Chemotherapy Pharmacodynamics and Neuroimaging and Neurocognitive Outcomes in Long-Term Survivors of Childhood Acute Lymphoblastic Leukemia

Kevin R. Krull, Yin Ting Cheung, Wei Liu, Slim Fellah, Wilburn E. Reddick, Tara M. Brinkman, Cara Kimberg, Robert Ogg, Deokumar Srivastava, Ching-Hon Pui, Leslie L. Robison, and Melissa M. Hudson

All authors: St Jude Children's Research Hospital, Memphis, TN

Published online ahead of print at www.jco.org on June 6, 2016.

Supported by the National Institute of Mental Health (Grant No. MH085849 to K.R.K.), the National Cancer Institute (Grant No. CA195547 to M.M.H. and L.L.R.), and by the American Lebanese Syrian Associated Charities.

Presented in part at the 51st Annual Meeting of the American Society of Clinical Oncology, Chicago, IL, May 29-June 2, 2015.

Authors' disclosures of potential conflicts of interest are found in the article online at www.jco.org. Author contributions are found at the end of this article.

Corresponding author: Kevin R. Krull, PhD, Department of Epidemiology and Cancer Control, St Jude Children's Research Hospital, 262 Danny Thomas Pl, MS 735, Memphis, TN 38105-3678; e-mail: kevin.krull@stjude.org.

© 2016 by American Society of Clinical Oncology

0732-183X/16/3422w-2644w/\$20.00

DOI: 10.1200/JCO.2015.65.4574

ABSTRACT

Purpose

To examine associations among methotrexate pharmacodynamics, neuroimaging, and neurocognitive outcomes in long-term survivors of childhood acute lymphoblastic leukemia treated on a contemporary chemotherapy-only protocol.

Patients and Methods

This longitudinal study linked pharmacokinetic assays collected during therapy to neurocognitive and brain imaging outcomes during long-term follow-up. A total of 218 (72.2%) of 302 eligible long-term survivors were recruited for outcome studies when they were more than 5 years post-diagnosis and older than 8 years of age. At long-term follow-up, survivors were an average of 13.8 years old and 7.7 years from diagnosis, and 51% were male. Neurocognitive testing, functional magnetic resonance imaging (MRI) during an executive function task, and structural MRI with diffusion tensor imaging were conducted. Generalized linear models were developed to identify predictors, and models were adjusted for age at diagnosis, sex, and parent education.

Results

Intelligence was within normal limits (mean, 98; standard deviation, 14) compared with population expectations (mean, 100; standard deviation, 15), though measures of executive function, processing speed, and memory were less than population means (all $P < .02$ after correction for false discovery rates). Higher plasma concentration of methotrexate was associated with a poorer executive function score ($P < .02$). Higher plasma methotrexate was also associated with higher functional MRI activity, with thicker cortices in dorsolateral prefrontal brain regions, and with white matter microstructure in the frontostriatal tract. Neurocognitive impairment was associated with these imaging findings as well. Associations did not change after adjustment for age or dose of leucovorin rescue.

Conclusion

Survivors of childhood acute lymphoblastic leukemia treated on contemporary chemotherapy-only protocols demonstrate executive dysfunction. A higher plasma concentration of methotrexate was associated with executive dysfunction as well as with a thicker cortex and higher activity in frontal brain regions, regions often associated with executive function.

J Clin Oncol 34:2644-2653. © 2016 by American Society of Clinical Oncology

INTRODUCTION

Contemporary treatment with intensified intrathecal and systemic chemotherapy has largely replaced cranial radiation therapy (CRT) for prophylaxis of the central nervous system in childhood acute lymphoblastic leukemia (ALL).¹ Because the 5-year survival rate approaches 95%, research has focused on identification of the source and reduction of neurotoxicity, which

continues to occur during active chemotherapy and may persist into long-term survivorship.² For survivors treated with chemotherapy only, neurocognitive problems remain relatively common, and risk factors reportedly include intrathecal and high-dose intravenous methotrexate.³ At an average of 21 years postdiagnosis, adult survivors of childhood ALL treated with chemotherapy only displayed severe impairment in complex cognitive processes (ie, executive function) at a rate eight times that observed in the general population;

these executive dysfunctions are more common than other deficits and usually manifest as impairments in cognitive flexibility, verbal fluency, and working memory.⁴ Given the long-term follow-up nature of that study and the change in therapy approaches over the past two decades, many of the patients were treated on low- or standard-risk protocols, and patients identified as high risk continued to receive CRT with relatively limited use of leucovorin rescue. In one of the first contemporary protocols that totally eliminated CRT, attention problems emerged by the end of therapy,⁵ though whether problems persist during long-term survivorship has not yet been determined.

Previous investigations to examine associations between chemotherapy and long-term neurocognitive outcomes have reported conflicting results.^{6,7} These contradictions may be due to inter-patient variability in pharmacokinetics of high-dose methotrexate (HDMTX).^{2,8} In these studies, chemotherapy drug dosage has been used as a surrogate for drug exposure, which may introduce measurement error and compromise the accuracy of results. Plasma HDMTX exposure is associated with acute neurotoxicity² and may provide more precision to understand sources of long-term neurocognitive and neuroimaging outcomes.

This study examined treatment-related pharmacologic (ie, chemotherapy dosage and exposure) and related biomarker (ie, plasma homocysteine) predictors of neurocognitive and brain imaging outcomes in long-term survivors of childhood ALL treated according to a single institutional protocol designed to eliminate the use of CRT. We hypothesized that higher exposures to methotrexate and related biomarkers would be associated with poorer executive function, which is a manifestation of neuroanatomical and functional integrity of frontal lobe brain regions that control complex cognitive performance. We also examined the functional and neuroanatomical state of frontal lobe brain regions.

PATIENTS AND METHODS

From 2000 to 2010, 408 children with ALL were treated at St Jude Children's Research Hospital (SJCRH) on the Total Therapy XV protocol ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00137111), NCT00137111).¹ To be eligible for the current long-term follow-up study, survivors must have been treated and observed at SJCRH and must have been more than 5 years from diagnosis and older than 8 years of age to reliably assess executive function abilities. Survivors were excluded (n = 53) if they received cranial radiation for CNS relapse or bone marrow transplantation, experienced a relapse or secondary cancer that required additional chemotherapy, had a pre-existing non-cancer-related neurodevelopmental or genetic disorder associated with cognitive impairment, had a subsequent brain injury unrelated to cancer, or were not proficient in English. Recruitment was not attempted for 18 patients who were discharged from pediatric follow-up care, were in foster care, or were in prison. Of the 302 eligible survivors, 218 (72.2%) participated in outcome studies (Fig. 1). There was no significant difference between participants and nonparticipants in demographics, risk stratum, or cumulative drug doses. This study was approved by the institutional review board at SJCRH, and informed consent/assent was obtained from all participants and/or parents.

