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RESEARCH ARTICLE

A pooled subgroup analysis of glucarpidase treatment in 86 pediatric, adolescent, and young adult patients receiving high-dose methotrexate therapy in open-label trials

Katherine A. Janeway[1] | Luis Gros[2] | Stefan Schwartz[3] | Claire Daugherty[4] | Eva Gallardo[5] | Christon Hill[4] | Emma Thomas[5] | Suzanne Ward[4] | Carmelo Rizzari[6,7]

[1]Dana-Farber/Boston Children's Cancer and Blood Disorders Center and Harvard Medical School, Boston, Massachusetts, USA

[2]Vall d'Hebron Research Institute and Department of Pediatric Hematology and Oncology, Vall d'Hebron University Hospital, Barcelona, Spain

[3]Department of Hematology, Oncology and Tumor Immunology, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität and Humboldt-Universität zu Berlin, Campus Benjamin Franklin, Berlin, Germany

[4]BTG International Inc., Conshohocken, Pennsylvania, USA

[5]Protherics Medicines Development Ltd., London, UK

[6]Unit of Pediatrics, Foundation IRCCS San Gerardo dei Tintori, Monza, Italy

[7]Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy

Correspondence
Carmelo Rizzari, Unit of Pediatrics, Foundation IRCCS San Gerardo dei Tintori, Via Pergolesi 33, 20900 Monza, Italy.
Email: carmelo.rizzari@gmail.com

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Abstract

Background: Delayed methotrexate elimination can occur in patients undergoing high-dose methotrexate cancer treatment. Effectiveness of glucarpidase for rapidly reducing methotrexate concentrations was shown in compassionate-use trials in patients aged 0–84 years.

Methods: We performed post hoc analyses of infants (≥28 days to <2 years), children (≥2 to <12 years), adolescents (≥12 to <15 years), and young adults (≥15 to <25 years) from four multicenter, open-label, single-arm, glucarpidase compassionate-use trials. Patients had toxic methotrexate levels due to delayed methotrexate elimination and/or renal dysfunction, and received glucarpidase (50 U/kg). The primary endpoint was clinically important reduction (CIR) in plasma methotrexate (methotrexate ≤1 μmol/L at all post-glucarpidase measurements) based on high-performance liquid chromatography.

Results: Among 86 patients included in efficacy analyses, CIR was achieved by zero of one infant (0.0%), five of 16 children (31.3%), seven of 24 adolescents (29.2%), and 26/45 young adults (57.8%). Median methotrexate reduction was 98.7% or higher in

Abbreviations: AE, adverse event; ALL, acute lymphoblastic leukemia; AUC, area under the concentration–time curve; CI, confidence interval; CIR, clinically important reduction; CTCAE, Common Terminology Criteria for Adverse Events; DAMPA, 2,4-diamino-N10-methylpteroic acid; HDMTX, high-dose methotrexate; HPLC, high-performance liquid chromatography; MTX, methotrexate; SD, standard deviation; ULN, upper limit of normal.

each group 15 minutes post-glucarpidase. Patients with pre-glucarpidase methotrexate less than 50 μmol/L (35/42, 83.3%) were more likely to achieve CIR than those with methotrexate 50 μmol/L or higher (1/37, 2.7%). The most common treatment-related adverse event was paresthesia, occurring in three adolescents (4.5%) and six young adults (5.2%). No other treatment-related adverse event occurred in 5% or higher of any age group.

Conclusion: After accounting for pre-glucarpidase methotrexate levels, glucarpidase efficacy at inducing CIR in pediatric/young adult patients was consistent, with efficacy observed in the overall study population (i.e., patients aged 0–84), and no unexpected safety findings were observed. These findings demonstrate glucarpidase (50 U/kg) is an effective and well-tolerated dose for pediatric, adolescent, and young adult patients.

KEYWORDS
acute kidney injury, delayed elimination, glucarpidase, methotrexate

1 | INTRODUCTION

Methotrexate (MTX) is an anti-folate therapy with a well-established role in the treatment of a diverse range of solid tumors and hematologic malignancies.[1,2] High-dose MTX (HDMTX) regimens—typically doses higher than 500 mg/m^2—may be used to treat cancers, including acute lymphoblastic leukemia (ALL), osteosarcoma, and lymphoma.[2–5] Due to the potential for lethal MTX toxicity, HDMTX must be administered with standardized supportive care,[2,6,7] including intravenous hydration and urinary alkalinization, appropriate administration of leucovorin rescue therapy to protect normal (nonmalignant) cells from injury, and avoidance of pharmacokinetic interactions between MTX and drug classes including proton-pump inhibitors, non-steroidal anti-inflammatory drugs, and β-lactam antibiotics.

MTX is primarily cleared by the kidneys, and delayed MTX elimination during HDMTX treatment due to renal impairment can leave high residual MTX levels, which can lead to severe toxicity.[8] In the setting of elevated serum creatinine and lower creatinine clearance, MTX toxicity can present as severe hepatotoxicity, bone marrow toxicity, mucositis, and worsening renal failure.[2,8–10]

In the European Union (EU), glucarpidase is indicated to reduce toxic plasma MTX concentrations in adults and children (aged ≥28 days) with delayed MTX elimination or at risk of MTX toxicity.[11] It was approved in the United States in 2012 for the treatment of toxic plasma MTX concentrations in patients with delayed MTX clearance due to impaired renal function.[12] Glucarpidase converts MTX into two non-cytotoxic metabolites (2,4-diamino-N10-methylpteroic acid [DAMPA] and glutamate).[8] Immunoassay methods commonly used to measure MTX do not distinguish between MTX and DAMPA, and overestimate true MTX concentrations after therapy with glucarpidase; therefore, high-performance liquid chromatography (HPLC) is more accurate and specific for measuring MTX levels in the presence of glucarpidase than immunoassay methods.[8]

