

Allergic Reactions to *E. coli* L-Asparaginase Do Not Affect Outcome in Childhood B-precursor Acute Lymphoblastic Leukemia

A Children's Oncology Group Study

Pierre Wacker, MD,* Vita J. Land, MD,† Bruce M. Camitta, MD,‡ Joanne Kurtzberg, MD,§ Jeanette Pullen, MD,|| Michael B. Harris, MD,¶ and Jonathan J. Shuster, PhD#

Summary: We describe the outcome of children with B-precursor acute lymphoblastic leukemia registered on Pediatric Oncology Group 8602 who switched to Erwinia asparaginase (ASP) due to an allergy to the *Escherichia coli* product. Between February 1986 and January 1991, children in complete remission after induction that included intramuscular *E. coli* ASP (6000 U/m² × 6) were randomized for consolidation. One regimen included intensive weekly intramuscular *E. coli* ASP (25,000 U/m²/wk × 24). In case of an allergic reaction to *E. coli* ASP, Erwinia ASP was substituted at the same dose and schedule. Of the 540 eligible patients, 408 switched to Erwinia ASP due to an allergic reaction. Allergic reactions were significantly associated with younger age, white race, and standard-risk acute lymphoblastic leukemia. Multivariate Cox analysis adjusting for these factors demonstrated no correlation between the switch per se or the timing of the switch and event-free survival.

Key Words: L-asparaginase, acute lymphoblastic leukemia, chemotherapy, allergy

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The antiproliferative activity of L-asparaginase (ASP) has been documented since 1953, when this action was first demonstrated in guinea pig serum.¹ Sensitive leukemic cells lack asparagine synthetase, become

deficient in asparagine, and lose asparagine-dependent protein synthesis. In the past 30 years, numerous studies have emphasized the importance of ASP in the treatment of childhood acute lymphoblastic leukemia (ALL).^{2–4} ASP is effective either as a single agent or as part of multidrug regimens for the treatment of ALL.^{5,6} The suggestion that the use of high-dose ASP during consolidation improved the prognosis of children with non-T-cell ALL⁵ led the Pediatric Oncology Group (POG) to test the effect of weekly high-dose ASP during the first 6 months of therapy in a large randomized study. Results of that study showed no effect of adding ASP on event-free survival (EFS). However, interpretation of this result was complicated by the development of allergic reactions in 75% of ASP-treated patients.^{7,8}

Allergic reactions to ASP, including fatal anaphylaxis, occur in up to 40% of patients, depending on the type of ASP used, the route of administration [intravenous vs. intramuscular (IM) injection], and the administration of concomitant therapy.^{9–14} Erwinia ASP has been administered safely in patients who experienced *Escherichia coli* ASP allergic reactions, but allergy to Erwinia ASP was observed in 22% of patients with prior *E. coli* ASP allergy.^{15–17} The effectiveness of ASP might be decreased in patients who develop allergic reactions.

In this manuscript, we describe the outcomes of a large cohort of children with B-precursor ALL, treated with intensive ASP during consolidation, who switched from *E. coli* ASP to Erwinia ASP owing to a clinical allergic reaction to *E. coli* ASP. Our data show that, although a high percentage of patients switched, neither the switch per se nor its timing affected EFS.

MATERIALS AND METHODS

Between February 1986 and January 1991, children 1 to 21 years of age with newly diagnosed B-precursor ALL were registered on POG 8602 (ALinC 14). Details of diagnostic criteria have been described previously.^{7,8} All patients had more than 25% lymphoblasts in their bone marrow. After informed consent was obtained, patients were stratified by risk group (Table 1) and

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From the *Hôpitaux Universitaires de Genève, Geneva, Switzerland;

†Children's Oncology Group, Washington, DC; ‡Midwest Children's Cancer Center, Milwaukee, WI; §Duke University Medical Center, Durham, NC; ||University of Mississippi Medical Center, Jackson, MS; ¶Tomorrows Children's Institute at Hackensack University Medical Center, Hackensack, NJ; and #COG Statistical Office at the University of Florida, Gainesville, FL.

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Reprints: Bruce M. Camitta, MD, Children's Oncology Group, 440 E. Huntington Dr, 3rd Floor, Arcadia, CA 91066 (e-mail: bcamitta@mcw.edu).

