Results of the Dana-Farber Cancer Institute ALL Consortium Protocol 95-01 for children with acute lymphoblastic leukemia

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The Dana-Farber Cancer Institute (DFCI) Childhood ALL Consortium Protocol 95-01 was designed to minimize therapy-related morbidity for children with newly diagnosed ALL without compromising efficacy. Patients participated in randomized comparisons of (1) doxorubicin given with or without dexrazoxane, a cardioprotectant (high-risk patients), (2) intensive intrathecal chemotherapy and cranial radiation (standard-risk patients), and (3) *Erwinia* and *Escherichia coli* asparaginase (all patients). Between 1996 and 2000, 491 patients (aged 0-18 years) were enrolled (272 standard risk and 219 high risk). With a median of 5.7 years of follow-up, the estimated 5-year event-free survival (EFS) for all patients was 82% ± 2%. Dexrazoxane did not have a significant impact on the 5-year EFS of high-risk patients ($P = .99$), and there was no significant difference in outcome of standard-risk patients based on type of central nervous system (CNS) treatment ($P = .26$). Compared with *E coli* asparaginase, *Erwinia* asparaginase was associated with a lower incidence of toxicity (10% versus 24%), but also an inferior 5-year EFS (78% ± 4% versus 89% ± 3%, $P = .01$). We conclude that (1) dexrazoxane does not interfere with the antileukemic effect of doxorubicin, (2) intensive intrathecal chemotherapy is as effective as cranial radiation in preventing CNS relapse in standard-risk patients, and (3) once-weekly *Erwinia* is less toxic than *E coli* asparaginase, but also less efficacious. (Blood. 2007;109:896-904)

Introduction

Over the last decade, investigators have reported favorable outcomes for children with acute lymphoblastic leukemia (ALL), with long-term event-free survival (EFS) rates for unselected patient populations of approximately 80%.[1-4] The Dana-Farber Cancer Institute (DFCI) Childhood ALL Consortium has conducted clinical trials in children with ALL since 1981.[5] Treatment strategies include intensive, multiagent induction therapy, early intensification with weekly, high-dose asparaginase, frequent pulses of vincristine and corticosteroids during continuation therapy, and for high-risk patients, doxorubicin during intensification. We have previously reported a 5-year overall EFS rate of 83% for children with ALL treated between 1991 and 1995.[1] Acute toxicities from our treatment approach include asparaginase-related allergic events and pancreatitis.[1] In long-term survivors, we have observed asymptomatic echocardiographic changes related to anthracycline exposure,[6,7] as well as short stature and learning disabilities of varying severity, presumably secondary to central nervous system (CNS)–directed therapy.[8,9]

The DFCI ALL Consortium Protocol 95-01 was open for enrollment between 1996 and 2000. The primary objective was to reduce therapy-related morbidity without compromising efficacy. Standard-risk (SR) patients participated in 2 randomizations designed to evaluate whether acute and late toxicities could be reduced: a comparison between 2 asparaginase preparations (*Erwinia* and *Escherichia coli* asparaginase) administered during induction and consolidation and a comparison between 1800 cGy cranial radiation and intensive intrathecal chemotherapy as CNS-directed therapy. High-risk (HR) patients also participated in the asparaginase randomization. Additionally, HR patients were randomized (1) to receive doxorubicin with or without the cardioprotective agent, dexrazoxane, and (2) to 2 different dosing schedules of cranial radiation (once-daily and twice-daily fractionation). The results of the HR radiation randomization have been reported previously.[10]

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Nonrandomized changes to Protocol 95-01, also designed to reduce toxicity compared with previous protocols, included a reduction in the number of doses of asparaginase during the consolidation phase (20 instead of 30 consecutive weekly doses) and a reduction in the cumulative dose of doxorubicin for HR patients (300 mg/m^2 instead of 360 mg/m^2). In addition, National Cancer Institute (NCI) age and leukocyte criteria were applied prospectively in Protocol 95-01.[11] Thus, some patients who would have been considered HR on prior DFCI Childhood ALL Consortium protocols (specifically children aged 1-2 years or 9-10 years and those with presenting leukocyte counts between 20 and 50×10^9/L) were treated on the less intensive SR arm of Protocol 95-01. We report here the treatment outcome for Protocol 95-01 at a median of 5.7 years of follow-up.

Patients, materials, and methods

Patients

Between January 1996 and September 2000, 498 children (aged 0-18 years) with newly diagnosed ALL (excluding mature B-cell ALL) were enrolled on Protocol 95-01. Informed consent was obtained from parents or guardians prior to instituting therapy. Four patients were ineligible because of incorrect diagnosis (mature B-cell leukemia; n = 1), pretreatment with corticosteroid (n = 1), infection with the HIV-1 (n = 1), and incorrect consent (consented for SR therapy but treated as HR patient; n = 1). Three other patients were enrolled initially, but subsequently withdrew permission to have data used for research purposes during the course of treatment. The remaining 491 patients were evaluable. Patients were enrolled from the following consortium institutions: DFCI/Children's Hospital (Boston, MA), Hospital Sainte Justine (Montreal, QC, Canada), University of Rochester Medical Center (Rochester, NY), McMaster Children's Hospital (Hamilton, ON, Canada), San Jorge Children's Hospital (San Juan, PR), Maine Children's Cancer Program and Barbara Bush Children's Hospital at Maine Medical Center (Portland, ME), Mount Sinai Medical Center (New York, NY), Le Centre Hospitalier de L'Universite Laval (Quebec City, QC, Canada), and Ochsner Clinic (New Orleans, LA). The institutional review boards of each participating institution approved the protocol prior to patient enrollment.

