Featured Article

Asparaginase Pharmacokinetics After Intensive Polyethylene Glycol-Conjugated L-Asparaginase Therapy for Children with Relapsed Acute Lymphoblastic Leukemia

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ABSTRACT

Purpose: **Asparaginase therapy is an important component in the treatment of children with acute lymphoblastic leukemia. Polyethylene glycol-conjugated asparaginase (PEG-ASNase) has significant pharmacological advantages over native *Escherichia coli* asparaginase. We investigated the pharmacokinetics of PEG-ASNase, presence of antibodies to PEG-ASNase, and concentrations of asparagine in serum and cerebrospinal fluid (CSF) in combination chemotherapy for relapsed pediatric acute lymphoblastic leukemia.**

Experimental Design: **Twenty-eight pediatric patients with relapsed medullary ($n = 16$) and extramedullary ($n = 11$) acute lymphoblastic leukemia were enrolled at three pediatric institutions and had at least two serum and CSF samples obtained for analysis. Patients received induction therapy (including PEG-ASNase 2500 IU/m^2 intramuscularly weekly on days 2, 9, 16, and 23) and intensification therapy (including PEG-ASNase 2500 IU/m^2 intramuscularly once on day 7). Serum samples were obtained weekly during induction and intensification. CSF samples were obtained during therapeutic lumbar punctures during induction and intensification.**

Results: **Weekly PEG-ASNase therapy resulted in PEG-ASNase activity of >0.1 IU/ml in 91–100% of patients throughout induction. During intensification, PEG-ASNase on day 7 resulted in PEG-ASNase activity >0.1 IU/ml in 94% and 80% of patients on days 14 and 21, respectively. Serum and CSF asparagine depletion was observed and maintained during induction and intensification in the majority of samples. PEG-ASNase antibody was observed in only 3 patients.**

Conclusions: **Intensive PEG-ASNase therapy in the treatment of relapsed acute lymphoblastic leukemia reliably results in high-level serum PEG-ASNase activity, and asparagine depletion in serum and CSF is usually achieved. Incorporation of intensive PEG-ASNase in future trials for recurrent acute lymphoblastic leukemia is warranted.**

INTRODUCTION

With complex, multiagent chemotherapy, children with acute lymphoblastic leukemia currently have a 70% 5-year relapse-free survival (1–6). The probability of survival is much lower for children with acute lymphoblastic leukemia who develop recurrence, with inferior outcome associated with bone marrow (*versus* isolated extramedullary site) relapse and short initial remission duration (7–17). Reinduction chemotherapy regimens for relapsed acute lymphoblastic leukemia usually includes a bacterial L-asparaginase (ASNase) enzyme derived from *Escherichia coli* or *Erwinia*. ASNase selectively kills leukemic cells by depleting circulating asparagine (Asn; Refs. 18–20). Because ASNase is a foreign protein, acute allergic reactions and silent immunity limit the efficacy of Asn depletion. Polyethylene glycol conjugated L-asparaginase (PEG-ASNase) is formed by covalent modification of *E. coli* ASNase with 5000 daltons units of monomethoxypolyethylene glycol. PEG-ASNase retains enzymatic activity but has reduced immunogenicity and a 5-fold greater half life compared with native *E. coli* ASNase (21–23). A randomized comparison of native *E. coli* ASNase and PEG-ASNase in children with previously untreated acute lymphoblastic leukemia showed that PEG-ASNase was associated with an improved prolonged serum ASNase activity, lower incidence of high titer antibodies to ASNase, and a more rapid clearance of lymphoblasts from the bone marrow (24). In children with relapsed acute lymphoblastic leukemia, weekly PEG-ASNase circumvented rapid systemic clearance seen in some relapsed patients and resulted in a higher remission induction rate (97% with weekly PEG-ASNase *versus* 82% with biweekly; Ref. 13). Comprehensive PEG-ASNase pharmacokinetics, including serum and cerebrospinal fluid (CSF) Asn and glutamine (Gln) levels, serum PEG-ASNase activity, and antibody formation to PEG-ASNase, in patients with recurrent acute lymphoblastic leukemia treated with weekly PEG-ASNase have not been investigated previously. To determine the pharmacokinetics of PEG-ASNase in children

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with recurrent acute lymphoblastic leukemia, we conducted a biological companion to a treatment study for relapsed acute lymphoblastic leukemia.