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TABLE 1. Risk Groups Definitions (POG 8602)

Age (y)	< 1	1-2	3-5	6-10	> 10
WBC level ($\times 10^9/L$)					
< 10	B	A	A	A	B
10-99.999	B	B	A	B	B
≥ 100	B	B	B	B	B
Liver or spleen below umbilicus; extramedullary leukemia	B	B	B	B	B

A indicates standard-risk; B, poor-risk.

immunophenotype. Induction, including IM *E. coli* ASP ($6000 \text{ U/m}^2 \times 6$), was identical for all patients.¹⁸ Patients who entered remission were randomized for consolidation therapy to 4 regimens: intermediate-dose methotrexate (6 doses every 3 wk) (regimen A); regimen A plus intensive IM ASP ($25,000 \text{ U/m}^2$ weekly from week 7 to week 30; 24 doses) (regimen B); regimen A plus cytosine arabinoside (regimen C); and intermediate-dose methotrexate plus cytosine arabinoside (every 12 wk for 6 doses) (regimen D). All patients received the same continuation therapy (daily mercaptopurine, weekly methotrexate, and pulses of vincristine plus prednisone), and central nervous system prophylaxis with triple intrathecal medications.^{7,8}

Allergy to ASP was diagnosed clinically by local investigators according to National Cancer Institute (NCI) cancer toxicity criteria and reported to the POG study coordinator. In case of a clinical allergic reaction to *E. coli* ASP, Erwinia ASP could be substituted at the same dose and schedule. In case of pancreatitis or diabetes (uncontrolled by insulin), ASP administration was discontinued altogether.

Patients

Between May 1987 and January 1991, 551 patients were randomized to regimen B (Table 2). Eleven patients were ineligible for analysis because no ASP was given (7) or for insufficient data (4).

Definitions

Complete remission was defined as $< 5\%$ marrow blasts with evidence of regeneration of normal hematopoiesis, no blasts in cerebrospinal fluid, absence of

TABLE 2. Patients Randomized to Regimen B of POG 8602

Stratum	Entered	Eligible
Standard-risk EPB	174	168
Standard-risk PB	114	113
Standard-risk NOS	8	7
Poor-risk EPB	155	154
Poor-risk PB	91	89
Poor-risk NOS	9	9
Total	551	540

Risk group by NCI criteria.

EPB indicates early pre-B; NOS, not otherwise specified; PB, pre-B.

symptoms and signs attributable to leukemia, and a normal clinical performance status. Central nervous system leukemia was diagnosed when blasts were observed on a cytospin examination of cerebrospinal fluid in which the white blood cell (WBC) count was $\geq 5 \text{ cells}/\mu\text{L}$. The NCI Consensus Risk Groups defines standard-risk as children 1 to < 10.00 years of age with $\text{WBC} < 50.0 \times 10^9/L$.

Statistical Considerations

This study is mainly descriptive. EFS is defined using the time from registration to the earliest of: failure (induction failure, relapse, second cancer, or remission death) or last clinical contact. Secondly, postconsolidation EFS (EFS/c) was used. Comparisons were conducted by 2-sided log-rank tests and by Cox proportional hazards tests as indicated. The association between patients who had allergic reactions and their clinical features was conducted by χ^2 analysis (all or none features) or by the 2-sided Wilcoxon rank test for age and WBC count.

RESULTS

Of the 540 eligible patients (302 males and 238 females), 408 (76%) switched from *E. coli* ASP to Erwinia ASP at different times (Tables 3, 4). Regimen B specified 24 ASP doses. The mean ASP doses received (SD) were $20.0 (\pm 6.3)$, $8.8 (\pm 8.1)$, and $11.2 (\pm 8.4)$ for total ASP, *E. coli* ASP, and Erwinia ASP doses, respectively (Table 3). There were similar frequencies of switching in males (74%) and females (78%) ($P = 0.30$). Children who switched were significantly younger than those who did not ($5.6 \pm 3.8 \text{ y}$ vs. $7.4 \pm 4.6 \text{ y}$; $P < 0.001$). White children switched more often than children from minorities (white 342/438; Black 30/50; and Hispanic 18/31) (white vs. Black $P = 0.005$; white vs. Hispanic $P = 0.012$). Children with standard-risk ALL had a 79% (290/369) frequency of switch compared with 69% (117/170) for children with poor-risk ALL ($P = 0.016$). WBC at diagnosis was similar in the switch and the no-switch groups.

