The Oncologist®

Consensus Guideline for Use of Glucarpidase in Patients with High-Dose Methotrexate Induced Acute Kidney Injury and Delayed Methotrexate Clearance

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Disclosures of potential conflicts of interest may be found at the end of this article.

ABSTRACT

Acute kidney injury due to high-dose methotrexate (HDMTX) is a serious, life-threatening toxicity that can occur in pediatric and adult patients. Glucarpidase is a treatment approved by the Food and Drug Administration for high methotrexate concentrations in the context of kidney dysfunction, but the guidelines for when to use it are unclear. An expert panel was convened to provide specific, expert consensus guidelines for the use of glucarpidase in patients who develop HDMTX-induced nephrotoxicity and delayed methotrexate excretion. The guideline provides recommendations to identify the population of patients who would benefit from glucarpidase rescue by more precisely defining the absolute methotrexate concentrations associated with risk for severe or life-threatening toxicity at several time points after the start of an HDMTX infusion. For an HDMTX infusion ≤24 hours, if the 36-hour concentration is above 30 μM, 42-hour concentration is above 10 μM, or 48-hour concentration is above 5 μM and the serum creatinine is significantly elevated relative to the baseline measurement (indicative of HDMTX-induced acute kidney injury), glucarpidase may be indicated. After a 36- to 42-hour HDMTX infusion, glucarpidase may be indicated when the 48-hour methotrexate concentration is above 5 μM. Administration of glucarpidase should optimally occur within 48–60 hours from the start of the HDMTX infusion, because life-threatening toxicities may not be preventable beyond this time point. *The Oncologist* 2018;23:52–61

Implications for Practice: Glucarpidase is a rarely used medication that is less effective when given after more than 60 hours of exposure to high-dose methotrexate, so predicting early which patients will need it is imperative. There are no currently available consensus guidelines for the use of this medication. The indication on the label does not give specific methotrexate concentrations above which it should be used. An international group of experts was convened to develop a consensus guideline that was specific and evidence-based to identify the population of patients who would benefit from glucarpidase.

INTRODUCTION

Nephrotoxicity induced by high-dose methotrexate (HDMTX) is a medical emergency because the renal excretion of methotrexate (MTX) is subsequently delayed, and the resulting prolonged exposure to high concentrations of the drug can cause severe and life-threatening toxicity. The recombinant bacterial enzyme glucarpidase, which is approved by the Food and

Table 1. Common high-dose MTX regimens

Indication	ALL	Osteosarcoma	Lymphoma
HDMTX dose	1–5 g/m^2	8–12 g/m^2	1–8 g/m^2
Infusion duration	24–36 h	4 h	2–6 h
Leucovorin start time	42 h	24 h	18–24 h
MTX monitoring times[a]	24 h, (36 h), 42 h, 48 h	24 h, 48 h, 72 h	24 h, 48 h, 72 h

[a]Most hospitals monitor plasma MTX concentrations until MTX ≤0.1–0.2 μM. In ALL patients, 36 h concentrations are occasionally monitored.
Abbreviations: ALL, acute lymphoblastic leukemia; HDMTX, high-dose methotrexate; MTX, methotrexate.

Drug Administration (FDA) for use in patients with delayed MTX excretion, is a rescue agent that cleaves MTX into inactive metabolites, providing an alternative route of elimination for the drug in patients with nephrotoxicity [1–6]. A task force was assembled to develop specific, evidence-based guidelines for the use of glucarpidase in patients who develop HDMTX-induced nephrotoxicity and delayed MTX excretion, and the recommendations of this working group are reported herein.

High-Dose Methotrexate Pharmacology

Methotrexate is an antifolate that can be administered over a broad dose range (from 20 to 33,600 mg/m^2) [7] but is only tolerable at higher doses when followed by the rescue agent leucovorin (5-formyltetrahydrofolate). HDMTX is commonly regarded as doses exceeding 500 mg/m^2 infused over 2–36 hours, requiring supportive care (e.g., hyperhydration) and leucovorin rescue. Doses and infusion times vary by indication (Table 1).

MTX is a competitive inhibitor of the enzyme dihydrofolate reductase (DHFR; Fig. 1) and blocks the conversion of dihydrofolate to its active, chemically reduced tetrahydrofolate form, thus depleting the intracellular pool of tetrahydrofolates, which are required cofactors (single-carbon donors) for the synthesis of methionine, thymidine, and purines. MTX is polyglutamated intracellularly, and in this form (MTXPG) can also directly inhibit enzymes in the purine/pyrimidine synthetic pathways. Thymidylate synthase is the only folate-requiring enzyme that oxidizes its tetrahydrofolate cofactor to dihydrofolate, and active synthesis of thymidine is necessary for MTX to deplete intracellular tetrahydrofolates.

The anticancer and toxic effects of MTX are highly schedule-dependent and are determined by the duration of exposure to a threshold concentration of the drug. The threshold concentration is tissue- and tumor-specific. Prolonged continuous exposure to low concentrations of MTX can cause severe myelosuppression and other severe toxicities. Leucovorin, the HDMTX rescue agent, provides an alternative source of tetrahydrofolate and counteracts MTX toxicity if administered, preferably within 42 hours of the start of the HDMTX infusion. Leucovorin must compete with MTX for cell entry and polyglutamation (Fig. 1), so it is less effective as a rescue agent at high MTX concentrations if it is not also present at an equipotent concentration.