The Total Therapy XV treatment schema is presented in Appendix Table A1 (online only). Children in the low-risk arm received HDMTX at 2.5 gm/m² per dose for four doses, 13 to 18 triple intrathecal treatments (MTX, hydrocortisone, and cytarabine), and oral dexamethasone pulses at 8 mg/m² per day for 5 days, in addition to other chemotherapeutic agents. Children in the standard-/high-risk arm received HDMTX at 5.0 gm/m²

Fig 1. CONSORT diagram. BMT, bone marrow transplant; CRT, cranial radiation therapy; SMN, second malignant neoplasm.

per dose for four doses, 16 to 25 triple intrathecal treatments, and oral dexamethasone pulses at 12 mg/m² per day for 5 days. A 10% loading dose of HDMTX was given over 1 hour, followed immediately by maintenance infusion for the next 23 hours. Leucovorin rescue (10 to 15mg/m² intravenously) started 42 hours after HDMTX and repeated every 6 hours for a total of five doses. Prophylactic CRT was not administered to any patient, including in the presence of CNS leukemia at diagnosis. CRT was included only for patients who experienced relapse; thus, no patients with CRT exposure were included in the current analyses.

Exposures to HDMTX and dexamethasone were measured as previously described.^{2,9,10} Blood samples were drawn before HDMTX infusion and at 6, 23, and 42 hours after the start of each HDMTX course. Blood was drawn before and 1, 2, 4, and 8 hours after morning dexamethasone dose on days 1 and 8 of reinduction I, which corresponded to weeks 7 and 8 of continuation therapy, respectively. Plasma homocysteine

concentrations were measured, as previously described,⁸ at baseline before HDMTX infusion and at 23 and 42 hours post-HDMTX for all four courses. Exposures to HDMTX, dexamethasone, and homocysteine were quantified as area under the curve (AUC).

All participants completed neurocognitive testing with certified examiners under the supervision of a board-certified clinical neuropsychologist. Testing procedures followed standard clinical guidelines, with a fixed test order and schedules controlled to reduce effects of interference and fatigue. The primary outcome of interest, executive function, was measured by the Delis-Kaplan Executive Function System,¹¹ which assessed cognitive flexibility, verbal fluency, working memory, organization, and problem solving abilities. Tests of intelligence (IQ),¹² processing speed,^{13,14} attention,¹⁵ memory,¹² and fine motor dexterity¹⁶ were also completed.

Participants underwent brain functional magnetic resonance imaging (fMRI) during completion of the Attention Network Test,¹⁷ which activates attention and executive function networks. Imaging was performed on a 3T scanner (Siemens Trio or Skyra MR; Siemens, Malvern, PA) by using a single-shot T2*-weighted echo planar imaging (EPI) pulse sequence (TR = 2.06 seconds; TE = 30 milliseconds; FOV = 192 mm; matrix = 64 × 64; slice thickness = 5 mm). Functional images were normalized to the Montreal Neurologic Institute brain template and smoothed with a Gaussian kernel of 6-mm full width at half maximum. The smoothed and normalized images were resliced to 2-mm isotropic resolution. Voxel-wise analyses with Family-wise error corrections were conducted with SPM8 (fil.ion.ucl.ac.uk/spm/).

Structural magnetic resonance imaging (MRI) included a T1-weighted set acquired using sagittal 3D MPRAGE sequence (TR = 1,560; TE = 2.75; TI = 900 milliseconds) with an imaging resolution of $0.98 \times 0.98 \times 1.1$ mm. The T1-weighted MRI set was processed with FreeSurfer software (<http://surfer.nmr.mgh.harvard.edu/>, Athinoula A. Martinos Centre for Biomedical Imaging) to assess cortical thickness.¹⁸ The MRI also included diffusion tensor imaging (DTI) to assess white matter microstructure, acquired using a double-spin echo EPI pulse sequence (TR = 10,000; TE = 100 milliseconds; b = 1000; $3 \times 1.8 \times 1.8$ mm; acquisition time, approximately 1.5 min each) with four acquisitions and 12 gradient directions. Measures of fractional anisotropy (FA), axial diffusivity (AD), and radial diffusivity (RD) were calculated within the frontal and parietal lobes and in the frontostriatal tract, which is associated with executive function. More detailed descriptions of the structural MRI processing and analysis are provided in the Appendix (online only).

Statistical Analysis

Neurocognitive performance was transformed into age-adjusted *z* scores by using national normative data. One-sample *t* tests compared performances between survivors and population norms, and only those measures on which performance differed from normative samples using a false discovery rate were examined for associations with predictors and brain imaging outcomes. Generalized linear models (GLMs) were conducted separately to identify pharmacologic and biomarker predictors of long-term neurocognitive outcomes, and models were adjusted for demographics (age at diagnosis, sex, education of parents). On the basis of prior literature, predefined pharmacologic predictors included HDMTX AUC, dexamethasone AUC, and number of triple intrathecal treatment doses. Biomarker predictor was homocysteine AUC, given its association with HDMTX. Because these predictors were identified a priori, correction for multiple GLM models was not conducted. Previous studies have reported the potential neuroprotective effect of leucovorin and reduction in dexamethasone clearance by asparaginase,¹⁰ so a sensitivity analysis of leucovorin dose and asparaginase exposure was conducted by adding them to the pharmacologic models to determine whether significant associations were affected. Interactions between demographic factors (sex and age at diagnosis) and treatment-related predictors were also explored.

Statistical contrasts were calculated for fMRI to identify attention and executive function networks that related to neurocognitive tests. Contrasts from each survivor were used as variables in second-level, random-effect analyses to identify patterns of brain activation. Our predefined pharmacologic and biomarker predictors were entered as covariates to identify

variables correlated with brain activity. Voxels were considered active for $P < .05$, corrected for multiple comparisons and with a minimum cluster size of five voxels (*T* threshold = 6.4).

Cortical thickness was calculated for predetermined brain regions by using the FreeSurfer software. Given the focus on executive function, we a priori identified the dorsolateral prefrontal cortex and anterior cingulate cortex as regions of interest. White matter microstructure, measured through the FA, AD, and RD DTI indices, was examined in frontal and parietal lobes, as well as in the frontostriatal tract, which is associated with executive function and which connects the striatum to dorsolateral and anterior cingulate cortices. Associations between cortical thickness and white matter microstructure, and predictors, were examined using the GLM, which was adjusted for current age. We also compared cortical thickness and DTI indices between survivors with and without impairment, defined as having at least two neurocognitive measures that were greater than 1.5 standard deviations less than population norms. Corrections were not made for multiple GLM models, because treatment predictors and brain regions of interest were identified a priori.

