Individualized high dose methotrexate for the treatment of malignancies in children and adolescents with a significant risk for methotrexate toxicities

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3	Children's Hospital of Pennsylvania
4	Dana Farber Cancer Institute

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1. GOALS AND OBJECTIVES

1.1 Primary Aim

To determine the incidence of successfully achieving peripheral blood concentration of methotrexate of 50-80μM in patients at high risk of drug toxicity after a 24 hour infusion of high-dose methotrexate treated according to our institutional treatment algorithm for individualized methotrexate dosing.

1.2 Secondary Aims

1.2.1 To determine the incidence of the following side effects in high risk patients receiving high dose methotrexate as a 24 hour infusion: nephrotoxicity, neurotoxicity, mucositis, hepatotoxicity, and myelosuppression.

1.2.2 To describe host polymorphisms that may identify risk of methotrexate toxicity or success of this institutional protocol.

1.2.3 To describe predictors of successfully achieving an optimum concentration of methotrexate at the end of a 24 hour infusion of HDMTX in patients at high risk of drug toxicity treated with this individualized high dose methotrexate protocol.

1.3 Hypothesis

At least eighty percent of patients with a high risk for or past history of significant methotrexate toxicity, receiving 24-hour high-dose methotrexate infusions per this institutional algorithm for individualized dosing will have an appropriately therapeutic but minimally toxic 24 hour methotrexate level between 50-80μM.

2. BACKGROUND

Methotrexate is a crucial chemotherapeutic agent used to treat children with acute lymphoblastic leukemia (ALL), and is a staple of treatment regimens both nationally and internationally[1]. In fact, cure rates for ALL approach 80%, in part due to the incorporation of methotrexate into treatment protocols[1]. While all serum concentrations of methotrexate have shown to be efficacious in ALL therapy, when doses above 1g/m^2 – considered high dose methotrexate (HDMTX) - are given, several leukemic cell methotrexate resistance mechanisms are readily overcome[2]. Most current protocols using HDMTX, including those run by the Children's Oncology Group (COG) now utilize 5g/m^2 given as a 24 hour infusion in non-Down syndrome leukemia patients[3]. Many studies have demonstrated the clinical benefits of HDMTX[4;5], even when directly compared to lower doses of methotrexate[6]. Recent data from the National Cancer Institute (NCI) sponsored COG trial AALL0232 compared the effectiveness of HDMTX and 6-MP against Capizzi escalating methotrexate plus PEG asparaginase (C-MTX/ASNase) in patients less than 30 years old with high-risk ALL (HR-ALL). Initial analysis showed that patients randomized to receive HDMTX had significantly higher 5-year event-free survival (EFS) compared to patients who received C-MTX/ASNase (82+ 3.4% versus 75.4 + 3.6%, p=0.006)[7]. This finding lead to all patients with HR-ALL being treated with HDMTX.

However, treatment with HDMTX can be associated with significant toxicity. The risk of morbidity and mortality from methotrexate toxicity increases when the drug is given at high dose. Toxicities are directly correlated with increased drug exposure and since methotrexate is cleared renally, patients with kidney dysfunction are at particularly high risk of complications[8]. In patients treated with HDMTX, 1.8% will experience renal dysfunction[9]. Patients with relapsed ALL are at increased risk of methotrexate toxicity due to prior exposure to nephrotoxic chemotherapy, as are patients with renal injury. Per current COG protocols, patients with certain features indicating a high risk of toxicity to methotrexate will either have their methotrexate arbitrarily reduced by 25%, or it will be omitted from their treatment regimen entirely reducing or eliminating the important anti-leukemic effects of the drug.

2.1 Methotrexate Pharmacokinetics

The pharmacokinetics of methotrexate vary greatly among patients, with a fixed dose resulting in a 7-fold variation in the range of plasma concentration[5]. This variability can also exist in individual patients on subsequent administrations of HDMTX[5]. Therapeutic drug monitoring has thus become a standard part of protocols in patients receiving HDMTX.

Typically pharmacokinetics of HDMTX are described using a two compartment model with four parameters (CL – clearance, V1 – central compartment volume of distribution, V2 peripheral compartment volume of distribution, and Q – intercompartmental clearance). Clearance (CL) of MTX ranges from 3.5-7.1 L/h, and is inversely proportional to the age of the patient. Among patients with the same total bodyweight (TBW), MTX clearance was 30% higher in children less than 10 years when compared with children greater than 10 years of age. The volume of distribution of central compartment (V_1) is 45% of TBW regardless of age[10].

The variation in pharmacokinetics also has important clinical implications. Prospective pharmacodynamic studies of patients with ALL receiving HDMTX have shown that those patients with median MTX plasma concentration at steady state (Cp_{ss}) less than 16μM had significantly lower rates of remaining in remissions compared to patients with Cp_{ss} greater than 16μM[5].

2.2 Polymorphisms

High-dose methotrexate administered as a 24 hour infusion is a standard therapy for patients with leukemia, types of lymphoma, and other malignancies. Though thousands of patients receive the same treatment, only a small subset will develop methotrexate toxicity. This suggests that individual variations in methotrexate metabolism may contribute to the development of toxicities.

Previous studies have identified several functional polymorphisms in both the folate cycle and methotrexate transporters now thought to play a significant role in the large variation in pharmacokinetics, response, and toxicity seen in patients who receive HDMTX[11-13]. For example, the solute carrier 19A1 (SLC19A1, also known as reduced folate carrier 1, RLF1), is the main mechanism by which MTX enters the cell[14]. The expression of SLC19A1 correlates with intracellular MTX concentration. Variations in the SLC19A1 gene have been shown to impact event-free survival[15]. The most common single nucleotide polymorphism (SNP) in *SLC19A1* results in the substitution of Arg with His at position 27. Recent data have shown that this SNP is also associated with HDMTX toxicity[12].