PATIENTS AND METHODS

Eligibility Criteria. Patients with an initial relapse of acute lymphoblastic leukemia at Children's Hospital and Regional Medical Center (Seattle, WA), Deaconess Hospital (Spokane, WA), and James W. Riley Hospital for Children (Indianapolis, IN) between May 1997 and May 2001 were considered for entry onto a prospective treatment protocol (CHMC 1001). Patients were eligible with any site of initial relapse (including isolated or combined bone marrow relapse and isolated or combined extramedullary relapse at any site) any time after initial remission regardless of prior therapy. Central nervous system (CNS) relapse was defined as ≥5 white blood cells/μl in the spinal fluid and a cytologic evaluation demonstrating leukemic blasts or clinical signs of cranial nerve involvement regardless of cell count. Eligible patients had to be 1–21 years old and have normal cardiac function. Patients with a history of allergy to PEG-ASNase, a history of prior pancreatitis associated with ASNase use, trisomy 21, or mature B-cell leukemia were excluded from the study. Patients with a history of hypersensitivity to native *E. coli* ASNase were eligible. Informed consent was obtained before study enrollment in accordance with local Institutional Review Boards.

Chemotherapy Treatment Plan. The details of the chemotherapy regimen, its toxicity, treatment after intensification, and outcome will be reported separately. Briefly, patients received induction and at least one course of intensification. Table 1 summarizes the chemotherapy doses and schedule of the regimen.

Table 1 Treatment schema

Induction	
Vincristine 1.5 mg/m^2 (2 mg maximum) i.v.	Days 0, 7, 14, 21
Dexamethasone 10 mg/m^2/day p.o.	Days 0–6, 14–20
Idarubicin 10 mg/m^2	Days 0–2
Pegaspargase 2500 IU/m^2 i.m.	Days 2, 9, 16, 23
Intrathecal cytarabine, methotrexate, hydrocortisone doses adjusted by age	Days 0, 14, 28 (and 7, 21 if CNS positive at relapse)
Intensification	
Vincristine 1.5 mg/m^2 (2 mg maximum) i.v.	Days 0, 7
Dexamethasone 10 mg/m^2/day p.o.	Days 0–4
Thioguanine 100 mg/m^2/day p.o.	Days 0–4
Methotrexate 1 g/m^2 i.v. over 36 h with leucovorin rescue starting at hour 48	Days 0, 1
Cytarabine 100 mg/m^2/day i.v.	Days 2–4
Pegaspargase 2500 IU/m^2 i.m.	Day 7
Intrathecal cytarabine, methotrexate, hydrocortisone doses adjusted by age	Days 0, 21 (and 7, 14 if CNS negative at relapse)
Etoposide 100 mg/m^2 i.v.	Days 21–25
Ifosfamide 1800 mg/m^2 i.v. with MESNA	Days 21–25

Abbreviations: i.v., intravenously; p.o., orally; i.m., intramuscularly; MESNA, 2-mercaptoethane sulfonic acid.

PEG-ASNase and Amino Acid Analyses. Blood samples were collected on days 0, 7, 14, 21, and 28 of induction and days 0, 7, 14, 21, and 28 of intensification. CSF samples were collected on induction days 0, 14, and 28 (and days 7 and 21 for patients with CNS relapse at study entry) and on intensification days 0 and 21 (and on days 7 and 14 for patients without CNS relapse at study entry). Blood and CSF samples were placed immediately in an ice-water bath. No inhibitors of ASNase enzymatic activity were added to the collection tubes. Blood was allowed to coagulate under these conditions. Serum was decanted from coagulated blood after refrigerated centrifugation. Both serum and CSF samples were stored at −70 C. A specimen transmittal form was used to record date and dose of PEG-ASNase and specimen collection time. Stored specimens were shipped to the reference laboratory by overnight mail on dry ice.

PEG-ASNase activity was measured by ammonia produced from Asn with a Nessler reaction. Reacted enzymatic activity solutions were placed in an ELISA plate, and the ELISA plate reader was used to read absorbance, calculate calibration line, and quantify specimens (23, 24).

