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# Preventing and Managing Toxicities of High-Dose Methotrexate

Scott C. Howard,[a] John McCormick,[b] Ching-Hon Pui,[c] Randall K. Buddington,[a] R. Donald Harvey[d]

[a]School of Health Studies, University of Memphis, Memphis, Tennessee, USA; Departments of [b]Pharmaceutical Sciences and [c]Oncology, St. Jude Children's Research Hospital, New York, New York, USA; [d]Department of Hematology and Medical Oncology, Emory University School of Medicine, Atlanta, Georgia, USA

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

## Abstract

High-dose methotrexate (HDMTX), defined as a dose higher than 500 mg/m$^2$, is used to treat a range of adult and childhood cancers. Although HDMTX is safely administered to most patients, it can cause significant toxicity, including acute kidney injury (AKI) in 2%–12% of patients. Nephrotoxicity results from crystallization of methotrexate in the renal tubular lumen, leading to tubular toxicity. AKI and other toxicities of high-dose methotrexate can lead to significant morbidity, treatment delays, and diminished renal function. Risk factors for methotrexate-associated toxicity include a history of renal dysfunction, volume depletion, acidic urine, and drug interactions. Renal toxicity leads to impaired methotrexate clearance and prolonged exposure to toxic concentrations, which further worsen renal function and exacerbate nonrenal adverse events, including myelosuppression, mucositis, dermatologic toxicity, and hepatotoxicity. Serum creatinine, urine output, and serum methotrexate concentration are monitored to assess renal clearance, with concurrent hydration, urinary alkalinization, and leucovorin rescue to prevent and mitigate AKI and subsequent toxicity. When delayed methotrexate excretion or AKI occurs despite preventive strategies, increased hydration, high-dose leucovorin, and glucarpidase are usually sufficient to allow renal recovery without the need for dialysis. Prompt recognition and effective treatment of AKI and associated toxicities mitigate further toxicity, facilitate renal recovery, and permit patients to receive other chemotherapy or resume HDMTX therapy when additional courses are indicated. *The Oncologist* 2016;21:1471–1482

**Implications for Practice:** High-dose methotrexate (HDMTX), defined as a dose higher than 500 mg/m$^2$, is used for a range of cancers. Although HDMTX is safely administered to most patients, it can cause significant toxicity, including acute kidney injury (AKI), attributable to crystallization of methotrexate in the renal tubular lumen, leading to tubular toxicity. When AKI occurs despite preventive strategies, increased hydration, high-dose leucovorin, and glucarpidase allow renal recovery without the need for dialysis. This article, based on a review of the current associated literature, provides comprehensive recommendations for prevention of toxicity and, when necessary, detailed treatment guidance to mitigate AKI and subsequent toxicity.

## Introduction

Methotrexate is an antimetabolite that interferes with the metabolism of folic acid. After entry into the cell, methotrexate is polyglutamated, binds dihydrofolate reductase (DHFR) with an affinity 1,000-fold greater than that of folate, and competitively inhibits conversion of dihydrofolate to tetrahydrofolate (Fig. 1). Tetrahydrofolate is essential for biosynthesis of thymidine and purines, which are needed for synthesis of DNA. Blockade of tetrahydrofolate synthesis by methotrexate leads to inability of cells to divide and to produce proteins. Methotrexate is an essential component of therapy for acute lymphoblastic leukemia (ALL) and is active against many types of cancer; this justifies its presence on the World Health Organization's list of essential medicines.

Methotrexate is administered at doses that range from 12 mg intrathecally and 20 mg/m$^2$ orally, intramuscularly, or intravenously as weekly maintenance chemotherapy for ALL to doses as high as 33,000 mg/m$^2$ intravenously for some other indications [1]. Doses of 500 mg/m$^2$ or higher given intravenously are defined as high-dose methotrexate (HDMTX) and are used to treat a variety of adult and pediatric cancers, including ALL, osteosarcoma, and lymphomas [2–4]. HDMTX therapy can cause significant toxicity, which not only leads to morbidity and occasional mortality but may also interrupt cancer treatment, potentially leading to inferior anticancer outcomes. To prevent unacceptable toxicity, it must be given with rigorously standardized supportive care [1], which differs across tumor types and treatment protocols (Table 1). When patients experience delayed methotrexate excretion, without timely recognition and treatment, the prolonged exposure to toxic methotrexate
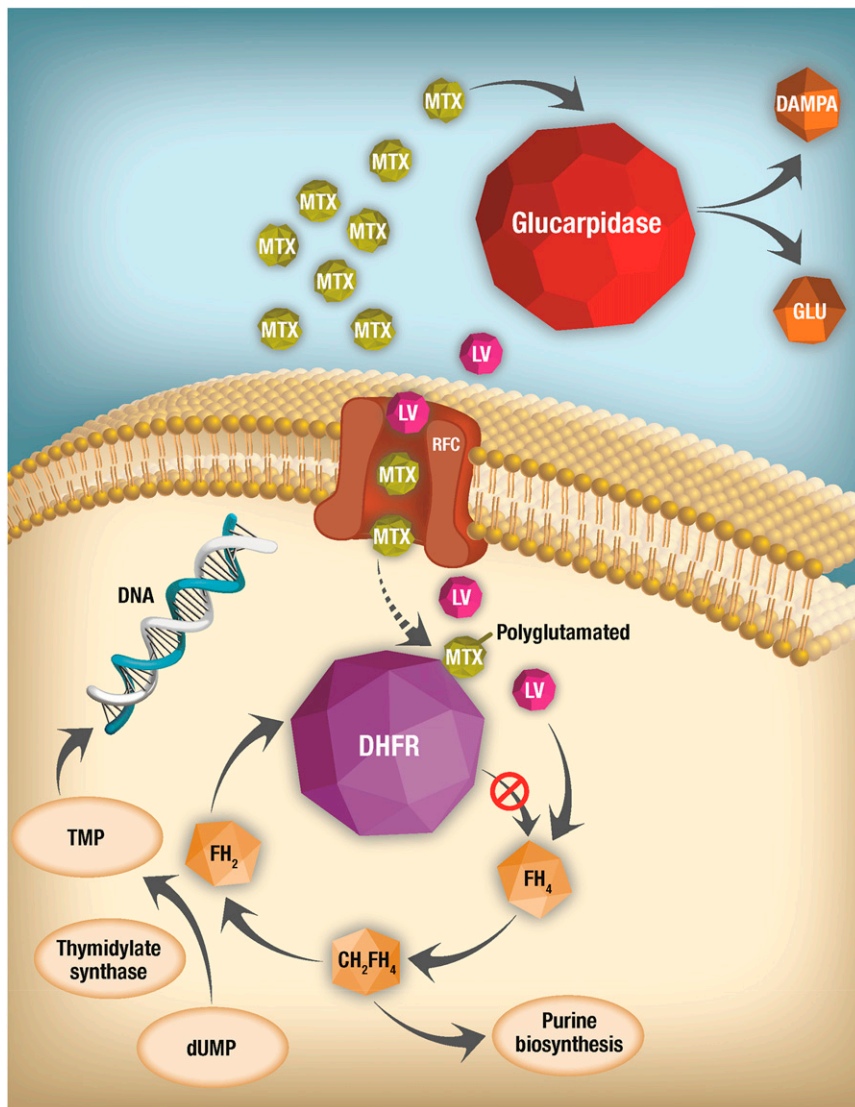
**Figure 1.** Mechanism and site of action of MTX and of rescue strategies for delayed MTX elimination. After MTX enters cells through the RFC, it is polyglutamated, then competitively and reversibly inhibits the activity of DHFR, thus preventing formation of $FH_4$ from $FH_2$. The lack of $FH_4$ inhibits DNA, RNA, and protein synthesis. LV enters cells through the RFC and allows formation of $FH_4$ despite the presence of MTX, which effectively rescues cells. However, when MTX elimination is impaired and it is present at very high concentrations, very high doses of LV are necessary to allow entry of a sufficient amount to rescue cells from MTX toxicity. Glucarpidase eliminates extracellular MTX by converting it to nontoxic DAMPA and therefore should always be given with LV to provide intracellular rescue even as the glucarpidase prevents further accumulation of intracellular MTX by removing it from the extracellular compartment.