Another example is found in individual variances in the enzyme methylenetetrahydrofolate reductase (MTHFR). Once MTX is transported into the cell, it is metabolized to polyglutamylated metabolites. These metabolites inhibit enzymes involved in the folic acid cycle, including MTHFR. MTHFR plays an important role in the folic acid cycle as it is responsible for catalyzing the reduction of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate[16]. A common cysteine to threonine substitution at *MTHFR* 677 results in lower enzyme activity and has been shown to be associated with decreased HDMTX clearance and increased toxicity[12]. SNPs associated with *SLCO1B1*, an organic anion transporter polypeptide, have also been shown be involved in MTX clearance[13].

2.3 Methotrexate Toxicity

While integral to improving the survival of patients with malignancy, HDMTX can also result in severe treatment-related complications. The main side-effects of HDMTX treatment include mucositis, hepatitis, renal dysfunction, prolonged myelosuppression, CNS toxicities such as seizures and stroke-like syndromes, and dermatitis[17;18]. In order to minimize these side effects, drug levels are closely monitored, and aggressive supportive care measures are standard practice. During HDMTX infusions, hyper-hydration and alkalization of urine promote efficient clearance of the drug. The tetrahydrofolic acid derivative leucovorin is given post-infusion to rescue normal DNA synthesis in non-leukemia cells minimizing myelosuppression[19]. Leucovorin dosing is typically based on post infusion methotrexate concentrations and increased for slow methotrexate clearance.

2.3.1 Nephrotoxicity

As methotrexate is excreted nearly exclusively by the kidneys, methotrexate induced nephrotoxicity is a serious potential complication of treatment[20]. The two main mechanisms of methotrexate induced nephrotoxicity involve damage to the renal tubules via either a direct toxic effect[21] or as a result of the precipitation of methotrexate metabolites[22;23]. The two main methotrexate metabolites, 7-hydroxy-methotrexate (7-OH-MTX) and 2,4-diamino-N^{10}-methylpteroic acid (DAMPA), are at least six times less soluble at acidic pH than methotrexate[22;24]. This decreased solubility prompted alkalinization of the urine to be a major component of methotrexate supportive care. In addition, alkalinization forces the ionization of methotrexate, a weak acid, making it more water soluble, preventing precipitation and trapping unchanged cleared methotrexate in urine for efficient elimination.

In patients receiving HDMTX, 1.8% will experience renal dysfunction resultant from HDMTX. This high risk group of patients has a mortality rate of 4.4%, greater than the mortality rate of 0.8% among patients who did not develop HDMTX induced renal dysfunction[9].

2.3.2 Neurotoxicity

Methotrexate toxicity can lead to severe neurological sequelae. Among children treated with methotrexate, 3-11% will experience either transient or chronic symptoms of neurotoxicity such as: seizures, hemiparesis, aphasia, headache, and altered mental status[25]. However, up to 76% of patients treated with HDMTX demonstrate leukoencephalopathic changes on magnetic resonance imaging (MRI)[26]. The prevalence of leukoencephalopathy increases with each subsequent course of HDMTX. Among patients who developed leukoencephalopathy following HDMTX, 10-56% had their symptoms recur when re-challenged with HDMTX[27-29].

2.3.3 Other side effects

Other common side effects of HDMTX include mucositis, hepatitis, and prolonged myelosuppression. These side effects can result in hospital admission for management of symptoms, as well as a delay in subsequent cycles of chemotherapy. Increased time between chemotherapy cycles may contribute to inferior outcomes[30;31]. Delayed clearance of MTX is thought to contribute to the development of these side effects, as the time of exposure to MTX is prolonged[19].

2.3.4 Management of Toxicity

As methotrexate is primarily cleared via renal excretion, patients with underlying renal dysfunction are at an increased risk of having delayed clearance. The elevated and prolonged exposure to methotrexate that results from delayed clearance makes patients more susceptible to developing other drug related complications such as mucositis and myelosuppression[32].

In an effort to minimize the nephrotoxic effect of HDMTX, patients concurrently receive leucovorin, hydration, and alkalinization[19]. If nephrotoxicity and delayed clearance develop despite these measures, two further options exist for lowering plasma methotrexate concentrations: dialysis and glucarbidase ($CPDG_2$). After dialysis plasma MTX concentrations decrease by a median of 52% (range 26-82%). Cases of rebound increases in MTX plasma concentrations by 10-221% have been reported. Patients are also subjected to the risks of dialysis including bleeding, infection, and electrolyte disturbances[8]. The total cost through hospital discharge for patients receiving dialysis can range from $94,000 to $140,000[33].

The enzyme $CPDG_2$, which hydrolyzes the terminal glutamate from naturally occurring folates and folate analogs such as methotrexate, is a well-tolerated alternative to lowering plasma MTX levels[3]. Within 15 minutes of administration, MTX plasma concentrations in patients with osteosarcoma, non-Hodgkin lymphoma, or ALL decreased by a median of 98.7% (range 95.6-99.6%). A rebound increase of 2.8-8.8% of the MTX concentration was seen in 60% of patients[9]. The drug cost of $CPDG_2$ for a 50kg patient is approximately $30,000, excluding costs associated with preparation, administration, and pharmacy overhead (personal communication, Brooke Bernhardt, PharmD, BCOP, COG pharmacist and clinical pharmacy specialist, Texas Children's Cancer Center). In addition, despite the fact that is recommended to consider CPDG2 in patients with sustained MTX concentrations of > 10 μM at 42-48 hours after the start of a MTX infusion, it is currently a highly regulated, difficult to obtain medication[8]. Although it is now commercially available, it requires special order from the manufacturer (http://www.btgplc.com/products/specialty-pharmaceuticals/voraxaze). In addition, minimal dosing is kept available by most tertiary care hospitals. Texas Children's Hospital, for example, keeps four 1000 unit vials on site. This is enough for one time dosing for an 80kg patient (personal communication, Amanda Berger, PharmD, Texas Children's Cancer Center).

