

Allergic Reactions and Antiasparaginase Antibodies in Children With High-Risk Acute Lymphoblastic Leukemia: A Children's Oncology Group Report

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BACKGROUND: The objectives of this study were to assess the incidence of clinical allergy and end-induction antiasparaginase (anti-ASNase) antibodies in children with high-risk acute lymphoblastic leukemia treated with pegylated (PEG) *Escherichia coli* ASNase and to determine whether they carry any prognostic significance. **METHODS:** Of 2057 eligible patients, 1155 were allocated to augmented arms in which PEG ASNase replaced native ASNase postinduction. *Erwinia chrysanthemi* (*Erwinia*) ASNase could be used to replace native ASNase after allergy, if available. Allergy and survival data were complete for 990 patients. End-induction antibody titers were available for 600 patients. **RESULTS:** During the consolidation phase, 289 of 990 patients (29.2%) had an allergic reaction. There were fewer allergic reactions to *Erwinia* ASNase than to native ASNase (odds ratio, 4.33; $P < .0001$) or PEG ASNase (odds ratio, 3.08; $P < .0001$) only during phase 1 of interim maintenance. There was no significant difference in 5-year event-free survival (EFS) between patients who received PEG ASNase throughout the entire study postinduction versus those who developed an allergic reaction to PEG ASNase during consolidation phase and subsequently received *Erwinia* ASNase ($80.8\% \pm 2.8\%$ and $81.6\% \pm 3.8\%$, respectively; $P = .66$). Patients who had positive antibody titers postinduction were more likely to have an allergic reaction to PEG ASNase (odds ratio, 2.4; $P < .001$). The 5-year EFS rate between patients who had negative versus positive antibody titers ($80\% \pm 2.6\%$ and $77.7\% \pm 4.3\%$, respectively; $P = .68$) and between patients who did not receive any ASNase postconsolidation and those who received PEG ASNase throughout the study ($P = .22$) were significantly different. **CONCLUSIONS:** The current results demonstrate differences in the incidence rates of toxicity between ASNase preparations but not in EFS. The presence of anti-ASNase antibodies did not affect EFS. **Cancer** 2015;000:000-000. © 2015 American Cancer Society.

KEYWORDS: asparaginase, childhood acute lymphoblastic leukemia, drug hypersensitivity, survival.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common cancer in children. Currently, cure rates surpass 85%.¹ Asparaginase (ASNase) is an enzyme that catalyzes the hydrolysis of the amino acid asparagine into aspartic acid and ammonia and has been a key chemotherapeutic agent in the treatment of ALL for more than 40 years. The first use of ASNase in treating children with ALL was reported in 1966, and the first use of ASNase derived from *Escherichia coli* was reported in 1967.²⁻⁴ It has been a mainstay of therapy ever since.

Despite the vast experience with ASNase, questions remain regarding the optimal preparation, dose, and dosing schedule. L-asparaginase comes from 2 bacterial sources, *E. coli* (native ASNase, Elspar; Lundbeck, Deerfield, Ill) and *Erwinia chrysanthemi* (*Erwinia* ASNase, Erwinase; Speywood Laboratories, Maidenhead, UK). Pegylation (PEG) of native ASNase (PEG ASNase, Oncaspar; Sigma-Tau Pharmaceuticals, Gaithersburg, Md) decreases proteolysis, increases drug half-life, and decreases immunogenicity with at least equivalent efficacy at an appropriate dose and schedule.^{5,6} It has been demonstrated in 2 large, multiinstitutional, cooperative group trials that *Erwinia* ASNase has less toxicity but inferior

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Additional Supporting Information may be found in the online version of this article.

DOI: 10.1002/cncr.29641, **Received:** April 23, 2015; **Revised:** July 17, 2015; **Accepted:** July 21, 2015, **Published online** Month 00, 2015 in Wiley Online Library (wileyonlinelibrary.com)

efficacy when used with the same dose and schedule as native ASNase, which is believed to be caused at least in part by the shorter half-life of *Erwinia* ASNase.^{7,8}

Herein, we report the experience of patients treated on arms containing PEG ASNase from a Children's Cancer Group (CCG) trial (Treatment of Patients With Acute Lymphoblastic Leukemia With Unfavorable Features; CCG-1961). We describe associations of allergic reactions and anti-ASNase antibodies as well as different preparations of ASNase with event-free survival (EFS). To our knowledge, this represents the largest cohort of PEG ASNase-treated patients reported to date.

MATERIALS AND METHODS

Between November 1996 and May 2002, 2057 eligible patients with newly diagnosed, high-risk ALL were enrolled onto CCG-1961. The patient characteristics of this cohort and treatment details were previously published.⁹ High-risk status was determined according to standard National Cancer Institute (NCI) criteria and included patients who were either older than 10 years or had a white blood cell count $>50,000$ cells/ μL . All patients received a standard 4-drug induction that included intramuscular native ASNase. Patients who had $<25\%$ blasts in a day-7 bone marrow aspirate were considered rapid early responders and were randomized to receive either standard-intensity and standard-duration (arm A), standard-intensity and increased-duration (arm B), increased-intensity and standard-duration (arm C), or increased-intensity and increased-duration (arm D) post-induction therapy. All slow early responders (those who had a day-7 bone marrow aspirate with $\geq 25\%$ blasts) received increased-intensity and increased-duration post-induction intensification and also were randomized to receive cyclophosphamide and either doxorubicin or idarubicin. Patients who were randomized to the increased-intensity arms were assigned 6 or 10 doses (arms C and D, respectively) of intramuscular PEG ASNase postinduction ($n = 1155$). The NCI and the institutional review boards of the participating institutions approved this protocol. Informed consent was obtained from the patients, their parents, or both before starting therapy.