Abbreviations: DAMPA, 4-deoxy-4-amino-*N*-10-methylpteroic acid; DHFR, dihydrofolate reductase; dUMP, deoxyuridine monophosphate; $FH_2$, dihydrofolate; $FH_4$, tetrahydrofolate; $FH_5$, tetrahydrofolate; LV, leucovorin; MTX, methotrexate; RFC, reduced folate carrier; TMP, thymidine monophosphate.

concentrations can lead to significant morbidity and mortality [1]. Aggressive monitoring and prompt intervention usually promote methotrexate excretion, prevent toxicity, and allow patients to receive subsequent HDMTX treatment [1, 5]. Here, we describe common toxicities, review supportive care strategies, explore approaches to manage toxicity, and suggest subsequent HDMTX therapy after treatment-related toxicity occurs.

## POTENTIAL TOXICITIES OF HIGH-DOSE METHOTREXATE

### Acute Kidney Injury
Despite appropriate supportive care measures during administration of HDMTX, acute kidney injury (AKI) develops in

2%–12% of patients [6]. The incidence depends on host factors, supportive measures used, and the dose and schedule of HDMTX. For example, 9.1% of HDMTX cycles in patients with lymphoma are complicated by AKI, compared with only 1.5% of cycles in patients with sarcomas [7, 8]. Nephrotoxicity caused by HDMTX arises through crystal nephropathy, which occurs when methotrexate and its metabolites precipitate within the renal tubules. Because methotrexate is acidic, drug crystals are not present in urine with an alkaline pH, as alkalinization greatly increases methotrexate solubility and excretion. Crystal-induced nephropathy initially manifests as an asymptomatic elevation in serum creatinine and then progresses to tubular necrosis and more severe renal injury.

**Table 1.** Selected protocols that include high-dose methotrexate for acute lymphoblastic leukemia and osteosarcoma

| Study, year [reference] | Methotrexate dose | Duration of methotrexate infusion (hours) | Leucovorin rescue dose | Time from start of methotrexate infusion to first leucovorin dose (hours) |
|---|---|---|---|---|
| **Acute lymphoblastic leukemia** | | | | |
| Takeuchi et al., 2002 [98] | 100-mg/m$^2$ bolus, then 500 mg/m$^2$ per hour | 4 | 15 mg every 6 hours × 8 doses | 28 |
| Linker et al., 2002 [99] | 220-mg/m$^2$ bolus, then 60 mg/m$^2$ per hour × 36 hours | 36 | 50 mg every 6 hours | 36 |
| Hill et al., 2004 [100] | 6 g/m$^2$ (age < 4 yr)  8 g/m$^2$ (age > 4 yr) | 10% bolus, remainder over 23 hours | 15 mg/m$^2$ every 3 hours, then every 6 hours when serum methotrexate < 2 × 10$^6$ $\mu$M | 36 |
| Pui et al., 2007 [58] | 2 g/m$^2$ | 2 | 10 mg/m$^2$ every 6 hours | 44 |
| Zhang et al., 2014 [101] | 3–5 g/m$^2$ | 24 | 15 mg/m$^2$ every 6 hours, then pharmacokinetically guided to serum methotrexate 0.1 $\mu$mol/L | 36 |
| **Osteosarcoma** | | | | |
| Souhami et al., 1997 [102] | 8 g/m$^2$ (age ≥12 yr)  12 g/m$^2$ (age <12 y) | Not specified | 12 mg/m$^2$ i.v. or 15 mg/m$^2$ p.o. every 6 hours for 10 doses | 24 |
| Fuchs et al., 1998 [103] | 12 g/m$^2$ (maximum, 20 g) | Not specified | 15 mg/m$^2$ every 6 hours for 12 doses | Not specified |
| Bacci et al., 2001 [104] | 12 g/m$^2$ (escalated to 14 g/m$^2$ if the 6-hour serum methotrexate was <1 $\mu$mol/L) | 6 | 15 mg every 6 hours for 11 doses | 24 |
| Goorin et al., 2003 [105] | 12 g/m$^2$ | 4 | 15 mg every 6 hours for 10 doses | 24 |
| Ferrari et al., 2005 [106] | 12 g/m$^2$ | 4 | 8 mg/m$^2$ every 6 hours for 11 doses | 24 |
| **Non-Hodgkin lymphoma** | | | | |
| Koller et al., 1997 [107] | 200 mg/m$^2$ over 30 min, then 800 mg/m$^2$ | 24 | 50 mg i.v. for one session, then 15 mg p.o. every 6 hours or as methotrexate concentrations define | 36 |
| Khouri et al., 1998 [108] | 200 mg/m$^2$ over 30 min, then 800 mg/m$^2$ | 24 | 50 mg i.v. for one session, then 15 mg p.o. every 6 hours or as methotrexate concentrations define | 36 |
| Thomas et al., 2004 [109] | 200 mg/m$^2$ over 30 min, then 800 mg/m$^2$ | 24 | 50 mg i.v. for one session, then 15 mg p.o. every 6 hours or as methotrexate concentrations define | 36 |
| **Primary central nervous system lymphoma** | | | | |
| Batchelor et al., 2003 [110] | 8 g/m$^2$ | 4 | Pharmacokinetically guided until serum methotrexate < 1 × 10$^6$ $\mu$M | 24 |
| Wright et al., 2015 [46] | 2,000/5,000 mg/m$^2$ | 24 | 15 mg/m$^2$ every 6 hours for a total of 5 doses | 24 |
| Dalia et al., 2015 [111] | 8 g/m$^2$ | Not specified | Not specified | Not specified |