The effectiveness of glucarpidase was demonstrated based on four compassionate-use, open-label, multicenter studies.[13–16] Subsequent pooled analysis of efficacy data from these four trials demonstrated that glucarpidase initiated a rapid, sustained reduction in MTX levels, with a clinically important reduction (CIR) observed in 59% of patients after a single glucarpidase dose.[9] Although pediatric patients were represented in the published pooled analysis, the analysis population comprised pediatric and adult patients (aged 0—84 years), with most patients (n = 89, 53%) aged 18 years or older.[9]

Case series have demonstrated the effectiveness of glucarpidase in pediatric patients,[17–19] evaluated risk factors, including Hispanic ethnicity, for pediatric patients requiring glucarpidase use,[20,21] and identified varying levels of awareness of glucarpidase across hematology-oncology centers, demonstrating a need for further information on the use of glucarpidase in pediatric settings.[22] Finally, consensus guidelines on the use of glucarpidase have been published,[8] and the web-based MTXPK.org tool has been developed to help identify patients with delayed MTX elimination,[23] both largely based on the clinical experience in pediatric patients.

To further characterize the efficacy and safety of glucarpidase in pediatric, adolescent, and young adult patients, we performed a pooled, post hoc subgroup analysis of the glucarpidase compassionate-use clinical trials.

2 | METHODS

2.1 | Study design

This was a pooled, post hoc analysis of data from four multicenter, open-label, single-arm, glucarpidase compassionate-use trials (referred to as studies 001, 002, 003, and 006; Table 1). Study design and inclusion criteria for the four constituent clinical trials have been published previously.[13–16] These studies were conducted in compliance with Institutional Review Board, Independent Ethics Committee or other local or institutional standards for research involving human subjects.[13–16] Written informed consent was obtained from

TABLE 1 Study inclusion criteria for delayed MTX elimination and glucarpidase dosing details.

Study 001[9,13] 29 centers	Study 002[9,14,16] 149 centers	Study 003[9,15] 48 centers	Study 006[9,14,16] 136 centers
Inclusion criteria			
MTX concentration (μmol/L) • >5 at ≥42 hours *OR* **MTX concentration (μmol/L)** • >1 at ≥42 hours • >0.4 at ≥48 hours *AND* **Renal impairment** • sCr >1.5 × ULN and/or • Urine output <500 mL/24 hours	**MTX concentration (μmol/L)** • ≥10 at ≥42 hours *OR* **MTX concentration (μmol/L)** • >2 SD at >12 hours, and **Renal impairment** • sCr ≥1.5 × ULN, or • CrCl ≤60 mL/min	**MTX concentration (μmol/L)** • >2 SD at >12 hours • >10 at >36 hours • >5 at >42 hours • >3 at >48 hours *AND* **Renal impairment** • sCr >1.5 × ULN and increasing during MTX, or • Decreased diuresis	**Osteosarcoma:** **MTX concentration (μmol/L)** • >50 at 24 hours • >5 at 48 hours **All other patients:** **MTX concentration (μmol/L)** • >10 at >42 hours *OR* **MTX concentration (μmol/L)** • >2 SD at >12 hours, and **Renal impairment** • sCr >1.5 × ULN, or • CrCl ≤60 mL/min
Glucarpidase treatment route and regimen			
50 U/kg IV: up to 2 doses based on MTX level; at least 24 hours apart Second dose given if MTX >0.1 μmol/L >24 hours after glucarpidase	50 U/kg IV; single dose, or second dose at 24 hours, or second dose based on MTX level, 48 hours apart; or 3 doses 4 hours apart Second dose given if MTX decreased by ≥90% (or by 1 log) but still ≥1 μmol/L. Alternately, at 48 hours if initial plasma MTX >100 μmol/L	50 U/kg IV; single dose with option for second dose based on pre-specified change in MTX level Second dose given if MTX decreased by >1 log but still >1 μmol/L	50 U/kg IV; single dose with second dose after 48 hours in patients with MTX level >100 μmol/L

Abbreviations: CrCl, creatinine clearance; IV, intravenous; MTX, methotrexate; sCr, serum creatinine; SD, standard deviation; ULN, upper limit of normal

the patient or their guardian prior to treatment. Patients included in this subgroup analysis were those aged 28 days or older to less than 25 years old.

Patients enrolled in these studies had toxic MTX levels due to delayed MTX elimination and/or renal dysfunction, and underwent intravenous treatment with glucarpidase rescue. Patients had delayed MTX elimination based on a serum MTX concentration of greater than 2 standard deviations (SD) above the MTX elimination curve nomogram at 12 hours following HDMTX infusion (or >0.4 to >50 μmol/L at later time points; see Table 1). Renal impairment criteria implemented across the studies included serum creatinine higher than 1.5 times the upper limit of normal (ULN), creatinine clearance less than or equal to 60 mL/min, and/or decreased urine output (<500 mL/24 h; Table 1). Creatinine clearance was calculated using the Schwartz formula for patients less than or equal to 12 years old and using the Cockcroft–Gault formula for patients aged older than 12 years. Patients received intravenous glucarpidase at an initial recommended dose of 50 U/kg. Subsequent doses of glucarpidase were permitted if MTX levels remained elevated (criteria for administration of subsequent doses are provided in Table 1). Leucovorin dosing was restricted to 2–4 hours before or after administration of glucarpidase depending on the study. Patients received intravenous hydration and bicarbonate to maintain urine output and alkaline urine pH, and leucovorin, according to the HDMTX treatment regimen being followed.

