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ORIGINAL ARTICLE

A prospective study of a simple algorithm to individually dose high-dose methotrexate for children with leukemia at risk for methotrexate toxicities

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Abstract

Purpose High-dose methotrexate (HDMTX) is critical to the successful treatment of pediatric acute lymphoblastic leukemia (ALL) but can cause significant toxicities. This study prospectively evaluated the effectiveness of a fixed algorithm which requires no real-time pharmacokinetic modeling and no previous patient exposure to HDMTX, to individualize HDMTX dosing for at-risk patients with the aim of avoiding methotrexate-related toxicities.

Methods We developed a simple algorithm to individualize HDMTX infusions with 0–2 rate adjustments based on methotrexate levels during the infusion. This was a prospective, open-label, study; eligible patients were identified and referred by their oncologist.

Results Fifty-four evaluable cycles of HDMTX (5 g/m^2 over 24 h) were administered to 22 patients. Blood samples were obtained in 21 patients to examine single nucleotide polymorphisms (SNPs) related to methotrexate disposition. Twelve (54.5%) subjects had a history of previous HDMTX toxicities including seven (31.8%) who previously required glucarpidase rescue and seven (31.8%) with an entry glomerular filtration rate < 80 ml/min/1.73 m^2. 107/110 (97.2%) of methotrexate levels were drawn properly and 100% of algorithm dosing instructions were performed correctly at the bedside. Thirty-five (64.8%) of all cycles and 24 of 33 (72.7%) cycles that required a dose-adjustment had an end 24-h methotrexate level (Cp_{ss}) within our goal range of 65 ± 15 µM with only 3 (5.6%) resulting in Cp_{ss} higher than goal. Grade 3/4 toxicities were rare; no patients developed > Grade 1 acute kidney injury.

Conclusion This algorithm is a simple, safe and effective method for individualizing HDMTX in pediatric patients with ALL.

Clinicaltrials.gov Registry NCT02076997.

Keywords Methotrexate · Children · Pharmacokinetics · Toxicity · Renal insufficiency

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Abbreviations

ABCC4	ATP-binding cassette sub-family C member 4
ALL	Acute lymphoblastic leukemia
AKI	Acute kidney injury
ALT	Alanine aminotransaminase
AST	Aspartate aminotransaminase
AUC	Area under the plasma concentration–time curve
BUN	Blood urea nitrogen
CBC	Complete blood count
CI	Confidence interval
COG	Children's Oncology Group
Cp_{ss}	Concentration in plasma at steady state
CTCAE	National Cancer Institute common terminology criteria for adverse events
FDA	US Food and Drug Administration

GFR	Glomerular filtration rate
$GPDG_2$	Carboxypeptidase G2
HDMTX	High-dose methotrexate
IQR	Interquartile range
MAF	Minor allele frequency
MTHFR	Methylenetetrahydrofolate reductase
MTRR	Methionine synthase reductase
NSAIDS	Non-steroidal anti-inflammatory drugs
PdL	Ponte di Legno consensus criteria
PK	Pharmacokinetics
SD	Standard deviation
SNP	Single nucleotide polymorphism
SLCO1B1	Solute carrier organic anion transporter family member 1B1

Introduction

Methotrexate is a critical component of the successful treatment of many hematologic, solid and central nervous system tumors [1]. High-dose (often defined as ≥ 1 g/m^2) methotrexate (HDMTX) is particularly important in the treatment of T-cell and high-risk and relapsed B-cell acute lymphoblastic leukemia (ALL), for which it is usually given as a 5 g/m^2 infusion over 24 h [2–5]. It has been well established that severity of methotrexate-related toxicities are directly proportional to the duration of methotrexate exposure [6–8] and therefore, while lower doses and shorter durations of methotrexate are typically well tolerated, HDMTX requires aggressive supportive care [8] with systemic hydration and urine alkalization, avoidance of concomitant medications which may interfere with methotrexate clearance, administration of post-dose rescue leucovorin and therapeutic drug monitoring. These now routine requirements during HDMTX infusions are designed to aid in avoiding abnormally high plasma and intracellular methotrexate concentrations which are known to lead to drug-related toxicities [8].

Even with supportive care, some patients receiving HDMTX experience significant toxicities [9]. Importantly, between 2 and 19% of patients will experience acute kidney injury (AKI) as a result of HDMTX [9, 10]. This high-risk group of patients has been shown to have a mortality rate of 4.4%, greater than the mortality rate of 0.8% among patients who did not develop HDMTX-induced renal dysfunction [11].

Life-threatening methotrexate-induced AKI has historically been treated with extracorporeal techniques. These interventions are limited by equivocal success, extensive resource utilization, patient morbidity and post-dialysis methotrexate concentration rebound [9, 12–14]. More recently, when the AKI is being caused by toxic methotrexate plasma concentrations in patients with delayed methotrexate clearance, the drug glucarpidase (Voraxaze®) can and has be utilized. Glucarpidase, which immediately inactivates methotrexate by hydrolyzing the terminal glutamate from naturally occurring folates, has been proven to be a safe and effective [15–17] but is almost prohibitively expensive [18]. Treatment of other methotrexate adverse events such as myelosuppression, neurotoxicity, mucositis and hepatotoxicity is almost entirely based on supportive care [9]. It is clear that prevention—rather than the treatment—of methotrexate toxicities is the most critical component of safe HDMTX administration.

With the exception of AKI, and perhaps Hispanic ethnicity [19], individual patient-related risk factors for impaired methotrexate clearance have been difficult to identify [20, 21]. Methotrexate clearance parameters vary as much as sevenfold among and even within patients receiving a similar fixed dose [22]. These phenomena, coupled with the importance of HDMTX in ALL, prompted investigators to explore methods of HDMTX personalization.