Because volume depletion and acidic urine are major risk factors for AKI, hyperhydration and urine alkalinization are mandatory during HDMTX treatment (discussed further in the section on supportive care measures) [7]. Drug-drug interactions can also contribute to delayed methotrexate excretion and subsequent nephrotoxicity [7]. Agents that pose the highest risk of adverse interaction are those that interfere with methotrexate clearance by competing for renal tubular secretion (Table 2) [1].

**Table 2.** Drugs that impair methotrexate clearance

| Agents | Mechanism of inhibition |
| --- | --- |
| Nonsteroidal anti-inflammatory drugs, penicillin and penicillin derivatives, salicylates, probenecid, gemfibrozil, trimethoprim-sulfamethoxazole | Direct inhibition of renal excretion |
| Amphotericin, aminoglycosides, radiographic contrast dyes | Nephrotoxicity that leads to decreased glomerular filtration with consequent inhibition of renal excretion |
| Proton-pump inhibitors | Unclear; potential inhibition of methotrexate BCRP-mediated renal transport |
| P-glycoprotein/ABCB1 inhibitors | Inhibition of methotrexate transport in multiple organs, including kidney |
| Levetiracetam, chloral hydrate | Unclear, potential competition for tubular secretion |

References [10–15].
Abbreviations: ABCB1, ATP-binding cassette B1; BCRP, breast cancer resistance protein, also known as ABCG2 (ATP-binding cassette) G2.

Careful reviews of all medications and close monitoring of all patients are warranted [9–15]. Whether prophylactic trimethoprim-sulfamethoxazole should be discontinued during HDMTX infusion is controversial. Although some protocols suggest deferring it until adequate HDMTX clearance is documented, no strong data support this approach.

## Other Toxicities

Acute kidney injury impairs the renal clearance of methotrexate, resulting in the accumulation of toxic concentrations and an increased risk for additional adverse events [7]. Prolonged renal dysfunction with increased systemic methotrexate exposure can cause myelosuppression, mucositis, hepatotoxicity, and, in severe cases, multiorgan failure [16]. Emesis occurs in 10%–30% of patients receiving HDMTX even when appropriate antiemetics are used; in this subgroup, antiemetics should be escalated to completely control vomiting and additional hydration provided to replace lost fluid [17]. The American Society of Clinical Oncology clinical practice guideline for antiemetic therapy classifies HDMTX as having low emetic risk and recommends dexamethasone to reduce the risk for nausea and vomiting. Because 5-HT3 antagonists are used almost universally, dexamethasone is often omitted [17]. Transient liver toxicity may include reversible chemical hepatitis in up to 60% and hyperbilirubinemia in 25% of courses, respectively [18]. In up to 15% of HDMTX courses, patients report transient disturbances of the central nervous system (CNS), and a subset of these experience significant symptoms, such as cortical blindness, hemiparesis, and seizure [19]. Chemical conjunctivitis occurs rarely and can be managed with local treatment; indeed, methotrexate can be safely administered intraocularly to control autoimmune diseases that affect the eye [20]. Pulmonary toxicity is observed in 0.5% of patients per year who receive weekly low-dose methotrexate, but rarely with HDMTX [18, 21–23]. Pulmonary function testing or other screening is not warranted in patients with cancer, in whom methotrexate-induced pneumonitis is rare, unless pre-existing lung compromise or other risk factors warrant surveillance.

Animal models, particularly rats, have been used to study toxicities of other therapeutics [24] and diseases, such as nonalcoholic steatohepatitis [25]. Rodent models have been used for pharmacokinetic modeling of methotrexate [26], exploring leucovorin rescue [27], and preclinical studies of investigational interventions to reduce toxicity (including pentoxifylline [28], amifostine [29], melatonin [30], and activators of peroxisome proliferator activator receptor $\alpha$ and $\gamma$ [31]). Critically, the mechanism of MTX crystal formation in the renal tubule was elucidated in monkeys [32], and the concept of enzymatic cleavage of MTX using glucarpidase was first described in a mouse model by Chabner et al. in 1972 [33].

## RISK FACTORS FOR TOXICITY DURING TREATMENT WITH HIGH-DOSE METHOTREXATE

Several patient-related factors can increase the risk for AKI [7]. Volume depletion is perhaps the most important and can result from fluid losses due to vomiting or diarrhea, adrenal insufficiency, or renal salt wasting [7]. Reductions in intravascular volume lead to renal hypoperfusion with subsequent decreased urine output [7]. Precipitation of methotrexate crystals occurs in acidic urine (pH < 5.5) when the concentration of methotrexate in the renal tubules exceeds $2 \times 10^{-3}$ molar. Intrarenal crystal formation can lead to tubule obstruction, direct toxic damage to the renal tubular epithelium (due to prolonged contact with methotrexate), and hypoperfusion from afferent arteriolar vasoconstriction, each of which independently can worsen AKI [34–36]. Polyuria leading to severe shifts in fluid balance has also been reported with methotrexate infusion. Although the mechanism remains unclear, patients with methotrexate-associated polyuria require especially close monitoring of fluid status and frequent adjustments in intravenous fluids to maintain fluid balance and prevent renal hypoperfusion [37].