RESULTS

Demographic, clinical, and treatment characteristics of survivors are listed in Table 1. Full-scale intelligence was within normal limits, though scores were significantly less than the population mean on six measures of executive function (ie, flexibility [two measures], fluency, working memory, organization/planning, and abstract reasoning; Table 2). Survivors also performed more poorly than population norms on measures of perceptual reasoning, attention, memory, and processing speed.

Higher MTX AUC and homocysteine AUC were associated with lower scores on measures of cognitive flexibility (Table 3). Higher MTX AUC was also associated with lower scores on verbal fluency, as well as on spatial memory span and numerical processing speed (Appendix Table A2, online only). Significant associations were seen when examined within each treatment risk arm, particularly in the standard-/high-risk arm (Fig 2; Appendix Fig A1, online only). The addition of leucovorin exposure into models did not change the significance of associations with MTX. Exposure to dexamethasone was not associated with executive function or other cognitive measures, and inclusion of antibodies to asparaginase in multivariable models did not affect this finding. Neither age at diagnosis nor sex significantly interacted with treatment exposures or biomarkers to affect executive function.

Higher MTX AUC was associated with increased brain fMRI activity in frontal and anterior cingulate cortices, as well as in the caudate nuclei and putamen (Fig 3). No areas of reduced brain activation were observed during the task. No other pharmacologic or biomarker predictors were associated with fMRI activity. Higher MTX AUC was also associated with thicker cortices in dorsolateral prefrontal regions (Fig 3), which may suggest a dose threshold effect for impact on brain structure, as well as higher axial diffusivity in the frontostriatal tract (Table 3), a tract that connects the caudate/putamen to dorsolateral prefrontal and anterior cingulate regions. Higher dexamethasone AUC was associated with thinner right anterior cingulate cortex ($r = -0.21$; $P = .008$) but not dorsolateral prefrontal cortex (Appendix Fig A2, online only) and was associated with DTI parameters of white matter microstructure in frontal lobes (Table 4). DTI parameters in frontal lobes and frontostriatal tract were associated with neurocognitive

Table 1. Survivor Demographics and Treatment Characteristics

Characteristic	% of Patients	Mean (SD)	Median (IQR)
Sex			
Male	51.2		
Female	48.8		
Race/ethnicity			
White	73.7		
Asian	1.9		
Black	12.7		
Hispanic	8.0		
Other	3.7		
Current age, years		13.8 (4.8)	13.0 (10.0-17.0)
Highest education, years		7.5 (4.0)	
Maternal education, years		13.7 (2.6)	
Paternal education, years		13.7 (3.1)	
Age at diagnosis, years		6.6 (4.5)	5.0 (3.2-8.7)
Time since diagnosis, years		7.7 (1.7)	7.5 (6.3-9.0)
Treatment risk stratum			
Low	57.3		
Standard/high	42.7		
Treatment			
Oral dexamethasone, mg/m ²		1,084.5 (307.3)	1,098.3 (968.1-1,246.1)
IV asparaginase, g/m ²			
<i>Erwinia</i> -asparaginase		396.4 (282.4)	269.3 (201.9-659.3)
L-asparaginase		273.4 (179.6)	240.4 (113.4-470.6)
Peg-asparaginase		12.6 (11.1)	10.1 (7.5-12.7)
IV cytarabine, g/m ²			
Standard dose		2.3 (2.1)	0.6 (0.6-4.3)
High dose		8.4 (3.0)	8.0 (8.0-8.0)
IV cyclophosphamide, g/m ²		2.6 (1.9)	1.0 (1.0-4.7)
IV daunorubicin, mg/m ²		49.5 (13.0)	50.0 (49.2-50.8)
IV doxorubicin, mg/m ²		109.8 (56.7)	62.9 (60.9-178.2)
IV leucovorin, mg/m ²		346.0 (200.3)	300.0 (200.0-400.0)
Methotrexate			
IV standard dose, g/m ²		4.0 (2.2)	3.7 (3.1-4.4)
IV high dose, g/m ²		15.7 (6.6)	14.3 (11.4-19.0)
IT, ml		170.3 (55.2)	189.5 (144.1-243.2)
IV vincristine dose, mg/m ²		58.9 (12.6)	63.2 (52.4-67.8)
IT chemotherapy, no. of counts		14.6 (4.1)	13.0 (12.0-17.0)

NOTE. With the exception of IT chemotherapy, all drugs are presented in cumulative doses. High-dose IV methotrexate was calculated separately from standard dose. High-dose IV methotrexate was defined as daily dose of more than 1 g/m² of IV methotrexate.

Abbreviations: IQR, intraquartile range; IT, intrathecal; IV, intravenous; SD, standard deviation.

impairment (Appendix Table A3, online only). These associations did not change after results were adjusted for current age.

DISCUSSION

This study presents novel neurocognitive and brain imaging outcomes in long-term survivors of childhood ALL, treated on a contemporary chemotherapy-only protocol, by using pharmacokinetic exposure and biomarker parameters to predict outcomes. Our study demonstrates that, at more than 5 years postdiagnosis, a substantial proportion of survivors experience neurocognitive dysfunction. Executive dysfunction is associated with exposure to HDMTX and the subsequent homocysteine response during cancer therapy. Plasma concentration of MTX was also associated with cortical thickness, higher functional brain activity, and DTI indices of white matter microstructure in frontal lobe regions, which are often associated with executive function. Survivors with neurocognitive impairment demonstrated differences in DTI indices in frontal but not parietal brain regions. This pattern may

suggest that frontal brain regions and executive functions are more prone to long-term pathology after HDMTX exposure. Although limited data are available on long-term outcomes in survivors of pediatric ALL treated according to contemporary chemotherapy-only protocols, our results are consistent with reports of processing speed deficits after chemotherapy⁷ as well as of executive dysfunction in long-term survivors treated with chemotherapy and cranial radiation.^{4,19,20} As adults, these long-term survivors have also demonstrated smaller volumes in cortical gray and white matter as well as in basal ganglia.^{19,21} Our results extend these findings to contemporary protocols and functional brain activation and demonstrate a connection to plasma methotrexate exposure.

