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Successful re-exposure to high-dose methotrexate after severely delayed methotrexate elimination and renal toxicity in children with acute lymphoblastic leukemia

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Abbreviations:

ALL	Acute lymphoblastic leukemia
AUC	Area under the Receiver Operating Characteristic (ROC) Curve
CI	Cumulative incidence
CNS	Central nervous system
CTCAE	Common Terminology Criteria for Adverse Events
DHFR	Dihydrofolate reductase
DME	Delayed MTX elimination
FA	Folinic acid
GEE	Generalized Estimating Equations
HDMTX	High-dose methotrexate
MTX	Methotrexate
OS	Overall survival
PTWG	Ponte di Legno International Toxicity Working Group
RR	Relapse rate

SAE	Serious Adverse Events

Abstract

High-dose methotrexate (HDMTX) is a cornerstone of contemporary treatment protocols for both pediatric and adult acute lymphoblastic leukemia (ALL); however, up to 4% of children and 15% of adults develop renal toxicity with severely delayed MTX elimination (DME). Evidence-based guidance on re-exposure after DME is lacking, and omission of further HDMTX may compromise anti-leukemic efficacy and potentially increase the risk of relapse. This study, conducted within the Ponte di Legno international toxicity working group, aimed to evaluate the safety of HDMTX re-challenge in pediatric patients after DME. National investigators from 12 countries provided case-level data on initial DME events and subsequent HDMTX re-exposures via structured questionnaires. Data from 189 patients treated for ALL who experienced DME were analyzed, of whom 143 were subsequently re-exposed to HDMTX. Clinical toxicities after the initial DME included gastrointestinal complications (vomiting, diarrhea, mucositis), infections, and neurological events (encephalopathy, seizures, MTX stroke-like syndrome). Laboratory toxicities comprised cytopenias and hepatic abnormalities. Two patients transiently required dialysis. DME led to chemotherapy modifications in 73% of the patients. After re-exposure, toxicities were similar in spectrum, self-limited, and non-fatal. Twenty children (14%) developed recurrent DME, including three with two additional episodes. Recurrent DME could not be predicted by clinical, pharmacokinetic, or demographic variables, nor by uniform MTX dose reduction during re-exposure. In conclusion, re-exposure to HDMTX following DME is feasible and generally well tolerated, although the risk of recurrence is increased. Re-challenge should be considered once renal function has normalized, with careful monitoring and individualized dose adjustment.

Introduction

Epidemiology, definition, clinical manifestations, and risk factors

High-dose methotrexate (HDMTX, defined as ≥ 500 mg/m^2) constitutes a fundamental part of most contemporary pediatric acute lymphoblastic leukemia (ALL) treatment protocols, as it significantly reduces the risk of central nervous system (CNS) relapse and improves overall survival (OS).[1-3] Owing to its potent antileukemic activity, HDMTX has also been incorporated into adult treatment regimens. [4]

HDMTX courses are usually administered at 3-5 g/m² over 24 hours, accompanied by intensive hydration and urine alkalinization, followed by folinic acid (FA) rescue to mitigate toxicity. Nevertheless, despite optimal supportive care and careful monitoring, up to 4% of the children and as many as 15% of adults receiving HDMTX develop renal toxicity resulting in severely delayed MTX elimination (DME).[5-10]

MTX enters cells primarily by active transport via the reduced folate carrier receptor and undergoes polyglutamylation, promoting intracellular retention. At higher serum concentrations, MTX can also cross the cell membrane by passive diffusion. By inhibiting dihydrofolate reductase (DHFR), MTX disrupts the synthesis of methionine, thymidine, and purines. During HDMTX therapy, monitoring of serum creatinine and MTX levels is crucial. Prolonged exposure to MTX and its metabolites can result in acute renal injury with reduced MTX clearance, leading to sustained, elevated plasma MTX concentrations, and enhanced systemic toxicities that may result in irreversible damage.[6,11,12] The severity of methotrexate-related toxicity is highly schedule-dependent and directly proportional to the duration of exposure (AUC); however, MTX concentrations show wide inter- and intra-individual variations, even when using the same dose and duration.[6]

Proposed mechanisms of MTX-induced renal dysfunction include direct tubular toxicity due to prolonged exposure to MTX and reduced renal perfusion due to arteriolar vasoconstriction.[6,11]

Several risk factors for delayed methotrexate elimination (DME) have been identified, including reduced renal function, acidic urine pH, inadequate hydration,[12] hypoalbuminemia,[13,14] and

concomitant drugs or food interfering with MTX clearance.[5,12,15] Population pharmacokinetic modeling has demonstrated that, beyond those known risk factors, patients' characteristics (age, sex, BMI, weight, and ethnicity) as well as polymorphisms in genes involved in MTX metabolism and transport, contribute to inter-individual variation in MTX pharmacokinetics.[16-19] Recently developed models that integrate early MTX and creatinine plasma measurements, alongside clinical and genetic variables, aim to personalize MTX dosing and reduce the risk of DME in children with ALL.[20-23]

The Ponte di Legno Toxicity Working Group (PTWG) defines severe DME as a rise in plasma creatinine >0.3 mg/dL or a 1.5-fold increase above baseline, with markedly elevated plasma MTX concentrations at key time points post-infusion: 36 hours >20 µmol/L, 42 hours >10 µmol/L, or 48 hours >5 µmol/L.[24] DME can be accompanied by gastrointestinal symptoms (vomiting, diarrhea), neurological complications, and other systemic toxicities; however, most patients remain initially asymptomatic and present with non-oliguric renal dysfunction characterized by an abrupt rise in serum creatinine during or shortly after MTX infusion, resulting in significantly elevated plasma MTX concentrations.[11,12,25,26] Early recognition of DME, followed by urgent intervention with plasma MTX-adapted FA rescue, and when indicated, glucarpidase administration, is therefore critical. Most ALL protocols include guidelines for prevention and management of DME, including criteria for glucarpidase use (Supplementary Table S1). However, the dilemma of **HDMTX re-exposure after severe DME** remains insufficiently addressed.

Although MTX-induced renal toxicity is generally reversible, and several studies have reported successful resuming once renal function has normalized,[8,20,27,28] many clinicians remain hesitant to re-challenge patients due to concerns about recurrence. If re-exposure is considered, MTX doses are often empirically reduced.[20,28] However, evidence-based guidelines for the timing, dosing, and safety of re-exposure are lacking. Consequently, clinical decisions are often based on institutional practice or physician discretion. For some patients, this results in permanent discontinuation of HDMTX, which may compromise treatment efficacy and increase relapse risk (RR).

Purpose

We conducted a retrospective, multinational study to evaluate the safety and tolerability of HDMTX re-exposure in pediatric patients with ALL following an episode of DME. Pharmacokinetic, clinical, and toxicity data were collected from 12 pediatric ALL working groups to support the development of international, evidence-based recommendations for HDMTX re-administration.

Methods

Patients

Inclusion criteria:

Children (≤18 years) with newly diagnosed ALL who developed severe DME following treatment with HDMTX. Only the most severe cases, meeting the PTWG criteria,[24] were included. Each case was independently reviewed twice to confirm eligibility.

Exclusion criteria:

Children with pre-existing renal dysfunction prior to the HDMTX course, therapy with Tyrosine Kinase Inhibitor, or insufficient data to apply the PTWG definition of DME.

Data capture and editing

Data on DME events were captured as Serious Adverse Events (SAEs) within the national pediatric ALL treatment protocols both prospectively and retrospectively. A detailed overview of the treatment protocols is provided in Supplementary Table S2. The study was conducted in accordance with the ethical standards of the Helsinki Declaration, and informed consent, approved by national ethics committees, was obtained for all participants before study registration. Primary national investigators completed detailed questionnaires (Supplementary Table S3) for each HDMTX course complicated by DME, documenting clinical, laboratory, and pharmacokinetic parameters, as well as comprehensive data on subsequent HDMTX courses.

Classification and data collected: DME events were classified as either first occurrences or subsequent events following HDMTX re-exposure. Data analysis comprised treatment details on MTX dosage, hydration, and concomitant chemotherapy, leukemia lineage, risk group, and treatment protocol. Laboratory data included baseline creatinine levels and serial MTX and creatinine values, blood counts, and biochemical profiles. Clinical toxicities (≥grade 3, per *Common Terminology Criteria for Adverse Events* (CTCAE)) during DME events and subsequent MTX courses were documented, for gastrointestinal, hepatic, CNS, and infectious complications. Finally, the impact on scheduled chemotherapy, resumption of treatment, and modifications was assessed.

Statistical Analysis

Outcome events: We examined the relationship between potential explanatory variables and the occurrence of severe DME at the index MTX re-exposure, and correlates of clinical and laboratory variables measured at the time of the index exposure, and the occurrence of DME at the next MTX exposure. We particularly focused on MTX dose, MTX level at 48 hours, and time to MTX level <0.25 μmol/L.

Analysis: The effect of categorical variables on the outcome was tested using Fisher's exact test. The effect of continuous variables was tested using the Wilcoxon rank sum test. For all variables, we computed the area under the ROC curve (AUC) and the Goodman-Kruskal gamma. Analyses were done with missing values imputed (mode for categorical variables and median for continuous variables) and with the exclusion of patients with missing values. Variables with 20% or more missing values were excluded. As an additional analysis, we examined the effects of the explanatory variables using the Generalized Estimating Equations (GEE) method, which accounts for possible dependence between multiple observations on the same patient. The GEE analysis was done in a logistic regression framework. We computed both raw p-values and adjusted p-values using the Benjamini-Hochberg adjustment for multiple testing. All analyses were univariate analyses examining one explanatory variable at a time. Multivariate analysis was not undertaken because of the small number of DME events.

Results

Study cohort

A total of 209 questionnaires were submitted from 12 countries. Twenty cases were excluded; 10 did not meet the PTWG criteria for severe DME, and 10 lacked essential data. The final cohort comprised 189 pediatric ALL patients, from 11 countries, treated between 1992 and 2022, who developed severe DME following HDMTX therapy (Figure 1, Supplementary Figure S1). Baseline patient characteristics are summarized in Table 1.

For the entire cohort, the OS rate was 84%, and the relapse rate (RR) was 17%. When stratified by treatment era, patients treated between 1995–2010 (n=100) had an OS of 83% and RR of 25%, whereas those treated between 2011–2022 (n=89) achieved an OS of 87% and RR of 8%.

First DME events (Table 2, Supplementary Table S4, Supplementary Figure S2)

More than half of the events (103 patients, 55%) occurred after the first HDMTX course, and an additional 15% occurred after the second of the 4-8 scheduled HDMTX courses. The remaining cases were distributed across later infusions, including some after the eighth course (Supplement Table S4). Glucarpidase was administered to 51 patients (27%).

Markedly elevated MTX plasma concentrations were first detected at 24 hours (>150 µmol/L) in 34% of patients, at 36 hours (>20 µmol/L) in 16%, at 42 hours (>10 µmol/L) in 18%, and at 48 hours (>5 µmol/L) in 30%. Most patients (77%) exhibited pathological MTX levels at multiple time points, and nearly all (n=169, 89%) had severely elevated 48-hour MTX levels (>5 µmol/L) (Figure 2).

The median time to reach a plasma MTX concentration <0.25 µmol/L was 192 hours (range: 48–476 hours). Additional analysis, including only patients who did not receive glucarpidase (n=136), revealed similar results with a median time of 190 hours (range 48–360 hours).

A significant negative correlation was found between the timing of DME onset and MTX clearance (p=0.0319): patients with DME detected at 24 hours, had a longer median time to MTX concentration <0.25 µmol/L (204 hours), compared with those whose DME occurred at 48 hours (170 hours) (Supplementary Figure S3).

Elevated serum creatinine was first observed 24 hours post-infusion, with a median relative increase of 1.58, and remained elevated at subsequent measurements. The peak increase occurred at a median of 54 hours, with a 2.51-fold rise. Renal function subsequently normalized, with creatinine returning to baseline after a median of 18 days (range 2–120 days).

Toxicities following the first DME event (Table 3, Supplementary Table S5)

Clinical toxicities were assessed in 100 patients (52%). Significant events (CTCAE grade≥3) included gastrointestinal toxicities: vomiting, diarrhea, and mucositis in 33%; infections in 28%; and neurological complications: encephalopathy, seizures, and MTX stroke-like syndrome in 7%. Significant laboratory toxicities were assessed in 78 patients (41%) and included: cytopenia in 41% and hepatic abnormalities (elevated bilirubin and transaminases) in 16%. Two patients required transient dialysis (for 6 and 16 days); both had clinical and laboratory profiles similar to the rest of the cohort. Two patients died following persistent pancytopenia after the DME event and HDMTX-related myelotoxicity. One died after sepsis with multi-organ failure, and one after stem cell transplantation performed for unrecovered bone marrow aplasia. All other laboratory abnormalities were transient.

