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ORIGINAL REPORT

Postinduction Dexamethasone and Individualized Dosing of *Escherichia Coli* L-Asparaginase Each Improve Outcome of Children and Adolescents With Newly Diagnosed Acute Lymphoblastic Leukemia: Results From a Randomized Study—Dana-Farber Cancer Institute ALL Consortium Protocol 00-01

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ABSTRACT

Purpose
We assessed the toxicity and efficacy of dexamethasone and a novel dosing method of *Escherichia coli* L-asparaginase (EC-Asnase) in children and adolescents with newly diagnosed acute lymphoblastic leukemia (ALL).

Patients and Methods
Patients achieving complete remission (CR) on Dana-Farber Cancer Institute ALL Consortium Protocol 00-01 were eligible for random assignment to 1) dexamethasone or prednisone, administered as 5-day pulses, every 3 weeks, and 2) weekly EC-Asnase, administered as a 25,000 IU/m^2 fixed dose (FD) or individualized dose (ID) starting at 12,500-IU/m^2, adjusted every 3 weeks based on nadir serum asparaginase activity (NSAA) determinations.

Results
Between 2000 and 2004, 492 evaluable patients (ages 1 to 18 years) enrolled; 473 patients (96%) achieved CR. Four hundred eight patients (86%) participated in the corticosteroid randomization and 384 patients (81%) in the EC-Asnase randomization. With 4.9 years of median follow-up, dexamethasone was associated with superior 5-year event-free survival (EFS; 90% *v* 81% for prednisone; $P = .01$) but higher rates of infection ($P = .03$) and, in older children, higher cumulative incidence of osteonecrosis ($P = .02$) and fracture ($P = .06$). ID EC-Asnase had superior 5-year EFS (90% *v* 82% for FD; $P = .04$), but did not reduce the frequency of asparaginase-related toxicity. Multivariable analysis identified both dexamethasone and ID EC-Asnase as independent predictors of favorable EFS.

Conclusion
There was no overall difference in skeletal toxicity by corticosteroid type; dexamethasone was associated with more infections and, in older children, increased incidence of osteonecrosis and fracture. There was no difference in asparaginase-related toxicity by EC-Asnase dosing method. Dexamethasone and ID EC-Asnase were each associated with superior EFS. Monitoring NSAA during treatment with EC-Asnase may be an effective strategy to improve outcome in pediatric ALL.

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INTRODUCTION

Corticosteroids and L-asparaginase are universal components of chemotherapy regimens for childhood acute lymphoblastic leukemia (ALL), although both are associated with significant adverse effects. Interest in substituting dexamethasone for prednisone in ALL treatment arose from studies suggesting that dexamethasone had more potent in vitro antileukemia activity, higher free-plasma levels, and enhanced CSF penetration.[1,2] Results of some randomized trials have indicated that dexamethasone was associated with superior event-free survival (EFS), whereas others have found no

difference in outcome.[3-6] Dexamethasone has been associated with increased toxicities, including higher rates of osteonecrosis, especially in older children and adolescents.[7] Thus, the optimal corticosteroid preparation and dosing are unknown, and may differ for patient subgroups.

Similarly, optimal dosing for asparaginase remains unclear. Asparaginase-associated toxicities, such as allergy, pancreatitis, and thrombosis, are a significant source of morbidity and may increase relapse risk in patients unable to receive all intended doses.[8] It has been suggested that serum asparaginase activity levels $\geq$ 0.10 IU/mL are required for therapeutic asparagine depletion.[9-14] However, interpatient variability in asparaginase activity levels is high among children receiving a fixed dose (FD; based on body surface) of *Escherichia coli* L-asparaginase (EC-Asnase).[9,12,15] We hypothesized that an individual dosing regimen, monitoring nadir serum asparaginase activity (NSAA) and adjusting the EC-Asnase dose to maintain a therapeutic level, would improve tolerability and outcome.

Dana-Farber Cancer Institute ALL Consortium Protocol 00-01 focused on optimizing the use of corticosteroids and EC-Asnase in children and adolescents with newly diagnosed ALL. The primary objectives of this study were to determine the relative toxicity, tolerability, and efficacy of 1) dexamethasone and prednisone administered during postinduction treatment and 2) weekly intramuscular EC-Asnase administered as the standard FD and a pharmacokinetically guided individualized dose (ID). In this article, we report the findings of Protocol 00-01.

PATIENTS AND METHODS

Patients

Patients ages 1 to 18 years with newly diagnosed ALL (excluding mature B-cell ALL) were eligible for enrollment. The protocol was approved by the institutional review boards of each participating institution (Appendix Table A1, online only).[16] Informed consent was obtained from parents or guardians for each patient before enrollment.

