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Polyethylene Glycol-Conjugated L-Asparaginase Versus Native L-Asparaginase In Combination With Standard Agents For Children With Acute Lymphoblastic Leukemia In Second Bone Marrow Relapse: A Children's Oncology Group Study (Pog 8866)

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Abstract

Administration of L-asparaginase is limited by hypersensitivity reactions mediated by anti-asparaginase antibodies. To overcome this problem, native *E. coli* L-asparaginase was conjugated to polyethylene glycol to formulate PEG-L-asparaginase, a preparation with decreased immunogenicity and increased circulating half-life. In early trials, PEG-L-asparaginase was tolerated by patients known to be hypersensitive to the native *E. coli* product. Between 1988-1992, the Pediatric Oncology Group (POG) conducted a Phase II, randomized trial to compare the efficacy and toxicity of PEG-L-asparaginase compared to native *E. coli* asparaginase in a standard reinduction regimen for children with acute lymphoblastic leukemia in second bone marrow relapse.

Methods—All patients (n=76) received standard doses of vincristine and prednisone. Non-hypersensitive patients (n = 34) were randomized to receive either PEG-L-asparaginase 2,500 IU/m²/dose intramuscularly on days 1 and 15 (Treatment I) or native *E. coli* asparaginase 10,000 IU/m²/dose intramuscularly on days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24, and 26 (Treatment II). Patients with a clinical history of an allergic reaction to unmodified asparaginase were directly assigned to treatment with PEG-L-asparaginase (n = 42). Asparaginase levels and anti-asparaginase antibody titers were monitored in all patients. Response and toxicity were scored using conventional criteria.

Results—The response rate for the total study population was 51% and there was no difference in response between the randomized groups, (p = 0.73, (exact χ^2 , two-sided). Toxicity was minimal. No unexpected or previously unreported adverse reactions occurred. Results of pharmacologic studies are reported and recommendations for dosing of PEG-L-asparaginase in hypersensitive patients are made.

Keywords

Asparaginase; Relapsed Acute Lymphoblastic Leukemia (ALL); Reinduction chemotherapy; Hypersensitivity

INTRODUCTION

L-asparagine is an essential amino acid for leukemic lymphoblasts which lack or have very low levels of L-asparagine synthetase and do not synthesize L-asparagine de novo, instead relying on L-asparagine supplied in the serum for survival.^{1,2} L-asparaginase (L-asp), an enzyme which catalyzes the hydrolysis of L-asparagine to L-aspartic acid and ammonia, depletes serum L-asparagine and has significant efficacy in the treatment of patients with acute lymphoblastic leukemia (ALL).³⁻⁵

L-asp, partially purified from guinea pig serum, was first used clinically in 1966 in an eight year old boy with multiply relapsed ALL who sustained a short clinical response to treatment.⁶ Subsequently, response rates of 30-65% were achieved with E. coli-derived drug tested as a single agent in phase I and II trials in children and adults.⁷⁻¹¹ Because of low myelosuppressive activity, lack of overlapping toxicities and non-cross resistance with other anti-tumor agents, L-asp was incorporated into combination chemotherapy protocols for the treatment of relapsed patients with ALL beginning in the late 1960s.¹²⁻¹⁹ In the late 1970s, L-asp was also added to the front-line therapy of patients with ALL, during induction and/or intensification phases and has become a mainstay of combination chemotherapy for ALL.^{2,20-24} Despite this widespread use, only one dose response study¹⁶ and two randomized trials evaluating efficacy have been conducted in over 30 years.^{2,20} Studies of plasma and cellular pharmacology are also lacking.

