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REVIEW



# *"Per aspera ad astra"*: the transformative contribution of glucarpidase to the improved management of delayed methotrexate elimination after high-dose therapy

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**ABSTRACT**

**Introduction:** High-dose methotrexate (HDMTX) is an important therapeutic tool for various malignancies; its use can be associated with severe treatment-emergent toxicities, particularly nephrotoxicity. These can disrupt anticancer treatment and increase morbidity and mortality. Glucarpidase (carboxypeptidase G2), a recombinant bacterial enzyme, rapidly converts toxic levels of circulating methotrexate (MTX) into nontoxic metabolites in patients with delayed MTX elimination and/or at risk of MTX toxicity. The reduction in MTX-associated toxicity and mortality can improve patients' outcomes. MTX elimination prevents the progression of renal toxicity and may minimize treatment disruptions by facilitating resumption of anticancer treatment, including HDMTX rechallenge. Currently, glucarpidase is underused in clinical practice, partly due to accessibility issues and uncertainty regarding treatment timings.

**Areas covered:** This review aims to provide clarity into the optimal application of glucarpidase by exploring its history and development, reviewing the clinical benefits reported in clinical trials and from real-world experiences, and critically considering recommendations for its administration.

**Expert opinion:** Glucarpidase is an invaluable tool in the management of MTX toxicity, allowing rapid and effective reduction of MTX toxic drug levels, especially in patients with compromised renal function. To update glucarpidase administration algorithms, research is needed to evaluate its efficacy in patients with moderate MTX elevations.

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Acute lymphoblastic leukemia; glucarpidase; high-dose therapy; methotrexate; non-Hodgkin lymphoma

## 1. Introduction

High doses of the antifolate agent methotrexate (MTX; equating to doses ≥500 mg/m$^2$ infused over 2–36 h), hereafter referred to as high-dose MTX (HDMTX), have been used for decades to treat various adult and pediatric malignancies [1–4]. Such malignancies include acute lymphoblastic leukemia, non-Hodgkin lymphoma, primary central nervous system (CNS) lymphoma, and osteosarcoma. Although HDMTX remains an important anticancer treatment, it is associated with severe and potentially life-threatening toxicities [1,3,4]. Renal toxicities, observed to varying degrees [5], represent one of the most prevalent classes of toxicity observed during HDMTX treatment [1,3,4]. Acute kidney injury (AKI), manifesting as an increase in serum creatinine and a decrease in glomerular filtration rate, occurs in up to 12% of adult and 8% of pediatric patients [1,3,4]. Other HDMTX-related toxicities include hematologic, neurologic, and hepatic adverse events, oral mucositis and gastrointestinal toxicity [3,6]. Consequently, AKI and other toxicities not only increase morbidity and mortality but may also lead to interruptions in, or withdrawal of, MTX treatment [3].

The primary route of elimination for MTX is via the kidneys, accounting for 70–90% of the overall clearance [4]. However, after HDMTX administration, MTX and its metabolites can accumulate within kidney tissue; this can result in crystal precipitation in the renal tubules, leading to nephrotoxicity and AKI [3,4,6,7]. When renal function is impaired, MTX elimination is delayed, prolonging drug exposure and further exacerbating nephrotoxicity [3,4].

Supportive measures (urine alkalinization, aggressive fluid hydration, and – in patients with volume overload – diuretics [8]) and folinic acid rescue (a biologically active derivative of folate) are standard approaches for mitigating HDMTX toxicities [3,4,9]. However, other interventions may be required in select patients, such as glucarpidase (the focus of this review) or hemodialysis [8].

Glucarpidase (carboxypeptidase G2) is a recombinant bacterial enzyme that converts MTX into nontoxic metabolites. Glucarpidase is used to rapidly reduce MTX levels in patients with delayed MTX elimination (DME) and/or those at risk of MTX toxicity [4,6,10]. While glucarpidase is highly effective [11,12], it seems to be underused in clinical practice. This could be due to factors such as cost, accessibility, and a historic lack of guidelines [4,8,13,14]. Such factors may have contributed to confusion among practicing clinicians over which patients may benefit, and when and how it should be administered. To provide insight into its optimal application, this review discusses the history and

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**Article highlights**

- High-dose methotrexate (HDMTX) remains an important therapeutic tool for various adult and pediatric malignancies, but it is associated with severe and potentially life-threatening toxicities, particularly nephrotoxicity.
- Glucarpidase rapidly reduces toxic levels of circulating MTX by converting it into nontoxic metabolites and is able to achieve >90% median reductions in plasma MTX concentrations within 15 min of administration.
- In the absence of randomized trials, real-world data in adults and children have provided further insights into the effectiveness and safety of glucarpidase as a rescue therapy.
- Expert consensus papers recommend glucarpidase administration as early as possible (within 48–60 h of HDMTX administration), as life-threatening toxicities may not be preventable beyond this point.
- Decision-making algorithms relying on consensus MTX thresholds and clinical cutoffs may be incomplete, with glucarpidase demonstrating efficacy in patients with moderate MTX elevations considered outside classical intervention thresholds.

development of glucarpidase, and considers the clinical benefits associated with the drug, based on clinical trial and real-world data. It also evaluates current recommendations for glucarpidase administration and explores potential future directions.

## 2. Glucarpidase discovery and development

The potential of bacterial carboxypeptidases to reduce plasma MTX levels and manage MTX-related toxicity was first described in 1972, in animal models evaluating the use of a non-recombinant carboxypeptidase G1 (Figure 1 [3,15–23]). Carboxypeptidase G2, the naturally occurring bacterial enzyme on which glucarpidase is based, was subsequently isolated and cloned from a *Pseudomonas* strain [16–18,24]. It was then sequenced, characterized, and expressed in *Escherichia coli* and *Pseudomonas putida* in the early 1980s, and its crystal structure determined in the late 1990s [16–18,24]. Carboxypeptidase G2 has distinct properties compared with other carboxypeptidases, including a high affinity for MTX and 5-methyl tetrahydrofolate (5-MTHF; an active metabolite of folinic acid) versus 5-formyltetrahydrofolate (folinic acid) [18].

These studies paved the way for the clinical development of glucarpidase, the commercial recombinant form of carboxypeptidase G2 produced in genetically modified *E. coli* [10]. Glucarpidase was first made available (off label) in the US and Europe in 1993 under a compassionate-use program. In the opinion of this review's authors, the Latin motto used in the title of this review '*Per aspera ad astra*' [3,15–23] (Figure 1) perfectly applies to the length of time taken for glucarpidase regulatory approvals, based on data from single-arm trials and compassionate-use studies, which eventually resulted in approval in several countries.

As a zinc peptidase, glucarpidase acts by rapidly metabolizing circulating (not intracellular) MTX and its active metabolite (7-hydroxymethotrexate) into two non-cytotoxic, systemically inactive metabolites (glutamate and 4-deoxy-4-amino-N10-methylpteroic acid [DAMPA]) [4,19,25,26], via hydrolyzation of the amide linkage (Figure 2 [4,8,18–20]). Clinical data have shown that, like carboxypeptidase G2, glucarpidase also rapidly hydrolyzes 5-MTHF [27]. DAMPA is eliminated by the kidneys [20] and the liver (via bile) [8] and is therefore eliminated more rapidly than MTX itself [19]. Thus, glucarpidase rapidly reduces toxic plasma MTX concentrations, relieves renal burden, and prevents further MTX-related nephrotoxicity.

