Shortening Infusion Time for High-Dose Methotrexate Alters Antileukemic Effects: A Randomized Prospective Clinical Trial

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A B S T R A C T

Purpose

To determine whether shortening the infusion duration of high-dose methotrexate (HDMTX; 1 g/m²) affects the in vivo accumulation of active methotrexate polyglutamates (MTXPG₁₋₇) in leukemia cells and whether this differs among major acute lymphoblastic leukemia (ALL) subtypes.

Methods

From June 2000 through October 2007, 356 children with ALL were randomly assigned to receive initial single-agent treatment with HDMTX (1 g/m²) as either a 24-hour infusion or a 4-hour infusion at two pediatric hospitals in the United States. The primary outcome measures were the accumulation of MTXPG₁₋₇ in leukemia cells and the antileukemic effects (eg, inhibition of de novo purine synthesis in bone marrow ALL cells, and decrease in circulating ALL cells).

Results

The 24-hour infusion resulted in significantly higher amounts of MTXPG₁₋₇ in bone marrow leukemia cells (median: 1,695 v 1,150 pmol/10⁹ cells, $P = .0059$), and better antileukemic effects. The 24-hour infusion had the greatest effect on MTXPG₁₋₇ accumulation in hyperdiploid ALL (median: 3,919 v 2,417 pmol/10⁹ cells, $P = .0038$); T-cell ALL exhibited smaller differences in MTXPG₁₋₇ but greater antileukemic effects with the longer infusion (median decrease in leukemia cells: 88.4% v 51.8%, $P = .0075$). In contrast, infusion duration had no significant impact on MTXPG₁₋₇ accumulation or antileukemic effects in ALL with the t(12;21)/(ETV6-RUNX1) chromosomal translocation.

Conclusion

Shortening the infusion time of HDMTX reduces accumulation of active methotrexate in leukemia cells and decreases antileukemic effects, with differing consequences among major ALL subtypes.

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INTRODUCTION

Cure rates for childhood acute lymphoblastic leukemia (ALL), the most common cancer in children, currently exceed 80%, yet more effective and less toxic therapy is needed because the number of children who are not cured of ALL exceeds the number of newly diagnosed cases of most types of childhood cancer.¹ High-dose methotrexate (HDMTX; 1 g/m² to 8 g/m²) is an important component of curative treatment of ALL (Data Supplement).²⁻⁶ The in vivo accumulation of active methotrexate polyglutamates (MTXPG₁₋₇) in leukemia cells is associated with methotrexate's antileukemic effects and has been a valuable end point to evaluate alternative therapeutic strategies.⁷⁻⁹ Prior studies have provided insights into the molecular mechanisms that underlie differ-

ences in MTXPG₁₋₇ accumulation in leukemia cells of different ALL subtypes.¹⁰⁻¹³

Strategies to overcome interpatient differences in MTXPG₁₋₇ accumulation have focused largely on altering the dose or duration of infusion of HDMTX. Indeed, it has been shown that higher doses of methotrexate can overcome the poor accumulation of MTXPG₁₋₇ in T-lineage leukemia cells, and this has become the standard of care for children with T-lineage ALL.^{8,14,15} The effect of HDMTX infusion duration has never been studied in patients with ALL. It has been suggested that HDMTX infused over 4 hours is less toxic than HDMTX infused over a longer time (ie, 24 to 36 hours) in patients with relapsed ALL or lymphomas.¹⁶⁻¹⁸ However, there have been no studies to determine whether shortening the duration of HDMTX infusions to

make treatment less toxic, would translate into suboptimal delivery of active methotrexate in leukemia cells or whether this would alter methotrexate's antileukemic effects. Furthermore, it is not clear whether altering methotrexate infusion time would have similar consequences for various major ALL subtypes.

In this prospective randomized clinical trial, our primary aim was to determine whether shortening the infusion duration of HDMTX (1 g/m²) affects the in vivo accumulation of MTXPG₁₋₇ in leukemia cells and whether this alters the antileukemic effects of methotrexate. To enhance generalizability of our findings, HDMTX was given as a single agent before the start of the conventional chemotherapy to patients with newly diagnosed ALL.