The most current widely adopted standard of HDMTX personalization occurs after HDMTX-related toxicities have occurred and consists of consideration between a fixed dose-reduction—usually in the range of 20–25%—or complete omission of future planned HDMTX cycles [23]. Since these methods have significant drawbacks, investigators have attempted to better individualize HDMTX. These protocols have demonstrated enormous improvement over the current standard. However, this work has been limited by logistical complexities (including the need for real-time complex pharmacokinetic modeling), applicability (such as the requirements for patients to have documented previous exposure and PK modeling to HDMTX and for patients to have normal renal function) and varying degrees of success [24, 25]. Based on prior work [25, 26], we developed a safety method of individualizing HDMTX which could be applied to patients at the bedside; which eliminated the need for complex, real-time PK calculations; allowed for the phenomenon that past methotrexate clearance does not predict future kinetics [24]; and which included patients with previous toxicities to methotrexate, including concurrent poor renal function. The aims of this pilot study were to evaluate the effectiveness and feasibility of this algorithm, as well as to explore potential clinical and pharmacogenetic characteristics that may be associated with individual success of this dosing method.

Materials and methods

Patient eligibility

Patients ≥ 1 year and < 23 years of age with any malignancy for which they were to receive HDMTX given as a 5 g/m^2 infusion over 24 h and had at least one of four additional

criteria were eligible. These four additional criteria were as follows: (1) a documented decrease in renal function as defined by a creatinine greater than 1.5× their baseline or a glomerular filtration rate (GFR) of < 65 ml/min/1.73 m^2; (2) a history of prior nephrotoxicity after receiving HDMTX as evidenced by an increase in creatinine to ≥ 1.5× baseline or the need for dialysis or glucarpidase; (3) a history of ≥ Grade 3 adverse event related to HDMTX (i.e. mucositis, myelosuppression, nephrotoxicity, hepatotoxicity) based on the NIH Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 [27]; (4) provider concern that the patient is at risk for MTX toxicity, examples of such could be: as a prior history of treatment with nephrotoxic chemotherapy, a history of HDMTX-related neurotoxicity or concomitant antimicrobial/antifungal therapy. Patients were excluded if there was the inability to draw labs appropriately for HDMTX plasma concentrations, if the patient was enrolled concurrently on a protocol which restricted the utilization of the study treatment intervention (i.e., individualizing the methotrexate dosing), if the patient had Trisomy 21, if the patient had > Grade 1 neurologic toxicity at the time of enrollment that was attributed to unresolved prior methotrexate toxicity and/or if patients had ≥ Grade 3 chronic kidney disease (GFR or creatinine clearance < 30 ml/min/1.73 m^2). A nuclear medicine GFR study was required for all patients to be done within 28 days of the first dose of study HDMTX. This trial was approved by the Institutional Review Board of Baylor College of Medicine (Houston, TX), conducted in accordance with the standards documented in the Declaration of Helsinki and registered with Clinicaltrials.gov as NCT02076997. Written informed consent and assent, as appropriate, were obtained in accordance with federal and institutional guidelines.

Study design

The primary purpose of this single arm study was to estimate the incidence of achieving a peripheral blood concentration of methotrexate at 24 h of 50–80 µM (65 ± 15 µM) in patients at high risk of drug toxicity, after a 24 h infusion of HDMTX, treated according to our institutional treatment algorithm for individualized methotrexate dosing. Once an eligible patient was enrolled and received their first dose of study HDMTX, if their primary oncologist assigned treatment protocol included additional doses of HDMTX, the subject could be "re-enrolled" on study to receive subsequent doses of study HDMTX as long as they continued to meet original eligibility criteria. Assuming a target success rate of 80%, we estimated that 50 evaluable infusions would provide a 95% confidence interval width of 22% (i.e. ± 11%). Methotrexate-related adverse events were collected to describe the safety of administering HDMTX per our algorithm. Feasibility of the algorithm was determined by the number of patients successfully being able to complete protocol therapy and the rate of errors encountered following the prescribed algorithm. While the protocol originally intended that the primary outcome would be the first infusion for each patient, and subsequent infusions would be considered repeated measures, experience with methotrexate pharmacokinetics in general and correlation analysis of data in this study indicate that outcomes of repeated infusions are uncorrelated [22, 24] and can reasonably be viewed as independent observations. Therefore, the sample size and analysis presented here are primarily based on the infusion as the experimental unit.

Drug administration

Five grams per meter squared of methotrexate was to be initially ordered and given as a 24-h continuous infusion as commonly administered in Children's Oncology Group (COG) protocols [3]. Briefly, after restricted medications were discontinued, all patients received intravenous (IV) hydration with D5 ¼ NS with $NaHCO_3$ 3 mEq/100 ml at 200 ml/m^2/h until urine pH ≥ 7.0 and ≤ 8.0 and urine specific gravity ≤ 1.010. Once parameters were met, patients received methotrexate 500 mg/m^2 in D5 ¼ NS with $NaHCO_3$ 3 mEq/100 ml (total volume 200 ml/m^2) IV over 60 min. This was followed immediately by methotrexate 4500 mg/m^2 in D5 ¼ NS with $NaHCO_3$ 3 mEq/100 ml (total volume 4600 ml/m^2) IV at 200 ml/m^2/h over 23 h. Post-methotrexate hydration and leucovorin rescue were administered and adjusted per published guidelines [8].