To evaluate the prognostic importance of switching, 2-sided log-rank comparisons of EFS and EFS/c were studied. EFS and EFS/c were similar in patients who switched and those who did not ($P = 0.78$ and 0.82 ; 2-sided, respectively). EFS and EFS/c were also similar in patients who experienced a grade IV allergic reaction and those who did not ($P = 0.38$ and 0.4 ; 2-sided, respectively) (Table 5). Multivariate Cox analysis, adjusting for race, NCI risk group, and age demonstrated no

TABLE 3. Descriptive Statistics (Planned ASP = 24 Doses)

	Mean	SD	Quartiles*
Total ASP	20.0	6.3	17, 24, 24
<i>E. coli</i> ASP	8.8	8.1	3, 5, 14
Erwinia ASP	11.2	8.4	1, 12, 19

*25th, median, 75th.

TABLE 4. Chronology of Switch (N = 408)

<i>E. coli</i> ASP Doses	No. Patients	Mean (Erwinia ASP)	SD
0	14	20.2	6.6
1	35	14.4	7.7
2	31	13.1	8.9
3	98	15.7	6.5
4	56	16.2	5.4
5	39	16.2	4.8
6	40	15.8	4.4
7	21	14.4	3.7
8	20	14.3	3.8
9	15	12.5	3.8
10	8	13.0	2.1
11	4	11.5	3.0
12	1	12.0	—
13	5	8.4	3.6
14	4	8.5	3.0
15	3	7.0	2.0
16	5	7.4	0.9
17	1	7.0	—
18	4	6.0	0.0
19	2	4.0	1.4
≥ 20	2	1.5	0.5

significant correlation between the switch and EFS (Table 6). Furthermore, in those who switched, EFS did not correlate significantly with the time of switch (data not shown).

DISCUSSION

In children with newly diagnosed B-precursor ALL, 98% to 99% achieve remission with multiagent induction chemotherapy containing ASP. Previous studies suggested a beneficial effect of high-dose ASP during subsequent consolidation.^{4,5} However, those studies were not randomized (used historical controls) or the benefit was of borderline significance ($P = 0.07$). The POG 8602 study failed to show an improvement in EFS for patients who received weekly ASP during consolidation. By contrast, the use of ASP in a regimen similar to this protocol improved EFS for patients with T-cell ALL and advanced stage T-cell non-Hodgkin lymphoma.⁶ As the presence of allergic reactions might alter the efficacy of therapy, the present report focused on the presence and timing of allergic reactions to ASP and their relationships to remission duration.

A wide range of clinical allergic reactions to ASP has been reported. Reactions include pain at the injection site, localized erythema to a generalized urticaria, tachypnea, and wheezing. Rarely, grade IV allergic reactions and/or death due to anaphylaxis are observed. The ASP pilot predicted that 70% of patients would have a clinical allergic reaction to *E. coli* ASP (N = 20 patients). Nonetheless, the phase III trial was conducted.

As allergy to ASP is associated with anti-ASP antibodies and a lower serum ASP activity, the presence of ASP allergy might be associated with a lower antileukemic effect.^{19,20} However, in our study, the presence of allergy to *E. coli* ASP had no significant correlation with the EFS/c, even in those patients who experienced early switch and/or grade IV allergic reactions. These results confirm another study in which patients with allergic reactions to *E. coli* ASP had high titers of anti-ASP antibodies but the presence of ASP antibodies had no effect on the 4-year EFS.²¹

Erwinia and *E. coli* ASP are not bioequivalent. Erwinia ASP has a shorter half-life than the *E. coli* product. Failure to find a benefit of ASP in our study might therefore reflect less effective asparagine depletion in patients who switched to Erwinia. In the present study, 2.6% of the patients (14/540) had switched to Erwinia before the first consolidation high-dose ASP. Moreover, 57% of the patients who switched did so during early consolidation (before the fifth dose of ASP). Nevertheless, patients who switched had similar EFS and EFS/c compared with patients who did not, regardless of the timing of the switch. A randomized EORTC study using a Berlin-Frankfurt-Munster-like regimen for children with ALL showed a lower 6-year EFS in children treated with Erwinia ASP compared with those treated with *E. coli* ASP (59.8% and 73.4%, respectively) ($P = 0.0004$).²² However, analysis of the EORTC study is complicated by an interaction between the ASP question and another randomization regarding mercaptopurine.