METHODS

Clinical Trial Design

Children 1 to 18 years of age, newly diagnosed with ALL and treated at St Jude Children's Research Hospital or Cook Children's Medical Center between 2000 and 2007, were enrolled on the St Jude total XV protocol.¹⁹ The diagnosis of ALL was made by immunophenotyping and by molecular and cytogenetic analyses.²⁰ For secondary analyses, patients were divided into five major ALL-subtypes based on genetic and immunophenotypic characteristics: T-lineage or B-lineage ALL with hyperdiploidy more than 50 chromosomes (B-hyperdiploid), with the t(12;21)/(ETV6-RUNX1) chromosomal translocation (also known as *TEL-AML1*), the t(1;19)/(TCF3-PBX1) translocation, or with none of these chromosomal aberrations (B-other).

Patients were randomly assigned to receive open-label preinduction therapy with intravenous HDMTX (1 g/m²) as either a 4-hour constant infusion or a 24-hour infusion (200 mg/m² over 5 minutes then 800 mg/m² over the next 23 hours 55 minutes). Intravenous leucovorin (50 mg/m²) was initiated 44 hours after start of methotrexate and repeated every 6 hours for 7 doses (15 mg/m² each). To ensure adequate hydration and urine alkalization (pH ≥ 6.5), intravenous saline with 5% dextrose and 40 mEq NaHCO₃/L was infused (200 mL/m²/h) for at least 2 hours before the HDMTX treatment and continued (100 mL/m²/h) for at least 24 hours after the MTX infusion; NaHCO₃ was given as needed to maintain a urine pH between 6.5 and 8.0. Patients with very high WBC counts were treated with urate oxidase to prevent tumor lysis syndrome and allopurinol was only used if patients had a history of allergy or G6PD deficiency. Standard remission induction therapy was not initiated until 3 days after start of the HDMTX infusion. The other components of the Total XV protocol have been previously described in detail.¹⁹ During treatment consolidation, all patients received four doses of HDMTX, 2.5 g/m² in the low-risk and 5 g/m² in the standard- and high-risk arms, with each dose infused over 24 hours and adjusted to achieve a steady-state plasma concentration of 33 μmol/L and 65 μmol/L, respectively, in the treatment arms. The protocol was approved by the institutional review board at St Jude Children's Research Hospital and at Cook Children's Hospital. Written informed consent and assent were obtained before treatment according to the Helsinki declaration. Patients with Down's syndrome or pre-existing renal failure and those who had received any antileukemic therapy before referral were ineligible for preinduction therapy with HDMTX. Because allopurinol inhibits the de novo purine synthesis, patients treated with allopurinol before or during the methotrexate infusion were excluded from the analysis of antileukemic effects. The CONSORT statement checklist (Data Supplement) and full treatment protocol (Data Supplement) are in the Data Supplement.

Plasma Pharmacokinetics of Methotrexate

Peripheral blood was drawn at 1, 4, 24, and 42 hours after start of the methotrexate infusion and concentrations of methotrexate in plasma were measured by a fluorescence polarization immunoassay (Abbott, TDxFLx System, Chicago, IL). Pharmacokinetic parameters were estimated using a two-compartment first-order model with the ADAPT II software, as previously described^{21,22}; pertinent parameters included the total area under the curve

from start to 42 hours after the infusion ($AUC_{(0 \text{ hours}-42 \text{ hours})}$), methotrexate clearance (mL/min per m²), and duration of exposure above 1 μmol/L.

Assessment of MTXPG₁₋₇ in Leukemia Cells

Bone marrow samples were obtained at diagnosis and 42 hours after start of the HDMTX infusion, immediately before leucovorin rescue. The intracellular concentrations of MTXPG₁₋₇ (pmol per 10⁹ cells) were measured in leukemia cells from the 42-hour bone marrow sample and peripheral blood as previously described (Text S3).^{8,23,24} Methotrexate, which contains one glutamate residue, is designated MTXPG₁.