## 3. Effectiveness and safety

Although no randomized, placebo-controlled trials were ever conducted for ethical reasons, the clinical efficacy/effectiveness and safety of glucarpidase have been reported in single-arm, open-label clinical trials and compassionate-use studies (summarized in Table 1 [5,20,21,23,27–30]). These studies, on which approvals were based, included patients with renal dysfunction or toxic MTX levels who received glucarpidase to overcome DME and/or HDMTX toxicity [5,20,21,23,27,28,30]. Patients were of varying ages, from young infants to elderly adults, and had both hematologic and solid neoplasms.

At the now clinically approved dose (single intravenous dose of 50 units/kg), glucarpidase was well tolerated, with only minor adverse events reported [20,21,23,27,28,30]. Across all studies, glucarpidase was associated with a >95% median reduction in plasma MTX concentrations, usually observed within 15 min of administration. These reductions lasted several days in most patients [21,28], despite a rebound effect caused by the release of intracellular MTX [20,23,27,30]. Patients with lower pre-glucarpidase MTX levels typically had better and more sustained responses than those with higher pre-dose levels [23,30]. Age appeared not to impact outcomes. A post hoc analysis of the compassionate-use data demonstrated the effectiveness of glucarpidase in children, adolescents, and young adults to be similar to that seen in the wider patient population (age range 0–84 years) after adjustment for pre-dose MTX levels [30]. With some exceptions, second doses of glucarpidase (where administered to patients with high pre-first-dose MTX concentrations) did not result in further marked reductions in MTX levels, mostly due to enzyme inhibition by the DAMPA metabolite [21,28].

Alongside reductions in plasma MTX levels, glucarpidase administration is associated with improvements in renal outcomes and rapid normalization/improvement in serum creatinine in most patients [5,20,21,23,27,29,30]. Younger patients appear to have better renal outcomes after glucarpidase treatment, compared with older individuals [23]. In some studies, additional cycles of MTX were administered after recovery of renal function, mostly without recurrence of nephrotoxicity [5,21,27].

Severe MTX-related toxicities and deaths were still seen after glucarpidase administration in some patients; however, in the absence of randomized trials, it is impossible to determine the direct impact of glucarpidase on these outcomes [5,20,21,23,27,28]. In a multivariate analysis of data from one of these studies, identified risk factors for severe MTX-related toxicity and mortality included severe MTX toxicity at the time of glucarpidase administration, inadequate initial folinic acid dosing, and delayed administration of glucarpidase (>96 h after the start of the MTX infusion) [27]. These results indicate the importance of patient selection, co-administration of supportive therapies including folinic acid, and early glucarpidase intervention.

Lastly, following a dose-confirmatory safety and pharmacokinetic trial in healthy volunteers in Japan [31], a phase 2 trial showed that glucarpidase (single intravenous dose of 50 units/kg) was both

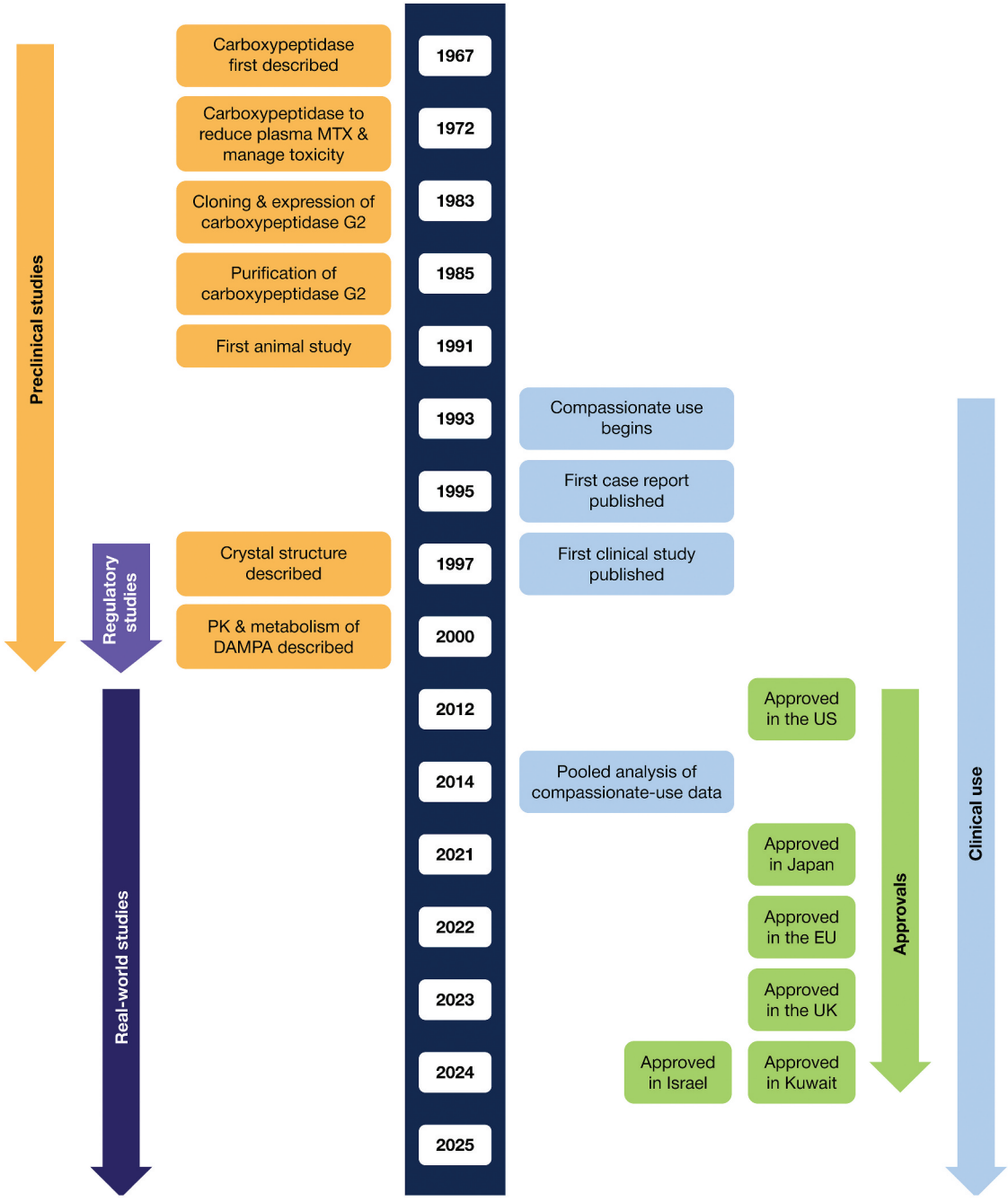


Figure 1. Timeline of the discovery, development, and approval of glucarpidase: '*Per aspera ad astra*.' [3,15–23].

Abbreviations: DME, delayed methotrexate elimination; DAMPA, 4-deoxy-4-amino-N10-methylpteroic acid; MTX, methotrexate; PK, pharmacokinetics.

well tolerated and efficacious in reducing MTX concentrations and mitigating toxicity in Japanese patients with DME after HDMTX [29].

## 4. Approvals and indications

Indications for glucarpidase vary by region. In the US, glucarpidase (approved in 2012; label last revised in 2019) is indicated to reduce toxic plasma MTX concentration (>1 µmol/L, timepoint not specified, but excluding patients with MTX concentrations within 2 standard deviations of the mean MTX excretion curve specific for the administered MTX dose, e.g. 42–48 h after start of MTX administration) in adult and pediatric patients with delayed MTX clearance due to impaired renal function [32]. In the EU, glucarpidase (approved in 2022; Summary of Product Characteristics last revised in 2024) is indicated to reduce toxic plasma MTX concentration in adults and children aged ≥28 days with DME or at risk of MTX toxicity [33]. Both the US and EU labels define DME as plasma MTX concentrations greater than two standard deviations of the mean MTX curve specific for the dose administered.