Of 2057 eligible patients enrolled on CCG-1961, 1067 patients were excluded from analyses for the following reasons: Philadelphia chromosome-positive disease, missing data on reaction status, or randomization to a standard-intensity arm. Data were complete for 990 patients in the PEG ASNase-containing arms who received single monthly doses of PEG ASNase (2500 IU/ m^2 per dose) during postinduction courses

before maintenance therapy. Patients received native ASNase (6000 IU/ m^2 per dose $\times 6$ per single dose of PEG ASNase) when PEG ASNase was unavailable. Patients who had adverse reactions to PEG ASNase or native ASNase received *Erwinia* ASNase (10,000 IU/ m^2 per dose every other day $\times 6$ doses per dose of PEG ASNase).

Institutions were required to submit details for all patients who experienced a reaction to any form of ASNase, defined according to CCG Toxicity and Complications Criteria, which are based on NCI Common Toxicity Criteria. According to the protocol, patient serum samples were collected at the end of induction and before delayed intensification phase 1 and maintenance to determine the presence of anti-ASNase antibodies. The methodology and definitions of positive and negative antibody titers have been previously published.¹⁰

First, we analyzed the incidence of allergic reactions to the 3 different forms of ASNase for patients enrolled on PEG ASNase-containing arms (arm 1). Then, allergic reactions to PEG ASNase were correlated with the presence or absence of end-induction anti-ASNase antibodies (arm 2). Because we believed that patients with high anti-ASNase antibody titers would be more likely to react to PEG ASNase exposure during the consolidation phase, a subanalysis was performed to examine patients who had positive end-induction antibodies and allergic reactions to PEG ASNase limited to the consolidation phase only. Next, end-induction anti-ASNase antibodies were correlated with EFS (arm 3). To determine whether patients who developed a severe allergic reaction to PEG ASNase could receive *Erwinia* ASNase as a substitute, we compared the EFS of patients who received PEG ASNase throughout the study and had no allergic reaction with the EFS of patients who developed an allergic reaction to PEG ASNase during consolidation phase and subsequently received *Erwinia* ASNase as a substitute (arm 4). Last, because a significant minority of patients did not receive any ASNase after consolidation because of a severe adverse event, the EFS of patients who received PEG ASNase throughout the study was compared with that of patients who received PEG ASNase during consolidation phase only and did not receive any form of ASNase thereafter (arm 5).

Statistical Methods

Study data for outcome analyses were frozen on March 28, 2008. All analyses were restricted to patients who were enrolled on the CCG-1961 treatment arms that contained PEG ASNase. EFS was defined as the time from randomization to either the first event (failure to achieve remission,

TABLE 1. The Number of Patients in Each Course by Reaction Status

Course of Therapy	No. of Patients (%)						Total
	<i>Erwinia</i> ASNase		PEG ASNase		Native ASNase		
	No Reaction	Allergic Reaction	No Reaction	Allergic Reaction	No Reaction	Allergic Reaction	
Consolidation	2 (66.7)	1 (33.3)	642 (71.4)	257 (28.6)	57 (64.8)	31 (35.2)	990
IM1	216 (91.9)	19 (8.1)	487 (78.7)	132 (21.3)	63 (72.4)	24 (27.6)	941
DI1	301 (94.7)	17 (5.4)	457 (95.2)	23 (4.8)	61 (96.8)	2 (3.2)	861
IM2	195 (92.4)	16 (7.6)	277 (89.6)	32 (10.4)	37 (100)	0 (0)	557
DI2	189 (97.4)	5 (2.6)	270 (98.2)	5 (1.8)	32 (94.1)	2 (5.9)	503

Abbreviations: *Erwinia* ASNase, *Erwinia chrysanthemi* asparaginase; DI, delayed intensification; IM, interim maintenance; native ASNase, *Escherichia coli* asparaginase; PEG ASNase, pegylated asparaginase.

TABLE 2. Sample Size, Odds Ratios, and Confidence Intervals for Each Comparison of Allergic Reactions by Preparation and Course

Course of Therapy	<i>Erwinia</i> ASNase/Native ASNase		<i>Erwinia</i> ASNase/PEG ASNase		PEG ASNase/Native ASNase	
	OR (95% CI)	No.	OR (95% CI)	No.	OR (95% CI)	No.
Consolidation	0.92 (0.08-11.11)	91	1.25 (0.11-14.29)	902	0.74 (0.46-1.17)	987
IM1	0.23 (0.12-0.45)	322	0.32 (0.2-0.54)	854	0.71 (0.43-1.18)	706
DI1	1.72 (0.39-7.69)	381	1.12 (0.59-2.13)	798	1.54 (0.35-6.67)	543
IM2	—	248	0.71 (0.38-1.33)	520	—	346
DI2	0.42 (0.08-2.27)	228	1.43 (0.41-5)	469	0.30 (0.06-1.59)	309

Abbreviations: *Erwinia* ASNase, *Erwinia chrysanthemi* asparaginase; CI, confidence interval; DI, delayed intensification; IM, interim maintenance; native ASNase, *Escherichia coli* asparaginase; OR, odds ratio; PEG ASNase, pegylated asparaginase.

relapse, second malignancy, or death) or the last contact. Estimates for EFS were calculated using the Kaplan-Meier method, and standard errors of the estimates were obtained using the method of Peto and Peto.¹¹ The log-rank test was used to compare survival curves among groups. Odds ratios (ORs) and their 95% confidence intervals (CIs) were estimated. Chi-square tests were used to analyze categorical variables. Statistical significance was defined as $P < .05$. All analyses were performed using SAS software (IBM Corporation, Armonk, NY). All graphics were generated using the R software package (version 2.8.1; R Foundation for Statistical Computing, Vienna, Austria; available at: <http://www.R-project.org>; Last Accessed July, 2002).