Study evaluations

During the 28-day screening, a patient history, physical examination and laboratory studies as well as a nuclear medicine GFR were obtained. Patients additionally consenting had peripheral blood obtained for single nucleotide polymorphism (SNP) analysis. During the methotrexate infusion, a plasma creatinine and methotrexate level were obtained at hours 2, 6–8 (depending on the hour 2 intervention), 24 (end-infusion), 36, 42, 48 and thereafter according to provider discretion. A physical exam, collection of vital signs and basic laboratory assessments were then collected within 12 h of the start of the HDMTX infusion and thereafter, at least weekly until 4 weeks from the infusion or the next infusion whichever occurred first.

Methotrexate concentration assessment and pharmacokinetic analysis

Measurements of methotrexate concentrations were performed within 2 h of collection in the clinical pathology laboratories at Texas Children's Hospital (Houston, TX).

Peripheral blood plasma was assessed using the Methotrexate II assay (Abbott Laboratories, Abbott Park, IL) which utilizes Fluorescence Polarization Immunoassay (FPIA) technology. The area under the concentration–time curve (AUC)—using the cubic spline method—was calculated for each administration of HDMTX using Stata SE v 15 pksumm (StataCorp LLC, College Station, TX) from time $0 \rightarrow \infty$ using concentrations collected over the first 48 h of the infusion.

Dose adjustment algorithm

Our algorithm (Fig. 1) has been previously described [28]. Briefly, adjustments were made to the methotrexate infusion rate based on a static, pre-determined, universal algorithm based on methotrexate levels drawn at hours 2 (safety check) and either 6 or 8 (presumed steady state)—depending on the hour 2 intervention. Adjustments were made targeting a Cp_{ss} of 65 µM ± 15 µM. Secondary to the fact that this was a safety algorithm, only decreases in the infusion rate (dose) were made. Therefore, algorithm adjustments were calculated based on the formula: $K_{0(\text{new})} = [C_{\text{ss(desired)}}/C_{\text{ss(measured)}}] \times k_{0(\text{original})}$ where K_0 is the rate of drug infusion and C_{ss} is the plasma concentration at steady state [29].

Toxicity assessment

Patient-specific toxicity data were collected and graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.03 [27] unless otherwise noted. HDMTX-induced severe nephrotoxicity and methotrexate-related stroke-like syndrome was assessed using the pediatric-specific Pointe de Legno (PdL) criteria [30].

Single nucleotide polymorphism (SNP) analysis

In consenting/assenting patients, 5–10 ml of peripheral blood was collected at the initiation of the first methotrexate infusion. Mononuclear cells were separated from whole blood using Ficoll-Hypaque centrifugation. Germline DNA was isolated from the enriched mononuclear cells using the QIAgen (Venlo, Netherlands) DNeasy kit per the manufacturer's instructions. Taqman SNP genotyping assays (Applied Biosystems, Foster City, CA) were utilized according to manufacturer's instructions to genotype the following SNPs: rs4149056 (*SLCO1B1*), rs1801133 and rs1801131 (*MTHFR*), rs7317112 (*ABCC4*), and rs1801394 (*MTRR*). Genotypes were categorized as homozygous for the major allele, heterozygous, or homozygous for the minor allele. The minor allele for each SNP was calculated within the study population and compared with several standard ancestral populations from the International HapMap Project.

Statistical analysis

Descriptive statistics were performed using mean and standard deviation (SD), median and interquartile range (IQR) as appropriate. Kendall's Tau was used to assess the correlation between a subject's 24-h Cp_{ss} during cycle 1 and their Cp_{ss} during cycle 2. Outcomes were compared between two groups for categorical variables using the Chi-square test or Fisher's exact test as appropriate. For continuous variables, the *t* test or Wilcoxon rank sum test was used. Unless otherwise specified, two-sided statistical tests were used and a *P* less than 0.05 was considered statistically significant. All analyses were conducted using SAS 9.4 (SAS Institute Inc. Cary, NC) and R version 3.4.1. Static patient characteristics were analyzed based on the number of enrolled eligible subjects as the sample size while analyses of cycles were analyzed assuming each cycle as an independent observation with the sample size, therefore, being the total number of cycles completed which were eligible for analysis.

Results

Patient characteristics

Between February 20, 2014 and November 20, 2106, 23 patients were enrolled on study; these patients received a total of 55 cycles of HDMTX. While inclusion criteria allowed inclusion of patients with any malignancy who were to receive a 5 g/m^2 infusion of methotrexate over 24 h, only leukemia patients ultimately enrolled. One patient, for his single HDMTX infusion, was errantly ordered to have a pre-infusion dose-reduction and therefore, both the subject and the subject's cycle were ineligible for evaluation (see CONSORT diagram, Supplemental Figure S1). Eligible subject ($n = 22$) demographics are described in Table 1. These subjects received a total of 54 eligible HDMTX cycles. The median age of subjects at the time of enrollment was 13 years old (IQR 9–15 years). Ninety-one percent ($n = 20$) of subjects had pre-B ALL. One subject had relapsed disease at the time of enrollment. While the median subject GFR at the time of screening of 98.5 ml/min/1.73 m^2 (IQR 78–118 ml/min/1.73 m^2) was within the normal range for children,[31] ten (45%) subjects had screening GFRs below the normal range and two (9%) met the acute kidney insufficiency definition of being at risk of renal dysfunction by the modified RIFLE criteria [32]. Subjects were enrolled for a variety of reasons, most commonly for a history of delayed MTX clearance or renal insufficiency. Twelve (54.5%) subjects entered the study with a history of previous significant methotrexate toxicities. Individual subject reasons for enrollment and pre-study methotrexate toxicity history are described in detail in Supplemental Table S1.