The major clinical and dose-limiting toxicity of L-asp therapy is the development of acute hypersensitivity reactions shortly after administration of drug.^{1,3,25-33} Patients exposed to E. coli-derived enzyme, have a cumulative 4% incidence of developing these reactions during induction and up to an 80% incidence of clinical allergic reactions if exposed to additional asparaginase in intensification³¹ or reinduction at the time of relapse.²⁵⁻³³ To address this problem, investigators at ENZON Inc, conjugated E. coli-derived L-asp to polyethylene-glycol to synthesize PEG-L-asparaginase (PEG-L-asp) in 1981³⁴⁻³⁶, hypothesizing that conjugation to PEG would decrease immunogenicity and increase the circulating half-life of the drug.³⁵ Phase I/II trials of PEG-L-asp given intravenously to adults and children with refractory hematological malignancies demonstrated that patients with known hypersensitivity to unmodified (native) E. coli-derived L-asp could tolerate infusions of PEG-L-asp without developing clinical allergic reactions.^{37,38} As hypersensitivity reactions were a major obstacle to completion of L-asp therapy in patients enrolled on Pediatric Oncology Group (POG) studies for newly diagnosed and relapsed ALL³¹, POG 8866 was designed as a randomized trial to compare the safety, efficacy and feasibility of administration of PEG-L-asp compared to native E. coli L-asp in children with ALL in second bone marrow relapse undergoing reinduction chemotherapy.

PATIENTS AND METHODS

Study design and patient population

Participating POG institutions enrolled patients on this study after obtaining approval of their local institutional review boards. Patients with ALL in second bone marrow relapse (M₃ marrow - >25% blasts) < 21 years of age at initial diagnosis, were eligible for inclusion in this study. Exclusion criteria included a life expectancy of less than 4 weeks, or

inadequate hepatic (alanine amino transferase >200 IU/L) or renal (creatinine >2 mg/dL) function. Patients with active CNS disease were allowed to enroll only if they and their treating physician felt that it was safe to withhold concomitant intrathecal chemotherapy. Written informed consent was obtained for each patient entered into the study according to federal and institutional guidelines.

Patients lacking a history of clinical allergy to native *E. coli*-derived L-asp were randomized to receive PEG-L-asp (Treatment I) or native *E. coli*-derived L-asp (Elspar; Treatment II) (Stratum 1). Patients with a history of clinical allergic reactions to native L-asp were directly assigned to treatment with PEG-L-asp (Treatment I) and analyzed in a separate stratum (Stratum 2). At the time of enrollment on study, the primary POG oncologist delivering care to the patient consulted with one of the study coordinators to assign hypersensitivity status for the patient based on information in the patient's medical history.

All patients had been previously treated with native *E. coli*-derived L-asp as part of their frontline, and in most cases first relapse, therapy. Forty of forty-two patients assigned to stratum 2 had experienced clinical hypersensitivity reactions to native *E. coli*-derived L-asp and had also been treated with *Erwinia*-derived L-asp. Eleven of these 40 patients (27.5%) also had allergic reactions to native *Erwinia*-derived L-asp and had been deemed ineligible to receive additional unmodified *E. coli* or *Erwinia* L-asp.

Treatment

Patients assigned to receive PEG-L-asp were given PEG-L-asp intramuscularly (IM) at a dose of 2500 IU/m² on days 1 and 15 (Treatment I). Patients assigned to native L-asp received unmodified *E. coli* L-asp (ELSPAR, Merck & Company) IM at a dose of 10,000 IU/m² IM on days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24, 26 (Treatment II). Both L-asp preparations were administered in combination with a standard induction regimen consisting of weekly vincristine (VCR) 1.5 mg/m²/dose intravenously (IV) on days 1, 8, 15, 22 (maximum dose = 2mg), and daily prednisone (PRED) 60 mg/m²/day days 1 through 28 (maximum dose = 60mg/day). Patients were observed through the study period, days 1-36. Patients developing clinical allergic reactions to Elspar while on study were allowed to cross over to PEG-L-asp although these patients were considered treatment failures at the time they developed the allergic reaction. After completion of the study period (day 36), patients responding to treatment could be maintained on PEG-L-asp, 2500 IU/m² IM, q 2 weeks, alone or in combination with other standard antileukemic agents.