RESULTS

Incidence of Allergic Reactions by Phase

The total number of patients who received 3 different forms of ASNase and the incidence of allergic reactions during each phase is summarized in Table 1. The likelihood of allergic reactions was similar with PEG ASNase, native ASNase, and *Erwinia* ASNase during all phases of therapy, with the exception of interim maintenance phase 1, in which the incidence of an allergic reaction to *Erwinia* ASNase was less likely than that for both native ASNase

(OR, 0.23; $P < .0001$) and PEG ASNase (OR, 0.32; $P < .0001$) (see Table 2).

End-Induction Anti-ASNase Antibody Status and Allergic Reactions to PEG ASNase

Of 600 patients who had antibody data available at the end of induction, 340 received PEG ASNase during subsequent phases. There were 97 patients (28.5%) with a positive antibody titer (>1.1) and 243 (71.5%) with a negative antibody titer (≤ 1.1). Patients who had a positive antibody titer were 2.41 times more likely to have an allergic reaction to PEG ASNase postinduction than patients who had a negative antibody titer (OR, 2.41; 95% CI, 1.49-3.89; $P < .001$). It is noteworthy that there were no significant demographic differences between patients who had antibody data and those who did not (Supporting Tables 1-4; see online supporting information), and this was consistent with patient demographic information reported for the entire study population.⁹

Subanalysis of Antibody Status and Allergic Reactions

There were 298 patients who had antibody titer measurements and received PEG ASNase during consolidation.

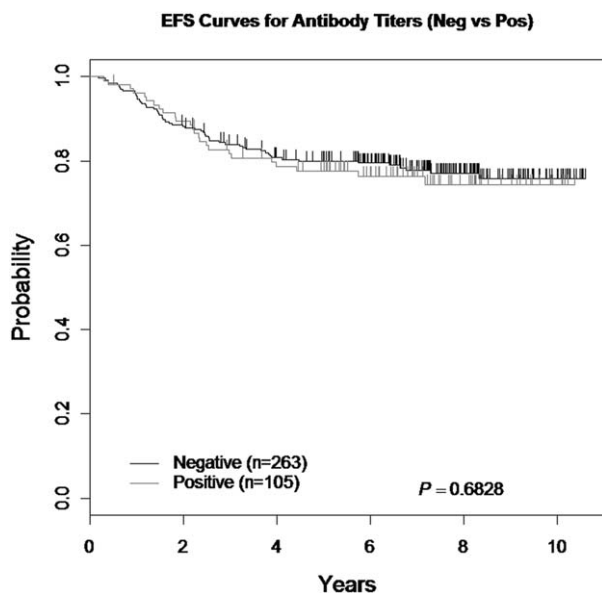


Figure 1. Event-free survival (EFS) is compared according to antibody status (negative vs positive).

Of these, 89 patients (29.9%) had an allergic reaction to PEG ASNase, and 88 (29.5%) had a positive antibody titer. Among those with positive antibody titers, 30 of 88 patients (34.1%) had an allergic reaction to PEG ASNase when we limited the time frame to the consolidation phase only (OR, 1.3; 95% CI, 0.78-2.3; $P = .33$). There was no statistically significant increase in risk for patients who had a positive antibody titer plus an allergic reaction when we limited the time frame to the consolidation phase alone.

End-Induction Antibody Status and EFS

Of 600 patients who had end-induction antibody data available, 368 patients were enrolled on PEG ASNase-containing arms (although not all received PEG ASNase). Of those 368 patients, 105 (28.5%) had a positive antibody titer, and 263 (71.5%) had a negative antibody titer. The 5-year EFS for patients who were randomized to a PEG ASNase-containing regimen and had a negative antibody titer was $80\% \pm 2.6\%$ versus $77.7\% \pm 4.3\%$ for patients who had a positive antibody titer ($P = .68$) (see Fig. 1).

Subanalysis of Antibody Status and EFS

Among 368 patients who had complete antibody titer data available, 67 did not receive PEG ASNase during consolidation. Of the remaining 301 patients, 88 (29.2%) had a positive antibody titer, and 213 (70.8%) had a negative antibody titer. The 5-year EFS for patients who received only PEG ASNase throughout therapy was

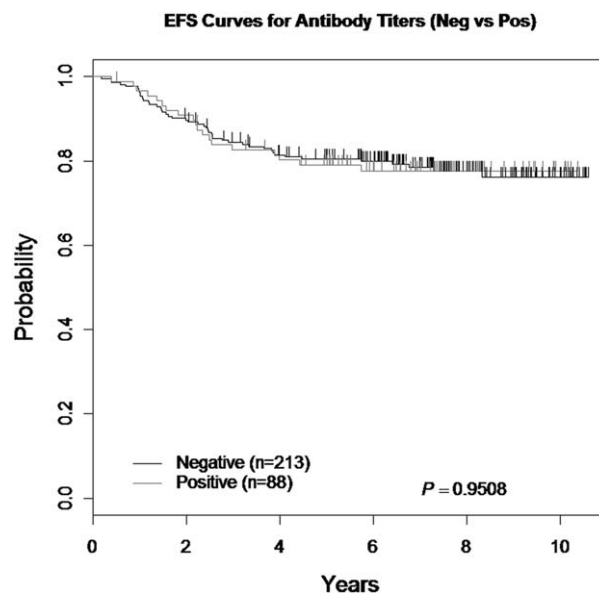


Figure 2. Event-free survival (EFS) is compared according to antibody status (subanalysis-received PEG-ASNase only).