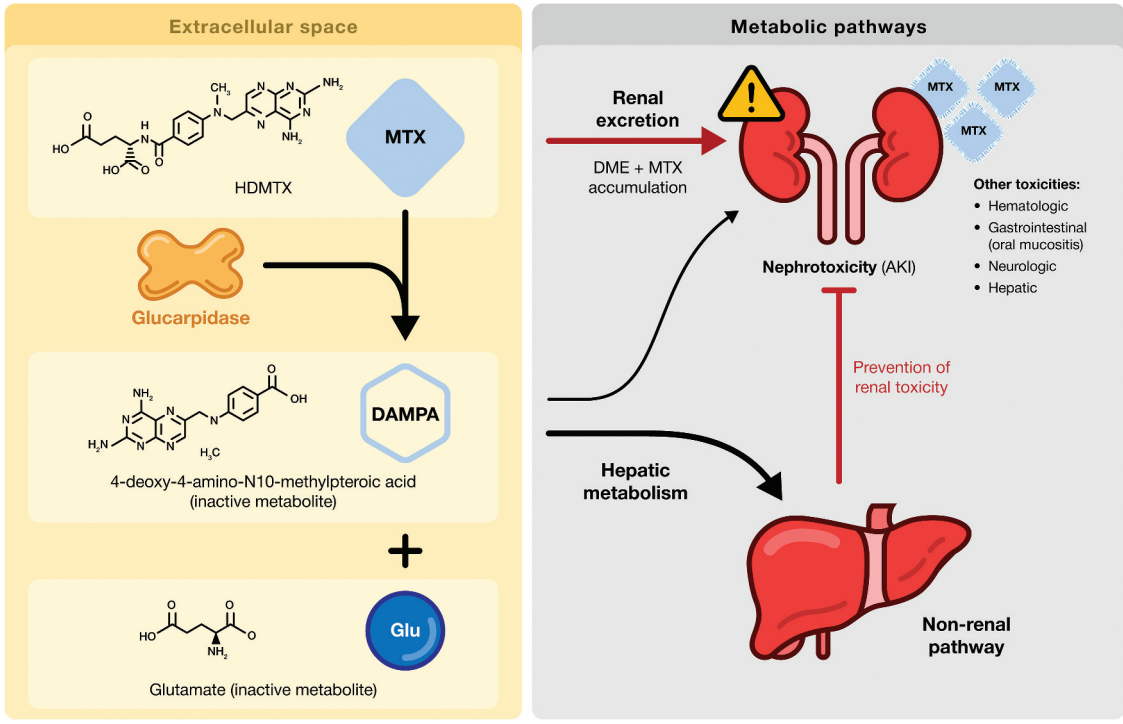


Figure 2. Mechanism of action of glucarpidase [4,8,18–20].

Abbreviations: AKI, acute kidney injury; DAMPA, 4-deoxy-4-amino-N10-methylpteroic acid; DME, delayed methotrexate elimination; Glu, glutamate; HDMTX, high-dose methotrexate; MTX, methotrexate.

By clearly defining the MTX threshold concentration for glucarpidase intervention (as >1 μmol/L), determining eligibility for treatment is simplified in the US labeling. In contrast, the EU label provides more complex, tabulated guidance on the threshold MTX concentrations that should prompt glucarpidase administration (Table 2) [33]. Although not specified in the US Prescribing Information, the EU label states that once the diagnosis of DME or risk for MTX toxicity is established, glucarpidase should be administered without delay; for patients with DME, the optimal window for administration is within, or no later than, 48–60 h from the start of HDMTX infusion. The EU label also provides recommendations for glucarpidase intervention based on MTX dose and infusion duration [33].

In both the US and the EU, the approved dosage of glucarpidase is 50 units/kg as a single intravenous injection over 5 min [32,33]. Renal dysfunction does not impact glucarpidase concentrations and does not necessitate dose adjustment [32–34].

## 5. Real-world data on glucarpidase in adults

Real-world data in adults with various malignancies have provided further insights into the effectiveness and safety of glucarpidase as a rescue treatment for DME and/or MTX toxicity (Table 3 [11,35,36]). In one of the largest studies reported to date, and the only one to utilize a control group, glucarpidase was given within 96 h of HDMTX administration to patients with MTX-induced AKI and mainly hematologic neoplasms [11]. Here, glucarpidase administration was associated with a 2.7-fold higher adjusted odds of renal recovery, faster time to renal recovery, and lower risk of selected extrarenal toxicities (grade ≥2 elevated transaminase and neutropenia) versus no administration [11]. Of note, as a multicenter, retrospective study, there were several limitations, including patient selection bias, heterogeneity in the supportive care protocols used, and variability in the indications for glucarpidase administration across the many centers included in the study.

Information derived from this study indicated particularly high odds of renal recovery if glucarpidase was administered within 60 h of HDMTX infusion [37]. From an oncology perspective, better renal recovery and tolerability could potentially enable HDMTX rechallenge and resumption of anticancer treatment. MTX rechallenge was consistently demonstrated in a small number of adults with lymphoma who required glucarpidase for toxic MTX levels after administration of HDMTX [38]. In this study, seven patients who were rechallenged with HDMTX after glucarpidase administration received a median of 5 (range, 1–7) additional MTX doses, with no patients requiring glucarpidase rescue during any of the subsequent HDMTX courses [38].

Some authors have questioned the need for glucarpidase, proposing that high-dose folinic acid alone might be sufficient to manage DME and toxicity [39,40]. However, as high doses of folinic acid administered shortly after HDMTX infusion might reduce progression-free survival and increase the risk of relapse, facilitating administration of a lower folinic acid dose through glucarpidase treatment has the potential to improve clinical outcomes [41,42].

Table 1. Main findings reported in clinical trials and compassionate-use studies investigating the safety and efficacy of glucarpidase over the past three decades [5,20,21,23,27–30].