Exposure to HDMTX was associated with problems in flexibility and fluency but not in working memory, organization, or abstraction skills. Impairments in working memory (17%) and abstraction (18%) were relatively less common than impairments in flexibility (27%) and fluency (24%), which suggests less variability in these scores; however, impairment in organization was common (58%), possibly because of excessive difficulty in the task. The ability of HDMTX to cross the blood-brain barrier and its

Table 2. Neurocognitive Outcomes of Survivors

Outcome	Mean (SD)	P*	% Impairment	95% CI	
				Lower	Upper
Executive function					
Flexibility					
Number letter switch	−0.55 (1.20)	< .001	27.14	21.13	33.16
Color word switch	−0.20 (1.00)	.01	14.15	9.38	18.92
Fluency					
Verbal fluency	−0.38 (0.99)	< .001	24.40	18.58	30.22
Categorical fluency	0.01 (1.07)	.86	14.35	9.60	19.11
Working memory					
Digit backward	−0.29 (1.00)	< .001	17.14	12.05	22.24
Spatial backward	−0.03 (0.98)	.66	12.38	7.93	16.84
Organization/planning					
Rey complex figure	−2.38 (2.40)	< .001	58.37	51.69	65.06
20 questions	−0.19 (1.03)	.02	18.27	13.02	23.52
Tower	−0.12 (0.85)	.08	10.43	6.30	14.55
Intelligence					
Full scale	−0.12 (0.91)	.09	10.58	6.40	14.76
Verbal					
Similarities	0.03 (0.96)	.69	10.58	6.40	14.76
Vocabulary	−0.10 (1.07)	.21	13.94	9.23	18.65
Perceptual					
Block design	−0.25 (0.98)	< .001	16.19	11.21	21.17
Matrix reasoning	−0.09 (0.93)	.20	10.95	6.73	15.18
Attention					
Detectability	−0.15 (1.16)	.09	10.19	6.06	14.33
Omission	−0.30 (1.53)	.01	15.05	10.17	19.93
Commission	−0.18 (1.16)	.06	11.17	6.86	15.47
Variability	−0.14 (1.18)	.13	15.05	10.17	19.93
Memory					
Digit forward	−0.38 (0.99)	< .001	20.95	15.45	26.46
Spatial forward	−0.21 (0.96)	.005	17.54	12.40	22.67
Processing speed					
Motor					
Dominant hand speed	−1.32 (1.54)	< .001	42.11	35.41	48.80
Visual					
Symbol search	−0.05 (1.04)	.51	13.27	8.69	17.85
Visual-motor					
Digit symbol	−0.41 (0.96)	< .001	17.62	12.47	22.77
Number sequencing	−0.21 (1.07)	.01	18.10	12.89	23.30
Letter sequencing	−0.34 (1.16)	< .001	20.48	15.02	25.93
Oral					
Word reading	−0.10 (0.93)	.19	11.11	6.83	15.39
Color naming	−0.13 (0.90)	.06	13.53	8.87	18.19

NOTE. Group means and SDs are represented in age-adjusted z scores referenced to nationally representative norms. Impairment is defined as a score falling less than the 10th percentile of the age-adjusted z score. P values represent comparison of the group average to the expected population value ($\mu = 0$; $\sigma = 1.0$) for specific tests. Abbreviation: SD, standard deviation.

*P value corrected for false discovery rate.

toxicologic profile when given in intensified doses establish it as a candidate for neurotoxicity in treatment of ALL. The HDMTX AUC provides a direct measure of systemic exposure. Although survivors received doses that were based on body surface area, the wide range of systemic exposure suggests an underlying inter-patient variability in metabolism of the drug.^{22,23} Research with whole-genome sequencing may help identify genetic variants that influence drug metabolism and yield additional insight into pharmacogenomic factors that affect long-term neurocognitive outcomes.

This study highlights exposure-response activation in frontal lobe regions of the brain. Higher HDMTX exposure was associated with increased fMRI activity in frontal and associated subcortical regions, which possibly suggests compensatory changes in brain function. Structural MRI analysis also demonstrated differences,

though thicker cortices in the dorsolateral prefrontal regions were observed in this study. DTI parameters suggested altered white matter microstructure in these same frontal brain regions in survivors exposed to higher concentrations of methotrexate. This pattern may suggest reduced cortical maturation, including less age-related synaptic pruning in cortical neurons and less white matter development in frontal brain regions.²⁴ These key regions of the brain, which govern executive functions, may be more susceptible to ALL treatment. HDMTX exposure was not associated with brain activation in parietal or temporal lobe brain regions. Previous studies have shown different brain regions with temporally distinct maturational trajectories characterized by white matter tracts within the frontal lobes.²⁵⁻²⁷ Collectively, these data suggest a multifactorial etiology to adverse neurocognitive outcomes in this population and may suggest that neuroanatomical

Table 3. Multivariable General Linear Models for Prediction of Executive Dysfunction

Parameter	Cognitive Flexibility: Number-Letter Switch*			Cognitive Flexibility: Color Word Switch*			Verbal Fluency			Working Memory			Organization			Abstraction		
	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P
Pharmacologic†																		
Total IT counts	0.02	0.03	.62	0.00	0.03	.89	0.04	0.03	.08	0.02	0.03	.56	−0.07	0.07	.27	0.00	.03	.90
Methotrexate AUC	−0.02	0.01	.02	−0.02	0.01	.08	−0.02	0.01	.01	−0.00	0.01	.75	0.01	0.02	.61	0.00	.01	.97
Dexamethasone AUC	0.00	0.00	.10	0.00	0.00	.62	0.00	0.00	.68	−0.00	0.00	.22	−0.00	0.00	.11	0.00	.00	.49
Biomarker†																		
Homocysteine AUC	−0.01	0.01	.05	0.00	0.01	.64	0.00	0.01	.58	0.00	0.01	.78	−0.00	0.01	.77	0.01	.01	.19

Abbreviations: AUC, area under curve; IT, intrathecal; SE, standard error.

*Models on each outcome were conducted separately for pharmacologic and biomarker risk factors because of confounding between the methotrexate AUC and the homocysteine AUC.

†Multivariable model on each neurocognitive outcome with adjustment for demographic risk factors (age at diagnosis, sex and highest education of parents).

changes induced by acute MTX neurotoxicity have long-term functional consequences and warrant attention from clinical, radiologic, and neuroscience perspectives.

Consistent with HDMTX exposure, elevated homocysteine during therapy was associated with poorer cognitive flexibility. Elevation in homocysteine in children with ALL is associated with acute neurotoxicities, such as seizure and leukoencephalopathy.^{2,9} Although treatment-related hyperhomocysteinemia is transient in most cases, temporary elevation could induce vascular injury or inflammation that affects glial or neuronal cell integrity and alters normal developmental trajectory of the brain. Variations in homocysteine levels might also be driven by pharmacogenetics, as shown through associations between methionine synthase polymorphisms and attention problems in ALL survivors.²⁸ The clinical implications of this finding include potential predictive serum markers for poor neurocognitive outcomes among individuals who may benefit from intervention to correct hyperhomocysteinemia. Future research should examine homocysteine

levels in long-term survivors, which, if elevated, may suggest a need for intervention with folate therapy and adjunctive pyridoxine and cobalamin supplementation.

