Escalating intravenous methotrexate improves event-free survival in children with standard-risk acute lymphoblastic leukemia: a report from the Children's Oncology Group

Yousif Matloub,[1] Bruce C. Bostrom,[2] Stephen P. Hunger,[3] Linda C. Stork,[4] Anne Angiolillo,[5] Harland Sather,[6] Mei La,[6] Julie M. Gastier-Foster,[7] Nyla A. Heerema,[8] Scott Sailer,[9] Patrick J. Buckley,[10] Blythe Thomson,[11] Catherine Cole,[12] James B. Nachman,[13] Gregory Reaman,[14] Naomi Winick,[15] William L. Carroll,[16] Meenakshi Devidas,[17] and Paul S. Gaynon[18]

[1]Division of Hematology-Oncology, Rainbow Babies & Children's Hospital, Case Western Reserve University, Cleveland, OH; [2]Children's Hospitals and Clinics of Minnesota, Minneapolis, MN; [3]The University of Colorado School of Medicine and the Children's Hospital, Aurora, CO; [4]Doernbecher Children's Hospital, Oregon Health & Science University, Portland, OR; [5]Children's National Medical Center, Washington, DC; [6]Operation Center, Children's Oncology Group, Arcadia, CA; [7]Laboratory Medicine, Nationwide Children's Hospital, Columbus, OH; [8]Department of Pathology, The Ohio State University, Columbus, OH; [9]University of North Carolina at Chapel Hill, Chapel Hill, NC; [10]Duke University Medical Center, Durham, NC; [11]Hematology-Oncology, Seattle Children's Hospital, University of Washington, Seattle, WA; [12]Princess Margaret Hospital for Children, Perth, Australia; [13]The University of Chicago Comer Children's Hospital, Chicago, IL; [14]Chair's Office, Children's Oncology Group, Bethesda, MD; [15]University of Texas Southwestern Medical Center, Dallas, TX; [16] New York University Cancer Institute, New York, NY; [17]Children's Oncology Group Statistics and Data Center, Gainesville, and University of Florida, Gainesville, FL; and [18]Division of Hematology-Oncology, Children's Hospital Los Angeles, Los Angeles, CA

Children's Cancer Group-1991 selected 2 components from the Children's Cancer Group studies shown to be effective in high-risk acute lymphoblastic leukemia and examined them in children with National Cancer Institute standard-risk acute B-precursor lymphoblastic leukemia. These were (1) vincristine and escalating IV methotrexate (MTX) without leucovorin rescue during the interim maintenance (IM) phases and (2) addition of a second delayed intensification (DI) phase. Eligible patients (n = 2078) were randomly assigned to regimens containing either oral (PO) MTX, PO mercaptopurine, dexamethasone, and vincristine or IV MTX during IM phases, and regimens with either single DI or double DI. Five-year event-free survival (EFS) and overall survival for patients on the PO MTX arms were 88.7% ± 1.4% and 96% ± 0.9% versus 92.6% ± 1.2% and 96.5% ± 0.8% for those on the IV MTX arms (*P* = .009, *P* = .66). Five-year EFS and overall survival for patients who received single DI were 90.9% ± 1.3% and 97.1% ± 0.8% versus 90.5% ± 1.3% and 95.4% ± 3.8% for those who received double DI (P = .71, *P* = .12). No advantage was found for a second DI; however, replacement of PO MTX, PO mercaptopurine, vincristine, and dexamethasone during IM with vincristine and escalating IV MTX improved EFS. (*Blood*. 2011;118(2):243-251)

Introduction

In the early 1980s the Berlin-Franfurt-Münster (BFM) investigators demonstrated the outcome benefit of postinduction intensification (PII) in children with acute lymphoblastic leukemia (ALL).[1] This same group later found that it also benefited children with lower-risk ALL.[2] This was confirmed by Children's Oncology Group (CCG) investigators in CCG-105 that showed a definite outcome improvement in children with intermediate-risk ALL who received a course of delayed intensification (DI) compared with those who did not; 5-year event-free survival (EFS) 77% versus 61% ($P = .001$).[3] This clinical trial was followed by CCG-1891, a modified BFM protocol, which tested the hypothesis that double DI (DDI) would result in superior outcomes compared with single DI (SDI) in children with intermediate-risk ALL that included patients with National Cancer Institute (NCI) standard-risk ALL (SR-ALL).[4,5] Six-year EFS and overall survival were 83% ± 2% and 91% ± 2% for the DDI regimen versus 79% ± 1% and 87% ± 2% for the SDI regimen ($P = .04$, $P = .17$).[6] There was, however, no clear EFS benefit to DDI among patients with M1 (< 5% blasts) BM on day 7 of induction (relative risk DI vs DDI = 1.16).

The CCG and other cooperative groups showed mixed results in different regimens evaluating the addition of IV methotrexate (MTX) to PII.[7-11] The CCG conducted a trial (CCG-1882) for high-risk patients with slow early response using an augmented BFM backbone. This regimen used escalating intermediate-dose IV MTX without leucovorin rescue and additional doses of vincristine and asparaginase during the 2 IM phases of therapy. In addition, it included a second DI and additional doses of vincristine and asparaginase during the consolidation and reconsolidation phases. Compared with the "standard" regimen with oral (PO) MTX and PO mercaptopurine (6-MP) in IM, the augmented regimen that included IV MTX in IM resulted in significantly improved 5-year EFS, 75% versus 55% ($P < .001$).[12] Notably, the advantage of the augmented regimen was most pronounced in patients 1-9 years of age. CCG-1922, a study for patients with NCI SR-ALL, showed that dexamethasone given during induction and maintenance achieved a higher EFS compared with prednisone. The outcome of patients who received dexamethasone and SDI on CCG-1922 was equivalent to that of those who received prednisone

Submitted December 10, 2010; accepted April 25, 2011. Prepublished online as *Blood* First Edition paper, May 11, 2011; DOI 10.1182/blood-2010-12-322909.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

Table 1. Details of therapy in the randomized regimens

Phase	Drug	Dose	Schedule
Induction (4 wk)	IT cytarabine	Age-adjusted*	Day 0
	Vincristine	1.5 mg/m^2 (2 mg max)	Days 0, 7, 14, 21
	Pegaspargase†	2500 U/m^2	Between days 3 and 5
	Dexamethasone	6 mg/m^2/d	Days 0-27
	IT MTX	Age-adjusted*	Days 7, 28
Consolidation (4 wk)	Vincristine	1.5 mg/m^2 (2 mg max)	Day 0
	Mercaptopurine	75 mg/m^2/d	Days 0-27
	IT MTX	Age-adjusted*	Days 7, 14, 21
IM-1 (8 wk), OS/OD arms	Vincristine	1.5 mg/m^2 (2 mg max)	Days 0, 28
	Dexamethasone	6 mg/m^2/d	Days 0-4, 28-32
	Mercaptopurine	75 mg/m^2/d	Days 0-49
	MTX	20 mg/m^2/d	Weekly for 8 doses
	IT MTX	Age-adjusted*	Day 28
IM-1 (8 wk), IS/ID arms	Vincristine	1.5 mg/m^2 (2 mg max)	Every 10 days for 5 doses
	IV MTX‡	100 mg/m^2/d, starting dose	Every 10 days for 5 doses
	IT MTX	Age-adjusted*	Day 30
DI-1 (8 wk)	Vincristine	1.5 mg/m^2 (2 mg max)	Days 0, 7, 14
	Pegaspargase	2500 U/m^2/dose	Day 3
	Dexamethasone	10 mg/m^2/d	Days 0-6, 14-20
	Doxorubicin	25 mg/m^2/d	Days 0, 7, 14
	Cytarabine	75 mg/m^2/d	Days 28-31, 35-38
	Cyclophosphamide	1000 mg/m^2	Day 28
	Thioguanine	60 mg/m^2/d	Days 28-41
	IT MTX	Age-adjusted*	Days 0, 28
IM-2(8 wk), OS/OD arms	Same as IM-1 with the addition of IT MTX on day 0		
IM-2 (8 wk), IS/ID arms§	Same as IM-1 with the addition of IT MTX on day 0		
DI-2 (8 wk), only OD/ID	Same as DI-1		
Maintenance (12-wk cycles)‖	Vincristine	1.5 mg/m^2 (2 mg max)	Days 0, 28, 56
	Dexamethasone	6 mg/m^2/d	Days 0-4, 28-32, 56-60
	Mercaptopurine	75 mg/m^2/d	Daily
	MTX (oral)	20 mg/m^2/dose	Weekly
	IT MTX	Age-adjusted*	Day 0

IT indicates intrathecal; MTX, methotrexate; IM, interim maintenance phase; OS, included regimen of PO MTX, mercaptopurine, vincristine, and dexamethasone (during the IM phases) and single delayed intensification; OD, included regimen of PO MTX, mercaptopurine, vincristine, and dexamethasone (during the IM phases) and double delayed intensification; IS, included regimen of IV MTX and vincristine (during the IM phases) and single delayed intensification; ID, included regimen of IV MTX and vincristine (during the IM phases) and double delayed intensification; and DI, delayed intensification phase.