2.2 | Assessments and endpoints

Plasma sampling for MTX central HPLC analysis was performed prior to glucarpidase dosing, and then periodically (according to the MTX treatment protocol/institutional standards) for up to 8 days post-glucarpidase treatment.

The primary efficacy endpoint was the proportion of patients achieving a CIR in plasma MTX concentration based on the central laboratory HPLC assay. Achievement of a CIR was defined as having plasma MTX concentration less than or equal to 1 μmol/L at all measurements after the first dose of glucarpidase. The proportion of patients with MTX rebound (defined as a rebound in MTX concentration of two times or higher the nadir MTX concentration and greater than 1 μmol/L above the nadir MTX concentration, following initial decrease of MTX concentration post-glucarpidase dosing) was also determined based on the central MTX HPLC assessments. Recovery of renal function was assessed based on changes in serum creatinine measured over time. Pharmacokinetic analyses of MTX and DAMPA were performed using central laboratory HPLC.

Safety was assessed based on the proportions of patients with adverse events (AEs; graded using Common Terminology Criteria for Adverse Events [CTCAE] v.3), and laboratory abnormalities that worsened by two or more CTCAE grades. The definition of treatment-emergent AEs included all confirmed treatment-emergent AEs plus all events with missing onset date.

2.3 | Statistical analysis

In this analysis, pediatric, adolescent, and young adult patients were defined by the following age groups: infants (patients aged ≥28 days to <2 years); children (patients aged ≥2 years to <12 years); adolescents (patients aged ≥12 years to <15 years); and young adults (patients aged ≥15 years to <25 years).

The primary analysis population (central HPLC population) comprised all pediatric and young adult patients who had one or more evaluations of MTX concentration from the HPLC assays after the first dose of glucarpidase. Sensitivity analyses were performed in the local MTX assay population, which comprised all patients who had one or more evaluations of MTX concentration from the local immunoassays after the first dose of glucarpidase.

For analysis of CIR, the number and percentage of patients who achieved a CIR were summarized with 95% confidence intervals (CI) using the Newcombe and Altman method.[24]

The renal evaluable population in which renal recovery was evaluated comprised patients with a pre-glucarpidase serum creatinine measurement and one or more post-glucarpidase serum creatinine measurement. The safety population comprised patients who received one or more doses of glucarpidase or who had evidence of follow-up after receiving the first dose of glucarpidase.

3 | RESULTS

3.1 | Patient demographics and clinical characteristics

There were 271 pediatric and young adult patients who met the study criteria, 86 of whom (31.7%) had one or more post-glucarpidase HPLC evaluations and were included in the central HPLC population. Of the 86 patients in the central HPLC population, 71 (83%) had documented evidence of impaired renal function and delayed MTX elimination pre-glucarpidase; eight of 86 (9%) had evidence of renal impairment but not delayed MTX elimination; two of 86 (2%) had evidence of delayed MTX elimination but not renal impairment; and five of 86 (6%) had no documented evidence of delayed MTX elimination or renal impairment. Patient demographics and clinical characteristics for the central MTX HPLC population are presented in Table 2. Approximately 75% of the patients were from North America, 21% from Europe, and a small number from Israel (2%) and Australia (2%). Over half of patients ($n = 47$, 55%) had a diagnosis of osteosarcoma. Demographic characteristics for patients who did not have one or more evaluations of MTX concentration from central HPLC (the local assay [non-HPLC] population) are presented in Table S1. Compared with the central MTX HPLC population, there were lower proportions of patients in the child and adolescent age groups with osteosarcoma in the local assay population (and higher proportions of patients with ALL and non-Hodgkin lymphoma).

3.2 | Outcomes in children, adolescents, and young adults

3.2.1 | MTX dosing and baseline clinical characteristics

Median intravenous MTX dose in the child, adolescent, and young adult age groups was 12.0, 12.0, and 8.0 g/m^2, respectively. Based on central MTX assessment, median pre-glucarpidase MTX concentration was 283.0, 57.8 and 36.4 μmol/L, respectively; and median pre-glucarpidase normalized creatinine clearance was 33.7, 44.8, and 46.4 mL/min/1.73 m^2, respectively. Hepatic impairment (defined as bilirubin >3 × ULN at the last pre-glucarpidase evaluation) was present in four (26.7%), five (23.8%), and six (15.0%) patients in the child, adolescent, and young adult age groups, respectively.

3.2.2 | Glucarpidase dosing

In each of the child, adolescent, and young adult age groups, the median time interval between MTX dosing (start of MTX infusion) and glucarpidase treatment was 3 days and the median glucarpidase dose was 50.0 U/kg. Most patients received either a single dose of glucarpidase or two doses.

3.2.3 | Reduction in serum MTX concentration following glucarpidase dosing

The proportion of patients achieving the primary efficacy endpoint of CIR based on central MTX HPLC concentrations is reported in Figure 1. Overall, a CIR was achieved by 38/86 patients (44.2%) in the central MTX HPLC population. A CIR was achieved by 31.3% of children (5/16), 29.2% (7/24) of adolescents, and 57.8% of young adults (26/45).