There is currently no standardized method of administering HDMTX to patients at risk for increased methotrexate toxicity. As a result, these patients are at risk of having HDMTX removed from their treatment regimen or suffering severe morbidity from its side-effects. Current COG protocols suggest postponing and/or eventually omitting HDMTX administration for impaired renal function, grade 3-4 mucositis, hepatitis, and/or prolonged myelosuppression. In patients who experience and fully recover from these effects, the current COG recommendations are to reduce subsequent doses of HDMTX. As methotrexate metabolism varies widely among individuals, a fixed dose-reduction without respect for individual patient characteristics results in patients being at risk for either not reaching a therapeutic level of methotrexate or experiencing further drug-related side effects.

2.4 Rationale for Treatment Design

The incorporation of HDMTX into treatment regimens for pediatric patients with ALL has improved cure rates now approaching 80%. HDMTX has proven to be effective in treating ALL, regardless of prognostic factors such as age, immunophenotype, and genetics[1]. HDMTX is also effective at treating other malignancies such as Burkitt's lymphoma and mixed-phenotype leukemia, among others. Recent data from the NCI-sponsored COG clinical trial AALL0232 has shown that patients with HR-ALL have significantly improved EFS when treated with HDMTX/6-MP as compared to C-MTX/ASNase[7]. As a result of this finding, patients with HR-ALL are no longer randomized to treatment during Interim Maintenance. Instead, all patients who are able receive HDMTX during interim maintenance.

Every effort should thus be made to ensure that patients can safely receive HDMTX. As effective as it is in treating leukemia, administration of HDMTX can result in serious side effects such as renal dysfunction, neurotoxicity, mucositis, prolonged myelosuppression, hepatitis, and dermatitis. The risk of methotrexate toxicity is increased in patients with impaired renal function. Due to underlying medical conditions and prior cytotoxic chemotherapy, a subset of patients with ALL is at an increased risk of developing severe methotrexate-related toxicity. There is currently no standardized method of administering HDMTX to patients at risk in increased methotrexate toxicity. As a result, these patients are at risk of having HDMTX removed from their treatment regimen or suffering severe morbidity from its side-effects.

Prior studies, mostly at the St. Jude Children's Research Hospital (SJCRH) have shown that

individualized methotrexate protocols can be safe and effective[34;35]. Wall and colleagues used real-time complex pharmacokinetic modeling to dose-adjust HDMTX in the treatment of patients with relapsed ALL.[36]. The group demonstrated that patients receiving HDMTX have an average clearance of 103ml/min/m^2 [34], which corresponded to a steady-state plasma concentration of (Cp_{ss}) of 65μM ± 10μM at 24 hours[35]. In addition, 58% of their patients required dose-adjustments to achieve a Cp_{ss} within this range. None of their patients experienced severe methotrexate-related toxicity. They found that the initial methotrexate clearance was the best predictor of high methotrexate Cp_{ss}[36].

Based largely on the previous work at SJCRH, we developed an individualized methotrexate treatment plan as an option to safely administer HDMTX, with a target Cpss of 65 μM to patients at risk for delayed clearance and subsequent toxicity. Other options for this population would include a 25% methotrexate dose reduction or complete omission of HDMTX from their treatment. Since 2007, Texas Children's Hospital (TCH) has given patients with past history of toxicities including renal toxicities individualized methotrexate with no known increase in toxicities based on evolving versions of this treatment plan (n =14 patients; ref: personal communications with faculty members of the Texas Children's Hospital Leukemia Program). Work by Aumente, et al, demonstrated that the Monte Carlo simulated MTX concentration and means over time show a peak methotrexate concentration near hour 2, with steady state occurring near hour 6[10]. Therefore, our treatment plan provides guidelines for HDMTX dose adjustments based on hour 2 and 6 methotrexate levels. In addition, these adjustments are based on a fixed, simple bed-side algorithm and thus avoid the need for complex pharmacokinetic modeling. Patients treated according to the algorithm may thus not need their dose altered. With this in mind, in addition to our eligibility criteria we will allow attendings to enroll patients as they deem necessary, since if the MTX Cp_{ss} is therapeutic no alterations to therapy will be made. In September, 2012, this treatment plan was formalized and approved for clinical use by the Texas Children's Cancer Center Chemotherapy Safety Committee.

In addition, despite the fact that MTX pharmacokinetics, tumor response and patient toxicities are known to vary greatly and it is known that several germline polymorphisms play a role in this variation, much controversy about the functional consequences of these polymorphisms still exists[11]. Our study, will, by definition, isolate a population of patients with a known increased propensity for certain MTX toxicities. Because the general population minor allele frequency (MAF) is known for many of the polymorphisms known to affect MTX metabolism, this study will provide a unique opportunity to compare rates of certain of these polymorphisms in our study population to that of the general population. This may help elucidate which polymorphisms truly correlate to deficiencies in MTX metabolism. In addition, we may be able to discover which polymorphisms describe a population best suited for individualized MTX dosing.

Though many genetic targets in the folate cycle and intracellular MTX transportation have already been identified, new target genes continue to be discovered. Given that the knowledge base in this field is rapidly expanding, the decision of which polymorphisms to be tested will be determined at the time of analysis, when the study accrual has been met. Common SNPs currently thought to be involved in methotrexate metabolism, pharmacodynamics and toxicity are listed below:

Polymorphism	SNP effect	Approximate MAF (%)	Clinical effect	Reference
MTHFR 677C>T	Reduced activity	15-25	Both increased and decreased risk of myelosuppression reported	[37;38]
SLC19A1 80G>A	Reduced activity	30-50	Increased hepatotoxicity	[39]
MTHFR 1298A>C	Reduced activity	15-20	Decreased myelosuppression	[40]
TYMS 28-bp triple repeat	Increased transcription	40-50	None reported	[41]

This research does not qualify for an IND as the use of HDMTX is per standard of care treatment of ALL, and the route of administration or dosage level does not significantly increase risk to the patient.