Modifications of therapy

The impact of DME on subsequent chemotherapy was evaluated in 172 patients (91%). Treatment modifications occurred in 126 patients (73%), and included treatment delays in 105 (61%) and chemotherapy regimen adaptations in 66 (38%). Among these, 51 patients (30%) received a reduced MTX dose in subsequent courses, and 15 (9%) had the next MTX course omitted or permanently discontinued.

Re-exposure to HDMTX (Table 3, Supplementary Table S6):

Of the 189 patients who experienced DME, 143 (80% of those scheduled for additional HDMTX courses) continued treatment and received between 1-7 further HDMTX infusions. The main reason for discontinuing HDMTX was physicians' concern regarding recurrent DME.

First MTX re-exposure

Most patients (81%) received > 50% of the planned MTX dose, while 62% received the full dose. MTX dosing during re-exposures was determined at the treating physician's discretion and

was not guided by algorithms or dose-adjustment models targeting specific plasma concentrations. Clinical toxicities were reported in 69 patients (48%) and included mucositis (n=3), neurological symptoms (n=3), and infectious complications (n=9). Laboratory toxicities were documented in 24 patients and included cytopenia (n=11) and elevated liver enzymes (n=2). Adverse events were less frequent than those observed after the first DME events, and all were transient and non-fatal.

Subsequent HDMTX re-exposures

Among re-exposed patients, 110 received two additional HDMTX courses, 86 received three, and 46 received more than three courses (Figure 1). During subsequent re-exposures, the proportion of patients receiving higher MTX doses gradually increased: the percentage of patients receiving >50% of the planned dose rose from 85% to 94%, and the percentage receiving the full dose rose from 65% to 88% across the second to fourth re-exposures (Table 4, Supplementary Figure S4). MTX doses administered during re-exposures were not influenced by the course number at which the initial DME occurred, or its severity, nor by pharmacokinetic or predictive modeling.

Toxicities following re-exposure to HDMTX

Clinical and laboratory toxicities observed after HDMTX re-exposures were generally mild and self-limiting, with no treatment-related deaths. Reducing MTX dose during re-exposure did not reduce the rate or severity of these toxicities.

Recurrent DME events after re-exposure

Across 387 re-exposures in 143 patients, 23 recurrent DME episodes occurred in 20 patients (14%). All events were clinically manageable, and most patients were able to continue HDMTX therapy without further complications. However, the cumulative incidence (CI) of recurrent DME increased with each successive course (Figure 3).

Recurrent DME events occurred after the first re-exposure in 8 patients (6%), after the second in 6 (5%), after the third in 7 (8%), and after the fourth in 2 (4%) (Table 3). Similar to initial DME

events, 70% of recurrent cases were characterized by pathologically elevated MTX plasma concentrations at 24 hours (Supplement Table S6).

Three patients experienced two recurrent DME episodes, occurring after the first through fourth re-exposures, at both full and reduced MTX doses (Supplementary table S7). All three subsequently continued HDMTX therapy without further complications.

Risk factor for additional DME events (Table 4, Supplementary Figure S5-8)

Multiple potential predictors of recurrent DME after re-exposure were evaluated. Variables related to the initial DME event included MTX course number, plasma MTX concentrations at defined time points, time to achieve MTX levels <0.25 μmol/L, relative increase in serum creatinine, time to creatinine normalization, glucarpidase use, and modifications to subsequent therapy. Re-exposure-related factors were also assessed, including MTX dose and course number, as well as plasma MTX and creatinine levels at multiple time points. In addition, demographic and treatment-related characteristics such as treatment protocol, sex, age, and risk group were analyzed.

None of these factors emerged as a statistically significant predictor of recurrent DME following re-exposure (Supp figure S5-7). Specifically, neither MTX dose reduction during subsequent courses nor delays in re-exposure reduced the risk of additional DME events. Overall, among all subsequent HDMTX courses, recurrent DME events occurred in 9.6% of those receiving < 3 g/m^2 MTX (5 of 52 courses) and in 5.4% of those receiving > 3 g/m^2 MTX (18 of 333 courses) (Supp figure S8).

Discussion

Severe DME is one of the most serious acute toxicities associated with HDMTX therapy. In this large international cohort, we evaluated 189 children with ALL who developed DME and assessed the feasibility and safety of subsequent HDMTX re-exposure.

The first severe DME events

Consistent with previous reports identifying the first HDMTX infusion as carrying the highest risk for DME,[8] more than half of the DME events in our cohort occurred after the initial MTX course. Possible explanations include stricter adherence to preventive measures in later courses, such as maintaining urine alkalinization and adequate hydration,[12] correcting hypoalbuminemia,[13] and avoiding drug or food interactions,[6,12,15] especially if there were some degrees of clinical or laboratory toxicities during the previous course. Another potential mechanism may be the persistence of an expanded folate pool from prior HDMTX courses, which could displace MTX from DHFR, resulting in reduced intracellular MTX levels and providing partial protection during subsequent infusions.[29]

Most patients (89%) exhibited pathological plasma MTX concentrations at 48 hours (MTX48), reaffirming this as the most sensitive time point for identifying DME. However, elevated MTX levels were already evident at 24 hours in 34% of patients and at 36 hours in 16%. When considering both early time points together, nearly half of all DME cases could have been detected before 48 hours. This underscores the importance of early and close monitoring, while MTX sampling at 36 and/or 42 hours may enable timely recognition and intervention.

Serum creatinine elevation, commonly the first indicator of DME, was observed in all patients, 24 hours post-infusion, peaking at a mean of 58 hours. This corresponds with sustained intracellular MTX accumulation and resultant tubular injury. Importantly, nephrotoxicity was always reversible, although recovery could be prolonged; MTX concentrations < 0.25 μmol/L were occasionally achieved only after 20 days, and full renal recovery could take up to four months. A statistically significant negative correlation was observed between the timing of DME onset and MTX clearance, with the slowest decline in serum MTX concentrations among patients whose DME developed within 24 hours after infusion. This may suggest that early-onset DME events are the most severe and nephrotoxic.

Toxicities following the first DME events were consistent with prior studies, including gastrointestinal, infections, and neurological complications in up to 30% of patients, as well as cytopenia and hepatotoxicity in up to 40%. Notably, DME events prompted modifications to subsequent chemotherapy in 73% of patients, primarily treatment delays (61%) and MTX dose reductions (38%).

As our cohort included patients treated under multiple protocols spanning three decades, and since most (80%) continue HDMTX therapy following DME, the potential long-term detrimental consequences of delaying or omitting MTX courses could not be directly assessed. Nonetheless, OS and RR in this cohort were comparable to those reported in the literature for children with ALL, suggesting that appropriate management and re-exposure strategies may mitigate the potential impact of DME on treatment outcomes.

Re-exposures to HDMTX

A total of 143 children were re-exposed to HDMTX following a prior DME event, and their subsequent courses were analyzed in detail. Clinical and laboratory toxicities during re-exposure were similar in type but less frequent than those observed after the initial DME episode; all were generally mild to moderate, self-limited, and without any treatment-related deaths.

The main reason for discontinuing HDMTX was the physician's concern about recurrence. Indeed, 20 patients (14%) experienced additional DME events, with three patients having two recurrences. The CI of recurrent DME increased with each re-exposure and reached 20% after the fourth re-exposure. This rate is considerably higher than that reported in the general pediatric ALL population (up to 4%), suggesting that a prior DME confers a markedly elevated risk of recurrence upon re-exposure. Beyond the known acute toxicities of DME, each event may further delay or compromise subsequent chemotherapy, as was demonstrated in our cohort.

Notably, in 70% of recurrent DME events, pathological MTX levels were detected first after 24 hours (>150 μmol/L), underscoring the critical importance of this time point for early detection and intervention.

Risk factors for additional DME events

To identify predictors of DME recurrence after re-exposure, we evaluated multiple variables related to the first DME event, subsequent re-exposures, and patient characteristics. However, none of these factors emerged as a statistically significant predictor of DME recurrence.

Reducing the MTX dose or delaying the next MTX course did not mitigate the risk of recurrence; in fact, 9.6% of courses at doses <3gr/m^2 were complicated by recurrent DME, compared to 5.4% of courses at doses >3gr/m^2. Similarly, none of the laboratory or pharmacokinetic parameters from the initial DME event, nor the patients' or ALL characteristics, reliably predicted recurrence.

Christensen et al.[28] reported successful resumption of HDMTX following DME by adjusting the MTX dose to target a steady-state plasma concentration of 65 μM, based on the clearance from the previous course. Likewise, Foster et al.[20] demonstrated that recurrence of DME might be prevented by individualizing 24-h HDMTX infusion rates according to real-time MTX concentrations measured 2 and 6–8 hours after infusion initiation. Implementing such adaptive pharmacokinetic algorithms could enhance the safety and efficacy of future HDMTX re-exposures.

Fifty-one patients (27%) received glucarpidase during their first DME event. As glucarpidase became more available only after 2008 and was approved in 2012, 65% of patients treated thereafter (n = 78) received it, indicating adherence of most pediatric ALL protocols to the international consensus guidelines for glucarpidase use in DME.[11] While glucarpidase effectively reduced plasma MTX concentrations and mitigated acute toxicities, it did not reduce the likelihood of recurrent DME upon re-exposure in our study.

Theoretically, genetic variants of MTX metabolism and clearance may increase susceptibility to DME recurrence. Polymorphisms in MTX transport genes (SLCO1B1, SLC19A1, SLCO1A2), and ATP-binding cassette transporters (ABCB1, ABCG2, ABCC2, ABCC4), have been associated with DME. Polymorphisms in polyglutamation pathway genes (FPGS and GGH), MTX target genes (MTHFR, ARID5B), and metabolism enzymes (GSTP1) have also been linked to elevated MTX concentrations and may predispose patients to DME recurrence.[30-32] However, none of these genes has been specifically validated as a predictor of DME recurrence following HDMTX re-exposure. Moreover, the applicability of these polymorphisms remains uncertain, particularly regarding whether interventions such as MTX dose reduction and stringent hydration and alkalization protocols could mitigate their effects. Future genome-wide association studies may elucidate genetic determinants of susceptibility to DME recurrence.

Limitations of the study

While data regarding DME occurrence were available for the entire cohort, information regarding other toxicities was incomplete. Furthermore, risk factors for the first DME could not be evaluated, as data were collected only for patients who developed DME, nor could we compare with the prevalence of other toxicities among patients without DME. Lastly, the extended study period of more than three decades, stemming from the rarity of DME, inevitably led to heterogeneity in treatment protocols and supportive care. Nevertheless, this prolonged observation period enabled the establishment of the largest international cohort of children with DME, providing the most comprehensive analysis currently available.

Conclusion and recommendations

Re-exposure to HDMTX after a severe DME event was achievable, did not result in unexpected toxicities, and adverse events were less frequent than those observed during prior HDMTX courses. However, 14% of re-exposed children experienced recurrent DME events. Upfront MTX dose reduction during re-exposure did not decrease the recurrence rate.

Given the central role of HDMTX in ALL therapy, we propose the following recommendations:

1. **Timing**: Re-exposure to HDMTX is feasible but should be undertaken only after full recovery of renal function.
2. **Monitoring**: As re-exposure carries an increased risk of recurrent DME, early and close monitoring of plasma MTX and creatinine levels, alongside strict control of modifiable risk factors, is essential.
3. **Dose adjustment**: Since uniform MTX dose reduction may not reduce recurrence, individualized dosing strategies should be implemented. Further studies validating the effectiveness of algorithms for MTX dose adjustment based on early plasma concentrations,[20] targeting a plasma concentration of 65 μM,[28] or employing pharmacokinetic modeling to predict the elimination profile,[23] may better support safe and effective re-exposure

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Table 1: study cohort n=189

Age, median (range)	10 (1-20.9 years)
Gender	
Female	68 (36%)
Male	121 (64%)
Risk group	
Standard Risk	50 (26%)
Medium Risk	58 (31%)
High Risk	80 (42%)
Not Assessed	1
Treatment protocol	
BFM	51 (27%)
DCOG	29 (15%)
NOPHO	109 (58%)
MTX dose at 1st severe DME	
5 gr/m^2	155 (82%)
8 gr/m^2	34 (18%)
Re-exposed	143 (76%, 80% of scheduled)
Additional severe DME	20 (14%)
Last follow-up status	
Alive	159 (84%)
Relapse	32 (17%)
Dead	28 (15%)
Lost to follow up	2 (1%)

Abbreviations: BFM, Berlin-Frankfurt-Münster; DCOG, Dutch Childhood Oncology Group; NOPHO, Nordic Society of Paediatric Haematology and Oncology; MTX, methotrexate; DME, delayed MTX elimination

Table 2: The first delayed MTX elimination (DME) event

MTX Course number n (%)			**Time to plasma MTX < 0.25** (hours)	Median: 192 (Q1-3; 160-234)
1st	103 (55%)		100-150	35 (22%)
2nd	29 (15%)		150-200	52 (32%)
>2nd	57 (30%)		200-250	47 (29%)
Concomitant IV chemotherapy n (%)			>250	27 (17%)
Single MTX	152 (80%)		**Time of maximal Creatinine** (hours)	Median: 48 (Q1-3; 36-72), Mean 58 (23-210)
Combined IV	37 (20%)		24	23 (15%)
Time of 1st pathological MTX level* (hours)			25-48	74 (48%)
24	65 (34%)		49-72	31 (20%)
36	30 (16%)		>72-120	27 (17%)
42	34 (18%)		**Time to baseline Creatinine** (days)	Median: 18 (Q1-3;10-30), Mean 23 (2-120)
48	56 (30%)		≤10	35 (26%)
Time of any pathological MTX (hours)			11-30	78 (58%)
24	65 (34%)		>30	21 (16%)
36	69 (49%)		**Relative increase in creatinine** ∞	Median (Q1,3)
42	106 (72%)		0-24 hours	1.58 (1.26, 2.07)
48	169 (93%)		0-36 hours	2.49 (1.91, 3)
Pre-hydration duration (hours)			0-42 hours	2.2 (1.8, 2.9)
4	111 (59%)		0-48 hours	2.43 (1.9, 3.2)
≥12	56 (30%)		0-54 hours	2.51 (2, 4)
NA	22 (11%)			

*Pathological MTX levels: 24 hours>150 µmol/L, 36 hours >20 µmol/L, 42 hours >10 µmol/L, or 48 hours >5 µmol/L

∞ The relative increase in creatinine level was measured from the time before starting IV high-dose MTX infusion to the highest creatinine level during this course.