Renal excretion of the parent drug is the primary route of elimination for HDMTX, accounting for approximately 70%–90% of MTX clearance [8]. MTX is a weak acid with limited solubility in acidic conditions (2 mM maximum solubility at pH 5). Alkalinization of the urine has a greater impact on MTX solubility in urine than fluid hydration [9]. MTX solubility increases 10-fold by increasing the pH from 6 to 7 [10]. MTX is secreted and not reabsorbed by renal tubules, but tubular secretion is saturated at higher plasma concentrations and only plays a minor role in MTX elimination during and immediately after the HDMTX infusion [11]. MTX is 60% protein-bound, and glomerular filtration is limited to free drug. Therefore, MTX clearance at high concentrations is less than the glomerular filtration rate (GFR). MTX is also metabolized in the liver by aldehyde oxidase to 7-hydroxymethotrexate, which accounts for 5%–10% of MTX elimination [8]. There is substantial interpatient and intrapatient variability in the clearance of MTX, which ranges 10-fold (depending on dose, age, and infusion time) in patients with normal renal function after an HDMTX infusion [12–15].

HDMTX-Induced Acute Kidney Injury and Delayed MTX Clearance

HDMTX-induced acute kidney injury (AKI) occurs during or shortly after the end of the infusion near the end of the steady-state plasma concentration (Fig. 2) [16]. The AKI is manifested as a rise in serum creatinine (decrease in GFR), but urine output is usually maintained (nonoliguric) [17]. MTX clearance declines in proportion to the decrease in GFR, resulting in prolonged exposure to high MTX concentrations that may exceed the capacity of standard doses of leucovorin to rescue from the toxicity of MTX. HDMTX-induced AKI that is associated with substantial reductions in MTX clearance occurs in 0.5%–1.0% of courses of 5 g/m^2 over 24 hours in children with acute lymphoblastic leukemia (ALL) [18, 19] and 1.8% of courses of 12 g/m^2 over 4 hours in children, adolescents, and young adults with osteosarcoma [20]. Approximately 2%–12% of adults treated with HDMTX develop nephrotoxicity [21]. HDMTX-induced AKI is reversible, and nearly all patients fully recover renal function. The GFR of most patients with HDMTX-induced AKI returns to baseline values after AKI, although this can take weeks [22, 23]. The long-term consequences of HDMTX-induced AKI have not been fully investigated, but it is possible that nephron loss occurs after such injury, resulting in subsequent chronic kidney disease, as has been shown in other models of AKI [24].

Possible mechanisms of HDMTX-induced AKI include pH-dependent precipitation of MTX in urine in the renal tubules [25–27], reduced renal perfusion from afferent arteriolar vasoconstriction, or uptake of MTX into the renal tubules with direct tubular toxicity [5, 9, 16]. The osteosarcoma HDMTX regimen (8–12 g/m^2 over 4 hours), which has a higher incidence of HDMTX-induced AKI, as noted above, yields an end-of-infusion plasma MTX concentration of approximately 1,000 μM (1 mM) [15, 28, 29], and the simultaneous urine MTX concentration is approximately 10,000 μM (10 mM) [8], which
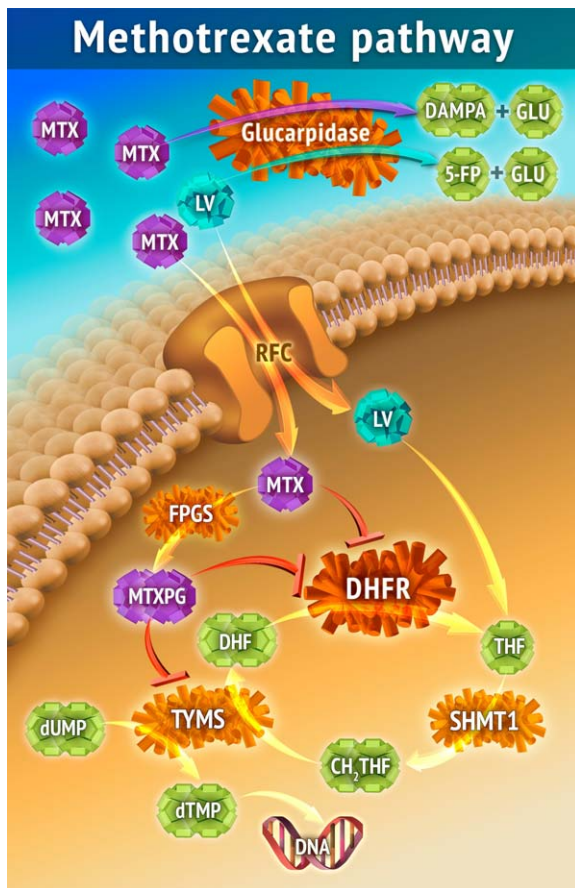
Figure 1. The cellular entry of MTX is mediated primarily by the ubiquitously expressed RFC, encoded by the human solute carrier family 19 member 1 (*SLC19A1*) gene on chromosome 21. Other influx and efflux mechanisms include ABC transporters (specifically ABCC1–4) and breast cancer resistance protein [79], but their role in MTX clearance and efficacy is less well documented. Passive diffusion plays a significant role during high-dose MTX (HDMTX) therapy. Most of the MTX that enters the liver re-enters the blood circulation by enterohepatic circulation via the ABCC transporters, and only a small portion is excreted into the bile. MTX interferes with the natural folate-homocysteine cycle and inhibits folate-dependent enzymes and pathways, including DHFR, TYMS, 5,10-methylene-tetrahydrofolate reductase, and purine de novo synthesis. This leads to a lack of reduced folate, inhibition of DNA synthesis, increased homocysteine and adenosine concentrations, and potentially life-threatening toxicities [32, 80, 81]. Leucovorin (folinic acid) is essential for cellular rescue after HDMTX, and insufficient intracellular concentrations of leucovorin after HDMTX may cause life-threatening toxicities. Intracellularly, the enzyme FPGS polyglutamates MTX by adding 2–6 glutamate residues, creating MTXPG, which increases intracellular retention as well as affinity for its target enzymes proportional to glutamyl chain lengths [82–86].