$80.4\% \pm 2.9\%$ and $79.1\% \pm 4.6\%$ ($P = .95$) for patients with negative and positive antibodies, respectively (see Fig. 2). Of the 65 patients who had an event, 46 (70.8%) had a negative antibody titer.

Allergic Reactions and EFS

When the survival of patients who received PEG ASNase throughout the study and had no allergic reaction was compared with the EFS of those who had an allergic reaction to PEG ASNase during consolidation and were switched to *Erwinia* ASNase in subsequent phases without an allergic reaction, the 5-year EFS was $80.8\% \pm 2.8\%$ and $81.6\% \pm 3.8\%$, respectively ($P = .66$) (Fig. 3). There were no demographic differences between patients who did and did not have allergic reactions.

PEG ASNase Versus No PEG ASNase Postconsolidation and EFS

There was no statistically significant difference in the 5-year EFS between patients who received PEG ASNase during consolidation only compared with patients who received PEG ASNase during all postinduction courses ($74.2\% \pm 6.5\%$ and $80\% \pm 2.7\%$, respectively; $P = .22$) (Fig. 4). There were no demographic differences between those who did and did not receive PEG ASNase throughout treatment.

DISCUSSION

ASNase has been an integral component of multiagent chemotherapeutic regimens for the treatment of ALL for

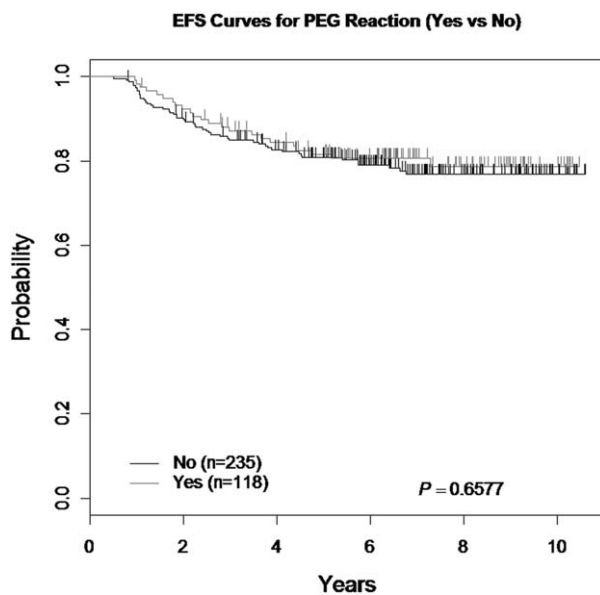


Figure 3. Event-free survival (EFS) is compared according to pegylated asparaginase (PEG ASNase) reaction status (yes vs no).

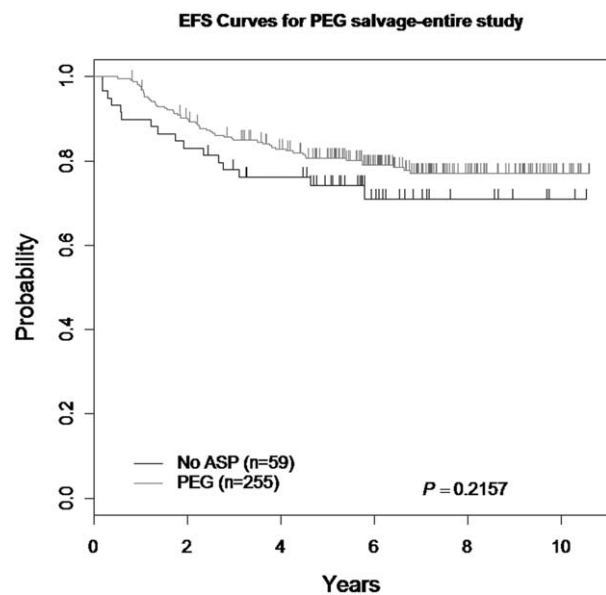


Figure 4. Event-free survival (EFS) is compared according to the receipt of pegylated asparaginase (PEG ASNase) versus no PEG ASNase postconsolidation.

over 40 years. To the best of our knowledge, CCG-1961 has been 1 of the largest studies conducted using PEG ASNase. We conducted a secondary analysis of allergic reactions to 3 different ASNase preparations used in the study and outcomes according to antibody status, allergic reactions to PEG ASNase, and postconsolidation receipt of PEG ASNase.

Incidence rates of hypersensitivity reactions to ASNase during consolidation in this study were consistent with reported rates ranging from 2% to 45%.^{10,12-16} *Erwinia* ASNase was associated with less allergic reactions compared with both PEG ASNase and native ASNase during interim maintenance phase 1 only. In all other phases of therapy, there was no significant difference in the incidence of allergic reactions. Possible explanations for these findings could be that too few patients received *Erwinia* ASNase or native ASNase for a meaningful comparison during consolidation; not many patients had allergic reactions to any form of ASNase during delayed intensification phases 1 and 2, because dexamethasone was administered concurrently; or, in interim maintenance phase 2, there few reactions, because the majority of allergic reactions occurred early during the course of treatment. The decreased incidence of reactions to *Erwinia* ASNase also may be related to its shorter half-life and different antigenic structures. We do know of reports indicating that antibodies to native ASNase measured by enzyme-linked immunosorbent assay do not cross-react to

Erwinia ASNase, so it is unlikely that the antibodies measured also react to *Erwinia* ASNase.^{17,18} It is likely that there is no significant difference in hypersensitivity reactions between forms of ASNase when treated on this protocol.