Study drug

PEG-L-asparaginase was formulated and supplied by ENZON, Inc (Piscataway NJ). Two sources of commercially available, native *E. coli* enzyme were conjugated to PEG during the study. Patients 1 through 61 received a preparation of PEG-L-asp (PEG-Merck) in which PEG was conjugated to Elspar manufactured by Merck while patients 62 through 76 received a preparation of PEG-L-asp in which PEG was conjugated to Lunase manufactured by Kyowahaka (Kyowo, Japan - PEG-KH). The amino acid sequences, protein structure and specific activities of PEG-Merck and PEG-KH were compared at the time of PEG-L-asp formulation and found to be nearly identical.

Pharmacologic assessments

Serum L-asp and anti-L-asparaginase antibody titers were intended to be monitored in all patients. Blood samples were scheduled to be obtained prior to L-asp administration and at 24, 48, 72, and 96 hours following the first dose of L-asp; and weekly thereafter for a total of 6 weeks (days 8, 15, 22, 29, and 36).

The L-asparaginase assay has been previously described.³⁹ Briefly, the assay involves a coupled enzymatic reaction in which L-aspartate formed from the hydrolysis of L-asparagine in the presence of L-asparaginase reacts with α -ketoglutarate in the presence of L-glutamic oxaloacetic transaminase to form oxaloacetate and L-glutamate. Malic dehydrogenase, using oxaloacetate as a substrate, oxidizes nicotinamide-adenine dinucleotide (NADH). The oxidation of NADH is measured spectrophotometrically. L-asparaginase half-life ($t_{1/2}$) and area under the curve (AUC) were calculated by standard methods.^{39,40}

Serum anti-L-asparaginase antibodies were measured by a competitive ELISA using native L-asp as the target antigen.^{40,41} The data are presented using ordinal levels and assigned arbitrary numeric values, e.g. low (0) to high (3), based on a reference standard of pooled serum with known high anti-L-asparaginase antibody levels.

Efficacy and safety monitoring

Responses were evaluated by examining bone marrow aspirates, peripheral blood, and cerebral spinal fluid on Day 29, with earlier evaluation for patients removed from study before completion of the planned therapy. Response criteria for bone marrow remission were:

Complete remission (CR)	MI 5% marrow blasts
Partial remission (PR)	M2 > 5 - 25% marrow blasts
No response (NR)	M3 > 25% marrow blasts
Progressive disease (PD)	> 25% increase in marrow, peripheral blood blasts, or rapidly progressive organomegaly

Patients were monitored for adverse events. Laboratory testing (complete blood counts with platelet count and differential, standard serum chemistry screens, and routine urinalysis) was performed pre-study, weekly during induction, and on day 36. The incidence of adverse events and the degree of change from baseline laboratory parameters were used to evaluate safety. Toxicity was graded according to National Cancer Institute Common Toxicity Criteria. Clinical hypersensitivity reactions to L-asparaginase were graded as follows:

hypersensitivity reactions to L-asparaginase were graded as follows:

Grade 0	No reaction
Grade 1	Mild local reaction (< 10cm, < 24 hours)
Grade 2	Urticaria
Grade 3	Bronchospasm, serum sickness, severe local reaction (>10cm, >24 hours)

Statistical considerations

Simple descriptive statistics were used to summarize the study results. Contingency tables were analyzed using the StatXact 9 software (Cytel Corp., Cambridge). Exact p-values were calculated and all comparisons were two-sided. We used an intent-to treat approach when analyzing subjects in the randomized stratum.

RESULTS

Patient Characteristics

Between 9/1988 and 5/1992, 76 patients were enrolled (Table I). Thirty-four patients had no prior history of allergy to L-asp and were randomized to receive PEG-L-Asp (n = 17) or native E. coli L-asp; Elspar (n = 17). Forty-two patients with prior hypersensitivity were assigned to the non-randomized strata and were treated with PEG-L-asp (Treatment I). There were three protocol violations during accrual: one patient was randomized to PEG-L-asp, but was later identified as previously hypersensitive; two patients were labeled incorrectly as previously hypersensitive and were non-randomly assigned to Treatment I (PEG-L-asp, stratum 2). Patients assigned to stratum 2 (prior hypersensitivity) were not randomized nor were they considered to be randomized by intent. All patients were analyzed according to assignment at the start of treatment.