Risk groups

Two patient risk groups were used to select therapy: SR and HR. Risk group was determined at the time of diagnosis. DFCI risk group criteria were redefined from previous studies to incorporate the NCI age and white blood cell (WBC) count criteria.[11] HR patients presented with one or more of the following pretreatment characteristics: (1) WBC count 50×10^9/L or higher; (2) age younger than 1.00 years or 10.00 years or older; (3) presence of leukemia blasts in a cytocentrifuged cerebrospinal fluid (CSF) specimen regardless of CSF WBC count (CNS-2 or CNS-3); (4) radiographic evidence of a mediastinal mass; or (5) T-cell immunophenotype. Patients with the Philadelphia chromosome, t(9;22)(q34;q11), were treated as HR patients, but received an allogeneic bone marrow transplant (BMT) during first remission. All other patients were classified as SR.

Therapy

Details of therapy are shown in Table 1. Therapy for all patients was discontinued after 24 months of continuous complete remission (CCR). The cumulative dose of doxorubicin was 60 mg/m^2 for SR patients and 300 mg/m^2 for HR patients. For SR and non-infant HR patients, CNS therapy commenced during the first cycle of intensification. During that cycle, asparaginase and prednisone were not administered. All patients received 4 doses of intrathecal chemotherapy over a 2-week period during CNS therapy. Additionally, 1800 cGy radiation was administered to those SR patients randomized to receive radiation and all HR patients. For infants, cranial radiation was delayed until 12 months of age. Infants (< 12 months at diagnosis) also received an additional month of intensive chemotherapy immediately after the remission induction phase, which included high-dose cytarabine and high-dose methotrexate, as previously described.[12] Otherwise, infants received therapy identical to other HR patients. Beginning in 1999, infants were enrolled on an international collaborative study instead of Protocol 95-01.

Asparaginase, *E coli* or *Erwinia*, was given weekly for 20 weeks during postinduction intensification. The asparaginase preparation was switched

Table 1. Therapy on DFCI ALL Consortium Protocol 95-01

Time frame	Treatment
Induction, 4 wk	Vincristine 1.5 mg/m^2 every week (maximum, 2 mg), days 0, 7, 14, 21
	Prednisone 40 mg/m^2/d, days 0-28
	Doxorubicin 30 mg/m^2/dose, days 0 and 1
	HR: randomized with/without dexrazoxane 300 mg/m^2, days 0 and 1
	Methotrexate 4 g/m^2 (8-24 h after doxorubicin) with leucovorin rescue
	Asparaginase (randomized *Erwinia* or *E coli*) 25 000 IU/m^2 IM × 1 dose, day 4
	IT cytarabine* × 1 dose, day 0
	TIT† × 1 dose, day 16
CNS therapy, 3 wk	SR: Randomized: TIT only (see "Randomizations") or 18-Gy hyperfractionated cranial radiation with IT methotrexate/cytarabine
	HR: 18-Gy cranial radiation (randomized hyperfractionated or daily fractions) with IT methotrexate/cytarabine
Intensification, 30 wk	Every 3 wk cycles:
	SR: Vincristine 2.0 mg/m^2 IV day 1 (maximum, 2 mg)
	Prednisone 40 mg/m^2/d orally, days 1-5
	Methotrexate 30 mg/m^2 IV or IM, days 1, 8, 15
	6-MP 50 mg/m^2/d orally, days 1-15
	Asparaginase (randomized): *Erwinia* 25 000 IU/m^2 IM weekly × 20 wk
	or
	E coli 25 000 IU/m^2 IM weekly × 20 weeks
	HR: same as SR patients, except prednisone dose higher (120 mg/m^2/d orally days 1-5), no methotrexate, doxorubicin 30 mg/m^2 day 1 of each cycle (cumulative dose of 300 mg/m^2), randomized to be given alone or with dexrazoxane 300 mg/m^2/dose
Continuation, until 24 mo CCR	Every 3 week cycles:
	SR: same as intensification, except no asparaginase
	HR: same as SR patients, except dose of prednisone (120 mg/m^2/d, days 1–5)

IM indicates intramuscular: IT, intrathecal; TIT, triple intrathecal chemotherapy (methotrexate, cytarabine, hydrocortisone): IV, intravenous; 6-MP: 6-mercaptopurine.

*IT cytarabine dosed according to age.[1] Patients with CNS leukemia at diagnosis (CNS-2 and CNS-3) received twice-weekly doses of IT cytarabine until CSF was clear of blast cells on 3 consecutive examinations. †TIT dosage according to age.[1]

after an allergic event. Because we have previously demonstrated that patients with a history of allergy to *E coli* asparaginase have a decreased half-life after allergy with other preparations,[13] we administered *Erwinia* to patients allergic to *E coli* patients twice weekly rather than using the investigational (once-weekly) dosing frequency administered to nonallergic patients as part of the randomized trial. Thus, patients allergic to *E coli* asparaginase were switched to twice-weekly *Erwinia* (25 000 IU/m^2/dose), and those allergic to *Erwinia* asparaginase were switched to weekly *E coli* (25 000 IU/m^2/dose) to complete 20 weeks of therapy. All patients were switched to weekly polyethylene glycol (PEG) asparaginase (2500 IU/m^2/dose) if they experienced a subsequent allergic event. Asparaginase therapy was held until resolution of mild pancreatitis or deep venous thrombosis and was stopped permanently after severe pancreatitis (abdominal pain for at least 72 hours with elevated pancreatic enzymes) or allergic events to all 3 preparations (*E coli*, *Erwinia*, and PEG).