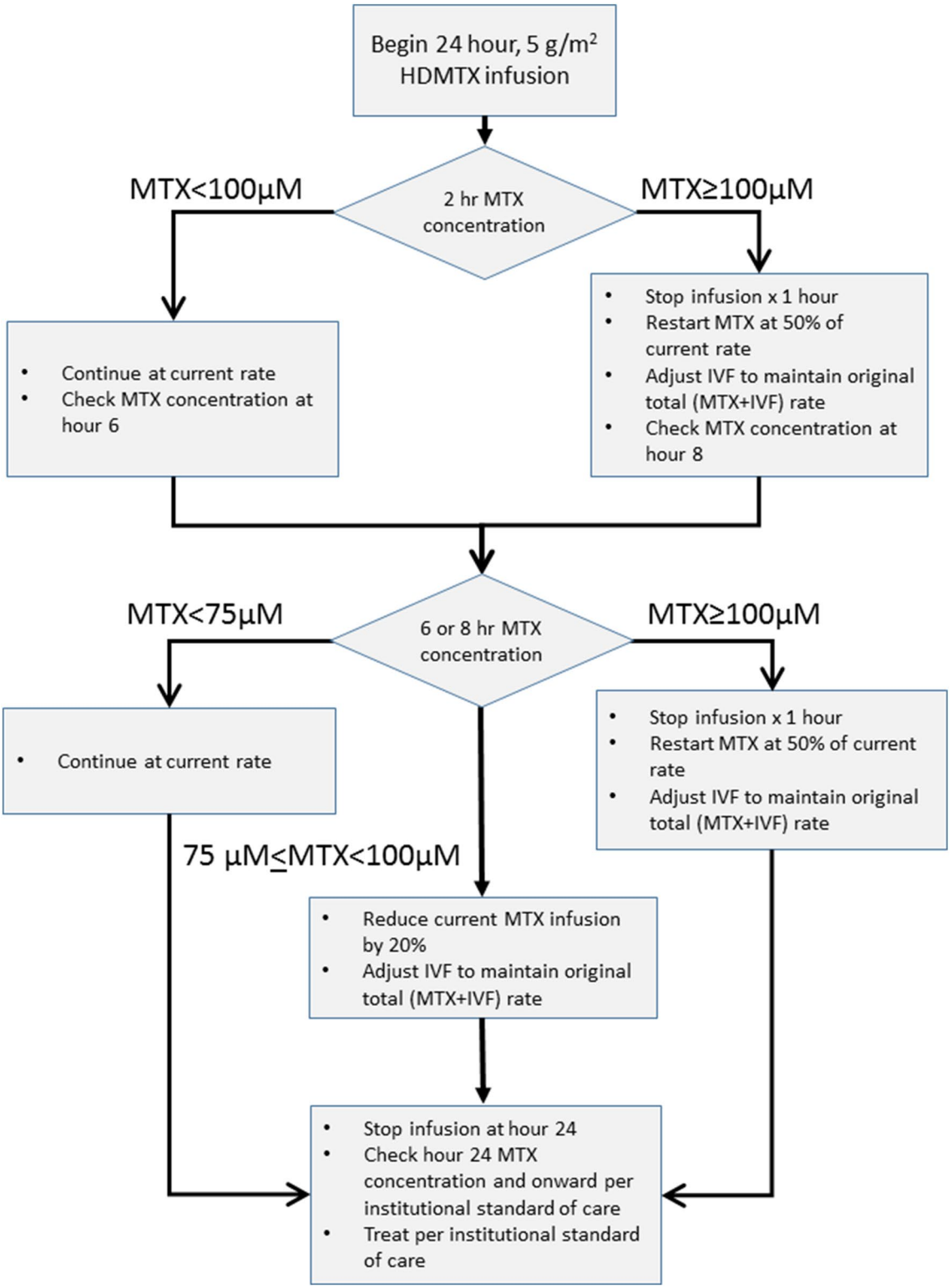

Fig. 1 Protocol algorithm. *MTX* methotrexate, *IVF* intravenous fluids

Table 1 Eligible subject demographics

	All subjects	Any Dose adjustment	
	Total ($n=22$)	N ($n=8$)	Y ($n=14$)
Age, median (IQR)	13 (1–19)	8 (1–16)	13.5 (8–19)
Sex			
Female, *n*	9 (41%)	2 (25%)	7 (50%)
Male, *n*	13 (59%)	6 (75%)	7 (50%)
Race			
Black, *n*	1 (4.5%)	0 (0%)	1 (7.1%)
Unknown, *n*	1 (4.5%)	0 (0%)	1 (7.1%)
White, *n*	20 (91%)	8 (100%)	12 (86%)
Ethnicity			
Hispanic, *n*	16 (73%)	6 (75%)	10 (71%)
Not Hispanic, *n*	6 (27%)	2 (25%)	4 (29%)
Cycles given, *n*	54 (100%)	14 (25%)	40 (74%)
# of cycles per patient, median (IQR)	2.5 (1–4)	1.5 (1–4)	3 (1–4)
GFR (ml/min/1.73 m^2), median (IQR)	98.5 (67–167)	111 (73–167)	84.5 (67–130)
ALL type			
T-cell ALL, *n*	2 (9.1%)	1 (13%)	1 (7.1%)
preB ALL, *n*	20 (91%)	7 (88%)	13 (93%)
Relapsed disease at time of enrollment?			
No, *n*	21 (95%)	7 (88%)	14 (100%)
Yes, *n*	1 (4.5%)	1 (13%)	0 (0%)