PEG-ASNase antibody was assayed by a modified indirect solid-phase ELISA (23, 24). The assay was done with a computer-controlled instrument from Dynatech Laboratories, Inc. Sera from patients who had high-titer antibodies PEG-ASNase was used to create a titration curve. The titers were compared with the pretreatment control serum from the same patient and negative control serum from a healthy volunteer. The assay had excellent linearity, reproducibility, and low detection limits, but the absorbance of control varied between assays. Day-to-day variation was corrected by expression of antibody titers as the ratio of sample over negative control for each assay. A positive antibody titer was defined as a ratio of serum antibody to the average control value of ≥1.5, because this ratio correlated with neutralization or rapid clearance of ASNase in our previous study (24).

Asn and Gln were assayed by modification of a reported high-performance liquid chromatography method in which amino acids are derivatized with phenylisothiocyanate (25). The derivatized samples were analyzed using a μC18 column by two-step gradient elution with a flow rate of 0.8 ml/min. The first step was composed of a 25-min linear gradient from 100% buffer A [0.05 M ammonium acetate (pH 6.8)] to 90% A and 10% of buffer B [0.1 M ammonium acetate (pH 6.8) and acetonitrile 50:50]. The second step consisted of a 10-min gradient from 90% A + 10% buffer B to 0% A + 100% B. Absorbance was followed at 254 nm. Calibration curves were used to quantify amino acid concentrations. The lowest limit of detection from the linear portion of the calibration lines was 0.01 μM for Asn and 1 μM for Gln (24).

Statistical Considerations. The principal goals of this study were to measure PEG-ASNase activity, serum Asn depletion, and CSF Asn depletion during induction and intensification. Because serum and CSF sampling were incomplete from all of the study subjects, comparisons of Asn concentrations were made by two-sided, two sample (rather than matched pair) *t* test assuming unequal variances using SPSS version 10.0 statistical package. The incidence of PEG-ASNase-associated

toxicities, including allergy, pancreatitis, and thrombosis, were reported descriptively.

RESULTS

Clinical Features of Patients. Table 2 outlines the clinical features of patients at time of original diagnosis and time of relapse. Although 30 patients enrolled on the therapeutic study, serum samples were obtained from 28 patients, and only these patients were analyzed for this report. Seventeen patients had bone marrow relapse (3 combined with CNS and 2 with testicle), and 11 had isolated extramedullary relapse (8 in CNS and 3 in other sites). All of the patients were treated previously with ASNase: 22 with native *E. coli* ASNase only, 3 with PEG-ASNase only, and 3 with both. All of the patients were either enrolled on Children's Cancer Group trials or institutional standard therapy based upon Children's Cancer Group trials. All of the patients received ASNase during at least two initial therapy phases: induction and delayed intensification. Twelve patients received ASNase during three initial therapy phases: induction and two delayed intensification courses. Two patients received ASNase during five initial therapy phases: induction, two interim maintenance courses, and two delayed intensification courses. Four had experienced hypersensitivity reactions to native *E. coli* ASNase. Details regarding the efficacy and toxicity of the therapeutic regimen will be reported separately. In brief, 86% (24 of 28) patients achieved a remission, 7% (2 of 28) patients died from infection during induction, and 7% (2 of 28) failed to achieve a remission despite receiving induction and intensification. Twenty-five of the initial 28 patients received intensification; reasons for not receiving intensification included death during induction (n = 2) and excessive toxicity during induction (n = 1).

ASNase Toxicity and Modifications of Therapy. During induction, 2 patients had adverse reactions to PEG-ASNase: CNS venous thrombosis (n = 1) and pancreatitis (n = 1). Seven patients had modifications of PEG-ASNase dosing during induction: 5 did not receive PEG-ASNase on day 23 due to severe infection (n = 4) or death due to infection before day 23 (n = 1), 1 patient did not receive PEG-ASNase on days 9, 16, or 23 due to pancreatitis after the initial dose, and 1 patient received native *E. coli* ASNase due to commercial unavailability of PEG-ASNase (no serum or CSF samples were obtained from this patient during induction). Twenty-two of the 25 patients who received intensification also received PEG-ASNase as scheduled on day 7 of intensification; PEG-ASNase was omitted due to thrombosis (n = 1) or pancreatitis (n = 1) during induction or commercial unavailability of PEG-ASNase (n = 1). During intensification, 3 additional patients had adverse reactions to PEG-ASNase, including hypersensitivity (n = 2) and pancreatitis (n = 1). The incidence of clinical hypersensitivity to PEG-ASNase was 0% during induction and 8% during intensification. All of the cases of PEG-ASNase-associated toxicity resolved spontaneously and without sequelae.