Measurement of De Novo Purine Synthesis

De novo purine synthesis (DNPS) was measured in bone marrow ALL cells, as previously described.²⁵ Percent change in DNPS from pretreatment to 42 hours after start of the methotrexate infusion was calculated as $100 \times (DNPS_{42H} - DNPS_{pre})/DNPS_{pre}$. The percentage of ALL cells in S phase was determined as previously described.⁹

Measurement of Antileukemic Effects

Circulating leukemia cells in peripheral blood were measured as WBC ($\times 10^9$ cells/L) immediately before the methotrexate infusion (WBC_{pre}) and 3 days after ($WBC_{day 3}$). The change in WBC was evaluable in 320 patients and the antileukemic response was calculated by two methods. First, the difference ($WBC_{\Delta day 3}$) between the expected and the actual drop in WBC at day 3 was determined by calculating the residual of the linear regression model generated from the association between $\log_{10}[(WBC_{pre})]$ and $\log_{10}[(WBC_{day 3})]$, as previously described (Data Supplement).⁹ This resulted in the following equation: $WBC_{\Delta day 3} = \log_{10}(WBC_{day 3}) - 0.4869 \times \log_{10}(WBC_{pre}) - 0.0285$. Second, the percent change in WBC from pretreatment to day 3 was calculated as $100 \times (WBC_{day 3} - WBC_{pre})/(WBC_{pre})$.

Random Assignment

Patients were enrolled and assigned to random assignment by pharmacists at St Jude Children's Research Hospital and Cook Children's Hospital using a computer software system which generated a block randomization scheme with a block size of 6. The random assignment was stratified according to ALL lineage (T- v B lineage ALL) and ploidy (hyperdiploid v nonhyperdiploid B-lineage ALL). The trial was open label.

Statistical Analyses

Sample size estimations for the primary end point, accumulation of MTXPG₁₋₇ in bone marrow leukemia cells, were based on pharmacokinetic data from our previous protocol (Total Therapy Study XIIIa) where children with ALL received HDMTX 1 g/m² infused over 24 hours.⁸ The study exceeded the planned sample sizes (randomly assigning 162, 42, and 28 patients in each subgroup) required to provide 90%, 79%, and 77% power to detect a two-fold differences in MTXPG₁₋₇ for nonhyperdiploid B lineage, hyperdiploid, and T lineage ALL patients, respectively. The analysis of a difference within the t(12;21)/(ETV6-RUNX1) and the t(1;19)/(TCF3-PBX1) subtypes was exploratory. Values are expressed as medians unless otherwise specified. Normally distributed variables were compared by *t*-test. Non-normally distributed variables were compared using a Wilcoxon rank-sum or a Kruskal-Wallis test. Categorical data were compared with a χ^2 test. Multivariable linear regression was performed to assess the association between $\log(MTXPG_{1-7})$ and covariates.

A proportional subdistribution hazards regression model was used to determine if accumulation of MTXPG₁₋₇ and $WBC_{\Delta day 3}$ were prognostic factors for relapse of leukemia.^{26,27} Disease outcome analyses were performed after a median follow-up of 4.6 years (range, 1.8 to 8.6 years). Leukemia relapse was the event of interest; second malignancy, failure to achieve a complete remission (CR), or death due to any cause while in CR were considered as competing events. Time to event was calculated from the date of initial CR to the date of leukemia relapse or to the date of the competing event. The time was set as zero for those who did not achieve CR. Patients who achieved CR and were still alive without any types of events were censored at the time of last follow-up. Patients were categorized into three groups based on the accumulation of MTXPG₁₋₇ in bone marrow cells; high accumulators representing the top quartile in each of the five ALL subtypes, intermediate accumulators representing the middle two quartiles, and poor accumulators representing

Fig 1. CONSORT flow chart depicting enrollment, random assignment, and analysis. HDMTX, high-dose methotrexate; IV, intravenous; MTXPG₁₋₇, total intracellular methotrexate polyglutamates; DNPS, de novo purine synthesis.

the bottom quartile (Data Supplement). A *P* value lower than .05 was considered as statistically significant. All statistical analyses were done using SAS 9.1 (SAS Institute, Cary, NC) or R 2.8.0 (RDevelopment Core Team, <http://www.r-project.org>).

RESULTS

Patients

Between June 2000 and October 2007, 356 children with ALL were randomly assigned to receive HDMTX (1 g/m²) as either a 24-hour infusion or a 4-hour infusion, before initiation of conven-

tional remission induction chemotherapy (Fig 1). There were no significant differences in demographic or biologic characteristics between the 180 patients randomly assigned to the 24-hour and the 176 to the 4-hour treatment groups (Data Supplement). Plasma methotrexate pharmacokinetics are depicted in the Data Supplement and summarized in Table 1.