This study did not find an association between other chemotherapy agents and neurocognitive function. The number of triple intrathecal injections was not associated with outcomes, though there was relatively little variability in this predictor. Recent studies have demonstrated an association between corticosteroids and neurocognitive impairment in ALL survivors when evaluated more than 10 years after diagnosis.^{29,30} In this study, dexamethasone exposure was not associated with neurocognitive outcomes, though serum dexamethasone levels were sampled at only one time point over the course of therapy. High-dose corticosteroids can lead to altered cortisol elevations through impaired feedback in the hypothalamic-pituitary-adrenal axis,³¹ and stress responses in this axis can produce adverse effects on brain function.³² Daily fluctuation in cortisol is a common limitation that deters meaningful clinical interpretation of such studies. Future studies should

Fig 2. Associations between methotrexate exposure, assessed as area under the curve, and executive function, stratified by low- and standard/high-risk treatment arms. Correlation coefficient (*P* value) for cognitive flexibility in low risk is -0.12 ($P = .23$) and in standard/high risk is -0.43 ($P < .001$). Correlation coefficient for verbal fluency in low risk is -0.12 ($P = .24$) and in standard/high risk is -0.27 ($P = .02$). Dashed line represents average score in the general population.

Fig 3. (Left) Brain activation during the attention network test (ANT) in survivors of childhood acute lymphoblastic leukemia. The ANT assesses sustained attention and executive function abilities. Collectively, the regions identified in green in the bilateral frontal cortex, caudate nuclei, left putamen, and anterior cingulate cortex represent areas where brain activation during the ANT was positively correlated with methotrexate exposure. (Right) Associations between methotrexate exposure (area under the curve) and cortical thickness, stratified by low-risk, standard-risk, and high-risk treatment arms. Correlation coefficients (*P* values) for the low-risk arm are as follows: left anterior cingulate, -0.01 ($P = .94$); right anterior cingulate, 0.01 ($P = .91$); left dorsolateral prefrontal, 0.13 ($P = .20$); and right dorsolateral prefrontal, 0.10 ($P = .31$). Correlation coefficients (*P* values) for the standard-risk/high-risk arm are as follows: left anterior cingulate, -0.15 ($P = .22$); right anterior cingulate, 0.04 ($P = .73$); left dorsolateral prefrontal, 0.24 ($P = .04$); and right dorsolateral prefrontal, 0.37 ($P = .002$).

Table 4. A

Treatment Variable	Frontostriatal Tract				Frontal Lobe				Parietal Lobe									
	FA		RD		FA		RD		FA		RD							
	Estimate (SE)	P*	Estimate (SE)	P*	Estimate (SE)	P*	Estimate (SE)	P*	Estimate (SE)	P*	Estimate (SE)	P*						
IT MTX	-4.2 × 10 ⁻⁵ (4.4 × 10 ⁻⁵)	.33	-4.6 × 10 ⁻⁵ (6.2 × 10 ⁻⁵)	.46	1.1 × 10 ⁻⁵ (6.6 × 10 ⁻⁵)	.86	4.7 × 10 ⁻⁶ (3.6 × 10 ⁻⁶)	.89	-1.1 × 10 ⁻⁴ (7.1 × 10 ⁻⁵)	.11	-6.8 × 10 ⁻⁵ (6.2 × 10 ⁻⁵)	.27	-3.9 × 10 ⁻⁶ (4.6 × 10 ⁻⁶)	.93	1.4 × 10 ⁻⁴ (6.2 × 10 ⁻⁵)	.024	-6.7 × 10 ⁻⁵ (5.8 × 10 ⁻⁵)	.25
MTX exposure	-1.2 × 10 ⁻⁴ (2.1 × 10 ⁻⁴)	.54	6.1 × 10 ⁻⁴ (2.9 × 10 ⁻⁵)	.042	5.3 × 10 ⁻⁴ (3.1 × 10 ⁻⁴)	.090	1.3 × 10 ⁻⁴ (1.7 × 10 ⁻⁴)	.43	4.0 × 10 ⁻⁶ (3.4 × 10 ⁻⁴)	.99	-1.3 × 10 ⁻⁴ (3.0 × 10 ⁻⁴)	.66	1.0 × 10 ⁻⁵ (2.2 × 10 ⁻⁴)	.96	-7.5 × 10 ⁻⁵ (3.0 × 10 ⁻⁴)	.80	-4.2 × 10 ⁻⁵ (2.8 × 10 ⁻⁴)	.88
DEX exposure	-2.9 × 10 ⁻⁶ (3.9 × 10 ⁻⁶)	.45	7.1 × 10 ⁻⁶ (5.5 × 10 ⁻⁶)	.20	8.6 × 10 ⁻⁶ (5.9 × 10 ⁻⁶)	.14	-2.6 × 10 ⁻⁷ (3.1 × 10 ⁻⁶)	.93	1.2 × 10 ⁻⁵ (6.3 × 10 ⁻⁶)	.044	7.7 × 10 ⁻⁶ (5.5 × 10 ⁻⁶)	.15	-3.8 × 10 ⁻⁶ (4.1 × 10 ⁻⁶)	.34	9.1 × 10 ⁻⁶ (5.2 × 10 ⁻⁶)	.081	7.4 × 10 ⁻⁶ (4.8 × 10 ⁻⁶)	.12
HVC exposure	-1.6 × 10 ⁻⁴ (1.6 × 10 ⁻⁴)	.33	-2.8 × 10 ⁻⁴ (2.3 × 10 ⁻⁴)	.24	-3.6 × 10 ⁻⁶ (2.5 × 10 ⁻⁴)	.98	-2.3 × 10 ⁻⁵ (1.3 × 10 ⁻⁴)	.86	-1.0 × 10 ⁻⁴ (2.7 × 10 ⁻⁴)	.71	-2.6 × 10 ⁻⁵ (2.4 × 10 ⁻⁴)	.91	7.2 × 10 ⁻⁵ (1.7 × 10 ⁻⁴)	.68	1.2 × 10 ⁻⁴ (2.4 × 10 ⁻⁴)	.61	-1.6 × 10 ⁻⁵ (2.2 × 10 ⁻⁴)	.94

NOTE: Higher FA, lower AD, and lower RD indicate better white matter microstructure; positive associations between MTX and AD in the frontostriatal tract suggest that higher exposure/doses of MTX are associated with poorer white matter microstructure.
Abbreviations: AD, axial diffusivity; DEX, dexamethasone; FA, fractional anisotropy; HYC, homocysteine; IT, intrathecal; MTX, methotrexate; RD, radial diffusivity; SE, standard error.
*Adjusted for current age.

evaluate interrelations among high-dose corticosteroid exposure during active treatment, changes in cortisol levels in response to stress, and their effects on neurocognitive outcomes in long-term ALL survivors.