Among the 86 patients in the central MTX HPLC population (including the infant patient), the CIR rate was higher in those with pre-glucarpidase central MTX concentration less than 50 μmol/L versus those with pre-glucarpidase central MTX concentration greater than or equal to 50 μmol/L (83.3% vs. 2.7%). Among patients with ALL, 80.0% (12/15) achieved CIR. Of the 14 patients with non-Hodgkin lymphoma, 50.0% (7/14) achieved CIR. The rate of achieving a CIR among patients with osteosarcoma was 29.8% (14/47 patients). There was a numerical trend for increasing rates of CIR with increasing pre-glucarpidase creatinine clearance, with the highest rate of CIR among patients with pre-glucarpidase normalized creatinine clearance greater than or equal to 60 mL/min/1.73 m^2 (12/22, 54.6%). Pediatric and young adult patients who achieved CIR were less likely to have hepatic impairment, based on the rate of CIR in patients with bilirubin levels higher than 3 × ULN (3/15, 20.0%) versus those with bilirubin less than or equal to 3 × ULN (31/62, 50.0%).

TABLE 2 Demographic and clinical characteristics by pediatric and young adult age subgroups in the central MTX HPLC population ($N = 86$).

	Central MTX HPLC population ($N = 86$)			
	Infant ≥28 days to <2 years ($N = 1$)	**Children ≥2 to <12 years ($N = 16$)**	**Adolescent ≥12 to <15 years ($N = 24$)**	**Young adult ≥15 to <25 years ($N = 45$)**
Demographic characteristics				
Age, years (range)	0.4	10 (5–11)	13 (12–14)	16 (15–24)
Female sex, *n* (%)	($n = 1$) 1 (100)	($n = 10$) 7 (70.0)	($n = 20$) 14 (70.0)	($n = 34$) 11 (32.4)
Weight, kg (range)	($n = 1$) 6.6	($n = 16$) 41.0 (18.8–64.0)	($n = 23$) 51.3 (30.0–98.0)	($n = 43$) 69.8 (30.0–134.0)
BSA, m^2 (range)	($n = 1$) 0.3	($n = 16$) 1.3 (0.8–1.7)	($n = 23$) 1.5 (1.1–2.3)	($n = 43$) 1.9 (1.3–2.7)
Tumor type, *n* (%)	($n = 1$)	($n = 15$)	($n = 21$)	($n = 43$)
ALL	1 (100)	4 (26.7)	1 (4.8)	9 (20.9)
NHL	0	1 (6.7)	3 (14.3)	10 (23.3)
Osteosarcoma	0	10 (66.7)	15 (71.4)	22 (51.2)
Other	0	0	2 (9.5)[a]	2 (4.7)[b]
Clinical characteristics				
MTX dose, g/m^2 (range)	($n = 1$) 4.0	($n = 16$) 12.0 (1.0–20.0)	($n = 24$) 12.0 (2.0–20.0)	($n = 43$) 8.0 (1.0–18.0)
First glucarpidase dose, U/kg (range)	($n = 1$) 52.3	($n = 15$) 50.0 (44.3–54.9)	($n = 21$) 50.0 (31.0–51.7)	($n = 43$) 50.0 (14.9–60.0)
Number of glucarpidase doses, *n* (%)	($n = 1$)	($n = 16$)	($n = 24$)	($n = 45$)
1	1 (100)	7 (43.8)	17 (70.8)	29 (64.4)
2	0	8 (50.0)	7 (29.2)	13 (28.9)
3	0	1 (6.3)	0	3 (6.7)
Time between MTX and first glucarpidase dose, days (range)	($n = 1$) 5	($n = 16$) 3 (2–4)	($n = 22$) 3 (2–6)	($n = 44$) 3 (2–9)
Baseline (pre-glucarpidase) central MTX concentration, μmol/L (range)	($n = 1$) 5.7	($n = 13$) 283.0 (1.3–849.1)	($n = 22$) 57.8 (0.1–708.4)	($n = 43$) 36.4 (0.03–337.4)
Normalized baseline (pre-glucarpidase) calculated creatinine clearance, mL/min/1.73 m^2 (range)	($n = 1$) 182.8	($n = 16$) 33.7 (21.9–156.2)	($n = 19$) 44.8 (13.8–173.0)	($n = 42$) 46.4 (16.8–130.1)
<15 mL/min/1.73 m^2, *n* (%)	0	0	1 (4.2)	0
≥15 to <30 mL/min/1.73 m^2, *n* (%)	0	6 (37.5)	2 (8.3)	8 (17.7)
≥30 to <60 mL/min/1.73 m^2, *n* (%)	0	5 (31.3)	10 (41.7)	24 (53.3)
≥60 mL/min/1.73 m^2, *n* (%)	1 (100)	5 (31.3)	6 (25.0)	10 (22.2)
Hepatic impairment, n (%)	($n = 1$)	($n = 15$)	($n = 21$)	($n = 40$)
Bilirubin >3 × ULN	0	4 (26.7)	5 (23.8)	6 (15.0)

Note: Data are median (range) unless otherwise stated.

Abbreviations: ALL, acute lymphoblastic leukemia; BSA, body surface area; HPLC, high-performance liquid chromatography; MTX, methotrexate; NHL, non-Hodgkin lymphoma; ULN, upper limit of normal.

[a]Medulloblastoma ($n = 1$); malignant myofibroblastic tumor ($n = 1$).

[b]Ewing sarcoma ($n = 1$); relapsed Hodgkin lymphoma ($n = 1$).