Patients with a history of toxicity with prior courses of HDMTX have higher risk for subsequent renal toxicity [1]. However, even when toxicity is severe, subsequent HDMTX courses can generally be administered safely after the patient recovers [5]. As many as 60% of adult cancer patients have some degree of renal dysfunction, which increases their risk for AKI [38]. Lower creatinine clearance (CrCl) before administration of HDMTX predicts renal toxicity, and both CrCl and serum creatinine concentration before HDMTX administration can be useful in predicting plasma methotrexate concentrations after infusion [8, 39]. Specific CrCl cutoff values for dose reduction or omission of subsequent HDMTX have not been established, with upper cutoffs for dose reduction starting at 50–60 mL/min and recommendations to omit further HDMTX when CrCl falls below 10–30 mL/min [40, 41]. Additional host factors that contribute to AKI risk include pre-existing nephropathy because of previous drug toxicity (e.g., from cisplatin) or

associated disease, metabolic derangements due to the presence of tumor, advanced age, and pharmacogenetic factors (such as hyperhomocysteinemia with concurrent relative or absolute folate deficiency) (Table 2) [9, 42]. In this regard, methotrexate clearance is also associated with polymorphisms of *SLCO1B1,* which encode a hepatic solute carrier organic anion transporter that mediates disposition of many medications, including methotrexate [43–45].

Delayed methotrexate excretion has been associated with extravascular fluid collections, including ascites, pleural effusions, or intracranial fluid; whether HDMTX should be deferred to a later date in such situations depends on the balance of risks and the benefits of deferral, but even more rigorous monitoring is warranted if one proceeds [46]. Pre-existing nephropathy is associated with substantial toxicity even with low doses of methotrexate [47], suggesting a need for even greater diligence during HDMTX administration in the presence of renal dysfunction [7, 48]. Finally, delays between recognition of toxicity and initiation of treatment can directly contribute to renal and systemic toxicities [6]. In the setting of AKI, the rise in serum creatinine values lags behind progressive intrinsic renal damage, such that precise measurement of function at a specific moment is difficult [49]. Instead, a decrease in urine output, positive fluid balance, or weight change may help identify patients with early AKI after HDMTX administration, even before creatinine increases. Nevertheless, in some cases irreversible damage to renal tubule epithelial cells may have already occurred before the onset of oliguria or detection of clinically significant increases in serum creatinine concentration.

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### SUPPORTIVE CARE MEASURES

In most patients with normal renal function, HDMTX can be given safely with the use of several supportive care strategies. These should include adjusting medications with potential interactions, vigorous hydration, and urinary alkalinization (target pH $\geq$ 7) before starting methotrexate infusions. The goal is to enhance the solubility and dilution of methotrexate in the urine and apply leucovorin rescue guided by serial serum methotrexate levels to protect against potentially lethal toxicity.

### Suspending Medications That Interfere With Methotrexate Clearance

All prescribed, over-the-counter, and nontraditional medications must be reconciled and documented before HDMTX administration begins. Because of their long half-life, some medications (e.g., naproxen sodium) can interfere with methotrexate elimination for many hours, with potentially disastrous delays in methotrexate elimination. Patients who

take such medications daily should be provided with an alternative starting the day before HDMTX and continuing until methotrexate clearance. Studies in mice documented inhibition of renal tubule methotrexate transporters by indomethacin and ketoprofen, with reduced methotrexate elimination and prolonged elevated levels and toxicity [24].

### Hydration

More than 90% of methotrexate is eliminated by the kidneys [1]. The use of fluids to promote high urinary flow rates and alkalinize the urine protects the kidney from injury during treatment with HDMTX [7]. Many pediatric protocols recommend at least 2 hours of hyperhydration of a minimum of 200 mL/m$^2$ per hour or 100–150 mL/m$^2$ per hour beginning 12 hours before the start of methotrexate infusion and continuing for 24–48 hours or longer if the patient has a history of methotrexate toxicity or develops delayed methotrexate elimination [1]. In adults, rates of 150–200 mL of bicarbonate-containing fluids per hour to a total of 2 L before HDMTX infusion are often used. Strict monitoring of fluid intake and output is recommended during and after administration of methotrexate.

### Urine Alkalinization

Methotrexate and its metabolites, including 7-OH-methotrexate and 4-deoxy-4-amino-*N*-10-methylpteroic acid (DAMPA), are poorly soluble at an acidic pH [1, 50]. An increase in urine pH from 6.0 to 7.0 increases the solubility of methotrexate and its metabolites by five- to eightfold, and alkalinization is imperative to reduce intratubular crystal formation (precipitation) [1, 50]. Thus, administration of fluids with 40 mEq/L sodium bicarbonate is recommended during and after HDMTX administration [1, 7]. A urine pH of 7 or greater should be required before administration of methotrexate to reduce crystal formation. It is also important to check urine pH values with each void during the infusion to ensure no extended periods of time with acidic urine, which could increase the risk for precipitation, nephrotoxicity, and delayed methotrexate elimination. The ability of the laboratory to process samples rapidly and notify clinicians when the pH decreases to 7 or less facilitates safer HDMTX administration. If a urine pH of 6.5 is identified, sodium bicarbonate at a dose of 12.5 mEq/m$^2$ is administered, and for urine pH <6.5, a dose of 25 mEq/m$^2$ is given; urine pH is measured hourly throughout HDMTX infusion because sometimes boluses of sodium bicarbonate must be repeated to achieve alkaline urine [51]. In patients with serum alkalosis and inadequate urine alkalinization, the carbonic anhydrase inhibitor acetazolamide (250–500 mg p.o. four times daily) may be added to directly alkalinize the urine by increasing renal excretion of sodium, water, and bicarbonate, without increasing serum pH [52].