Doses of methotrexate and 6-mercaptopurine were adjusted during the intensification and continuation phases to maintain absolute phagocyte nadirs of 0.500 to 0.750 $\times$ 10^9/L and platelet nadirs of 75 to 100 $\times$ 10^9/L. Doses were reduced for mucositis and transaminitis. Maximum doses were 40 mg/m^2/wk for methotrexate and 75 mg/m^2/d for 6-mercaptopurine.

Randomizations

Patients were eligible to participate in the following randomizations.

Asparaginase. To determine whether *Erwinia* asparaginase was associated with decreased toxicity, both SR and HR patients were randomized to receive 20 weekly doses of either *Erwinia* or *E coli* asparaginase, both administered intramuscularly at a dose of 25 000 IU/m^2. Asparaginase randomization met its target accrual in December 1998; thereafter, all patients were directly assigned to receive *E coli* asparaginase.

Dexrazoxane. To determine whether dexrazoxane prevented cardiac dysfunction due to doxorubicin, HR patients were randomized to either receive or not receive dexrazoxane 300 mg/m^2/dose immediately prior to each dose of doxorubicin (30 mg/m^2/dose) during induction and consolidation therapy.

CNS treatment (SR patients). To determine if intensive intrathecal therapy without cranial radiation was associated with fewer or less severe late neurocognitive sequelae, SR patients were randomized to receive either intensive triple intrathecal chemotherapy (methotrexate, cytarabine, and hydrocortisone, dosed according to age) without radiation or 1800 cGy cranial radiation delivered in twice-daily fractions (90 cGy twice daily) with less frequent doses of intrathecal therapy (methotrexate and cytarabine, dosed according to age). Patients randomized to receive intensive intrathecal therapy (no radiation) received 4 doses over 2 weeks during the CNS phase, then every 9 weeks for a total of 6 doses, then every 18 weeks until the completion of all treatment. Patients randomized to receive cranial radiation received 4 doses of intrathecal chemotherapy over 2 weeks concurrent with cranial radiation, then every 18 weeks until the completion of all chemotherapy. SR girls between 2 and 9 years of age and with presenting leukocyte counts less than 20 $\times$ 10^9/L were directly assigned to receive intensive intrathecal therapy (no radiation).[1]

Cranial radiation dosing (HR patients). To determine whether hyperfractionated radiation was associated with a decreased incidence of neuropsychologic sequelae, HR patients, including infants, were randomized to receive either twice-daily fractions of 90 cGy (hyperfractionated) or once-daily fractions of 180 cGy (conventional).[10]

All randomizations were performed centrally and occurred following enrollment. Patients who declined randomization were directly assigned to the standard arm of the declined randomization (*E coli* asparaginase, doxorubicin alone, cranial radiation for SR patients, and daily fractions of cranial radiation for HR patients).

Immunophenotype, cytogenetics, and DNA index

Bone marrow cells from diagnostic aspirates were examined for cell-surface antigens using standard indirect immunofluorescence assays and cultured for cytogenetic analyses, as described previously.[12] Ploidy status was determined by counting modal chromosome number on karyotypic analysis or measuring DNA index using flow cytometric techniques.[14]

Statistical analysis

Fisher exact tests[15] were used to compare presenting characteristics among patient groups. Outcome events were death during induction therapy, failure to achieve complete remission (defined as persistent leukemia at day 30 or persistent marrow hypoplasia at day 51 after diagnosis), death during remission, and relapse. EFS was the time from CR to the first outcome event; induction failure and induction deaths were considered events at time zero. Leukemia-free survival (LFS) was the time from CR to relapse; induction failure was considered a relapse at time zero. Overall survival (OS) was the time from start of treatment to death from any cause. EFS, LFS, and OS were estimated using the Kaplan-Meier method,[16] and the Greenwood formula was used to calculate standard errors.[17] Univariate analyses of differences in EFS, LFS, and OS were conducted with log-rank tests. Multiple regression was conducted using Cox proportional hazards models to assess prognostic factors for EFS, LFS, and OS.[18] Adjustments were not made for multiple comparisons. $P \leq .05$ were deemed statistically significant, unless otherwise stated.

Results

Patient characteristics

The presenting clinical characteristics of the 491 evaluable patients are summarized in Table 2. Of the 491 patients, 272 (55%) were classified as SR and 219 (45%) as HR (including 14 infants). Age at diagnosis ranged from 13 days to 17.9 years with a median of 4.6 years. Initial WBC count ranged from 0.7 to 1175 $\times$ 10^9/L, with a median leukocyte count of 10.6 $\times$ 10^9/L. A total of 434 patients (88%) had B-lineage ALL, 52 (11%) had T-ALL, and 5 (1%) did not have complete information available regarding immunophenotype.

Treatment results

The outcome of the 491 patients is presented in Table 3. Median follow-up was 5.7 years. Of the 491 patients, 480 (98%) achieved CR, 7 (1.4%) had persistent leukemia at the end of the first month (and were considered induction failures), and 4 patients (0.8%) died during induction therapy. Three patients (0.6%) died in first CR, all of whom were in the HR group. Seventy-eight patients (16%) had a relapse and 399 (81%) remained in CCR. The estimated 5-year EFS ($\pm$ SE) was 82% $\pm$ 2% (Figure 1A) and the 5-year OS was 90% $\pm$ 1%. The 5-year EFS estimate according to risk group was 86% $\pm$ 2% for SR patients and 76% $\pm$ 3% for HR patients ($P = .01$; Figure 1B and Table 3). The 5-year LFS and OS estimates according to risk group were 87% $\pm$ 2% and 95% $\pm$ 1% for SR patients and 78% $\pm$ 3% and 84% $\pm$ 3% for HR patients, including infants ($P = .03$ for LFS comparison and $P < .01$ for OS comparison; Table 3).