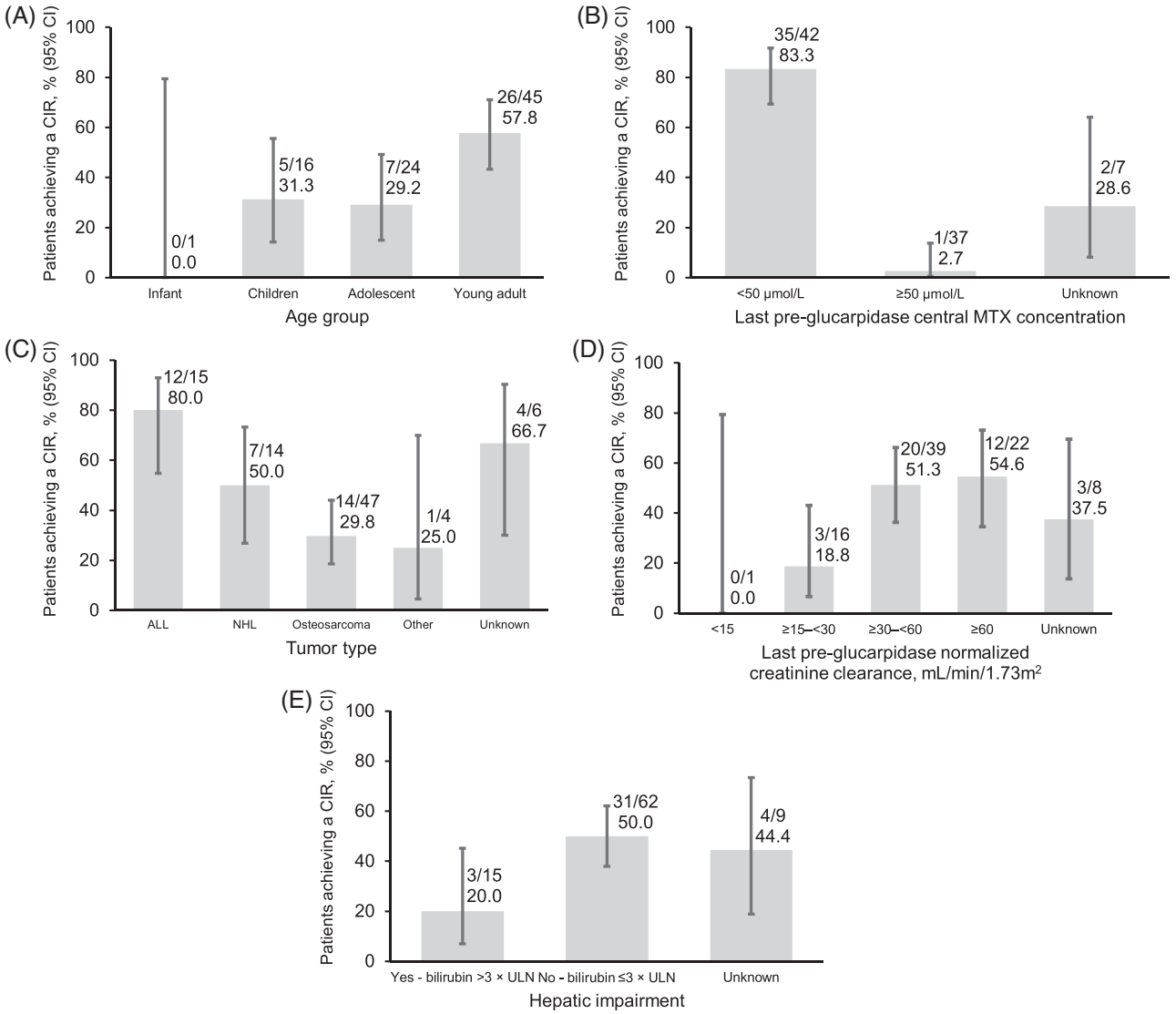

FIGURE 1 Clinically important reduction in MTX concentration in the (A) pediatric and young adult age groups, (B–E) and by selected subgroups (central MTX HPLC population [$N = 86$]). 95% CIs were calculated using the Newcombe and Altman method; creatinine clearance was calculated using the Schwartz formula for patients 12 years old and younger and using the Cockcroft–Gault formula for patients aged older than 12 years. ALL, acute lymphoblastic leukemia; CI, confidence interval; CIR, clinically important reduction; HPLC, high-performance liquid chromatography; MTX, methotrexate; ULN, upper limit of normal.

The median percentage change from baseline in MTX concentration over time (from the last pre-glucarpidase MTX measurement) is shown by age group in Figure 2. In the child, adolescent, and young adult age groups, the percentage reduction from pre-glucarpidase MTX concentration was higher than 95% at all time points following glucarpidase administration (except at the 240-minute time point in the child age group), and occurred within the first 15 minutes post-dosing. The proportion of patients who had MTX concentration rebound in the child, adolescent, and young adult age groups was 25.0% (3/12), 33.3% (7/21), and 18.4% (7/38), respectively.

Response data based on local MTX immunoassay were available in 241 patients. The proportion of infants, children, adolescents, and young adults with reduction in MTX concentration to less than or equal to 1 μmol/L as measured by local MTX assay was 60.0% (3/5), 46.3% (37/80), 44.8% (26/58), and 55.1% (54/98), respectively (Table S2).

3.2.4 | Pharmacokinetics and metabolism of DAMPA

Pharmacokinetic parameters for MTX and DAMPA based on central HPLC analyses are presented in Table S3 by age group. The mean DAMPA area under the concentration–time curve from 0 to 2 hours (AUC_{0-2}) in the child, adolescent, and young adult age groups was approximately 6—10-fold higher than the corresponding mean MTX AUC_{0-2}, consistent with the rapid conversion of MTX to DAMPA following glucarpidase administration. The mean half-life of DAMPA was approximately 10 hours across groups.

