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Outcome of pediatric patients with acute lymphoblastic leukemia/lymphoblastic lymphoma with hypersensitivity to pegaspargase treated with PEGylated *Erwinia* asparaginase, pegcrisantaspase: A report from the Children's Oncology Group

Rachel E. Rau[1,2], **ZoAnn Dreyer**[1], **Mi Rim Choi**[3], **Wei Liang**[3], **Roman Skowronski**[3], **Krishna P. Allamneni**[3], **Meenakshi Devidas**[4], **Elizabeth A. Raetz**[5], **Peter C. Adamson**[6], **Susan M. Blaney**[1], **Mignon L Loh**[7], and **Stephen P. Hunger**[6]

[1]Division of Pediatric Hematology/Oncology, Texas Children's Cancer Center, Baylor College of Medicine, Houston, Texas

[2]Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, Texas

[3]Jazz Pharmaceuticals, Palo Alto, California

[4]Department of Biostatistics, Colleges of Medicine, Public Health and Health Professions, University of Florida, Gainesville, Florida

[5]Department of Pediatrics, University of Utah, Salt Lake City, Utah

[6]Department of Pediatrics and the Center for Childhood Cancer Research, Children's Hospital of Philadelphia and the Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania

[7]Department of Pediatrics, University of California School of Medicine, San Francisco, California

Abstract

Background—*Erwinia* asparaginase is a Food and Drug Administration approved agent for the treatment of acute lymphoblastic leukemia (ALL) for patients who develop hypersensitivity to *Escherichia coli* derived asparaginases. *Erwinia* asparaginase is efficacious, but has a short half-life, requiring six doses to replace one dose of the most commonly used first-line asparaginase, pegaspargase, a polyethylene glycol (PEG) conjugated *E. coli* asparaginase. Pegcristantaspase, a recombinant PEGylated *Erwinia* asparaginase with improved pharmacokinetics, was developed for patients with hypersensitivity to pegaspargase. Here, we report a series of patients treated on a pediatric phase 2 trial of pegcrisantaspase.

Correspondence: Rachel E. Rau, Division of Pediatric Hematology/Oncology, Texas Children's Cancer Center, Baylor College of Medicine, Houston, TX. rerau@bcm.edu.

Orcid: *Rachel E. Rau* http://orcid.org/0000-0003-4096-6603

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Procedure—Pediatric patients with ALL or lymphoblastic lymphoma and hypersensitivity to pegaspargase enrolled on Children's Oncology Group trial AALL1421 (Jazz 13-011) and received intravenous pegcrisantaspase. Serum asparaginase activity (SAA) was monitored before and after dosing; immunogenicity assays were performed for antiasparaginase and anti-PEG antibodies and complement activation was evaluated.

Results—Three of the four treated patients experienced hypersensitivity to pegcrisantaspase manifested as clinical hypersensitivity reactions or rapid clearance of SAA. Immunogenicity assays demonstrated the presence of anti-PEG immunoglobulin G antibodies in all three hypersensitive patients, indicating a PEG-mediated immune response.

Conclusions—This small series of patients, nonetheless, provides data, suggesting preexisting immunogenicity against the PEG moiety of pegaspargase and poses the question as to whether PEGylation may be an effective strategy to optimize *Erwinia* asparaginase administration. Further study of larger cohorts is needed to determine the incidence of preexisting antibodies against PEG-mediated hypersensitivity to pegaspargase.

Keywords

acute lymphoblastic leukemia; asparaginase; hypersensitivity

1 Introduction

L-Asparaginase is an essential component of multiagent chemotherapy for the treatment of childhood acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma (LLy).[1–4] There are two asparaginase preparations approved by the US Food and Drug Administration (FDA) and currently available in the United States: (1) pegaspargase, a PEGy-lated form (having polyethylene glycol [PEG] attached) of the native *Escherichia coli* asparaginase, and (2) *Erwinia* asparaginase, isolated from *Erwinia chrysanthemi*.