Creatinine units: mmol/l, MTX units: µmol/L - **Abbreviations:** NA, not assessed; MTX, methotrexate

Table 3: HDMTX courses with the first delayed MTX elimination (DME) event & re-exposures to HDMTX

	First DME N=189	1st re-exposure N=143	2nd re-exposure N=110	3rd re-exposure N=86	4th re-exposure N=46
MTX dose					
100%*	189 (100%)	88 (62%)	71 (65%)	76 (88%)	38 (83%)
69-90%		28 (19%)	22 (20%)	5 (6%)	5 (11%)
≤ 50%		27 (19%)	17 (15%)	5 (6%)	3 (6%)
Time to MTX (<0.25)	Mean 198 hours (48-476)	Mean: 67.7 hours (42-249)	Mean: 64 hours (42-192)	NA	NA
Time to baseline creatinine (days)	Mean 23 days (3-120)	Mean 3.8 days (0-24)		NA	NA
Clinical Toxicities CTCAE grade ≥ 3	Assessed in 100 patients	Assessed in 69 patients	Assessed in 50 patients	NA	NA
GI	33 (33%)	0	0		
Mucositis	13 (13%)	3 (4%)	0		
CNS	7 (7%)	3 (4%)	1 (2%)		
Infectious	28 (28%)	9 (13%)	8 (16%)		
Laboratory toxicities	Assessed in 78 patients	Assessed in 24 patients	Assessed in 35 patients	NA	NA
Hematological	32 (41%)	11 (46%)	10 (29%)		
Liver	9 (12%)	2 (8%)	2 (6%)		
**DME		N=8 (6%)	N=6 (5%)	N=7 (8%)	N=2 (4%)
MTX % dose	100%	100%	4: 100%, 2: >50%	5:100%, 2:50%	One:80% One:50%
Further re-exposure to MTX	143 (76%)	5 (62%)	3 (50%)	3 (43%)	2 (100%)
Death	2	0	0	0	0

*5gr/m^2 (155 patients; 82%), 8gr/m^2 (34 patients; 18%)

**Data on additional DME after re-exposure were provided for 132 patients (92%)

Creatinine units: mmol/l, MTX units: μmol/L

Abbreviations: HDMTX, high dose methotrexate; DME, delayed MTX elimination; CTCAE, Common Terminology Criteria for Adverse Events; GI, gastrointestinal; CNS, central nervous system

Table 4 Univariate analysis: risk factors for additional delayed MTX elimination (DME) upon re-exposure

Categorical Variable	Values	Total	DME post re-exposure N (%)	Fisher's exact test Raw P-value	AUC	GAM
Gender	F	132	11 (8.33%)	0.266	0.561	0.624
	M	231	12 (5.19%)			
Current Re-Exposure Number	0**	130	8 (6.15%)	0.820	0.557	0.579
	1	105	6 (5.71%)			
	2	82	7 (8.54%)			
	3	46	2 (4.35%)			
Treatment Protocol	NOPHO	270	20 (7.41%)	0.508	0.568	0.700
	DCOG	55	2 (3.64%)			
	BFM	38	1 (2.63%)			
Risk Group	SR	136	7 (5.15%)	0.729	0.544	0.567
	MR	143	10 (6.99%)			
	HR	80	6 (7.50%)			
Glucarpidase	No	274	15 (5.47%)	0.211	0.560	0.641
	Yes	86	8 (9.30%)			
Modification of therapy	No	219	15 (6.85%)	0.667	0.526	0.556
	Yes	144	8 (5.56%)			
Continuous Variable	**Total (no NA values)**	**DME post-re-exposure Median [min, max**		**Wilcoxson test raw p-value**	**AUC (without imputation)**	**GAM (without imputation)**
		No	**Yes**			
Age	363	6.5 [1, 20.9]	8 [2, 19.3]	0.873	0.510	0.510
MTX course number*	363	1 [1, 8]	1 [1, 7]	0.179	0.574	0.640
MTX level 24*	324	134 [10, 623]	145 [42, 300]	0.451	0.547	0.548

Maximal increase in creatinine *	310	2.608 [0.774, 8.897]	3.045 [1.745, 5.644]	0.164	0.591	0.592
Time of maximal Creatinine (hours)*	308	48 [23, 210]	48 [24, 180]	0.687	0.474	0.470
Maximal creatinine*	314	90 [24, 333]	118 [46, 333]	0.269	0.572	0.573
Number of Re-Exposures	363	4 [1, 9]	3 [1, 9]	0.399	0.551	0.564
MTX dose (gr/m^2) current exposure	362	5 [0.5, 8]	5 [2.5, 8]	0.510	0.510	0.518
MTX48 level	340	0.8 [0.1, 79]	1.88 [0.2, 28.14]	0.356	0.559	0.559
Time to MTX <0.25	307	66 [42, 476]	84 [48, 258]	0.549	0.539	0.541

**Current Re-Exposure Number = 0 signifies the exposure of the initial DME event

* At the exposure of the initial DME event

Creatinine units: mmol/l, MTX units: μmol/L

Abbreviations: M, Male; F, Female; NOPHO, Nordic Society of Paediatric Haematology and Oncology; DCOG, Dutch Childhood Oncology Group; BFM, Berlin-Frankfurt-Münster; MR, medium risk; SR, standard risk; HR, high risk; AUC, area under the curve; GAM, generalized additive model.

Legends to the figures:

Figure 1: study cohort

Figure 2. Time points of the first and subsequent pathological plasma methotrexate (MTX) levels

Pathological plasma methotrexate (MTX) levels persisted across multiple time points following MTX infusion. Pathological MTX levels at 24 hours are shown in green, at 36 hours in pink, at 42 hours in orange, and at 48 hours in yellow. The majority of patients (89%) exhibited pathological MTX levels 48 hours after MTX infusion.

Figure 3: The cumulative incidence of additional severe delayed MTX elimination (DME) after re-exposure to HDMTX

The cumulative incidence of additional DME increases with each additional exposure.

* 143 patients received 1st re-exposure, 12 were excluded from analysis due to missing full DME data.

Figure 1

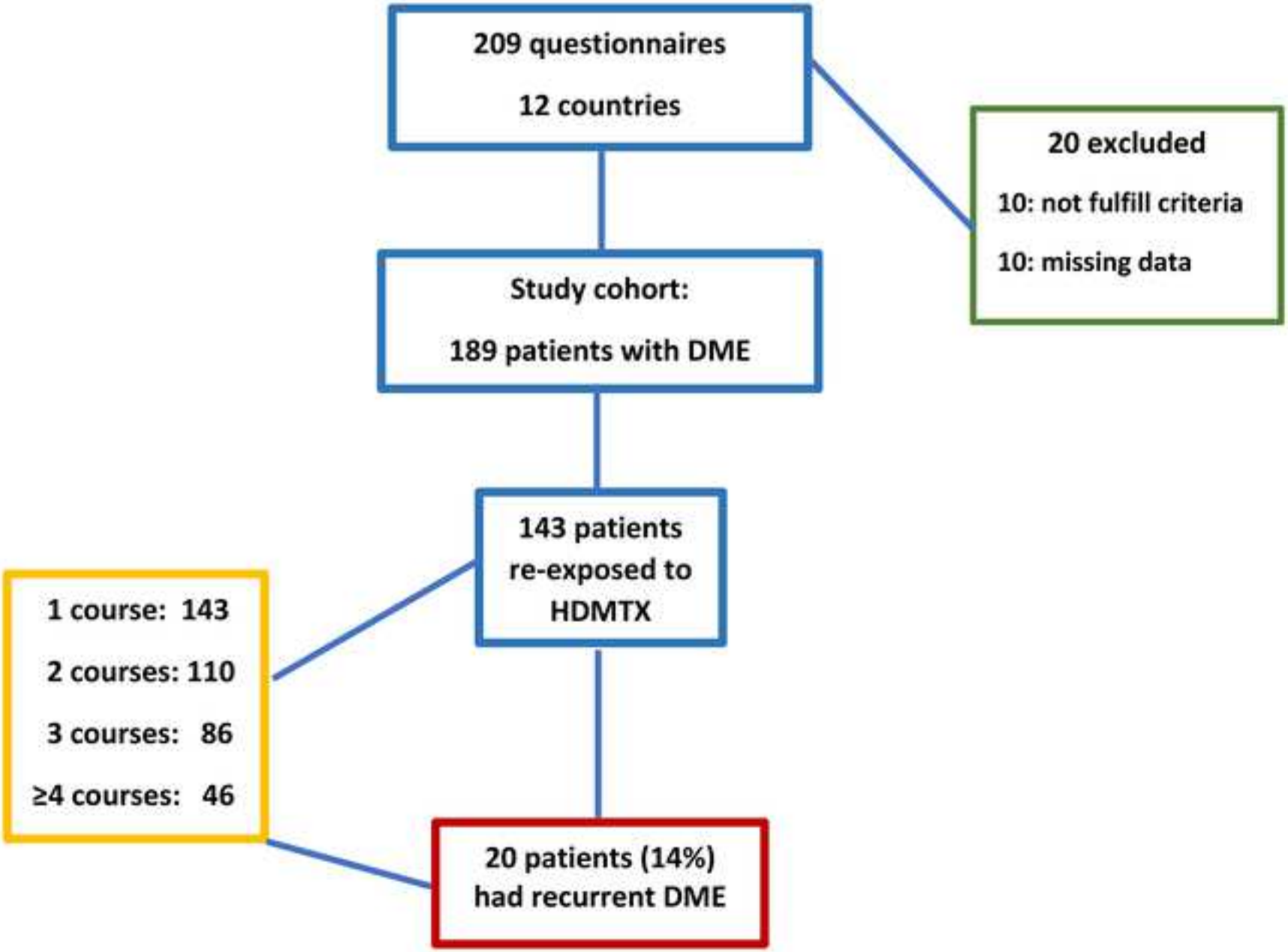
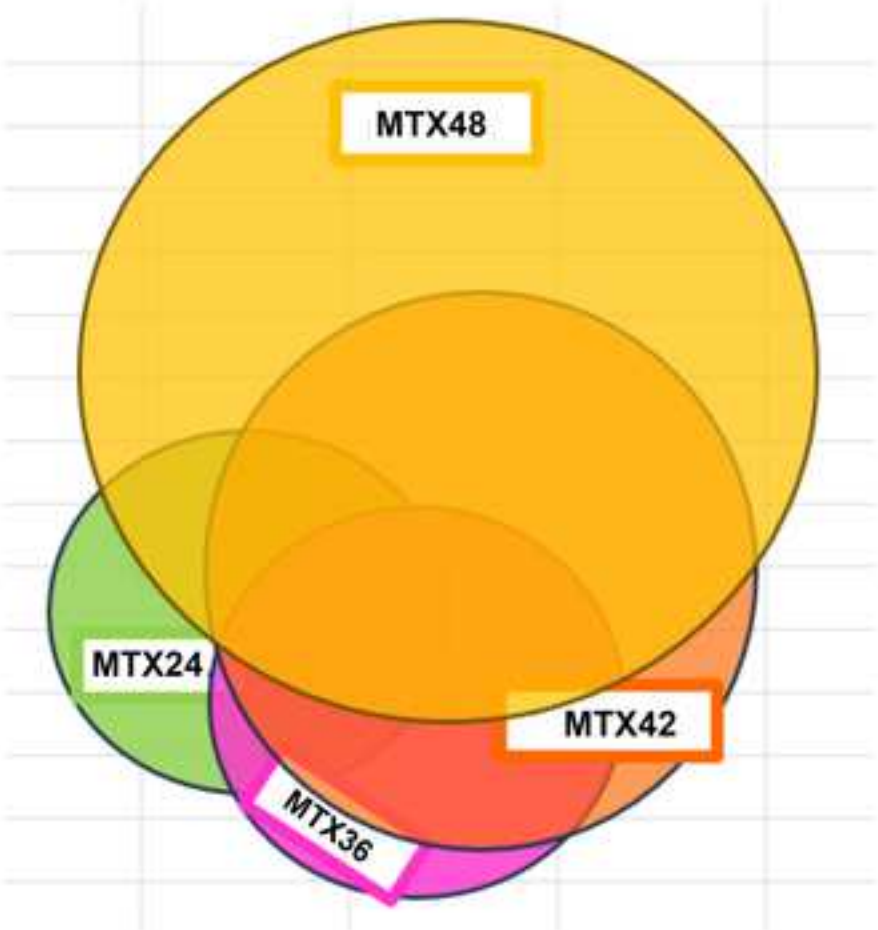