Several limitations should be considered in interpretation of these results. Serum assays for pharmacokinetics and biomarker parameters were conducted at specific cycles or at a single time point, out of a wide treatment window of 2.5 to 3 years. The effect of these parameters may interact with other treatment agents or procedures (eg, intrathecal methotrexate). Still, the parameters should reflect prototypical responses to treatment over the course of therapy. Other on-therapy pharmacologic agents and physiologic responses, such as biomarkers of oxidative stress and inflammation, were not evaluated in this study. The MTX AUC was associated with one of three DTI indices in the frontostriatal tract, which by itself is relatively weak evidence of impact. However, associations across multiple imaging parameters make this impact more convincing. Finally, neurocognitive impairment is a multifactorial process that may be affected by health behaviors and chronic health conditions, such as cardiovascular and endocrine complications that were not considered in this analysis. These chronic health conditions generally occur at ages much later than the relatively young age at follow-up (mean, 13.8 years) in this study.

Limitations notwithstanding, this study demonstrates an association between pharmacokinetics and biomarker response of HDMTX and neurocognitive and brain imaging outcomes in long-term survivors of childhood ALL treated on a contemporary chemotherapy-only protocol. Future prospective studies should

include a robust infrastructure that allows for coordinated data collection at prediagnosis, active treatment, posttreatment, and long-term survivorship phases to ascertain predictive makers for cognitive impairment in ALL survivors.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Kevin R. Krull, Yin Ting Cheung, Leslie L. Robison, Melissa M. Hudson

Provision of study materials or patients: Ching-Hon Pui, Melissa M. Hudson

Financial support: Kevin R. Krull, Melissa M. Hudson, Leslie L. Robison

Administrative support: Kevin R. Krull, Leslie L. Robison, Melissa M. Hudson

Collection and assembly of data: Kevin R. Krull, Slim Fellah, Wilburn E. Reddick, Cara Kimberg, Robert Ogg, Deokumar Srivastava, Ching-Hon Pui, Leslie L. Robison, Melissa M. Hudson

Data analysis and interpretation: Kevin R. Krull, Yin Ting Cheung, Wei Liu, Slim Fellah, Tara M. Brinkman, Robert Ogg, Ching-Hon Pui, Leslie L. Robison, Melissa M. Hudson

Manuscript writing: All authors

Final approval of manuscript: All authors

REFERENCES

- Pui CH, Campana D, Pei D, et al: Treating childhood acute lymphoblastic leukemia without cranial irradiation. *N Engl J Med* 360:2730-2741, 2009
- Bhojwani D, Sabin ND, Pei D, et al: Methotrexate-induced neurotoxicity and leukoencephalopathy in childhood acute lymphoblastic leukemia. *J Clin Oncol* 32:949-959, 2014
- Landier W, Bhatia S, Eshelman DA, et al: Development of risk-based guidelines for pediatric cancer survivors: The Children's Oncology Group long-term follow-up guidelines from the Children's Oncology Group late effects committee and nursing discipline. *J Clin Oncol* 22:4979-4990, 2004
- Krull KR, Brinkman TM, Li C, et al: Neurocognitive outcomes decades after treatment for childhood acute lymphoblastic leukemia: A report from the St Jude lifetime cohort study. *J Clin Oncol* 31:4407-4415, 2013
- Conklin HM, Krull KR, Reddick WE, et al: Cognitive outcomes following contemporary treatment without cranial irradiation for childhood acute lymphoblastic leukemia. *J Natl Cancer Inst* 104:1386-1395, 2012
- Spiegler BJ, Kennedy K, Maze R, et al: Comparison of long-term neurocognitive outcomes in young children with acute lymphoblastic leukemia treated with cranial radiation or high-dose or very high-dose intravenous methotrexate. *J Clin Oncol* 24:3858-3864, 2006
- Kadan-Lottick NS, Brouwers P, Breiger D, et al: Comparison of neurocognitive functioning in children previously randomly assigned to intrathecal methotrexate compared with triple intrathecal therapy for the treatment of childhood acute lymphoblastic leukemia. *J Clin Oncol* 27:5986-5992, 2009
- Rühs H, Becker A, Drescher A, et al: Population PK/PD model of homocysteine concentrations after high-dose methotrexate treatment in patients with acute lymphoblastic leukemia. *PLoS One* 7:e46015, 2012
- Kishi S, Griener J, Cheng C, et al: Homocysteine, pharmacogenetics, and neurotoxicity in children with leukemia. *J Clin Oncol* 21:3084-3091, 2003
- Yang L, Panetta JC, Cai X, et al: Asparaginase may influence dexamethasone pharmacokinetics in acute lymphoblastic leukemia. *J Clin Oncol* 26:1932-1939, 2008
- Delis DC, Kaplan E, Kramer JH. Delis-Kaplan Executive Function System. Psychological Corp, San Antonio, TX, 2001
- Wechsler D. Wechsler Abbreviated Scale of Intelligence. Psychological Corp, San Antonio, TX, 1999
- Wechsler D. Wechsler Intelligence Scale for Children (ed 4). Harcourt Assessment, San Antonio, TX, 2003
- Wechsler D. Wechsler Adult Intelligence Scale (ed 4). Pearson, San Antonio, TX, 2008
- Conners CK. Conners' Continuous Performance Performance Test II. Multi-Health Systems, North Tonawanda, NY, 2001
- Strauss E, Sherman EM, Spreen O. A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary (ed 3). New York, NY, Oxford University Press, 2006
- Fan J, McCandliss BD, Fossella J, et al: The activation of attentional networks. *Neuroimage* 26:471-479, 2005
- Reuter M, Schmansky NJ, Rosas HD, et al: Within-subject template estimation for unbiased longitudinal image analysis. *Neuroimage* 61:1402-1418, 2012
- Zeller B, Tamnes CK, Kanellopoulos A, et al: Reduced neuroanatomic volumes in long-term survivors of childhood acute lymphoblastic leukemia. *J Clin Oncol* 31:2078-2085, 2013
- Schuitema I, de Sonnevile L, Kaspers G, et al: Executive dysfunction 25 years after treatment with cranial radiotherapy for pediatric lymphoid malignancies. *J Int Neuropsychol Soc* 21:657-669, 2015
- Schuitema I, Deprez S, Van Hecke W, et al: Accelerated aging, decreased white matter integrity, and associated neuropsychological dysfunction 25 years after pediatric lymphoid malignancies. *J Clin Oncol* 31:3378-3388, 2013
- Yang L, Hu X, Xu L: Impact of methylenetetrahydrofolate reductase (MTHFR) polymorphisms on methotrexate-induced toxicities in acute lymphoblastic leukemia: A meta-analysis. *Tumour Biol* 33:1445-1454, 2012
- Dulucq S, Laverdière C, Sinnett D, et al: Pharmacogenetic considerations for acute lymphoblastic leukemia therapies. *Expert Opin Drug Metab Toxicol* 10:699-719, 2014
- Chechik G, Meilijson I, Ruppin E: Neuronal regulation: A mechanism for synaptic pruning during brain maturation. *Neural Comput* 11:2061-2080, 1999
- Gogtay N, Giedd JN, Lusk L, et al: Dynamic mapping of human cortical development during childhood through early adulthood. *Proc Natl Acad Sci USA* 101:8174-8179, 2004