*IT cytarabine was adjusted for age as follows: 1-1.99 years, 30 mg; 2-2.99 years, 50 mg; > 3 years, 70 mg. IT MTX was adjusted for age as follows: 1-1.99 years, 8 mg; 2-2.99 years, 10 mg; > 3 years, 12 mg.

†Asparaginase preparation: pegylated asparaginase; *Erwinia* asparaginase replaced pegaspargase after severe allergic reactions.

‡IV MTX initial dose was 100 mg/m^2; dose was escalated by 50 mg/m^2 every 10 days (± 2 days) for 4 doses, to toxicity.

§Starting dose of IV MTX is two-thirds maximum tolerated dose in IM-1.

‖Total duration of treatment for boys was 38 months and girls was 26 months.

and DDI in CCG-1891, raising the question of whether replacement of prednisone with dexamethasone and the administration of an additional DI phase were additive or redundant interventions.[13] The CCG-1991 SR-ALL trial was opened for accrual in 2000. Children enrolled in this trial received "standard" CCG-modified BFM backbone therapy with dexamethasone used as the sole corticosteroid during steroid-containing phases of therapy. CCG-1991 had 2 primary aims for patients with NCI SR-ALL and rapid early response to therapy. The first was to determine in a randomized fashion the benefit of a second DI when added to a dexamethasone backbone. The second was to compare outcome with 2 different treatments during two 8-week IM phases: escalating-dose IV MTX without leucovorin and vincristine versus standard PO MTX, PO 6-MP, vincristine, and dexamethasone.

Methods

CCG-1991 opened in June 2000 and completed accrual in February 2005. Eligible patients were ≥ 1 and < 10 years of age with presenting white blood cell (WBC) count < 50×10^9/L. Diagnosis of ALL required a BM aspirate with > 25% L1 or L2 lymphoblasts by French-American-British morphology, negative histochemical staining for myeloperoxidase, and reactivity with monoclonal antibodies to B-lineage– or T-lineage–associated differentiation antigens, as previously described.[14,15] Patients with L3 morphology or with t(8;14), t(8;22), or t(2;8) were excluded. Patients with systemic corticosteroid exposure for > 48 hours during the preceding month were ineligible. Patients with standard-risk T-cell ALL were initially eligible for enrollment, and, if they met the randomization eligibility criteria, they were randomly assigned to the 4 regimens mentioned below. However, interim study analysis by the Children's Oncology Group (COG) Data Monitoring Committee in March 2004 showed an inferior outcome of patients with T-cell immunophenotype enrolled on CCG-1991 than did patients with B-precursor ALL and patients with T-cell ALL enrolled on more-intensified COG protocols. Therefore, CCG-1991 was closed to further accrual of patients with T-cell ALL.

Treatment protocol

The NCI and institutional review boards of all participating CCG institutions approved the study. Written informed consent was obtained from parents or guardians according to the guidelines of the National Institutes of Health and the Declaration of Helsinki. Patients received 3-drug induction therapy with intrathecal (IT) cytarabine for 1 dose, IV vincristine, PO

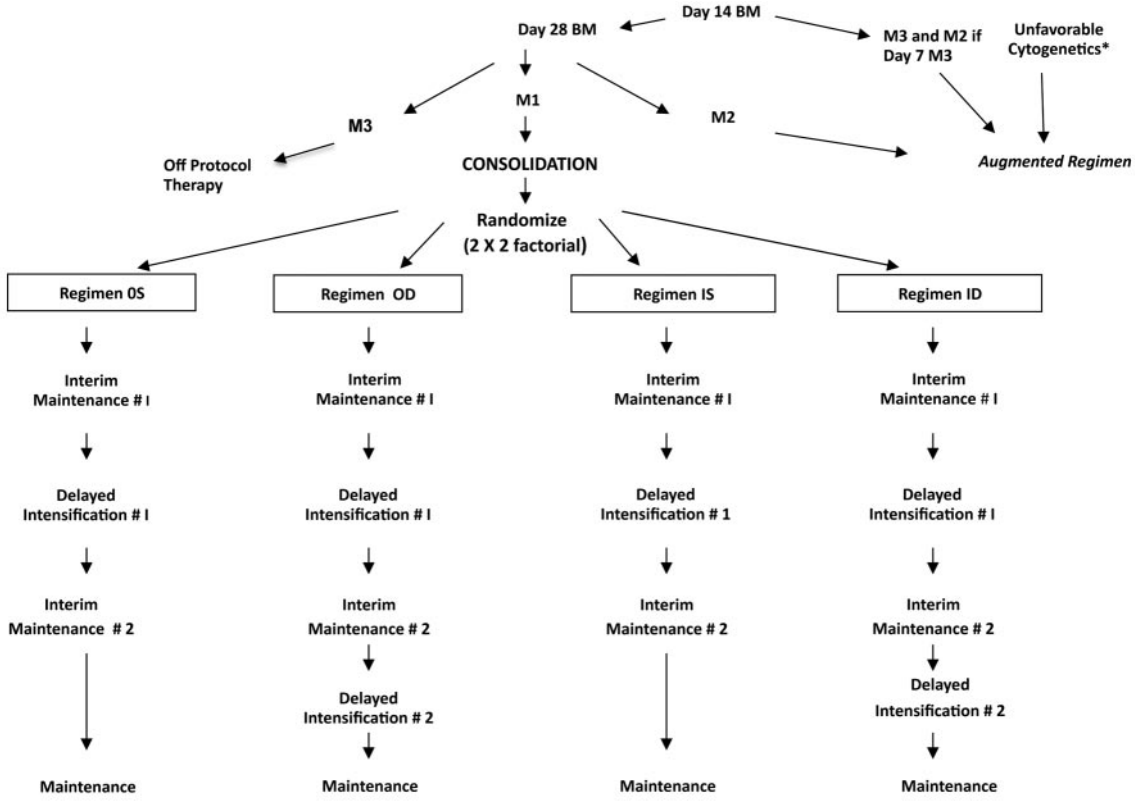

Figure 1. Protocol schema. OS, included regimen of oral (PO) methotrexate (MTX), mercaptopurine, vincristine, and dexamethasone (during the interim maintenance [IM] phases) and single delayed intensification; OD, included regimen of PO MTX, mercaptopurine, vincristine, and dexamethasone (during the IM phases) and double delayed intensification; IS, included regimen of IV MTX and vincristine (during the IM phases) and single delayed intensification; ID, included regimen of IV MTX and vincristine (during the IM phases) and double delayed intensification. *t(4;11)(q21;q23)(q34;q11), t(9;22), balanced t(1;19)(q23;p13), and hypodiploidy with < 45 chromosomes.