3. STUDY ENROLLMENT AND PATIENT ELIGIBILITY

3.1 Study Enrollment

3.1.1 IRB approval

Approval for the use of this protocol by the individual institution's Human Subjects Review Committee must be obtained, in accordance with the institutional assurance policies of the U.S. Department of Health and Human Services.

3.1.2 Study Enrollment

Patients may be enrolled on study once all eligibility requirements for the study have been met. Patients will be enrolled by calling Kathy McCarthy at 832-824-4804 Mon-Fri from 9am-5pm central time. At enrollment patients will be assigned an individual identification number.

3.1.3 Timing

Study enrollment must take place prior to patients receiving methotrexate dose modifications.

3.2 Patient Eligibility Criteria

INCLUSION CRITERIA

3.2.1 Age

Patients must be ≥365 days and <23 years of age at the time of enrollment.

3.2.2 Diagnosis

Patients with any malignancy who will receive HDMTX given as a 5 g/m2 infusion over 24 hours and a history of ≥1 of the following:

- Documented decreased renal function, as defined as Creatinine greater than 1.5 x baseline or GFR <65ml/min/1.73m^2.
- History of prior nephrotoxicity with HDMTX as evidence by increased creatinine to 1.5 x baseline or need for dialysis or carboxypeptidase
- History of Grade 3 adverse event (AE) related to HDMTX (mucositis, myelosuppression, nephrotoxicity, hepatotoxicity) based on the NIH Common Terminology Criteria for Adverse Events (CTCAE) version 4.0
- Provider concern patient is at risk for MTX toxicity, such as a prior history of treatment with nephrotoxic chemotherapy, history of HDMTX-related neurotoxicity, or antimicrobial/antifungal therapy

EXCLUSION CRITERIA

3.2.3 Unable to draw labs for HDMTX serum concentration

3.2.4 Enrollment on a protocol (COG or other) which restricts proposed dose modifications

3.2.5 Patients with Trisomy 21

3.2.6 Patients with > grade 1 neurologic toxicity at the time of enrollment that is attributed to unresolved prior methotrexate toxicity

3.2.7 Patients with ≥ grade 3 chronic kidney disease at enrollment (eGFR or CrCl < 30ml/min/1.73m^2)

REGULATORY

3.2.8 Informed consent for participation in this study must be obtained within 30 days from the start of HDMTX on this protocol and prior to any research procedures from the patient and/or the patient's legally authorized guardian in accordance with institutional policies approved by the U.S. Department of Health and Human Services

3.2.9 All institutional, FDA, and NCI requirements for human studies must be met.

4. TREATMENT PLAN

4.1 Overview of Treatment Plan

Our prospective study will enroll patients who will receive HDMTX given as a 5g/m^2 24-hour infusion. Enrolled patients will be receiving HDMTX as determined by their primary provider. Patients are eligible to be enrolled at any time point during their treatment course, and may participate during any one or more of their multiple HDMTX administrations.

Concomitant Medication Restrictions:

1. **Sulfamethoxazole and Trimethoprim (SMX-TPM); synonyms: Bactrim, Co-trimoxazole**
2. **Non-steroidal anti-inflammatory medications (NSAIDS)**
3. **Penicillins**
4. **Proton pump inhibitors**
5. **Aspirin-containing medications**

Hold the above medications on the day of HDMTX infusion and for at least 72 hours after the start of the HDMTX infusion and until the MTX level is less than 0.4μM (In the presence of delayed clearance continue to hold these medications until MTX level is less than 0.1 μM):

As per the Children's Oncology Group (COG) and institutional guidelines, restricted medications should be stopped (i.e. 1. Sulfamethoxazole and Trimethoprim (SMX-TPM); synonyms: Bactrim, Co-trimoxazole; 2. Non-steroidal anti-inflammatory medications (NSAIDS); 3. Penicillins; 4. Proton pump inhibitors; 5. Aspirin-containing medications), and prior to receiving HDMTX patients will receive IV hydration with D5 ¼ NS with $NaHCO_3$ 3 mEq/100 mL at 200 mL/m^2/hr for a minimum of 2 hours, until urine pH ≥ 7.0 and ≤ 8.0 and urine specific gravity ≤1.010.

Once these parameters are met, between hours 0-1 of the protocol patients will receive methotrexate 500 mg/m^2 in D5 ¼ NS with $NaHCO_3$ 3 mEq/100 mL at 200 mL/ m^2 IV over 60 minutes. This will be followed immediately by Hours 1 to 24 Methotrexate 4500 mg/m^2 in D5 ¼ NS with $NaHCO_3$ 3 mEq/100 mL 4600 mL/ m^2 IV at 200 mL/m^2/hr for 23 hours.

Dose adjustments will be made based on the following tables. Adjustments should be made as soon as possible after MTX levels checked are resulted. Interventions are expected to be done within 120 minutes of sending the MTX level.