It is believed that allergic reactions to ASNase occur as a result of antibodies, which cause significant toxicity and neutralize the enzyme's activity.^{19,20} Although there are different methods for measuring the presence of anti-ASNase antibodies, the current standard is the enzyme-linked immunosorbent assay method.^{15,21} In our study, end-induction antibodies were associated with an increased incidence of clinical allergy to PEG ASNase during subsequent treatment phases, but they did not affect 5-year EFS. In a subanalysis of patients who had positive end-induction antibody titers and a subsequent allergic reaction, there was no statistically significant increase in the risk of an allergic reaction during consolidation versus later phases of therapy. We expected that patients who had positive antibody titers would have a higher incidence of allergic reactions during consolidation than in later phases of therapy because the overall presence of anti-ASNase antibodies would have a statistically significant association with the development of allergic reactions. However, this was not the case. It is possible that the incidence of allergic reactions, when limited to the consolidation phase, was not statistically significant because of the prednisone taper, which continued into

consolidation, although the steroids were stopped over a week before patients received additional ASNase. Furthermore, repeated exposure to PEG ASNase may have increased the likelihood of an allergic reaction occurring when patients had anti-ASNase antibodies. In addition, the presence of anti-ASNase antibodies did not result in inferior 5-year EFS, as has been suggested in the literature.^{13,15}

There have been various studies investigating the clinical efficacy of different preparations of ASNase in the treatment of childhood ALL. In a pair of studies that compared native ASNase with PEG ASNase (Dana Farber Cancer Institute [DFCI] 91-01 and CCG-1962), there was no difference in survival between the 2 groups, but those studies were not powered to examine this question.^{14,15} In a trio of studies that compared native ASNase with *Erwinia* ASNase (DFCI 95-01, European Organization for Research and Treatment of Cancer [EORTC]-Children's Leukemia Group study 58881, and the National University Hospital Ma-Spore ALL study), native ASNase improved survival compared with *Erwinia* ASNase when given on the same schedule and at the same dose.^{7,8,22} However, it is known that *Erwinia* ASNase has a shorter half-life, and it is possible that the difference in pharmacokinetics and the relative underdosing of *Erwinia* ASNase caused inferior survival in the DFCI and EORTC studies. Vrooman et al examined the outcomes of patients on DFCI ALL Consortium study 00-01 who developed allergies to native ASNase and were switched to *Erwinia* ASNase. The dose of native ASNase in that study was 25,000 IU/m² per dose given weekly, which was substituted with *Erwinia* ASNase 25,000 IU/m² per dose given twice weekly. Those authors observed neither an increase in toxicity nor a difference in EFS.²³ Willer et al performed a retrospective review of patients who initially received native ASNase but developed high anti-ASNase antibody levels (>200 AU/mL) and discovered that they did not have an increase in ASNase activity as measured by the indo-oxin method after switching to PEG ASNase, although there was a significant increase in ASNase activity when patients were switched to *Erwinia* ASNase.²⁴ Because of potentially decreased efficacy with *Erwinia* ASNase based on the DFCI and EORTC study results, we analyzed the survival data for patients who were switched to *Erwinia* ASNase postconsolidation because of an allergic reaction to PEG ASNase and observed no difference in 5-year EFS compared with patients who received PEG ASNase throughout the study. Previously published data on patients enrolled on CCG-1961 who had neutralizing antibodies to native ASNase indicated

that patients can be receive *Erwinia* ASNase and still retain good activity of the enzyme.²⁵ These data suggest that 6 doses of intramuscular *Erwinia* ASNase at 10,000 IU/m² per dose may be an adequate substitute for intramuscular PEG ASNase 2500 IU/m² and can replace PEG ASNase in patients who develop antibodies and/or clinical allergies to native ASNase.

Because a small but significant number of patients did not receive any ASNase postconsolidation on account of serious adverse events (eg, anaphylaxis, thrombosis, pancreatitis, etc), we compared the 5-year EFS of such patients with the EFS of patients who received PEG ASNase throughout the study, and we observed no difference. Although we are tempted to speculate that this may indicate that ASNase is unnecessary after consolidation, the numbers of patients were small, and this finding should be investigated in the future. More recently, reviews of patients who could not receive further ASNase on frontline studies (UKALL 2003)²⁶ and in relapsed patients (ALLR3)²⁷ revealed no difference in EFS or overall survival when comparing those who could not continue to receive ASNase versus those who did continue. Of course, these reflect different populations of patients on different protocols; however, as more evidence emerges, we believe that this should be investigated further.

Here, we have demonstrated that: 1) *Erwinia* ASNase is associated with less hypersensitivity compared with PEG ASNase and native ASNase during interim maintenance phase 1 only; 2) the presence of end-induction anti-ASNase antibodies is associated with an increased risk of allergic reaction in subsequent phases; 3) the presence of end-induction anti-ASNase antibodies does not affect EFS; 4) patients with an allergy to PEG ASNase do not have poorer EFS when it is substituted with *Erwinia* ASNase; and 5) there may be no difference in EFS between patients who did not receive any form of ASNase postconsolidation and those who received PEG ASNase throughout the study.

There are a few limitations to this study. First, these analyses were done retrospectively and were not the endpoints that were defined for analysis. Second, current protocols no longer use native ASNase during induction, which may alter the rates of antibodies formed against ASNase during induction and allergic reactions to ASNase in subsequent courses. Third, we were unable to ascertain whether patients who had allergic reactions or other adverse effects to ASNase that caused the discontinuation of ASNase received other therapies that may have obviated the need for ASNase.

The current generation of Children's Oncology Group studies uses PEG ASNase. Although allergic reactions are likely to continue, the results from this study suggest that patients would be able to receive *Erwinia* ASNase without affecting outcome. Although a substantial amount of information regarding ASNase and its use in the treatment of childhood leukemia exists, there is still much to be learned. We acknowledge that these analyses were not the initial aims of the study, but they may help in asking specific questions about ASNase in future clinical trials.