Asparaginase randomization

Table 4 summarizes the result of the asparaginase randomization. A total of 286 patients participated in the randomized comparison of *E coli* and *Erwinia* asparaginase; patients received a single intramuscular dose during remission induction followed by 20 weekly injections during postremission consolidation. Fewer patients randomized to receive *Erwinia* asparaginase experienced an asparaginase-related toxicity (10% of *Erwinia* versus 24% of *E coli* patients, $P < .01$; Table 4). However, significantly more patients randomized to *Erwinia* experienced a relapse at any site (19% versus 10% of *E coli*–randomized patients, $P = .02$), including relapses involving the CNS (6% versus 1%, $P < .01$). With a median follow-up of 6.5 years for randomized patients, the 5-year EFS for *Erwinia*-randomized patients was 78% $\pm$ 4% compared

Table 2. Patient characteristics and outcome on Protocol 95-01

	Total	5-y EFS ± SE	*P*
All patients	491	82 ± 2	—
DFCI risk group			.01
Standard	272	86 ± 2	
High	219	76 ± 3	
NCI risk group, non-infants*			.19
Good-risk pre-B	299	86 ± 2	
Poor-risk pre-B	121	70 ± 4	
Good-risk T	12	83 ± 11	
Poor-risk T	40	85 ± 6	
Age			<.01
Younger than 1.00 y	14	42 ± 13	
1.00-9.99 y	385	84 ± 2	
10.00-18.00 y	92	75 ± 5	
WBC count			<.01
Less than 20.000 × 10^9/L	318	87 ± 2	
20.000-49.999 × 10^9/L	76	71 ± 5	
50.000-99.999 × 10^9/L	43	79 ± 6	
100.000 × 10^9/L or higher	54	66 ± 7	
Sex			.26
Male	274	79 ± 3	
Female	217	84 ± 3	
Immunophenotype			.57
B-lineage	434	81 ± 2	
T-cell	52	85 ± 5	
CNS at diagnosis			.16
CNS-1	403	83 ± 2	
CNS-2	49	72 ± 7	
CNS-3	12	75 ± 13	
Traumatic	19	68 ± 11	
Down syndrome			.33
No	477	82 ± 2	
Yes	14	71 ± 12	
Ploidy in 309 assessable patients			.12
Hyperdiploid 50 or greater	82	86 ± 4	
Hyperdiploid less than 50	23	73 ± 9	
Diploid	134	84.3	
Pseudodiploid	54	71 ± 6	
Hypodiploid	16	73 ± 12	

*NCI age/WBC criteria[11] used to define good-risk group (children 1 to <10 yr old with a leukocyte count <50 × 10^9/L) and poor risk group (all others) for both B-precursor and T-cell patients.

with 89% ± 3% for *E coli*–randomized patients ($P = .01$; Figure 2A). The difference in EFS remained significant when stratified by risk group ($P = .02$).

Dexrazoxane randomization

A total of 205 HR patients were randomized to receive doxorubicin with or without the potential cardioprotectant, dexrazoxane, during the induction and consolidation phases of therapy. The 5-year EFS for patients randomized to receive doxorubicin with dexrazoxane was 76% ± 4%, compared with 77% ± 4% for those randomized to receive doxorubicin alone ($P = .99$; Figure 2B).

HR patients randomized to doxorubicin alone were more likely than those randomized to dexrazoxane to have elevations of troponin T, a serum marker of cardiomyocyte damage, during therapy (50% versus 21%, respectively; $P < .001$), as reported previously.[19] There were no reported episodes of congestive heart failure in any patient.

CNS randomization

Fifteen of the 491 (3%) patients experienced a CNS relapse, including 3 isolated CNS relapses (0.6%) and 12 combined marrow and CNS relapses (2.4%). Isolated CNS relapses occurred in 2 of 272 SR patients (0.7%) and one of 219 HR patients (0.5%). Combined marrow/CNS relapses occurred in 6 SR (2.2%) and 6 HR (2.7%) patients.

A total of 164 SR patients participated in the randomization comparing 2 CNS-directed treatments: intensive triple intrathecal chemotherapy without radiation or 1800 cGy cranial radiation with less frequent intrathecal therapy. For the 83 patients randomized to receive intrathecal therapy only, there were 9 marrow-only relapses (10.8%) and 5 relapses involving the CNS (6%), including 2 isolated CNS relapses (2.4%). For the 81 patients randomized to receive cranial radiation, there were 8 marrow-only relapses (9.9%), and no isolated or combined CNS relapses (0%). The 5-year EFS for those randomized to intrathecal therapy alone was 83% ± 4% compared with 86% ± 4% for those patients randomized to receive cranial radiation ($P = .26$; Figure 2C).

Ninety-four SR female patients were directly assigned to receive intrathecal therapy only based on age (2-9 years) and very low presenting leukocyte count (< 20 × 10^9/L) because such patients had been successfully treated without radiation on previous DFCI ALL Consortium protocols.[1] The 5-year EFS for this subset of patients was 88% ± 3%, with CNS relapses observed in 3 (3.2%) patients (all of which were combined marrow/CNS).

