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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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.

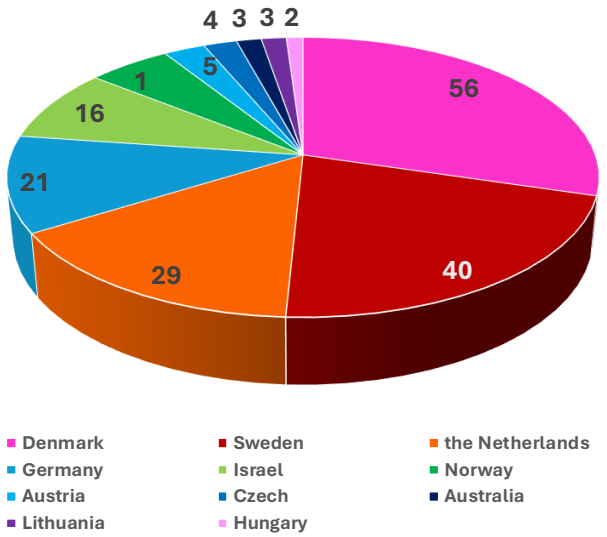
Supplementary Table S7: Multiple delayed MTX elimination (DME) events

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

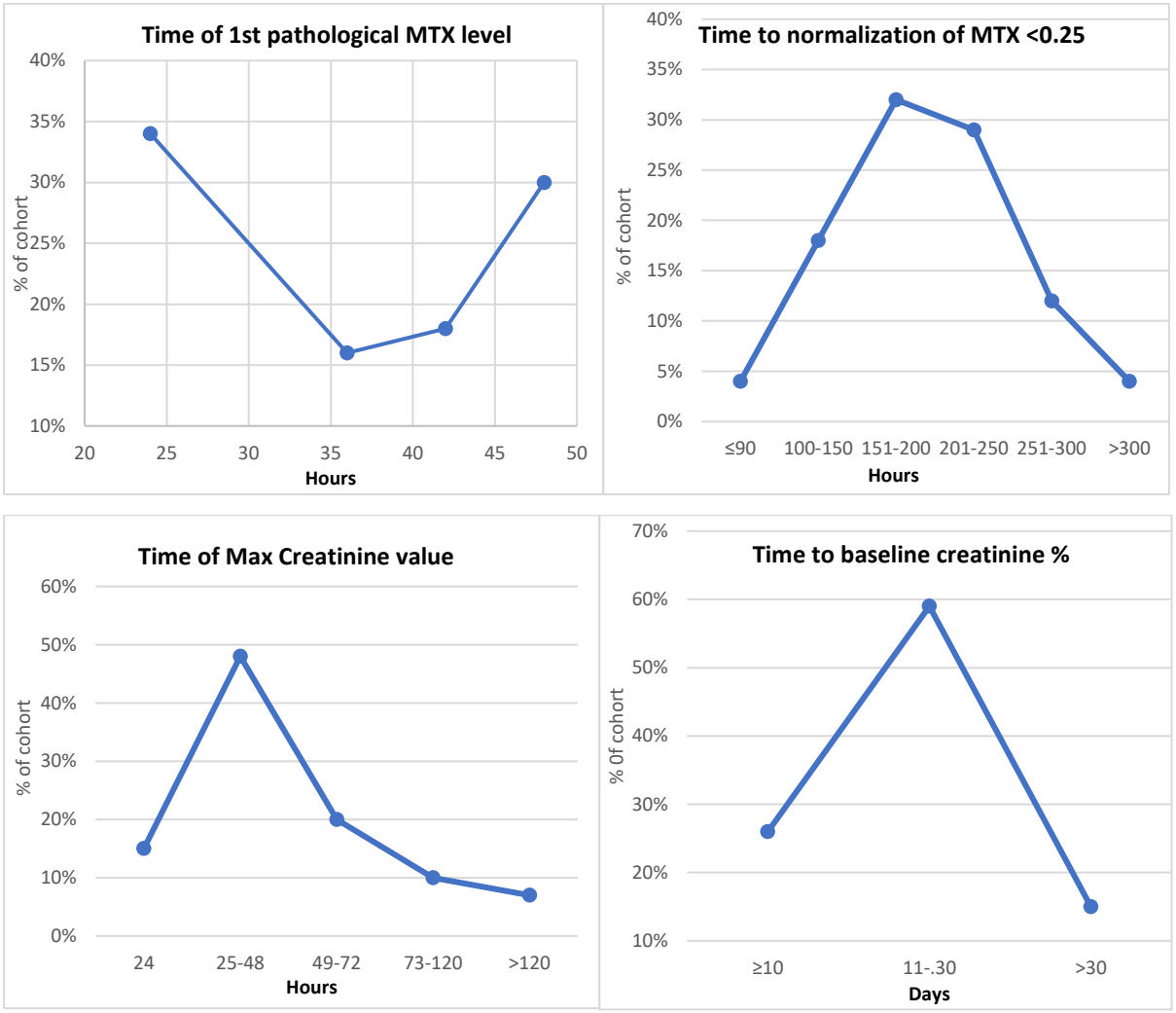
Abbreviations: MR, medium risk; HR, high risk; MTX, methotrexate; DME, delayed MTX elimination;