Risk Groups

Patients were stratified into risk groups for treatment. Standard risk was defined as: patients ages 1 to 9.99 years, presenting WBC count less than 50,000/μL, B-precursor phenotype, no mediastinal mass, and diagnostic spinal fluid without blasts (CNS1) or with blasts but fewer than five leukocytes per high-power field. All other patients were defined as high risk. Patients with Philadelphia chromosome (Ph+) were considered to be high risk but were eligible for allogeneic hematopoietic stem-cell transplantation in first complete remission (CR). Patients with *MLL* gene rearrangement received an additional postinduction intensification cycle and then continued as high risk.[17]

Therapy

Details of treatment are summarized in Table 1. All patients received prednisone during induction. Patients with persistent morphologic leukemia after 1 month were removed from study and received alternative therapy.[18] Corticosteroids were permanently discontinued in the setting of symptomatic, radiographically confirmed osteonecrosis and were temporarily held for bone fracture (resumed when the fracture healed). EC-Asnase was held until resolution of mild/moderate pancreatitis or thrombosis and was permanently discontinued after severe pancreatitis. Patients with allergic reactions to EC-Asnase of any severity, including local reactions, were switched to *Erwinia* asparaginase (25,000 IU/m^2) two times per week.[16] Patients were switched to weekly intramuscular polyethylene glycol–asparaginase on allergy to or unavailability of *Erwinia* asparaginase. Asparaginase was permanently discontinued after allergy to all available preparations. Therapy for all patients was discontinued after 24 months of continuous CR.

Randomizations

Patients who achieved CR were eligible for two randomized comparisons. Patients who declined participation in random assignments and Ph+ patients were directly assigned to receive prednisone and FD EC-Asnase.

Corticosteroid Randomization

Standard-risk patients were randomly assigned to receive either dexamethasone 6-mg/m^2/day or prednisone 40 mg/m^2/day, administered as 5-day pulses every 3 weeks beginning at week 7, until the completion of therapy. High-risk patients received either dexamethasone 18-mg/m^2/day or prednisone 120 mg/m^2/day during the 30-week intensification phase, and then the same corticosteroid dose as standard-risk patients during the continuation phase.[8,19]

Asparaginase Randomization

During intensification, patients were scheduled to receive 30 weekly doses of IM EC-Asnase. Patients were randomly assigned to receive either FD (25,000 IU/m^2) or ID, based on monitoring NSAA. The initial dose for the ID arm was 12,500 IU/m^2, with subsequent doses adjusted to maintain NSAA between 0.10 and 0.14 IU/mL using the algorithm in Table 1.

Samples for monitoring NSAA were obtained from all patients just before administering the second and fourth EC-Asnase doses and every 3 weeks thereafter. Procedures for sample collection, processing, storage, and determining NSAA have been previously described.[16] Only samples obtained 7 days after the prior dose were considered evaluable for dose adjustment and statistical analysis. EC-Asnase antibodies were assayed as previously described in samples obtained every 6 weeks from all patients and when NSAA was below the level of detectability ($<$ 0.025 IU/mL) in ID patients.[20,21] ID patients with NSAA less than 0.1 IU/mL on successive determinations despite dose adjustment or when coupled with EC-Asnase antibody positivity were considered to have "silent inactivation" and were switched to *Erwinia*- or polyethylene glycol–asparaginase. Clinical allergic reaction was the only planned indication for switching asparaginase preparation for FD patients.

Toxicity Assessment

Toxicity data were prospectively collected. For the corticosteroid randomization, the primary toxicity end points were symptomatic osteonecrosis and fracture, confirmed by radiographic imaging. A diagnosis of bacteremia or fungemia was based on positive blood culture; invasive fungal disease was diagnosed by radiographic imaging, plus/minus culture results. Asparaginase-associated toxicities included allergy, thrombosis (radiographically confirmed), and pancreatitis. Pancreatitis cases were classified by duration of clinical signs/symptoms as mild/moderate ($<$ 72 hours in duration) or severe ($\geq$ 72 hours).

Minimal Residual Disease

End-induction marrow aspirates were collected prospectively for patients with B-precursor immunophenotype and were analyzed for minimal residual disease by a polymerase chain reaction–based methodology.[22] Results were not used to adjust treatment.

Statistical Methods

Descriptive statistics are presented as frequencies and percentages. The 5-year cumulative incidence (CumInc) of osteonecrosis and fracture were estimated using the CumInc utility in the cmprsk package in R and tested using the Gray test, with relapse and death in remission identified as competing risks. Frequencies of infection and asparaginase toxicities were identified and analyzed with the Fisher's exact test. The study was designed to test for differences in the rate of asparaginase-related toxicity between FD and ID and of bony morbidity (osteonecrosis and fracture) between dexamethasone and prednisone. Four hundred three patients were calculated to provide 90% power to detect a difference between 36% and 21% in asparaginase-related toxicity using a two-sided .05 level test and 80% power to detect a difference between 16% and 28% incidence of bony morbidity using a two-sided .05 level test.

