need, enabling oncologists to limit the duration and extent of patient's exposure to toxic methotrexate concentrations, thereby increasing confidence in administering high-dose methotrexate where needed.

Conclusion

Glucarpidase caused a clinically important 99% or greater sustained reduction of serum methotrexate levels and provided noninvasive rescue from methotrexate toxicity in renally impaired patients.

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Figure 1. Methotrexate (MTX) concentrations (median [25th and 75th percentiles]) after treatment with glucarpidase in 156 of 169 efficacy-evaluable patients who had preglucarpidase (baseline [BL]) and postglucarpidase MTX measurements by high-performance liquid chromatography.

Figure 2. Rapid and sustained clinically important reduction (RSCIR) in methotrexate (MTX) concentration was achieved in 59% (95% confidence interval 51–67%) of 140 patients with preglucarpidase MTX concentrations greater than 1 μ mol/L.

Figure 3. Mean $\pm$ SD serum creatinine concentrations in patients with preglucarpidase (baseline) and postglucarpidase measurements.

Table 1
Inclusion Criteria and Key Protocol Characteristics of the Four Studies Analyzed

Study Designation and No. of Participating Centers	Study Time Frame	Inclusion Criteria						Renal Impairment Criteria		Initial Glucarpidase Dose, Leucovorin Regimen, and Serum Sampling Times for Methotrexate
		Methotrexate Concentration (μmol/L) by Assessment Time Point						Renal Impairment Criteria		
		>12 h	>24 h	>36 h	>42 h	>48 h	sCr (x ULN) or CrCl (mL/m ² /min)	Urine output		
Study 001 (29 centers) ²⁷	January 2000 to August 2003	--	--	--	>5 or >1	>0.4	sCr >1.5	<500 mL per 24 h	<ul style="list-style-type: none">50 U/kg; second dose in patients with methotrexate concentration >0.1 μmol/L >24 hours after dose 1.LV was not to be administered within 4 hours prior to glucarpidase dosing. Following glucarpidase dosing, LV was to be subsequently dosed at a level determined by the serum MTX concentration (based on local assay), but at no less than 15 mg/m² every 6 hours until two MTX measurements, 24 hours apart, were <0.1 μmol/L.MTX sampling times: prior to glucarpidase, at 15 min postglucarpidase, then at 1 hr, 4 hrs, and every 24 hrs until methotrexate <0.1 μmol/L.	
Study 002 (149 centers) ^{26, 31}	November 1993 to May 2004	>2 SD(¹)	--	--	10	--	sCr 1.5 or CrCl 60	--	<ul style="list-style-type: none">50 U/kg; second dose in patients with decrease in methotrexate concentration 90% (or 1 log) but 1 μmol/L at 24 h after first dose, or second dose 48 h after first dose in patients with preglucarpidase methotrexate concentrations >100 μmol/L(²)LV was to be administered as an IV dose of 1000 mg/m² every 6 hours and, if possible, not administered 2 hours prior to and 2 hours following the administration of glucarpidase. Following the postglucarpidase LV dose of 250 mg/m² every 6 hours for a total of 48 hours, the dose of LV was to be based on local laboratory measurements of plasma MTX concentrations.MTX sampling times: prior to glucarpidase, at 15 min, 30 min, 1 hr, and 2 hrs postglucarpidase, then daily.	
Study 003 (48 centers) ²⁸	March 1997 to March 2002	>2 SD(¹)	--	>10	>5	>3	sCr >1.5 and increasing during MTX	Decreased d diuresis	<ul style="list-style-type: none">50 U/kg; second dose in patients with decrease in methotrexate >1 log but still >1 μmol/L.Because of the potential for interaction of glucarpidase and LV, LV administration was to be stopped 4 hours prior to glucarpidase dosing and resumed 1 hour following the final glucarpidase dose. During the first 24 hours after glucarpidase administration, LV was to be administered at a dose of 100 mg/m² every 6 hours for 4 doses followed by an accelerated rescue regimen for 5 days	

Study Designation and No. of Participating Centers	Study Time Frame	Inclusion Criteria						Initial Glucarpidase Dose, Leucovorin Regimen, and Serum Sampling Times for Methotrexate	
		Methotrexate Concentration (µmol/L) by Assessment Time Point							
		>12 h	>24 h	>36 h	>42 h	>48 h	Renal Impairment Criteria		
							sCr (x ULN) or CrCl (mL/m ² /min)	Urine output	
									<ul style="list-style-type: none">MTX sampling times; prior to glucarpidase, then at 15 min, 30 min, 1 hr, and 2 hrs postglucarpidase.
Study 006 (136 centers) ²⁶	June 2004 to April 2007	>2 SD(1)	>50	--	>10	>5	sCr > 1.5 or CrCl 60	--	<ul style="list-style-type: none">50 U/kg; second dose at 48 h in patients with pre-glucarpidase methotrexate concentration >100 µmol/L.Prior to glucarpidase administration, patients were to be maintained on LV administered IV, either at a dose of 1,000 mg/m2 every 6 hours, or in accordance with local standard practice. LV was not to be administered 2 hours prior to and 2 hours following the administration of glucarpidase. Following glucarpidase administration, LV was to be administered as an IV dose of 250 mg/m2 every 6 hours for a total of 48 hours, after which the LV dose could be adjusted based on local laboratory measurements of plasma MTX concentrations.MTX sampling times; prior to glucarpidase, at 15 min, 1 hr, and 2 hrs postglucarpidase, then daily for 8 days.