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

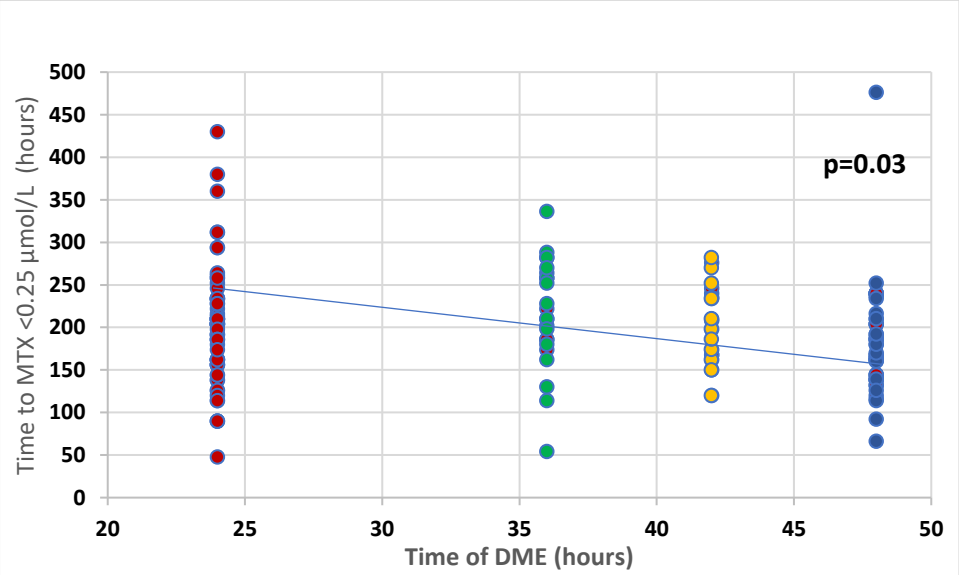


Supplementary Figure S2: 1st DME



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

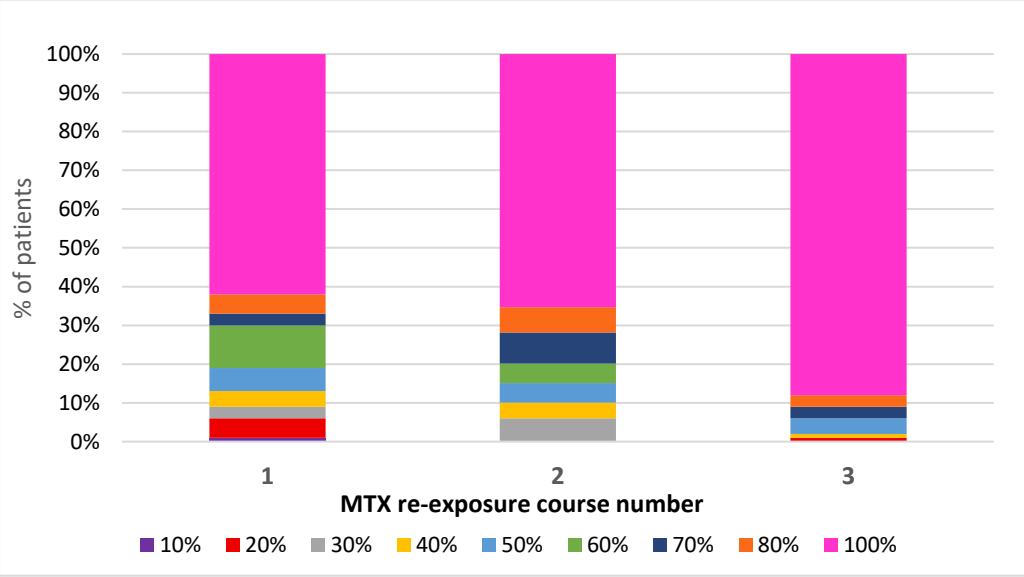


Time of DME	Time to plasma MTX<0.25µmol/L (hours)					
	Min	1st quartile	Median	Mean	3rd quartile	Max
24 hours	48	157.5	**204**	**204.9**	234	430