A subset of 12 patients with prior hypersensitivity enrolled on this study had been heavily exposed to asparaginase therapy during front line treatment on POG 8602 where they had been randomized to Regimen B - intensification with L-asp. Ten of these 12 patients (83%) developed a hypersensitivity reaction to native E. coli L-asp and were directly assigned to treatment I.

Demographic characteristics for each treatment group are summarized in Table 2. The mean age at the start of treatment was 9.18 ± 4.19 years (range 1-18 years). Forty-seven (62%) were male. Fifty (66%) were Caucasian, 13 (17%) were African American, 9 (11.8%) were Hispanic and 4 were another racial ethnic group. There were no significant differences between the 2 treatment groups related to gender, age, racial background or prior hypersensitivity status.

Efficacy Results

Two patients refused therapy and were not evaluable for response (NE refused treatment). Treatment responses were analyzed by intent-to-treat in 74 patients (Table 3). There were 31 (41%) complete response (CR) and 8 (10%) partial responses (PR). The overall response rate (CR + PR) was 51%. There were no significant differences in response for the two treatment groups in the randomized stratum (two-sided exact p-value = 0.73). Four patients randomized to receive native E. coli L-asp were crossed over to PEG-L-asp. Of these, one patient achieved a CR with PEG-L-asp therapy after experiencing progressive disease on E. coli asp; this patient was considered an E. coli treatment failure. One patient entered the study with active CNS disease and achieved a CR in the marrow and CNS on Treatment I, PEG-L-asp. Twenty-seven responding patients continued therapy with biweekly PEG-L-asp for 1-35 months. Eventually these patients discontinued PEG-L-asp therapy to go to bone marrow transplantation or because of relapse.

Safety Results

Thirty-three patients developed asparaginase-related, grade 3 or 4 toxicities although no unexpected or unusual toxicities were encountered (Table 4). There were no statistically significant differences in toxicities encountered in the randomized or nonrandomized patients treated with PEG-L-asp versus unmodified L-asp. For the randomized stratum, 2 of 27 patients treated with E. coli asparaginase experienced a grade 3 or 4 allergic reaction compared to 0 of 17 patients treated with PEG-L-asp (exact p = 0.49). Patients previously allergic to native L-asp, directly assigned to PEG-L-asp (stratum 2) tolerated PEG-L-asp well. Three patients experienced clinical allergic reactions, two had grade 1 and one had grade 2, there were no grade 3 or 4 reactions. Patients with reactions responded to treatment with diphenhydramine. No patient experienced prolonged or recurrent hypersensitivity

reactions. No additional adverse reactions were observed in 27 responding patients who continued PEG-L-asparaginase every two weeks as palliative maintenance therapy for 1-35 months.

Plasma L-asparaginase Pharmacology and Anti-L-asparaginase Antibody Formation

Results of pharmacologic assessments of the subset of study patients in whom sufficient samples were collected to permit analysis are shown in Table 5. The $T_{1/2}$ of PEG-L-asparaginase was 2.34 ± 2.27 days in hypersensitive patients (directly assigned) and 5.00 ± 3.4 days in nonhypersensitive patients (randomized). Hypersensitive patients also had a smaller area under the curve (AUC) for PEG-L-asparaginase than non-hypersensitive patients (4.88 ± 4.13 vs 8.58 ± 5.76 days, respectively). Fewer patients in the hypersensitive group (treated with PEG-L-asparaginase) had detectable L-asparaginase levels at days 8 (40%) or 15 (20%) compared to the non-hypersensitive group, (treated with either PEG-L-asparaginase or native E. coli asparaginase, (92% and 69%, respectively) demonstrating more rapid clearance of asparaginase in the hypersensitive group. Hypersensitive patients had a higher baseline and achieved-during-therapy antibody titers than non-hypersensitive patients. As shown in Table 6, patients with higher anti-L-asparaginase antibody titers were less likely to obtain a CR or PR on study.