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Table 1. Therapy on DFCI-ALL Consortium Protocol 00-01

Phase/Duration	Treatment
Induction	VCR 1.5 mg/m^2 once per week (maximum 2 mg), days 0, 7, 14, 21
4 weeks	Prednisone 40 mg/m^2 per day, days 0 to 28
	DOX 30 mg/m^2/dose, days 0 and 1; (HR patients: with dexrazoxane 300 mg/m^2/dose)
	MTX 4 mg/m^2 (8-24 hours after doxorubicin) with leucovorin rescue
	EC-Asnase 25,000 IU/m^2 IM × one dose
	IT cytarabine, day 0,*† IT MTX/cytarabine/hydrocortisone × one dose, day 14; IT on day 28 (counting as first IT of CNS phase)
CNS therapy	VCR 2 mg/m^2 (maximum 2 mg) day 1; 6-MP 50 mg/m^2/d orally at bedtime, × 14 days
3 weeks	SR patients: IT MTX/cytarabine/hydrocortisone two times per week × four doses
	HR patients: IT MTX/cytarabine two times per week × four doses; also DOX 30 mg/m^2 day 1, with dexrazoxane 300 mg/m^2; cranial irradiation‡
Intensification	Every 3 week cycles:
30 weeks	VCR 2 mg/m^2 (maximum 2 mg), day 1; 6-MP 50 mg/m^2 per day orally at bedtime × 14 doses; MTX 30 mg/m^2 (1 mg/kg if < 0.6 m^2) IV or IM once per week
	Corticosteroid, randomized:
	Dexamethasone 6 mg/m^2 per day, divided two times per day (3 mg/m^2 per dose), days 1-5 of each 3-week cycle, or
	Prednisone 40 mg/m^2 per day divided two times per day (20 mg/m^2 per dose), days 1-5 of each 3-week cycle
	EC-Asnase randomized:
	Fixed-dosing: 25,000 IU/m^2 IM, once per week × 30 weeks, or
	Individualized dosing: 12,500 IU/m^2 IM (starting dose)§, once per week × 30 weeks

NSAA (IU/mL)	Change in subsequent doses
< 0.025	Increase by 80%, send urgent EC-Asnase antibody
0.025 to < 0.05	Increase by 60%
0.05 to < 0.08	Increase by 40%
0.08 to < 0.1	Increase by 20%
0.1 to < 0.14	No change
0.14 to < 0.20	Decrease by 20%
> 0.20	Decrease by 40%

	IT MTX/cytarabine/hydrocortisone at start of a cycle every 9 weeks × 6 doses, then every 18 weeks through completion of therapy (at start of a cycle)
	HR patients: same as above, except higher corticosteroid dose (prednisone 120 mg/m^2 per dose, days 1-5, or dexamethasone 18 mg/m^2 per dose, days 1-5), DOX 30 mg/m^2 day 1 of each cycle (cumulative dose 300 mg/m^2) with dexrazoxane 300 mg/m^2/dose, no weekly MTX IV/IM until doxorubicin completed, and IT therapy of MTX/cytarabine every 18 weeks
Continuation	Every 3 week cycles:
74 weeks	SR: same as intensification except no asparaginase
	HR: same as SR patients, including lower corticosteroid dose of dexamethasone 6 mg/m^2 per day and prednisone 40 mg/m^2 per day, days 1-5

Abbreviations: 6-MP, 6-mercaptopurine; ALL, acute lymphoblastic leukemia; bid, twice per day; DFCI, Dana-Farber Cancer Institute; DOX, doxorubicin; EC-Asnase, *E coli* asparaginase; HR, high risk; IM, intramuscular; IT, intrathecal; IV, intravenous; MTX, methotrexate; NSAA, nadir serum asparaginase activity; SR, standard risk; VCR, vincristine.
*Dosed according to age.
†Patients with CNS leukemia at diagnosis (CNS-2 and CNS-3) received IT cytarabine two times per week until CSF was clear of blast cells on three consecutive examinations.
‡Only HR patients received cranial radiation, 12 Gy cranial radiation delivered as 1.5 Gy daily fractions for 8 days, except for patients classified as CNS-2 or CNS-3 at diagnosis or during induction therapy who received 18 Gy delivered as 1.8-Gy fractions for 10 days.
§Asparaginase dose-adjustments were based on NSAA determinations. Percentage change was from most recent dose. Starting dose was 12,500 IU/m^2. Minimum dose was 6,000 IU/m^2. Maximum dose was 25,000 IU/m^2.

For overall protocol results, EFS was defined as the time from diagnosis to first outcome event (induction failure, induction death, death during remission, or relapse). Overall survival (OS) was calculated from the time of diagnosis to death from any cause. EFS and OS were estimated using Kaplan-Meier methodology, and the Greenwood formula was used to construct 95% CI, which were truncated at 100%. The log-rank test was used to compare EFS and OS between groups. Multivariable regression analysis was conducted using Cox proportional hazards models (Appendix, online only).

Analyses of both randomizations were by intention-to-treat for all patients achieving CR. Because the analysis of patients participating in the randomizations included only those alive and in CR at the start of the intensification phase, a landmark approach defined at this time-point was used for EFS estimates. EFS and OS were planned secondary end points of the randomized comparisons.

No adjustments were made for performing multiple statistical tests. *P* values are two-sided and values less than .05 were considered significant.

RESULTS

Patient Characteristics

Between September 2000 and December 2004, 498 patients (ages 1 to 18 years) were enrolled, 492 of whom were considered evaluable (five were subsequently found ineligible and one withdrew consent; Fig 1; Table 2). Median follow-up was 4.9 years.