209 questionnaires
12 countries
20 excluded
10: not fulfill criteria
10: missing data
Study cohort:
189 patients with DME
143 patients
re-exposed to
HDMTX
1 course: 143
2 courses: 110
3 courses: 86
≥4 courses: 46
20 patients (14%)
had recurrent DME

Figure 2

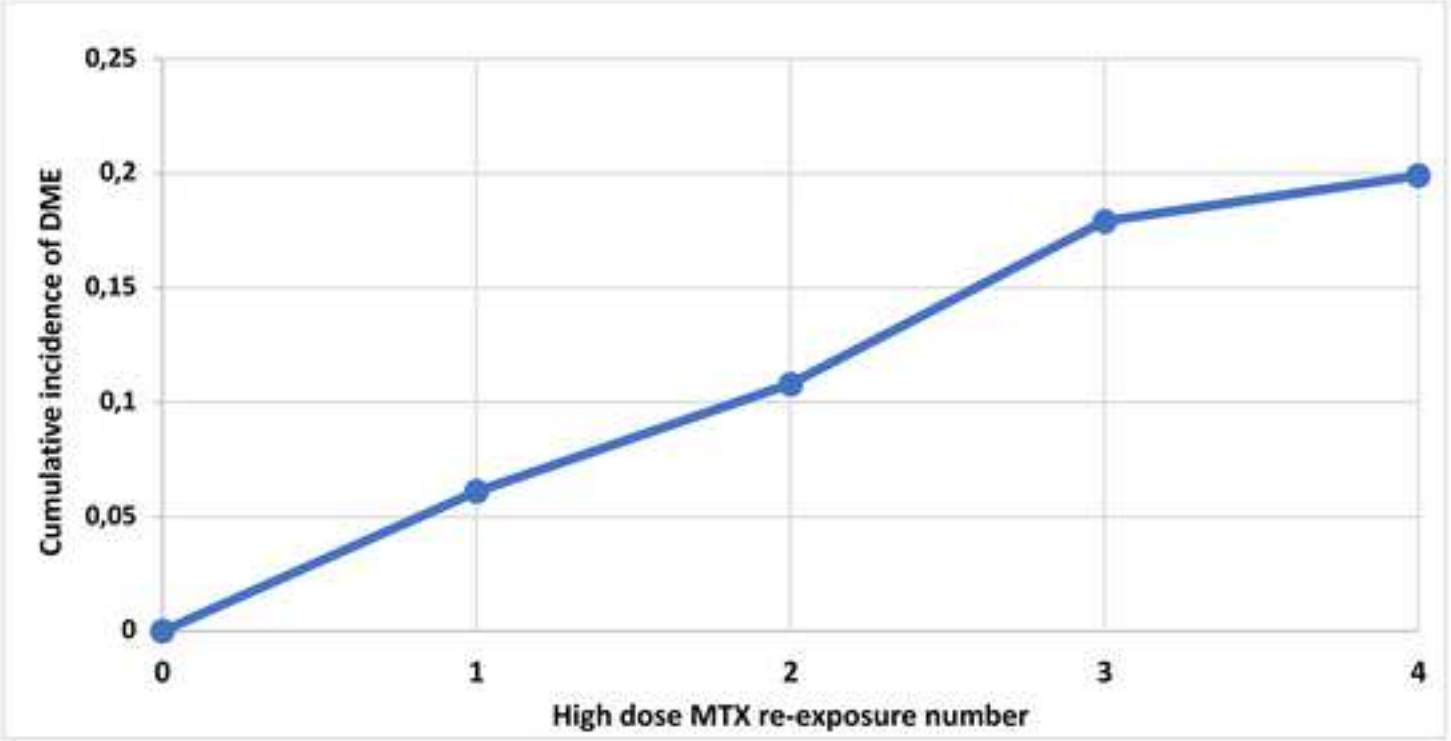


	24 hours	36 hours	42 hours	48 hours
First pathological plasma MTX levels*	**65 (34%)**	**30 (16%)**	**34 (18%)**	**56 (30%)**
Persistence of pathological plasma MTX levels	**39 (21%)**			
	46 (24%)			
	42 (22%)			
		63 (33%)		
		61 (32%)		
			96 (51%)	
Pathological plasma MTX levels all cohort	**65 (34%)**	**69 (36%)**	**106 (56%)**	**169 (89%)**

* First pathological MTX plasma levels:

MTX24 > 150 µmol/L; MTX36 > 20 µmol/L; MTX42 > 10 µmol/L; MTX48 > 5 µmol/L

Figure 3



		Additional DME	Cumulative incidence
*Number of children in the 1st re-exposure	131	8	6.10%
Number of children in the 2nd re-exposure (and no DME at the 1st re-exposure)	99	5	10.80%
Number of children in the 3rd re-exposure (and no DME at the 1st and 2nd re-exposure)	76	6	17.90%
Number of children in the 4th re-exposure (and no DME at the 1st, 2nd, and 3rd re-exposure)	40	1	20.10%

Supplementary tables and figures **Pages**

Supplementary table S1: Prevention and management of delayed MTX elimination (DME)) in the different pediatric ALL protocols:

Protocol	Avoidance	Management	Glucarpidase use
DCOG*	Avoidance of drugs that compromise renal function, e.g., aminoglycosides, can decrease the clearance of MTX and lead to systemic toxicity. Due to MTX metabolism interactions, cotrimoxazole should not be given at least 6 days prior to beginning HDMTX therapy, and should be resumed only after ending protocol M. Requirements for the administration of HD-MTX: No severe infection, no mucositis. No effusions. Renal function is within normal limits. ASAT/ALAT < 10x upper normal limit for age. Urine alkalinization: urine pH between 7 and 8, before, during, and for at least 48 hours after the start of MTX-infusion. Hyperhydration. Determination of the MTX serum level: 24 and 48 hours after the beginning of the MTX infusion. LEUKOVORIN Folinic Acid 15 mg/m2/dose, i.v., 42, 48, 54 hours after beginning of Methotrexate infusion.	T24 Creatinine, if increased ≥50% from baseline, take extra MTX level(s). T48 creatinine and MTX level If T48< 0.4 µM: stop hyperhydration and alkalinization, and give 1 dose (T54) of leucovorin. If T48: 0.4-1 µM: continue hyperhydration, alkalinization, and leucovorin (same dose) If T48 > 1 µM: Hyperhydration should be increased to 4000 ml/ m²/day. Continue alkalinization and increase the dose of leucovorin (using the formula: MTX level in µM/1uM X 15 mg/m2/dose) (every 6 Hours): MTX-level Dosage LCV Rescue 0,25 - 1,0 µM: continue 15 mg/m² > 1,0 - 2,0 µM: 30 mg/m² > 2,0 - 3,0 µM: 45 mg/m² > 3,0 - 4,0 µM: 60 mg/m² > 4,0 -5,0 µM: 75 mg/m² > 5,0 µmol/l : etc Measure the MTX level every 24 hrs until T72 hr (or later time point) < 0.25 uM	In case of excessively high MTX levels (24-hour>120 µM, and 36-hour levels > 30 µM or 42-hour levels >10 µM and/or T48>5 µM). Especially in the case of reduced kidney function. No leucovorin 2 hours before and after glucarpidase. MTX levels within 48 hours after glucarpidase should be measured with LC-MSMS instead of the immunoassays.
BFM**	Adequate clinical condition and no serious infection according to the assessment of the investigator - Normal renal function. Dose adjustments of HD-MTX are recommended in the case of reduced creatinine clearance. - No urinary obstruction - Stable blood counts: Granulocytes ≥ 500/µl, Platelets ≥ 50 000/µl - GOT/GPT < 10 x upper normal limit - Bilirubin < 3 x upper normal limit with normal direct bilirubin If transaminases	Delayed MTX excretion should be suspected in case of: - onset of diarrhea during the MTX infusion, - heavy vomiting during the MTX infusion, - significant increase in serum creatinine over the baseline value 24 hours after the start of the MTX infusion and/or if – MTX levels are: ≥ 150 µmol/l at 24h, ≥ 3 µmol/l at 36h, ≥1 µmol/l at 42h, ≥ 0.4 µmol/l	In case of excessively elevated MTX levels and significantly reduced kidney function, the use of carboxypeptidase G2 should be considered. Since there are only insufficient data on any benefit of using carboxypeptidase rather than an intensified Leucovorin rescue, there are currently no absolute indications for the use of carboxypeptidase except for kidney failure with anuria. Please contact the study coordination

	are between 10 and 20 times of the upper normal limit, wait 36 to 48 hours and check to ensure that the levels are decreasing. If GOT and/or GPT are ≥ 20 times of normal upper limits, contact the national study coordinator for further recommendations. Avoid the administration of cotrimoxazol, nonsteroidal anti-inflammatory medications, and penicillins simultaneously to HD-MTX and as long as the MTX level is not less than 0.25 µmol/l. Avoid sun exposure (also solarium) during HD-MTX-containing treatment elements. 1/10 (500 mg/m2) of the total MTX dose should be infused over 30 min as a loading dose, immediately followed by the remaining 9/10 of the dose (4,500 mg/m²) given by continuous IV infusion over 23.5 h. Leucovorin (LCV) rescue: 15 mg/m2 i.v. of the racemic product or 7.5 mg/m² of the levo-product 42, 48, and 54 hrs after the start of the MTX infusion. The LCV dose depends on the MTX plasma level.	at 48h or > 0.25 µmol/l at 54h: the following procedure applies: If the MTX level at the end of the MTX infusion (at 24 h) exceeds 150 µmol/l and/or there is a significant increase in serum creatinine over the baseline value, the following procedure applies: Increase of hydration to 4500 ml/m²/24 h, Securing a urine pH of > 7,0, - Strict balancing of fluids, frequent controls of electrolytes, - Determination of the level of MTX at the 36th hour and immediate administration of 30 mg/m² LCV i.v., in case the level exceeds 3 µmol/l. Administration of LCV every 6 hours, adaptation of the dosage of LCV to the level of MTX as determined 6 hours before. If the MTX serum level at 54 hours is still ≥0.25µmol/l, hydration and the administration of LCV rescue every 6 hours must be continued, and it is necessary to perform additional measurements in intervals of 6 to 12 hours, until the serum concentration of MTX has decreased below 0,25 µmol/l. Serum Concentration of MTX Dosage LCV Rescue (every 6 Hours) 0,25 - 1,0 µmol/l: 15 mg/m² > 1,0 - 2,0 µmol/l: 30 mg/m² > 2,0 - 3,0 µmol/l: 45 mg/m² > 3,0 - 4,0 µmol/l: 60 mg/m² > 4,0 -5,0 µmol/l:75 mg/m² > 5,0 µmol/l ----mg LCV i.v. all 6 h = Serum MTX Concentration [µmol/l] x KG [kg]. With doses of leucovorin of more than 600 mg/m2, or respectively, 20 mg/kg, administration of	center in case of doubt. Carboxypeptidase G2 (Glucarpidase, CPG2, Voraxaze®) for the treatment of MTX excretion disorder is approved as orphan drug and available for use with named patients. The manufacturer recommends a single dose of 50 IU/kg to be administered as an i.v. bolus over 5 min. However, there are currently no clinical dose-finding studies. Leucovorin should not be administered at least 4 h before and 4 h after carboxypeptidase administration. Leucovorin rescue should subsequently be continued at a dose of 100 mg/m2/single dose every 6 hours for another 24 h, then at doses depending on MTX levels (see Table 3), or at least for a week, even if levels have already decreased to below 0.25 µmol/l. Important: The MTX metabolite 4-amino-4-deoxy-N10-methylpteroic acid (DAMPA), which occurs in large quantities during carboxypeptidase treatment, is recorded by many widely used immunoassays for determining MTX levels, and consequently artificially high levels may be measured. HPLC allows reliable measurements, but this procedure is only offered by very few laboratories.