A. Interventions at 2 hours

If MTX level is:	**<100µM**	**≥100µM**
MTX administration intervention	·Continue unchanged	·Stop infusion for 1 hour ·At restart time; restart MTX at 50% reduction from original infusion rate; therefore, restart at 100 mL/m^2/hr
Fluid intervention	·Continue unchanged	·At restart time; begin D5 ¼ NS with NaHCO3 3 mEq/100 mL at 100 mL/m^2/hr)
MTX level specific lab instructions	·Check 6 hour MTX level and again at 24, 36, 42 and 48 hours per COG algorithm#	·Check 8 hour MTX level and then per the COG algorithm# at 24, 36, 42 and 48 hours*

B. Interventions at 6 hours (*8 hours if 2 hour level was ≥100µM)

If MTX level is:	**<75µM**	**≥75µM/<100µM**	**≥100µM**
MTX administration intervention	·Continue Unchanged	·Reduce MTX infusion 20% from **MOST RECENT** infusion rate; Therefore, if (select A OR B): A) 2 hr MTX level was <100 µM and therefore, infusion rate was 200 mL/m^2/hr then restart at 160 mL/m^2/hr B) 2 hr MTX level was ≥100 µM and therefore, infusion rate was 100 mL/m^2/hr then restart at 80 mL/m^2/hr	·Stop infusion for 1 hour ·At restart time; restart MTX at 50% reduction from **MOST RECENT** infusion rate; Therefore, if (select A OR B): A) 2 hr MTX level was <100 µM and therefore, infusion rate was 200 mL/m^2/hr then restart at 100 mL/m2/hr B) 2 hr MTX level was ≥100 µM and therefore, infusion rate was 100 mL/m^2/hr then restart at 50 mL/m^2/hr
Fluid intervention	·Continue unchanged	Select A OR B: A) If 2 hr MTX level was <100 µM and therefore, there was no previous additional fluid order then begin D5 ¼ NS with NaHCO3 3 mEq/100 mL at 40 mL/m^2/hr B) If 2 hr MTX level was ≥100 µM and therefore, additional D5 ¼ NS with NaHCO3 3 mEq/100 mL was already running at 63.5 mL/m^2/hr then increase this fluid to run at 120 mL/m^2/hr	Select A OR B A) If 2 hr MTX level was <100 µM and therefore, there was no previous additional fluid order then begin D5 ¼ NS with NaHCO3 3 mEq/100 mL at 100 mL/m^2/hr B) If 2 hr MTX level was ≥100 µM and therefore, additional D5 ¼ NS with NaHCO3 3 mEq/100 mL was already running at 100 mL/m^2/hr then increase this fluid to run at 150 mL/m^2/hr

MTX level specific lab instructions	·Check MTX level again at 24, 36, 42 and 48 hours per COG algorithm[#]	·Check MTX level again at 24, 36, 42 and 48 hours per COG algorithm[#]	·Check MTX level again at 24, 36, 42 and 48 hours per COG algorithm[#]

[#]see COG algorithm on page 21

Subjects will come off protocol therapy for infusion delays longer than 2 hours. In addition, the total methotrexate infusion time, on this study, should be no longer than 26 hours. Infusions should only be extended past 24 hours to accommodate stops for other med infusions. Increases in rate of MTX infusion to ensure completion within this time frame are **not** allowed.

The interventions in this protocol occur at the 2 and 6 hour (or 8 hour if the 2 hour level was ≥100μM) time points. After the 24hr HDMTX infusion is complete, the individualized protocol will end and patients will remain on study but be off of the protocol therapy. After this time, management of MTX levels will continue according the treating physician's discretion using standard supportive care guidelines. This is elaborated below in Appendix B, suggested supportive care guidelines based on the COG algorithm. Interventions for toxicity are at the discretion of the treating provider as patients will be off treatment protocol at the 24hr time point. Glucarpidase should be administered based on the treating physician's discretion, with the recommendation that it be administered prior to the development of grade 4 nephrotoxicity and prior to 96 hours after the start of the HDMTX infusion. Administration of $CPDG_2$ after these time points have been shown to be statistically associated with the development of ≥ grade 4 toxicity[3].

Patients may be re-enrolled on this protocol therapy again for subsequent HDMTX infusions without the need for re-consent. In order to re-enroll, call the study coordinating center as outlined in Section 3.1 PRIOR to the cycle in which the dose modification protocol will be implemented.

The active length of study participation for each patient is 4 weeks after the last high-dose methotrexate infusion or until the next cycle of chemotherapy begins (which ever occurs first). Therefore, the maximum amount of active time on study would be 2.5 months. This would include 4 doses of HDMTX given every other week plus a 4 week follow-up period to assess for side effects.

4.2 Study Endpoints

After the initial monitoring of MTX levels and creatinine during the MTX infusion, patients will be followed for the development of HDMTX related toxicity for 4 weeks after their infusion or until their next cycle of chemotherapy begins, whichever is shorter.

5. EVALUATIONS/MATERIAL AND DATA TO BE ACCESSIONED

At Study Entry

5.1 Physical Exam

5.2 Laboratory Tests Prior to Treatment

- A maximum of 28 days prior to the first dose of HDMTX on this protocol a nuclear medicine GFR must be performed.

- A maximum of 7 days prior to the first dose of HDMTX on this protocol the following labs will be collected (Week 0 labs): CBC, Chem 10, AST, ALT, total and direct bilirubin, and 10 mL in a purple top (minimum of 5mL will be accepted) of additional blood for MTX transportation genetic analysis. Sample for MTX transportation genetic analysis is optional.

5.3 Demographic information

Age, race, leukemia type, cytogenetics of leukemia, leukemia risk stratification, disease status, protocol treated according to, concomitant medication, reason for enrollment on protocol and type of adverse event with prior HDMTX.

5.4 Laboratory Tests During Treatment

- Methotrexate levels and creatinine at times 2, 6 (or 8) and 24 hours are required for research purposes. Additional methotrexate levels and creatinine should be performed per institutional standards. Methotrexate levels and creatinine at hours 36 (if methotrexate levels are ≥150 micromolar), 42, 48 and every 6-12 hours thereafter until methotrexate is considered cleared (e.g. ≤0.4 micromolar at hour 48 and/or <0.1 micromolar thereafter) is highly recommended but considered optional. An example of routine post-methotrexate monitoring is outlined in Appendix B.
- The following labs should be drawn at Week 1 and Week 2 and repeated weekly until either the next dose of HDMTX is administered or 4 weeks have passed since the last dose of HDMTX: CBC, Creatinine, BUN, AST, ALT, total and direct bilirubin.