Overall Results

Four hundred seventy-three patients (96%) achieved CR, 13 patients (3%) had persistent leukemia at the end of the first month, and three patients (0.6%) died during induction (two patients from infection/sepsis and one from necrotizing colitis). Three patients

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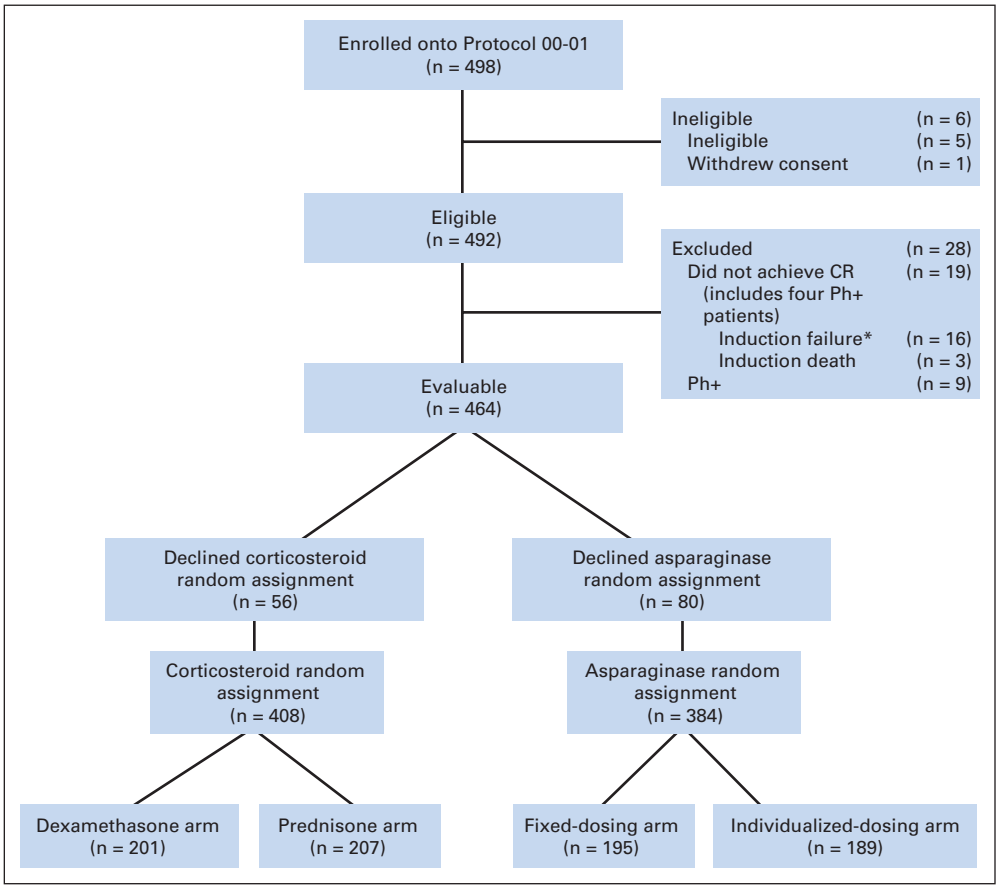

Fig 1. Dana-Farber Cancer Institute Acute Lymphoblastic Leukemia (ALL) Consortium Protocol 00-01 flow diagram. Patients with newly diagnosed ALL were enrolled (N = 498); 492 patients were considered evaluable. Of these 492 patients, 473 patients achieved complete remission (CR). Of the 473 patients who achieved CR, 408 patients (86%) participated in the corticosteroid random assignment and 384 (81%) in the asparaginase random assignment. Ph, Philadelphia chromosome. (*) Includes 13 patients with persistent leukemia at end of first month and three patients who did not recover blood counts by day 49.

(0.6%) failed to recover blood counts by day 49 of treatment and were removed from protocol. Of those who achieved CR, 69 patients (15%) relapsed and five patients (1%) died while in first CR as a result of infection/sepsis (n = 3) and complications of hematopoietic stem-cell transplantation (two Ph+ patients). No second malignant neoplasms have been reported. The estimated 5-year EFS and OS for all 492 patients were 80% (95% CI, 76% to 84%) and 91% (95% CI, 88% to 93%), respectively (Fig 2A).

Of the 473 patients who achieved CR, 408 patients (86%) participated in the corticosteroid randomization (prednisone, 207; dexamethasone, 201) and 384 patients (81%) participated in the asparaginase randomization (FD, 195; ID, 189). Presenting characteristics did not differ significantly between treatment arms.

Corticosteroid Randomization

Osteonecrosis. Of the 408 randomly assigned patients, 23 patients (6%) had symptomatic osteonecrosis (six patients at more than one site). Osteonecrosis was more frequently diagnosed in patients 10 to 18 years old than in younger patients (5-year CumInc, 14% *v* 3.5%; $P = .0004$). Five-year osteonecrosis CumInc did not differ by sex ($P = .15$).

The overall incidence of osteonecrosis did not differ by corticosteroid type ($P = .25$; Appendix Table A2, online only). However, for patients 10 to 18 years, dexamethasone was associated with a higher risk of osteonecrosis (5-year CumInc, 23% *v* 5% for patients randomly assigned to prednisone; $P = .02$). Osteonecrosis CumInc did not differ by corticosteroid type in children 1 to 9.99 years old ($P = .43$). For high-risk patients ages 1 to 9.99 years randomly assigned to dexamethasone, osteonecrosis CumInc was 3%, similar to that of standard-risk patients and significantly less than older high-risk patients (5-year CumInc, 23%; $P < .01$). For high-risk patients randomly assigned to prednisone, there was no significant difference in osteonecrosis CumInc in those patients 1 to 9.99 years old (0%) versus patients 10 to 18 years old (5%; $P = .92$).