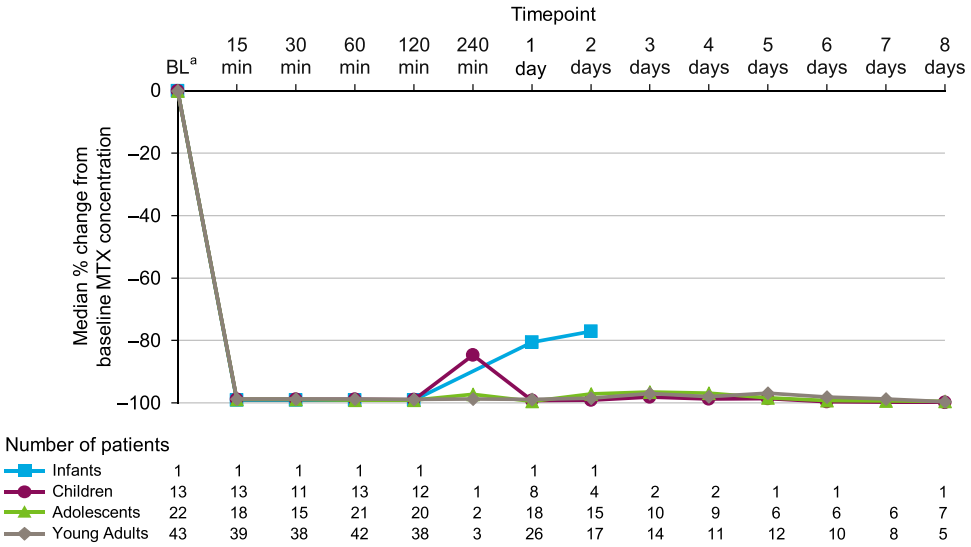

FIGURE 2 Median percentage change from pre-glucarpidase MTX concentration by age group over time (central MTX HPLC assessment). [a]Baseline was the last assessment prior to the first dose of glucarpidase. BL, baseline; HPLC, high-performance liquid chromatography; MTX, methotrexate.

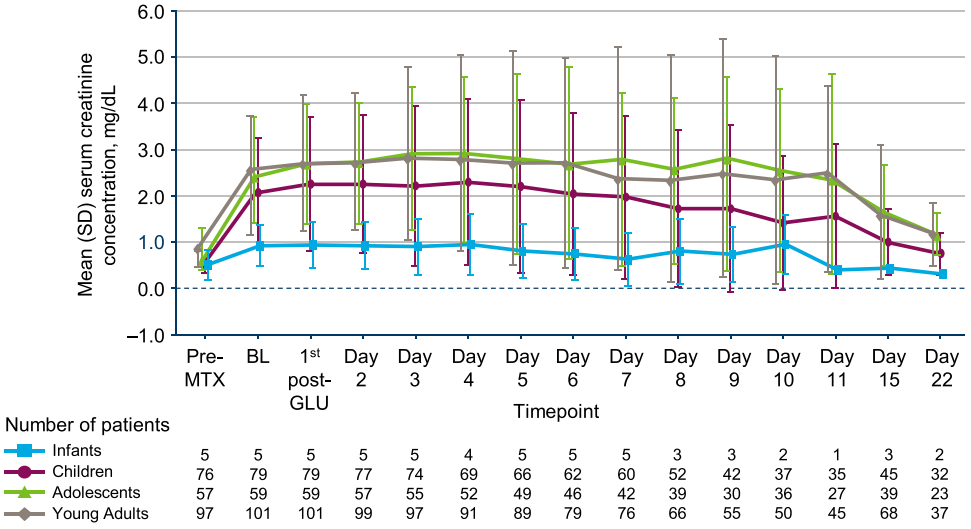

FIGURE 3 Mean serum creatinine concentration by age group over time (renal evaluable population). The renal evaluable population included patients with a baseline serum creatinine measurement and one or more post-baseline serum creatinine measurements. Pre-MTX is the last assessment prior to first dose of MTX (for patients who had a pre-glucarpidase measurement and one or more post-glucarpidase measurements). Baseline was the last assessment prior to the first dose of glucarpidase. BL, baseline; GLU, glucarpidase; MTX, methotrexate; SD, standard deviation.

3.2.5 | Recovery of renal function

Mean serum creatinine concentrations over time in the renal evaluable population are shown by age group in Figure 3. At the time of the last assessment, mean (SD) reductions from pre-glucarpidase baseline serum creatinine levels were similar in the child, adolescent, and young adult age groups (−1.16 [1.34], −1.15 [1.64], and −1.15 [1.55] mg/dL, respectively), demonstrating that renal function returned to baseline levels over the course of 22 days post-glucarpidase dosing.

3.3 | Summary of safety

Table 3 provides a summary of safety among the 271 pediatric and young adult patients who received one or more doses of glucarpidase or had evidence of follow-up after the first dose of glucarpidase. Treatment-related AEs occurred in seven of 83 children (8.4%), 11/67 (16.4%) adolescents, and 17/115 (14.8%) young adults. The most commonly occurring treatment-related AE was paresthesia, occurring in three adolescents (4.5%) and six young adults (5.2%). No other

TABLE 3 Summary of safety by age group.