dexamethasone, intramuscular pegylated asparaginase, and IT MTX for 2 doses. Details of therapy are listed in Table 1. Marrow status was determined by local institutions; patients with $\geq 5\%$ BM blasts on day 7 were reassessed with aspirates on day 14. Randomization required $< 25\%$ blasts (M1 or M2 marrow status) by day 14, remission status with $< 5\%$ blasts (M1) at day 28 of induction, and no unfavorable leukemia cytogenetics [t(4;11)(q21;q23), t(9;22)(q34;q11), balanced t(1;19)(q23;p13), or hypodiploidy with < 45 chromosomes]. Patients with unfavorable cytogenetics, M3 BM ($> 25\%$ blasts) on day 7 induction and M2 (5%-25% blasts) on day 14, or M3 BM on day 14 were nonrandomly assigned to receive more intensive therapy, the Augmented Regimen. This stratification was based on the results of the predecessor study (CCG-1952) that showed that these patients had an inferior outcome than did the other subgroups.[16] Randomization occurred between days 21 and 28 of the consolidation phase of therapy for those who met the randomization criteria. Eligible patients were assigned randomly in a 2×2 factorial design to 1 of 4 treatment regimens, as shown in Figure 1: regimen OS included PO MTX, 6-MP, vincristine, and dexamethasone (during the IM phases) and SDI; regimen OD included PO MTX, 6-MP, vincristine, and dexamethasone (during the IM phases) and DDI; regimen IS included IV MTX and vincristine (during the IM phases) and SDI; and regimen ID included IV MTX and vincristine (during the IM phases) and DDI. All patients received 2 IM courses regardless of the number of courses of DI. Girls were treated for 2 years and boys for 3 years from the start of IM 1. Patients with CNS-1 (cerebrospinal fluid [CSF] WBC count $< 5/\mu L$, without blasts) and CNS-2 (CSF WBC count $< 5/\mu L$ with blasts present) status or traumatic taps received standard IT and systemic therapy. Patients with CNS-3 (CSF WBC count $\geq 5/\mu L$ with blasts present) were nonrandomly assigned to regimen OD with 1800 cGy of cranial irradiation given during consolidation. The protocol required patients with biopsy-proven testicular involvement to receive 2400 cGy of testicular irradiation during consolidation. This report presents the outcome of the random assignment of patients with B-precursor SR-ALL with a rapid early response. Patients with T-cell ALL and those nonrandomly assigned to receive the augmented regimen will be described in separate reports.

BM and cytogenetic evaluations

Cytogenetic evaluation was performed on diagnostic BM samples at local institutions with the use of standard techniques and nomenclature.[17] The CCG Cytogenetics Committee centrally reviewed each karyotype. *ETV6/RUNX1* expression was analyzed by RT-PCR in the CCG ALL Reference Laboratory on blasts from the first thousand subjects. RT-PCR was performed with the conditions and primers reported by Shurtleff et al[18] Local institutions determined BM blast percentage of aspirates on induction days 7, 14, and 28, as has been standard on CCG protocols.

Relapse definitions

BM relapse was defined as an M3 BM after achieving initial remission. Combined relapses included those with simultaneous recurrence in BM and extramedullary site(s). CNS relapse was diagnosed when CSF contained ≥ 5 WBC count/μL with morphologically identifiable blasts on a cytospin preparation; BM aspirations showed normal trilineage hematopoiesis with M1 status, testes were normal to palpation, and no other extramedullary sites of disease were identified.

Statistical methods and analyses

Data current as of October 2008 are used in this report. Patients in remission at the end of consolidation were randomly assigned in a 2×2 factorial design that included a double randomization to test the relative benefits of SDI versus DDI and PO MTX versus escalating-dose IV MTX without leucovorin rescue. Outcome analyses used life table methods and associated statistics. The primary endpoints examined were EFS and overall survival from the time of randomization. Events included relapse at any site, death in remission, or a second malignant neoplasm, whichever occurred first.

Table 2. Presenting features of the randomly assigned patients with B-precursor acute lymphoblastic leukemia

	PO MTX, n	IV MTX, n	*P*	SDI, n	DDI, n	*P*
Age						
< 2 y	85	82		79	88	.76
2-5 y	733	720	.55	726	727	
6-9 y	218	240		232	226	
Sex						
F	475	454	.32	451	478	.29
M	561	588		586	563	
Ethnicity						
White	705	719		721	703	
Black	40	36		36	40	
Hispanic	196	203	.99	194	205	.26
Asian	28	27		21	34	
Other	67	57		65	59	
WBC count						
$< 20 \times 10^9$/L	856	867	.92	871	852	.14
$> 20 \times 10^9$/L	176	175		162	189	
CNS status						
CNS-1	799	831		809	829	
CNS-2	32	35	.08	32	35	.87
TLP^+	25	12		17	20	
Cytology						
*ETV6/RUNX1**	176	182	.95	182	176	.58
Trisomy 4 and 10†	117	133	.30	132	118	.47
Down syndrome	44	31	.15	42	33	.33
BM day 7						
MI	495	470		491	474	
M2	298	319	.45	300	317	.45
M3	216	227		218	225	
BM day 14						
MI	532	579	.45	556	555	.06
M2	18	18		10	22	

PO indicates oral; MTX, methotrexate; SDI, single delayed intensification phase; DDI, double delayed intensification phase; WBC, white blood cell; and TLP^+, traumatic lumbar punctures with lymphoblasts present in the spinal fluid.

*Patients (n = 1041) were analyzed for the presence of *ETV6/RUNX1* transcript.

†Patients (n = 1330) were evaluated for the presence of trisomies 4 and 10.

Patients who had not had an event were censored at the time of last contact. Life table estimates were calculated by the Kaplan-Meier method, and standard errors of the estimate were obtained by the Peto method. The log-rank test was used to compare survival curves between groups. Five-year estimates of survival rates are presented in this report. The chi-square test for homogeneity of proportions was used to compare baseline patient clinical characteristics.

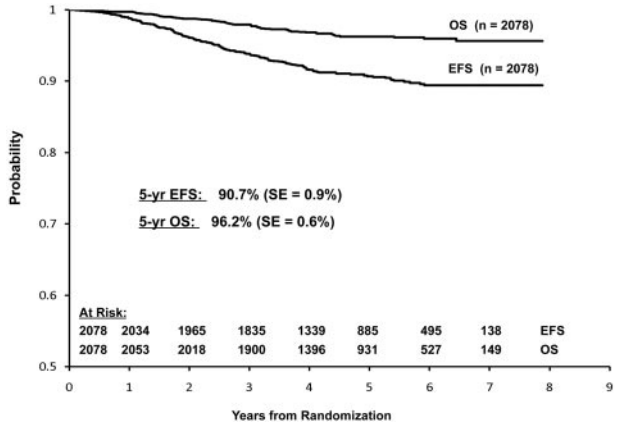

Figure 2. Event-free survival (EVS) and overall survival (OS) in randomly assigned patients with B-precursor acute lymphoblastic leukemia.