Fracture. Fifty-six patients (14%) were diagnosed with a bone fracture (10 patients with more than one fracture). Five-year CumInc in patients ages 10 to 18 years was 19% compared with 12% for younger patients ($P = .06$). There was no difference in fracture CumInc by corticosteroid randomization ($P = .89$). For patients 10 to 18 years old, fracture CumInc for those patients randomly assigned to dexamethasone was 29% compared with 10% for those randomly assigned to prednisone ($P = .06$).

Infection. Postinduction infections were documented in 60 patients (15%), including 12 (13%) of 91 patients ages 10 to 18 years and 48 (15%) of 317 patients ages 1 to 9.99 years ($P = .74$). The infection rate differed by corticosteroid type; 22 patients (11%) randomly assigned to prednisone had ≥ one infection compared with 38 patients (19%) randomly assigned to dexamethasone ($P = .03$; Appendix Table A2). The infection rate was higher for patients 10 to 18 years old in the dexamethasone arm compared with the prednisone arm ($P = .004$), but for patients 1 to 9.99 years old, infection rate did not significantly differ by corticosteroid type ($P = .27$). There was one infection-related death in a child randomly assigned to prednisone

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Table 2. Patient Characteristics and 5-Year EFS Survival Rates of Children and Adolescents With Newly Diagnosed ALL Treated on DFCI ALL Consortium Protocol 00-01 (N = 492)

Characteristic	Total No. of Patients	5-Year EFS (%)	95% CI	*P*
All evaluable patients	492	80	76 to 84	
DFCI risk group				.001
Standard risk	282	85	80 to 89	
High risk	210	74	68 to 80	
Age, years				.008
1-9.99	380	83	79 to 87	
10-18	112	72	63 to 80	
Median	4.75			
Range	1-17.8			
WBC count, $\times 10^9$/L				.007
< 50,000	391	83	78 to 87	
≥ 50,000	101	72	63 to 81	
Median	11.4			
Range	0.9-865.1			
Sex				.04
Male	264	77	71 to 82	
Female	228	85	80 to 90	
Immunophenotype				.01
B lineage	443	82	78 to 85	
T cell	49	69	56 to 82	
CNS status at diagnosis				.68
CNS 1	410	80	76 to 84	
CNS 2	60	84	74 to 93	
CNS 3	17	81	62 to 100	
Missing	5			
Down syndrome				.34
Yes	13	92	76 to 100	
No	479	80	76 to 84	
End-induction MRD*	186			< .0001
Low	168	87	81 to 92	
High	18	61	35 to 79	
Cytogenetics†	405			
TEL/AML1	98	91	85 to 97	
High hyperdiploid	110	88	81 to 95	
t(1;19)	14	100		
t(9;22)	14	31	6 to 56	
MLL rearrangement	14	48	21 to 75	

NOTE. Bone marrow cells from diagnostic aspirate samples were examined for cell-surface antigens using direct immunofluorescence assays and were cultured for cytogenetic analyses. Screening for the following translocations by FISH and/or PCR was performed at each participating institution when diagnostic marrow sample was available: *TEL/AML1*, *MLL* gene rearrangements, and *BCR-ABL*.

Abbreviations: ALL, acute lymphoblastic leukemia; DFCI, Dana-Farber Cancer Institute; EFS, event-free survival; FISH, fluorescent in situ hybridization; MRD, minimal residual disease; PCR, polymerase chain reaction.

*Includes only B-precursor patients with evaluable MRD results (n = 186).

†Includes only patients with evaluable cytogenetic, FISH, and/or PCR results (n = 405).