5.5 Host Polymorphisms

The literature on SNPs influencing the pharmacokinetics and pharmacodynamics of methotrexate is rapidly evolving[11]. Based on current knowledge, at minimum we will assay each participant who consents for the SLC19A1 80G>A, TYMS 28-bp triple repeat, MTHFR 677C>T and MTHFR 1298A>C SNPs. In addition, technologies for genotype analysis are constantly changing and improving. However, for the above SNPs multiple technologies have been discovered and validated[12]. Our samples will be batched, and the most appropriate technique for genotyping at the time of analysis will be performed. Our collaborator, Dr. Donald W. (Will) Parsons has vast expertise in various genotyping methods including TaqMan, restriction enzyme, and Sanger sequencing.

5.5.1 Genotyping Methods

More than one genotyping technique may need to be used for different samples. Dr. Parsons' lab has experience in this field, and will be able to perform the necessary technique to achieve reproducibility in all samples. When choosing a method we will consider, cost, amount of DNA

needed, and availability of technique.

5.5.2 DNA Extraction and Distribution
Five to 10 mL of peripheral blood will be collected for genotyping purposes prior to the initiation of the methotrexate infusion, during the gathering of additional baseline laboratories. At this time additional baseline labs are routinely collected and peripheral blood mononuclear cells will be optimally present prior to methotrexate/chemotherapy induced myelosuppression. Mononuclear cells will be separated from whole blood using Ficoll-Hypaque centrifugation. DNA will be isolated from the enriched mononuclear cells using the QIAgen DNeasy kit per the manufacturer's instructions. To avoid DNA loss or degradation DNA samples will be stored in Axygen or comparable tubes. Based on prior experience with ALL samples, we expect an average of 18.5mcg of DNA per sample. Most current genotyping techniques require 5-20ng per assay.

5.6 Other information to be collected

- Ordered HDMTX dose
- date/time of 24hr infusion start
- initial HDMT infusion rate with HDMTX concentration
- date/time of each change in HDMTX infusion rate and rate at which new infusion is set (if applicable)
- date/time of 24hr infusion start and stop for concomitant medications
- date/time of completion of HDMTX infusion
- toxicities experienced during infusion,
- hour at completion of monitoring
- total MTX given
- total leucovorin given
- all MTX levels resulted and time each MTX level was drawn.

5.7 After Treatment Adverse effects

Toxicities will be assessed until the next cycle of chemotherapy is administered or until 4 weeks after the administration of HDMTX, whichever time period is shorter. Each grade $\geq$3 adverse event will be collected for the following methotrexate related toxicities: mucositis, myelosuppression, hepatotoxicity (AST, ALT, GGT, bilirubin). All adverse events will be collected for neurotoxicity and nephrotoxicity. If multiple occurrences of a toxicity take place during a single course, please list a separate Max Grade This Occurrence for each occurrence.

The International Conference on Harmonization (ICH) Guideline for Good Clinical Practice E6(R1) defines an adverse event (AE) as:

"Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product."

A Serious Adverse Event (SAE) is any AE that: 1. Results in death; 2. Is immediately life threatening; 3. Results in hospitalization – either initial or prolonged; 4. Results in persistent or significant disability/incapacity; 5. Results in congenital anomaly or birth defect; 6. Results in required intervention to prevent permanent impairment or damage or 7. Results in another serious

important medical event.

For the purposes of this study, it is not required to report SAEs which result in admissions to the hospital for simple febrile neutropenia or prolongation of a hospitalization secondary to delayed methotrexate clearance.

All SAEs should be identified and reported locally per institutional standards.

All unanticipated problems involving risks to subjects or others (UPIRSOs) should be identified and reported locally per institutional standards.

All reportable AEs and SAEs recorded during this study will be summarized. The incidence of treatment-emergent AEs (new or worsening from baseline) will be summarized by primary system organ class, severity based on CTCAE, version 4.0, type of adverse event and relationship to methotrexate. Deaths reportable as a Serious Adverse Event (SAE) and non-fatal SAEs will be listed by patient and tabulated by primary system organ class and type of adverse event. Any other information collected (e.g. start/end dates and duration of AE, severity or relatedness to methotrexate) will be listed as appropriate.

All reportable SAEs and UPIRSOs should be reported to the Principal Investigator Jennifer Foster at jhfoster@txch.org and the Research Coordinator Kathy McCarthy at ksmccart@texaschildrens.org within 5 business days.

5.8 Shipping of samples

Submit materials to:

Linna Zhang
1102 Bates St, Suite 1220.07
Houston, TX 77030
Ph 832-824-4592

6. DRUG INFORMATION

See the consent document for toxicities. All other information is available on the COG website in the manual titled —Drug Information for Commercial Agents used by the Children‘s Oncology Group https://members.childrensoncologygroup.org/prot/reference_materials.asp under Standard Sections for Protocols. Also see drug package inserts for added information on toxicities.

7. CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

7.1 Removal from Protocol Therapy

7.1.1 ≥ Grade 3 renal or neurologic toxicity

7.1.2 Parent/patient or provider request

7.1.3 Inability to analyze methotrexate levels at given time points. Patients will come off of study secondary to the inability to make dose modification decisions if methotrexate levels at required time points are not available within 120 minutes of drawing the level. If this occurs, treatment decisions will be up to the treating physician. Options would include: discontinuing the methotrexate infusion, altogether or calculating a 25% total dose reduction (as suggested by COG) and stopping the infusion when the total dose given reaches the newly calculated reduced dose.

7.1.4 Methotrexate infusion interrupted for longer than 2 hours.

7.2 Off Study Criteria

7.2.1 Lost to follow-up

7.2.2 4 weeks after the end of methotrexate infusion or start of next chemotherapy, which ever is shorter.