Table 2 Patient characteristics

Characteristic	Number/value
Number of patients	28
Sex	
Male	18
Female	10
Prior ASNase hypersensitivity	4
Median age at relapse, years	7.9
(range)	(3.9–18.3)
Immunophenotype	
B cell	24
T cell	3
Null cell	1
Median initial remission	36
Duration, months (range)	(5–45)
Site of relapse	
Medullary	17
Isolated	11
With CNS	3
With testicle	2
Extramedullary only	11
CNS	8
Other extramedullary site	3

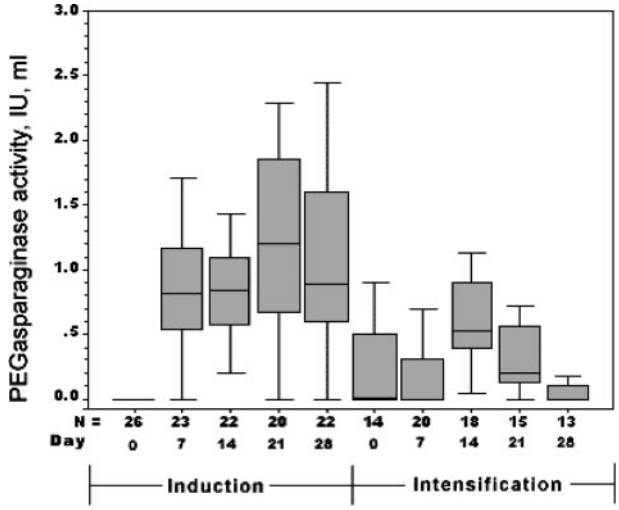

Fig. 1 Box and whisker plot of serum PEG-ASNase activity during induction and intensification. *Center* of box represents median, *box* encloses 50% of values, and *whiskers* enclose all values excluding outliers. PEG-ASNase was given on days 2, 9, 16, and 23 induction and day 7 of intensification.

Pharmacokinetics of PEG-ASNase. Fig. 1 and Table 3 show the median serum PEG-ASNase activity over time. During induction, median PEG-ASNase activity 5 days after the prior dose was 0.8–1.2 IU/ml. Therapeutic serum PEG-ASNase activity, defined as either $>$ 0.03 IU/ml or $>$ 0.1 IU/ml, was maintained throughout induction at 91–100% of patients at each time point. Only 2 patients did not maintain therapeutic serum PEG-ASNase activity levels ($>$ 0.1 IU/ml) throughout induction. The first patient (treated with native *E. coli* ASNase during initial therapy without clinical hypersensitivity) did not receive PEG-ASNase on day 23, had no detectable serum PEG-ASNase activity on day 28, and developed hypersensitivity to PEG-ASNase during intensification. This patient did not have PEG-ASNase antibody. The second patient (treated with native *E. coli* ASNase during initial therapy without clinical hypersensitivity) had undetectable serum PEG-ASNase activity throughout induction, never achieved Asn depletion, had detectable serum PEG-ASNase antibody, and was 1 of only 2 patients with refractory leukemia.

Table 3 Serum PEG-ASNase, serum amino acids, and CSF amino acids during induction and intensification

	Induction					Intensification				
Pharmacokinetic value	Day 0	Day 7	Day 14	Day 21	Day 28	Day 0	Day 7	Day 14	Day 21	Day 28
Median PEG-ASNase, IU/ml	0.00	0.81	0.84	1.20	0.89	0.01	0.00	0.53	0.20	0
>0.03 IU/ml	2/26	22/23	22/22	19/20	20/22	6/14	7/20	18/18	13/15	4/12
>0.1 IU/ml	1/26	22/23	22/22	19/20	20/22	6/14	6/20	17/18	12/15	3/12
Median serum Asn, μM	28.1	2.0	2.8	3.6	3.0	2.8	2.1	4.0	1.4	2.0
<3 μM	3/23	14/21	11/21	8/16	9/19	7/12	10/18	8/17	6/11	7/10
Median CSF Asn, μM	4.5	2.8	1.3	1.1	0.5	1.7	1.0	1.1	1.2	ND
<3 μM	8/25	6/10	16/24	6/8	15/20	8/12	9/10	9/11	8/13	
Median serum Gln, μM	488	93	110	76	79	360	168	101	46	176
<100 μM	1/23	10/21	9/21	9/16	9/19	3/11	7/18	7/17	6/11	4/10
Median CSF Gln, μM	613	875	590	672	597	770	629	592	530	ND
<100 μM	0/24	0/9	0/21	0/7	0/18	0/12	0/11	0/11	0/13	
PEG-ASNase Ab ratio ≥1.5	1/25	0/23	1/22	1/20	1/21	2/14	1/20	1/18	1/15	1/10

