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Studies of the Safety, Pharmacokinetics and Immunogenicity of Repeated Doses of Intravenous Staphylococcal Protein A in Cynomolgus Monkeys

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Abstract: Three Good Laboratory Practice safety studies were performed with intravenous injections of highly purified staphylococcal protein A (SPA) in cynomolgus monkeys, in support of a clinical development programme utilizing this protein as an immunomodulator. These studies established a no-observable-adverse-effect level (NOAEL) for up to 12 weekly doses of SPA, as well as toxicokinetic profiles for SPA, evaluation of antiproduct antibodies and biomarkers to better characterize the pharmacodynamic response to SPA. Biomarkers included neopterin, C-reactive protein (CRP), troponin I and the change in the blood absolute lymphocyte count (ALC) 24 hr after SPA dosing. The transient decrease in ALC noted at 24 hr after dosing was similar to that seen in human Phase 1 trials. The majority of active-treated monkeys developed antibodies against SPA. C_{max} was not affected by development of antidrug antibodies (ADAs), and after the first dose was 87 (SD 19) ng/mL, 330 (SD 84) ng/mL and 1191 (SD 208) ng/mL for 5, 25 and 100 μg/kg doses, respectively. The development of ADAs increased plasma clearance of SPA. By the sixth weekly dose, the AUC was decreased by 76%, 54% and 66% for the 5, 25 and 100 μg/kg dose groups, respectively. These results indicate that SPA can be administered intravenously to non-human primates without observable toxicity at weekly doses of up to 100 μg/kg.

Staphylococcal protein A (SPA) is an immune-modulating virulence protein produced by many strains of *Staphylococcus aureus*. In addition to binding to the immunoglobulin (Ig)G Fc region, SPA also binds with high affinity to the heavy chain CDR2 domain of the 20–30% of human or monkey immunoglobulins that utilize the VH3 gene to code CDR2. Thus, SPA also binds to the IgM antigen receptor on all Vh3 B cells [1,2], resulting in its description as a 'B-cell superantigen'. Parenteral doses of SPA reduce disease activity in the collagen-induced arthritis model and exposure of murine or human macrophages to complexes formed by SPA and homologous immunoglobulin induced a 'regulatory' macrophage phenotype characterized by decreased IL-12 and increased IL-10 secretion, as well as decreased TNF-alpha secretion in response to endotoxin [3].

In the 1990s, the Food and Drug Administration (FDA) approved of a medical device containing SPA covalently linked to silica beads (PROSORBA®, Cypress Bioscience, Inc., San Diego, CA, USA) for the plasma-adsorption treatment of patients with refractory rheumatoid arthritis or with immune thrombocytopenia [4,5]. Some investigators reported that SPA could leach from the column, and thus, patients might be exposed to small amounts of SPA in returned plasma. It was hypothesized that patient exposure to SPA might contribute to PROSORBA® efficacy [6–8]. However, the molecular nature of any SPA released, the magnitude of patient exposure and the human pharmacokinetic (PK) and pharmacodynamic activity of SPA remained uncharacterized.

Prior pharmacology and immunology studies with SPA have reported that at exposures 10–1000 times higher than those used in these studies, the injection of commercially produced protein A causes immune complex formation, complement activation, peripheral B-cell depletion and mast-cell degranulation in mouse, rat and rabbit animal models [7,9–11]. Clinical toxicities reported after PROSORBA® column treatments include fever, chills, rigours, headache and joint pain, as well as complement activation and vasculitis [12,13]. Only one published toxicology study with protein A was identified, a non-GLP pilot study dosing six monkeys for 28 days [14].

In 2006, Protalex Inc. initiated IND-enabling studies to investigate intravenous SPA for the treatment of autoimmune diseases, which led to two Phase 1 clinical trials [15]. The three GLP safety studies reported here were performed in cynomolgus monkeys. The monkey was seen as the most appropriate safety species, as the binding of SPA to monkey peripheral blood mononuclear cells (PBMCs) (T cells 0%; monocytes >90%; B cells 18–30%) is similar to that seen with human cells, in contrast to results with rabbit and mouse PBMCs where much lower binding frequencies were observed (data not shown).

The first two studies were performed prior to the first-in-man trial for this drug product and established the pharmacokinetic profile of SPA in the safety species, evaluated standard in life, laboratory and pathological safety end-points, as well as product immunogenicity. The third trial, performed after two single-dose healthy volunteer Phase 1 studies,

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incorporated additional pharmacodynamic biomarkers which were informative in the prior Phase 1 clinical trials and also evaluated a more extended dosing schedule.