Because it is a large, bacteria-derived protein, exposure to asparaginase has the capacity to illicit an immune response. Hypersensitivity is the most common toxicity observed with these drugs and has been reported in up to 60% of patients treated with intensive schedules of native *E. coli* asparaginase.[5–10] Conjugation of native *E. coli* asparaginase to polyethylene glycol reduces the immunogenicity of the enzyme; however, clinical hypersensitivity still occurs in a subset of patients with reported incidence rates ranging from 3 to 24%.[7,11–14] Patients with hypersensitivity usually have high titer serum immunoglobulin G (IgG) and immunoglobulin E (IgE) antibodies to asparaginase and, in the majority of cases, these antibodies neutralize the effect of the enzyme and hence its therapeutic effect.[1,9,10,14–17] The benefit of intensive asparaginase treatment compared with less intensive regimens has been demonstrated in numerous studies.[5,10,18,19] Additionally, while most patients with antiasparaginase antibodies have clinical hypersensitivity, a subset experiences subclinical hypersensitivity, or “silent inactivation,” in which they develop neutralizing antibodies, yet have no overt signs of an immune reaction. This condition is associated with a lack of adequate depletion of serum asparagine and inferior outcomes.[9,14,20,21]

Erwinia chrysanthemi derived L-asparaginase has minimal antigenic cross-reactivity with asparaginase derived from *E. coli*,[22–24] which allows it to be used after a hypersensitivity reaction to *E. coli* derived asparaginase has occurred. The Children's Oncology Group (COG) study AALL07P2 found that intramuscular (IM) *Erwinia* asparaginase given as six doses on a Monday/Wednesday/Friday schedule was well tolerated by patients who had experienced hypersensitivity to pegaspargase and achieved nadir serum asparaginase activity (SAA) above the 0.1 international units (IU)/ml threshold, the level most commonly correlated with complete depletion of asparagine.[25] Based on these and other data, the FDA approved *Erwinia* asparaginase for use following hypersensitivity to pegaspargase. Intravenous (IV) administration of the same dose and schedule of *Erwinia* asparaginase has also been explored, though fewer patients receiving IV *Erwinia* asparaginase achieved SAA levels ≥0.1 IU/ml at 48 and 72 hr after dosing compared to IM administration.[26]

While *Erwinia* asparaginase has proven to be a safe and effective alternative to *E. coli* PEG–asparaginase, the thrice weekly delivery schedule is burdensome. A product with a reduced frequency-dosing regimen would provide important benefits. Additionally, hyper-sensitivity reactions occurred in 11% of patients during the first course *Erwinia* asparaginase in AALL07P2[25] and 23% of patients during the first course of the IV Erwinaze trial.[26] Thus, preparations with reduced immunogenic potential would provide important therapeutic benefits. To this end, pegcrisantaspase (JZP-416), a novel PEGylated recombinant *E. chrysanthemi* L-asparaginase, was developed.

A phase I dose escalation study conducted in asparaginase-naïve adults aged 18–50 years with relapsed or refractory hematological malignancies demonstrated that pegcrisantaspase administered IV every 2 weeks for two doses at both 500 IU/m^2 and 750 IU/m^2 achieved SAA levels ≥0.1 IU/ml 14 days after administration.[27] Furthermore, the drug was well tolerated with no infusion-related or hypersensitivity reactions. Overall, the adverse events (AEs) reported in the initial clinical study were consistent with the known safety profile of L-asparaginase in this patient population.

The efficacy and favorable toxicity profile in the adult trial supported the feasibility of testing the agent in a population of pediatric patients with ALL/LLy who had developed a hypersensitivity reaction to pegaspargase. Here, we describe the results of the COG AALL1421 pediatric Phase 2 study of pegcrisantaspase.

2 Patients and Methods

2.1 Patients

Patients with ALL or LLy, age >1 year and ≤21 years of age with a history of prior ≥ grade 2 allergic reaction (according to the National Cancer Institute's Common Terminology Criteria for Adverse Events [CTCAE] v 4.03) to pegaspargase with ≥1 remaining scheduled doses of pegaspargase were eligible to enroll on COG AALL1421 (Jazz 13-011). Patients who had previously received *Erwinia* asparaginase were excluded. The study was approved by institutional review boards at the individual institutions. Informed consent was obtained according to Department of Health and Human Services Guidelines and in accordance with the Declaration of Helsinki.