Abbreviations: 5-FP, 5-formylpteroate; ABC, ATP-binding cassette; ABCC, ABC subfamily C; CH₂THF, 5,10-Methylenetetrahydrofolate; DAMPA, 4-deoxy-4-amino-N10-methylpteroic acid; DHF, dihydrofolate; DHFR, dihydrofolate reductase; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; FPGS, folylpolyglutamate synthetase; GLU, glutamate; LV, leucovorin, MTX, methotrexate; MTXPG, polyglutamated methotrexate; RFC, reduced folate carrier; SHMT1, serine hydroxymethyltransferase 1; THF, tetrahydrofolate; TYMS, thymidylate synthase.

approximates the solubility limit of MTX at pH 7. The timing of HDMTX-induced AKI and the effectiveness of alkalinization and volume expansion (fluid hydration) administered prior to, during, and after a HDMTX infusion at preventing HDMTX-
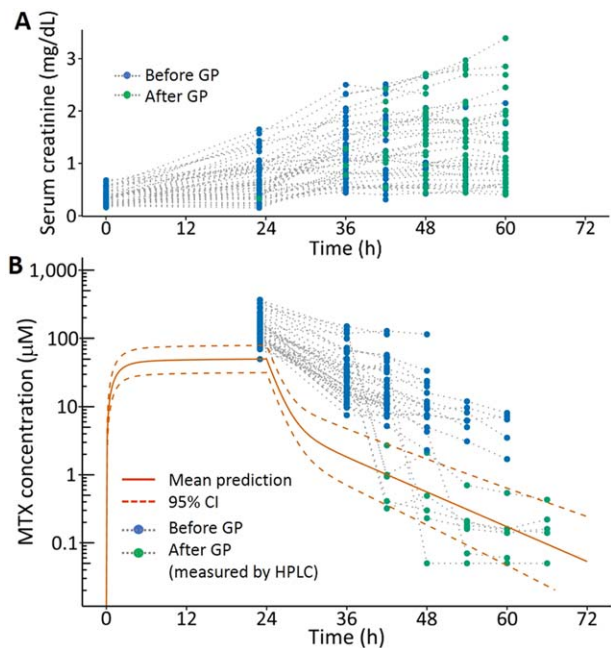
Figure 2. Change in serum creatinine **(A)** and plasma methotrexate **(B)** concentrations relative to the start of the high-dose methotrexate infusion (time 0) in pediatric patients with acute lymphoblastic leukemia (ALL) receiving 5 g/m² over 24 hours on the Nordic Society of Paediatric Haematology and Oncology ALL-2008 protocol who also received GP. Their measurements prior to glucarpidase (blue) and after glucarpidase (green) are shown relative to the predicted mean and 95% CI for patients receiving the same MTX dose (orange lines).

Abbreviations: CI, confidence interval; GP, glucarpidase; HPLC, high pressure liquid chromatography; MTX, methotrexate.

induced AKI supports the view that MTX precipitation plays a role in this toxicity.

Documenting normal renal function prior to administering HDMTX and serial monitoring of serum creatinine before and at the end of HDMTX infusion are mandatory to detect HDMTX-induced AKI as early as possible. An increase in serum creatinine of more than 50% within 24–36 hours from the pre-HDMTX baseline value has a sensitivity of 0.32 and a specificity of 0.99 for predicting delayed MTX elimination [16]. However, serum creatinine is a suboptimal biomarker of AKI, as creatinine rise may lag significantly from the time of the renal insult. Elevated plasma MTX concentration may indicate HDMTX-induced AKI prior to a significant change in creatinine. Thus, clinicians administering HDMTX should be familiar with the expected plasma MTX concentration at the various time points after infusion (supplemental online Tables 1 and 2). After recovery of renal function (normal GFR), HDMTX therapy can generally be safely administered at full dose in pediatric and adult patients who previously experienced HDMTX-induced AKI or received glucarpidase [18, 19, 30].

STRATEGIES TO PREVENT HDMTX-INDUCED AKI

In early studies of HDMTX, severe toxicity occurred in approximately 10% of patients with a 6% toxic mortality rate [31]. The incidence of severe, life-threatening toxicity after HDMTX therapy has been reduced to less than 1% by the implementation of supportive care measures to prevent HDMTX-induced AKI,

including alkalinization of urine, fluid hydration with frequent monitoring of serum creatinine, and serial monitoring of plasma MTX concentrations to determine the dose and duration of leucovorin rescue [19, 32–34]. Urine pH should be documented to be above 7 prior to the start of a HDMTX infusion and should be maintained at this level until the plasma MTX concentration drops below the solubility threshold. Urinary flow prior to, during, and after a HDMTX infusion should be maintained at least at 2,500 mL/m^2 per day (supplemental online Table 3) [35]. If urinary flow drops below 2,000 mL/m^2 per day, there is a higher risk for delayed MTX clearance [36].

The purpose of alkalinization of the urine and fluid hydration is to maximize the solubility of MTX in urine. These measures do not enhance MTX clearance, which is largely dependent on glomerular filtration at high plasma MTX concentrations. Once the plasma MTX concentration drops below 10 μM after infusion, the urine concentration is estimated to be an order of magnitude below the drug's solubility limit at pH 6; thus, alkalinization and hydration are less important as the plasma MTX concentration continues to decrease to the target level (typically ≤0.1 or 0.2 μM) at which leucovorin rescue can be stopped in patients with normal renal function.