26. Carey ME, Haut MW, Reminger SL, et al: Reduced frontal white matter volume in long-term childhood leukemia survivors: A voxel-based morphometry study. *AJNR Am J Neuroradiol* 29:792-797, 2008
27. Reddick WE, Glass JO, Johnson DP, et al: Voxel-based analysis of T2 hyperintensities in white matter during treatment of childhood leukemia. *AJNR Am J Neuroradiol* 30:1947-1954, 2009
28. Krull KR, Bhojwani D, Conklin HM, et al: Genetic mediators of neurocognitive outcomes in survivors of childhood acute lymphoblastic leukemia. *J Clin Oncol* 31:2182-2188, 2013
29. Drozdowicz LB, Bostwick JM: Psychiatric adverse effects of pediatric corticosteroid use. *Mayo Clin Proc* 89:817-834, 2014
30. Edelmann MN, Ogg RJ, Scoggins MA, et al: Dexamethasone exposure and memory function in adult survivors of childhood acute lymphoblastic leukemia: A report from the SJLIFE cohort. *Pediatr Blood Cancer* 60:1778-1784, 2013
31. Dresler M, Genzel L, Kluge M, et al: Off-line memory consolidation impairments in multiple sclerosis patients receiving high-dose corticosteroid treatment mirror consolidation impairments in depression. *Psychoneuroendocrinology* 35:1194-1202, 2010
32. Andreotti C, Root JC, Ahles TA, et al: Cancer, coping, and cognition: a model for the role of stress reactivity in cancer-related cognitive decline. *Psychooncology*, 24:617-623, 2014

Apply for Quality Oncology Practice Initiative (QOPI) Certification to Join More Than 260 Certified Practices

Certification signifies that the practice or institution has:

- A commitment to provide quality cancer care
- The resources, programs, and services sufficient to accomplish and sustain that commitment
- Participated in a voluntary comprehensive site assessment against clearly specified national standards of care
- Succeeded in meeting the standards and objectives of the Certification Program

Learn more at qopi.asco.org/certification.html.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Chemotherapy Pharmacodynamics and Neuroimaging and Neurocognitive Outcomes in Long-Term Survivors of Childhood Acute Lymphoblastic Leukemia

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or jco.ascopubs.org/site/ifc.

Kevin R. Krull

No relationship to disclose

Yin Ting Cheung

No relationship to disclose

Wei Liu

No relationship to disclose

Slim Fellah

No relationship to disclose

Wilburn E. Reddick

No relationship to disclose

Tara M. Brinkman

No relationship to disclose

Cara Kimberg

No relationship to disclose

Robert Ogg

No relationship to disclose

Deokumar Srivastava

No relationship to disclose

Ching-Hon Pui

No relationship to disclose

Leslie L. Robison

No relationship to disclose

Melissa M. Hudson

No relationship to disclose

Acknowledgment

We thank the laboratory of Mary Relling at St Jude Children's Research Hospital for providing results of pharmacokinetic studies conducted during the therapeutic trial.

Appendix

Methods: Brain Image Processing

The T1-weighted molecular resonance imaging (MRI) set was processed with the FreeSurfer software (<http://surfer.nmr.mgh.harvard.edu/>). This processing included removal of nonbrain tissue, normalization to the Talairach space, segmentation of the subcortical white matter and deep gray matter volumetric structures, intensity normalization, tessellation of the gray/white matter boundary, topology correction, and surface deformation according to intensity gradients.¹⁸ In all, the FreeSurfer software provides parcellations for 34 cortical and seven subcortical regions for each hemisphere. Procedures for the measurement of cortical thickness have been validated against manual measurements and have been demonstrated to show good test-retest reliability across scanner manufacturers and across field strengths.¹⁸

For the diffusion tensor imaging (DTI) set, voxel-wise tensor calculations were performed with the Diffusion II toolkit under SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/>). All four DTI acquisitions were corrected for eddy current drift, distortions, and slight misalignments during the acquisition before the diffusion tensors for each voxel were calculated. Reorientation of the b matrix was not performed. The Eigen values derived from the imaging tensor were used to calculate the parameter maps of fractional anisotropy (FA), axial (AD), and radial (RD) diffusivity. Parameter maps were registered to the patient T1 using FLIRT from the FMRIB Software Library (<http://fsl.fmrib.ox.ac.uk/fsl/>). The FreeSurfer normalization was then used to bring the cortical and subcortical labels from the Desikan-Killiany Atlas back to the patient T1 space. Probabilistic fiber tracking was performed for individual seed points within the rostral middle frontal and the caudate, putamen, and pallidum regions identified with FreeSurfer. Fiber tracking was performed with the PROBTRAX2 routine in FMRIB Software Library using 5,000 permutations for each seed. Tracks were computed separately for each of the three subcortical regions to the rostral middle frontal regions, and only tracks between the two regions were kept. Any pixel found in any of the three individual pathways was kept to represent the frontostriatal tract. The parameter values of FA, RD, and AD were then extracted from each voxel within the identified tract, and mean values were calculated. Mean diffusivity is a weighted average of the AD and RD measures and, as such, does not provide any additional information beyond that contained in the other two measures. Additionally, mean diffusivity lacks the specificity provided by the individual diffusivity measures.

A more coarse grain analysis of the white matter was performed by using a previously defined region of interest within the deep white matter in which white matter damage, leukoencephalopathy, is most likely to develop.²⁷ The patient T1 images were spatially normalized to the Talairach space by using FNIRT from FMRIB Software Library. The inverse of this normalization was combined with the inverse of the registration between the DTI and patient T1 in the tractography to move the regions of interest into patient space. The previously defined region of interest²⁷ was divided into frontal and parietal sections by using a coronal plane at the midpoint between the most anterior extent of the genu and the most posterior extent of the splenium of the corpus callosum. The voxels of the region of interest that were anterior to this coronal plan were identified as frontal, and those voxels posterior to the plane were identified as parietal. These regions were then overlain on the DTI parameter maps in the patient space, and mean values were extracted. In none of these analyses was spatial smoothing applied.