Table 2 Key outcomes of $n=54$ subject-cycles

	Total ($n=54$)	Dose-adjusted cycles		p value
		N ($n=21$)	Y ($n=33$)	
Total MTX given, g/m^2, median (IQR)	4.5 (4.2–5)	5 (5–5)	4.3 (3.8–4.5)	< 0.0001
Cp_{ss} (μM) (MTX 24 h), mean (SD)	57 (14)	54 (16)	61 (12)	0.085
Time to clearance[a], h, median (IQR)	48 (48–84)	48 (48–60)	48 (48–84)	0.199
Cleared at 48 h, *n*	33 (61%)	15 (71%)	18 (55%)	0.215
AUC (μM × h/l), median (IQR)	2022 (992–2883)	1646 (992–2507)	2141 (1741–2883)	< 0.0001
Cycle delay[b], *n*	20 (63%)	6 (55%)	14 (67%)	0.501
Any grade hematological toxicity, *n*	47 (87%)	18 (86%)	29 (88%)	0.817
Grade 3/4 hematological toxicity, *n*	39 (72%)	17 (81%)	22 (67%)	0.253
Any grade non hematological toxicity, *n*	38 (70%)	15 (71%)	23 (70%)	0.892
Grade 3/4 non hematological toxicity, *n*	16 (30%)	6 (29%)	10 (30%)	0.892
Any grade AKI, *n*	2 (3.7%)	0 (0%)	2 (6%)	0.516
Grade 3/4 AKI, *n*	0 (0%)	0 (0%)	0 (0%)	

[a]Time to clearance defined as time from the start of the methotrexate infusion until the methotrexate plasma concentration is < 0.4 μM at 48 h or is < 0.1 μM thereafter, whichever occurs first

[b]Cycle delay is defined as the inability to start the next cycle of HDMTX secondary to toxicities unresolved from the previous HDMTX cycle as determined by the provider

Algorithm outcomes

Major algorithm outcomes are described in Table 2. Consistent with the literature [22, 24], in patients who were on protocol therapy for ≥ 2 cycles ($n=17$), there was no correlation between a subject's 24-h Cp_{ss} during cycle 1 and their Cp_{ss} during cycle 2 (Kendall's Tau = −0.02, $P=0.93>0.05$, Supplemental Figure S2). The mean 24-h Cp_{ss} of evaluable infusions on protocol was 57 μM (SD 14 μM). Of the 54 infusions, 35 [63%, 95% confidence interval (CI) 50–75%] were within our goal range of 50–80 μM—outside of our goal of 80% of cycles having a 24-h Cp_{ss} within our target

range. Three infusions (5.6%) ended with a Cp_{ss} higher than the goal. Among dose-adjusted infusions ($n=33$), 24 (72%, 95% CI: 56–85%) were within the goal range and only one (3%) was higher than the goal. Seventeen of the 54 total cycles (31%) and eight of the 33 dose-adjusted cycles (24%) had a Cp_{ss} lower than the goal, with the lowest Cp_{ss} overall being 27.4 µM and the lowest dose-adjusted Cp_{ss} being 33.8 µM.

A majority of subjects ($n=14$, 63.6%) and infusion cycles ($n=33$, 61.1%) required at least one dose adjustment and nine (40.1%) subjects needed a dose adjustment on all infusions. Five (22.7%) subjects had a mixture of adjusted and non-adjusted cycles. While most adjusted cycles required only a single modification, one infusion required dose adjustments based on both hour 2 and hour 8 levels. Non-dose adjusted cycles provided 5 g/m^2 of methotrexate per infusion, while the median dose of methotrexate administered during dose adjusted cycles was 4.3 g/m^2 (IQR 3.8–4.5 g/m^2). In the end, despite receiving a lower administered dose of methotrexate ($p<0.001$), because of the effect of early high concentrations, the mean AUC of dose-adjusted cycles was significantly greater than that in non-dose adjusted cycles (2190 µM × h/l vs. 1696 µM × h/l, $p<0.001$). However, dose adjustment generally collapsed concentration–time curves and there was no significant difference in the end 24-h Cp_{ss} levels between dose adjusted and non-dose adjusted cycles (61 µM vs. 54 µM, $p=0.21$) (Fig. 2 and Supplemental Figure S3).

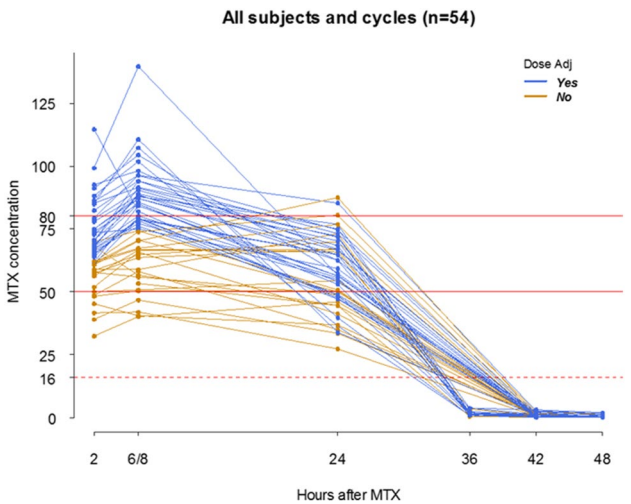

Fig. 2 Plasma methotrexate concentrations over time for each cycle. Blue lines represent cycles during which a dose adjustment was made at any time in the methotrexate administration rate per protocol and straw lines represent those infusions during which no dose adjustments were made. Goal end 24 h Cp_{ss} range is identified by solid red lines at 50 µM and 80 µM. The dotted red line at 16 µM represents the Cp_{ss} if one falls below which, is known to increase the risk of relapse