LEUCOVORIN RESCUE

Leucovorin and its primary circulating metabolite, 5-methyltetrahydrofolate, prevent the potentially severe and life-threatening toxicities from HDMTX by providing a source of intracellular tetrahydrofolates that enter the folate cycle downstream of DHFR, which is inhibited by MTX. However, these exogenous tetrahydrofolates must compete with MTX for cellular uptake via the reduced folate carrier and, once in the cell, for polyglutamylation, which enhances intracellular retention of folates and affinity for the target enzymes (Fig. 1). Therefore, leucovorin rescue is less effective at high MTX concentrations, especially when the MTX concentration exceeds 10 μM for 48 hours or longer. The leucovorin dose must be increased in proportion to the MTX concentration when MTX clearance is delayed due to HDMTX-induced AKI.

Timing of MTX measurements and the dose and initiation of leucovorin rescue vary across different treatment regimens (Table 1) [9]. Leucovorin rescue usually starts 24–42 hours after the start of the HDMTX infusion and must not be delayed beyond 42–48 hours, even if the HDMTX infusion does not finish at the planned time. Delaying the start of leucovorin rescue beyond 48 hours after the start of the HDMTX infusion significantly increases the risk for severe MTX toxicity [37]. Standard doses of leucovorin can be administered orally, but higher doses used when the MTX concentration is elevated must be administered intravenously because the absorption of leucovorin and other folates from the intestinal tract is carrier-mediated and saturable at doses above 40 mg.

GLUCARPIDASE BACKGROUND

Glucarpidase (carboxypeptidase G$_2$ or Voraxaze, BTG plc, London, UK, www.btgplc.com) is a recombinant bacterial enzyme that inactivates MTX and folates by hydrolyzing the glutamate moiety (Fig. 3). Carboxypeptidase G$_1$ (CPG$_1$) was isolated from *Pseudomonas stutzeri* in the 1970s. Studies in mice and in a small number of patients revealed that administration of CPG$_1$ after MTX dosing prevented subsequent toxicity [38]; however,
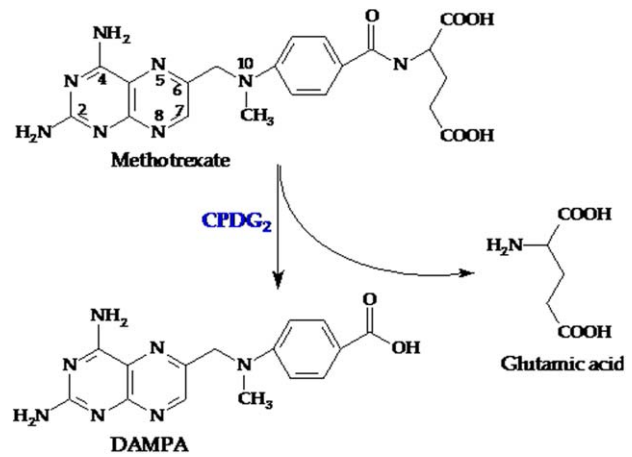
Figure 3. Methotrexate is cleaved into two noncytotoxic metabolites, DAMPA and glutamic acid, by CPDG$_2$.
   Abbreviations: CPDG$_2$, glucarpidase; DAMPA, 4-deoxy-4-amino-N10-methylpteroic acid.

the bacterial source was subsequently lost [39]. In the 1980s, carboxypeptidase G$_2$ (CPG$_2$) was isolated and purified from the *Pseudomonas* strain RS-16 and cloned into *Escherichia coli* [40, 41]. Glucarpidase rapidly lowers the MTX concentration by cleaving MTX into two noncytotoxic metabolites, 4-deoxy-4-amino-N10-methylpteroic acid (DAMPA) and glutamate, which are eliminated primarily by the liver (through the bile) and not by the kidney [4, 42].

Glucarpidase is provided as a lyophilized powder in vials containing 1,000 units and is administered in a single intravenous dose of 50 units/kg. Dose-finding studies of glucarpidase were not conducted in humans, but this recommended dose has been shown to be safe and effective [43]. Glucarpidase may not be routinely stocked in pharmacies because of its high cost and infrequent use, so the drug should be ordered as soon as the need for its use is anticipated (https://www.btgplc.com/products/specialty-pharmaceuticals/voraxaze-glucarpidase/). Adverse reactions related to glucarpidase, including nausea/vomiting, hypotension, paresthesia, flushing, and headache (mostly grade ≤2, according to the NCI Common Terminology Criteria for Adverse Events, version 3), were recorded each in less than 3% of patients [6]. Despite the potential immunogenicity related to the bacterial source of glucarpidase, hypersensitivity reactions were reported in <1% of patients, but 17% of patients receiving one or two doses of glucarpidase developed antiglucarpidase antibodies [6, 18, 44, 45].

GLUCARPIDASE PHARMACOLOGY

The pharmacokinetics of glucarpidase, which is an 83 kDa protein, were studied in the absence of MTX in eight healthy volunteers and in four subjects with severe renal dysfunction [46]. The molecular weight exceeds the threshold for glomerular filtration, so renal excretion is not likely to play a role in drug elimination. In normal volunteers, the clearance was 7.5 mL/min; the volume of distribution was 3.6 L, which is comparable to plasma volume; and the half-life was 6 or 9 hours (depending on the drug assay method) and was not substantially different in patients with renal dysfunction [43]. Due to the large molecular size of glucarpidase, it does not enter cells or cross the blood-brain barrier.

Because of the limited distribution volume of glucarpidase, metabolism of tissue and intracellular MTX is reliant on diffusion of the drug back into circulation. Glucarpidase cannot directly rescue the intracellular effects of MTXPG, although it can limit further kidney damage. Thus, leucovorin is still required after glucarpidase to protect normal cells from MTX toxicity [47].