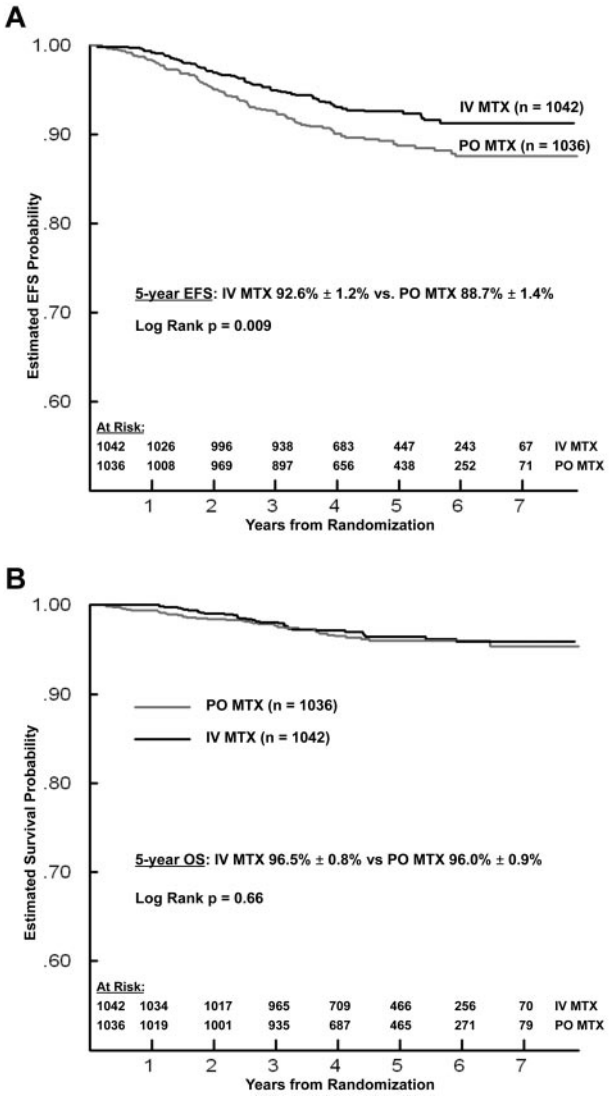

Figure 3. Event-free survival (EFS) and overall survival (OS) by route of methotrexate (MTX) administration. (A) EFS by route of MTX administration in the interim phases. (B) OS by route of MTX administration in the interim maintenance phases. PO indicates oral.

Results

Patient characteristics

A total of 3054 patients from 109 institutions were entered on study between June 1, 2000, and January 31, 2005. Twenty-eight patients were found to be ineligible. Three patients were found to have acute myelogenous leukemia; 2 patients had B-cell ALL, 1 had ALL as a second malignancy, 14 had consent deficiencies, 1 patient's parent withdrew the study consent; 1 was not registered within the required period; 1 received the first IT chemotherapy beyond the allowed 72-hour window before starting systemic chemotherapy; 1 patient was enrolled before 1 year of age; 1 was found ineligible because of a WBC count $>50 \times 10^9$/L; 1 received systemic corticosteroids for longer than 48 hours within 30 days of diagnosis; 1 started treatment before registration; and 1 was registered in error. Four-hundred fifty-six parents/guardians decided not to sign the second consent or to remove their children from the study. Two hundred eighty-three patients were found to

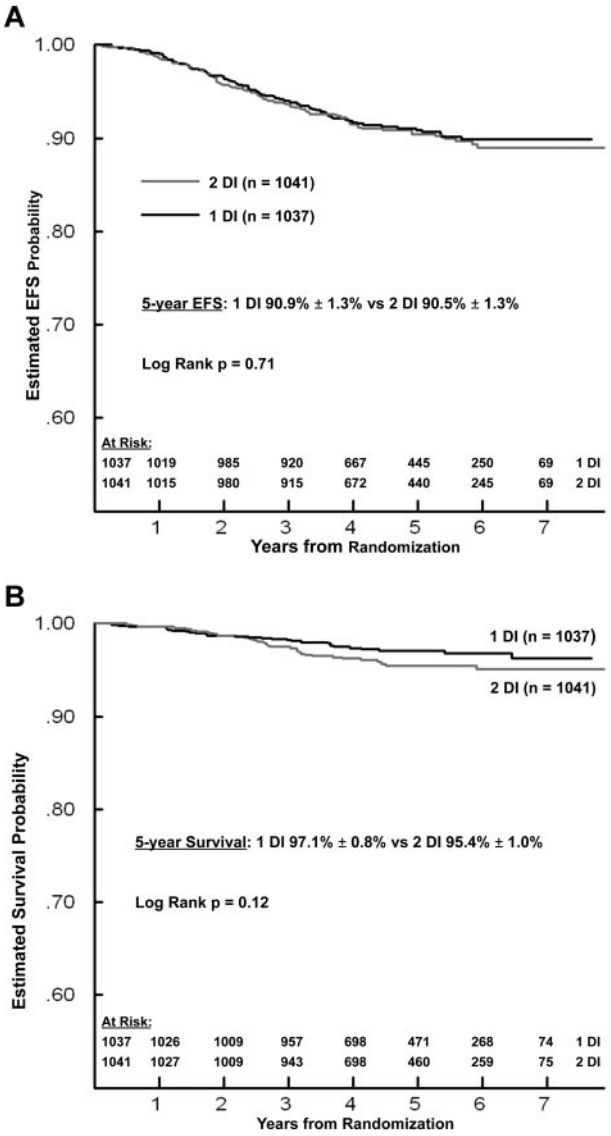

Figure 4. Event-free survival (EFS) and overall survival by number of delayed intensification (DI) phases. EFS (A) and overall survival (B) by number of DI phases.

have high-risk features and so were nonrandomly assigned to the Augmented Regimen. Thirty-five patients were found to have CNS leukemia at diagnosis and were assigned to the OD regimen with cranial irradiation as directed by the study.

Twenty-six patients were determined to be ineligible for randomization. Reasons for the latter were marrow evaluations either unavailable or erroneously reported (n = 10), second consent deficiencies (n = 9), too early randomization (n = 4), and off-protocol therapy at end of induction (n = 3). Among eligible randomly assigned patients with B-precursor ALL, 512 patients were randomly assigned to the OS arm, 524 patients to the OD arm, 525 to the IS arm, and 517 to the ID arm. Presenting features of these patients are listed in Table 2.

Table 3. Distribution of events in the randomly assigned patients with B-precursor acute lymphoblastic leukemia

	PO MTX, n	IV MTX, n	SDI, n	DDI, n
Isolated BM relapse	43	51	42	52
Isolated CNS relapse	26	11	21	16
BM + CNS	10	5	8	7
BM + EM	4	3	2	5
Testicular	7	0	4	3
Other EM	6	2	5	3
Death as first event	5	1	3	3
Total	101	73	85	89

PO indicates oral; MTX, methotrexate; SDI, single delayed intensification phase; DDI, double delayed intensification phase; and EM, extramedullary site relapse.

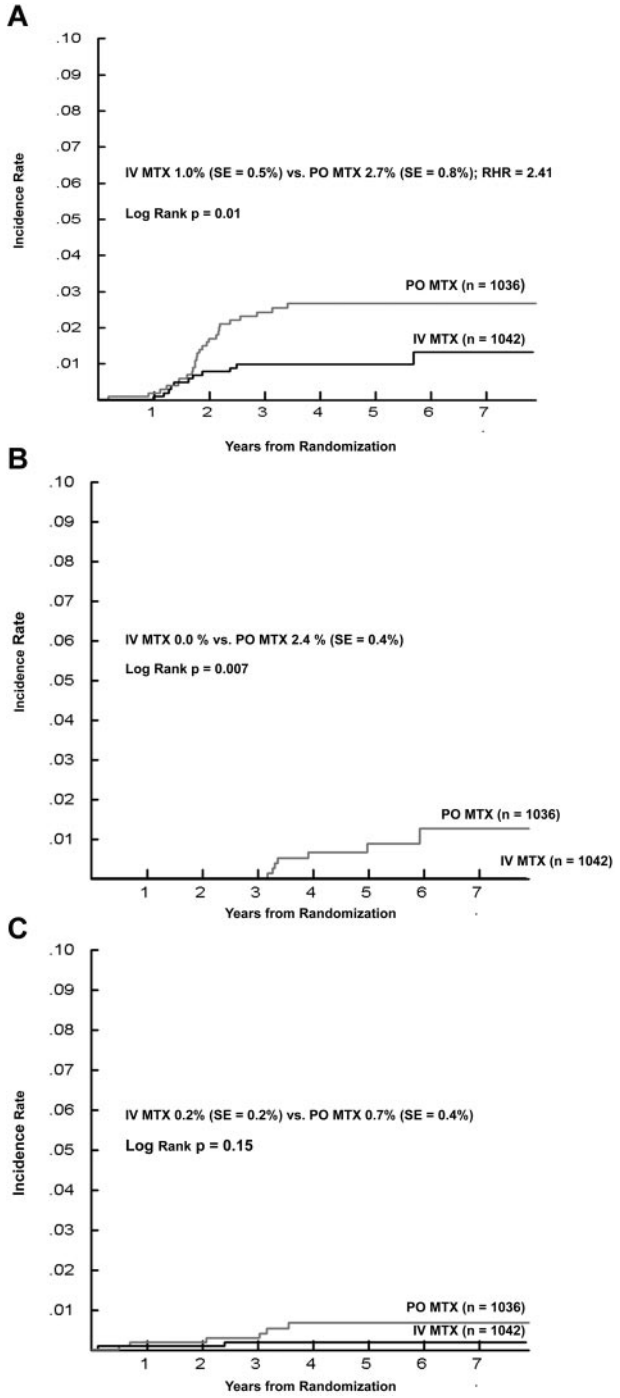

Figure 5. Five-year cumulative incidence of isolated relapse by route of methotrexate (MTX) administration during the interim maintenance phases. Five-year cumulative incidence of isolated CNS relapse (A), isolated testicular relapse (B), and isolated non-CNS nontesticular extramedullary relapse (C) by route of MTX administration during the interim maintenance phases. PO indicates oral; RHR, relative hazard ratio.