2.2 Treatment plan

Prior to treatment, patients were required to have a documented SAA level below the lower limit of quantification (defined as <0.013 IU/ml) as per the analytical method. Patients received pegcrisantaspase via 1-hr IV infusion as a replacement for each remaining scheduled dose of pegaspargase. The study was designed to include a limited dose confirming phase followed by a part 2 expansion phase. The starting dose was 750 IU/m^2 with a possible escalation to 1,000 IU/m^2. The proportion of subjects with a Day 15 SAA level ≥0.1 IU/ml was planned as the primary efficacy endpoint. Originally, part 2 of the study was planned to test the null hypothesis of true response rate (defined as the proportion of subjects with a day 15 SAA level ≥0.1 IU/ml) of ≤70% against an alternative hypothesis of ≥85% at the significance level of 0.05 with 90% power. The study was suspended and then stopped before completion of part 1, and therefore, part 2 was not initiated. Patients continued all other chemotherapy according to their treatment regimen.

2.3 Determination of SAA

Eight to 10 blood samples were scheduled for collection from each patient during course 1, prior to each pegcrisantaspase dose, at end of the infusion, at 3, 5, and 25 hr after start of the infusion and at days 8, 11, 15, 22, and 29, provided the subsequent dose of pegcrisantaspase had not been administered on days 22 and 29. For each subsequent dose, blood samples were obtained at predose, end infusion, and days 8, 11, 15, 22, and 29 after infusion. Asparaginase activity was determined by a coupled enzymatic assay, as previously reported.[25]

2.4 Immunologic studies

Possible mechanisms for the hypersensitivity-like reactions or increased clearance of asparaginase activity observed in three of the four patients enrolled in the study were investigated in two nonclinical studies. Samples of study drug pegcrisantaspase, pegaspargase, and host cell proteins were tested for functional complement activation in vitro (ELISA to assess the activation of complement markers C4a, Bb, C3a, and C5a in normal human serum).

Available serum samples from patients enrolled in the study were also assessed for the presence of anti-PEG IgM and IgG antibodies. A conventional double antigen-bridging ELISA method was used to assay for anti-PEG IgM and a direct detection ELISA method was used to assay for anti-PEG IgG.

3 Results

3.1 Patient outcome

A total of four patients were enrolled on study, none of whom completed protocol therapy (Table 1). Two patients withdrew due to hyper-sensitivity reactions to the first dose of pegcrisantaspase and one discontinued treatment due to failure to achieve therapeutic SAA levels following the first dose of pegcrisantaspase. One patient completed cycle 1 and received two subsequent doses of pegcrisantaspase with-out complication. Due to the

unexpectedly high frequency of hypersensitivity reactions, the study was permanently closed to accrual.

3.2 Case summaries

Patient #1 was a 9-year-old male with B-cell ALL (B-ALL) who experienced a grade 3 hypersensitivity reaction to his second dose of pegaspargase requiring diphenhydramine and hydrocortisone 1 week prior to his first dose of pegcrisantaspase. He received pegcrisantaspase IV 750 IU/m^2 over 1 hr and tolerated the infusion without complication. However, an SAA level obtained on day 8 following infusion was below the limit of detection (Table 2), prompting removal from the study due to potential lack of study drug efficacy. The patient subsequently received IM *Erwinia* asparaginase, which was well tolerated and resulted in therapeutic SAA levels 48 hr after dosing.

Patient #2 was a 14-year-old male with relapsed B-ALL and a history of grade 3 allergic reaction to pegaspargase during his initial therapeutic regimen approximately 5.5 years prior to enrollment on AALL1421; no pegaspargase was administered following relapse. The patient received IV pegcrisantaspase 750 IU/m^2 over 1 hr. The infusion was well tolerated and SAA levels remained above the 0.1 IU/ml threshold through day 15 of the study (Table 2). The patient subsequently received two additional courses, which were also well tolerated and resulted in therapeutic SAA levels at day 15. The peak SAA level occurred at 1-hr postinfusion start for of each of the three infusions. He experienced no AEs attributed to pegcrisantaspase, but was terminated from the study after the third course due to study suspension.

Patient #3 was a 20-year-old female with T-cell LLy and a history of grade 3 allergic reaction to her forth dose of pegaspargase approximately 6 weeks prior to enrollment on AALL1421. She began treatment with pegcrisantaspase IV 750 IU/m^2; however, approximately 3 min into the infusion developed chest tightness and facial erythema with mild swelling. The infusion was stopped and IV diphenhydramine was administered. The patient then experienced difficulty breathing and developed a nonproductive cough. Subcutaneous epinephrine was administered as well as IV methyl prednisolone leading to immediate resolution of all symptoms. Levels of SAA were not obtained in this patient as she experienced anaphylaxis after only approximately 7% of the intended dose was administered. She was discontinued from the study due to anaphylaxis.