Glucarpidase rapidly metabolizes circulating MTX and reduces plasma MTX concentrations by >95% within 15 minutes of administration [18, 42, 45]. This catalytic effect on circulating MTX persists for 48 hours, but there can be a rebound of plasma MTX concentration as the activity of glucarpidase wanes and MTX redistributes to circulation from tissues [21] (Fig. 4). However, the rebound plasma MTX concentration is typically substantially lower than the preglucarpidase MTX level [23]. Glucarpidase has a higher affinity for MTX (K_m 8 mM) than for leucovorin (K_m 120 mM) and 5-methyltetrahydrofolate (K_m 35 mM), but in vivo the AUC_{0-3h} of 5-MeTHF drops by >90% after administration of glucarpidase [40, 41].

Intravenously administered glucarpidase mediates rapid degradation of MTX and, as such, may have a role in limiting further nephrotoxicity; however, by itself, glucarpidase does not impact the normalization of kidney dysfunction [23]. Successful intrathecal administration of glucarpidase has been reported in a limited number of patients who experienced an accidental intrathecal MTX overdose [44, 48]; in contrast, in this emergency scenario, intrathecal administration of leucovorin should strictly be avoided [49].

INDICATIONS FOR GLUCARPIDASE THERAPY

Treatment with glucarpidase is indicated for patients with HDMTX-induced AKI and delayed MTX elimination leading to potentially toxic plasma MTX concentrations. The FDA has approved a dose of 50 units/kg for the treatment of toxic plasma methotrexate concentrations (>1 micromole per liter) in patients with delayed methotrexate clearance due to impaired renal function. But the limitations of use indicate that it should not be used in patients who exhibit the expected clearance of methotrexate (plasma methotrexate concentrations within two standard deviations of the mean methotrexate excretion curve specific for the dose of methotrexate administered) or those with normal or mildly impaired renal function because of the potential risk of subtherapeutic exposure to MTX [6].

The goal of this task force was to identify the population of patients who would benefit from glucarpidase rescue by more precisely defining the absolute MTX concentrations that put patients at risk for severe or life-threatening toxicity at specific time points after the start of the HDMTX infusion based on reported experience with HDMTX infusions. The duration of time above the threshold MTX concentration for various tissues was considered when determining the concentration cutoffs at each time point. We provide guidance for many of the time points that are routinely monitored in clinical practice (Fig. 5). Consulting with an experienced oncologist, nephrologist, or clinical pharmacist who is familiar with managing patients treated with HDMTX infusions is recommended if the patient has prior HDMTX-induced AKI or a GFR <75 mL/min/1.73 m². Administration of glucarpidase should optimally occur within 48–60 hours from the start of the HDMTX infusion, because
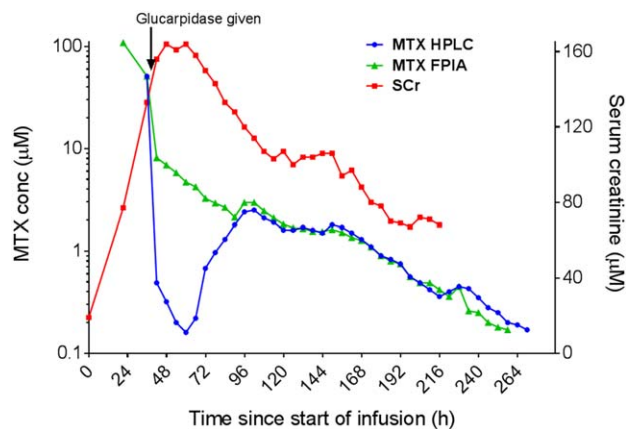
Figure 4. Concentrations of MTX and SCr in a patient enrolled on the Nordic Society of Paediatric Haematology and Oncology acute lymphoblastic leukemia 2008 protocol treated with glucarpidase following a 5 g/m² dose of MTX over 24 hours. The HPLC measurement of MTX (blue line) is more accurate than the FPIA measurement of MTX (green line) for the 48 hours following the administration of glucarpidase at hour 41. The HPLC measurement shows a rebound of the MTX concentrations from hour 60 to hour 96. The SCr increased during the 24-hour infusion and remained high for several days following the glucarpidase administration. The FPIA and HPLC measurements were performed by Stein Bergan at the Department of Pharmacology, Rikshospitalet, Oslo, Norway.

Abbreviations: conc, concentration; FPIA, fluorescence polarization immunoassay; HPLC, high pressure liquid chromatography; MTX, methotrexate; SCr, serum creatinine.