Table 4. Outcome of randomly assigned patients with B-precursor acute lymphoblastic leukemia with *ETV6/RUNX1* and favorable trisomies

	5-year event-free survival mean ± SD, %	*P*	5-year overall survival, mean ± SD, %	*P*
DT$^+$ vs DT$^-$ ($n_1 = 250$, $n_2 = 807$)	95.4 ± 1.8 vs 88.7 ± 1.5	.002	98.7 ± 1.0 vs 95.5 ± 1.0	.04
TEL$^+$ vs TEL$^-$ ($n_1 = 358$, $n_2 = 503$)	93.2 ± 2.2 vs 90.0 ± 2.2	.06	98.6 ± 1.0 vs 95.2 ± 1.6	.004
DT$^+$ and/or TEL$^+$ vs DT$^-$ and TEL$^-$ ($n_1 = 606$, $n_2 = 199$)	94.2 ± 1.4 vs 89.0 ± 3.4	.009	98.7 ± 0.7 vs 95.8 ± 2.2	.003

DT indicates trisomy 4 and trisomy 10; and TEL, *ETV6/RUNX1*.

Treatment outcome

The overall 5-year EFS and overall survival for the eligible patients with B-precursor ALL randomly assigned were 90.7% ± 0.9% and 96.0% ± 0.6%, Figure 2. There was no difference in outcome by sex; 5-year EFS and overall survival in boys were 90.4% ± 1.3% and 96.4 ± 0.8% versus 90.9% ± 1.4% and 96.1% ± 0.9% in girls ($P = .47$, $P = .98$). There was, however, a significant improvement in 5-year EFS (92.6% ± 1.2% vs 88.7% ± 1.4%; $P = .009$; Figure 3A) for patients randomly assigned to the IV MTX-based IM (IS and ID) compared with the PO MTX-based arms (OS and OD). The 5-year overall survival rates were comparable for the IV and PO MTX-based regimens (Figure 3B). In contrast, the addition of a second DI phase provided no benefit with 5-year EFS and overall survival of 90.9% ± 1.3% and 97.1% ± 0.8% for the SDI regimen and 90.5% ± 1.3% and 95.4% ± 1.0% for the DDI regimen ($P = .71$, $P = .12$; Figure 4A-B).

Eighty-two relapses occurred among 1037 patients randomly assigned to the SDI (OS + IS) arms, and 86 relapses among 1041 patients randomly assigned to the DDI (OD + ID) arms. Ninety-six relapses occurred in 1036 patients randomly assigned to the arms that used PO MTX in IM (OS + OD), and 72 patients relapsed among the 1042 randomly assigned to the IM with IV MTX (IS + ID). Details of the pattern of relapses and deaths on the randomized regimens are listed in Table 3. The IV MTX-based IM regimens had no obvious effect on BM relapse, but extramedullary events were greatly reduced compared with the PO MTX arms; CNS was 11 versus 26 (1% vs 2.5%) and testicular was 0 vs 7 (0% vs 0.7%). IV MTX eliminated CNS relapses in girls and testicular relapses in boys. The 5-year cumulative risk of isolated CNS, testicular, and other extramedullary relapses in the IV MTX versus PO MTX arms are shown in Figure 5A-C. The advantage of IV MTX was seen in both girls (5-year EFS 93.1% ± 1.7% vs 88.8% ± 2.1%, $P = .02$; relative hazard ratio, 1.7) and boys (5-year EFS 92% ± 1.6% vs 88.6% ± 2%, $P = .13$) but reached conventional levels of statistical significance only in girls.

Outcome analyses among subsets

CNS status. Sixty-seven patients were found to have CNS-2 status at study entry. Their 5-year EFS and overall survival were 77% ± 8.3% and 92.5% ± 5.2%, respectively. Relapses in the CNS were double those in the BM in this subset of patients: 9 versus 4. Thirty-five patients had CNS-3 disease at enrollment; 25 were nonrandomly assigned to the OD arm with 1800 cGy of cranial irradiation; 5 patients were treated on the augmented regimen; 5 received nonstudy therapy. The 5-year EFS and overall survival for patients with CNS-3 disease were 80.0% ± 9.9% and 93.3% ± 6.2%, respectively. Thirty-seven patients had traumatic lumbar punctures with lymphoblasts present in the spinal fluid. Their 5-year EFS and overall survival were 90.2% ± 7.6% and 97% ± 4.4%, respectively, similar to those of the overall group of randomly assigned patients.

Testicular disease. Fifteen patients with B-precursor ALL had testicular involvement at diagnosis. Seven of these patients were randomly assigned to the PO MTX arms and 8 to the IV MTX arms. Fourteen patients are alive in first remission; the 15th patient is alive after sustaining a BM relapse. Only 4 patients, including the patient with BM relapse, received testicular irradiation, as dictated by protocol therapy for patients with testicular involvement.

Favorable cytogenetics. One thousand three hundred thirty patients were analyzed for the presence of trisomy 4 and trisomy 10 (DT, and 1041 patients were evaluated for the presence of *ETV6-RUNX1* fusion transcript. Twenty-four percent of patients were positive for DT, and 41% were positive for *ETV6-RUNX1*. Among the randomized subset, 23.6% had DT, and 41.6% had *ETV6-RUNX1*. There was a statistically significant difference in EFS and overall survival between patients with DT and patients without DT and also between patients who had *ETV6-RUNX1* and patients without *ETV6-RUNX1*, as shown in Table 4. There was no apparent benefit of IV MTX in the EFS or overall survival in patients with *ETV6-RUNX1* fusion transcript or DT.

Table 5. Comparison of incidence of common grades 3/4 nonhematologic toxicities according to number of delayed intensification phases

	Induction DI/DDI, %	*P*	Consolidation DI/DDI, %	*P*	IM-1 DI/DDI, %	*P*	DI-1 DI/DDI, %	*P*	IM-2 DI/DDI, %	*P*	DI-2 DDI, %	*P*	Maint DI/DDI, %	*P*
Allergic	0/0.10	.50	0/0	—	0.10/0.10	1.00	0.30/0.30	1.00	0/0	—	0.53	—	0/0	—
Coagulation	5.32/6.15	.41	0.39/0.48	.74	0.10/0.10	.75	1.49/1.60	.83	0/0	—	1.58	—	0.10/0	.51
Hepatic	5.32/5.67	.72	9.03/11.67	.05	12.28/13.60	.38	4.16/4.21	.96	11.44/11.41	.98	5.16	—	9.65/8.74	.49
Infections	7.64/9.04	.25	6.99/7.14	.90	4.52/4.73	.82	9.51/9.42	.94	4.45/4.93	.62	10.43	—	5.24/6.29	.32
Neurologic*	3.87/3.94	.93	3.30/3.66	.65	1.96/3.45	.04	1.09/2.81	.005	0.81/1.95	.03	2.00	—	0.82/0.85	.94
Seizures	0.87/0.29	.07	0.10/0.19	.50	0.59/0.49	.77	0.10/0.40	.18	0.40/0.10	.19	0.53	—	0/0	—
Leuko†	0/0	—	0/0	—	0/0	—	0/0.20	.25	0/0	—	0	—	0.10/0	.51
Pancreatitis	0.19/0.29	.50	0.19/0.10	.50	0/0.10	.50	0.59/0.20	.15	0.10/0	.50	0	—	0.10/0	.51
Renal	0.19/0.38	.35	0.10/0	.50	0/0	—	0.20/0	.25	0/0	—	0	—	0/0	—

DI indicates single delayed intensification phase; DDI, double delayed intensification phase; IM, interim maintenance phase; and leuko, leukoencephalopathy.
*Excluding seizures and leukoencephalopathy.