7.2.3 Death

8. Data Monitoring

Texas Children's Cancer Center, where we will be conducting our research, is part of the Dan L. Duncan Cancer Center (DLDCC). The NCI-designated Dan L. Duncan Cancer Center at Baylor College of Medicine is a consortium made up of several institutions across the Texas Medical Center. As part of the DLDCC, we will have access to various shared resources, including a Clinical Trials Support Unit. For our data monitoring, we will utilize the Data Safety and Monitoring Committee (DSMC) through the DLDCC.

8.1. Stopping rules

8.1.1. Two patients with HDMTX induced nephropathy necessitating dialysis or administration of carboxypeptidase.
8.1.2. Two patients achieving a 24 hour Cp_{ss} of <20μM.
8.1.3. Two patients needing intensive care admission for HDMTX related toxicities.
8.1.4. Any death attributed to HDMTX toxicity.

If any of the above criteria are met then the study will be stopped, the incident(s) will be reviewed, and the study will be amended to address the incident(s) prior to potentially re-opening.

9. Statistical Considerations

9.1 Statistical Design

Aim 1 1a) Calculate the incidence of success with the new protocol (achieving an end infusion

peripheral blood MTX concentration between 50-80 μM)

1b) Explore potential changes in incidence of success (achieving an end infusion peripheral blood MTX concentration between 50-80 μM) between sequential administrations of HDMTX. The primary endpoint and sample size calculation refer to the first cycle for each patient.

Aim 2 2a) Calculate the frequency of adverse effects after each infusion. Success rate over multiple cycles within individual patients will be analyzed using generalized estimating equations for binary outcomes. This method will also be applied to the frequency of adverse events and to the assessment of factors associated with outcome. Continuous and ordinal/non-normal adverse effects will be compared via paired t-test or Wilcox sign rank test respectively.

2b) Describe the genotype of various methotrexate metabolizing SNPs in each subject. The polymorphisms of subjects in this study will be compared to published minor allele frequencies and when appropriate will be stratified by race/ethnicity. The purpose of this analysis will be to describe trends away from the major allele frequency in patients known to either have experienced or who are at high risk for developing adverse effects to HDMTX.

2c) Examine predictors of success of achieving a CP_{ss} of 50-80 μM. Variables in our analysis will include (but not be limited to) age, sex, disease type, race/ethnicity and measure of pre-MTX renal function. An analysis both with and without SNP genotype will be performed secondary to the likelihood that race/SNP correlations will interfere with SNPs being a truly independent variable. We will initially use chi-square test for group variables and t-test for continuous variables. Logistic regression will be used for multivariable analysis to control for confounders and estimation of adjusted odds ratios. $P<0.05$ will be considered statistically significant.

9.2 Patient Accrual and Expected Duration of Trial

Patients will be continually accrued until a target of 50 methotrexate infusions requiring any protocol-prescribed dose adjustments are reached. The expected duration of accrual for the trial is 2-2.5 years.

9.3 Statistical Analysis Methods

Sample size with power justification:

Sample size is estimated on primary study objective of estimating the true rate of success with the new protocol. Success is defined as 80% of infusions achieving 24hr HDMTX end infusion MTX values of 50-80μM. A sample size of 50 methotrexate infusions per the algorithm produces a two-sided 95% confidence interval with a width equal to 0.22 when the sample proportion is 80%. We will be 95% confident that the true success rate in the study would be between 69% and 91%.

Analysis:
Aim 1a) Overall change in the incidence of success with the new protocol (achieving end infusion MTX levels 50-80μM) and

1b) change in incidence of success at various infusion times during interim maintenance and interim maintenance-like cycles in which patients are administered HDMTX every 2 weeks, as well as

1c) Success rate over multiple cycles within individual patients will be analyzed using generalized estimating equations for binary outcomes. This method will also be applied to the frequency of adverse events and to the assessment of factors associated with outcome. Continuous and ordinal/non-normal adverse effects will be compared via paired t-test or wilcox sign rank test respectively.

Aim 2) To examine predictors of success (achieving CP_{ss} of 50-80μM) we will initially use chi-square

test for grouped variable and t-test for continuous variables. Logistic regression will be used for multivariable analysis to control for confounders and estimation of adjusted odds ratios. P<.05 will be considered statistically significant.