Abbreviations: CSF, cerebrospinal fluid, ND, not done, Gln, glutamine, Ab, antibody.

The median interval between induction day 23 PEG-ASNase dose and the start of intensification was 15 days (range, 7–26 days). Intensification did not start until recovery of blood counts, except in the case of persistent leukemia. Residual therapeutic PEG-ASNase activity (before the scheduled day 7 of intensification dose of PEG-ASNase) persisted in 43% and 30% of patients on days 0 and 7 of intensification, respectively. After a single dose of PEG-ASNase on day 7 of intensification, most patients maintained therapeutic PEG-ASNase activity on days 14 and 21 (87–100% were >0.03 IU/ml, and 80–94% were >0.1 IU/ml). The median PEG-ASNase activity was lower during intensification compared with during induction, reflecting less frequent dosing (Fig. 1).

Antibodies to PEG-ASNase. Table 3 shows the incidence of PEG-ASNase antibody during induction and intensification, defined as a PEG-ASNase antibody ratio ≥1.5. During induction and intensification, immunological reaction to PEG-ASNase was uncommon. Serum PEG-ASNase antibody was only observed in 2 patients during induction and in 1 additional patient during intensification (combined incidence, 11%). The 3 patients who developed PEG-ASNase antibody had received only two courses of ASNase during their initial treatment for acute lymphoblastic leukemia. Neither of the 2 patients with clinical hypersensitivity had a positive PEG-ASNase antibody ratio.

Serum and CSF Asn and Gln Concentrations. Table 3 and Fig. 2 show the median serum Asn concentration over time. Serum Asn was reduced significantly from day 0 of Induction compared with days 7, 14, 21, and 28 during induction and days 0, 7, 14, 21, and 28 during intensification ($P \leq 0.002$ for all comparisons). Serum Asn levels were <3 μM in 47–67% and 47–70% of patients in each time point sampled during induction and intensification, respectively. Table 3 also shows Gln depletion, defined as serum levels <100 μM, was also observed at 42–56% and 27–55% of patients in each time point sampled during induction and intensification, respectively.

Table 3 and Fig. 3 show the median CSF Asn concentration over time. Median CSF Asn was progressively lower during induction. The frequency of CSF Asn depletion <3 μM also increased from 60% at day 7 to 75% on days 21 and 28 of induction. CSF Asn depletion <3 μM was maintained in 62–90% of patients at each time point sampled during intensification. Table 3 also shows that CSF Gln did not decrease in any patient.

DISCUSSION

This is the first report in children with recurrent acute lymphoblastic leukemia to evaluate the pharmacokinetics and pharmacodynamics of intensive PEG-ASNase therapy including serum PEG-ASNase activity, serum PEG-ASNase antibody formation, and serum and CSF concentrations of Asn and Gln. Previous reports have suggested that serum ASNase activity either >0.03 IU/ml (13, 19) or >0.1 IU/ml (24, 26, 27) are necessary to achieve therapeutic Asn depletion. In addition, a clinical study in relapsed acute lymphoblastic leukemia patients demonstrated that PEG-ASNase trough activity levels were sig-