36 hours	54	183	**210**	**215**	261	336
42 hours	120	168	**198**	**201**	235	282
48 hours	66	142	**170**	**181**	207	476

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

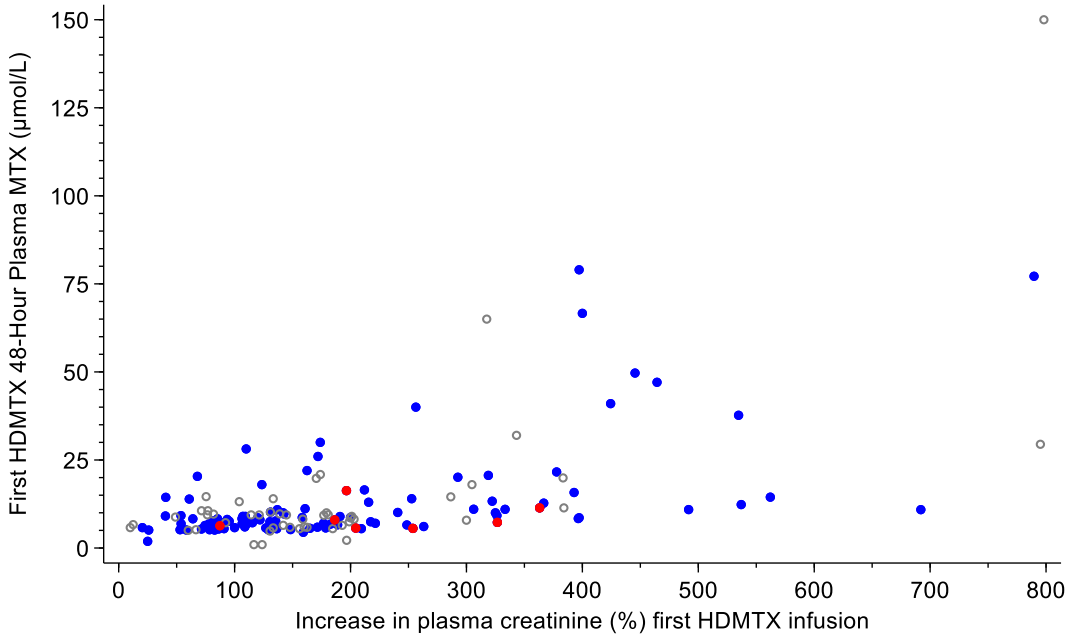
A statistically significant negative correlation was found between the timing of DME onset and MTX clearance, as indicated by the time to plasma MTX<0.25 µmol/L.