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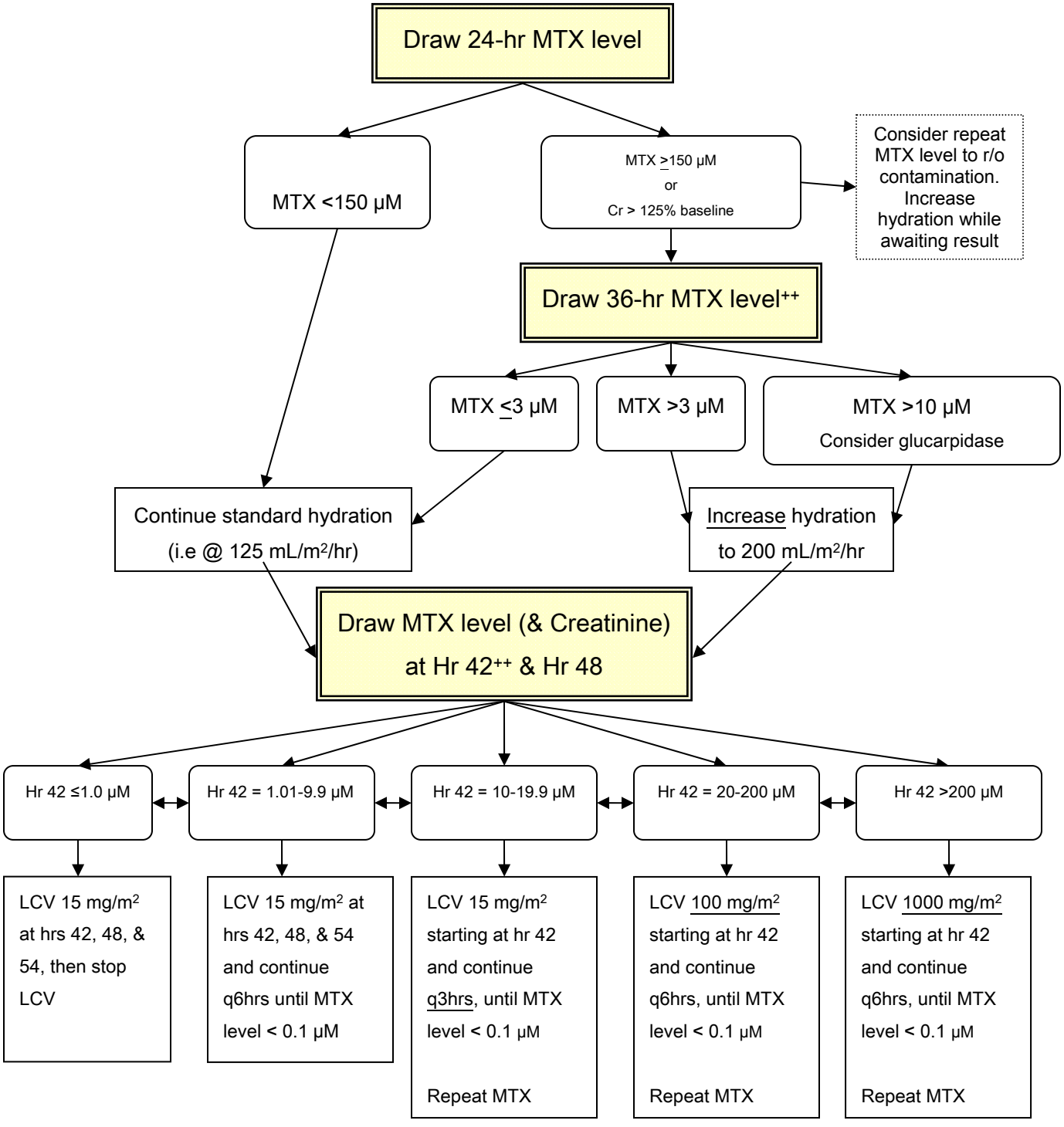
APPENDIX A

Schedule of Assessments											
		Hours after methotrexate infusion starts (Week 0)							Weeks		
Procedures	**S**	**0**	**2**	**6** [1]	**24**	**36‡**	**42‡**	**48**[2]**‡**	**1**	**2**[3]	**Protocol section**
Blood for SNP analysis		X									5.5.2
Nuclear eGFR	X										5.2
CBC with diff and platelets		X							X	X	5.2
BUN		X							X	X	5.2
AST/ALT/T&D* bilirubin		X							X	X	5.2, 5.4
Creatinine		X	X	X	X	X	X	X	X	X	5.4
Methotrexate level			X	X	X	X	X	X			4.1

S: Screening; Week 0: Week of high dose methotrexate administration; [1]Hour 6 or 8 depending on 2 hour methotrexate level (Sec 4.1); ‡ highly recommended but optional; should be done per institutional standards of post-methotrexate monitoring; [2] Continue 48 hour labs per algorithm (recommended but optional) (See appendix B); [3] Repeat Week 2 labs weekly until the next dose of methotrexate or 30 days since last dose of methotrexate; *T&D: Total and Direct

APPENDIX B



++ If the level is high at hour 36 or 42, but then the patient "catches up" and the level falls to the expected values of **≤1.0** and/or **≤ 0.4 µM** at hours 42 and 48, respectively, resume standard leucovorin and hydration as long as urine output remains satisfactory.

Suggested post HDMTX infusion guidelines based on standard COG guidelines.

36 hr MTX level (if needed)+	**42 hr MTX level**	**48 hr MTX level**	**Hydration and leucovorin rescue**
>3 µM			• Increase hydration to 200 mL/m^2/hr.
>10 µM			• Increase hydration to 200 mL/m^2/hr. • Consider glucarpidase
	≤1.0 µM	≤ 0.4 µM	• No further leucovorin after Hr 54, no further MTX levels after Hr 48
	1.01-9.9 µM	0.41-5.9 µM	• Continue leucovorin at 15 mg/m^2 q 6 hours after Hr 54 until plasma MTX is < 0.1 µM • Repeat MTX levels Q 12 hrs
	10-19.9 µM	6-9.9 µM	• Leucovorin at 15 mg/m^2 at Hr 42 then continue Q 3 hours until plasma MTX is < 0.1 µM • Repeat MTX levels Q 12 hrs • Consider glucarpidase
	20-200 µM	10-100 µM	• Leucovorin at 100 mg/m^2 at Hr 42 then continue q 6 hours until plasma MTX is < 0.1 µM • Repeat MTX level Q 12 hrs • Consider glucarpidase
	>200 µM	>100 µM	• Leucovorin at 1000 mg/m^2 at hr 42 and continue q 6 hours until plasma MTX is < 0.1 µM • Repeat MTX level Q 12 hrs • Consider glucarpidase

Concomitant Medication Restrictions:

1. **Sulfamethoxazole and Trimethoprim (SMX-TPM); synonyms: Bactrim, Co-trimoxazole**
2. **Non-steroidal anti-inflammatory medications (NSAIDS)**
3. **Penicillins**
4. **Proton pump inhibitors**
5. **Aspirin-containing medications**

Hold the above medications on the day of HDMTX infusion and for at least 72 hours after the start of the HDMTX infusion and until the MTX level is less than 0.4µM (In the presence of delayed clearance continue to hold these medications until MTX level is less than 0.1 µM):