		the individual dose as a 1 h infusion because of the high calcium content. In case of a relevant reduction of creatinine clearance, the HD-MTX blocks should be postponed or reduced after appropriate diagnostics, if necessary. Please contact the study coordination center concerning further procedure. If antibiotic therapy becomes necessary during high-dose MTX treatment, interaction with MTX excretion and the potential nephrotoxic effect of, for instance, aminoglycosides must be taken into account.	
NOPHO***	2008: HD-MTX (High-dose methotrexate. Start hydration 4-12 hours before the MTX-24-hour continuous infusion. Plasma MTX concentrations are measured at hours 24, 36, 42, 48, 66, and from then on at least every 24 hours. CF-Rescue (Leucovorin/Rescuvolin) 15 mg/m² slow i.v. push, given at 42 hours from the start of MTX infusion and every 6th hour until P-MTX concentration is <0.2 µmol/l. Before the start of HDM: Renal function is within normal limits. ASAT/ALAT < 10x upper normal limit for age.	Hydration should be increased from 3000 to 4500 ml/ m²/day if plasma 42-hour > 1 µmol/l or plasma creatinine increase ≥50% from baseline. Concentration of MTX Dosage LCV Rescue (every 6 Hours): 0,25 - 1,0 µmol/l: 15 mg/m² > 1,0 - 2,0 µmol/l: 30 mg/m² > 2,0 - 3,0 µmol/l: 45 mg/m² > 3,0 - 4,0 µmol/l: 60 mg/m² > 4,0 -5,0 µmol/l:75 mg/m² > 5,0 µmol/l ----mg LCV i.v. all 6 h = Serum MTX Concentration [µmol/l] x KG [kg].	Indications for GPDG2 therapy: in case of excessively high MTX levels (e.g. 24 24-hour> 250 µM, 36-hour levels > 30 µM, or 42-hour levels >10 µM). Not least in the case of reduced kidney function (50% increase in plasma creatinine) or anuria. Treatment with GPDG2 may also be indicated in case of severe acute neurotoxicity during treatment with HDMTX. Treatment with GPDG2 should always be discussed with a pediatric oncologist, who is familiar with HDMTX therapy and should optimally take place within 48 hours (max 60 hours) from the start of the MTX-infusion, since the risk of life-threatening toxicities may not be reversible beyond this time point.

Abbreviations:

* DCOG: PROTOCOL ALL-11 Treatment study protocol of the Dutch Childhood Oncology Group for children and adolescents (1-19 years) with newly diagnosed acute lymphoblastic leukemia ** BFM: AIEOP-BFM ALL 2009-2017 International Collaborative treatment protocol for children and adolescents with Acute Lymphoblastic Leukemia *** NOPHO: The Nordic Society of Paediatric Haematology and Oncology ALL 2008 protocol

Supplementary Table S2: ALL treatment protocols

Protocol	Number of patients	MTX dose	Planned MTX courses	Number of Re-exposed
BFM	51	5 gr/m^2	SR, MR=4 HR=2	30 (59%)
BFM 95-2003	4	5 gr/m^2		4 (100%)
BFM 2009	39	5 gr/m^2		21(54%)
BFM 2017	8	5 gr/m^2		5 (62%)
DCOG-protocol ALL-11 (2012-2019)	29	5 gr/m^2	SR, MR=4 HR=3	22 (76%)
NOPHO- 1992-2000	81			67 (83%)
SR, MR	50	5 gr/m^2	8	43 (86%)
HR	31	8 gr/m^2	4	24 (77%)
NOPHO 2008	28	5 gr/m^2	SR, MR=8 HR=3	24 (86%)
Summary	189			143 (76%)

Abbreviations:

BFM, Berlin-Frankfurt-Münster; DCOG, Dutch Childhood Oncology Group; NOPHO, Nordic Society of Paediatric Haematology and Oncology; SR, standard risk; MR, medium risk; HR, high risk.

Supplementary Table S3: Severely delayed MTX elimination questionnaire:

INTERNATIONAL COLLABORATIVE LEUKEMIA PROJECT

RETROSPECTIVE INVESTIGATION OF CHILDREN WITH ALL WHO DEVELOPED

SEVERELY DELAYED MTX ELIMINATION

BASIC INFORMATION AND PATIENT DEMOGRAPHICS

1. ALL Study group: _____

Codes: [1] AIEOP, [2] ANZCHOG, [3] BFM, [4] BFM-Austria, [5] CoALL, [6] COG, [7] CPH, [8] DCOG, [9] DFCI, [10] EORTC, [11] FRALLE, [12] Hungary, [13] Israel, [14] JPLSG, [15] NOPHO, [16] St. Jude, [17] TPOG, [18] UKALL.

2. Patient ID/study Number: ☐☐☐☐☐☐☐☐☐☐

3. Date of Birth: ☐☐ ☐☐ ☐☐☐☐

D D M M Y Y Y Y

4. BSA at ALL diagnosis: _____m^2 Weight _____kg Height _____cm

5. Gender: ☐ Codes: [1] Male, [2] Female

6. Race: ☐ Codes: [1] White [2] Black [3] Asian [4] American Indian/Alaska Native/Eskimo

[5] Middle Eastern [6] Hispanic [7] Unknown [9] : _____

7. Cancer predisposition syndrome: ☐ If yes, please specify: _____

Codes: [1] Yes, [2] No, [3] Down syndrome, [9] Unknown

8. Date ALL diagnosis: ☐☐ ☐☐ ☐☐☐☐

D D M M Y Y Y Y

9. Lineage: ☐ Codes: [1] B-lineage, [2] T-lineage, [3] Bilineage, [4] Other - specify_____, [9] Unknown

10. Treatment protocol: _____ Risk group (defined by treatment protocol): _____

11. Date of Complete morphological remission: ☐☐ ☐☐ ☐☐☐☐

D D M M Y Y Y Y

DETAILS ON THE MTX COURSE WITH SEVERELY DELAYED MTX ELIMINATION:

Definition of DME:

An increase in plasma creatinine of ≥0.3 mg/dl or a relative increase of 1.5-fold above baseline, together with severely elevated plasma MTX concentrations at one or more of the following time-points after initiation of the MTX infusion: (This definition is independent of HDMTX dose)

24 hour MTX >150 µM

36 hours >20 µM

42 hours >10 µM

48 hours >5 µM

12. Date of start of HDMTX course with the delayed elimination: ☐☐ ☐☐ ☐☐☐☐

D D M M Y Y Y Y

13. Date of the previous HDMTX administration:

(= the latest HDMTX infusions given prior to the MTX infusion with DME) ☐☐ ☐☐ ☐☐☐☐

D D M M Y Y Y Y

First course ☐

14. Total number of MTX infusions given to the patient prior to the infusions with DME: ☐

[9] Unknown

15. Dose of MTX used in the infusion with DME: ☐ gr
[9] Unknown

BSA at HDMTX infusion: _____m²

16. Was the patient treated with other chemotherapeutic drugs (besides 6MP) that were administered in the same treatment course (from 12 hrs before to 72 hours later) as the HDMTX (e.g, VCR, Cyclophosphamide, Ifosfamide, PEG-Asparaginase, etc)?

Codes: [1] sole drug, [2] in combination, [9] Unknown ☐

17. If MTX was given in combination with other chemotherapy within the same course, with which other drugs?

☐☐ ☐☐ ☐☐ ☐☐☐☐

Codes: [1] VCR, [2] Cytarabine, [3] Cyclophosphamide, [4] Asparaginase, [5] Ifosfamide, [6] Anthracyclines,

[7] Other specify: _____, [9] Unknown

18. Concomitant oral drugs administered during HDMTX course:

☐☐ ☐☐ ☐☐☐☐

Codes: [1] NSAIDS, [2] Quinolones [3] Proton pump inhibitors, [4] antifungal, please specify _____ [5] other, please specify _____

19. Which kind of intrathecal (IT) was given during the HDMTX course? ☐

Codes: [1] MTX, [2] TIT (MTX-Steroid-Cytarabine), [3] No IT treatment, [4] Other specify: _____ [9] Unknown

20. When was the IT chemotherapy given? ☐

Codes: [1] **Before** the MTX infusion, [2] **During** the HDMTX infusion, [3] **After** the end of the HDMTX infusion, [9] Unknown

21. Duration of hydration prior to the MTX infusion: ☐ hours

22. Volume of hydration prior to the MTX infusion: ☐ ml/hour

23. Type of hydration: ☐

Codes: [1] NACL 0.9% GLUC5%+ Bicarbonate, [2] NACL 0.3% GLUC5%+ Bicarbonate, [3] NACL 0.9% GLUC5%,

[4] NACL 0.3% +GLUC5%, [5] Other, specify_____, [9] Unknown

24. Time of the day when the MTX infusion was started: ☐

Codes: [1] Morning 8-12, [2] afternoon 13-17, [3] evening 18-22, [9] Unknown

25. 6MP daily dose during the HDMTX course: ☐

Codes: [1] as per protocol [2] not given (3) other, please specify dose_____ [9] Unknown

26. When was leucovorin rescue started: ☐

Codes: [1] as per protocol [2] not given (3) other, please specify hours post starting HDMTX_____ [9] Unknown

27. Cumulative leucovorin dose given in the HDMTX course with DME: ☐ mg [9] Unknown

* If Isovorin or Levofolinate were given, please write their cumulative dose.

28. **Plasma MTX concentrations:**

Hours after start of the MTX infusion:	**24hr**	**36hr**	**42hr**	**48hr**	**54hr**	**66hr**
Plasma MTX (μM) measured by immune-assay:						
Plasma MTX (μM) HPLC:						

Time to plasma MTX concentration <0.25 µM: _____hours from start of the HDMTX

29. Was Glucarpidase (Carboxypeptidase) administered? ☐

Codes: [1] yes, [2] no, [9] Unknown

30. If yes:

At what dose: ☐ (IU)

At what hour after the start of the MTX infusion: ☐ hour [9] Unknown

How many doses of Carboxypeptidase were administered? ☐

Codes: [1]1, [2] 2, [3] 3, [4] other, specify—[9] unknown

Laboratory Parameters: *(If the parameter was not measured, please write "N.A. ")*

31. GFR before MTX therapy: ☐ ml/min [9] unknown

- How was it measured? _____

Codes: [1] 24 hrs urine collection [2] 12 hrs urine collection [3] calculated formula [4] other, specify-------- [9] unknown

- Was not measured ☐

32. Creatinine levels:

0 hr (Base line)	24 hr	36 hr	42 hr	48 hr	54 hr	66 hr	Units:

33. Time of Maximum creatinine level (hr): _____ hours after start of the HDMTX infusion.

34. Maximum creatinine level: _____

35. Anuria/oliguria (less than 1 ml/kg/hr): Yes □ No □ Unknown □

36. Did the patient undergo dialysis? Yes □ No □ Unknown □

37. Time of returning to baseline creatinine level as before HDMTX or lowest creatinine level within 2 months from the event: _____ (days from maximum creatinine value).

Never ☐ Unknown ☐

38. **Laboratory:**

	Units	Value **before start** of the MTX infusion:	**Most abnormal** value within 14 days after the MTX infusion:	Date of abnormal value
Haemoglobin				
Thrombocytes				
WBC				
Neutrophils				
Bilirubin				
Alanine aminotransferase				
Albumin				
CRP				

CLINICAL TOXICITY:

39. Were these symptoms/clinical findings* present at diagnosis of DME or within 14 days?
*in severity that required intervention (CTCAE 3-4)

		CTCAE 3 definition
Vomiting:	Yes □ No □ Unknown □	>=6 episodes (separated by 5 min) in 24 hrs; tube feeding, TPN Indicated or hospitalization
Diarrhea:	Yes □ No □ Unknown	>=7 stools/ d; incontinence; hospitalization indicated, severe increase in ostomy output compared to baseline; limiting in ADL.
Mucositis:	Yes □ No □ Unknown □	Severe pain; interfering with oral intake

CNS toxicity: Yes □ No □ Unknown □

If yes, what kind of CNS toxicity? ☐

Codes: [1] MTX stroke-like-syndrome, [2] PRESS, [3] Seizures, [4] Encephalopathy, [5] other, specify-------- [9] unknown

Infection/ fever that required antibiotic therapy: Yes □ No □ Unknown □

40. Death because of MTX-related toxicity? Yes □ No □

41. Other significant symptoms: _____

MODIFICATIONS OF LEUKEMIA TREATMENT DUE TO DME:

42. Was the next chemotherapy course delayed because of toxicity post-HD-MTX infusion?

Yes ☐ No ☐

43. When did the patient return to the ALL treatment after the HD-MTX infusion with DME?

☐☐ ☐☐ ☐☐☐☐

D D M M Y Y Y Y

44. Was 6MP administration paused, or was the dose reduced because of the MTX-induced toxicity?

6MP dose reduced ☐ 6MP paused ☐ No ☐ Unknown ☐

RE-EXPOSURE TO HIGH-DOSE MTX

45. Was the patient re-exposed to high-dose MTX? Yes ☐ No ☐

46. If no, why was the patient not re-exposed?

☐ No further high-dose MTX infusions scheduled according to the ALL protocol.

☐ Permanent renal insufficiency (Increased plasma creatinine or/and decreased GFR).