The lack of a better EFS for patients treated with ASP during consolidation could be due to the inability of many patients (25%) to receive the planned 24 ASP doses. However, in the present study, patients who were administered the planned ASP doses had similar EFS to those who were not, even after stratification for risk groups. A retrospective analysis of ASP toxicity in adults

TABLE 5. EFS and EFS After Consolidation (EFS/c) (Log-rank Test, 2-sided)

	No			Yes			<i>P</i>
	N	Fail	Expected	N	Fail	Expected	
a) Switch vs. no switch							
EFS	132	42	40.4	408	127	128.6	0.78
EFS/c	128	38	36.8	397	116	117.2	0.82
b) Grade IV allergic reactions vs. no grade IV allergic reaction							
EFS	495	157	153.8	45	12	15.2	0.38
EFS/c	481	143	140	44	11	14.0	0.40

TABLE 6. Prognostic Significance of Erwinia ASP Use (Cox Model)

	Estimated Hazard Ratio	95% Confidence Limits	P
EFS	1.08	0.77, 1.51	0.69
EFS/c	1.07	0.74, 1.56	0.72

with lymphoblastic leukemia showed no difference in leukemia-free survival between groups of patients who received various numbers of doses of ASP.²³

The frequency of allergic reactions observed (75%) was higher than generally reported (5% to 20%).^{3,4,17} The higher rate in our patients when compared with the Dana Farber Cancer Institute experience might reflect our use of ASP in both induction and consolidation versus consolidation alone in Dana Farber Cancer Institute studies. More intensive postremission treatment might also decrease the frequency of allergic reactions to ASP. However, this could not explain the lower frequency of allergic reactions in our higher risk patients, because they received the same treatment as the standard-risk children.

Race differences in ASP allergy have not been reported, probably because of the low numbers of patients analyzed and also the lower frequency of ASP allergy reported in other studies. In our study, white children had a higher frequency of allergic reactions than either Black or Hispanic patients. The explanation for these data is not immediately apparent but could involve variations in HLA genes or other immunoregulatory factors.

Our data do not support the use of repeated ASP during consolidation therapy of children with B-precursor ALL. However, owing to the potential mitigating factors discussed above, it is possible that less immunogenic or longer acting forms of ASP might be more effective. Further studies of ASP in patients at higher risk of relapse are currently in progress.

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APPENDIX

Participating Institutions and NCI Support (POG 8602)

Institution	Grant Number
Alberta Children's Hospital	
All Children's Hospital	
Baylor College of Medicine	CA-03161
Boston Floating Hospital	
Cancer Center of Hawaii	
Carolinas Medical Center	CA-69177
Children's Hospital (San Diego)	
Children's Hospital Greenville System	CA-69177
Children's Hospital Michigan	CA-29269
Children's Hospital New Orleans/LSU CCOP	
Children's Memorial Hospital (Chicago)	CA-07431
Christ Hospital	CA-07431
City of Hope	
Cleveland Clinic	
Cook County Children's Hospital	
Cross Cancer Institute	
Dartmouth Hitchcock	CA-29293
Duke University	CA-15525
East Carolina University	CA-69177
Emory University	CA-20549
Fairfax Hospital	CA-28476
Fitzsimmons Army Medical Center	
Hackensack Medical Center/Tomorrow's Children's Institute	
Hurley Medical Center	CA-29691
Joe DiMaggio Children's Hospital	
Johns Hopkins University	CA-28476
Keesler AFB Hospital	CA-15898
Madigan Army Medical Center	
Massachusetts General Hospital	CA-29293
McGill University/Montreal Children's Hospital	CA-33587
Medical College Virginia	
Medical University of South Carolina	CA-69177
Miami Children's Hospital	
Midwest Children's Cancer Center	CA-32053
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Nemours Children's Clinic-Orlando	
Nemours Children's Clinic-Jacksonville	
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Presbyterian Hospital	CA-69177
Rhode Island Hospital	CA-29293
Roswell Park Cancer Institute	CA-28383
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Sacred Heart Hospital	
San Antonio MPC & BDC	
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St Christopher's Hospital	
St Johns Hospital	CA-29691
St Jude Children's	CA-31566
St Louis University/Cardinal Glennon Children's Hospital	
St Mary Hospital	
Stanford University	CA-33603
SUNY Syracuse	
Sutter Community Hospital	
Tampa Children's Hospital	
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UC-Davis	
UC-San Diego	CA-28439
University of Alabama-Birmingham	CA-25408

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Institution	Grant Number
University of Arizona	CA-33603
University of Arkansas	
University of Florida	
University of Kansas	
University of Miami	
University of Mississippi Medical Center	CA-15989
University of Missouri	CA-05587
University of New Mexico	
University of South Alabama	
University of South Florida	
University of Vermont	CA-29293
University of Virginia	
UT-Galveston	CA-03161
UT-San Antonio	
Via Christi Regional Medical Center	
Wake Forest University School of Medicine	CA-53128
Walter Reed Army Medical	
Warren Clinics	CA-11233
Washington University	CA-05587
West Virginia University, Charleston	CA-15525
West Virginia University, Morgantown	CA-15525
Yale University	CA-69428