### Leucovorin

For more than 30 years, leucovorin rescue has been a cornerstone of HDMTX treatment (Fig. 2) [18]. Leucovorin is particularly effective in the prevention of myelosuppression, gastrointestinal toxicity, and neurotoxicity during treatment with HDMTX. Chemotherapy protocols that include HDMTX also include recommendations for the timing, dose, and duration of leucovorin administration to protect normal cells
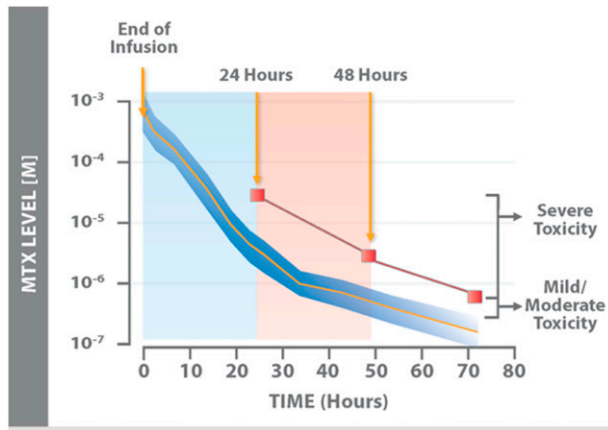
**Figure 2.** Nomogram for the expected time-dependent decrease in serum MTX levels after completion of MTX infusion. Nomogram for expected serum MTX levels as a function of time from the end of a 6-hour infusion of methotrexate at a dose of 7.5 g/m$^2$. The dark blue area represents ±2 SD from the mean (orange line). Values above the red line indicate impending or severe toxicity. Adapted from [112] with permission.
   Abbreviation: MTX, methotrexate.

from injury (Table 1) [1, 18]. Because leucovorin effectively neutralizes the effects of methotrexate, it must not be started too early because it would then reduce not only toxicity but also anticancer efficacy. In this regard, if a patient is taking leucovorin at the time HDMTX treatment is scheduled to begin, the leucovorin should be discontinued and the HDMTX deferred until the following day.

### Other Supportive Care Measures
Other supportive care measures can be tailored according to individual patient risk factors. For instance, HDMTX doses can be reduced in patients with pre-existing renal dysfunction or severe toxicity after a prior course of HDMTX, and serum methotrexate levels can be measured early (e.g., at hour 6 of a 24-hour infusion) to make sure that there is no excessive accumulation [7]. During treatment with HDMTX, patients must also minimize exposure to other potential nephrotoxins, including those listed in Table 2 [7, 10].

### MONITORING DURING TREATMENT WITH HIGH-DOSE METHOTREXATE
The pharmacokinetics of methotrexate dictate the degree of supportive care and monitoring needed after treatment. After a HDMTX infusion with a fixed dose and duration, plasma concentrations can vary widely between patients and within a patient on different cycles. Plasma protein binding, effusions, renal function, and, to a lesser degree, hepatic function can all contribute to peak concentrations after infusion. Serial methotrexate concentrations are obtained, with the primary focus on values that easily fit within the leucovorin nomogram timeframe (i.e., 42 hours and later) (Fig. 2). However, pharmacokinetic modeling data from Evans et al. [53] show that values above 10 $\mu$M at 24 hours after the start of the infusion confers a high risk for toxicity.
   Methotrexate is primarily eliminated by the kidney, so renal function must be assessed before, during, and after each course of HDMTX. Currently used measures of kidney function include serum creatinine, urine output, urine pH, and blood urea nitrogen [54]. A rise in serum creatinine concentration and other parameters above normal values indicates potential renal dysfunction and delayed methotrexate elimination [2]. Incorporation of clinical decision support in the electronic health record can alert clinicians of acute changes in serum creatinine or prescription of medicines that may delay elimination of methotrexate. Automated early warnings may facilitate prompt interventions, such as increasing the rate of intravenous fluids, substitution of an alternative drug that does not interfere with methotrexate clearance, and, in extreme cases, even stopping the methotrexate infusion early to prevent toxicity. Treatment protocols that include HDMTX sometimes outline strategies for dose reduction in patients with reduced creatinine clearance [1]. The utility of alternate biomarkers for earlier detection of renal injury is an area of active study [55].
   Plasma methotrexate concentrations should be monitored closely to detect any delay in methotrexate clearance during each cycle of therapy [56]. Depending on the regimen, plasma methotrexate assays may be appropriate at 24, 48, and 72 hours after the start of methotrexate infusion [57]. Other protocols may require serum methotrexate measurements at 36 hours (i.e., 12 hours after the end of a 24-hour infusion) or at 42 hours from the start [58]. Importantly, leucovorin doses are adjusted according to plasma methotrexate concentrations, and hydration and alkalization can be fine-tuned to optimize safety [1]. Serum methotrexate concentrations should be monitored with ongoing adjustments in hydration, alkalinization, and leucovorin rescue until the target of less than 0.05–0.1 $\mu$M is reached [1]. Plasma methotrexate monitoring is a reliable indicator specifically of nephrotoxicity but may be a limited predictor of other toxicities [59]. However, in centers where methotrexate levels cannot be monitored, assiduous monitoring of urine pH and output, serum creatinine, and twice-daily examination of mucosal membranes for evidence of inflammation can allow safe administration of HDMTX for most patients; indeed, this practice in Recife, Brazil, where methotrexate levels were not available, allowed for safe administration of hundreds of courses of HDMTX [60].

### MANAGEMENT OF SPECIFIC TOXICITIES ASSOCIATED WITH HIGH-DOSE METHOTREXATE

### Nephrotoxicity
Aggressive supportive care measures are needed when AKI occurs after HDMTX. Continuing to administer alkalinized i.v. fluids with addition of acetazolamide when needed to keep urine pH > 7 maximizes methotrexate elimination and reduces further crystal formation in nephrons. Increasing infusion rates to the maximum tolerated amount (≥3 L/m$^2$ per day) is recommended to maximize urine output. Attention to fluid balance, frequent symptom assessment, pulmonary examination, pulse oximetry, and chest radiography or echocardiography of patients at risk for heart failure allow aggressive hydration with minimal risks. Although pleural effusions may occur with very aggressive hydration, the risk-benefit relationship favors continued hydration in most cases, as delayed methotrexate elimination in most patients is primarily driven by renal dysfunction.