Accumulation of MTXPG₁₋₇ in Leukemia Cells

Among all patients, the 24-hour infusion produced significantly higher amounts of MTXPG₁₋₇ in leukemia cells (1,695 pmol/10⁹ cells) compared to the 4-hour infusion (1,150 pmol/10⁹ cells; *P* = .0059; Fig

Table 1. MTX Pharmacokinetics

Plasma MTX Exposure Variable	24-Hour Infusion (n = 145)		4-Hour Infusion (n = 129)		P*
	Median	Range	Median	Range	
4-hour concentration, μM	11.8	4.7-43.5	67.7	25.3-130.2	< .001
23-hour concentration, μM	9.5	2.7-23.9	0.26	0.03-4.12	< .001
42-hour concentration, μM	0.17	0.04-1.92	0.07	0.07-1.02	< .001
Time above 1 μM , hours	29.5	26.7-42.0	11.8	7.4-41.3	< .001
MTX clearance, $\text{mL}/\text{min}/\text{m}^2$	123	40-196	111	36-252	.009
$\text{AUC}_{0 \rightarrow 42\text{h}}$, $\mu\text{M} \times \text{h}$	294	184-914	327	144-1,012	.0005

Abbreviations: MTX, methotrexate; $\text{AUC}_{0 \rightarrow 42\text{h}}$, area under the concentration curve from start to 42 hours after the methotrexate infusion.
*P is calculated with Wilcoxon rank-sum test.

2A). The difference remained significant after adjusting for cell lineage and ploidy ($P = .0011$). After adjusting for ALL subtype in a multiple linear regression analysis, the 24-hour infusion remained significantly associated with higher accumulation of total MTXPG_{1-7} ($P < .001$; Table 2). The accumulation of MTXPG_{1-7} was significantly higher with the 24-hour infusion within the B-lineage ALL (1,861 ν 1,342 $\text{pmol}/10^9$ cells, $P = .0049$); the trend was similar in the T-lineage ALL (433 ν 314 $\text{pmol}/10^9$ cells, $P = .18$). Within specific B-lineage genetic subtypes, the 24-hour infusion resulted in a significantly higher amount of intracellular MTXPG_{1-7} in hyperdiploid ALL (3,919 ν 2,417 $\text{pmol}/10^9$ cells, $P = .0038$) and in the B-other ALL subtype (2,210 ν 1,576 $\text{pmol}/10^9$ cells, $P = .048$; Data Supplement). The median MTXPG_{1-7} also tended to be higher after the 24-hour infusion in B-lineage ALL with the $t(1;19)/(TCF3-PBX1)$ (525 ν 349 $\text{pmol}/10^9$ cells, $P = .10$), whereas the difference in B-lineage ALL with the $t(12;21)/(ETV6-RUNX1)$ did not approach statistical significance (858 ν 680 $\text{pmol}/10^9$ cells, $P = .58$; Data Supplement). With either infusion duration, the accumulation of MTXPG_{1-7} was significantly higher in hyperdiploid ALL than in any other ALL subtypes, and lowest in B-lineage ALL with the $t(1;19)/(TCF3-PBX1)$ and in T-ALL (Data Supplement). For patients in whom accumulation of MTXPG_{1-7} was measured at four time points in circulating ALL cells, the 24-hour infusion produced a significantly higher amount of MTXPG_{1-7} which was evident by the 24-hour time point (460 ν 314 $\text{pmol}/10^9$ cells, $P = .033$).

Inhibition of DNPS in Leukemic Cells

The percent inhibition of DNPS was high, with a median of -91.8% (quartiles, -84% to -97%) in the 24-hour group and -89.1% (quartiles, -74% to -96%) in the 4-hour group; statistically significant in favor of the 24-hour infusion ($P = .021$; Fig 2B). This difference in inhibition of DNPS between the 24-hour and 4-hour infusion groups was also significant after adjusting for ALL lineage and ploidy ($P = .044$). Infusion duration ($P = .012$) and ALL subtype ($P = .042$) were the only factors significantly related to the percent inhibition of DNPS in a multivariable model (Data Supplement).