Patient #4 was a 7-year-old male with B-ALL who experienced an allergic reaction to his third dose of pegaspargase. One week after this allergic reaction, he began treatment with pegcrisantaspase IV 750 IU/m^2. Approximately 17 min after the start of the infusion, the patient began coughing and experienced erythema of the face and ears and reported tongue and throat discomfort. The infusion was paused and the symptoms resolved without medical intervention. The infusion was resumed approximately 24 min later at half the initial starting rate. After 36 min, the patient became irritable and developed lip pruritus and edema as well as circumoral, eyebrow, and nasal erythema. The infusion was stopped and IV diphenhydramine, hydrocortisone, and ranitidine were administered and subsequently, all symptoms resolved. Before discontinuation of the infusion, he received approximately 62% of the intended dose. His SAA level 1 hr after the start of the infusion was 0.04 IU/mL and

on study day 2, the level was below the limit of detection. The subject was removed from protocol therapy due to infusion-related reaction. He subsequently received IV *Erwinia* asparaginase as a replacement for the remaining pegaspargase doses. He tolerated this agent without allergic symptoms and had SAA levels within therapeutic range at 48 hr after dosing.

3.3 Results of immunologic studies

Clinical samples from all four patients enrolled in the study were analyzed for evidence of complement activation and the presence of anti-PEG antibodies (IgG and IgM). Significant complement activation with 100% reduction of C3H50 and 80% reduction of C5H50 was observed for samples from Patient #1 who had increased asparaginase clearance. Mild complement activation with 20–30% reduction of C3H50 and C5H50 was observed for samples from patients #3 and #4 who had AEs of anaphylaxis and infusion-related reaction, respectively. No complement activation was observed in samples from patient #2.

Thus, the assessment of functional complement activation in vitro by pegcrisantaspase, pegaspargase, and host cell proteins in normal human sera indicated that that there was no direct activation of complement by these test substances; however, the indirect activation of complement in serum samples from three patients was observed.

Anti-PEG IgM antibodies were detected in the sera of three patients (patients #2, #3, and #4). Two of these patients had either experienced hypersensitivity-like reactions or increased clearance of asparaginase (patients #3 and #4). However, the anti-PEG IgM antibodies were only detected at low optical density in the serum samples taken at screening and all postscreening samples were negative for anti-PEG IgM. Anti-PEG IgM antibodies were not detected in the serum from patient #1.

Anti-PEG IgG antibodies were detected in the sera of three patients, representing the 3 patients who experienced hypersensitivity-like reactions or increased clearance of asparaginase activity (patients #1, #3 and #4), at all of the time points sampled, including samples taken prior to dosing with pegcrisantaspase (Table 3). For patient #2, no anti-PEG IgG antibodies were detected in any of the serum samples.

4 Discussion

While pegcrisantaspase was well tolerated in a phase I trial for asparaginase-naïve adults with hematologic malignancies, hypersensitivity occurred in three of the four treated patients in this pediatric trial. Immunologic analysis demonstrated the presence of anti-PEG IgG antibodies in all three hypersensitive patients. A fourth patient tolerated three doses of pegcrisantaspase without any clinical complications, met the pharmacological endpoint of nadir SAA at 15 days after each infusion, and had no evidence of anti-PEG IgG antibodies. Anti-PEG IgM antibodies were also detected at low optical density in three patients at screening, including the patient who did not have hyper-sensitivity or accelerated clearance, and all patients were negative for IgM post-pegcrisantaspase infusion, thus the association of the transient presence of anti-PEG IgM antibodies with *Erwinia* asparaginase in this cohort is uncertain. None of the hypersensitive patients had detectable anti-*Erwinia* asparaginase

antibodies, and two received subsequent native *Erwinia* asparaginase without showing any evidence of clinical hypersensitivity and attained therapeutic nadir SAA 48–72 hr after drug administration. Together, these findings show that preexisting anti-PEG IgG antibodies present in three of the four pegaspargase-hypersensitive patients mediated clinically relevant immune-mediated reactions to pegcrisantaspase.