Table 6. Comparison of incidence of grades 3/4 common nonhematologic toxicities according to route of methotrexate administration

	Induction PO/IV, %	*P*	Consolidation PO/IV, %	*P*	IM-1 PO/IV, %	*P*	DI-1 PO/IV, %	*P*	IM-2 PO/IV, %	*P*	DI-2 PO/IV, %	*P*	Maint PO/IV, %	*P*
Allergic	0.10/0	.50	0/0	—	0.10/0.10	1.00	0.10/0.50	.11	0/0	—	0.62/0.43	.51	0/0	—
Coagulation	5.59/5.88	.78	0.29/0.58	.26	0.20/0	.25	1.59/1.50	.88	0/0	—	2.29/0.85	.06	0.11/0	.50
Hepatic	6.36/4.63	.08	10.67/10.04	.64	17.63/8.20	< .0001	7.23/1.10	< .0001	14.20/8.62	.0001	7.48/2.78	.001	8.40/10.00	.23
Infections	7.62/9.06	.23	7.08/7.05	.98	4.11/5.14	.27	10.60/8.32	.08	6.29/3.08	.0008	11.43/9.40	.31	5.99/5.52	.66
Neurologic*	3.28/4.53	.14	3.39/3.57	.83	2.15/3.26	.12	1.39/2.51	.07	1.22/1.54	.54	2.49/1.50	.27	0.74/0.94	.63
Seizures	0.39/0.77	.19	0.19/0.10	.50	0.10/0.99	.006	0.30/0.20	.51	0/0.51	.03	0.21/0.85	.18	0/0	—
Leuko	0/0	—	0/0	—	0/0	—	0/0.20	.25	0/0	—	0/0	—	0/0.10	.50
Pancreatitis	0.39/0.10	.19	0.19/0.10	.50	0.10/0	.50	0.59/0.20	.15	0/0.10	.50	0/0	—	0.11/0	.50
Renal	0.19/0.39	.34	0.10/0	.50	0/0	—	0.20/0	.25	0/0	—	0/0	—	0/0	—

PO indicates oral; IM, interim maintenance phase; DI, delayed intensification phase; and Leuko, leukoencephalopathy.
*Excluding seizures and leukoencephalopathy.

Toxicity. Protocol therapy was well tolerated in all the treatment regimens. Toxicities were graded according to the Common Toxicity Criteria Version 2 (NCI). The incidence of grades 3 and 4 common nonhematologic toxicities in the different phases of therapy in the 4 randomized regimens is listed in Tables 5 and 6. No major differences in toxicity were expected in the different treatment phases in the SDI versus DDI regimens, because the patients received the same therapy except for the number of DI phases. The incidence of common toxicities during the second DI phase was similar to that in the first. The same was true for the first maintenance course after 1 or 2 DI phases. There were, however, noticeable differences, mainly elevation of the hepatic transaminases, between the MTX arms. These were higher in the IM and DI phases of the PO MTX arms. This is probably because of the combination of PO MTX and 6-MP. These were carried over to the subsequent DI phases. The incidence of seizures was low in all regimens but was relatively higher in the IV MTX arms. In addition, patients on the PO MTX arms had a higher number of mean hospital days during the DI phases of therapy (Table 7).

Discussion

PII of therapy was first incorporated in the treatment of children with ALL by Riehm et al of the BFM Group.[1] Its benefit has been apparent over several decades for various risk groups of children with ALL.[2,3,6,19] However, further intensification of postinduction therapy can be associated with considerable increase in toxicity and morbidity. In the CCG-189, study referred to in the "Introduction," high-grade pancreatic, gastrointestinal, and coagulation toxicities were significantly more common among patients on the DDI than on the SDI regimen. The incidence of serious infections was more than double in the DDI regimen than in the other regimens. There was a 4-fold increase in the requirements for red cell transfusions and 6-fold increase in the requirements for platelet transfusions compared with the SDI treatment arm. Furthermore, mean number of days of hospitalization for the DDI arm was nearly double that of the SDI regimen, 15 ± 15 days versus 8 ± 11 days; $P = .0001$.[6]

This increase in burden of therapy with augmented PII has to be justified by an improvement in outcome. Less drastic modifications of therapy without prolonging the overall duration of treatment may accomplish the same goals for certain subgroups of patients. The best arm of CCG-1922, the CCG-1891 successor study for children with NCI SR-ALL, achieved the same outcome as the DDI arm of CCG-1891 with the use of only a SDI, and a dexamethasone rather than prednisone backbone; 6-year EFS and overall survival were 83.4% ± 3.4% versus 83.2% ± 2.0% ($P = .86$) and 93.0% ± 2.3% versus 91.7% ± 0.1.5% ($P = .78$).[13]

CCG-1991 was designed to test the previously mentioned elements of PII, such as an extra phase of DI and escalating dose IV MTX without leucovorin rescue during IM. It also used dexamethasone as the sole corticosteroid therapy. The IM phases on CCG-1991 were not identical to the IM phases used in CCG-1882 or CCG-1961, because they did not include asparaginase. Prednisone was the steroid used during induction in previous CCG trials for patients with SR-ALL, except for CCG-1922 in which it was given in the experimental arms. CCG-1991 evaluated in a prospective randomized manner the value of a second DI in a dexamethasone-only BFM-based protocol in children with SR-ALL. The main differences between CCG-1991 and CCG-1891 were the use of dexamethasone in induction, DI and maintenance versus only during DI in CCG-1891, 2 IM phases for all patients in CCG-1991 versus only in those receiving DDI in CCG-1891, and the use of the pegylated preparation of asparaginase versus its native *Escherichia*

Table 7. Hospital days during the different phases of therapy

	MTX									
Phase	PO, mean	No.*	IV, mean	No.*	*P*	SDI, mean	No.*	DDI, mean	No.*	*P*
Induction	9.21	1035	9.33	1037	.67	9.35	1034	9.18	1038	.53
Consolidation	2.11	1030	2.09	1036	.91	2.19	1030	2.01	1036	.39
IM-1	1.22	1020	0.98	1013	.15	1.14	1019	1.06	1014	.61
DI-1	4.56	1010	2.70	999	< .0001	3.80	1011	3.47	998	.19
IM-2	0.98	988	0.97	976	.96	0.87	991	1.08	973	.11
DI-2	4.80	482	2.74	468	< .0001	—	—	3.79	950	—
Maintenance course 1	1.19	954	1.09	963	.48	1.17	978	1.11	939	.69

MTX indicates methotrexate in interim maintenance; PO, oral; SDI, single delayed intensification; DDI, double delayed intensification; IM, interim maintenance phase; and DI, delayed intensification phase.
*Number of patients with available data about hospital days.

coli form in CCG-1891. The positive contribution of dexamethasone in induction has repeatedly been shown in previous clinical trials for the treatment of childhood ALL.[13,19-22] Pegylated asparaginase has been shown to have a superior effect on early marrow response in induction compared with the native *E coli* preparation in similar BFM-based studies.[23] CCG-1991 showed that for children with SR-ALL and rapid early response to induction, a second DI phase did not provide any outcome benefit. However, the experimental arm with vincristine and escalating dose of IV MTX, without leucovorin rescue in the IM phases, showed an EFS advantage compared with the standard arm. The latter regimen was also shown to have a favorable toxicity profile. Unlike previous studies, sex was not found a prognosticator for outcome on CCG-1991.[24,25] EFS was inferior for boys on CCG-1891 and CCG-1952, whereas EFS was similar for both sexes on CCG-1922.[6,13,26] The 2 former protocols used prednisone as the steroid backbone, whereas in CCG-1922 one-half the patients received dexamethasone. Perhaps the equivalent outcome of males and females on CCG-1991 reflects use of dexamethasone throughout treatment.