The frequency of relapses involving the CNS in nonirradiated patients varied depending on asparaginase randomization, with more CNS relapses occurring with *Erwinia* asparaginase. For the 30 SR patients randomized to both intrathecal therapy only and *Erwinia* asparaginase, there were 5 CNS relapses (including one isolated CNS relapse), compared with no CNS relapses (isolated or combined) in the 22 patients randomized to both intrathecal therapy only and *E coli* asparaginase ($P = .06$). For SR girls directly assigned to intrathecal therapy only, 2 of the 3 CNS relapses (all combined) were in patients who received *Erwinia* asparaginase.

Toxicities

Four of the 491 patients (0.8%) experienced an induction death and 3 (0.6%) experienced a remission death. Induction deaths were due to

Table 3. Results of Protocol 95-01 for 491 children with ALL

	Total	SR	HR*
No. of patients	491	272	219
Percent of total	100	55	45
Induction failures	7	0	7
Induction deaths	4	2	2
Complete remissions, no. (%)	480 (98)	270 (99)	210 (96)
Relapses, no. (%)	78 (16)	39 (14)	39 (18)
BM only	56	26	30
CNS only	3	2	1
CNS + BM	12	6	6
Testis	2	0	2
Testis + BM	3	3	0
Other†	2	2	0
Remission deaths	3	0	3
Continuous remission	399	231	168
5-y EFS ± SE, %	82 ± 2	86 ± 2	76 ± 3
5-y LFS ± SE, %	83 ± 2	87 ± 2	78 ± 3
5-y OS ± SE, %	90 ± 1	95 ± 1	84 ± 3

Median follow-up time was 5.7 y.
*Includes HR patients and infant very HR patients.
†Other sites of relapse: ocular only (1), pelvic mass (1).

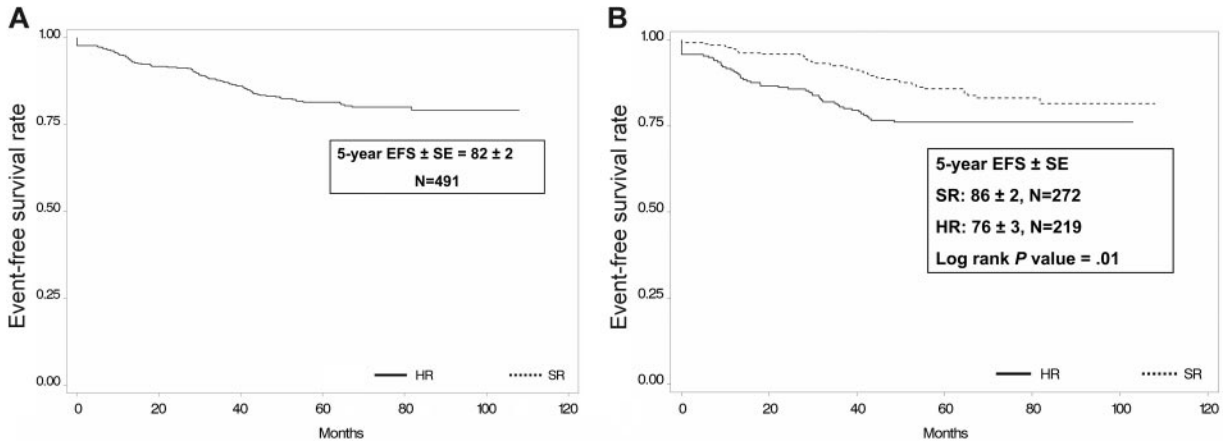

Figure 1. EFS for total group and subgroups of patients. (A) EFS of all 491 patients. With a median follow-up of 5.7 years, the 5-year EFS ± SE for all 491 patients treated on Protocol 95-01 was 82% ± 2%. (B) EFS for SR and HR patients. The 5-year EFS ± SE for SR patients (n = 272) was 86% ± 2% compared with 76% ± 3% for HR patients (n = 219; $P = .01$).

sepsis (n = 2), severe pneumonia (n = 1), and typhlitis (n = 1). Remission deaths were due to sepsis (n = 2) and development of a hemophagocytic lymphohistiocytic-like syndrome soon after the completion of chemotherapy (n = 1). One patient developed a malignant melanoma in a nonirradiated site (lower extremity) after 5 years of CR; that lesion was completely excised, and the patient remains alive and well. No other second malignancies have been observed.

Asparaginase-related toxicities were observed in 21% of the 491 patients. The most frequent toxicities included allergic reactions (13%), pancreatitis (5%) and non–CNS-related thromboses (3%). No patient experienced a symptomatic CNS thrombosis or bleed. Patients aged 10 to 18 years were more likely to experience an asparaginase-related toxicity compared with those younger than 10 years (29% versus 19%, $P = .03$), including a higher incidence of pancreatitis (11% versus 4%) and thromboses (11% versus 2%) but not allergic events (8% versus 14%).