Extracorporeal techniques to remove excessive methotrexate have been used and are logical on the basis of the distribution of methotrexate in serum and its limited protein binding (58%) [61]. However, results have been mixed; because of ethical considerations, studies have lacked suitable control patients who did not receive dialysis and have been confounded by differing concomitant interventions (e.g., leucovorin doses, glucarpidase use) [1, 62]. Furthermore, retrospective analyses of differing approaches without control groups, including plasmapheresis, charcoal hemoperfusion, high-flux hemodialysis, conventional hemodialysis, and peritoneal dialysis make it difficult to identify one optimal extracorporeal strategy. High-flux hemodialysis is likely to be the most effective based on technique and flow rates and reduced methotrexate concentrations during a 6-hour period in one series, whereas peritoneal dialysis is unlikely to be effective [63]. Unfortunately, even when hemodialysis is effective, many patients experience a rebound in serum methotrexate concentrations of 10%–220% of the postprocedure values [64]. Complications of dialysis must also be considered, especially in critically ill patients who are at higher risk for electrolyte abnormalities, bleeding at catheter sites, and cardiac arrest. Rapid institution of extracorporeal methods is critical if they are used, but the variable results and rebound rise in methotrexate concentrations necessitate continuous monitoring and repeat dialysis as needed. In all cases, high doses of leucovorin should be administered until methotrexate has been completely eliminated; in very ill patients, continuing it for another day or two thereafter is warranted. Leucovorin is removed by dialysis, and so it should be redosed afterward [65].

## Hepatotoxicity

Hepatotoxicity after HDMTX is much less common than with the lower, long-term oral methotrexate dosing that is used in patients with rheumatoid arthritis, who are at risk for liver fibrosis and require regular monitoring of liver enzyme levels [66]. Indeed, almost all patients have elevations of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values after HDMTX, but these laboratory findings have no clinical significance and require no adjustment of subsequent courses of HDMTX because most cases are transient and reversible and do not lead to chronic liver disease [67]. Measurement of AST, ALT, and bilirubin before each course of HDMTX is advisable to assure there is no evidence of hepatic inflammation or dysfunction that could be worsened by methotrexate [68]. Risk factors for hepatotoxicity in long-term methotrexate dosing include alcohol and hepatitis B and C virus infection [69–71]. Although these have not been documented to complicate HDMTX, avoiding alcohol and controlling hepatitis infection before HDMTX is warranted to minimize risk. Conversely, existing steatohepatitis may increase methotrexate toxicity [25].

## Neurotoxicity

CNS toxicity may occur after HDMTX, and concurrent intrathecal treatment, cranial irradiation, infiltration of malignant cells, or concomitant CNS toxins increase the risk, as well as confound the etiology. Depending on the population studied, up to 11% of patients may have CNS events, including confusion, seizures, somnolence, and headaches with or without radiographic evidence of leukoencephalopathy [72]. For example, 3% of pediatric ALL patients had acute encephalopathy, and pharmacokinetic parameters did not predict the onset [72]. Symptoms typically occur within 24 hours, often resolve spontaneously, and rarely have long-term sequelae [19, 73]. Elevated ratios of methotrexate to leucovorin at 42 hours may predict those at risk for CNS toxicity, and polymorphisms in genes associated with neurodevelopment (e.g., *TRIO*, *PRKG1*, *ANK1*, *COL4A2*, *NTN1*, and *ASTN2*) may also increase risk [19].

A potential mechanism of neurotoxicity is the accumulation of adenosine after MTX-induced reductions in purine synthesis [74]. The finding of increased adenosine in the CNS in patients with toxicity led some investigators to evaluate 1-hour 2.5-mg/kg infusions of aminophylline in pediatric ALL patients on the basis of their ability to displace adenosine from central receptors [75]. In the 6 patients treated, 4 had complete resolution of symptoms that had not improved after other measures (e.g., corticosteroids), and 2 had persistent nausea but no other symptoms. However, no definitive studies demonstrate the efficacy of aminophylline in treating or preventing methotrexate-induced neurotoxicity. Patients who develop CNS toxicity should have all potential neurotoxins discontinued and magnetic resonance imaging examination should be performed, particularly if symptoms do not improve within 24 hours of onset.

## Mucositis

Oral mucositis can become a dose-limiting toxicity, require the use of opioids, increase infectious risk, and lead to chemotherapy delays. The biological processes associated with mucositis ultimately result in mucosal barrier injury, based on a systematic cascade of cellular and tissue interactions involving the endothelium, extracellular matrix, metalloproteinase, submucosal reactions, and connective tissue [76–78]. Mucositis after HDMTX is caused by cellular damage to rapidly dividing epithelial cells along the entire gastrointestinal tract; inadequate or delayed leucovorin rescue can lead to impaired epithelial cell growth and regeneration.

Grade IV mucositis is an oncologic emergency and is associated with infections, the need for parenteral nutrition, increased use of health care resources, delayed chemotherapy, and even death [77]. A variety of methods have been used to prevent or treat oral mucositis, including ice chips [79, 80], glutamine and *N*-acetylcysteine, benzydamine hydrochloride, benzydamine hydrochloride, and prostaglandin E1 and E2, but none has proven benefits in patients receiving prolonged infusions of HDMTX [78, 81]. Palifermin, a recombinant human keratinocyte growth factor that stimulates growth of epithelial cells, reduces the incidence of mucositis after HDMTX [82, 83]. Interventions to prevent and treat mucositis have recently been reviewed, but none is standard practice in patients receiving HDMTX [84, 85].

Small animal models, mainly rats, have been used to investigate the mechanisms of gastrointestinal methotrexate toxicity [86, 87], the effect on the resident microbiome [88], and a variety of approved and experimental interventions.

## Myelosuppression

A dose-limiting toxicity of HDMTX in the absence of leucovorin is severe, prolonged myelosuppression; however, the frequency

and severity of cytopenias with pharmacokinetically guided leucovorin is low. When elimination is delayed because of third spacing and fluid accumulations or as a result of renal injury, neutropenia and thrombocytopenia may be severe [89]. When myelosuppression occurs, standard therapies for febrile neutropenia and transfusions are provided as clinically indicated. The only known strategies to prevent myelosuppression are to prevent delayed methotrexate elimination by avoiding interacting medications around the time of infusion and draining effusions before treatment (or delaying HDMTX until effusions resolve) and to ensure optimal leucovorin dosing.