Antileukemic Effects

Three hundred twenty patients were evaluable to assess the influence of infusion duration on methotrexate's antileukemic effects, measured as the decrease of circulating ALL cells over 3 days. Among all patients, the 24-hour infusion produced greater

antileukemic effects than the 4-hour infusion, with a significant difference in mean $\text{WBC}_{\Delta\text{day } 3}$ ($P = .038$; Fig 2C). Within individual ALL subtypes, the 24-hour infusion produced a better response, especially in T-ALL when measured as either $\text{WBC}_{\Delta\text{day } 3}$ ($P = .055$) or percent change in circulating leukemia cells ($-88.4\% \nu -51.8\%$; $P = .0075$). The better antileukemic effect achieved by the 24-hour infusion was also evident when adjusted for the effect of ALL subtypes and WBC at diagnosis in a multivariable model ($P = .03$; Table 3). When the concentration of MTXPG_{1-7} in bone marrow ALL cells was included in the model, MTXPG_{1-7} accumulation was more strongly associated with $\text{WBC}_{\Delta\text{day } 3}$ than was the infusion duration.

MTXPG₁₋₇ Accumulation As Predictor of Relapse

Low accumulation of MTXPG_{1-7} in ALL cells was significantly associated with a higher risk of relapse when compared to intermediate (hazard ratio [HR], 3.3; 95% CI, 1.2 to 9.1; $P = .018$) or high accumulation of MTXPG_{1-7} (HR, 3.6; 95% CI, 1.0 to 12.5; $P = .047$) and this difference remained significant after adjusting for disease risk group and treatment arm (Fig 3, Table 4). Consistent with prior findings⁹ the antileukemic effect induced by methotrexate ($\text{WBC}_{\Delta\text{day } 3}$) was also associated with risk of relapse in both the univariate analysis (HR, 6.1; 95% CI, 1.2 to 32.0; $P = .032$) and in the multivariate analysis after adjusting for disease risk group (HR, 5.9; 95% CI, 1.3 to 26.3; $P = .02$).

DISCUSSION

The current clinical trial was undertaken because shortening the intravenous infusion time for HDMTX is being explored to reduce toxicity in ALL treatment and to decrease the duration of hospitalization and reduce health care costs. The 24-hour infusion of HDMTX (1 g/m^2) produced significantly higher in vivo concentrations of MTXPG_{1-7} in bone marrow leukemia cells, when compared to a 4-hour infusion. The time of exposure to methotrexate above 1 $\mu\text{mol}/\text{L}$ was 2.5-fold longer for the 24-hour infusion compared to the 4-hour infusion and significantly related to the level of MTXPG_{1-7} in leukemia cells (Data Supplement), consistent with the hypothesis that due to saturation of methotrexate uptake and metabolism, longer exposure to moderate concentrations (eg, 1.0 μM) is advantageous compared to short exposure to very high

Fig 2. Accumulation of total intracellular methotrexate polyglutamates (MTXPG₁₋₇) in bone marrow acute lymphoblastic leukemia (ALL) cells and antileukemic effects. (A) Accumulation of total MTXPG₁₋₇ (pmol/10⁶ cells) grouped by the MTX infusion length (4 hour or 24 hour) and the ALL lineage (B-cell ALL [B-ALL] or T-cell ALL [T-ALL]). The significance level comparing 4-hour versus 24-hour infusion length in all patients, B-ALL, and T-ALL are $P = .0059$, $P = .0049$, and $P = .18$, respectively. Medians, quartiles, nonoutlier range (defined as 1.5 times the interquartile range), and outliers (plus signs) are depicted. (B) Percent change in de novo purine synthesis (DNPS) induced by MTX in bone marrow leukemia cells 42 to 44 hours after start of the infusion. The significance level comparing 4-hour versus 24-hour infusion length in all patients, B-ALL, and T-ALL are $P = .021$, $P = .081$, and $P = .24$, respectively. (C) Leukemic response expressed as residual in WBC from day 0 to day 3 grouped by the MTX infusion length (4 hour or 24 hour) and the ALL lineage (B-ALL or T-ALL). The significance level comparing 4-hour versus 24-hour infusion length in all patients, B-ALL, and T-ALL are $P = .038$, $P = .66$, and $P = .038$, respectively. Medians, quartiles, nonoutlier range (defined as 1.5 times the interquartile range), and outliers (plus signs) are depicted.