| Design | Patient population (age) | MTX dose | Outcomes | Reference |
|---|---|---|---|---|
| Compassionate use (reported again in 2004 literature review) | 20 patients with osteosarcoma, lymphoma, or gastric cancer and MTX-induced renal dysfunction (0.3–60 years) | NR | • Serum MTX by HPLC decreased by 95.6%–99.6% within 15 min (median 98.7%); rebound in 60% of patients (2.5%–8.8% of initial levels); no further decreases with second or third doses<br>• Serum creatinine returned to normal at a median of 22 days<br>• HDMTX rechallenge in 5/20 patients (full dose, $n = 1$; 20%–75% dose, $n = 4$), with no nephrotoxicity<br>• Deaths in 2/20 patients; mild-to-moderate MTX toxicity in 18/20 patients | Widemann et al. [21,59] |
| Phase 2 trial/ compassionate use | 82 patients with ALL, NHL, osteosarcoma, or other malignancy and acute MTX toxicity with renal failure (0.9–71.8 years) | 1–12 g/m$^2$ | • Serum MTX by HPLC decreased by 97% within 15 min in 65 evaluable patients; ongoing decrease during follow-up; no further decreases with second dose ($n = 9$)<br>• 4 patients died due to myelosuppression and septic complications 7–22 days after MTX infusion | Buchen et al. [28] |
| Clinical trial | 43 patients with ALL, lymphoma with or without CNS involvement, or solid tumors and renal dysfunction with DME after HDMTX (18–78 years) | 0.9–12.1 g/m$^2$ | • Serum MTX by HPLC decreased by >97% within a median of 15 min in 24 evaluable patients; decrease sustained during follow-up (rebound increases did not exceed 1.2 µmol/L in 23/24 patients); small additional decrease with second dose ($n = 3$)<br>• Serum creatinine improved or normalized in 40/43 patients (93%)<br>• One patient received a low dose and demonstrated a markedly reduced pharmacologic effect<br>• 10/43 patients (23%) died due to complications of HDMTX; 9 patients (21%) died due to causes unrelated to HDMTX; 8/43 patients (19%) experienced grade 3/4 renal toxicity<br>• Patients with ≥3 contributory risk factors for DME had poorer survival than those with <3 risk factors (HR 3.64; 95% CI 1.14–17.54) | Schwartz et al. [20] |
| Compassionate use | 100 patients with osteosarcoma, NHL, ALL, or other diagnosis and HDMTX-induced renal dysfunction (0.3–82 years) | Median 7.7 g/m$^2$ (range 0.4–12 g/m$^2$) | • Serum MTX by HPLC decreased by >98% within 15 min; no further decreases with second or third doses<br>• Serum creatinine normalized at a median of 22 days in 70% of patients<br>• HDMTX rechallenge in 12/88 surviving patients after renal recovery, with no further toxicity<br>• 12/100 patients (12%) died, of which 6 (6%) were related to MTX<br>• Grade 4 toxicity before glucarpidase, inadequate initial folinic acid dose, and glucarpidase given >96 h after start of MTX all associated with grade 4/5 toxicity | Widemann et al. [27] |
| Pooled analysis of four compassionate-use studies | 476 patients with osteosarcoma, NHL, ALL, primary CNS lymphoma, or other diagnosis and AKI after HDMTX (0–84 years) | 12 g/m$^2$ (43 patients), 4 g/m$^2$ (103 patients) | • ≥95% median reduction in serum MTX by HPLC in 136/156 patients, at a median of 15 min post-glucarpidase; CIR in 83/140 patients (59%) with pre-glucarpidase MTX >1 µmol/L<br>• Patients with pre-glucarpidase MTX <50 µmol/L more likely to achieve CIR than those with MTX ≥50 µmol/L<br>• Of 410 patients with grade ≥2 creatinine values, 262 (64%) recovered to grade 0/1, with median time to recovery of 12.5 days; better renal recovery in younger patients | Widemann et al. [23] |

(*Continued*)

Table 1. (Continued).

| Design | Patient population (age) | MTX dose | Outcomes | Reference |
|---|---|---|---|---|
| Pooled analysis of four compassionate-use studies (pediatric data) | 86 infants (≥28 days to <2 years), children (≥2 to <12 years), adolescents (≥12 to <15 years), and young adults (≥15 to <25 years) with osteosarcoma, NHL, ALL, or other diagnosis receiving HDMTX (0.4–24 years) | 1–20 g/m$^2$ | • ≥98.7% median reduction in serum MTX by HPLC in each age group at 15 min; CIR in 36/86 patients (44.2%): 0/1 infants (0%), 5/16 children (31.3%), 7/24 adolescents (29.2%), and 26/45 young adults (57.8%)<br>• Patients with pre-glucarpidase MTX <50 µmol/L (35/42; 83.3%) more likely to achieve CIR than those with MTX ≥50 µmol/L (1/37; 2.7%)<br>• Serum creatinine returned to baseline values in all patients over 22 days | Janeway et al. [30] |
| Two phase 2 trials | 19 Japanese patients with osteosarcoma, NHL, ALL, or other diagnosis and DME after HDMTX (1–79 years) | NR | • In the first trial, serum MTX decreased by 99.1% at 20 min; CIR rate was 76.9% (10/13 evaluable patients)<br>• In the second trial, serum MTX by HPLC decreased by a median of 98.9% at 20 min; CIR in all four patients<br>• MTX rebound observed after 48 h, but levels remained below 1 µmol/L in most patients<br>• After initial increase, serum creatinine returned to baseline values on day 11 and decreased below baseline by day 21 in all evaluable patients | Ogawa et al. [29] |

Note: CIR = MTX ≤1 µmol/L at all post-glucarpidase measurements.
Abbreviations: AKI, acute kidney injury; ALL, acute lymphoblastic leukemia; CI, confidence interval; CIR, clinically important reduction; CNS, central nervous system; DME, delayed methotrexate elimination; HDMTX, high-dose methotrexate; HPLC, high-performance liquid chromatography; HR, hazard ratio; MTX, methotrexate; NHL, non-Hodgkin lymphoma; NR, not reported.

Table 2. Recommendations for intervention with glucarpidase [33].

| MTX dose | ≤1 g/m$^2$ | 1–8 g/m$^2$ | 8–12 g/m$^2$ |
|---|---|---|---|
| **Infusion duration, hours** | 36–42 | 24 | ≤6 |
| **Hours after start of MTX infusion** | **Threshold plasma MTX concentration, µM** | | |
| 24 | – | –* | ≥50 |
| 36 | – | ≥30 | ≥30 |
| 42 | – | ≥10 | ≥10 |
| 48 | ≥5 | ≥5 | ≥5 |
| ***Further guide for patients receiving short infusion regimens*** | | | |
| MTX dose | 3–3.5 g/m$^2$ | 5 g/m$^2$ | |
| **Hours after start of MTX infusion** | **Threshold plasma MTX concentration, µM** | | |
| 24 | ≥20 | – | |
| 36 | – | ≥10 | |
| 48 | ≥5 | ≥6 | |

Note: *Start supportive care when ≥120 µM.
Abbreviation: MTX, methotrexate.

In a retrospective analysis of severe toxicities after HDMTX, glucarpidase was given to 41 patients with AKI [36]. These patients exhibited higher peak serum MTX levels compared with patients who did not require glucarpidase and, despite glucarpidase treatment, experienced hematologic and extra renal toxicities (leading to the death of two patients) [36]. These findings suggest that whilst glucarpidase may reduce free MTX levels, this may help to prevent but not reverse organ injury, and therefore that the timing of glucarpidase administration is important. This study also underscores the need for prospective trials to exclude the potential bias of glucarpidase recipients representing only the most severe cases and clarify the role of glucarpidase in HDMTX toxicity management.

In a separate pharmacoeconomic analysis of real-world data, glucarpidase administration was associated with a shorter hospital stay and lower inpatient and 90-day mortality rates, compared with no administration, in patients with lymphoma or leukemia treated for presumed MTX toxicity by various methods, including dialysis [35]. Further, patients who received glucarpidase within 3 days of admission spent less time in hospital and intensive care than those who received treatment beyond 3 days, indicating the importance of timely intervention. The authors of this analysis proposed that the shorter hospital stay may partially offset the high cost of glucarpidase.