☐ Concern for delayed MTX elimination in the next high-dose MTX infusion.

☐ Other: _____

- If the patient **was not re-exposed** to HDMTX, please proceed to question **75.**

47. Number of HDMTX infusions (re-exposure) after the course with DME: _____

48. Re-exposure to HDMTX

Re-exposure HDMTX no.	HDMTX dose (mg)		Plasma MTX concentrations			Time to MTX conc.<0.25 uM *Obligatory
	Measured by immune assay	Measured by HPLC	23	42 *Obligatory	48	
1						
2						
3						
4						
5						
6						
7						

Please report the following details regarding the 1st and 2nd re-exposure to HDMTX:

FIRST RE-EXPOSURE to HIGH-DOSE MTX:

49. When was HDMTX re-introduced? ☐☐ ☐☐ ☐☐☐☐

D D M M Y Y Y Y

50. Was 6MP given? ☐

Codes: [1] yes, [2] no [9] unknown

If 6MP was given, was the dose modified during the re-exposure with HDMTX?

6MP dose reduced □ No change in dose □ Unknown □

51. What was the BSA? ☐ m²

52. GFR before first re-exposure to high-dose MTX: ☐ ml/min Not measured ☐

53. Duration of hydration prior to the MTX infusion: ☐ hours

54. The volume of hydration prior to the MTX infusion: ☐ ml or ml/hour

55. Type of hydration: ☐

Codes: [1] NACL 0.9% GLUC5%+ Bicarbonate, [2] NACL 0.3% GLUC5%+ Bicarbonate, [3] NACL 0.9% GLUC5%, [4] NACL 0.3% + GLUC5%, [5] Other, specify_____, [9] Unknown

56. Was Glucarpidase administered after re-exposure to MTX? Yes □ No □ Unknown □

57. **Plasma creatinine** concentration:

0 hr (base line)	23 hr	36 hr	42 hr	48 hr	54 hr	66 hr	Units:

Time of maximum creatinine level (hr): _____ hours after start of the HDMTX infusion.

Maximum plasma creatinine level: _____

Time of returning to baseline creatinine level as before HDMTX or lowest creatinine level within 2 months from the event: _____ (days from maximum creatinine value).

Never ☐ Unknown ☐

58. **Laboratory: FIRST RE-EXPOSURE to HIGH-DOSE MTX:**

	Unit	Values **before the start** of the MTX infusion:	**Most abnormal** value within **14 days** after the MTX infusion:	Date:
Haemoglobin				
Thrombocytes				
WBC				
Neutrophils				
Bilirubin				
Alanine aminotransferase				
Albumin				
CRP				

59. Were these symptoms/clinical findings* present at diagnosis of DME or within 14 days?
*in severity that required intervention (CTCAE 3-4)

CTCAE 3 definition

Vomiting: Yes □ No □ Unknown □ >=6 episodes (separated by 5 min) in 24 hrs; tube feeding, TPN indicated or hospitalization

Diarrhea: Yes □ No □ Unknown >=7 stools/ d; incontinence; hospitalization indicated, severe increase in ostomy output compared to baseline; limiting in ADL.

Mucositis: Yes □ No □ Unknown □ Severe pain; interfering with oral intake

CNS toxicity: Yes □ No □ Unknown □

If yes, what kind of CNS toxicity? ☐

Codes: [1] MTX stroke-like-syndrome, [2] PRESS, [3] Seizures, [4] Encephalopathy, [5] other, specify-------- [9] unknown

Infection/ fever that required antibiotic therapy: Yes ☐ No ☐ Unknown ☐

60. Death because of MTX-related toxicity? Yes ☐ No ☐

61. Was the next chemotherapy course delayed because of toxicity post-HD-MTX infusion?
Yes ☐ No ☐

62. Date of return to treatment with chemotherapy other than 6MP: ☐☐ ☐☐ ☐☐☐☐
D D M M Y Y Y Y

SECOND RE-EXPOSURE to HIGH-DOSE MTX:

63. When was HDMTX re-introduced? ☐☐ ☐☐ ☐☐☐☐
D D M M Y Y Y Y

64. What was the 6MP dose during the MTX infusion? ☐ mg/day

65. Duration of hydration prior to the MTX infusion: ☐ hours

66. The volume of hydration prior to the MTX infusion: ☐ ml/hour

67. Type of hydration: ☐

Codes: [1] NACL 0.9% GLUC5%+ Bicarbonate, [2] NACL 0.3% GLUC5%+ Bicarbonate, [3] NACL 0.9% GLUC5%, [4] NACL 0.3% + GLUC5%, [5] Other, specify_____, [9] Unknown

68. Was Glucarpidase administered after the 2nd re-exposure to MTX? Yes □ No □ Unknown □

69**. Plasma creatinine**:

0 hr (base line)	23 hr	36 hr	42 hr	48 hr	54 hr	66 hr	Units:

Time of maximum creatinine level (hr): _____ hours after start of the HDMTX infusion.

Maximum plasma creatinine level: _____

Time of returning to baseline creatinine level as before HDMTX or lowest creatinine level within 2 months from the event: _____ (days from maximum creatinine value).

70. **Laboratory SECOND RE-EXPOSURE WITH HIGH-DOSE MTX:**

	Unit	Values **before the start** of the MTX infusion:	**Most abnormal** value within **14 days** after the MTX infusion:	Date:
Haemoglobin				
Thrombocytes				
WBC				
Neutrophils				
Bilirubin				
Alanine aminotransferase				
Albumin				
CRP				

71. Were these symptoms/clinical findings* present at diagnosis of DME or within 14 days?
*in severity that required intervention (CTCAE 3-4)

CTCAE 3 definition

Vomiting: Yes □ No □ Unknown □ >=6 episodes (separated by 5 min) in 24 hrs; tube feeding, TPN indicated or hospitalization

Diarrhea: Yes □ No □ Unknown >=7 stools/ d; incontinence; hospitalization indicated, severe increase in ostomy output compared to baseline; limiting in ADL.

Mucositis: Yes □ No □ Unknown □ Severe pain; interfering with oral intake

CNS toxicity: Yes □ No □ Unknown □

If yes, what kind of CNS toxicity? ☐

Codes: [1] MTX stroke-like-syndrome, [2] PRESS, [3] Seizures, [4] Encephalopathy, [5] other, specify-------- [9] unknown

Infection/ fever that required antibiotic therapy: Yes □ No □ Unknown □

72. Death because of MTX-related toxicity? Yes ☐ No ☐

73. Was the next chemotherapy course delayed because of toxicity post-HD-MTX infusion?

Yes ☐ No ☐

74. Date of return to treatment with chemotherapy other than 6MP: ☐☐ ☐☐ ☐☐☐☐

D D M M Y Y Y Y

FOLLOW UP

75. Date of Last Follow-Up: ☐☐ ☐☐ ☐☐☐☐

D D M M Y Y Y Y

76. Status: ☐

Codes: [1] Alive, [2] Dead, [3] relapse, [4] Lost to Follow-Up

Note: if Status = [2] Dead, then Date of the Last Follow-Up=Date of Death

77. If relapse

Please state the date of relapse ☐☐ ☐☐ ☐☐☐☐

D D M M Y Y Y Y

Please state the localization of the relapse ☐

Codes: [1] BM, [2] CNS, [3] Testis, [4] Combined, [5] other, specify_____, [9] unknown

78. If dead, please state cause of death: ☐

Codes: [1] ALL, active disease [2] Second Malignancy, active disease 3] Therapy-related, non-SCT AND non-MTX related

[4] MTX-related toxicity [5] SCT-related [6] Accident [7] Other [8] Unknown

If code = 3, 4 or 6, please specify: _____

Supplementary table S4: The 1st delayed MTX elimination (DME) events n=189

1st DME	
MTX course number	Patients n (%)
1	103 (55%)
2	29 (15%)
3	16 (8%)
4	16 (8%)
5	11 (6%)
>5	14 (7%)
MTX dose (gr/m^2)	
3.5	1
5	154 (82%)
8	34 (18%)
MTX timing	NA: 49 (25%)
Morning	45 (24%)
Afternoon	86 (46%)
Evening	9 (5%)
Single MTX	152 (80%)
Combined with other chemotherapy	37 (20%)
IT	
MTX	156 (82%)
MTX-Hydrocortisone	2 (1%)
TIT	31 (16%)
Timing of IT	NA: 113 (60%)
Before IV MTX	47 (25%)
During	27 (14%)
After	2 (1%)

Duration of pre-hydration (hours)	Mean 7 (range 4-18) NA:22 (12%)
4	111 (59%)
12	44 (23%)
18	12 (6%)
*DME timing / 1st pathological MTX values (hours)	
24	65 (34%)
36	30 (16%)
42	34 (18%)
48	56 (30%)
Time of any pathological MTX levels (hours)	
24	65 (34%)
36	69 (36%)
42	106 (56%)
48	169 (89%)
=1 abnormal MTX level	39 (20%)
>1	145 (77%)
MTX plasma values (µmol/L)	Median, (Q1-3) range
MTX24	130 (89, 186) 7.3-623
MTX36	20 (14, 31) 0.83-216
MTX42	13 (10,18) 0.32-181
MTX48	9 (6, 13) 0.21-150
MTX54	5.5 (4, 10) 0.1-77
Time to MTX < 0.25 µmol/L (hours) NA: 32 (17%)	Median: 192 (Q1-3; 160-234), mean 198, range: 48-476 hours
≤90	6 (3%)
100-150	29 (15%)
150-200	52 (27%)
200-250	47 (25%)
250-300	20 (11%)

>300	7 (4%)
Glucarpidase use	51 (27%)
Dose (u/kg)	Mean: 44 (range 15-63 u/kg)
Number of doses	1=47 (92%), 3=1 (2%)
Relative increase in creatinine levels	Median (Q1-3)
0-24 (n=99)	1.58 (1.26-2.07)
0-36 (n=82)	2.49 (1.9-3)
0-42 (n=38)	2.2 (1.78-2.89)
0-48 (n=81)	2.43 (1.92-3.22)
0-54 (n=37)	2.51 (2-3.95)
Time of maximal Creatinine (hours) NA: 38 (20%)	Median: 48 (Q1-3; 36-72), mean 58, range: 23-210
24	23 (12%)
25-48	74 (39%)
49-72	31 (17%)
73-120	16 (8%)
>120	11 (6%)
Time to baseline Creatinine (days) NA: 55 (29%)	Median: 18 (Q1-3;10-30), mean 23, range 2-120
≤10	35 (26%)
11-30	78 (58%)
>30	21 (16%)

*Calculated **relative increase** in creatinine level from baseline before MTX infusion.

*Creatinine units: mmol/l, MTX units: μmol/L

Supplementary Table S5: Toxicities after the first delayed MTX elimination (DME) event:

Clinical Toxicities (CTCAE≥3)	Assessed in 100 patients (52%)
Gastrointestinal	33 (33%)
Vomiting	22 (22%)
Diarrhea	6 (6%)
Mucositis	13 (13%)
Infectious	28 (28%)
CNS	7 (7%)
Encephalopathy	2
Seizures	4
MTX SLS	1
Laboratory Toxicities (CTCAE≥3)	Assessed in 78 patients (41%)
Hematological	Cytopenia: 32 (41%), Persistent SAA = 1
Hepatic	9 (12%)
Dialysis[€]	2 (3%)
Death	*2 (1%)
Modifications of therapy	Assessed in 172 patients (91%)

Any modification	126 (73%)
Delay	105 (61%)
Chemotherapy regimen adaptations	66 (38%)

*One died due to sepsis, and one died after complications of stem cell transplantation performed due to persistent bone marrow aplasia

€ For 6 and 16 days

Abbreviations:

DME, delayed MTX elimination; CTCAE, Common Terminology Criteria for Adverse Events; CNS, central nervous system; MTX, methotrexate; SLS, stroke-like syndrome; SAA, severe aplastic anemia

Supplementary Table S6: Re-exposure to HDMTX;

N=143 patients (80% of those scheduled for further MTX) for a total of 387 courses of HDMTX

Reasons for no re-exposure N=46			**MTX 2nd re-exposure N=110**	
No further MTX courses per protocol	11		Dose gr/m^2	N (%)
Concern for DME	14		≤ 1.5	7 (6%)
Death	1		2-4	28 (25%)
Relapse	1		5	59 (54%)
Other	1		>5	15 (14%)
NA	18		Dose %	
Number of re-exposures	Number of patients		≤ 30%	7 (6.5%)
1	33		40-50%	10 (9%)
2	24		60-70%	14 (13%)
3	40		80-90%	7 (6.5%)
4	12		100%	71 (65%)
>4	34		Time to MTX <0.25 (mean 64, range 42-192 hours)	Median 54 (Q1-2, 48-67)
MTX 1st re-exposure N=143			Max Creatinine levels (hours)	Mean 45.2 (range 13-80)
Dose (gr/m2)	N (%)		Time of Max Creatinine	Median 24 (Q1-3, 23-42) mean: 31, range 0-147
≤ 1.5	12 (8%)		Time to normalization of Creatinine levels (days)	Median 1(Q1-3, 1-31.5), mean: 16 (range 0-66)
2-4	38 (27%)		**Laboratory toxicities (documented in 35 patients)**	
5	69 (48%)		Hematological	10