	Main safety population[a]			
	Infant ≥28 days to <2 years (*N* = 6)	Children ≥2 to <12 years (*N* = 83)	Adolescent ≥12 to <15 years (*N* = 67)	Young adult ≥15 to <25 years (*N* = 115)
TEAE[b,c]	0 (0)	7 (8.4)	11 (16.4)	17 (14.8)
Grade ≥3	0 (0)	3 (3.6)	3 (4.5)	3 (2.6)
Grade ≥4	0 (0)	3 (3.6)	1 (1.5)	2 (1.7)
TEAEs occurring in more than one patient				
Paresthesia	0 (0)	0 (0)	3 (4.5)	6 (5.2)
Flushing	0 (0)	0 (0)	3 (4.5)	3 (2.6)
Feeling hot	0 (0)	0 (0)	2 (3.0)	2 (1.7)
Burning sensation	0 (0)	1 (1.2)	1 (1.5)	2 (1.7)
Headache	0 (0)	2 (2.4)	1 (1.5)	1 (0.9)
Hypoesthesia	0 (0)	2 (2.4)	0 (0)	1 (0.9)
Tremor	0 (0)	1 (1.2)	1 (1.5)	1 (0.9)
Nausea	0 (0)	1 (1.2)	0 (0)	1 (0.9)
Vomiting	0 (0)	0 (0)	2 (3.0)	0 (0)
Hypersensitivity	0 (0)	0 (0)	1 (1.5)	1 (0.9)
Hemoglobin decreased	0 (0)	2 (2.4)	0 (0)	0 (0)
Platelet count decreased	0 (0)	2 (2.4)	0 (0)	0 (0)
White blood cell count decreased	0 (0)	2 (2.4)	0 (0)	0 (0)
Pruritus	0 (0)	0 (0)	0 (0)	2 (1.7)
Rash	0 (0)	0 (0)	0 (0)	2 (1.7)
Hypotension	0 (0)	1 (1.2)	1 (1.5)	0 (0)
Any TEAE[b]	5 (83.3)	76 (91.6)	57 (85.1)	102 (88.7)
Any Grade ≥3 AE[b,d]	4 (66.7)	55 (66.3)	38 (56.7)	70 (60.9)
Any Grade ≥4 AE[b,d]	2 (33.3)	36 (43.4)	20 (29.9)	35 (30.4)
Serious AE[b]	2 (33.3)	38 (45.8)	21 (31.3)	38 (33.0)
AEs with outcome of death	1 (16.7)	6 (7.2)	2 (3.0)	9 (7.8)

Note: Data are the number of patients with ≥1 event, *n* (%).
Abbreviations: AE, adverse event; TEAE, treatment-emergent AE.
[a]Only includes patients who have evidence of follow-up post-glucarpidase.
[b]Includes all TEAEs plus all events with missing onset date.
[c]Excludes AEs where relationship to treatment was unknown.
[d]Excludes AEs where severity grade was unknown.

treatment-related AE occurred in greater than or equal to 5% of patients in any age group. Grade ≥3 and grade ≥4 treatment-related AEs occurred in fewer than 5% of patients in any age group.

AEs of any causal relationship and with a fatal outcome occurred in six patients (7.2%) in the child age group, two patients (3.0%) in the adolescent age group, and nine patients (7.8%) in the young adult age group. AEs with a fatal outcome are listed in Table S4. AEs with a fatal outcome that occurred more than once were death (not otherwise specified; *n* = 3), multiorgan failure (*n* = 2), and infection (*n* = 3).

A summary of patients who had laboratory abnormalities that worsened by at least two CTCAE grades during the studies is reported in Table S5. In the child, adolescent, and young adult age groups, worsening elevations in aspartate aminotransferase occurred in 10.6%, 14.3%, and 7.7% of patients, respectively; worsening elevations in alanine aminotransferase occurred in 11.0%, 17.2%, and 11.4% of patients, respectively, and worsening elevations in bilirubin occurred in 9.9%, 12.5%, and 5.9%, respectively. Across the age groups, worsening reductions in potassium levels and hematologic parameters,

including hemoglobin levels, and leukocyte, neutrophil, and platelet counts, occurred relatively frequently.

3.4 | Outcomes in infant patients

There was one patient in the central HPLC population aged 28 days or older to less than 2 years old. Outcomes for this single patient are summarized here. The female infant patient, who had a diagnosis of ALL, received an intravenous MTX dose of 4.0 g/m^2. Based on central MTX assessment, the patient had a pre-glucarpidase MTX concentration of 5.7 μmol/L and pre-glucarpidase normalized creatinine clearance of 182.8 mL/min/1.73 m^2. The patient received a single glucarpidase dose of 52.3 U/kg, with 5 days between MTX dosing and treatment with glucarpidase. The patient did not achieve a CIR in MTX. The percentage reduction in MTX concentration was 99.1% during the first 15–120 minutes post-glucarpidase, but MTX levels rebounded to a 77.1% reduction compared to pre-glucarpidase levels over days 1–2. At the time of the last assessment, mean (SD) reduction from pre-glucarpidase baseline serum creatinine levels was −0.38 (0.04) mg/dL.

There were six infant patients who received one or more doses of glucarpidase or had evidence of follow-up after the first dose of glucarpidase (i.e., who were included in the safety population). There were no treatment-related AEs among the six infants. AEs of any causal relationship and with a fatal outcome occurred in one patient (16.7%) in the infant age group.

4 | DISCUSSION

In this post hoc age subgroup analysis of glucarpidase compassionate-use clinical trials, glucarpidase had an immediate (i.e., within 15 minutes) and sustained effect on reducing MTX concentrations in pediatric and young adult patients with delayed MTX elimination and/or renal dysfunction. While the proportion of patients achieving a CIR generally increased with age, the initial median percentage reduction in MTX was greater than 95% in all age groups. Pharmacokinetic data based on central HPLC analyses of MTX and DAMPA were also consistent with rapid conversion of MTX to DAMPA following the administration of glucarpidase. There were no unexpected safety findings observed in the pediatric and young adult patient population, and no clear relationship between age group and the incidence of treatment-related AEs. These observations in the pediatric and young adult subgroup were generally consistent with observations in the overall study population (i.e., patients aged 0–84 years old),[9] suggesting that no glucarpidase dose adjustment is required in these patients.