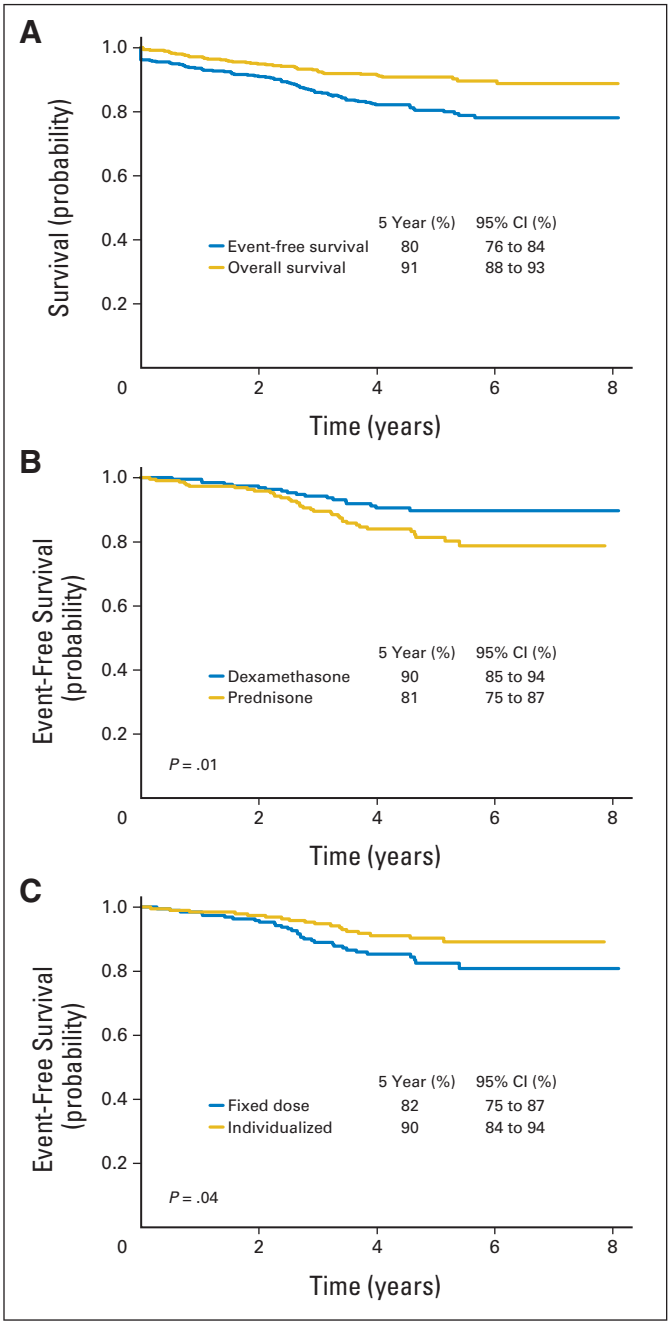

Fig 2. (A) Event-free survival (EFS) and overall survival (OS) for all patients. With a median follow-up of 4.9 years, the 5-year EFS for 492 evaluable patients was 80% (95% CI, 76% to 84%) and the 5-year OS was 91% (95% CI, 88% to 93%). (B) EFS results of corticosteroid randomization. The 5-year EFS for patients randomly assigned to prednisone was 81% (95% CI, 75% to 87%) compared with 90% (95% CI, 85% to 94%) for those randomly assigned to dexamethasone (P = .01). (C) EFS results of asparaginase randomization. The 5-year EFS for patients randomly assigned to fixed-dosing of asparaginase was 82% (95% CI, 75% to 87%) compared with 90% (95% CI, 84% to 94%) for those randomly assigned to individualized dosing (P = .04).

(from invasive fungal disease) and no infection-related deaths among patients on the dexamethasone arm.

EFS and OS. The estimates of EFS and OS included only patients who were alive and in CR at the start of intensification. The 5-year EFS was 81% (95% CI, 75% to 87%) for patients on the prednisone arm and 90% (95% CI, 85% to 94%) for dexamethasone-arm patients (P = .01; Fig 2B). The trend toward superior EFS with dexamethasone was observed in subsets defined by age, risk group, phenotype, and other characteristics (Table 3). Sites of relapse are listed in Appendix Table A3 (online only). There was no significant difference in 5-year OS (prednisone arm, 94%; dexamethasone arm, 95%; P = .31).

Asparaginase Randomization

NSAA and asparaginase antibodies. Data pertaining to NSAA and asparaginase antibody determinations are summarized in Table 4. A

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Table 3. Five-Year Event-Free Survival for the Randomly Assigned Study Arms

Patient Group	Corticosteroid Random Assignment						Asparaginase Random Assignment					
	Dexamethasone			Prednisone			Fixed Dose			Individualized Dose		
	No. of Patients	5-Year EFS (%)	95% CI	No. of Patients	5-Year EFS (%)	95% CI	No. of Patients	5-Year EFS (%)	95% CI	No. of Patients	5-Year EFS (%)	95% CI
All evaluable patients	201	90	85 to 94	207	81	75 to 87	195	82	75 to 87	189	90	84 to 94
Risk group												
SR	114	89	83 to 95	121	84	76 to 91	110	86	76 to 92	115	89	81 to 93
HR	87	91	84 to 97	86	78	69 to 87	85	78	66 to 86	74	93	84 to 97
Age, years												
1-9.99	153	91	86 to 95	164	82	76 to 89	151	85	77 to 90	147	90	83 to 94
10-18	48	88	77 to 98	43	77	64 to 89	44	72	55 to 84	42	90	76 to 96
WBC count, $\times 10^9$/L												
$<$ 50,000	159	91	86 to 96	163	84	77 to 90	152	84	76 to 89	155	89	83 to 94
$\geq$ 50,000	42	87	76 to 98	44	73	59 to 87	43	77	61 to 87	34	93	75 to 98
Sex												
Male	100	87	79 to 94	89	78	69 to 86	112	77	67 to 84	90	87	77 to 92
Female	101	93	88 to 99	118	86	79 to 94	83	90	81 to 95	99	93	86 to 97
Immunophenotype												
T cell	22	96	87 to 100	17	65	42 to 87	20	74	49 to 88	15	100	
B lineage	179	89	84 to 94	190	83	77 to 94	175	83	76 to 89	174	89	83 to 93
Cytogenetics												
TEL/AML1	43	100		40	85	73 to 97	41	88	77 to 99	45	95	89 to 100
High hyperdiploid*	44	95	87 to 100	52	83	71 to 95	47	88	77 to 98	38	89	76 to 100

Abbreviations: EFS, event-free survival; HR, high risk; SR, standard risk.
*High hyperdiploidy: 51-65 chromosomes.

total of 2,536 evaluable NSAA samples were obtained from 95% of the 384 patients randomly assigned to the asparaginase arm. In both arms, median NSAA at each time-point increased progressively during the initial 6 weeks of treatment and then reached steady-state (not shown). Overall median steady-state NSAA was significantly higher on the FD arm. The percentage of NSAA determinations within the 0.10 to 0.14 IU/mL target range was similar for both arms, but there were fewer samples with high NSAA ($>$ 0.14 IU/mL) on the ID arm. The percentage of patients who tested positive for EC-Asnase antibodies was similar for the two arms ($P = .24$).