>5	23 (16%)		Liver	2
Dose% of scheduled	N (%)		CRP	0
≤ 30%	12 (8%)		**Clinical Tox CTCAE grade ≥ 3 (documented in 50 patients)**	
40-50%	15 (10%)		Mucositis	0
60-70%	21 (14%)		CNS	1
80-90%	7 (5%)		Infectious	8
100%	88 (62%)		Death	0
Time to MTX <0.25, mean 67.7 (42-249 hours)	Median 60 hours (Q1-Q3; 54-70)		**2nd DME post 2nd re-exposure** (1 also after 1st re exposure)	**N=6***
≤54	47 (47%)		Timing of 2nd DME (hours)	
60-80	39 (39%)		24	5
81-120	8 (8%)		48	1
>120	6 (6%)		Glucarpidase use	no
NA	43 (43%)		**MTX 3rd re-exposure**	**N=86**
Max Creatinine levels	Mean 50 (range 16-146)		Dose%	
Time of Max Creatinine (hours)	Mean 34.5 (0-192), Median 36 (Q1-3, 23-48)		≤50%	5 (6%)
Time to normalization of Creatinine (days) NA=24	Mean 3.8 (0-24), Median 1 (Q1-3, 0-24)		60-90%	5 (6%)
Glucarpidase use	N=2		100%	76 (88%)
Laboratory toxicities (documented in 24 patients)			**DME post 3rd re-exposure**	**N=7 (8%)**
Hematological	11 (46%)		Timing of 2nd DME (hours)	
Liver	2 (8%)		24	4

CRP	3 (12%)		42	1
Clinical Tox CTCAE grade ≥ 3 (documented in 69 patients)			48	2
GI	0		**MTX 4th re-exposure**	**N=46**
Mucositis	3 (4%)		Dose%	
CNS	3 (4%)		≤50%	3 (6%)
Infectious	9 (13%)		60-90%	5 (11%)
Death	0		100%	38 (83%)
2nd DME post 1st re-exposure N=8 (6%)			**DME post 4th re-exposure**	**N=2 (4%)**
Timing of 2nd DME (hours)			Timing of 2nd DME (hours)	
24	6		24	1
42	1		42	1
48	1			

Creatinine units: mmol/l, MTX units: µmol/L

Abbreviations:

MTX, Methotrexate; DME, delayed MTX elimination; NA, not assessed; MAX, maximal; Cre, creatinine; CRP, C-reactive protein; GI, gastrointestinal; CNS, central nervous system.

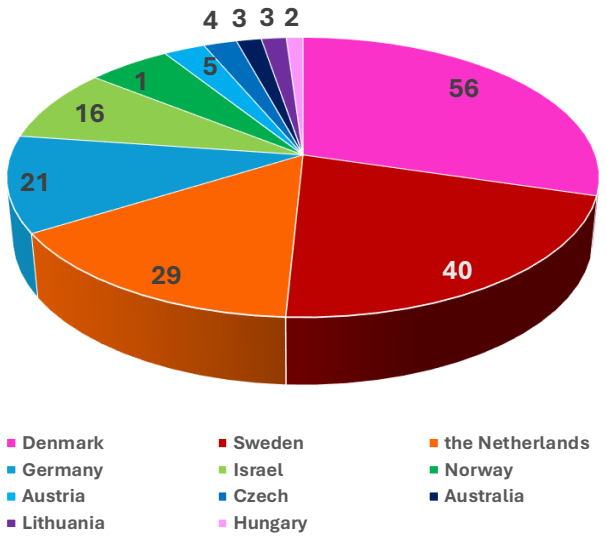
Supplementary Table S7: Multiple delayed MTX elimination (DME) events

Risk group	MTX course with 1st DME	MTX dose 1st DME	MTX course with 2nd DME	MTX dose 2nd DME	MTX course with 3rd DME	MTX dose 3rd DME	Additional MTX courses given post-3rd DME
MR	1st	5 gr/m^2	4th	2.5gr/m^2	5th	2.5gr/m^2	2
MR	1st	5 gr/m^2	2nd	5 gr/m^2	4th	5 gr/m^2	3
HR	1st	8 gr/m^2	2nd	8 gr/m^2	3rd	8 gr/m^2	1

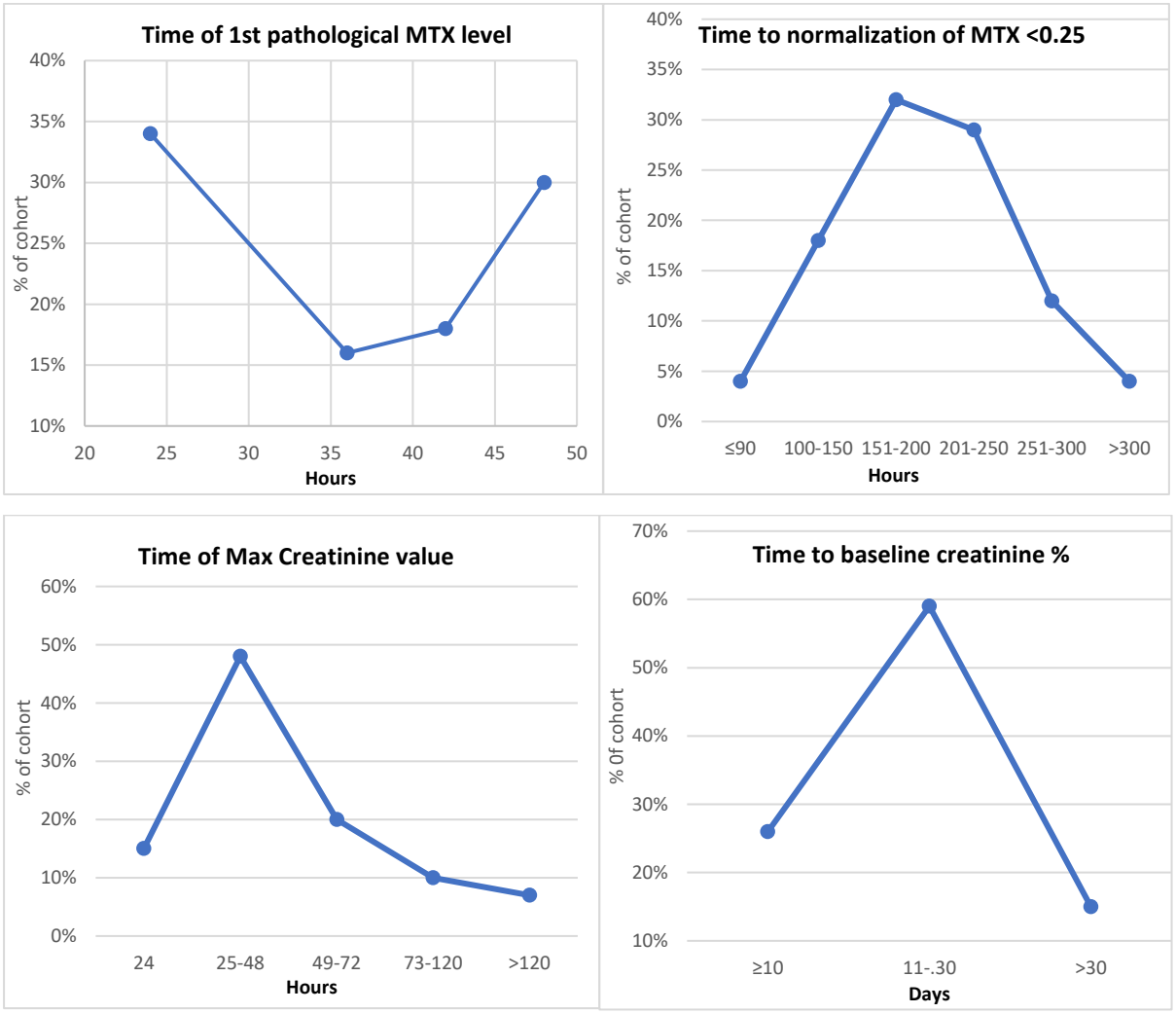
Abbreviations: MR, medium risk; HR, high risk; MTX, methotrexate; DME, delayed MTX elimination;

Supplementary Figure S1: Participating countries

Denmark	56
Sweden	40
the Netherlands	29
Germany	21
Israel	16
Norway	10
Austria	5
Czech	4
Australia	3
Lithuania	3
Hungary	2
Sum	189

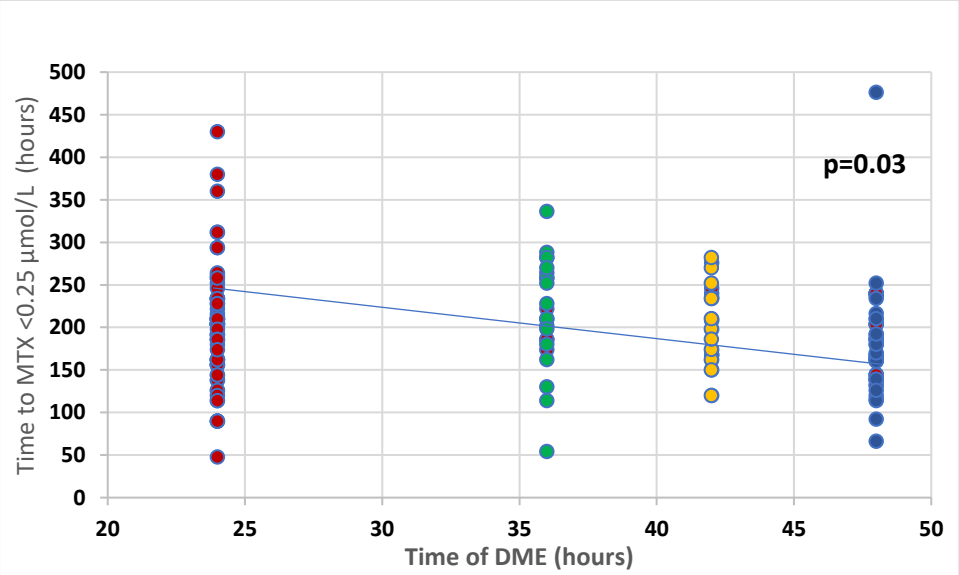


Supplementary Figure S2: 1st DME



Creatinine units: mmol/l, MTX units: µmol/L

Supplementary Figure S3: Time to plasma MTX level < 0.25 µmol/L according to timing of DME

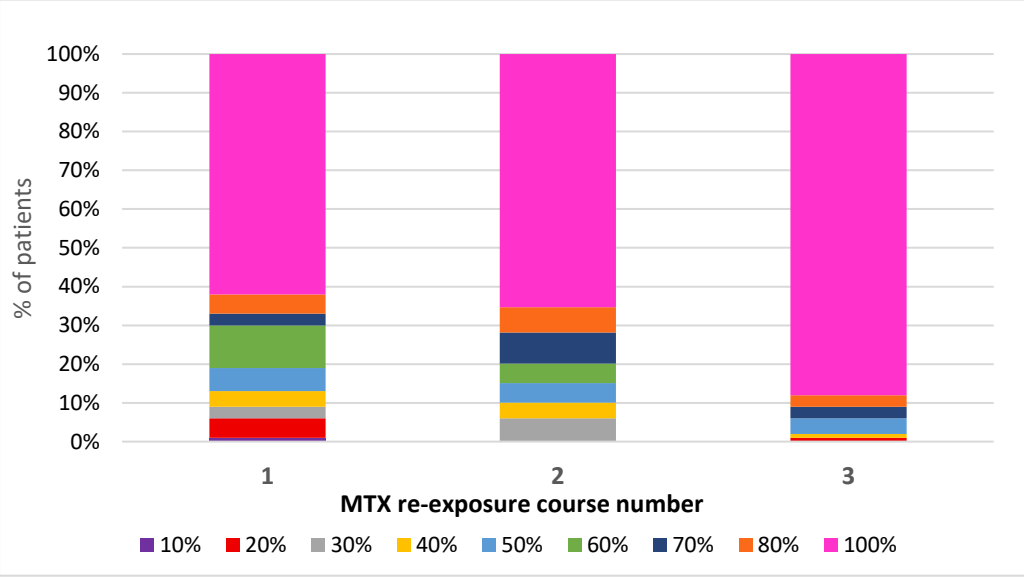


Time of DME	Time to plasma MTX<0.25µmol/L (hours)					
	Min	1st quartile	Median	Mean	3rd quartile	Max
24 hours	48	157.5	**204**	**204.9**	234	430
36 hours	54	183	**210**	**215**	261	336
42 hours	120	168	**198**	**201**	235	282
48 hours	66	142	**170**	**181**	207	476

Spearman correlation: rho=-0.1665, **p=0.0397**, Stuart-Kendall correlation: tau-c = -0.1329, **p=0.0319**