Study patient #3 had no history of prior hypersensitivity to unmodified asparaginase, was randomized to treatment with native L-asparaginase and experienced a dose-limiting, clinical hypersensitivity reaction on day 8 of therapy. Strikingly, his serum L-asparaginase level fell after the L-asparaginase dose on day 5, concomitant with a rise in his anti-L-asparaginase antibody titers illustrating the phenomenon of “silent hypersensitivity” (accelerated clearance of asparaginase from the serum without clinical symptomatology). Although this patient was scored as a treatment failure for the randomized arm, he crossed over to therapy with PEG-L-asparaginase. Serum L-asparaginase titers rose to therapeutic levels with the first and second doses of PEG-L-asparaginase. This patient had progressive disease after therapy with unmodified L-asparaginase, but achieved a CR after two doses of PEG-L-asparaginase.

DISCUSSION

L-asparaginase is an essential component of induction and consolidation chemotherapy for patients with newly diagnosed and relapsed ALL. Unfortunately, it frequently cannot be delivered as prescribed because of clinical hypersensitivity reactions to the native bacterial preparations. Additional patients, previously exposed to asparaginase therapy, may develop anti-asparaginase neutralizing antibodies in the absence of clinical symptoms of allergy, a circumstance coined “silent hypersensitivity.” In these patients, accelerated clearance of asparaginase diminishes or ablates the pharmacologic effects of the drug.

Modification of E. coli L-asparaginase by conjugation to polyethylene glycol results in an asparaginase product with decreased immunogenicity and extended half-life. Phase I and II trials of PEG-L-asparaginase demonstrated that it was tolerated by patients who had experienced dose-limiting clinical allergic reactions to native L-asparaginase and that it had equivalent efficacy and less immunogenicity compared to native L-asparaginase.^{19,37,38} The current study was designed to compare, in a randomized trial, the efficacy and toxicity of PEG-L-asparaginase compared to native L-asparaginase in a standard reinduction regimen for pediatric patients with ALL in second bone marrow relapse. Patients also received vincristine and prednisone. Anthracyclines were not included because the majority of patients had previously received maximum recommended cumulative doses of these drugs.

More than half of the patients eligible for enrollment on this study had had a prior clinical hypersensitivity reaction to unmodified L-asparaginase making it impractical to meet initial accrual objectives for the randomized stratum in a timely fashion. A contemporaneous front-line ALL study (POG 8602) included a regimen with L-asparaginase intensification (Regimen B).²⁴ Many

patients eligible for the 8866 study had therefore received prolonged prior therapy with L-asparaginase, and many of those had demonstrated hypersensitivity reactions.³¹ These patients may have been over-represented in the study population because they did not receive adequate asparaginase therapy in the front-line due to clinical intolerance of L-asparaginase, or increased clearance of L-asparaginase due to formation of anti-L-asparaginase neutralizing antibodies. However, there was no relationship of the amount or duration of L-asparaginase therapy to outcome in the POG 8602 trial.⁴² Conversely, it is possible that patients with the worst asparaginase-related toxicities during initial treatment were not entered onto this study thereby decreasing the risks of toxicities. We have no evidence that this occurred.

L-asparaginase has been isolated from various microorganisms including gram negative bacteria, mycobacteria, yeasts, and molds, as well as from plants and from the plasma of certain vertebrates.^{1,5,8} *E. coli* produces two L-asparaginases, EC-1 and EC-2. However, only the EC-2 enzyme has substantial anti-tumor activity.^{43,44} The two most widely used bacterial enzymes are derived from *E. coli* and *Erwinia chrysanthemi*.¹ The pharmacokinetic profiles of asparaginases derived from these two bacterial sources are different, with the *Erwinia*-derived product having a shorter $t_{1/2}$ than the *E. coli*-derived product.^{39,40,44} Recent trials, including one randomized trial⁴⁵, in newly diagnosed patients with ALL has demonstrated decreased efficacy and toxicity of the native *Erwinia* product as compared to the native *E. coli* product in newly diagnosed patients.⁴⁵⁻⁴⁸ This supports the hypothesis that achievement and maintenance of a therapeutic serum asparaginase level represents a valid surrogate for clinical efficacy and provides a rationale for pharmacologic monitoring in clinical trials.