Toxicities

Toxicities experienced by subjects on our protocol were generally expected for those receiving HDMTX (Table 2). Twenty of the 32 (63%) cycles, which ended with the expectation that the subject would receive a subsequent cycle of HDMTX, required a delayed start (median 7 days, range 7–14 days) for toxicity recovery. A majority of cycles resulted in higher-grade (Grade 3 and 4) hematologic toxicities. However, a minority of cycles resulted in higher-grade non-hematologic toxicities. Five cycles (9.3%) resulted in Grade ≥ 3 mucositis. Ten cycles (18.5%) resulted in ≥ Grade 3 hepatotoxicity—defined as ≥ Grade 3 AST, ALT and/or bilirubin. A single subject cycle (1.9%) resulted in a Grade 3 neurotoxicity of stroke-like syndrome. This subject's toxicity fully resolved within 30 days of completing HDMTX therapy. No subjects experienced higher-grade AKI. Only two subjects experienced and two cycles (3.7%) resulted in any grade AKI (both of which were Grade 1 and met the PdL definition of HDMTX-induced severe nephrotoxicity, but both of which fully resolved within 30 days of completing HDMTX therapy). Both subjects who did experience Grade 1 AKI on our protocol had a history of glucarpidase use with previous HDMTX.

Re-challenging a patient after they have required glucarpidase rescue from HDMTX is an area of interest and research. A recent comprehensive review of outcomes of such a re-challenge was published by Christiansen, et al. and noted that while it was generally safe to do so, only a single patient out of 20 studied received and tolerated a full 5 g/m^2 of methotrexate after requiring glucarpidase previously; of the other patients, seven were not re-challenged (two secondary to not requiring further HDMTX and five at the discretion of the provider), nine were ordered a pre-infusion dose reduction of between 50–75% by provider discretion and the final three received intra-infusion dose reductions of 66, 32 and 25%, respectively [33]. In our study, seven subjects (31.8% of eligible subjects) had a previous history of glucarpidase requirements with HDMTX therapy prior to enrollment. Details comparing these subjects' pre- and post- protocol enrollment HDMTX experience are detailed in Table 3. These seven subjects received 18 HDMTX cycles on protocol therapy. Their median pre-protocol HDMTX GFR was 78 ml/min/1.73 m^2 (range 67 ml/min/1.73 m^2 to 130 ml/min/1.73 m^2, Table 1). They received a mean methotrexate dose of 4.1 g/m^2 (range 2.6–4.7 g/m^2), had a mean 24-h Cp_{ss} of 62.8 µM (range 33.79–85.4 µM) and had a median AUC exposure of 2320 µM × h/l (range 1961–2882 µM × h/l). Twelve (66.7%) cycles cleared "on time" (at 48 h post-start of infusion) with a median clearance of 48 h (range 48–186 h). On protocol therapy, these cycles had a median peri-treatment creatinine increase of 6% (range 0–51%) with 16 (88.9%) cycles resulting in no

Table 3 Comparison for $n = 7$ subjects between pre-enrollment HDMTX cycles which required glucarpidase use (G) for toxicity rescue and post-enrollment HDMTX cycles on protocol therapy (P)

Subject (ID)	1 (6)		2 (12)		3 (15)		4 (17)		5 (19)		6 (20)		7 (23)	
Cycle type	G	P	G	P	G	P	G	P	G	P	G	P	G	P
Number of cycles	1	3	1	2	1	2	1	3	1	3	1	2	1	3
Number of dose adjustments during cycle	N/A	1 (1)	N/A	1 (1)	N/A	1 (1)	N/A	1 (1)	N/A	1 (1–2)	N/A	1 (1)	N/A	1 (1)
Total MTX delivered/cycle (g/m^2)	5	4.08 (3.8–4.15)	5	4.1 (4-4.2)	5	4.35 (4.3–4.4)	5	4.6 (4.5–4.7)	5	3.4 (2.6–3.5)	5	3.9 (3.4–4.4)	5	3.9 (2.4–4.4)
% Delivered MTX dose decrease	N/A	18 (17–24)	N/A	18 (16–20)	N/A	13 (12–14)	N/A	8 (7–10)	N/A	32 (30–48)	N/A	22 (12–32)	N/A	22 (12–52)
24 h Cp_{ss}	219	67 (54.9–74.8)	140.3	71 (56.5–85.4)	129.9	66.2 (59.3–73.2)	148.9	66.4 (56.3–68.5)	167	56.8 (47.6–57.3)	94.2	52.8 (33.8–72)	140.8	70.4 (39.7–74.9)
Creatinine increase (% from baseline)	223	6 (0–50)	125	16 (8–23)	228	6 (3–9)	207	0 (0–4)	150	4 (0–10)	200	29 (7–51)	176	9 (0–25)
Time to MTX clearance (hrs)	264	48 (48–84)	133	117 (48–186)	132	84 (84)	267	48 (48)	96	48 (48)	292	66 (48–84)	240	48 (48–96)
PICU admission	Y	N	N	N	N	N	N	N	N	N	N	N	N	N
Non-hematological grade 3/4 toxicities	N, V, ↓K, ↓Ca, E, ↑T	↑T (3 cycles)	N, V, F/N	None	N, A	M (1 cycle)	None	None	M	None	↑T	↑T (1 cycle)	↑T	↑T (1 cycle)