Toxicity. There was no difference in the frequency of asparaginase-associated toxicity between the two arms (Table 4) or in the proportion of patients completing $\geq$ 25 weeks of asparaginase (FD, 88%; ID, 87%).

Significantly more patients on the ID arm were switched from EC-Asnase to another asparaginase preparation (Table 4). The percentage of patients for whom the preparation was changed in response to clinical allergy was similar for the two arms (FD, 20%; ID, 21%). However, the preparation was switched for 19 ID patients (10%) because of silent inactivation. Seventeen of these patients (90%) had at least one NSAA $\geq$ 0.1 IU/mL after switching preparation. Conversely, 18 FD patients (9%) never had NSAA $\geq$ 0.1 IU/mL with EC-Asnase, never developed clinical allergies, and never changed preparations.

EFS and OS. Twenty-nine patients (15%) on the FD arm experienced relapse compared with 17 ID patients (9%). Five-year EFS was 82% (95% CI, 75% to 87%) for FD and 90% (95% CI, 84% to 94%) for ID patients ($P = .04$; Fig 2C). The trend toward superior EFS with ID was observed in subsets defined by age, risk group, phenotype, and other characteristics (Table 3). There was no significant difference in 5-year OS by arm (FD, 93%; 95% CI, 88% to 96%; ID, 96%; 95% CI, 92% to 98%; $P = .18$).

For patients with at least one NSAA $\geq$ 0.1 IU/mL with EC-Asnase, 5-year EFS was 85% (95% CI, 78% to 90%) for the FD arm compared with 90% for the ID arm (95% CI, 83% to 95%; $P = .16$). For patients with persistently low NSAA with EC-Asnase (maximum NSAA $<$ 0.1 IU/mL), 5-year EFS was 73% (n = 36; 95% CI, 52% to 86) on the FD arm and 91% on the ID arm (n = 51; 95% CI, 79% to 97%; $P = .058$). Five-year EFS for patients with persistently low EC-Asnase NSAA who never changed preparations was similar on both arms (FD: n = 18; 76%; 95% CI, 38% to 92%; ID: n = 12; 78%; 95% CI, 35% to 94%; $P = .99$). Five-year EFS for the 19 ID patients who switched to a different preparation for silent inactivation was 95% (95% CI, 68% to 99%).

We also explored the outcome of patients based on asparaginase antibody status. There was no significant difference in 5-year EFS in antibody-negative patients who never developed EC-Asnase clinical allergy (FD: 83%; 95% CI, 72% to 90%; ID: 88%; 95% CI, 78% to 92%; $P = .38$). For the 54 FD patients who developed antibody-positivity but never developed EC-Asnase clinical allergy, 5-year EFS was 84% (95% CI, 70% to 92%) compared with 93% (95% CI, 82% to 97%) for similar patients on the ID arm (n = 63; $P = .12$). Of note, zero (0%) of 54 antibody-positive/EC-Asnase allergy–negative patients on the FD arm changed preparations, compared with 22 (35%) of 63 of such patients on the ID arm, including all 19 patients who were switched for silent inactivation (Appendix).

Randomization Interactions

We investigated differences in CumInc of osteonecrosis and of fracture between the randomized asparaginase arms and found no statistically significant differences. No significant differences in asparaginase toxicities (clinical allergy, pancreatitis, or thrombosis) were found based on corticosteroid randomization.

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Table 4. Asparaginase Toxicities, Activity, Antibody Positivity, and Relationship With EFS

Characteristic	Fixed Dose		Individualized Dose		*P*
	No. of Patients	%	No. of Patients	%	
Patients randomly assigned	195		189		
EC-Asnase dose, IU/m^2	25,000		17,500*		
Any toxicity	63	32	59	31	.83
EC-Asnase clinical allergy	39	20	40	21	.80
Pancreatitis	10	5	12	6	.66
Thrombosis	16	8	13	7	.70
Completed ≥ 25 week asparaginase	172	88	164	86	.76
Patients with evaluable NSAA data	186	95	180	95	
No. of evaluable NSAA samples	1,235		1,301		
Overall steady-state NSAA, IU/mL					< .001
Median	0.11		0.08		
Range	< 0.025-1.78		< 0.025-0.74		
Maximum NSAA in each patient					.05
≥ 0.10 IU/mL	150	81	129	72	
< 0.10 IU/mL	36	19	51	28	
EC-Asnase antibody determinations	180		181		.24
Positive test	64	36	76	42	
Asparaginase preparation changed from EC-Asnase	43	22	64	34	.01
EC-Asnase clinical allergy	39	20	39	21	
Silent inactivation	N/A	—	19	10	
Other	4	2	6	3	
Overall 5-year EFS, %					.04
EFS	82		90		
95% CI	75 to 87		84 to 94		
5-year EFS of other subsets, %					
Maximum NSAA ≥ 0.10 IU/mL					
EFS	85		90		
95% CI	78 to 90		83 to 95		
Maximum NSAA < 0.10 IU/mL					.16
EFS	73		91		
95% CI	52 to 86		79 to 97		
Maximum NSAA < 0.10 IU/mL, did not change asparaginase preparation					.058
EFS	76		78		
95% CI	38 to 92		35 to 94		
Changed asparaginase for silent inactivation					.99
EFS	N/A		95		
95% CI			68 to 99		