Table 3. Main findings reported in key, real-world adult and pediatric studies investigating the safety and efficacy of glucarpidase [11,12,35,36,43–46].

| Design | Patient population (age) | MTX dose | Outcomes | Reference |
|---|---|---|---|---|
| ***Adult studies*** | | | | |
| Retrospective study of Medicare claims | Patients with select lymphomas or leukemias and MTX toxicity treated with glucarpidase ($n = 30$), dialysis ($n = 58$), or any non-glucarpidase method ($n = 701$) (mean 68.6–70.0 years) | NR | • Patients treated with glucarpidase had a shorter LOS (14.7 vs. 21.9/40.2 days), and lower inpatient (3.3% vs 20.8%/50.6%) and 90-day (16.7% vs 37.6%/58.6%) mortality than patients not treated with glucarpidase (all/dialysis)<br>• There were no significant differences in 30-day mortality or 90-day readmission rates between the glucarpidase and non-glucarpidase groups<br>• Patients who received glucarpidase within 3 days of admission spent on average ~8.1 fewer days in ICU than patients who received glucarpidase later in their hospital stay (0.8 vs. 8.9 days), and had a shorter overall average LOS (10.0 vs. 21.7 days) | Demiralp et al. [35] |
| Retrospective cohort study | 486 patients with ALL/LL or NHL and AKI (39–67 years) | Median 3 g/m$^2$ | • 41 patients with AKI received glucarpidase: most developed extrarenal toxicity, and two patients died | Medrano et al. [36] |
| Retrospective study utilizing a TTE framework | 708 patients (209 received glucarpidase) with primary CNS lymphoma, ALL, other lymphoma or leukemia, osteosarcoma, or other solid tumor and MTX-induced AKI (51–71 years) | ≥1 g/m$^2$ (median 3.5 g/m$^2$) | • Glucarpidase use was associated with a 2.70-fold higher adjusted odds of kidney recovery (95% CI 1.69–4.31) versus no glucarpidase use<br>• Patients treated with glucarpidase had a faster time-to-kidney recovery (aHR 1.88; 95% CI 1.18–3.33), and lower risk of grade ≥2 neutropenia (aOR 0.50; 95% CI 0.28–0.91) and grade ≥2 transaminitis (aOR 0.50; 95% CI 0.28–0.91) after 7 days<br>• There was no difference in time-to-death (aHR 0.76; 95 CI 0.49–1.18) | Gupta et al. [11] |
| ***Pediatric studies*** | | | | |
| Retrospective chart review | 20 pediatric patients with ALL, osteosarcoma, or lymphoma and HDMTX-induced AKI (4.1–20.4 years) | 3.3–12 g/m$^2$ | • Serum MTX by HPLC decreased by median of 99.6% at first MTX measurement; median time to complete MTX excretion was 355 h<br>• Median time to renal recovery (return of serum creatinine to ≤1.5× baseline) was 21 days<br>• 13/20 patients underwent rechallenge with HDMTX (full dose, $n = 4$; 50–75% dose, $n = 9$); median time to rechallenge was 28 days after initial HDMTX; 1 patient had nephrotoxicity on rechallenge<br>• No treatment-related deaths | Christensen et al. [43] |
| Retrospective chart review | 26 pediatric patients with osteosarcoma, ALL, or other cancers and HDMTX-induced AKI (4.0–20.4 years) | 2.5–12.4 g/m$^2$ | • Serum MTX by HPLC decreased by a median of 99.4% at first MTX measurement in patients who received glucarpidase<br>• All patients recovered renal function, with median time to recovery of ~18 days | Scott et al. [44] |
| Retrospective study | 47 pediatric patients with ALL and DME/ compromised renal function (1–17.9 years) | 5 g/m$^2$ | • Serum MTX by HPLC decreased by a median of 99.9% at first MTX measurement ($n = 8$)<br>• Renal function (serum creatinine and/or creatinine clearance) normalized in all reported patients ($n = 35$); median time to normalization was 16 days<br>• All patients, except two who received glucarpidase in their last HDMTX course, continued their treatment according to the protocol with further HDMTX courses (3–5 g/m$^2$)<br>• MTX elimination time was correlated with body surface area and inversely correlated with maximum creatinine increase<br>• No deaths associated with DME | Svahn et al. [45] |

(*Continued*)

Table 3. (Continued).

| Design | Patient population (age) | MTX dose | Outcomes | Reference |
|---|---|---|---|---|
| Retrospective cohort study | 15 pediatric patients with ALL, osteosarcoma, or other malignancy and DME and/or acute renal failure (3.3–16.9 years) | 0.4–12 g/m$^2$ | • Serum MTX by HPLC decreased by median of 92.6% at first MTX measurement; median MTX elimination time was 193 h; six patients had a CIR<br>• Renal function (serum creatinine and/or creatinine clearance) normalized in all patients<br>• HDMTX rechallenge in 11/15 patients (9 at a reduced dose) after renal recovery, with no serious toxicity | Vila et al. [46] |
| Retrospective study | 42 pediatric patients with ALL or NHL and DME or HDMTX-induced AKI (1–18 years) | 1–5 g/m$^2$ | • Serum MTX by immunoassay decreased by a median of 72.53% at first MTX measurement; median elimination time for MTX and its metabolites was 216 h<br>• Renal function normalized (return of serum creatinine to ≤1.5× baseline) in all but one patient (who died); median time to recovery of renal function was 18 days<br>• HDMTX rechallenge in 22/42 patients (at 40%–100% of recommended dose; four patients at full dose) after renal recovery, with no DME; all 22 patients completed all planned cycles of HDMTX<br>• One MTX-related death | Peccatori et al. [12] |

Note: CIR = MTX ≤1 µmol/L at all post-glucarpidase measurements.

Abbreviations: aHR, adjusted hazard ratio; AKI, acute kidney injury; ALL, acute lymphoblastic leukemia; aOR, adjusted odds ratio; CI, confidence interval; CIR, clinically important reduction; CNS, central nervous system; DME, delayed methotrexate elimination; HDMTX, high-dose methotrexate; HPLC, high-performance liquid chromatography; ICU, intensive care unit; LL, lymphoblastic lymphoma; LOS, length of (hospital) stay; MTX, methotrexate; NHL, non-Hodgkin lymphoma; NR, not reported; TTE, target trial emulation.

## 6. Real-world pediatric data and other reports of glucarpidase use

Real-world data in children, including from an Italian multicenter study reporting experience over an 8-year observation period [12], also support the effectiveness and safety of glucarpidase, as summarized in Table 3 [12,43–46]. Overall, these studies demonstrated that glucarpidase administration results in rapid and sustained reductions in plasma MTX levels and subsequent recovery of renal function. Multiple studies reported decreases in serum MTX levels >90% by the first MTX measurement [43–46], with the authors stating that glucarpidase was preferred over dialysis-based strategies in terms of both speed and effectiveness of MTX elimination [12,43]. Reports have shown that, when glucarpidase availability is limited, hemodialysis can be used to facilitate gradual clearance of MTX [47–49]. Although dialysis can be used, MTX clearance is slow and efficacy can be limited. If required, intermittent high-efficiency hemodialysis should be used [50].

Following glucarpidase treatment, the median time to recovery of renal function reported in these studies ranged from 16 to 21 days [12,43–45]. Where it has been previously reported that delays in administering glucarpidase, beyond 96 h after the initiation of HDMTX, increase the risk of grade 4 or 5 toxicity [27], all of the patients in these studies received glucarpidase within this timeframe (ranging from 26 to 95 h) [12,43–46], highlighting the importance of rapid access. HDMTX rechallenge was also reported, with good tolerability and completion of all planned cycles of chemotherapy [12]. Patients who underwent HDMTX rechallenge were able to do so at full dose (45/47 rechallenged patients [45], 4/13 rechallenged patients [43] and 4/22 rechallenged patients [12] across separate studies), whilst others who were rechallenged received a reduced HDMTX dose at ≥40% of the full dose. Treatment completion may optimize the likelihood of achieving a good clinical outcome, but only if patients can be rechallenged at a clinically effective dose. Single-dose glucarpidase administration tended to only be associated with infrequent, low-grade adverse events.

A retrospective analysis of glucarpidase dose variability (13–90 units/kg) in 26 pediatric patients revealed no statistically significant relationship between glucarpidase dosage and an immediate decrease in MTX plasma concentration or time to recovery of serum creatinine [44]. As this study did not assess whether these unapproved doses resulted in a sustained reduction of plasma MTX concentrations, or include an assessment of rebound, further studies are required to evaluate whether a glucarpidase dose below the standard and recommended dose of 50 units/kg in pediatric oncology patients is similarly effective in producing a profound and sustained decrease of MTX plasma concentration, without compromising efficacy.