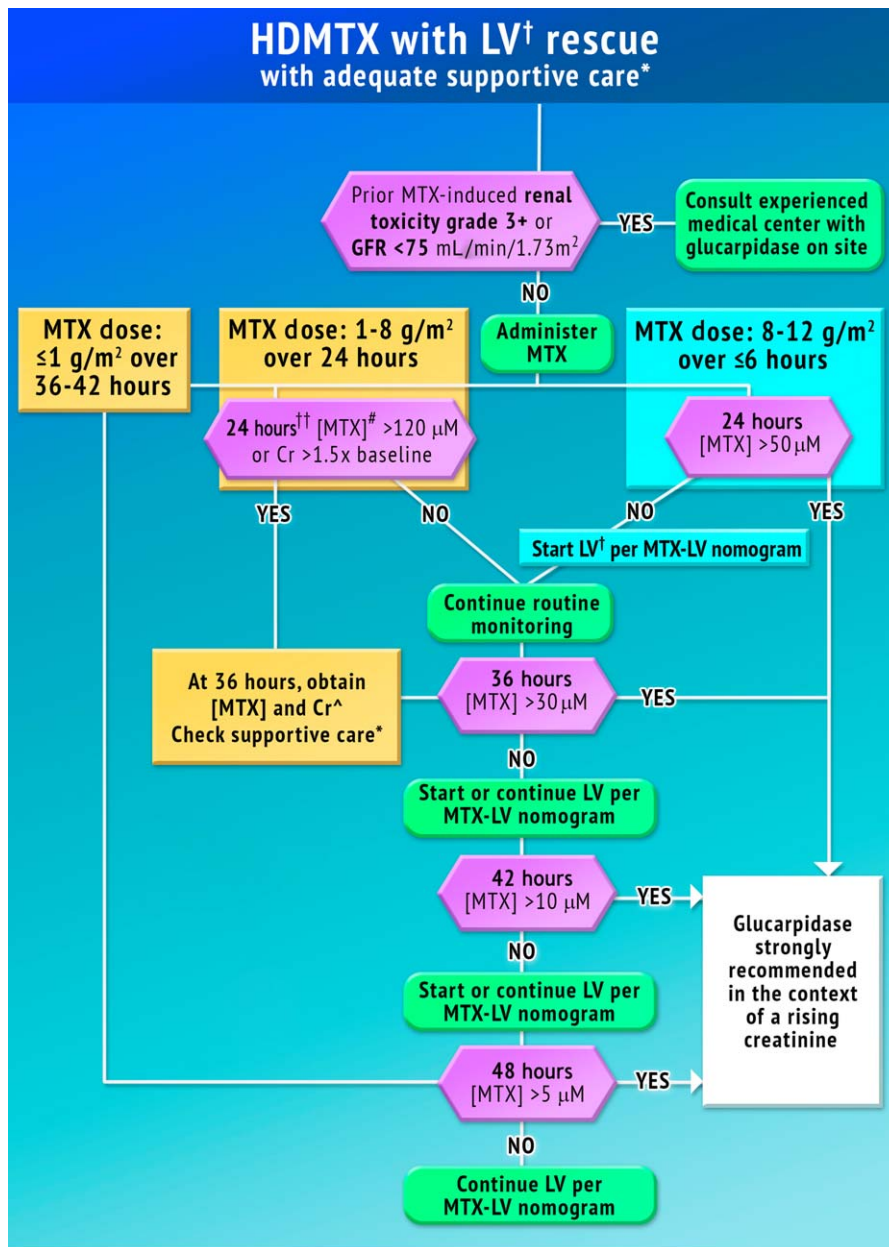life-threatening toxicities may not be preventable beyond this time point.

1–8 g/m² MTX Infused over 24–42 Hours

For a 24-hour infusion, a plasma MTX concentration >120 µM (54.5 µg/mL) at the end of the infusion or a creatinine increase ≥50% over baseline warrants additional monitoring at 36 hours. If the 36-hour MTX concentration is above 30 µM (13.6 µg/mL), the 42-hour MTX concentration is above 10 µM (4.54 µg/mL), or the 48-hour concentration is above 5 µM (2.27 µg/mL) and the serum creatinine is elevated relative to the baseline measurement (indicative of HDMTX-induced AKI), glucarpidase may be indicated. After a 36–42 hour HDMTX infusion, administration of glucarpidase may be indicated when the 48-hour MTX concentration is above 5 µM.

8–12 g/m² MTX Infused over ≤6 Hours

A plasma MTX concentration >1500 µM (681 µg/mL) at the end of the infusion warrants additional monitoring at 24 hours. If the 24-hour concentration is above 50 µM (22.7 µg/mL), the 36-hour concentration is above 30 µM (13.6 µg/mL), the 42-hour MTX concentration is above 10 µM (4.54 µg/mL), or the 48-hour concentration is above 5 µM (2.27 µg/mL) and the serum creatinine is elevated relative to the baseline measurement (indicative of HDMTX-induced AKI), administration of glucarpidase may be indicated.

There is a potential for falsely elevated plasma MTX concentration during or shortly after the end of the HDMTX infusion due to contamination if the specimen is drawn from the same lumen of the central venous catheter through which the drug was infused. If the plasma MTX concentration is elevated, but

HDMTX with LV† rescue
with adequate supportive care*

Prior MTX-induced renal toxicity grade 3+ or GFR <75 mL/min/1.73m² —YES→ **Consult experienced medical center with glucarpidase on site**

NO

Administer MTX

MTX dose: ≤1 g/m² over 36-42 hours

MTX dose: 1-8 g/m² over 24 hours

MTX dose: 8-12 g/m² over ≤6 hours

24 hours†† [MTX]# >120 μM or Cr >1.5x baseline

24 hours [MTX] >50 μM

YES / NO / NO / YES

Start LV† per MTX-LV nomogram

Continue routine monitoring

At 36 hours, obtain [MTX] and Cr^ Check supportive care*

36 hours [MTX] >30 μM —YES→

NO

Start or continue LV per MTX-LV nomogram

42 hours [MTX] >10 μM —YES→

NO

Start or continue LV per MTX-LV nomogram

48 hours [MTX] >5 μM —YES→

NO

Continue LV per MTX-LV nomogram

Glucarpidase strongly recommended in the context of a rising creatinine

Figure 5. Treatment with glucarpidase may be indicated in the case of excessively high MTX concentrations and rising creatinine. Time points indicated are after the start of the infusion and are not required measurement times. Guidance is provided for time points that are routinely measured in clinical practice. Renal toxicity grade 3+ is according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 4. Concentrations in μM can be converted to μg/mL by dividing by 2.2005. 120 μM = 54.5 μg/mL, 50 μM = 22.7 μg/mL, 30 μM = 13.6 μg/mL, 10 μM = 4.54 μg/mL, and 5 μM = 2.27 μg/mL.
  *Urine pH >7, urine output >2.5L/m² per day, emesis control.
  †Hours are indicated after infusion start.
  Abbreviations: Cr, serum creatinine; GFR, glomerular filtration rate; HDMTX, high-dose methotrexate; LV, leucovorin (folinic acid, citrovorum factor, 5-methyltetrahydrofolate); MTX, methotrexate; [MTX], plasma methotrexate concentration.

the serum creatinine is normal, the measurement should be repeated with a new blood sample.