Prognostic factors

Significant univariate predictors of outcome included age, presenting leukocyte count, and DFCI risk group (Table 2). Ploidy was assessed in 309 patients and was not a significant predictor of outcome. In a subset analysis including 176 patients known to be TEL/AML1$^-$ by polymerase chain reaction (PCR),[20] results were similar (data not shown). A proportional hazards model was conducted including all 491 eligible patients, with covariates of age, sex, race, presenting WBC count, immunophenotype, CNS status at diagnosis, and ploidy. Significant independent predictors of inferior EFS (with hazard ratios and 95% CLs) included age younger than 12 months (hazard ratio 5.12 [2.41, 10.89], $P < .001$), age older than 10 years (hazard ratio 1.74 [1.06, 2.84], $P = .03$), and presenting leukocyte count higher than 50×10^9/L (hazard ratio 1.71 [1.27, 2.25] for WBC count 50-100 $\times 10^9$/L and 2.84 [1.60, 5.04] for WBC count $> 100 \times 10^9$/L at diagnosis, $P < .001$). The hazard ratio for T-cell phenotype was 0.47 [0.21, 1.05] ($P = .06$), suggesting that T-cell patients may have had a lower risk of an event compared with B-lineage patients. When a similar proportional hazards analysis was conducted including the 286 patients who participated in the asparaginase randomization, assignment to weekly *Erwinia* asparaginase was also an independent predictor of inferior outcome (along with age < 12 months and elevated leukocyte counts), with a hazard ratio of 2.00 [1.12, 3.57] ($P = .02$). Age older than 10 years was no longer an independent outcome predictor ($P = .65$) in that analysis.

Table 4. Outcome of asparaginase randomization

	E coli	*Erwinia*	*P*
Total n	147	139	—
Any toxicity, %	24	10	<.01
Allergy, %	14	6	.03
Pancreatitis, %	6	2	.14
Thrombosis, %	5	1	.17
Relapse, %	10	19	.02
Marrow only	7	11	.41
CNS involved	1	6	<.01
5-y EFS, %	89 ± 3	78 ± 4	.01

— indicates not applicable.

Discussion

DFCI ALL Consortium Protocol 95-01 was designed to minimize therapy-related morbidity for children with newly diagnosed ALL without compromising efficacy. The protocol focused on strategies to minimize toxicities, especially those related to doxorubicin, CNS-directed treatments, and asparaginase. Additionally, risk group criteria were liberalized so that fewer patients were treated on the more intensive HR arm (40% of patients considered HR on Protocol 95-01 compared with 60% of patients on previous protocols). With the exception of the use of weekly *Erwinia* asparaginase instead of *E coli* asparaginase, the potentially less toxic treatments did not adversely affect EFS. The estimated 5-year EFS rate of 82% ± 2% is similar to that observed on the predecessor DFCI Childhood ALL Consortium Protocol 91-01 (5-year EFS 83% ± 2%)[1] and compares favorably with results for similar groups of unselected patients with ALL treated contemporaneously by other investigators.[2,21,22] Favorable outcomes were observed for patients considered historically to be at higher risk of relapse, including adolescents (5-year EFS, 75%) and those with T-cell disease (5-year EFS, 84%). This regimen did not require any hospitalizations for chemotherapy administration after remission induction and resulted in a relatively low remission death rate of 0.6%.

Anthracyclines such as doxorubicin are effective agents for ALL and other pediatric malignancies, but their use in children has been associated with progressive, late cardiotoxicity. We and others have observed asymptomatic echocardiographic abnormalities in up to 50% of long-term survivors of childhood ALL who received a

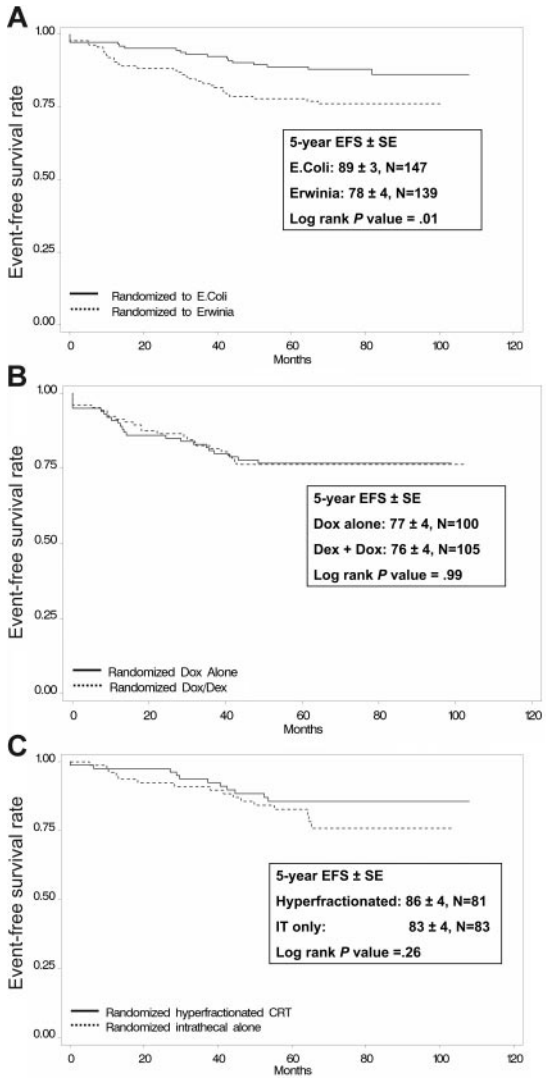

Figure 2. EFS results based on asparaginase, dexrazoxane, and CNS randomization. (A) EFS results of asparaginase randomization. The 5-year EFS for patients randomized to *Erwinia* asparaginase was 78% ± 4% compared with 89% ± 3% for those randomized to *E coli* asparaginase ($P = .01$). (B) EFS results of dexrazoxane randomization (HR patients only). The 5-year EFS for patients randomized to doxorubicin with dexrazoxane was 76% ± 4% compared with 77% ± 4% for those randomized to doxorubicin alone ($P = .99$). (C) EFS results of CNS randomization (SR patients only). The 5-year EFS for patients randomized to intrathecal therapy only (no radiation) was 83% ± 4% compared with 86% ± 4% for those randomized to 18-Gy cranial radiation ($P = .26$).