FUNDING SUPPORT

This research was supported by Children's Oncology Group (COG) Chair's grant U10 CA98543-08, Children's Oncology Group National Clinical Trials Network (COG NCTN) Group Operations Center grant U10 CA180886, Statistics and Data Center grant U10 CA98413-08, and COG NCTN Statistics and Data Center grant U10 CA180899.

CONFLICT OF INTEREST DISCLOSURES

Dr. Gaynon is a consultant/advisor to Jazz Pharmaceuticals, Sigma Tau Pharmaceuticals, Incyte Corporation, and Novartis and has received honoraria and research funding from Sigma Tau Pharmaceuticals.

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SUPPORTING TABLE 1. Demographics and Antibody Data (Include vs Excluded)

Table of sex by incl_antbdy			
sex	incl_antbdy		
Frequency Percent Row Pct Col Pct	Antibody Exclude	Antibody Include	Total
M	450 38.96 67.06 57.18	221 19.13 32.94 60.05	671 58.10
F	337 29.18 69.63 42.82	147 12.73 30.37 39.95	484 41.90
Total	787 68.14	368 31.86	1155 100.00

Table of cnsdx by incl_antbdy			
cnsdx(CNS DISEASE AT DX)	incl_antbdy		
Frequency Percent Row Pct Col Pct	Antibody Exclude	Antibody Include	Total
CNS-3	38 3.45 66.67 5.13	19 1.73 33.33 5.29	57 5.18
CNS-2	71 6.45 68.93 9.58	32 2.91 31.07 8.91	103 9.36
CNS-1	632 57.45 67.23 85.29	308 28.00 32.77 85.79	940 85.45
Total	741 67.36	359 32.64	1100 100.00
Frequency Missing = 55			

Table of testes by incl_antbdy			
testes(Testes Rating)	incl_antbdy		
Frequency Percent Row Pct Col Pct	Antibody Exclude	Antibody Include	Total
Normal	419	207	626
	65.37	32.29	97.66
	66.93	33.07	
	97.22	98.57	
Enlarged	12	3	15
	1.87	0.47	2.34
	80.00	20.00	
	2.78	1.43	
Total	431	210	641
	67.24	32.76	100.00
Frequency Missing = 514			

Table of down by incl_antbdy			
down(Down Syndrome)	incl_antbdy		
Frequency Percent Row Pct Col Pct	Antibody Exclude	Antibody Include	Total
DS	21	10	31
	1.82	0.87	2.69
	67.74	32.26	
	2.67	2.72	
No DS	765	358	1123
	66.29	31.02	97.31
	68.12	31.88	
	97.33	97.28	
Total	786	368	1154
	68.11	31.89	100.00
Frequency Missing = 1			

Table of LINEAGE by incl_antbdy			
LINEAGE(Immunophenotyping - lineage)	incl_antbdy		
Frequency Percent Row Pct Col Pct	Antibody Exclude	Antibody Include	Total
B	543	253	796
	47.01	21.90	68.92
	68.22	31.78	
	69.00	68.75	
T	164	82	246
	14.20	7.10	21.30
	66.67	33.33	
	20.84	22.28	
Other	47	21	68
	4.07	1.82	5.89
	69.12	30.88	
	5.97	5.71	
Unknown	33	12	45
	2.86	1.04	3.90
	73.33	26.67	
	4.19	3.26	
Total	787	368	1155
	68.14	31.86	100.00

Table of CALLA by incl_antbdy			
CALLA(CALLA STATUS)	incl_antbdy		
Frequency Percent Row Pct Col Pct	Antibody Exclude	Antibody Include	Total
CALLA-	188 17.47 67.38 25.65	91 8.46 32.62 26.53	279 25.93
CALLA+	545 50.65 68.38 74.35	252 23.42 31.62 73.47	797 74.07
Total	733 68.12	343 31.88	1076 100.00
Frequency Missing = 79			

SUPPORTING TABLE 2. Demographics by Anti-ASNase Antibody (Yes vs No)

The FREQ Procedure, Statistics for Table of CALLA by Antibody

Table of race_collapsed by Antibody			
race_collapsed	Antibody		
Frequency Percent Row Pct Col Pct	Negative	Positive	Total
White	186 50.54 70.19 70.72	79 21.47 29.81 75.24	265 72.01
Hispanic	49 13.32 73.13 18.63	18 4.89 26.87 17.14	67 18.21
Black	18 4.89 81.82 6.84	4 1.09 18.18 3.81	22 5.98
Other	10 2.72 71.43 3.80	4 1.09 28.57 3.81	14 3.80
Total	263 71.47	105 28.53	368 100.00

Table of sex by Antibody			
sex	Antibody		
Frequency Percent Row Pct Col Pct	Negative	Positive	Total
M	158 42.93 71.49 60.08	63 17.12 28.51 60.00	221 60.05
F	105 28.53 71.43 39.92	42 11.41 28.57 40.00	147 39.95
Total	263 71.47	105 28.53	368 100.00

Table of cnsdx by Antibody			
cnsdx(CNS DISEASE AT DX)	Antibody		
Frequency Percent Row Pct Col Pct	Negative	Positive	Total
CNS-3	14	5	19
	3.90	1.39	5.29
	73.68	26.32	
	5.47	4.85	
CNS-2	22	10	32
	6.13	2.79	8.91
	68.75	31.25	
	8.59	9.71	
CNS-1	220	88	308
	61.28	24.51	85.79
	71.43	28.57	
	85.94	85.44	
Total	256	103	359
	71.31	28.69	100.00
Frequency Missing = 9			