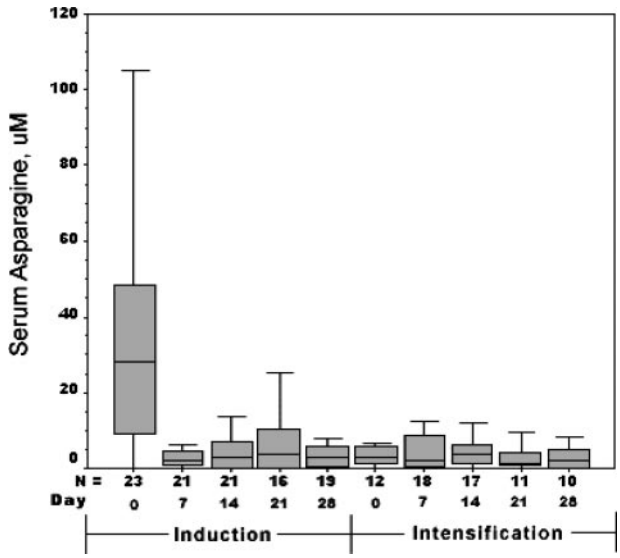

Fig. 2 Box and whisker plot of serum asparagine concentration during induction and intensification. *Center* of box represents median, *box* encloses 50% of values, and *whiskers* enclose all values excluding outliers.

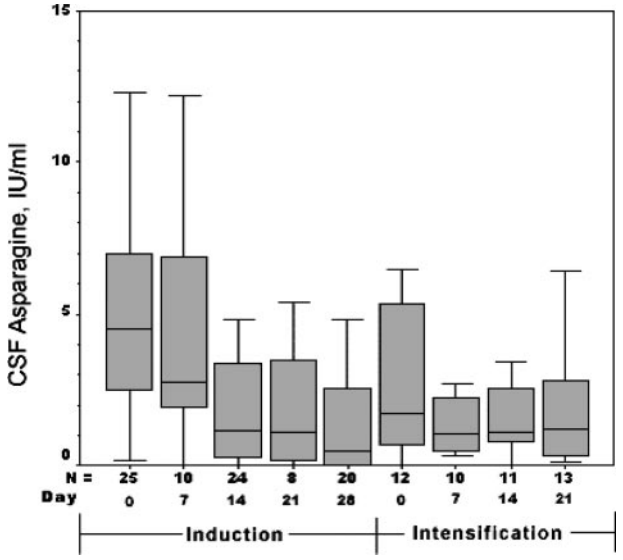

Fig. 3 Box and whisker plot of CSF asparagine concentration during induction and intensification. *Center* of box represents median, *box* encloses 50% of values, and *whiskers* enclose all values excluding outliers.

nificantly higher in patients who achieve a second remission (0.75 IU/ml) *versus* the nonresponding patients (0.45 IU/ml; Ref. 13). Furthermore, recent population analyses from newly diagnosed patients with high-risk acute lymphoblastic leukemia (Children's Cancer Group 1961) demonstrated that >0.4 IU/ml ASNase concentrations in serum were associated with 90% deamination of both Gln and Asn, whereas ≤0.4 IU/ml were associated with less amino acid deamination: 68% and 75%, respectively (28). These pharmacodynamic evaluations correlated higher ASNase levels with greater Asn and Gln deamination and with improved remission induction rates in recurrent acute lymphoblastic leukemia patients. In the current study, incorporated into an intensive regimen for recurrent acute lymphoblastic leukemia, weekly PEG-ASNase during induction and an additional dose during intensification nearly always resulted in therapeutic PEG-ASNase activity using either target level.

Despite median PEG-ASNase activity of 0.8–1.2 IU/ml, most serum and CSF samples had detectable Asn concentrations, in contrast to reports of <0.2 μM or undetectable concentrations by others (26, 29–32). Other groups who have reported undetectable serum or CSF Asn concentrations after ASNase therapy use different methods of specimen processing, including deproteination after specimen collection (26, 29–31) or addition of an ASNase inhibitor (32). Asselin *et al.* (32) demonstrated that postsampling deamination of Asn occurs unless ASNase activity is inhibited by assaying matched samples with and without the addition of an ASNase inhibitor. We have consistently found low residual Asn concentrations in some patient samples despite high levels of ASNase activity. Our method of specimen processing rapidly chilled the sample but did not use deproteination or an ASNase inhibitor. Samples with low but detectable Asn concentration do not appear to be an artifact of our assay methodology, because our results have been confirmed by blinded analysis in another laboratory (33). In addition, low but detectable concentrations of CSF Asn after i.v. PEG-ASNase have been reported recently by Appel *et al.* (34) Therefore, our findings of residual Asn in CSF (Fig. 3) have been reported elsewhere. Furthermore, isolated hepatic perfusion studies suggest that the liver is capable of increasing Asn input into the circulation in response to Asn depletion (35). Therefore, it is possible that an equilibrium low concentration of Asn can be present in the blood when the systemic input balances the rate of enzymatic depletion.