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%

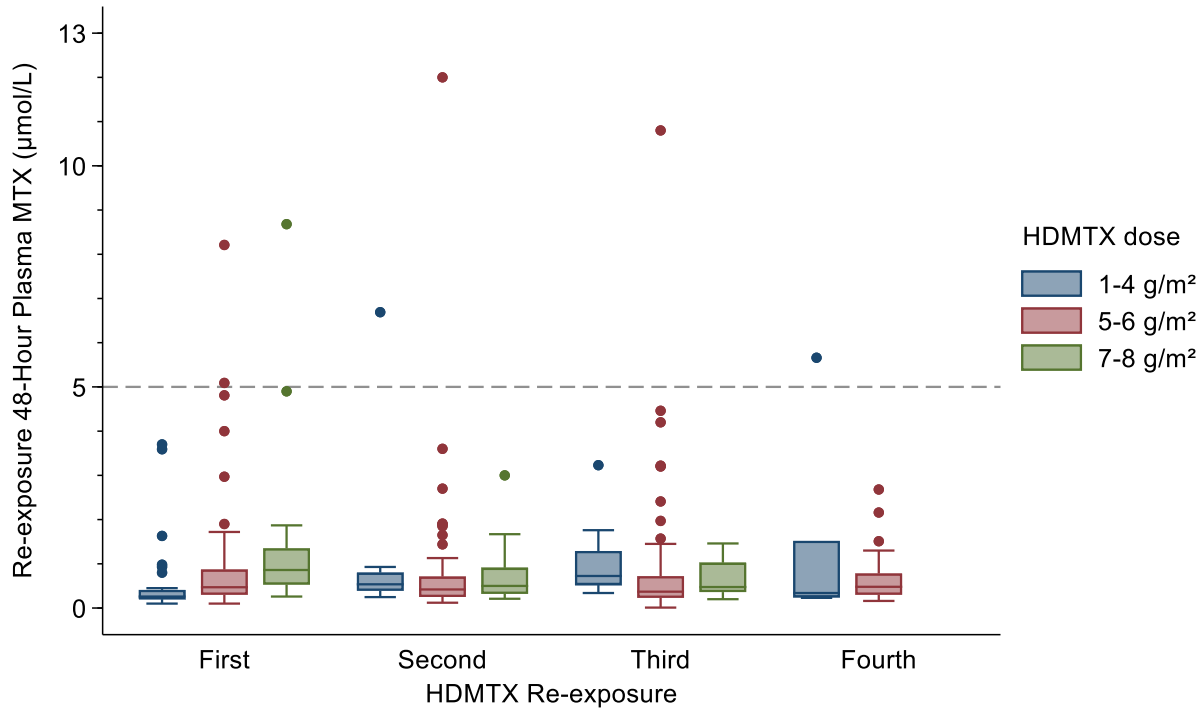
Supplementary Figure S5: Relative increase in creatinine and MTX48 levels at 1st DME and MTX elimination at re-exposure; p=ns



- **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

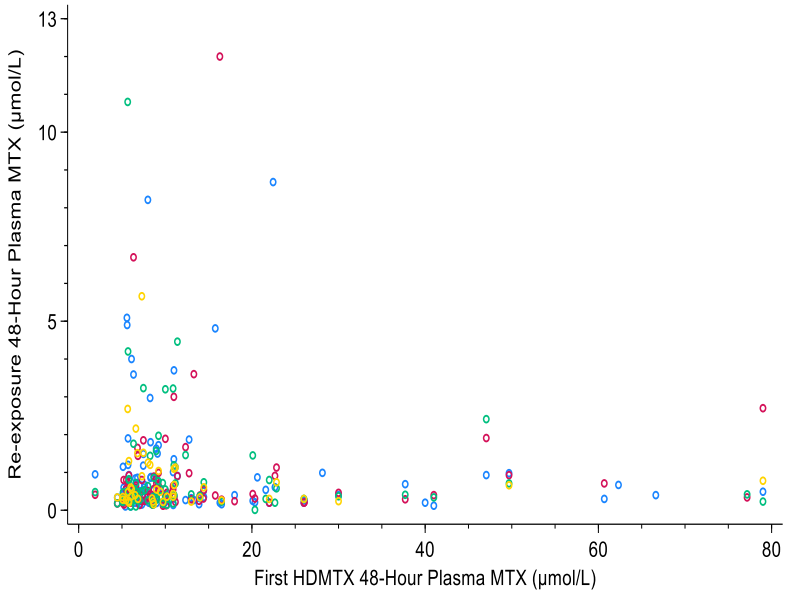


Figure S7a

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.