## GLUCARPIDASE

Enzymatic cleavage of MTX using glucarpidase (a recombinant bacterial carboxypeptidase G2) was first described in 1972 [33]. Glucarpidase was approved by the U.S. Food and Drug Administration in 2012 for patients with delayed methotrexate elimination or AKI and plasma methotrexate concentrations >1 $\mu$mol/L [50]. Glucarpidase cleaves methotrexate into DAMPA and glutamate, two nontoxic metabolites, and thus provides an enzymatic method to rapidly remove methotrexate in patients with renal dysfunction (Fig. 1). A single dose of glucarpidase (50 U/kg i.v. over 5 minutes) reduces plasma methotrexate concentrations by 97% or more within 15 minutes [50]. However, despite the decrease in the magnitude and duration of systemic exposure to methotrexate after glucarpidase, it has no effect on intracellular methotrexate concentrations [6, 16]. Therefore, just as with dialysis, the coadministration of high-dose leucovorin is required to protect cells from toxic methotrexate concentrations until renal function recovers sufficiently to clear any residual methotrexate as it is released from cells (Fig. 1). In fact, after glucarpidase administration, leucovorin should be continued until methotrexate concentrations have been maintained at close to undetectable levels for several more days. Leucovorin should not be administered within 2 hours before or after a dose of glucarpidase because, like methotrexate, leucovorin is a substrate for glucarpidase. Hydration and urine alkalinization should also be continued in patients requiring glucarpidase [50].

Within 48 hours of glucarpidase administration, only a chromatographic (high-performance liquid chromatography) method can reliably measure methotrexate concentrations because the DAMPA produced by enzymatic breakdown of methotrexate cross-reacts with methotrexate in the standard immunoassay and artificially elevates the level [50]. The long half-life of DAMPA (approximately 9 hours) precludes use of immunoassays for several days after glucarpidase administration.

Correct timing of leucovorin dosing relative to glucarpidase is essential and is based on pharmacologic principles (Fig. 3) [1]. Glucarpidase is confined to the plasma; therefore, interstitial and intracellular leucovorin concentrations are not directly affected by glucarpidase. Glucarpidase is given as a single i.v. infusion over 5 minutes and has a half-life of 5.6 hours. Leucovorin should be given 2–3 hours after glucarpidase, then every 3–6 hours at doses guided by the methotrexate concentration (pharmacokinetically guided dosing; Fig. 3) [1]. Reduction of the plasma methotrexate
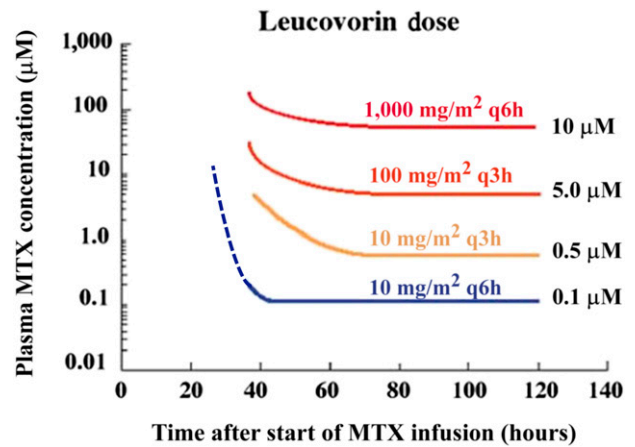


**Figure 3.** Pharmacokinetically guided leucovorin rescue based on plasma MTX levels after high-dose MTX. Leucovorin dosing must be increased dramatically when plasma MTX levels are elevated above 5 $\mu$M at 42 hours after the start of the MTX infusion because leucovorin must compete with MTX to enter cells via the reduced folate carrier and the goal of leucovorin rescue is to achieve a high intracellular concentration of leucovorin. In color are the recommended doses of leucovorin based on the plasma MTX concentration at each time point after the start of the MTX infusion. For example, if at hour 60 the MTX concentration is 100 $\mu$M, it falls above the red line and the recommended leucovorin dose would be 1,000 mg/m$^2$ every 6 hours. If at 100 hours the methotrexate concentration decreases to 3 $\mu$M (above the yellow line, below the orange line), then the recommended leucovorin dose would decrease to 10 mg/m$^2$ every 3 hours. The dotted lines indicate extrapolated values based on modeling and clinical trial experience following the original publication [113].

Abbreviation: MTX, methotrexate.

concentration reduces the competition between methotrexate and leucovorin for intracellular access. Therefore, the combination of a lower plasma methotrexate level and the transient presence of glucarpidase probably enhances the intracellular transport of leucovorin [90]. Leucovorin is continued for 48 hours after glucarpidase at the dose appropriate for the preglucarpidase methotrexate concentration [1]. It is important to note that in the presence of very high methotrexate concentrations, no plasma leucovorin concentration may be sufficiently high to reverse intracellular toxicity because of competition with the shared active transport mechanism. Urgent hemodialysis plus glucarpidase and very-high-dose leucovorin are warranted to reduce mortality [91].

> Reduction of the plasma methotrexate concentration reduces the competition between methotrexate and leucovorin for intracellular access. Therefore, the combination of a lower plasma methotrexate level and the transient presence of glucarpidase probably enhances the intracellular transport of leucovorin.

Many curable cancers require multiple courses of HDMTX therapy. If doses are skipped or delayed, treatment outcomes may be adversely affected [1]. For patients with delayed methotrexate clearance, the early use of glucarpidase rescue