cumulative dosage of doxorubicin exceeding 300 mg/m.[7,23-25] In a prior study, we compared 2 doxorubicin infusion strategies (continuous infusion and bolus) and did not observe a significant difference in late cardiac dysfunction.[26] On Protocol 95-01, SR patients received a cumulative doxorubicin dosage of only 60 mg/m^2, presumably putting them at low risk for late cardiac effects. For HR patients, we reduced the cumulative doxorubicin dosage to 300 mg/m^2 to minimize late cardiac toxicity.[24] Also, HR patients were randomized to receive doxorubicin alone or with dexrazoxane, a bis-dioxopiperazine compound that acts as a free radical scavenger.[25] Dexrazoxane has been shown to prevent acute cardiotoxicity in adults with cancer who receive anthracycline,[27,28] but there have not been any published prospective studies of pediatric patients treated with this agent. We have reported previously that HR patients treated with doxorubicin alone were more likely than those who received dexrazoxane to have elevated troponin T samples during therapy (50% versus 21%; $P < .001$), suggesting that dexrazoxane prevented acute cardiac injury.[19] Here, we report that there was no significant difference in EFS between HR patients who received dexrazoxane and those who did not (76% ± 4% versus 77% ± 4%, $P = .99$). Although longer follow-up is necessary to determine whether dexrazoxane prevented late echocardiographic changes, these data suggest that dexrazoxane has a cardioprotective effect without compromising antileukemic efficacy.

Late effects related to CNS-directed treatments have been a continuing concern in long-term survivors of childhood ALL. Late neurotoxicity has been attributed most frequently to cranial radiation, although the role of intrathecal and systemic chemotherapy in the development of neurocognitive impairments remains controversial.[29-31] Because of concerns regarding neurocognitive sequelae in younger patients, as well as the risk of radiation-induced second malignancies,[32] we have attempted to reduce exposure to radiation, especially in patients at lower risk. On a trial we conducted between 1987 and 1991, SR boys (but not SR girls) had a higher incidence of CNS relapse when radiation was deleted from their treatment without any other changes to their treatment.[33] On Protocol 95-01, we demonstrated that an increase in the number and frequency of intrathecal chemotherapy doses substituted adequately for cranial radiation in SR patients. The CNS relapse rate (isolated and combined) of nonirradiated SR patients receiving *E coli* asparaginase was 3%, the same rate that was observed for SR patients (most of whom received radiation) on our previous trial, Protocol 91-01.[1] These results are similar to the experience of others who have reported that alternative CNS-directed treatments, including the use of high-dose antimetabolite therapy or intensive intrathecal chemotherapy or both, can be as effective as cranial radiation in preventing CNS relapses in patients with lower- and intermediate-risk disease.[3,34,35] Having randomized children with ALL to radiation or not, neurocognitive testing of long-term survivors (currently ongoing) will be especially informative regarding the relative late toxicities of 1800 cGy cranial radiation and intensive intrathecal chemotherapy.

For HR patients, we have investigated alternative radiation strategies, including the use of hyperfractionated radiation. For HR patients treated between 1987 and 2000 (including those treated on Protocol 95-01), there was no significant difference in EFS between conventional (once-daily) and hyperfractionated (twice-daily) 1800 cGy radiation nor any differences in intelligence, academic achievement, visuospatial reasoning, or verbal learning, suggesting that hyperfractionated radiation provided neither significant survival advantage nor neurocognitive benefit.[10] A lower dose (1200 cGy) of radiation may provide adequate antileukemic efficacy,[36] but the late neurocognitive effects and second tumor risks associated with this dose have not yet been elucidated. Currently, we are treating the majority of patients, including most HR patients, with intensive IT chemotherapy instead of cranial radiation, reserving radiation (administered at 1200 cGy) only for the subset (~20% of all patients) considered at highest risk of subsequent CNS relapse, including those with CNS-leukemia at diagnosis (CNS-3), very high presenting leukocyte counts ($> 100 \times 10^9$/L), or T-cell phenotype.

The results of the asparaginase randomization demonstrate that changes in a regimen, made in an attempt to reduce toxicity, can significantly affect EFS. Asparaginase is an important and universal component of multiagent chemotherapy for childhood ALL, but it is also associated with various toxicities, including allergy, pancreatitis, and thromboembolic complications.[1] The antigenically distinctive asparaginase derived from *Erwinia caratova* has