GLUCARPIDASE ADMINISTRATION RECOMMENDATIONS
Leucovorin should be dosed according to the standard guidelines (Fig. 6) until glucarpidase can be given. Some regimens use the following equation to calculate the leucovorin dose (in mg) rather than the leucovorin nomogram in Figure 6 if the

plasma MTX concentration is >5 μM: plasma MTX concentration (μM) × body weight (kg). If glucarpidase is administered within 2 hours of the last dose of leucovorin, it will cleave the leucovorin as well as the MTX. Leucovorin should not be administered until 2 hours after a dose of glucarpidase because it may interfere with glucarpidase-mediated metabolism of MTX.

Leucovorin and MTX are racemic mixtures of two stereoisomers, and only the levo (L) enantiomer is recognized by most
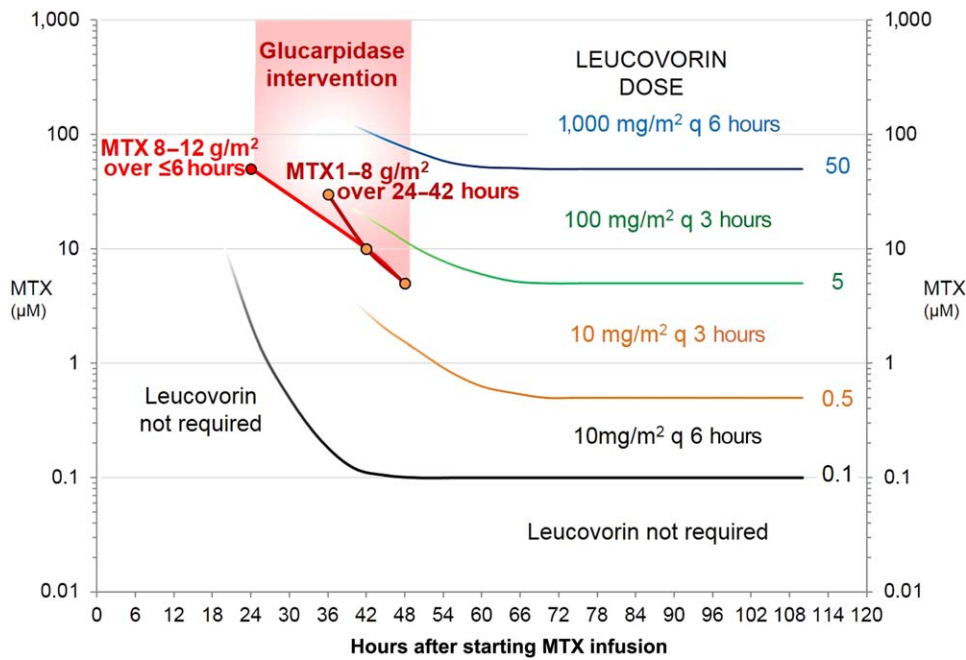
Figure 6. Standard leucovorin nomogram used in the U.S. Glucarpidase use may be indicated when MTX concentrations are within the shaded area.
Abbreviation: MTX, methotrexate.

folate carriers and enzymes that utilize folates as a cofactor or are inhibited by MTX or MTXPG. The active levo enantiomer of leucovorin (levoleucovorin calcium) is FDA-approved for use as a MTX rescue agent. Glucarpidase cleavage of MTX and tetrahydrofolates is less stereo-specific because the cleavage site does not involve the chiral carbon. Glucarpidase should be administered according to the package insert. If less than the full dose of 50 units/kg is all that is available, any amount that is available should be given, as lower doses may be effective [22]. Leucovorin rescue should be reinitiated no sooner than 2 hours after glucarpidase administration. Repeat administration of glucarpidase within 48 hours of the first dose during the same MTX course is not recommended due to decreased efficacy.

MEASUREMENT OF MTX AFTER GLUCARPIDASE THERAPY

Most clinical laboratories measure plasma MTX with an immunoassay method and not by a more specific high pressure liquid chromatography (HPLC) method [50]. Immunoassay methods detect MTX and its metabolites DAMPA and, to a varying extent, 7-hydroxymethotrexate. After glucarpidase administration, essentially all circulating MTX is converted into the nontoxic metabolite DAMPA and other metabolites [4] (Fig. 3), but an immunoassay method that does not distinguish between MTX and DAMPA overestimates the true MTX concentration. With an HPLC method that chromatographically separates MTX from DAMPA, the plasma MTX concentration usually drops by >95% by 15 minutes after glucarpidase. The half-life of DAMPA is approximately 9–10 hours, resulting in immunoassay interference and an inability to accurately quantify the MTX concentration for approximately 48 hours after glucarpidase. This limitation of the MTX immunoassay methods after glucarpidase therapy should be recognized and communicated between clinical and laboratory personnel. Another factor to consider is the

potential rebound of MTX concentrations more than 48 hours after glucarpidase administration (Fig. 4) due to release of MTX from tissue stores; therefore, continued monitoring of MTX concentrations and administration of leucovorin is very important in these patients [23, 42, 51, 52].

GUIDELINES FOR LEUCOVORIN USE AFTER GLUCARPIDASE THERAPY

Due to the large quantities of calcium, the infusion time of leucovorin at doses >200–500 mg (or the patient's body surface area (BSA) × 50) should be over 1–2 hours. To avoid excessive calcium concentrations, leucovorin can be substituted with calcium levofolinate or disodium levofolinate, which contain the active L-form of leucovorin, allowing the dose of leucovorin to be reduced by 50% compared with the racemic form. Disodium levofolinate can be given as a bolus dose and thus gives a faster clinical effect. Treatment with leucovorin should be continued until the plasma MTX concentration is below the threshold prescribed by the treatment protocol (e.g., below 0.1–0.2 μM). The minimal single dose of leucovorin is 15 mg/m² per dose. The minimal single dose of calcium or disodium levofolinate is 7.5 mg/m² per dose.