Anecdotally, some evidence from both adult and pediatric studies suggest that lower doses of glucarpidase may reduce plasma MTX to levels that can be managed with folinic acid rescue and other supportive measures [14,44,51–54]. This approach has the potential to reduce costs, but there may be a risk of under-dosing, resulting in inadequate efficacy of anticancer treatment, and prolonged exposure to high, toxic MTX concentrations [20,55]. Administration of fixed doses of glucarpidase, regardless of weight, has been reported to be effective among adult patients with lymphoma [56]. Although outside of the approved use, successful intrathecal administration of glucarpidase to manage accidental intrathecal MTX

Table 4. Summary of expert consensus recommendations for glucarpidase use [4,8].

| | Ramsey et al. (2018) [4] | Bielack et al. (2024) [8] |
|---|---|---|
| Factors to consider | • MTX clearance rate (high serum MTX levels)<br>• Renal function<br>• Age and risk of AKI from other nephrotic medications | • Evidence and/or risk* of MTX toxicity<br>• Renal function<br>• Clinical signs |
| General indication for intervention | • HDMTX-induced AKI<br>• DME leading to potentially toxic plasma MTX concentrations | • Adult/child (age ≥28 days)<br>• DME<br>• At risk of MTX toxicity |
| MTX and creatinine thresholds for intervention | • Timepoint plasma MTX concentrations, based on infusion protocol (with increased serum creatinine relative to baseline):<br>*1–8 g/m$^2$ MTX over 24 h: >30 µmol/L at 36 h; >10 µmol/L at 42 h; >5 µmol/L at 48 h*<br>*8–12 g/m$^2$ MTX over ≤6 h: >50 µmol/L at 24 h; >30 µmol/L at 36 h; >10 µmol/L at 42 h; >5 µmol/L at 48 h* | • Plasma MTX concentrations two standard deviations above mean expected concentrations (determined using the MTXPK.org tool)<br>• Timepoint plasma MTX concentrations:<br>*MTX >50 µmol/L at 24 h; >30 µmol/L at 36 h; >10 µmol/L at 42 h; >5 µmol/L at 48 h* |
| Glucarpidase dose | • Single dose, 50 units/kg | • Single dose, 50 units/kg<br>• IV injection over ≥5 min |
| Timing of glucarpidase | • Optimally, within 48–60 h from the start of HDMTX infusion | • At the earliest opportunity (ideally, within 48–60 h of MTX infusion start)<br>• If no local stocks are available, have arrangements in place to access within <24 h (ideally in <12 h) |
| Post-dose monitoring of MTX | • Most hospitals monitor plasma MTX until levels are ≤0.1–0.2 µmol/L<br>• Preferably use HPLC method (immunoassays can overestimate MTX concentrations)<br>*Note: MTX levels can rebound from ~48 h after glucarpidase administration due to MTX release from tissue stores. Continued monitoring of MTX concentrations and administration of folinic acid is important in these patients* | • Monitor until MTX levels are undetectable<br>• Use HPLC-based assay<br>*Note: MTX levels can rebound from ~48 h after glucarpidase administration; levels are usually lower than pre-intervention and are not likely to be clinically relevant* |
| Folinic acid use after glucarpidase | • Doses >200–500 mg (or BSA × 50) over 1–2 h<br>• Stop ≥2 h prior to, and resume ≥2 h after, glucarpidase dosing<br>• Continue until MTX concentration is below threshold prescribed by treatment protocol | • Stop ≥2 h prior to, and resume ≥2 h after, glucarpidase dosing<br>• Continue until MTX is undetectable in serum |

*Risk factors include age/frailty, excess body weight, presence of pleural effusion and ascites, sepsis, fever/infection, tumor lysis, diabetes or hypoalbuminemia, Down syndrome, and concomitant use of drugs that are nephrotoxic or interfere with MTX elimination.

Abbreviations: AKI, acute kidney injury; BSA, body surface area; DME, delayed methotrexate elimination; HDMTX, high-dose methotrexate; HPLC, high-performance liquid chromatography; IV, intravenous; MTX, methotrexate.

overdose has also been demonstrated in both animal models and in a limited number of case reports and case series [57–59]. As noted in a recent consensus paper, without further evidence, the use of lower glucarpidase doses is not recommended until unequivocal, solid data become available [60].

## 7. Glucarpidase: when and for whom?

In light of the limited availability of societal guidelines [61], two expert consensus papers have been developed that put forward recommendations for the optimal use of glucarpidase; the key points from these papers are presented in Table 4 [4,8]. The earlier of the two expert consensus papers (published in 2018) identifies patients who may benefit from glucarpidase, by defining absolute MTX concentrations associated with the risk of severe or potentially fatal toxicity at predefined timepoints after MTX infusion for individuals with evidence of AKI [4]. The more recent, European expert consensus paper (published in 2024) also recommends glucarpidase intervention based on MTX levels, while additionally considering renal function, clinical signs, and evidence and/or risk of MTX toxicity [8]. This panel of European experts also recommended that glucarpidase should be considered when plasma MTX levels are two standard deviations above the mean expected MTX plasma concentration (e.g. as determined via a software-based tool named MTXPK.org, described below), or above predefined thresholds at specific timepoints after MTX infusion, particularly in the presence of impaired renal function [8]. A most recently published expert consensus paper from Italy provides detailed recommendations specifically tailored for the pediatric setting [60]. The panel recommended glucarpidase administration for patients with significant DME, or DME accompanied by AKI (defined as a ≥50% rise in serum creatinine within 24–48 h after HDMTX), and specified DME threshold concentrations of ≥120 µM for long infusions of MTX or ≥50 µM for short infusions at 24 h; ≥30 µM at 36 h; ≥10 µM at 42 h; and ≥5 µM at 48 h [60]. All expert consensus papers advocate for glucarpidase being administered as early as possible and within 48–60 h of HDMTX administration, as life-threatening toxicities may not be preventable beyond this point [4,8,60]. Avoidance of drugs, including loop diuretics, and foods that can interfere with MTX clearance, is also advised; loop diuretics in particular can worsen volume depletion in patients without fluid overload and further impair renal

Table 5. Drugs and foods that may impact MTX clearance [4,6,26,62].

| Drugs and foods that may impact MTX clearance | Examples |
|---|---|
| Drugs | • Proton pump inhibitors<br>• Asparaginase inhibitors<br>• Non-steroidal anti-inflammatory drugs and acetylsalicylic acid (aspirin)<br>• Certain antibiotics (e.g. beta-lactams, sulfonamides, vancomycin)<br>• Tyrosine kinase inhibitors<br>• Probenecid and weak organic acids (e.g. pyrazoles)<br>• Immunosuppressants<br>• Radiographic iodinated contrast agents<br>• Loop diuretics<br>• Other nephrotoxic drugs |
| Foods and supplements | • Liquorice<br>• Acidic beverages (e.g. cola, fruit juice)<br>• Vitamins<br>• Glutamine |

Abbreviation: MTX, methotrexate.

function in patients with DME (Table 5) [4,8,26,62]. Also, hypoalbuminemia, a side effect of concomitant asparaginase administration in patients with leukemia, can cause a reduced glomerular filtration rate and increased free MTX concentration, resulting in additional toxicities such as stomatitis and myelotoxicity [62].