The covalent attachment of PEG is a widely used strategy to increase the half-life and reduce the immunogenicity and antigenicity of therapeutic agents. Initially thought as nonimmunogenic, over 30 years ago Richter and Akerblom clearly demonstrated the development of anti-PEG antibodies after exposure to PEG-conjugated allergens in both murine and human experiments.[28,29] Further, a number of studies have been published documenting anti-PEG antibodies in normal individuals who have never been exposed to PEGylated pharmaceutical agents, likely attributable to exposure to PEG-diols, which are increasingly present in processed foods and cosmetics.[29–32] However, the clinical significance of anti-PEG antibodies has been debated in the literature. In the Richter and Akerblom study, during hyposensitization with PEG-modified ragweed extract and honey bee venom, the patients showed an anti-PEG antibody response, however the responses were deemed of no clinical significance.[29] Additionally, while anti-PEG antibodies have been detected in some patients with hepatitis C treated with PEGylated interferons, no clinically significant hypersensitivity developed.[31,33] Conversely, in gout patients receiving a PEG-conjugated porcine uricase, pegloticase, anti-PEG antibodies were detected in up to 40% of treated patients and were associated with a loss of therapeutic response.[34–36] Regarding the role of anti-PEG antibodies in pegaspargase hypersensitivity, Armstrong et al. analyzed the banked serum of 28 pediatric ALL patients treated with pegaspargase on the ALL Berlin-Frankfurt-Münster Study Group 2000 trial without clinical hypersensitivity, including 15 with undetectable asparaginase activity, and 16 treated with unmodified asparaginase, eight of which had low asparaginase activity levels. They found a strong association between the presence of anti-PEG antibodies and rapid clearance of the drug, whereas no such association could be demonstrated with anti-asparaginase antibodies, or between anti-PEG antibodies and asparaginase activity levels in patients treated with unmodified asparaginase.[30]

The results observed in these four patients enrolled in COG AALL1421 provide evidence for the role of anti-PEG IgG antibodies in the development of clinically relevant hypersensitivity reactions to pegcrisantaspase and suggest that anti-PEG antibodies rather than antiasparaginase antibodies may mediate a proportion of the pegaspargase-hypersensitivity reactions occurring in pediatric patients with ALL/LLy. As there should be no cross-reactivity between the asparaginase portion of pegcrisantaspase and pegaspargase,[22] the PEG moiety, common to both, may be the antigenic target of the immune-mediated reaction. Similarly, in a study in which adults with phenylketonuria were given a single dose of recombinant PEGylated phenylalanine ammonia lyase, all 42 participant developed anti-PEG antibodies of varying titers. Two of these patients received PEGylated contraceptives 15 and 40 days after study drug and had hypersensitivity reactions, implicating a PEG-mediated immune reaction in these cases.[37]

It is interesting to note that while three hypersensitive subjects reacted to pegcrisantaspase within weeks of their allergic reactions to pegaspargase, the patient on this study who did not experience a hypersensitivity reaction to pegcrisantaspase had not been exposed to pegaspargase for 5.5 years. It is possible this subject's hypersensitivity to pegaspargase was mediated by antiasparaginase antibodies, but this discrepancy in timing also raises the possibility that while PEG can be immunogenic, anti-PEG-mediated immune reactions may not be associated with the development of durable immunologic memory.

The small number of patients treated limits the ability of this study to determine the extent to which anti-PEG antibodies contribute to hypersensitivity reactions and silent inactivation of pegaspargase. Recently, Schore et al. reported the presence of anti-PEG antibodies in 11 of the 72 patients treated on the COG trial AALL07P4 comparing pegaspargase to calaspargase pegol, a PEGylated *E. coli* derived asparaginase with a succinimidyl carbamate linker rather than the succinimidyl succinate linker used in pegaspargase. Of the 18 patients who experienced an anaphylactic reaction to either pegaspargase or calaspargase pegol, four had detectable anti-PEG antibodies.[38] While the combined existing data indicate some contribution of the PEG moiety, further detailed analysis of large populations of pegaspargase-treated patients is needed to determine the actual incidence of anti-PEG-mediated pegaspargase hypersensitivity.