All numbers are mean and range

ID subject study identification number, *G* glucarpidase cycles, *P* cycles on study protocol therapy, *PICU* pediatric intensive care unit, *Y* yes, *N* no, *N/A* not applicable, *N* nausea, *V* vomiting, ↓*K* hypokalemia, ↓*Ca* hypocalcemia, *E* encephalopathy, ↑*T* increased transaminases, *F/N* febrile neutropenia, *A* anorexia, *M* mucositis

AKI and two (11.1%) cycles resulting in Grade 1 AKI and HDMTX-induced severe nephrotoxicity. No other major unexpected (and very few Grade 3 and 4) toxicities were experienced during any of the cycles. These minor toxicities are in stark comparison to toxicities experienced prior to treatment on study during cycles which required glucarpidase. During glucarpidase, subjects' median Cp_{ss} was 140.8 μM (range 94.2–219 μM); their median peri-treatment creatinine increase was 200% (range 125–228%) and their median time to clearance was 240 h (range 96–292 h) resulting in many serious (Grade 3 and 4) toxicities including significant electrolyte disturbances, nausea, vomiting, anorexia, mucositis and hepatotoxicity.

SNP analysis

Leukemia-free peripheral blood was collected on 21 of the 22 eligible patients for SNP analysis (Supplemental Figure S1) of five SNPs known from the literature to be critical in methotrexate metabolism and previously described as being associated with methotrexate toxicity (Supplementary Table 2). Analysis was performed on five SNPs previously described as important in methotrexate metabolism, in order if they might be associated either with previous significant (Grade 3 or 4) toxicities from HDMTX or if they may be associated with obtaining the most benefit from participation in our protocol. Overall, there was no significant association between minor allele frequency and prior HDMTX toxicity or benefit from dose modification for any SNPs analyzed. However, among subjects who required at least one dose adjustment on study ($n=13$), those who carried one or two copies of the minor allele of the C677T methylenetetrahydrofolate reductase (MTHFR) gene appeared to be more likely to have "success" on this protocol than those who did not carry a minor allele (OR 6.7, 95% CI 0.49–91.3), when "success" was defined as a subject who had an end 24-h Cp_{ss} between 50 and 80 μM in > 50% of their cycles on protocol therapy.

Discussion

Despite not meeting our primary statistical goal of at least 80% of cycles having a 24 h Cp_{ss} of 65 μM ± 15 μM, our study evaluating the ability of our bedside algorithm to individualize the dose of HDMTX administered as a 24-h infusion, showed that patients with a high-risk of HDMTX toxicity can safely, easily and effectively receive HDMTX per our protocol. Were they not enrolled on our protocol, these patients would have either received a fixed dose reduction or would have had the drug omitted from their treatment plan entirely if consortium guidelines/generally prescribed standards of care were followed [23].

While occasionally effective, fixed dose reductions and omissions do not allow for a patient to receive the benefits of a maximally tolerated dose of HDMTX and further, do not ensure that a patient receives a small enough dose of methotrexate to be safe. For these reasons, investigators at St. Jude Children's Research Hospital (SJCRH) have been exploring patient-specific HDMTX personalization. In SJCRH Therapy for Relapsed/Refractory ALL Study 15 (R15), investigators used a real-time two-compartment model coupled to a Bayesian algorithm to adjust the ordered 5 g/m^2 methotrexate infusion rates of 24 children with relapsed ALL. Infusion rates were adjusted up or down at hours 1 and 6 of a 24-h infusion to a target Cp_{ss} of 65 ± 10 μM (the predicted Cp_{ss} of 5 g/m^2 of methotrexate infused over 24 h in patients with normal renal function and an average creatinine clearance of 103 ml/min/m^2) [25]. During the study, they adjusted 58% of their infusions, resulting in a final administered dose of between 2.854 and 6.7 g/m^2 with no major toxicities noted and a mean Cp_{ss} of 68 μM [25]. While highly effective, this method required real-time, complex pharmacokinetic calculations and communication to the provider. In SJCRH Total Therapy for ALL Study XV (TTXV), investigators used the same PK modeling used in R15 to order individualized doses of HDMTX based on a patient's past methotrexate clearance [24]. While the authors noted that this approach reduced the number of extreme Cp_{ss} values and reduced delayed excretion, they also noted that only 63% of their patients had Cp_{ss} values of 65 μM ± 20% and that this was no different than conventional dosing methods ($p=0.43$) [24].

Our algorithm attempted to treat at-risk patients, eliminate the need for real-time PK analysis and the need to understand a patient's past methotrexate clearance parameters while also accounting for the observation that a patient's past methotrexate clearance does not precisely predict their future elimination kinetics [22, 24]. Other published studies have utilized 65 μM ± 10 μM [25] or 65 μM ± 20% (65 μM ± 13 μM) [24]. We chose 65 μM ± 15 μM to account for our expected sample size and to the fact that our study was a safety study, and therefore, a slightly more liberal range with no different clinical significance was considered appropriate. Despite the fact that methotrexate pharmacokinetics is usually described using either a 2 [24, 25] or 3 [6] compartment model, we presumed that during a continuous infusion, a single compartment model could be approximated and that at steady state, the plasma concentration is directly proportional to the rate of administration presuming the clearance is unchanged [29].