Leucovorin rescue should be restarted 2 hours after the administration of glucarpidase at the dose based on the MTX level prior to glucarpidase therapy. Because most institutions do not use an HPLC assay to specifically quantify MTX levels after glucarpidase administration, leucovorin rescue will be based on the MTX plasma concentration measured with the clinical immunoassay method, which measures MTX and its metabolites. Leucovorin should be administered for at least 48 hours after glucarpidase because of the MTX reentering the bloodstream from the tissues (Fig. 4).

DISCUSSION

Alternatives to Glucarpidase Therapy

There are scant data to directly compare the efficacy of glucarpidase with other modalities of lowering MTX plasma concentrations [53], including intermittent and continuous hemodialysis, peritoneal dialysis, charcoal hemoperfusion, and increasing elimination via enterohepatic circulation using enteral binding agents like oral cholestyramine. Hemodialysis and hemodiafiltration can clear MTX that is free in the plasma. Given the relatively high volume of distribution and protein binding of MTX, a rebound of free MTX occurs when dialysis is stopped [54–56]. In situations in which glucarpidase is not available, when patients have very high MTX concentrations, the risks of hemodialysis and/or high dose continuous veno-venous hemodiafiltration are low compared with the potential benefit of providing enhanced MTX clearance. Thymidine, which counteracts the effects of MTX through the restoration of DNA synthesis, did show promising results in clinical trials in a small number of patients but has been discontinued and is unavailable for investigational use [5].

High-dose leucovorin rescue has over the years been used for delayed MTX clearance when CPG$_1$ and CPG$_2$ were not easily available [57]. It is still the best option when glucarpidase is not available within 60 hours from the start of the HDMTX infusion. However, unnecessarily high doses of leucovorin should be avoided, as treatment failures in association with higher leucovorin doses or "over-rescue" have been reported in pediatric patients with ALL and osteosarcoma [58, 59]. Leucovorin is a storage vitamin, and excessive rescue could thus interfere with the MTX efficacy at the next HDMTX course [60]. Therefore, glucarpidase has increasingly come to the fore in the management and prevention of HDMTX-induced AKI.

Effect of Age on MTX-Induced Nephrotoxicity

Adults generally do not tolerate HDMTX as well as children, and MTX pharmacokinetic parameters are dependent on age [12, 61, 62]. The available pharmacokinetic data in adults are not as abundant as in children. One protocol reduces the MTX dose based on the GFR in elderly patients with central nervous system lymphoma, which resulted in similar toxicities compared with patients with normal renal function [63]. However, in most contemporary adult ALL protocols, there is no dose reduction. In a retrospective analysis including 649 HDMTX treatment cycles in 194 adult patients, advanced age was significantly associated with delayed MTX elimination and grade 2–4 renal toxicity [64]. In addition, elderly cancer patients are more likely to have some degree of baseline renal dysfunction that can be exacerbated by MTX [65]. Of 749 patients aged 1–45 years old treated by the Nordic Society of Paediatric Haematology and Oncology (NOPHO) ALL-2008 protocol, the 61 adult standard- or intermediate-risk patients who received 8 courses of HDMTX each and the 31 high-risk patients who received 6 courses demonstrated similar toxicities to the children treated on the same protocol [19].

Concomitant Medications

As MTX-induced nephrotoxicity is closely tied to MTX clearance, reducing the risk of AKI from other nephrotoxic medications is imperative. Each potential nephrotoxic medication should be carefully evaluated, and the risk and benefits of stopping this medication should be individualized. Studies in pediatrics have clearly shown that the AKI can nearly be prevented with judicious use of nephrotoxic medications [66]. In addition, increased exposure to 7-hydroxymethotrexate has been reported in association with pantoprazole [67]. Concomitant use of medications that can interfere with MTX elimination, such as proton pump inhibitors and nonsteroidal anti-inflammatory drugs, should be avoided [5, 68–75]. Even food (e.g., licorice [76]) and beverages (soft drinks, often sweetened with licorice extract) with low pH have been suspected to affect the MTX clearance. Since the introduction of 5-hydroxytryptamine (5HT3) receptor antagonists, emesis is not a problem during HDMTX and nowadays unlikely to be linked to AKI.

Trimethroprim-sulfamethoxazole is generally used as *Pneumocystis jiroveci* prophylaxis during ALL therapy [77], and there has been a worry that it could interfere with MTX pharmacokinetics and/or efficacy. However, it does not seem to interfere with HDMTX pharmacokinetics [78].

FURTHER RESEARCH NECESSARY

Given the paucity of published pharmacokinetic data in adults, it is difficult to determine whether the present recommendations, which are primarily based on pediatric experiences, adequately apply to adult patients. Thus, pharmacokinetic data in adult patients should be prospectively collected and analyzed and include patients with HDMTX-induced AKI and patients receiving glucarpidase treatment. These data are being collected in adults in Denmark (personal communication with Nina Toft, Adult NOPHO ALL-2008 coordinator). Additional studies on the effect of comedications on HDMTX pharmacokinetics are also needed, because existing data are based mostly on single case reports.

CONCLUSION

The expert panel was able to come to consensus, providing specific MTX concentrations above which glucarpidase is strongly recommended. Implementation of the recommendations in this guideline may help reduce the incidence of life-threatening HDMTX-induced toxicities.

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DISCLOSURES

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