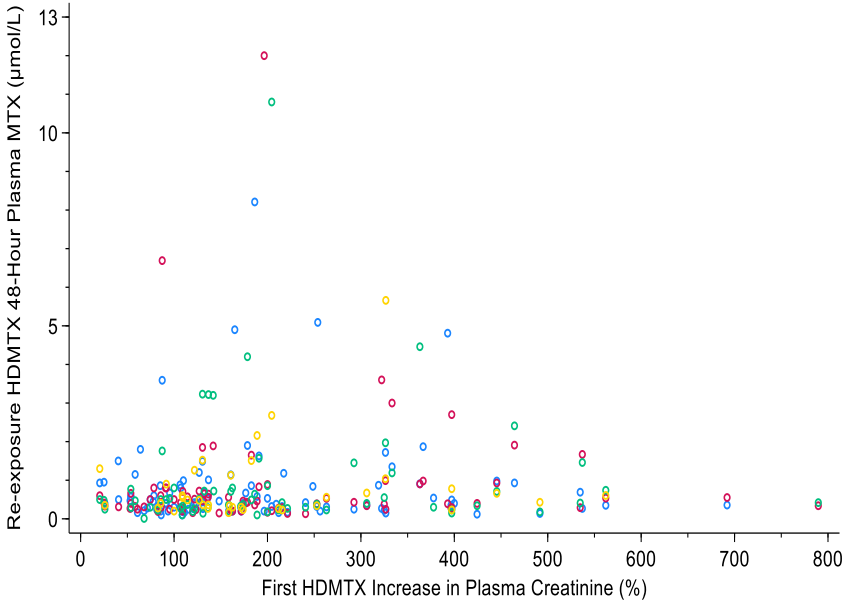
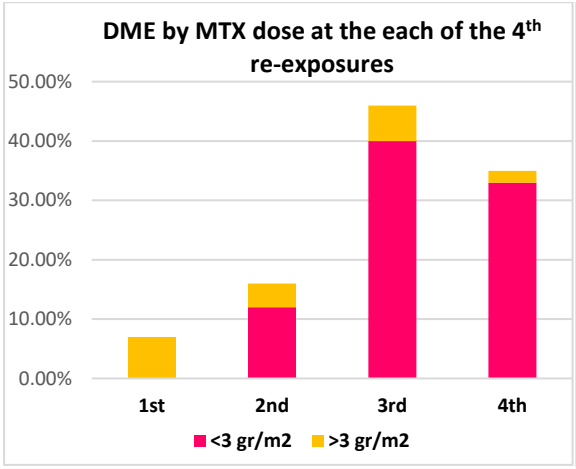


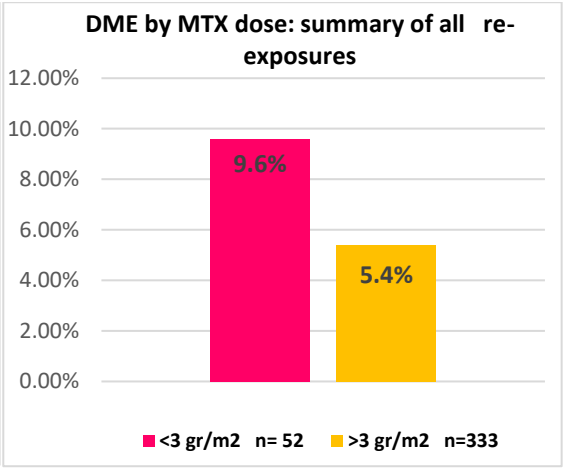
Fig S7b

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.