As endorsed in the European consensus guidelines [8], a free-to-access, online, clinical decision support tool (MTXPK.org) has been developed, based on population pharmacokinetic modeling and Bayesian estimation. MTXPK.org helps physicians decide when to administer glucarpidase for patients receiving HDMTX, by providing estimates of when a patient may reach the MTX concentration threshold required for intervention [63,64]. Since its introduction in 2019, MTXPK.org has been improved by remodeling in a more diverse population including both adults and children with a range of malignancies [63]. Because MTXPK.org is closely aligned to the EU label [33], it enables proof of in-label glucarpidase administration according to European recommendations [33].

To avoid interactions and maximize the likelihood of achieving a good outcome, all expert consensus papers recommend that glucarpidase be given in conjunction with folinic acid, but not at the same time, as folinic acid is a competitive substrate of glucarpidase [4,8,60]. Consequently, it is advised that folinic acid should be stopped ≥2 h before, and restarted ≥2 h after, glucarpidase administration. A recent article provides further guidance on this topic and emphasizes the importance of minimizing the risk of folinic acid over-rescue and folinic acid-related adverse events [65]. Folinic acid is commonly administered as a racemic calcium folinate; however, as only the *L*-isomer is clinically active, and the *D*-isomer accumulates in the body, there are challenges regarding treatment timings. Furthermore, excessive use of folinic acid may limit the efficacy of HDMTX (as discussed previously) and potentially induce hypercalcemia, although this latter scenario may be averted if alternative, less commonly available formulations (calcium levofolinate [containing only the *L*-isomer] or sodium levofolinate [containing no calcium]) are used. If only racemic calcium-based folinic acid is available, it is recommended that a reduced dose is used, followed by glucarpidase [65].

## 8. Post-glucarpidase management

Post-HDMTX infusion, and before and after glucarpidase administration, therapeutic monitoring of plasma MTX levels is recommended, along with regular assessment of renal function, according to the labels and expert consensus recommendations [4,8,32,33]. After receipt of glucarpidase, continuation of folinic acid rescue (to be restarted ≥2 h post-glucarpidase administration) and supportive measures for at least 48 h (to counter MTX rebound) is recommended until plasma MTX concentrations are reduced to undetectable or close to undetectable levels [4,8,32,33]. In all patients, folinic acid should be given periodically, according to standard guidelines, at doses guided by the plasma MTX concentration.

As MTX and its metabolite DAMPA have similar structures, overestimation of MTX levels occurs when immunoassays are used for therapeutic monitoring whilst DAMPA is present [4,8]. Although not widely available, high-performance liquid chromatography mass spectrometry provides a reliable analysis of MTX levels and should therefore be preferred for at least the first 48–72 h after glucarpidase administration [4,8].

## 9. Limitations of available evidence and challenges in the clinical application of glucarpidase

Whilst single-arm, open-label clinical trials and compassionate-use studies have demonstrated the clinical efficacy and safety of glucarpidase, there are important limitations to the available evidence. Inherently, these studies included heterogenous patient populations which may be susceptible to selection biases. In particular, glucarpidase is often given to patients with greater severity of illness or DME compared with patients who did not receive glucarpidase. In a retrospective analysis, data collection was often incomplete across multiple study sites. For example, missing information regarding concomitant treatment with cytotoxic chemotherapy limited the ability to attribute toxicity solely to MTX [27]. Although the recommended glucarpidase dose is 50 units/kg, doses administered varied across the reported studies. Typically, patients received a single administration of glucarpidase at a dose of 50 units/kg, yet reported doses ranged from 13 to 90 units/kg

[44], and some patients received second and third doses of glucarpidase, with differing criteria for additional doses between studies. Despite the recommended dose of 50 units/kg, lower doses were sometimes given due to supply shortages, or off-label institutional practices of capping the dose. Of note, reduced dosing of glucarpidase cannot be recommended and should be restricted to well-designed clinical studies.

These studies do not facilitate the structured, controlled comparisons afforded by prospective randomized controlled studies, with strictly defined study protocols, participant selection, standardized outcome measures, and comparator arms. However, it would not be ethical to conduct a randomized controlled trial in patients with severe DME. Consequently, robust and larger estimates of the benefit of glucarpidase intervention are clearly constrained based on the data derived from such studies.

Due to the cost and infrequency of treatment, glucarpidase stocks may not be maintained locally. Therefore, the drug can only be administered in a timely manner if the necessary infrastructure is in place to facilitate a rapid supply of the drug, ideally within 12–24 h. Efforts to provide an early definition of who may benefit from glucarpidase, the optimal time to administer the drug, and the potential effectiveness of reduced dosages could help to improve the cost-effectiveness of treatment and increase worldwide access. However, such suggestions cannot be recommended outside the licensed dosages until better structured evidence becomes available.

## 10. Practical guidance on optimal clinical use of glucarpidase

In Europe, glucarpidase is indicated to reduce toxic plasma MTX concentration in adults and children (aged 28 days and older) with delayed MTX elimination or at risk of MTX toxicity [66]. To determine when glucarpidase should be administered and to account for all MTX doses and infusion durations that could be administered to a patient, it is recommended to utilize local treatment protocols or guidelines if available, especially to adopt consistent decision-making pathways. Intervention with glucarpidase is recommended when plasma MTX levels are greater than two standard deviations of the mean expected MTX excretion curve. The administration of glucarpidase should optimally occur within 60 h from the start of the HDMTX infusion, because life-threatening toxicities may not be preventable beyond this timepoint [66].

Expert consensus recommendations advocate for the administration of glucarpidase within 48–60 h of HDMTX initiation [4,8,60], but particularly within 96 h to mitigate the risk of grade 4 or 5 toxicity [27]. Further, a recent consensus publication from Italy notes that, given the FDA/EMA approved dosage for glucarpidase (50 units/kg) and the lack of evidence, dose capping of glucarpidase is not advisable [60].

The approved glucarpidase dose is 50 units/kg, administered as a single intravenous dose infused over 5 min, and no dose adjustment is required for the pediatric population [32,33]. Repeated dosing of glucarpidase is generally not recommended within 48 h of the first dose during the same HDMTX course due to reduced efficacy [4]. Folinic acid rescue, initiated 24–42 h after the start of the HDMTX infusion, is recommended but should not be administered within 2 h before or after glucarpidase to avoid interference with glucarpidase-mediated metabolism of MTX [4]. Intensive fluid hydration (≥2.5 L/m$^2$/day) and urine alkalinization (pH ≥7) are important standard supportive measures [8].

If available, high-performance liquid chromatography-based assays are preferred for post-glucarpidase monitoring of MTX concentrations, as immunoassays overestimate MTX levels due to interference from the DAMPA metabolite [4]. Where immunoassays only are available, the folinic acid dosage during the 48 h post-glucarpidase should be based on MTX levels prior to glucarpidase administration due to the effect of DAMPA interference [33]. It should be anticipated that MTX levels may rebound more than 48 h post-glucarpidase due to the release of MTX from tissue stores, although rebound levels are typically substantially lower than before the administration of glucarpidase [4,8,23].

## 11. Conclusions

Despite its limited availability in some countries, and the direct treatment costs, glucarpidase use in clinical practice appears to be increasing [1,48]. Where glucarpidase is currently available, effective planning is required to ensure its appropriate administration and avoid treatment delays [1,48].

Since its use in clinical studies and compassionate-use programs in the early 1990s, glucarpidase has progressively transformed the management of DME and HDMTX-associated toxicity. When used in conjunction with optimized supportive measures and folinic acid rescue, timely administration leads to rapid and sustained reductions in plasma MTX levels, improvements in renal outcomes, and maintenance of disease outcomes in specific settings, by contributing to a safe continuation of the treatment journey for affected patients [12]. Although glucarpidase is an expensive tool for reducing MTX levels and managing/preventing MTX toxicity, its administration has been reported to improve patient survival, reduce hemodialysis use, and shorten the length of hospital stay [13,35]. Crucially, completion of planned chemotherapy protocols, with HDMTX rechallenge, has also been reported following glucarpidase administration, maximizing the likelihood of a favorable long-term clinical outcome for the affected patients.