On our study, patients with CNS-2 disease at presentation had an inferior outcome to those who had a CNS-1 status, 5-year EFS and overall survival were 76.8% ± 8.3% and 92.5% ± 5.2% versus 91.4% ± 1.0% and 96.4% ± 0.7%, respectively ($P \leq .0001$ for EFS; $P = .20$ for overall survival). This finding is similar to that found in the predecessor study (CCG-1952) in which patients with CNS-2 disease at diagnosis had an inferior outcome to those with CNS-1 status; 5-year EFS and overall survival were 74.0% ± 4.3% and 89.4% ± 3.1% versus 82.5 ± 0.9% and 93.5 ± 0.6%, respectively ($P = .001$ for EFS; $P = .20$ for overall survival).[26,27] However, patients with CNS-2 status on CCG-1991 had a better outcome than the similar cohort on CCG-1952, perhaps reflecting the beneficial effect of dexamethasone or the use of escalating IV MTX during IM or both in half the patients. Notably, the incidence of isolated CNS relapses in patients with CNS-2 status randomly assigned to the IV MTX regimens was less than a third the rate for patients randomly assigned to the PO MTX-containing regimens (2 of 35 vs 7 of 32). The 5-year EFS for patients with CNS-2 status treated on the IV MTX regimens was higher than for patients on the PO MTX regimens, 85.5% ± 8.7% versus 67.3% ± 14.5% ($P = .09$). Patients with CNS-2 status had an inferior outcome than patients with CNS-3 disease in both CCG-1991 and in its predecessor study, CCG-1952. A probable explanation of this finding is that all patients with CNS-3 disease received cranial irradiation during consolidation as dictated by protocol therapy. Unlike the recent practice of some cooperative groups, the CNS-2 cohort in CCG-1952 and -1991 did not receive additional IT chemotherapy.[28-31] Patients with CNS-2 status have not uniformly had an inferior outcome than patients with CNS-1 status. When treated on the BFM study group protocols, these patients have had a similar outcome than patients who had CNS-1 status.[31,32] Patients enrolled on these protocols receive much higher doses of IV MTX with leucovorin rescue in the early phases of therapy. Nonetheless, escalating IV MTX as given in IM phases on CCG-1991 appears to provide protection against recurrence for patients with CNS-2 status. Clearly, additional therapy is needed to improve the outcome of patients with CNS-2 disease treated on a standard CCG-1991 backbone. As previously mentioned, IV MTX during the IM phases of therapy greatly improved the EFS for these patients in CCG-1991. The outcome of patients with CNS-3 disease at presentation was inferior to the randomized group despite the administration of cranial irradiation during consolidation. This is consistent with the results of several collaborative group studies.[24,25,33,34] The outcome of CNS-3 on CCG-1991, however, compares favorably with the outcome of this group of patients in the aforementioned studies. Some cooperative groups have found that patients whose initial lumbar puncture is traumatic with lymphoblasts present in the spinal fluid correlates with an increased risk of CNS relapse and adverse outcome.[31,35,36] The cohort with traumatic lumbar puncture in CCG-1991 had a 5-year EFS and overall survival of 90.2% ± 7.6% and 97% ± 4.4%, respectively, with no additional CNS-directed therapy. The uniform use of dexamethasone in our study may have contributed to this excellent outcome, because it has been shown that dexamethasone has a superior CNS penetration and a longer CSF half-life than prednisolone.[37]

Finally, CCG-1991 seems to have provided optimal therapy for patients with favorable genetics, that is, trisomy 4 and trisomy 10, and *ETV6/RUNX1* fusion transcript whose 5-year overall survival is almost 99%. These results compare favorably with previous studies.[24,25,33,38] In light of the excellent outcomes achieved on CCG-1991, the current COG standard regimen for patients with B-precursor SR-ALL and rapid early response incorporates 1 DI phase and 2 IM phases of IV-escalating MTX.

Acknowledgments

We thank all the patients and families who participated in this trial. We also thank all the COG staff, institutional investigators, and their clinical research staff.

This study was supported in part by the Children's Oncology Group (chairman's grants CA-13539, CA-98543, and CA-98413) from the National Cancer Institute, National Institutes of Health.

Y.M. is the Angie Fowler Chair of Adolescent & Young Adult Cancer; S.P.H. is the Ergen Family Chair in Pediatric Cancer; L.C.S. is the Robert Neerhout Professor of Pediatrics; and C.C. is the Stan Perron Chair of Paediatric Hematology and Oncology.

Authorship

Contribution: Y.M. participated in the design of the study, chaired the study, and wrote the manuscript; B.C.B. participated in the design of the study and co-chaired the study; S.P.H. is a member of the study committee, participated in the running of the study, and edited the manuscript; L.C.S. participated in the design of the study and edited the manuscript; A.A. is a study committee member and reviewed the manuscript; H.S. participated in the design of the study and performed the statistical analyses; M.L. and M.D. performed the statistical analyses and edited the manuscript; J.M.G.-F. performed the molecular analyses and reviewed the manuscript; N.A.H. performed the cytogenetic analyses and reviewed the manuscript; S.S. is a study committee member, reviewed radiation therapy guidelines, and reviewed the manuscript; P.J.B. is a study committee member and reviewed pathology slides; B.T. and C.C. are study committee members and reviewed the manuscript; J.B.N. participated in the study design and reviewed the manuscript; G.R. edited the manuscript; N.W. and W.L.C. were involved in running the study and reviewed the manuscript; and P.S.G. participated in the design and running of the study and edited the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Yousif Matloub, Rainbow Babies & Children's Hospital, Pediatric Hematology/Oncology, Cleveland, OH 44106; e-mail: yousif.matloub@uhhospitals.org.