been used effectively to treat patients who have experienced an allergic reaction to the *E coli* preparation. The results of Protocol 95-01 indicated that *Erwinia* asparaginase, when dosed once weekly for 20 weeks during consolidation, was significantly less toxic and less efficacious than *E coli* asparaginase. Both of these findings—reduced toxicity and decreased efficacy—may have been due to the once-weekly dosing schedule. Because *Erwinia* asparaginase has a shorter half-life than the enzyme derived from *E coli*,[13] patients receiving once-weekly *Erwinia* asparaginase likely did not experience continuous serum asparagine depletion for the full 20 weeks of consolidation, whereas those receiving *E coli* asparaginase did. When we chose a once-weekly dosing schedule for *Erwinia* asparaginase, we hypothesized that intermittent asparagine depletion over a 20-week period might be associated with fewer toxicities but still provide sufficient antileukemic control. The results of the randomization indicate that this hypothesis was incorrect. Thus, continuous serum asparagine depletion may be associated with better EFS than intermittent depletion. It is possible that with a different dosing schedule, such as twice weekly, *Erwinia* asparaginase would lead to continuous asparagine depletion and be as efficacious as *E coli* asparaginase. However, even when given 3 times per week during induction and delayed consolidation (reinduction), *Erwinia* asparaginase was associated with lower toxicity, but higher induction failure and relapse rates when compared with *E coli* asparaginase in a randomized trial conducted by the European Organization for Research and Treatment of Cancer (EORTC).[37] Another asparaginase preparation, PEG asparaginase, is formed by the polyethylene glycosylation of the native *E coli*–derived enzyme, resulting in a longer circulating half-life,[13] lower incidence of antiasparaginase antibody formation,[38] and lower frequency of allergic reactions.[1] We are currently assessing the relative efficacy and toxicity of intramuscular *E coli* asparaginase and intravenous PEG asparaginase within the context of a randomized trial.

Our results also suggest that asparaginase plays a role in preventing CNS relapses, especially in nonirradiated patients. Asparaginase does not penetrate into the CNS, but CSF asparagine depletion has been observed after the systemic administration of asparaginase.[38-40] We observed a significantly higher CNS relapse rate for patients randomized to *Erwinia* asparaginase. Also, although SR patients who received cranial radiation did not experience any CNS relapses regardless of the asparaginase type they received, we observed more relapses involving the CNS in nonirradiated SR patients who received *Erwinia* asparaginase instead of *E coli* asparaginase, emphasizing that the efficacy of any single component of therapy (eg, intensive intrathecal therapy) must be considered within the context of the entire treatment regimen (eg, type of asparaginase).

In addition to type of asparaginase, the other independent predictors of outcome included age and presenting leukocyte count. Other groups have reported that high levels of minimal residual disease (MRD) early in therapy (determined by either flow cytometric or PCR-based techniques) are associated with an inferior prognosis.[41-44] We are currently using end of induction MRD levels (measured using a real-time quantitative PCR technique)[45] to risk-stratify patients, intensifying therapy for those patients with higher levels of MRD.

Many other groups have reported that patients with high hyperdiploidy, especially those with trisomies of chromosomes 4, 10, and 17, have an excellent outcome.[46-48] We did not confirm this finding on Protocol 95-01, although our results may have been limited by the relatively small number of patients with available ploidy data (63% of enrolled subjects). These results may also reflect differences in treatment between our regimen and those of other groups, including the intensity of dosing of antimetabolite agents, such as methotrexate. In contrast, we have previously reported *TEL/AML1*$^+$ patients had an excellent outcome on Protocol 95-01 (89% EFS compared with 80% for *TEL/AML1*$^-$ patients).[20] *TEL/AML1*$^+$ lymphoblasts demonstrate extreme in vitro sensitivity to asparaginase,[49] which is used intensively in our regimen. Interestingly, *TEL/AML1* status was not an independent predictor of outcome on Protocol 95-01,[20] suggesting that other factors (such as presenting age and WBC count) should be considered when treating these patients. Thus, despite the relatively favorable outcome of these patients, we are not using *TEL/AML1* status to risk-stratify patients in our current clinical trial.

Patients with T-cell ALL had a very good outcome on Protocol 95-01, including those with high presenting WBC counts ($> 50\,000$) or age > 10 years (Table 2). In fact, T-cell phenotype, often considered a high-risk feature, was associated with a trend toward more favorable outcome in multivariate analysis. We have previously reported that children with T-cell ALL treated between 1981 and 1995 treated on our trials fared at least as well as patients with B-precursor disease.[50] The 5-year EFS of T-ALL patients on this protocol ($85\% \pm 5\%$) compares favorably with results reported for T-ALL patients treated contemporaneously on other regimens (5-year EFS rates 60%-70%),[2,3] suggesting that our high-risk regimen, including consolidation with doxorubicin and weekly asparaginase, may be especially beneficial for this patient subset. The Pediatric Oncology Group previously reported that the inclusion of high-dose asparaginase during consolidation improved outcome for patients with T-ALL,[51] and preliminary results of another trial run by the same group (which used a DFCI high-risk backbone, with the addition of high-dose methotrexate) also appear to be favorable.[52]

Although the results of Protocol 95-01 suggest that therapy can be modified to reduce toxicity without compromising efficacy, treatment-related morbidity remains a major issue. Moreover, a sizable number of patients are still not cured by currently available therapies. It is essential that new agents be identified to treat ALL more effectively and with less morbidity. Targeted therapies, such as imatinib in *BCR-ABL*$^+$ leukemia, flt3-inhibitors in *MLL*-rearranged ALL, and NOTCH1 inhibitors in T-cell ALL, hold promise as leukemia-specific agents that may be used in conjunction with or even replace more toxic agents. Additionally, identification of biologically distinctive subtypes of leukemia may supplement or replace clinical factors (such as age and leukocyte count) to more precisely determine risk-based therapy, reserving more intensive or novel therapies for those patients at highest risk of relapse.

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Authorship

Contribution: A.M., B.A., R.B., L.C., C.H., Y.S., M.S., S.E.L., V.K.D., S.E.S, and L.B.S. all helped in designing the study protocol, contributed patients to the study, and helped in analyzing the data and writing the manuscript; H.J.C. helped in designing the study protocol, in analyzing the data, and in writing the manuscript; and D.E.L., D.S.N., and R.D.G. helped in designing the study protocol, in analyzing the data, and in writing the manuscript.

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