Data from this study continued to demonstrate tremendous intra-patient methotrexate clearance variability with no correlation noted between cycle 1 and cycle 2 Cp_{ss} in subjects receiving ≥ 1 cycle on our protocol (Kendall's Tau = −0.02, $p=0.93$, Supplemental Figure S1). In our study, 72.7% of dose-adjusted infusions were in the target

range of 65 μM ± 15 μM. Of note, when including infusions that did not require a dose adjustment, 63% (95% CI 50–75%) of infusions were in the target range despite the fact that only adjustments downward were made. Only three infusions (5.6%) ended with a Cp_{ss} higher than goal, with no Cp_{ss} higher than 87.67 μM—well below any toxic range. In addition, the lowest overall Cp_{ss} was 27.4 μM with the lowest dose-adjusted Cp_{ss} being 33.8 μM—both well above the 16 μM mark known to be a risk factor for relapsed disease [34, 35].

Our algorithm was logistically practical and simple to follow. During our study, 97.2% (107/110) of the required blood draws were completed properly (Supplemental Figure S4). Furthermore, there were no errors in algorithm directed dose adjustments (Supplemental Figure S4), ultimately leading to the ability to provide a median of 90% of the ordered dose of 5 g/m^2 to high-risk patients, including those with significant previous toxicities (including the need for glucarpidase) and renal insufficiency. This is 20% more methotrexate (median 4.4 g/m^2 vs. 3.75 g/m^2) than would be administered with a simple fixed dose reduction of 25% as recommended by cooperative group studies. In addition, even in those who were dose reduced, the AUC was equal or higher than what is expected from a normal patient getting 5 g/m^2 which should eliminate the fear that using our protocol would potentially "under-dose" patients who met criteria for intra-infusion dose-adjustments. From a clinical pharmacology perspective, this finding also underscores the generally poor relationship between dose administered and exposure achieved in children receiving HDMTX and augments the argument that HDMTX should be given using a method that targets a cycle-specific AUC rather than one which uses a static pre-determined dose to be infused.

The patients on our study were able to safely receive HDMTX with minimal toxicity. The median clearance was "on-time" at 48 h, cycle delays were infrequent, and Grade ≥ 3 hematologic and non-hematologic toxicities were minimal and within expected ranges. Previous reports of pediatric leukemia protocols have noted a Grade ≥ 3 HDMTX-induced hepatotoxicity incidence between 20 and 27% [36, 37], comparable to our occurrence of 18.5%. Similar reports of Grade ≥ 3 mucositis note an incidence between 9 and 13% [3, 10], comparable to our occurrence of 9.2%; and reports of Grade ≥ 3 HDMTX-induced neurotoxicity note an incidence between 4 and 7% [38, 39], comparable to our occurrence of 1.9%. Of note, despite enrolling subjects with poor renal function, none of the patients on our study, including those with past history of severe HDMTX-induced AKI, experienced ≥ grade 3 renal toxicity. The only renal toxicity noted was grade 1 AKI seen in 3.7% patient-cycles. This low incidence and low-Grade AKI is in stark contrast and is far superior to other reports of HDMTX-induced AKI in pediatric leukemia patients which note incidences of Grade ≥ 1, Grade ≥ 2 and Grade ≥ 3 of 12–18.5% [9, 10], 8% [23] and 1.8–3.6% [8, 40] respectively.

Our study did not identify any demographic risk factors which would potentially identify patients who would most benefit from the administration of this algorithm. However, in an exploratory analysis of subjects who required at least one dose adjustment on study ($n = 13$), those who carried one or two copies of the minor allele of the C677T *MTHFR* gene appeared to be more likely to have "success" on this protocol than those who did not carry a minor allele when "success" was defined as a subject who had an end 24-h Cp_{ss} between 50 and 80 μM in > 50% of their cycles on protocol therapy. Given our small sample size on this pilot trial, these results were not statistically significant; however, the strong suggested effect size indicates that this SNP may play a role in HDMTX effectiveness in this population. The minor allele C677T *MTHFR* SNPs is known to confer a *MTHFR* enzyme with lower activity than its wild-type counterpart [41, 42]. As such, while the exact biological effect is not completely known [43], there have been associations reported between those who carry the minor allele and a higher 24-h methotrexate level in children with ALL [44]. It is logical that those who are genetically susceptible to less efficient clearance of methotrexate would benefit most from an individualizing protocol such as ours. However, this concept would have to be confirmed in a larger study powered to answer such a question.

The limitations of this study revolved around the fact that it was carried out at a single institution with a small number of patients. While inclusion criteria allowed inclusion of any patient receiving 5 g/m^2 of methotrexate over 24 h, only leukemia patients enrolled, and therefore, these results should not be generalized to children with other disease states. In addition, to treat patients according to our algorithm, an institution must have the capability to run methotrexate levels in real-time and get the results back within 2 h. Furthermore, although the study was powered to answer our primary question, larger studies, potentially with modified goals, are needed to more precisely demonstrate the overall utility of this algorithm. A larger and longer multi-site study, which was able to enroll both leukemia and lymphoma patients, would be better positioned to analyze generalizability, could be powered to better analyze characteristics of patients (including the SNPs of such patients) who would be best suited to receive HDMTX using this algorithm and could follow patients long enough to interpret any decreases (or increases) in efficacy receiving HDMTX on this algorithm produced versus the current standard of care.

Conclusion

Our work produced a simple and safe bedside algorithm for individually adjusting 24-h HDMTX infusion rates based on real-time methotrexate concentrations at hours 2

(safety check) and 6–8 (steady state). The algorithm is an option for any practitioner to safely administer HDMTX to patients with a history of methotrexate toxicities or who are at risk for toxicities. Incorporation of this algorithm should be considered in future larger clinical trials for pediatric patients receiving HDMTX as a 24-h infusion.

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Compliance with ethical standards

Conflict of interest No authors have any conflicts of interest to report.

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