Perhaps the most immediate implication of this study and the emerging anti-PEG antibody literature is that strategies other than PEGylation may be needed to optimize the *Erwinia* asparaginase treatment schedule for pegaspargase-hypersensitive patients. The use of alternative “stealth” polymers such as chitosan, poly(carboxybetaine), and poly(glycerol),for example, could be explored.An alternative strategy that has been suggested for pegaspargase sensitive patients with anti-PEG antibodies is to first saturate preexisting anti-PEG antibodies with free, low molecular weight PEG prior to administration of subsequent pegaspargase.[39] However, the safety and efficacy of this strategy would need to be tested.

In conclusion, in this phase 2 study of pegcrisantaspase for the treatment of pegaspargase-hypersensitive pediatric ALL patients, pegcrisantaspase-induced hypersensitivity reactions, mediated by anti-PEG antibodies, occurred in three of the four treated patients. Our results support the findings of Armstrong et al., implicating anti-PEG antibodies in the development of pegasparagase-induced hypersensitivity in a subset of pediatric ALL/LLy patients, and warrant further study in additional, larger cohorts. This study also highlights the need to explore alternative strategies to extend the half-life of *Erwinia* asparaginase for use after hypersensitivity reactions to pegaspargase.

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Abbreviations

AE	adverse events
ALL	acute lymphoblastic leukemia
COG	Children's Oncology Group
FDA	Food and Drug Administration
Ig	immunoglobulin
IM	intramuscular
IU	international units
IV	intravenous
LLy	lymphoblastic lymphoma
PEG	polyethylene glycol
SAA	serum asparaginase activity

Table 1

Patient information

Parameter	Patient 1	Patient 2	Patient 3	Patient 4
Sex	M	M	F	M
Age (years)	9	14	20	7
Disease	B ALL	B ALL	T LLy	B ALL
Relapse/progression	No	Ye s	No	No
Number of prior pegaspargase doses	2	2	4	3
Time since exposure to pegaspargase	1 week	5.5 years	6 weeks	1 week
Hypersensitivity grade	N/A	N/A	3 (anaphylaxis)	2
Percentage of course 1 dose received	100%	100%	<10%	62%

N/A, not applicable.

Table 2

Summary of SAA levels for all subjects

Subject			Patient 1 SAA IU/ml	Patient 2 SAA IU/ml	Patient 3 SAA IU/ml	Patient 4 SAA IU/ml
Course	Day	Time point				
0	0	Screening	<0.013	<0.013	<0.013	<0.013
1	1	Predose	<0.013	<0.013	ND	ND
1	1	1 hr post	0.67	0.50	ND	0.04
1	1	3 hr post	0.56	0.44	No sample	ND
1	1	5 hr post	0.52	0.41	No sample	ND
1	2	Visit 2	0.21	0.36	No sample	<0.013
1	8	Visit 3	<0.013	0.28	No sample	No sample
1	11	Visit 4	No sample	0.24	No sample	No sample
1	15	Visit 5	No sample	0.26	No sample	No sample
2	1	1 hr post	N/A	0.69	N/A	N/A
2	8	Visit 3	N/A	0.51	N/A	N/A
2	11	Visit 4	N/A	0.33	N/A	N/A
2	15	Visit 5	N/A	0.34	N/A	N/A
2	22	Visit 6	N/A	0.23	N/A	N/A
2	29	Visit 7	N/A	0.21	N/A	N/A
3	1	Predose	N/A	0.03	N/A	N/A
3	1	1 hr post	N/A	0.44	N/A	N/A
3	8	Visit 3	N/A	0.32	N/A	N/A
3	11	Visit 4	N/A	0.22	N/A	N/A
3	15	Visit 5	N/A	0.22	N/A	N/A
3	22	Visit 6	N/A	0.17	N/A	N/A
3	29	Visit 7	N/A	0.12	N/A	N/A

SAA, serum asparaginase activity level; ND, not determined; N/A, not applicable.

Table 3

Titration assay results for anti-PEG IgG

Subject	Sample	End point titer
Patient 1	Screening	1:10
	Predose	1:40
	Day 11	1:10
	Day 13	1:20
	Final visit (day 29)	1:10
Patient 3	Screening	1:80
	Predose	1:80
	Day 15	1:80
Patient 4	Screening	1:40
	Predose	1:20
	Day 11	1:20
	Day 13	1:40
	Early termination visit	1:40