The primary efficacy endpoint in this analysis of a CIR required patients to achieve an MTX concentration less than or equal to 1 μmol/L at all measurements taken post-glucarpidase. Patients with a very high MTX concentration prior to glucarpidase administration are unlikely to be able to achieve a reduction below 1 μmol/L given the high initial concentration of MTX and negative feedback on MTX elimination due to high concentrations of DAMPA.[15,16] However, the initial reduction in MTX concentration of higher than 95% observed in all age groups is clinically meaningful and relevant owing to the decreased exposure to toxic effects of MTX. While a CIR was the predefined primary efficacy endpoint in the constituent glucarpidase compassionate-use trials in this analysis, future studies should establish whether other objective measures of glucarpidase efficacy are more clinically meaningful and/or better suited to monitoring the effectiveness of glucarpidase in clinical practice.

Compared with the overall study population, there were differences in the characteristics of patients across the four age groups in this analysis, most notably the proportion of patients with osteosarcoma in the pediatric and young adult subgroups. Over half (55%) of the pediatric and young adult population had osteosarcoma, whereas 32% of the overall population had osteosarcoma.[9] Osteosarcoma was the most common tumor type in the child, adolescent, and young adult age groups. Non-Hodgkin lymphoma was more common in the older age groups (adolescent and young adult) than in the younger age groups (infant and child). The observed general increase in the rate of achieving CIR with increasing age could be attributed to a greater proportion of younger patients with osteosarcoma who hence received higher MTX doses, resulting in correspondingly higher pre-glucarpidase MTX concentrations, and thus making CIR more difficult to achieve for these patients. The analysis by pre-glucarpidase MTX concentration—where patients with pre-glucarpidase MTX concentrations less than 50 μmol/L were more likely to achieve CIR than patients with pre-glucarpidase MTX concentrations greater than or equal to 50 μmol/L, regardless of age group—suggests that pre-glucarpidase MTX concentration rather than age was prognostic of achieving a CIR. Efficacy data based on local immunoassay detection of MTX also supported the efficacy of glucarpidase in reducing MTX concentrations in pediatric and young adults.

Efficacy findings in this subgroup analysis are consistent with studies of glucarpidase use in pediatric oncology patients where glucarpidase rapidly reduced MTX plasma concentrations and restored renal function.[17,19] Similarly in these studies, reductions in MTX levels as measured using HPLC were larger than those measured using immune-based methods.[17,19]

The safety profile of glucarpidase in the pediatric and young adult population was in line with previous observations, with a similar pattern for the types and frequency of treatment-related safety events observed and no unexpected safety findings.[13–16,25]

A limitation of this subgroup analysis is the small sample size, particularly within the infant age group, which limits comparison of efficacy and safety in the infant population versus other age groups. In addition, sample size in the central HPLC population and renal recovery population was relatively small, limiting interpretation of findings in these analyses. Furthermore, differences in the baseline characteristics of patients across the age groups, including tumor type and the corresponding dose of MTX received, are potential confounders for comparison between age groups. A final limitation is that the formation of anti-glucarpidase antibodies and their impact on the pharmacological efficacy of glucarpidase were not assessed in this analysis.

Published clinical data have demonstrated that although formation of anti-glucarpidase antibodies was more likely following repeated glucarpidase dosing, there was no association with glucarpidase-related AEs.[25]

5 | CONCLUSIONS

While the rate of CIR was lower in patients with pre-glucarpidase MTX concentration greater than or equal to 50 μmol/L versus those with pre-glucarpidase MTX concentration less than 50 μmol/L, the median percentage reduction in MTX concentration was higher than 95% at the first post-glucarpidase measurement across each of the four age groups evaluated. After accounting for pre-glucarpidase MTX concentration, the efficacy of glucarpidase for inducing CIR in MTX levels was in line with observations in the overall population (i.e., patients aged 0–84 years; 59% rate of CIR),[9] with no unexpected safety findings observed in this patient population. This demonstrates that glucarpidase, dosed at 50 U/kg, is an effective and well-tolerated rescue agent for pediatric, adolescent, and young adult patients.

AUTHOR CONTRIBUTIONS

Conception and design of the study: Katherine A. Janeway, Luis Gros, Stefan Schwartz, Emma Thomas, Claire Daugherty, and Carmelo Rizzari. Data acquisition: Stefan Schwartz. Data analysis and interpretation: Katherine A. Janeway, Luis Gros, Stefan Schwartz, Claire Daugherty, Eva Gallardo, Christon Hill, Emma Thomas, Suzanne Ward, and Carmelo Rizzari. Drafting/revising the manuscript for critically important intellectual content: Katherine A. Janeway, Luis Gros, Stefan Schwartz, Claire Daugherty, Eva Gallardo, Christon Hill, Emma Thomas, Suzanne Ward, and Carmelo Rizzari. All authors read and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

Katherine A. Janeway reports fees for consulting from Bayer and Ipsen; and honoraria from Takeda and Foundation Medicine. Luis Gros reports no relevant disclosures of interest. Stefan Schwartz has received honoraria as an advisory board member and received a research grant from BTG International Inc. Claire Daugherty is a consultant biostatistician for BTG International Inc. Eva Gallardo and Emma Thomas are employees of Protherics Medicines Development Ltd. Christon Hill and Suzanne Ward are employees of BTG International Inc. Carmelo Rizzari has received honoraria as an advisory board member and speaking fees from BTG International Inc.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Katherine A. Janeway https://orcid.org/0000-0001-6000-3594
Emma Thomas https://orcid.org/0000-0002-4225-096X
Carmelo Rizzari https://orcid.org/0000-0002-4828-3893

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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