Abbreviations: EC-Asnase, *E coli* asparaginase; EFS, event-free survival; N/A, not applicable; NSAA, nadir serum asparaginase activity.
*Median dose.

A total of 361 patients participated in both randomizations. When proportional hazard regression modeling was restricted to these patients, dexamethasone and ID EC-Asnase were both independent predictors of favorable EFS, without indication of an interaction (dexamethasone: hazard ratio, 0.49; $P = .02$; ID: hazard ratio, 0.52; $P = .04$).

DISCUSSION

Our study demonstrates that postinduction dexamethasone and ID of L-asparaginase improve EFS in pediatric patients with newly diagnosed ALL.

A novel aspect of our trial was the investigation of ID of EC-Asnase.[23] We demonstrate the feasibility of adjusting the dose of EC-Asnase based on measurement of NSAA in the context of a large multi-institutional study. ID was associated with superior EFS, but did not decrease asparaginase-associated toxicity, increase the proportion of patients able to complete the planned treatment course of asparaginase, or lead to higher (potentially more therapeutic) steady-state NSAA with EC-Asnase.

One possible explanation for this result was that silent inactivation was prospectively identified on the ID but not the FD arm. ID patients with silent inactivation switched asparaginase preparations, but on the FD arm preparation was changed only for clinical allergy. FD patients with low EC-Asnase NSAA (maximum < 0.1 IU/mL) who never switched preparations had a 5-year EFS of 76% compared with 95% for the ID patients who switched preparations for silent inactivation. We and others have previously demonstrated that patients with EC-Asnase hypersensitivity and antibody-positivity achieved therapeutic NSAA after switching preparations.[16,24]

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Furthermore, we demonstrate that patients with silent inactivation also achieve therapeutic NSAA with change in preparation (17 of 19 ID patients with silent inactivation had at least one therapeutic NSAA after switching preparations). Our results suggest that prospectively monitoring for the development of silent inactivation (and not just clinical allergy) and changing asparaginase preparation may improve outcome. In our trial that is currently enrolling, we are monitoring NSAA in real time and switching asparaginase preparation in patients with presumed silent inactivation (two consecutive nondetectable NSAA measurements).

Our results also support the use of dexamethasone instead of prednisone during postinduction treatment phases. There is controversy regarding whether dexamethasone is superior to prednisone. Two trials showed dexamethasone superiority[3,4] whereas others demonstrated no differences in outcome by corticosteroid type.[5,6] We demonstrate that dexamethasone was associated with better EFS in all examined patient subsets.

The beneficial impact of dexamethasone in reducing relapse risk comes at a cost of increased toxicity, primarily in older children and adolescents. Osteonecrosis can lead to severe pain, joint damage, and articular collapse and can necessitate invasive procedures.[7,25-31] Dexamethasone was associated with an increased risk of osteonecrosis and fracture only in patients ages 10 to 18 years. Neither corticosteroid type nor cumulative dose of corticosteroid affected the rate of osteonecrosis in younger patients (ages 1 to 9.99 years). We and others have reported that dexamethasone was associated with higher rates of infection and death when given during induction in conjunction with an anthracycline.[32,33] In this article, we demonstrate that dexamethasone was associated with a higher risk of infection during postinduction treatment, particularly in older children and adolescents, but that risk did not result in a higher toxic death rate.

A limitation of this study is that detailed subgroup analyses of EFS and OS were limited by sample size. In addition, analyses of outcome based on NSAA and asparaginase antibody status were exploratory.

In summary, there was no overall difference in skeletal toxicity by corticosteroid type. Dexamethasone was associated with a higher infection rate and, in older children, increased incidence of osteonecrosis and fracture. In addition, we found no difference in asparaginase-related toxicity by EC-Asnase dosing method. Dexamethasone and pharmacokinetically guided individualized dosing of asparaginase were each associated with favorable EFS. Importantly, dexamethasone was associated with a better outcome in younger patients (1 to 10 years old) without any increased risk in skeletal toxicity. Considering its beneficial impact in reducing relapse risk, we continue to use dexamethasone in postinduction therapy for all age groups. Future studies should focus on minimizing the toxicity of dexamethasone in older pediatric patients without compromising efficacy. Our results suggest that pharmacokinetic monitoring during treatment with EC-Asnase to identify patients with silent inactivation may be an effective strategy to improve the outcome of children and adolescents with ALL.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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