PEG-ASNase antibody was rare, occurring in only 3 patients at any time during induction or intensification. Therefore, neutralizing PEG-ASNase antibody formation did not explain the persistence of serum or CSF Asn. The incidence of PEG-ASNase antibody was similar to our previous observation in newly diagnosed acute lymphoblastic leukemia patients treated with PEG-ASNase: 3% during induction, 9% during first delayed intensification, and 18% during second delayed intensification (24). In addition, there did not appear to be a correlation between the number of previous ASNase-containing courses of therapy and the incidence of PEG-ASNase antibody. However, the small sample size and uniform prior exposure to at least two courses of ASNase-containing therapy limited the ability to correlate the incidence of PEG-ASNase antibody with prior ASNase therapy.

The threshold level of serum or CSF Asn depletion necessary to achieve antileukemia activity is not clear. An analysis of serum Asn after PEG-ASNase treatment in patients with early bone marrow relapse of acute lymphoblastic leukemia (Children's Cancer Group 1941) demonstrated an association between serum Asn depletion and induction success (36). In that study, patients received PEG-ASNase 2500 IU/m^2 IM every 2 weeks (37). Serum Asn concentration <3 μM on day 14 of induction, 12 days after the first dose of PEG-ASNase (20 patients), was associated with an improved remission rate (86%) compared with serum Asn concentration ≥3 μM (10 patients), remission rate 40%. Serum PEG-ASNase activity was not associated with the rate of remission success. However, Abshire *et al.* (13) demonstrated an association between higher mean serum ASNase activity in relapsed acute lymphoblastic leukemia patients who achieve a remission (0.75 IU/ml) compared with those who did not achieve remission (0.45 IU/ml). The high-remission induction rate and median PEG-ASNase activity attained in the present study precludes a similar analysis of serum Asn, serum PEG-ASNase activity, and remission induction. Similarly, the small sample size limits the power to detect differences in the risk of leukemic relapse and Asn depletion.

Our study has several limitations. First, the patient sample size is relatively small. However, uniform relapse status at study enrollment, universal prior exposure to either native *E. coli* ASNase or PEG-ASNase, and intensive measurement of both serum and CSF Asn and Gln concentrations distinguish this report from the literature. Second, the timing of serum PEG-ASNase activity measurement during induction was not a trough value but rather 5 days after the last dose. Absorption of PEG-ASNase after intramuscular injection is slow, with a peak level achieved at 5 days (23). Because of the prolonged half-life of PEG-ASNase (5.5–6 days; Refs. 23, 24), it is unlikely that trough PEG-ASNase activity was dramatically lower. The per-

sistence of serum PEG-ASNase activity 7 and 14 days after the single dose of PEG-ASNase during intensification suggested that adequate ASNase activity would be maintained at the trough. Third, complete serum and CSF data were not available for all of the patients at all of the time points, limiting the correlation between serum PEG-ASNase activity and Asn depletion. Fourth, 25% of patients did not receive all of the prescribed doses of PEG-ASNase during induction, reducing the effect of intensive, weekly PEG-ASNase therapy. Fifth, the time between the fourth dose of PEG-ASNase during induction and the start of intensification varied. The variable residual PEG-ASNase activity on days 0 and 7 of intensification compromised the analysis of PEG-ASNase pharmacokinetics during intensification.

These results allow us to conclude that intensive PEG-ASNase can be incorporated into an intensive reinduction regimen for recurrent acute lymphoblastic leukemia. Weekly intramuscular treatment during induction and a single dose during intensification reliably resulted in high serum PEG-ASNase activity and usually resulted in Asn depletion in serum and CSF. Despite uniform prior exposure to native *E. coli* ASNase or PEG-ASNase, hypersensitivity reactions to PEG-ASNase were rare, as were other ASN-associated toxicities, such as pancreatitis and thrombosis. This favorable activity and side-effect profile supports routine use of intensive PEG-ASNase therapy in the treatment of recurrent acute lymphoblastic leukemia.

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