Table of testes by Antibody			
testes(Testes Rating)	Antibody		
Frequency Percent Row Pct Col Pct	Negative	Positive	Total
Normal	149	58	207
	70.95	27.62	98.57
	71.98	28.02	
	99.33	96.67	
Enlarged	1	2	3
	0.48	0.95	1.43
	33.33	66.67	
	0.67	3.33	
Total	150	60	210
	71.43	28.57	100.00
Frequency Missing = 158			

Table of down by Antibody			
down(Down Syndrome)	Antibody		
Frequency Percent Row Pct Col Pct	Negative	Positive	Total
DS	8 2.17 80.00 3.04	2 0.54 20.00 1.90	10 2.72
No DS	255 69.29 71.23 96.96	103 27.99 28.77 98.10	358 97.28
Total	263 71.47	105 28.53	368 100.00

Table of svonst by Antibody			
svonst(Survival Status)	Antibody		
Frequency Percent Row Pct Col Pct	Negative	Positive	Total
Alive	224 60.87 71.79 85.17	88 23.91 28.21 83.81	312 84.78
Died	39 10.60 69.64 14.83	17 4.62 30.36 16.19	56 15.22
Total	263 71.47	105 28.53	368 100.00

Table of LINEAGE by Antibody			
LINEAGE(Immunophenotyping - lineage)	Antibody		
Frequency Percent Row Pct Col Pct	Negative	Positive	Total
B	188 51.09 74.31 71.48	65 17.66 25.69 61.90	253 68.75
T	56 15.22 68.29 21.29	26 7.07 31.71 24.76	82 22.28
Other	12 3.26 57.14 4.56	9 2.45 42.86 8.57	21 5.71
Unknown	7 1.90 58.33 2.66	5 1.36 41.67 4.76	12 3.26
Total	263 71.47	105 28.53	368 100.00

Table of CALLA by Antibody			
CALLA(CALLA STATUS)	Antibody		
Frequency Percent Row Pct Col Pct	Negative	Positive	Total
CALLA-	69 20.12 75.82 27.82	22 6.41 24.18 23.16	91 26.53
CALLA+	179 52.19 71.03 72.18	73 21.28 28.97 76.84	252 73.47
Total	248 72.30	95 27.70	343 100.00
Frequency Missing = 25			

The NPAR1WAY Procedure

Table of RERSER by Antibody			
RERSER(RER VS SER)	Antibody		
Frequency Percent Row Pct Col Pct	Negative	Positive	Total
RER	157 42.66 71.04 59.70	64 17.39 28.96 60.95	221 60.05
SER	106 28.80 72.11 40.30	41 11.14 27.89 39.05	147 39.95
Total	263 71.47	105 28.53	368 100.00

SUPPORTING TABLE 3. Table Demographics: PEG Reaction (Yes vs No)

The FREQ Procedure: Statistics for Table of Sex by PEG Reaction

Table of race_collps by PEG Reaction			
race_collps	PEG Reaction		
Frequency Percent Row Pct Col Pct	No	Yes	Total
White	153 43.22 64.83 64.83	83 23.45 35.17 70.34	236 66.67
Hispanic	54 15.25 71.05 22.88	22 6.21 28.95 18.64	76 21.47
Black	16 4.52 80.00 6.78	4 1.13 20.00 3.39	20 5.65
Other	13 3.67 59.09 5.51	9 2.54 40.91 7.63	22 6.21
Total	236 66.67	118 33.33	354 100.00
Frequency Missing = 395			

Table of sex by PEG Reaction			
sex	PEG Reaction		
Frequency Percent Row Pct Col Pct	No	Yes	Total
M	136 38.42 65.38 57.63	72 20.34 34.62 61.02	208 58.76
F	100 28.25 68.49 42.37	46 12.99 31.51 38.98	146 41.24
Total	236 66.67	118 33.33	354 100.00
Frequency Missing = 395			

The FREQ Procedure: Statistics for Table of svonst by PEG Reaction

Table of cnsdx by PEG Reaction			
cnsdx(CNS DISEASE AT DX)	PEG Reaction		
Frequency Percent Row Pct Col Pct	No	Yes	Total
CNS-3	13	3	16
	3.82	0.88	4.71
	81.25	18.75	
	5.80	2.59	
CNS-2	21	11	32
	6.18	3.24	9.41
	65.63	34.38	
	9.38	9.48	
CNS-1	190	102	292
	55.88	30.00	85.88
	65.07	34.93	
	84.82	87.93	
Total	224	116	340
	65.88	34.12	100.00
Frequency Missing = 409			

Table of testes by PEG Reaction			
testes(Testes Rating)	PEG Reaction		
Frequency Percent Row Pct Col Pct	No	Yes	Total
Normal	127	64	191
	64.14	32.32	96.46
	66.49	33.51	
	96.95	95.52	
Enlarged	4	3	7
	2.02	1.52	3.54
	57.14	42.86	
	3.05	4.48	
Total	131	67	198
	66.16	33.84	100.00
Frequency Missing = 551			

Table of down by PEG Reaction			
down(Down Syndrome)	PEG Reaction		
Frequency Percent Row Pct Col Pct	No	Yes	Total
DS	13 3.68 100.00 5.53	0 0.00 0.00 0.00	13 3.68
No DS	222 62.89 65.29 94.47	118 33.43 34.71 100.00	340 96.32
Total	235 66.57	118 33.43	353 100.00
Frequency Missing = 396			