NOTES: sCr = serum creatinine concentration; SD = standard deviation; CrCl = creatinine clearance; MTX = methotrexate; LV = leucovorin; ULN = upper limit of normal

¹ Greater than 2 SD above the methotrexate elimination curve nomogram.

² A small number of patients enrolled early in Study 002 received per-protocol 3 doses of glucarpidase at 4 hour intervals.

Table 2

Patients with Data Available for Analysis in the Four Studies

Variable	Pooled Studies	Study 001	Study 002	Study 003	Study 006
No. of patients who received glucarpidase(¹)	476	44	214	69	149
No. (%) of patients with confirmed efficacy data available(²)	169 (35.5)	28 (63.6)	84 (39.3)	30 (43.5)	27 (18.1)
No. (%) of patients who had renal data analyzed(³)	447 (93.9)	44 (100.0)	205 (95.8)	58 (84.1)	140 (94.0)

¹ Patients confirmed to have received glucarpidase.
² Patients with at least 1 postglucarpidase methotrexate concentration measured by high-performance liquid chromatography.
³ Patients who received glucarpidase and had at least 1 postglucarpidase sCr or CrCl measurement

Table 3
Baseline Demographic and Clinical Characteristics of the Patients Included in the Efficacy Analysis

Characteristic	Pooled Studies ^a (n=169)	Study 001 (n = 28)	Study 002 (n = 84)	Study 003 (n = 30)	Study 006 (n = 27)
Age (yrs), median (range)	20 (0 to 84)	51 (18 to 75)	17 (0.4 to 82)	17 (5 to 67)	16 (5 to 84)
Age (yrs)					
<12	17 (10.1)	0	10 (11.9)	4 (13.3)	3 (11.1)
12–18	55 (34.5)	0	35 (41.7)	9 (30.0)	11 (40.7)
18–65	73 (43.2)	25 (89.3)	29 (34.5)	10 (33.3)	9 (33.3)
65	16 (9.5)	3 (10.7)	8 (9.5)	1 (3.3)	4 (14.8)
NR	8 (4.7)	0	2 (2.4)	6 (20.0)	0
Sex					
Female	40 (23.7)	NR	30 (35.7)	NR	10 (37.0)
Male	59 (34.9)	NR	42 (50.0)	NR	17 (63.0)
NR	70 (41.4)	28 (100)	12 (14.3)	30 (100)	0
Tumor Type					
Acute lymphoblastic leukemia	30 (17.8)	9 (32.1)	9 (10.7)	9 (30.0)	3 (11.1)
Non-Hodgkin's lymphoma	40 (23.7)	12 (42.9)	14 (16.7)	8 (26.7)	6 (22.2)
Primary central nervous system lymphoma	14 (8.3)	5 (17.9)	3 (3.6)	2 (6.7)	4 (14.8)
Osteosarcoma	54 (32.0)	0	38 (45.2)	4 (13.3)	12 (44.4)
Other	13 (7.7)	2 (7.1)	7 (8.3)	2 (6.7)	2 (7.4)
NR	18 (10.7)	0	13 (15.5)	5 (16.7)	0
Median (range) preglucarpidase serum creatinine concentration (n=156)	2.7 mg/dL (0.7–10.2)	--	--	--	--
Median (range) time between methotrexate and first glucarpidase dose (n=157)	3 days (2–12)	--	--	--	--
No. (%) of patients who received a second or third dose of glucarpidase during treatment	51 (30.2%)	--	--	--	--

^p Pooled numbers of patients differ for some characteristics, as data were not recorded in some individual studies included in the data pool. NR = data not recorded.

Table 4

Shifts in Serum Creatinine Concentration CTCAE Grade from Baseline (Preglucarpidase) to Last Assessment in 147 Evaluable Patients^a

Baseline CTCAE Grade	CTCAE Grade 0	CTCAE Grade 1	CTCAE Grade 2	CTCAE Grade 3
Grade 0 (n=1)	0 (0)	1 (0.7)	0 (0)	0 (0)
Grade 1 (n=5)	4 (2.7)	1 (0.7)	0 (0)	0 (0)
Grade 2 (n=73)	27 (18.4)	25 (17.0)	20 (13.6)	1 (0.7)
Grade 3 (n=56)	15 (10.2)	25 (17.0)	12 (8.2)	4 (2.7)
Grade 4 (n=12)	3 (2.0)	7 (4.8)	2 (1.4)	0 (0)
All grades (n=147)	49 (33.3)	59 (40.1)	34 (23.1)	5 (3.4)

Data are no. (%) of patients.

^a Includes patients who had a baseline and at least one postbaseline value for serum creatinine concentration on day 22 after glucarpidase administration.