References

1. Riehm H, Langermann HJ, Gadner H, Odenwald E, Henze G. The Berlin Childhood Acute Lymphoblastic Leukemia Therapy Study, 1970-1976. *Am J Pediatr Hematol Oncol.* 1980;2(4):299-306.
2. Henze G, Fengler R, Reiter A, Ritter J, Riehm H. Impact of early intensive reinduction therapy on event-free survival in children with low-risk acute lymphoblastic leukemia. *Haematol Blood Transfus.* 1990;33:483-488.
3. Tubergen D, Gilchrist G, O'Brien A, et al. Improved outcome with delayed intensification for children with acute lymphoblastic leukemia and intermediate presenting features. *J Clin Oncol.* 1993;11(3):527-537.
4. Mastrangelo R, Poplack D, Bleyer A, Riccardi R, Sather H, D'Angio G. Report and recommendations of the Rome workshop concerning poor-prognosis acute lymphoblastic leukemia in children: biologic bases for staging, stratification, and treatment. *Med Pediatr Oncol.* 1986;14(3):191-194.
5. Smith M, Arthur D, Camitta B, et al. Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia [see comments]. *J Clin Oncol.* 1996;14(1):18-24.
6. Lange B, Bostrom BC, Cherlow JM, et al. Double-delayed intensification improves event free survival for children with intermediate-risk acute lymphoblastic leukemia: a report from the Children's Cancer Group. *Blood.* 2002;99(3):825-833.
7. Abromowitch M, Ochs J, Pui CH, Fairclough D, Murphy SB, Rivera GK. Efficacy of high-dose methotrexate in childhood acute lymphocytic leukemia: analysis by contemporary risk classifications. *Blood.* 1988;71(4):866-869.
8. Evans WE, Relling MV, Rodman JH, Crom WR, Boyett JM, Pui CH. Conventional compared with individualized chemotherapy for childhood acute lymphoblastic leukemia. *N Engl J Med.* 1998; 338(8):499-505.
9. Lange BJ, Blatt J, Sather HN, Meadows AT. Randomized comparison of moderate-dose methotrexate infusions to oral methotrexate in children with intermediate risk acute lymphoblastic leukemia: a Childrens Cancer Group study. *Med Pediatr Oncol.* 1996;27(1):15-20.
10. Jurgens H, Janka G, Ibrahim M, Tonert C, Winkler K, Gobel U. Prognostic significance of exposure to intermediate dose methotrexate in children with standard risk ALL: the COALL 82/85 experience. *Haematol Blood Transfus.* 1992;34:338-342.
11. Leblanc T, Auclerc MF, Landman-Parker J, et al. Impact of HD-MTX on the outcome of children with intermediate-risk ALL: results from the FRALLE93: a randomized study. *Blood.* 1998; 92(suppl 1):399a.
12. Nachman JB, Sather HN, Sensel MG, et al. Augmented post-induction therapy for children with high-risk acute lymphoblastic leukemia and a slow response to initial therapy. *N Engl J Med.* 1998;338(23):1663-1671.
13. Bostrom BC, Sensel MR, Sather HN, et al. Dexamethasone versus prednisone and daily oral versus weekly intravenous mercaptopurine for patients with standard-risk acute lymphoblastic leukemia: a report from the Children's Cancer Group. *Blood.* 2003;101:3809-3817.
14. Uckun FM, Muraguchi A, Ledbetter JA, et al. Biphenotypic leukemic lymphocyte precursors in CD2+CD19+ acute lymphoblastic leukemia and their putative normal counterparts in human fetal hematopoietic tissues. *Blood.* 1989;73(4):1000-1015.
15. Pui CH, Behm FG, Crist WM. Clinical and biologic relevance of immunologic marker studies in childhood acute lymphoblastic leukemia. *Blood.* 1993; 82(2):343-362.
16. Cho R, Stork L, Gaynon PS, Sather H, La M, Hutchinson R. Early marrow response in CCG-1952: the prognostic impact of poor day 14 marrow response is powerful and only partially reversible with intensified therapy [abstract]. *Blood* (*ASH Annual Meeting Abstracts*). 2005;106(11): Abstract 255.
17. Heerema N, Sather H, Reaman G, et al. Cytogenetic studies of acute lymphoblastic leukemia: clinical correlations results from the Children's Cancer Group. *J Assoc Genetic Technol.* 1998; 24:206-212.
18. Shurtleff SA, Buijs A, Behm FG, et al. TEL/AML1 fusion resulting from a cryptic t(12;21) is the most common genetic lesion in pediatric ALL and defines a subgroup of patients with an excellent prognosis. *Leukemia.* 1995;9(12):1985-1989.
19. Veerman AJ, Hahlen K, Kamps WA, et al. Dutch Childhood Leukemia Study Group: early results of study All VI (1984-1988). *Haematol Blood Transfus.* 1990;33:473-477.
20. Mitchell CD, Richards SM, Kinsey SE, Lilleyman J, Vora A, Eden TOB. Benefit of dexamethasone compared with prednisolone for childhood acute lymphoblastic leukaemia: results of the UK Medical Research Council ALL97 randomized trial. *Br J Haematol.* 2005;129(6):734-745.
21. Kamps WA, Bokkerink JP, Hakvoort-Cammel FG, et al. BFM-oriented treatment for children with acute lymphoblastic leukemia without cranial irradiation and treatment reduction for standard risk patients: results of DCLSG protocol ALL-8 (1991-1996). *Leukemia.* 2002;16(6):1099-1111.
22. Silverman LB, Gelber RD, Dalton VK, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. *Blood.* 2001;97(5):1211-1218.
23. Avramis VI, Sencer S, Periclou AP, et al. A randomized comparison of native Escherichia coli asparaginase and polyethylene glycol conjugated asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukemia: a Children's Cancer Group study. *Blood.* 2002;99(6):1986-1994.
24. Pui CH, Pei D, Sandlund JT, et al. Long-term results of St Jude Total Therapy Studies 11, 12, 13A, 13B, and 14 for childhood acute lymphoblastic leukemia. *Leukemia.* 2010;24(2):371-382.
25. Salzer WL, Devidas M, Carroll WL, et al. Long-term results of the pediatric oncology group studies for childhood acute lymphoblastic leukemia 1984-2001: a report from the Children's Oncology Group. *Leukemia.* 2010;24(2):355-370.
26. Stork LC, Matloub Y, Broxson E, et al. Oral 6-mercaptopurine vs oral 6-thioguanine and veno-occlusive disease in children with standard risk acute lymphoblastic leukemia: report of the Children's Oncology Group CCG-1952 clinical trial. *Blood.* 2010;115(14):2740-2748.
27. Matloub Y, Lindemulder S, Gaynon PS, et al. Intrathecal triple therapy decreases central nervous system relapse but fails to improve event-free survival when compared with intrathecal methotrexate: results of the Children's Cancer Group (CCG) 1952 study for standard-risk acute lymphoblastic leukemia, reported by the Children's Oncology Group. *Blood.* 2006;108(4):1165-1173.
28. Chauvenet AR, Martin PL, Devidas M, et al. Antimetabolite therapy for lesser-risk B-lineage acute lymphoblastic leukemia of childhood: a report from Children's Oncology Group Study P9201. *Blood.* 2007;110(4):1105-1111.
29. Pui CH, Sandlund JT, Pei D, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Total Therapy Study XIIIB at St Jude Children's Research Hospital. *Blood.* 2004; 104(9):2690-2696.
30. Pui CH, Mahmoud HH, Rivera GK, et al. Early intensification of intrathecal chemotherapy virtually eliminates central nervous system relapse in children with acute lymphoblastic leukemia. *Blood.* 1998;92(2):411-415.
31. Burger B, Zimmermann M, Mann G, et al. Diagnostic cerebrospinal fluid examination in children with acute lymphoblastic leukemia: significance of low leukocyte counts with blasts or traumatic lumbar puncture. *J Clin Oncol.* 2003;21(2):184-188.
32. te Loo DM, Kamps WA, van der Does-van den Berg A, van Wering ER, de Graaf SS. Prognostic significance of blasts in the cerebrospinal fluid without pleiocytosis or a traumatic lumbar puncture in children with acute lymphoblastic leukemia: experience of the Dutch Childhood Oncology Group. *J Clin Oncol.* 2006; 24(15):2332-2336.
33. Schmiegelow K, Forestier E, Hellebostad M, et al. Long-term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia. *Leukemia.* 2010;24(2):345-354.
34. Mitchell C, Richards S, Harrison CJ, Eden T. Long-term follow-up of the United Kingdom medical research council protocols for childhood acute lymphoblastic leukaemia, 1980-2001. *Leukemia.* 2010;24(2):406-418.
35. Gajjar A, Harrison PL, Sandlund JT, et al. Traumatic lumbar puncture at diagnosis adversely affects outcome in childhood acute lymphoblastic leukemia. *Blood.* 2000;96(10):3381-3384.
36. Mahmoud HH, Rivera GK, Hancock ML, et al. Low leukocyte counts with blast cells in cerebrospinal fluid of children with newly diagnosed acute lymphoblastic leukemia. *N Engl J Med.* 1993;329(5):314-319.
37. Balis FM, Lester CM, Chrousos GP, Heideman RL, Poplack DG. Differences in cerebrospinal fluid penetration of corticosteroids: possible relationship to the prevention of meningeal leukemia. *J Clin Oncol.* 1987;5(2):202-207.
38. Kamps WA, van der Pal-de Bruin KM, Veerman AJ, Fiocco M, Bierings M, Pieters R. Long-term results of Dutch Childhood Oncology Group studies for children with acute lymphoblastic leukemia from 1984 to 2004. *Leukemia.* 2010;24(2):309-319.