Table 2. Treatment and Biological Variables Significantly Related to Leukemia Cell Accumulation [$\log(\text{MTXPG}_{1-7})^*$] in Multivariate Analysis

Variable	Coefficient	SE	P*
Infusion time, 4 hour v 24 hour	-0.39	0.11	.0006
ALL subtype †	—	—	< .001
Hyperdiploid	1.0	—	—
B-other	-0.48	0.15	—
t(12;21)/(ETV6-RUNX1)	-1.08	0.16	—
t(1;19)/(TCF3-PBX1)	-1.61	0.22	—
T-ALL	-1.91	0.20	—
MTX clearance (mL/min/m ²)	-0.005	0.002	.0082

NOTE. Treatment and biological variables significantly related to $\log(\text{MTXPG}_{1-7})$ in bone marrow leukemia cells (multiple linear regression analysis) are shown. The coefficients represent the estimated difference in $\log(\text{MTXPG}_{1-7})$ for a one-unit difference in the variable, adjusted for the other variables in the linear-regression model. Cells in S-phase, patient sex, age, race, and WBC at diagnosis were not included in the multiple linear regression model as they were not significant in univariate analyses.

Abbreviations: $\log(\text{MTXPG}_{1-7})$, the logarithm of total intracellular methotrexate polyglutamates; SE, standard error of the coefficient; ALL, acute lymphoblastic leukemia; T-ALL, T-cell ALL; MTX, methotrexate.

*Multivariate regression analysis.

†Hyperdiploid ALL is the reference group.

concentrations of methotrexate.²⁸⁻³¹ Based on the current results, and using a pharmacokinetic model of systemic and cellular disposition of methotrexate,^{22,32} simulations indicate that approximately 2 g/m² of methotrexate infused over 4 hours would be required to achieve leukemia cell MTXPG₁₋₇ accumulation comparable to 1 g/m² infused over 24 hours, thus representing a potential alternative if short infusions are desirable (Data Supplement).

We have previously shown that the intracellular accumulation of MTXPG₁₋₇ in leukemia cells influences the antileukemic effects of HDMTX.^{8,9} In this study, the 24-hour infusion produced significantly greater inhibition of DNPS (a known mechanism of methotrexate) in ALL cells,^{33,34} and produced better antileukemic effects (WBC_{Δday 3}) when compared to the 4-hour infusion. When both infusion duration and MTXPG₁₋₇ accumulation were included in a multivariate model, MTXPG₁₋₇ was more strongly associated with antileukemic effects (WBC_{Δday 3}), indicating that it was the higher MTXPG₁₋₇ accumulation achieved by the 24-hour infusion that was responsible for better antileukemic effects. Moreover, in vivo MTXPG₁₋₇ accumulation was

Table 3. Multivariate Regression Analysis of Variables Potentially Associated With MTX's Antileukemic Effects (WBC_{ΔDay3})

Variable	P*	
	Without MTXPG ₁₋₇	With MTXPG ₁₋₇
Treatment arm, 24 hours v 4 hours	.03	.13
ALL subtype	.12	.008
WBC at diagnosis	.14	.040
MTXPG ₁₋₇	—	.008

NOTE. Covariates that were not significantly associated with WBC_{ΔDay3} in a univariate analysis did not enter in the multiple regression analysis: cells in S-phase, % inhibition of de novo purine synthesis, MTX clearance, patient sex, age, and self-reported race.

Abbreviations: MTX, methotrexate; MTXPG₁₋₇, total intracellular methotrexate polyglutamates; ALL, acute lymphoblastic leukemia.

*Multivariate regression analysis.

Fig 3. Cumulative incidence of leukemic relapse. Illustrates the cumulative incidence of risk of relapse with patients categorized into three groups on the basis of the accumulation of total intracellular methotrexate polyglutamates (MTXPG₁₋₇) in bone marrow acute lymphoblastic leukemia (ALL) cells. High MTXPG accumulation represented the top quartile in each of the five ALL subtypes, intermediate MTXPG accumulation represented the middle two quartiles, and low MTXPG accumulation represented the bottom quartile.

related to risk of leukemic relapse. This supports the use of MTXPG₁₋₇ accumulation as a phenotype to identify genome variations associated with ALL treatment effects.²³

The level of MTXPG₁₋₇ in leukemia cells differed significantly among ALL subtypes with either infusion length. Although our study was not designed to compare the effect of infusion duration within individual ALL molecular subtypes, our findings indicate that infusion length has a more pronounced effect in some ALL subtypes (ie, T-ALL, hyperdiploid B-lineage ALL) and suggests that it may be possible to treat certain subtypes with the shorter infusion without compromising treatment efficacy (ie, t(12;21)/(ETV6-RUNX1)).