This review began with the Latin motto '*per aspera ad astra*,' which translates to 'through difficulties to the stars,' a phrase that eloquently captures the long journey of this drug from its first recombinant production to its now broad availability for clinical administration. '*Per aspera*' relates to the initial pioneering years of glucarpidase compassionate-use and single-arm trials. During this period, physicians and patients were hesitant over the use of a drug supported by limited data, despite its promise. Today, the drug has moved toward the '*ad astra*' ('to the stars') stage, given the data from large, international real-world studies in adults and children

with various malignancies. We now benefit from better understanding of the importance of the precise and timely administration of glucarpidase, as well as its short- and long-term effects, expanding its applications beyond the prevention of single DME or AKI episodes.

While HDMTX remains a very important chemotherapy strategy for many cancers, the associated risks of severe acute and subacute side effects are important to fully prevent and recognize. The approval of glucarpidase by several competent authorities facilitates the wide and prompt availability of the drug and allows clinicians to more safely negate the pitfalls of HDMTX administration.

It is our hope that additional, upcoming evidence emerging from ongoing studies will provide further insights into the ongoing use of glucarpidase, thus improving and refining its administration.

## 12. Expert opinion

Recent advances in the clinical use of glucarpidase compel the pediatric and adult hematology and oncology community to reexamine long-standing assumptions regarding the management of DME. Historically, glucarpidase administration has been guided by algorithms relying on arbitrary MTX thresholds and clinical cutoffs. These criteria, developed largely from expert consensus rather than robust prospective data, have shaped practice in the last decade.

Emerging evidence, particularly the recent study performed by Gupta et al. [11], partially challenges this traditional framework and suggests that our current decision pathways may underestimate the potential benefit of earlier or more flexible intervention.

In fact, in the Gupta et al. study, glucarpidase-treated patients were more likely to have comorbidities (such as hypertension or diabetes mellitus), to have received concomitant nephrotoxic medications, and to have increased 24-h MTX levels and initial AKI severity than patients who did not receive glucarpidase [11]. Incomplete adjustment for these severity-of-illness covariates may clearly lead to an underestimation of the magnitude of the beneficial effects of glucarpidase on clinical outcomes.

Data suggest that patients with moderate elevations in MTX levels, previously considered below classical intervention thresholds, may still experience meaningful reductions in morbidity when glucarpidase is administered in a timely manner. Gupta et al. reported that more than half of the patients who received glucarpidase had 24-h MTX levels <50 µM, which is below the consensus threshold recommended for glucarpidase rescue after MTX infusions (8–12 g/m$^2$) over ≤6 h [11]. Nevertheless, glucarpidase administration was associated with improved renal and extrarenal outcomes compared with non-glucarpidase-treated patients [11]. As such, our perspective would be that glucarpidase may prove beneficial for patients with serum MTX levels <50 µM. It remains to be determined whether these real-world observations are a true reflection of glucarpidase benefit in patients with moderate MTX elevations or are also a feature of earlier intervention and improved supportive care.

The implications for real-world outcomes are substantial. Glucarpidase is a life-saving drug in the setting of severe MTX toxicity with renal impairment, but its clinical value might extend beyond the 'extreme' scenarios in which it is currently used. Current consensus cutoff criteria may not fully encapsulate which patients will benefit from glucarpidase treatment (including those with 24-h MTX <50 µM after short MTX infusions). The hypothesis that there may be an indication for glucarpidase use even in the context of moderate MTX elevations, however, still needs to be rigorously demonstrated.

An evidence-based approach is required to update existing decision-making algorithms.

Several barriers hinder widespread adoption of more proactive glucarpidase use. These include logistical delays in drug procurement, the limited availability of timely MTX assays, which are not affected by glucarpidase metabolites, and concerns about treatment costs in resource-constrained settings. Moreover, the absence of standardized, data-driven criteria for intermediate-risk patients continues to create clinical uncertainty.

Addressing these gaps will require harmonized protocols, improved laboratory methodologies, and clearer decision-support tools that can be implemented across diverse care environments.

From a research standpoint, the field is at a pivotal moment. There is a pressing need for prospective studies, including, when feasible, randomized trials. Early-intervention strategies must be validated and the best therapeutic window for glucarpidase needs to be even more precisely identified. Whilst it would not be ethical to conduct randomized trials in patients with severe DME, randomization may allow us to delineate the benefits of glucarpidase in patients with less severe DME, for whom the risk–benefit balance has remained less well proven in the absence of controlled data.

Parallel efforts should focus on predictive modeling and biomarker development to identify patients at high risk before toxicity escalates, enabling more personalized MTX management.

The approved dose of glucarpidase (50 units/kg) is supported by a body of clinical data demonstrating safety and efficacy in the treatment of DME. Although studies have documented reductions in plasma MTX concentration in patients who have received lower doses of glucarpidase [14,28,44,51–54], further research is needed to determine whether reduced glucarpidase doses are able to achieve sustained MTX reductions and prevent rebound effects. Of note, typically designed dose-finding studies would not be feasible in an emergency setting. To successfully implement glucarpidase dose reductions, additional research is required to identify which patients are most suitable for reduced doses, and which biomarkers (e.g. plasma MTX levels) offer the most accurate data to gauge the optimal dose. Given that glucarpidase is an effective and very well-tolerated treatment for DME when administered in a timely manner at the approved dose (50 units/kg), and there is a risk of ongoing exposure to MTX and potentially life-threatening toxicities associated with insufficient treatment, the approved dose should be favored until such well-designed studies can present robust data to support lower doses.

The future use of glucarpidase will depend on our ability to refine its role within evolving supportive-care

frameworks, and we anticipate a more standardized, evidence-based approach that integrates early risk stratification, real-time pharmacokinetic monitoring, and routine access to glucarpidase. Such advancements may substantially reduce MTX-related complications and shorten hospitalizations.

In conclusion, glucarpidase represents a key tool in the management of MTX toxicity in pediatric and adult patients with hematologic and oncologic disease, especially in those with compromised renal function where dosages and schedules have been thoroughly defined by the competent regulatory authorities. Even if the increasing amount of data reported over the last decades has bridged some of the existing gaps in the understanding of its use, knowledge in several areas requires expanding, such as updating the administration algorithms in patients with moderate MTX elevations. A new generation of high-quality clinical studies will be essential to better define its use, to clarify its utility across the full spectrum of DME severity, and to guide the evolution of more precise MTX-based therapy with an improved safety profile.

## Declaration of interests

N Peccatori has received speaker fees from SERB. S Schwartz has served as a consultant for Amgen, Pfizer, and SERB SAS; has received speaker honoraria from Akademie für Infektionsmedizin e.V., Amgen, CSi Hamburg GmbH, Pfizer, and SERB SAS; has received a research grant from Protherics Medicines Development Ltd.; has received travel grants from Amgen, Pfizer, and SERB SAS. C Rizzari has received institutional research grants for independent pharmacologic and clinical studies in the field of high-dose methotrexate toxic effects; has received speaker fees and travel support from Amgen, Clinigen, Jazz, SERB, and Servier. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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## Author contributions

N Peccatori, S Schwartz, and C Rizzari were responsible for the conceptualization, data curation, and methodology. N Peccatori and C Rizzari were responsible for the investigation. C Rizzari was responsible for the supervision. All authors contributed to the original draft, participated in the review and editing process, and approved the final version of the manuscript.

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