Most L-asparaginases can hydrolyze L-glutamine as well as L-asparagine, although the K_m for L-glutamine is significantly lower than that for L-asparagine.¹ Hydrolysis of L-glutamine, which produces glutamic acid (monosodium glutamate), may contribute to clinical toxicity, especially neurotoxicity.⁴⁹ When PEG-L-asparaginase was first tested in the clinic, many investigators were concerned that the increased half-life would increase the severity of asparaginase-related toxicities. This was not seen in our patients. However, all of these patients were previously exposed to native L-asparaginase and demonstrated increased clearance of PEG-L-asparaginase ($t_{1/2}$ 2-5 days) as compared to patients naïve to the drug ($t_{1/2}$ 14-28+ days).⁴⁰ This question cannot be answered definitively by this study and would need to be addressed in a study of asparaginase-naïve patients.

All patients hypersensitive to unmodified asparaginase tolerated at least 2 doses of PEG-L-asparaginase given during the induction phase of this study. Twenty-seven patients continued on PEG-L-asparaginase as maintenance therapy for 1-35 months without clinical reactions. Pharmacokinetic studies were not extended to the latter patients. This raises the question as to whether the use of PEG-L-asparaginase in newly diagnosed patients would decrease the development of both clinical and silent hypersensitivity thereby increasing the efficacy of asparaginase therapy.

In this study, PEG-L-asparaginase demonstrated equivalent efficacy and toxicity as compared to native asparaginase in the patients eligible for randomization. However, the numbers were small and confidence intervals large. Some activity was also demonstrated in those with known hypersensitivity to native asparaginase. Increased clearance and decreased pharmacologic efficacy of PEG-L-asparaginase was observed in patients known to have experienced a clinical allergic reaction to unmodified asparaginase preparations prior to study entry. The majority of these patients did not maintain adequate serum asparaginase levels on the current (q 2 weeks) dosing schedule. Patients known to be allergic to unmodified asparaginase might benefit from a weekly dosing schedule if subsequently treated with PEG-L-asparaginase. In a randomized trial in relapsed patients undergoing reinduction

therapy for ALL in first relapse (all of whom were previously exposed to native asparaginase), weekly PEG was more effective than bi-weekly PEG-asp.⁵⁰

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Table 1

Patient Accrual, Treatment, and Prior Hypersensitivity Status

	(PEG-L-asp)	(native E. coli L-asp)	
	Directly Assigned - hypersensitive	Randomized - nonhypersensitive	
	Treatment I	Treatment I	Treatment II
Total Patients (n = 76)	42	17	17
<u>Hypersensitivity history:</u>			
None	2		
Unknown	1		
E. coli Grade 2	23		
E. coli Grade 3 or 4	16		
Erwinia Grade 1 or 2	7		
Erwinia Grade 3 or 4	4		
Enrolled after POG 8602B	10	1	1

Table 2

Demographic Characteristics

	Treatment I PEG-L-Asp		Treatment II Native E. coli Asp
	Directly Assigned	Randomized	Randomized
	Hypersensitive	Nonhypersensitive	Nonhypersensitive
N	42	17	17
Age (years)			
At diagnosis	5.79 +/-3.60	5.18 +/-3.66	6.55 +/- 5.00
Range	1-13	0-12	0-17
At study entry	8.55 +/-4.09	8.47 +/-3.87	10.03 +/-4.76
Range	2-16	2-16	1-18
Gender			
Male (%)	27 (64)	10 (59)	11 (65)
Female (%)	15 (36)	7 (41)	6 (35)

*
= Mean +/- standard deviation

Table 3

: Response to Therapy*

	Treatment I PEG-L-Asp		Treatment II Native E. coli Asp
	Directly Assigned	Randomized	Randomized
	Hypersensitive	Non-hypersensitive	Non-hypersensitive
Total n = 76	42	17	17
Response **	CR + PR rate I vs. II, $p = 0.43$ (exact χ^2)		
CR (%)	16 (38)	7 (41)	8 (47)
PR (%)	5 (12)	3 (18)	0 (0)
NR/PD (%)	20 (48)	6 (35)	9 (53)
NE (%)	1 (2)	1 (6)	0 (0)

* = Dependent on intent to treat.