T-ALL is known to accumulate MTXPG₁₋₇ less avidly than B-lineage ALL, due in part to lower folypolyglutamate synthetase

activity and to overexpression of efflux transporters that facilitate the export of methotrexate from T-ALL cells.^{11,12} In vitro studies indicate that an infusion duration beyond 4 hours might overcome resistance to methotrexate induced by efflux transporters, but this has never been tested in vivo.³⁵ We found that in T-ALL, the 24-hour infusion produced higher MTXPG₁₋₇ accumulation in bone marrow ALL cells and was associated with significantly better antileukemic effects, compared to the shorter infusion. Consistent with this finding, recent improvements in outcome of patients with T-ALL have been attributed in part to the use of higher doses of HDMTX (eg, 5 g/m²).^{14,19}

Hyperdiploid ALL has a favorable prognosis, and recent efforts have focused on strategies to reduce toxicity (eg, by shortening methotrexate infusion duration) as a way to further improve treatment. We found that even though the concentration of MTXPG₁₋₇ was relatively high after the 4-hour infusion, significantly higher accumulation was observed with the 24-hour infusion, indicating that reducing infusion duration should be done with caution in patients with hyperdiploid ALL.

The t(12;21)/(ETV6-RUNX1) is found in approximately 25% of pediatric patients with B-lineage ALL, and has been associated with an excellent outcome (5-year event-free survival, 86% to 98%).^{12,19,36-38} We previously reported that these leukemia cells overexpress the methotrexate export transporter ABCG2 and accumulate lower MTXPG₁₋₇.¹² This study did not reveal higher intracellular accumulation of MTXPG₁₋₇ in t(12;21)/(ETV6-RUNX1) ALL with the 24-hour infusion compared to the 4-hour infusion. Interestingly, the pretreatment DNPS rate was lower in the t(12;21)/(ETV6-RUNX1) ALL compared to other ALL subtypes, and patients with this genetic subtype had antileukemic effects (WBC_{Δday 3}) comparable to other ALL subtypes despite lower MTXPG₁₋₇ accumulation. These findings suggest that infusion of HDMTX over 4 hours may be adequate for patients with the t(12;21)/(ETV6-RUNX1), consistent with a recent abstract from the Pediatric Oncology Group that showed similar event-free survival in patients with t(12;21)/(ETV6-RUNX1) who were randomly assigned to receive either four courses of HDMTX 1 g/m² infused over 24 hours or 2 g/m² infused over 4 hours during consolidation therapy.³⁹

In summary, this study indicates that infusion duration is an important determinant of the intracellular accumulation of active methotrexate in ALL cells in vivo, with more prominent effects in

Table 4. Univariate Hazard Analysis of the Risk of Relapse and Multivariate Hazard Analysis After Adjusting for Disease Risk Group

Parameter	Univariate Analysis			Multivariate Analysis		
	Hazard Ratio	95% CI	P	Hazard Ratio	95% CI	P*
ALL risk groups	—	—	—	—	—	—
Intermediate/high v low	6.0	2.3 to 15.4	.0002	—	—	—
WBC _{ΔDay3}	6.1	1.2 to 32.0	.032	5.9	1.3 to 26.3	.020
MTXPG ₁₋₇ accumulation groups	—	—	—	—	—	—
Low MTXPG ₁₋₇ v high MTXPG ₁₋₇	3.6	1.0 to 12.5	.047	3.9	1.1 to 14.3	.039
Low MTXPG ₁₋₇ v intermediate MTXPG ₁₋₇	3.3	1.2 to 9.1	.018	4.4	1.5 to 12.5	.006
Log(MTXPG ₁₋₇)	0.7	0.5 to 1.0	.025	0.7	0.5 to 1.0	.050

Abbreviations: ALL, acute lymphoblastic leukemia; MTXPG₁₋₇, total intracellular methotrexate polyglutamates.

*The P value after adjusting for risk group in a multivariate analysis using the proportional subdistribution hazards regression model. Because of correlation between the covariates, they were not analyzed at the same time in the multivariate model. Because ALL risk group classification includes presenting age, ALL subtype, and WBC at diagnosis these parameters were not analyzed as single covariates in the model. Treatment arm was not an independent predictor of risk of relapse and including it in the model did not alter the significance of MTXPG₁₋₇ accumulation groups (data not shown).

certain subtypes of ALL, indicating that this must be considered when contemplating changes in treatment to reduce costs or toxicity.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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