**Table 3.** Selected studies that used glucarpidase

| Parameter | Widemann et al., 1995 [90] | Widemann et al., 2010 [6], Widemann et al., 1997 [64] | Buchen et al., 2005 [95] | Pharmacodynamic study (unpublished)[a] |
|---|---|---|---|---|
| Patients (n) | 43 | 262 | 65 | 68 |
| Cancer types | ALL, NHL, PCNSL | Primarily osteosarcoma | Primarily osteosarcoma | Primarily osteosarcoma |
| Inclusion criteria: MTX concentration ($\mu$M) | >5 or >1 at 42 hours; >0.4 at 48 hours | >2 SDs above standard elimination curve at >12 hours; ≥10 at 42 hours | >2 SDs above standard elimination curve at >12 hours; >10 at 36 hours; >5 at 42 hours; >3 at 48 hours | >2 SDs above standard elimination curve at >12 hours; >50 at 24 hours; >10 at 42 hours; >5 at 48 hours |
| Inclusion criteria: renal | Serum Cr ≥ 1.5 times ULN and urine output < 500 mL/24 hours | Serum Cr ≥1.5 times ULN; CrCl ≤ 60 mL/m$^2$ | Serum Cr ≥ 1.5 times ULN; decreased diuresis | Serum Cr ≥ 1.5 times ULN; CrCl ≤ 60 mL/m$^2$ |
| Median age (range), yr | 52 (10–78) | 17 (0–82) | 16 (0–72) | 20 (2–84) |
| Median pretreatment MTX concentration (range), $\mu$M | 5 (0.4–166), n = 24 patients[b] | 35 (1–849); n = 70 patients | 12 (0.5–902); n = 65 patients | 40 (3–708); n = 23 patients |
| Median time from start of MTX to first glucarpidase dose (range), d | 2 (1–7) | 3 (1–9) | 2 (1–7) | 2 (1–6) |
| Median glucarpidase doses (range), g/m$^2$ | 3 (0.9–12) | 5.5 (0.4–19) | 1–12 | 6.7 (1–20) |
| Median reduction in MTX levels after glucarpidase administration | 98.9% within 15 min (n = 28)[c] | 93.8% within 15 min (n = 84)[c] | 97% within 15 min (n = 30)[c] | 99.3% within 15 min (n = 27)[c] |
| Toxicities associated with glucarpidase | Potential acute infusion reaction, type I or III hypersensitivity | Type I or III hypersensitivity | Potential acute infusion reaction, type I or III hypersensitivity | Type I hypersensitivity |
| Toxicities associated with MTX (%) | Hematologic (70.5) Hepatic (45.5) Mucositis (43.2) Renal (29.5) | Renal (35.5) Stomatitis (32.2) Gastrointestinal (16–27) | Hepatic (45.5) Gastrointestinal (45.5) Renal (30) | Renal (38.9) Gastrointestinal (29.5) Stomatitis (27.5) |

[a]Unpublished; data on file, BTG International Inc., West Conshohocken, PA.
[b]Not all patients had measurements for every parameter.
[c]Patients whose MTX levels were measured by high-performance liquid chromatography.
Abbreviations: ALL, acute lymphoblastic leukemia; Cr, creatinine; CrCl, creatinine clearance; MTX, methotrexate; NHL, non-Hodgkin lymphoma; PCNSL, primary central nervous system lymphoma; ULN, upper limits of normal.

can facilitate a return to acceptable renal function that allows safe administration of subsequent HDMTX courses. Christensen et al. [5] reviewed the clinical courses of 1,141 pediatric oncology patients who received a total of 4,909 courses of HDMTX (≥1 g/m$^2$) at St. Jude Children's Research Hospital from 1998 through 2010 and identified 20 (1.8% of patients, 0.4% of HDMTX courses) who developed AKI and delayed methotrexate excretion and required glucarpidase. All patients had a return to baseline creatinine values, none died of methotrexate toxicity, and 13 of 20 received a total of 39 subsequent courses of HDMTX, which was well-tolerated in all cases [5]. In a pooled analysis of efficacy data from four multicenter, single-arm compassionate-use clinical trials, Widemann et al. [43] showed that glucarpidase resulted in a 99% or greater sustained reduction of serum methotrexate concentrations in renally impaired patients. Of concern is the declining glomerular function later in life among childhood cancer survivors who received methotrexate and other nephrotoxic chemotherapeutics; thus, prevention of AKI is preferable to managing it [92].

**Efficacy of Glucarpidase**
A series of compassionate-use studies that used glucarpidase in conjunction with standard management approaches for patients with signs of renal toxicity were published by Widemann and colleagues about their 15-year experience with glucarpidase for methotrexate toxicity in 492 cancer patients between November 1993 and June 2009 (Table 3) [6, 50, 64, 93–95].

Widemann and colleagues [6] reported additional experience with glucarpidase, leucovorin, and thymidine in 100 patients treated with 1–3 doses of glucarpidase and standard leucovorin rescue. An initial cohort of 35 patients received thymidine by continuous infusion. Thereafter, thymidine was restricted to patients with prolonged methotrexate exposure (>96 hours) or with substantial methotrexate toxicity. Plasma methotrexate concentrations decreased by 99% within 15 minutes after the first glucarpidase dose [6]. This analysis underscores the importance of early leucovorin dose adjustment and timely glucarpidase administration. Of 12 deaths, 6 were considered directly related to methotrexate because patients experienced grade 4 myelosuppression (n = 5), grade 3 or 4 mucositis (n = 4), sepsis (n = 5), and toxic epidermal necrolysis (n = 2). All 6 patients had received thymidine. Predictors of grade 4 and 5 toxicity included the presence of grade 4 toxicity before glucarpidase administration, inadequate initial increase in leucovorin dosing, and administration of glucarpidase more than 96 hours after the start of the methotrexate infusion. The other patient deaths were attributed primarily to rapid cancer progression. The major risk factors for severe toxicity

are preexisting toxicity, inappropriate leucovorin increase, and delayed glucarpidase administration [6].

## CONCLUSION

HDMTX can be safely administered to patients with normal renal function using hyperhydration, urine alkalization, and pharmacokinetically guided leucovorin rescue. Successful management of methotrexate toxicity requires timely recognition of delayed methotrexate elimination and renal dysfunction. In particular, rising serum creatinine concentration or decreased urine output after HDMTX indicates a medical emergency. Increased hydration, high-dose leucovorin, and glucarpidase (when necessary) effectively reduce serum methotrexate concentrations and protect cells from methotrexate, but these measures must be administered as early as possible to prevent further toxicity, facilitate renal recovery, and allow patients to resume HDMTX therapy after normalization of renal function [96, 97].

## AUTHOR CONTRIBUTIONS

**Conception/Design:** Scott C. Howard, John McCormick, Randall K. Buddington, R. Donald Harvey

**Provision of study material or patients:** Scott C. Howard, Ching-Hon Pui, R. Donald Harvey

**Collection and/or assembly of data:** Scott C. Howard, Ching-Hon Pui, Randall K. Buddington, R. Donald Harvey

**Data analysis and interpretation:** Scott C. Howard, John McCormick, Ching-Hon Pui, Randall K. Buddington, R. Donald Harvey

**Manuscript writing:** Scott C. Howard, John McCormick, Ching-Hon Pui, R. Donald Harvey

**Final approval of manuscript:** Scott C. Howard, John McCormick, Ching-Hon Pui, Randall K. Buddington, R. Donald Harvey

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