Fig A1. Associations between methotrexate exposure, assessed as area under the curve, and executive function, stratified by low- and standard/high-risk treatment arms. Correlation coefficient for spatial memory span in low risk was -0.21 ($P = .04$) and in standard/high risk was -0.55 ($P < .001$). Correlation coefficient for numerical processing speed in low risk was -0.17 ($P = .08$) and in standard/high risk was -0.46 ($P < .001$).

Fig A2. Associations between dexamethasone exposure, assessed as area under the curve, and cortical thickness in the left and right dorsolateral prefrontal cortex (top; $r = -0.14$, $P = .08$ and $r = -0.11$, $P = .17$, respectively) and left and right anterior cingulate cortex (bottom; $r = -0.05$, $P = .60$ and $r = -0.21$, $P = .008$, respectively).

Table A1. Schema of Total XV Treatment Protocol for Acute Lymphoblastic Leukemia and Time Points of Data Collection

Treatment Phase	Upfront Window (4 days)	Induction (6-7 weeks)	Consolidation (8 weeks)	Reintensification (1 week)	Reinduction I (7-9 weeks)	Reinduction II (17-19 weeks)	Continuation (120-146 weeks)
Low risk	HDMTX + LV, IT ARA-C	PRED, VCR, DNR, ASP, CYCLO, ARA-C, MP, IT MHA	HDMTX + LV, IT MHA, MP*	NA	DEX, VCR, ASP, DOX, IT MHA†	DEX, VCR, ASP, DOX, IT MHA	MP, DEX, VCR, MTX, IT MHA
Standard/ high risk				DEX, ARA-C, VP16, ASP, IT MHA		DEX, VCR, ASP, DOX, IT MHA, HDARA-C	DEX, DOX, VCR, MP, ASP, IT MHA, MTX, CYCLO, ARA-C

Abbreviations: ASP, L-asparaginase; ARA-C, cytarabine; CYCLO, cyclophosphamide; DEX, oral dexamethasone; DOX, doxorubicin; DNR, daunorubicin; HDARA-C, high-dose cytarabine; HDMTX, high-dose methotrexate; IT ARA-C, intrathecal cytarabine; IT MHA, intrathecal methotrexate + hydrocortisone + cytarabine; LV, leucovorin; MP, mercaptopurine; MTX, methotrexate; NA, not available; PK, pharmacokinetics; PRED, prednisolone; VP16, etoposide; VCR, vincristine.

*HDMTX and homocysteine PK measurements.

†Oral DEX PK measurements.

Table A2. Multivariable General Linear Models That Predict Nonexecutive Function Cognitive Domains

Cognitive Domain*																																						
Memory						Attention				Perceptual				Processing Speed																								
Digit Forward						Spatial Forward				Omissions				Block Design				Dominant hand			Digit Symbol			Number Sequencing			Letter Sequencing											
Parameter	Estimate	SE	P			Estimate	SE	P			Estimate	SE	P			Estimate	SE	P			Estimate	SE	P			Estimate	SE	P										
Pharmacologic																																						
Total IT counts	0.02	0.03	.49			0.03	0.02	.27			-0.03	0.04	.56			0.00	0.03	.93			-0.00	0.04	.91			-0.01	0.02	.65			0.04	0.03	.15			0.00	0.03	.98
Methotrexate AUC	-0.01	0.01	.27			-0.02	0.01	.003			-0.02	0.01	.13			-0.01	0.01	.35			0.01	0.01	.50			-0.01	0.01	.21			-0.02	0.01	.02			-0.01	0.01	.48
Dexamethasone AUC	-0.00	0.00	.64			0.00	0.00	.65			0.00	0.00	.99			0.00	0.00	.81			0.00	0.00	.28			0.00	0.00	.21			0.00	0.00	.81			-0.00	0.00	.26
Biomarker																																						
Homocysteine AUC	-0.00	0.00	.96			0.00	0.01	.47			0.00	0.01	.61			-0.01	0.01	.20			0.00	0.01	.87			-0.00	0.00	.68			-0.01	0.01	.09			0.00	0.01	.92

Abbreviations: AUC, area under curve; IT, intrathecal; SE, standard error.

*Multivariable models on each neurocognitive outcome with adjustment for demographic risk factors (age of diagnosis, sex, and highest education of parents). Models on each outcome were conducted separately for pharmacologic and biomarker risk factors because of confounding between the methotrexate AUC and the homocysteine AUC.

Table A3. Association Between Neurocognitive Impairment and Magnetic Resonance Imaging Outcomes

Outcome	Impaired (n = 56)		Not Impaired (n = 115)		P*
	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)	
Diffusion tensor imaging: frontal lobes					
Fractional anisotropy	0.37 (0.03)	0.37 (0.29-0.46)	0.38 (0.02)	0.39 (0.32-0.46)	.005
Axial diffusivity	1.07 (0.07)	1.06 (0.97-1.49)	1.06 (0.04)	1.06 (0.97-1.15)	.51
Radial diffusivity	0.60 (0.06)	0.59 (0.52-0.96)	0.58 (0.03)	0.59 (0.51-0.67)	.03
Diffusion tensor imaging: frontostriatal tract					
Fractional anisotropy	0.38 (0.04)	0.39 (0.21-0.45)	0.40 (0.03)	0.40 (0.34-0.45)	.01
Axial diffusivity	1.13 (0.05)	1.13 (1.06-1.31)	1.13 (0.04)	1.13 (1.04-1.29)	.82
Radial diffusivity	0.62 (0.06)	0.61 (0.53-0.93)	0.60 (0.04)	0.60 (0.51-0.75)	.03
Diffusion tensor imaging: parietal lobes					
Fractional anisotropy	0.40 (0.03)	0.41 (0.33-0.46)	0.41 (0.03)	0.41 (0.33-0.51)	.29
Axial diffusivity	1.19 (0.05)	1.19 (1.07-1.31)	1.18 (0.04)	1.18 (1.07-1.33)	.34
Radial diffusivity	0.63 (0.05)	0.62 (0.53-0.78)	0.61 (0.04)	0.61 (0.51-0.73)	.12
Magnetic resonance imaging: cortical thickness					
Dorsolateral prefrontal	2.68 (0.18)	2.69 (2.23-3.00)	2.73 (0.19)	2.73 (1.94-3.16)	.13
Anterior cingulate	2.86 (0.17)	2.85 (2.54-3.24)	2.91 (0.21)	2.94 (2.13-3.45)	.07

NOTE. Impairment is based on having two or more neurocognitive tests that fall more than 1.5 SDs less than the age-adjusted population normative data.

Abbreviation: SD, standard deviation.

*P value is based on Wilcoxon test.