** CR = complete response; PR = partial response; NR = no response; PD = progressive disease; NE = not evaluable as defined in Methods.

Table 4

Asparaginase-Related Adverse Reactions Toxicities for all patients, worst degree of toxicity reported among 74 patients evaluable for toxicity.

Toxicity	Grade				
	None	1	2	3	4
Fibrinogen	53	10	3	7	3
PT	71	2	2	1	0
PTT	60	7	5	3	1
Allergic Reaction	63	7	2	4	0
Fever (Drug)	66	10	0	0	0
Nausea	73	1	0	0	0
Bilirubin	65	7	0	2	2
SGOT/SGPT	38	16	12	10	0
Glucose	73	0	0	1	2
Amylase	74	0	1	0	1
Fibrinogen	15	0	0	2	0
PT	16	1	0	0	0
PTT	14	0	3	0	0
Allergic Reaction	15	1	1	0	0
Fever (Drug)	15	2	0	0	0
Nausea	17	0	0	0	0
Bilirubin	14	1	0	1	1
SGOT/SGPT	10	5	0	2	0
Glucose	17	0	0	0	0
Amylase	17	0	0	0	0
Fibrinogen	8	4	1	3	1
PT	13	1	2	1	0
PTT	10	3	0	3	1
Allergic Reaction	15	0	0	2	0
Fever (Drug)	13	4	0	0	0
Nausea	16	1	0	0	0
Bilirubin	15	1	0	1	0
SGOT/SGPT	7	3	5	2	0

Toxicity	Grade				
	None	1	2	3	4
Glucose	15	0	0	1	1
Amylase	17	0	0	0	0
Fibrinogen	30	6	2	2	2
PT	42	0	0	0	0
PTT	36	4	2	0	0
Allergic Reaction	33	6	1	2	0
Fever (Drug)	38	4	0	0	0
Nausea	42	0	0	0	0
Bilirubin	36	5	0	0	1
SGOT/SGPT	21	8	7	6	0
Glucose	41	0	0	0	1
Amylase	40	0	1	0	1

Table 5

Serum L-asp Levels and Antibody Titers *

	Treatment I PEG-L-Asp		Treatment II Native E. coli Asp
	Directly Assigned	Randomized	Randomized
	Hypersensitive	Nonhypersensitive	Nonhypersensitive
<u>AUC</u>	4.88 + 4.13 (n=31)	8.58 + 5.76 (n=11)	ND **
<u>t ½</u>	2.34 + 2.27 (n=17)	5.00 + 3.4 (n=7)	ND **
<u>Serum L-asp Detectable</u>			
Day 7	4/10 (40%)	5/6 (83%)	7/7 (100%)
Day 14	2/10 (20%)	3/6 (50%)	6/7 (86%)
<u>Antibody Titers</u>			
Baseline	1.48 + 1.18	1.21 + 1.48	0.25 + 0.62
Highest	2.45 + 1.06	1.57 + 1.50	1.25 + 1.35

* The data in this table represents actual therapy delivered, not intent-to-treat.

** ND = not done.

Table 6

Relationship Between Response and Antibody Titers

Patient Group	Treatment I (PEG-L-asp)		Treatment II (E. coli L-asp)	
	Assigned (n = 30)	Randomized (n = 12)	Assigned (n = 30)	Randomized (n = 12)
Antibody Level*	R**	NR***	R	NR
High # (%)	11(37)	15(50)	2(17)	3(25)
Low # (%)	3(10)	1(3.3)	6(50)	1(8)

High antibody titers were those scored as 3; low were those scored 0-2.

* Antibody titer = highest titer of antibody during the study, including the pre-study sample.

** R = responding patients (CR or PR by the end of study)

*** NR = non-responding patients (NR or PD).