The NPAIR1WAY Procedure

Table of LINEAGE by PEG Reaction			
LINEAGE(Immunophenotyping - lineage)	PEG Reaction		
Frequency Percent Row Pct Col Pct	No	Yes	Total
B	161	79	240
	45.48	22.32	67.80
	67.08	32.92	
	68.22	66.95	
T	54	28	82
	15.25	7.91	23.16
	65.85	34.15	
	22.88	23.73	
Other	12	9	21
	3.39	2.54	5.93
	57.14	42.86	
	5.08	7.63	
Unknown	9	2	11
	2.54	0.56	3.11
	81.82	18.18	
	3.81	1.69	
Total	236	118	354
	66.67	33.33	100.00
Frequency Missing = 395			

Table of CALLA by PEG Reaction			
CALLA(CALLA STATUS)	PEG Reaction		
Frequency Percent Row Pct Col Pct	No	Yes	Total
CALLA-	54	26	80
	16.12	7.76	23.88
	67.50	32.50	
	24.11	23.42	
CALLA+	170	85	255
	50.75	25.37	76.12
	66.67	33.33	
	75.89	76.58	
Total	224	111	335
	66.87	33.13	100.00
Frequency Missing = 414			

Table of RERSER by PEG Reaction			
RERSER(RER VS SER)	PEG Reaction		
Frequency Percent Row Pct Col Pct	No	Yes	Total
RER	158 44.76 66.67 67.23	79 22.38 33.33 66.95	237 67.14
SER	77 21.81 66.38 32.77	39 11.05 33.62 33.05	116 32.86
Total	235 66.57	118 33.43	353 100.00
Frequency Missing = 396			

**SUPPORTING TABLE 4. Table Demographics: Subanalysis of Antibody Data
(Data Included vs Excluded)**

Table of race_collps by anbdy_301sub			
race_collps	anbdy_301sub		
Frequency Percent Row Pct Col Pct	301 Include	854 Exclude	Total
White	217 18.79 28.52 72.09	544 47.10 71.48 63.70	761 65.89
Hispanic	55 4.76 21.74 18.27	198 17.14 78.26 23.19	253 21.90
Black	17 1.47 22.97 5.65	57 4.94 77.03 6.67	74 6.41
Other	12 1.04 17.91 3.99	55 4.76 82.09 6.44	67 5.80
Total	301 26.06	854 73.94	1155 100.00

Table of sex by anbdy_301sub			
sex	anbdy_301sub		
Frequency Percent Row Pct Col Pct	301 Include	854 Exclude	Total
M	183 15.84 27.27 60.80	488 42.25 72.73 57.14	671 58.10
F	118 10.22 24.38 39.20	366 31.69 75.62 42.86	484 41.90
Total	301 26.06	854 73.94	1155 100.00

Table of cnsdx by anbdy_301sub			
cnsdx(CNS DISEASE AT DX)	anbdy_301sub		
Frequency Percent Row Pct Col Pct	301 Include	854 Exclude	Total
CNS-3	18 1.64 31.58 6.12	39 3.55 68.42 4.84	57 5.18
CNS-2	28 2.55 27.18 9.52	75 6.82 72.82 9.31	103 9.36
CNS-1	248 22.55 26.38 84.35	692 62.91 73.62 85.86	940 85.45
Total	294 26.73	806 73.27	1100 100.00
Frequency Missing = 55			

Table of testes by anbdy_301sub			
testes(Testes Rating)	anbdy_301sub		
Frequency Percent Row Pct Col Pct	301 Include	854 Exclude	Total
Normal	171 26.68 27.32 99.42	455 70.98 72.68 97.01	626 97.66
Enlarged	1 0.16 6.67 0.58	14 2.18 93.33 2.99	15 2.34
Total	172 26.83	469 73.17	641 100.00
Frequency Missing = 514			

Table of down by anbdy_301sub			
down(Down Syndrome)	anbdy_301sub		
Frequency Percent Row Pct Col Pct	301 Include	854 Exclude	Total
DS	10 0.87 32.26 3.32	21 1.82 67.74 2.46	31 2.69
No DS	291 25.22 25.91 96.68	832 72.10 74.09 97.54	1123 97.31
Total	301 26.08	853 73.92	1154 100.00
Frequency Missing = 1			

Table of LINEAGE by anbdy_301sub			
LINEAGE(Immunophenotyping - lineage)	anbdy_301sub		
Frequency Percent Row Pct Col Pct	301 Include	854 Exclude	Total
B	210 18.18 26.38 69.77	586 50.74 73.62 68.62	796 68.92
T	65 5.63 26.42 21.59	181 15.67 73.58 21.19	246 21.30
Other	15 1.30 22.06 4.98	53 4.59 77.94 6.21	68 5.89
Unknown	11 0.95 24.44 3.65	34 2.94 75.56 3.98	45 3.90
Total	301 26.06	854 73.94	1155 100.00

Table of CALLA by anbdy_301sub			
CALLA(CALLA STATUS)	anbdy_301sub		
Frequency Percent Row Pct Col Pct	301 Include	854 Exclude	Total
CALLA-	71 6.60 25.45 25.27	208 19.33 74.55 26.16	279 25.93
CALLA+	210 19.52 26.35 74.73	587 54.55 73.65 73.84	797 74.07
Total	281 26.12	795 73.88	1076 100.00
Frequency Missing = 79			

Table of RERSER by anbdy_301sub			
RERSER(RER VS SER)	anbdy_301sub		
Frequency Percent Row Pct Col Pct	301 Include	854 Exclude	Total
RER	179 15.51 25.64 59.47	519 44.97 74.36 60.84	698 60.49
SER	122 10.57 26.75 40.53	334 28.94 73.25 39.16	456 39.51
Total	301 26.08	853 73.92	1154 100.00
Frequency Missing = 1			