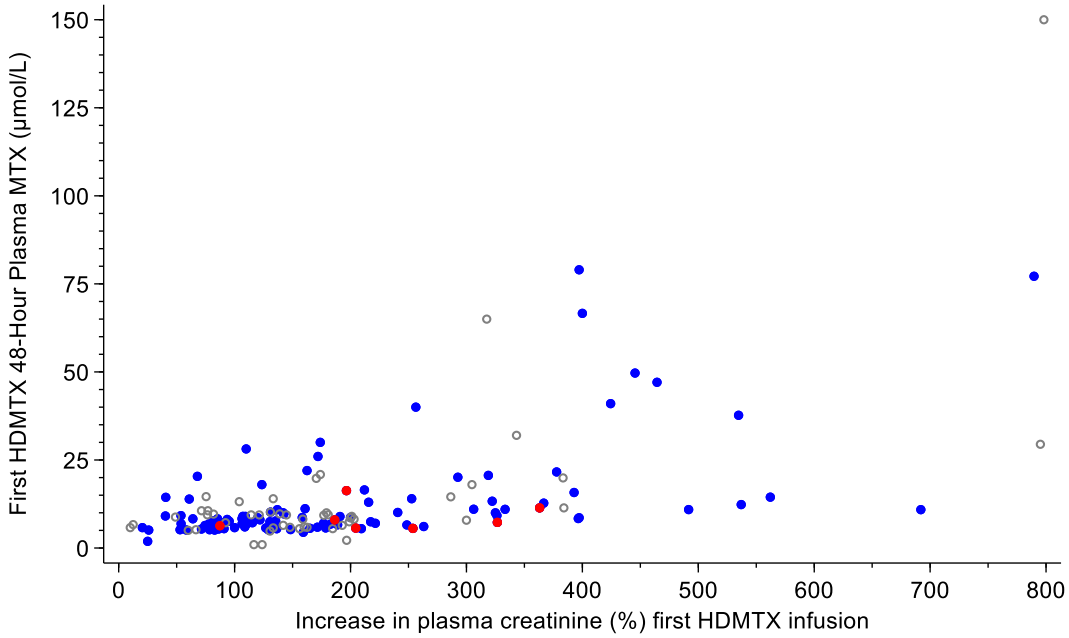
A statistically significant negative correlation was found between the timing of DME onset and MTX clearance, as indicated by the time to plasma MTX<0.25 µmol/L.

Supplementary figure S4: MTX dose at re-exposure by MTX course number



	Re-exposure course number		
MTX dose at re-exposure	1st	2nd	3rd
10%	1%	0	0
20%	5%	0	1%
30%	3%	6%	0
40%	4%	4%	1%
50%	6%	5%	4%
60%	11%	5%	0
70%	3%	8%	3%
80%	5%	7%	3%
100%	62%	65%	88%

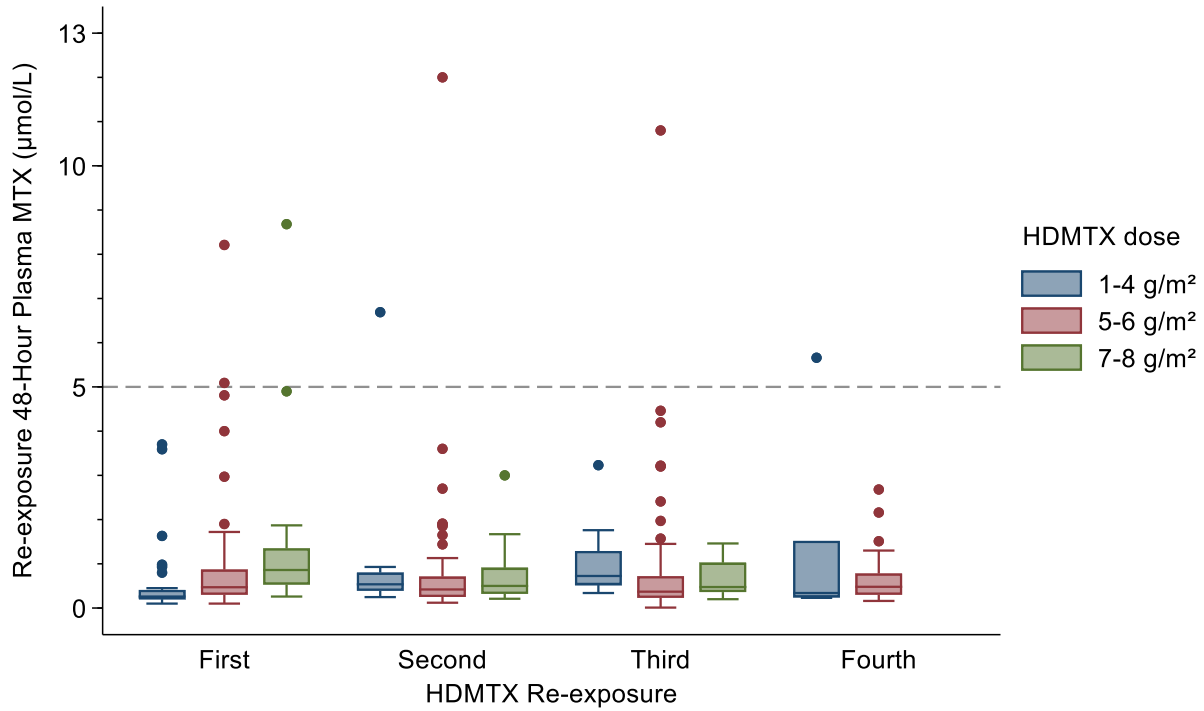
Supplementary Figure S5: Relative increase in creatinine and MTX48 levels at 1st DME and MTX elimination at re-exposure; p=ns



- **Blue dots**: patients who were re-exposed to HDMTX and had normal MTX elimination.
- **Red dots:** patients who were re-exposed to HDMTX and had delayed MTX elimination with plasma 48-hour MTX >5 µmol/L in the re-exposure (n=7)
- **Grey circles:** patients who were not re-exposed to HDMTX.

Lack of association between the relative increase in plasma creatinine (x-axis) and 48-hour plasma MTX concentrations during the first HDMTX infusion (y-axis), and additional DME during re-exposure to HDMTX.

Supplementary Figure S6: Plasma 48-hour MTX at re-exposure; p=ns



Plasma 48-hour MTX concentrations in the first, second, third, and fourth HDMTX re-exposure at several MTX doses.

Boxes represent the interquartile range (IQR), with the median indicated by a line. Whiskers extend to the most extreme data values within 1.5 × IQR from Q1 and Q3. Full circles represent outliers.

There was a lack of association between the dosage or the number of the re-exposed MTX courses and the 48-hour plasma MTX level.

Supplementary Figure S7: Increase in plasma creatinine and MTX48 levels at 1st DME and plasma MTX48 at re-exposures

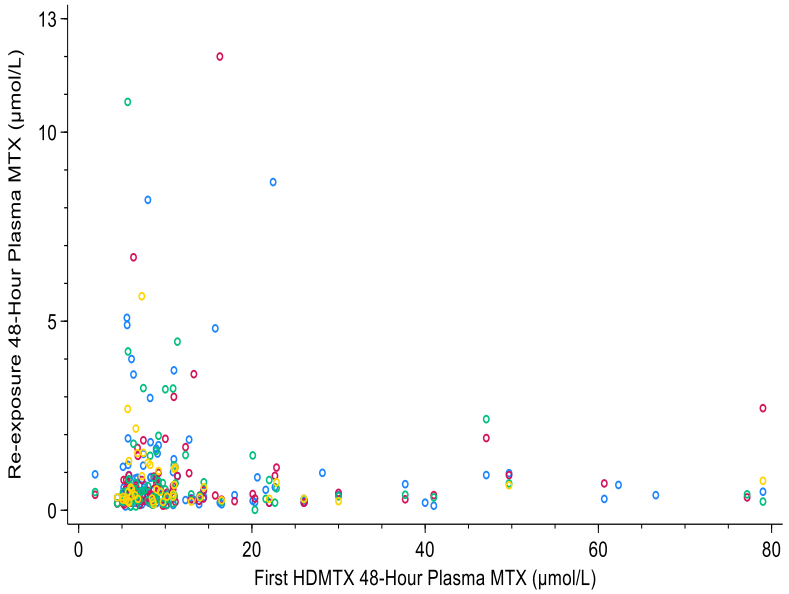


Figure S7a

Plasma 48-hour MTX in the first HDMTX infusions (x-axis) and re-exposure to HDMTX (y-axis). Circles represent patients in the first (blue), second (red), third (green), and fourth (yellow) re-exposure HDMTX infusion, p=ns.

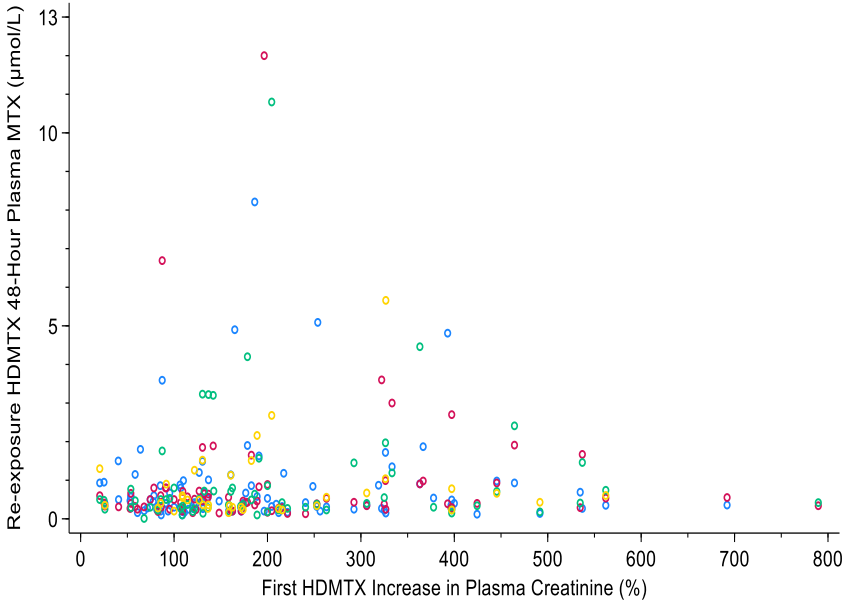
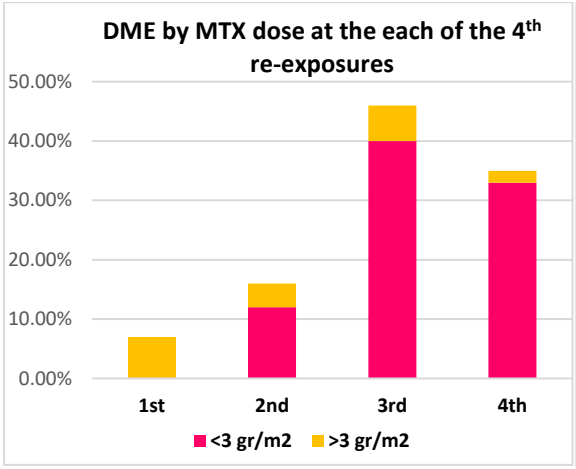


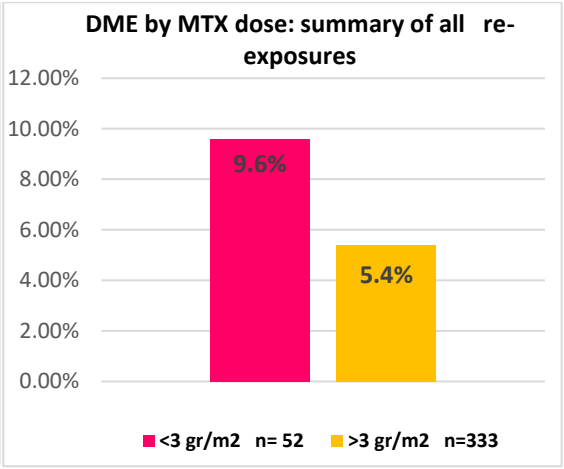
Fig S7b

Percentage increase in plasma creatinine in the first HDMTX infusions and re-exposure to HDMTX 48-hour plasma MTX. Circles represent patients in the first (blue), second (red), third (green), and fourth (yellow) re-exposure HDMTX infusion groups, p = ns.

Lack of association between 48-hour plasma MTX at re-exposures and 48-hour plasma MTX at first DME (S7a) or the relative increase in plasma creatinine at first DME (Figure S7b).

Supplementary Figure S8: DME by MTX dose at re-exposures





MTX dose	<3 gr/m^2	>3 gr/m^2
1st	0 (0/27)	7% (8/116)
2nd	12% (2/17)	4% (4/93)
3rd	40% (2/5)	6% (5/81)
4th	33% (1/3)	2% (1/43)

MTX dose	<3 gr/m^2 n= 52	>3 gr/m^2 n=333
DME	9.6% (5/52)	5.4% (18/333)

Reduced MTX dose at re-exposure did not reduce recurrent DME rates.