Materials and Methods

Drug product. A highly purified form of 47 kDA native SPA was isolated from *Staphylococcus aureus* culture medium using Good Manufacturing Practices. SPA was harvested from bulk cultures of *Staphylococcus aureus*, Strain A676. Following microfiltration, three chromatographic purifications steps were followed by final formulation and microfiltration to produce bulk drug product. Product identity and purity (>98%) were determined by size exclusion chromatography–high-performance liquid chromatography (SEC-HPLC), reversed-phase HPLC and sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE). The product met FDA requirements for endotoxin content by limulus assay, residual DNA and for bioburden. Enterotoxin B content measured by enzyme immunoassay (EIA) was undetectable.

Following the first two monkey safety studies, a sensitive EIA was developed to detect non-SPA staphylococcal-host cell protein (S-HCP). This immunoassay was developed by raising antibodies against lysates of a related strain of *Staphylococcus* without the gene for SPA. Using this assay, the drug product was found to have approximately 800 ppm (0.08%) S-HCP. After changes in downstream chromatographic purification, the residual S-HCP was reduced 80 times to <10 ppm. This 'second-generation' drug substance (SPA gen2) was used to prepare drug product for the third monkey safety study (1541-07115), except for one comparison group treated with the original drug substance. The SPA gen2 product was also used for all subsequent human trials, where it was found to be far less reactogenic and cause less elevations of C-reactive protein than the original drug substance [15].

Study in cynomolgus monkeys. All studies were conducted under GLP at GeneLogic Laboratories, Gaithersburg, MD, USA and were approved by their Institutional Animal Care and Use Committee and in accordance with provisions of the United States Department of Agriculture (USDA) Animal Welfare Act, the Public Health Service Policy on Humane Care and Use of Laboratory Animals. All monkeys were acclimated prior to study and had two negative tuberculin tests. After the in-life study phase, monkeys were killed with barbiturates injection, in accordance with USDA guidelines. Monkeys were injected at weekly intervals via the saphenous vein, alternating between left and right. Retains of all dosing solutions were analysed for dose confirmation. Drug was diluted in a volume of 5 mL of sterile saline and injected over 20 sec. In Study 1541-07115 (the third study), one group received 25 μg/kg injections subcutaneously (s.c.), which were rotated between four intrascapular sites, which were evaluated and scored using the Draize scale [16] for erythema and oedema at 1 hr after injection, then daily for 6 days.

Cageside observations included observation for mortality, moribundity, general health and signs of toxicity. Clinical observations included evaluation of skin and fur characteristics, injection site, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, and somatomotor and behaviour patterns. Monkeys had *ad libitum* access to monkey chow, and daily qualitative food consumption was evaluated using the following scale: poor = 1–4 biscuits eaten, fair = 5–8 biscuits eaten, good = 9–12 biscuits eaten. In-life observations also included electrocardiograms and blood pressure, urinalysis, chemistry and haematology, and serial body-weights. Ophthalmologic observations were conducted using indirect ophthalmoscopy and slit-lamp biomicroscopy (as needed).

Pathology included organ gross examination and weights, and histopathology of selected organs or organs with abnormal gross appearance. Monkeys were killed at 5–7 days (main groups) or 5 weeks (recovery groups) after administration of the final dose.

In study 1541-07115, two biomarkers of immune reactogenicity were evaluated: serum C-reactive protein (CRP) and neopterin. Troponin I was also assessed as a measure of any potential cardiac muscle damage. An additional measure, also identified during a Phase 1 single-dose clinical study, was analysed as a pharmacodynamic marker of SPA activity. This marker was the Absolute Lymphocyte Count (ALC) and the ALC per cent change observed after SPA dosing. The ALC per cent change was derived from complete blood counts (CBCs) obtained prior to and 24 hr after the first and last dosing with SPA.

Table 1 provides an overview of the three cynomolgus studies conducted. Study 1541-04032 was considered a pilot study.

Pharmacokinetic and immunogenity assays. In the second two studies, plasma SPA concentrations were measured with a qualified ELISA method with a lower limit of quantification (LLQ) of 2 ng/mL. Antiproduct antibodies were measured using a validated ELISA method at Prevalere Life Sciences. In brief, this method utilized SPA bound to wells on a polystyrene plate. After blocking of non-specific binding of IgG by SPA with 20% non-primate serum, a 1/100 dilution of samples, negative controls or calibration samples were incubated in the wells. Rabbit anti-SPA was used for positive controls and calibration samples. Bound anti-SPA antibodies were detected using biotinylated pig anti-rabbit or anti-monkey Ig, after incubation with streptavidin–horse radish peroxidase (HRP) conjugate and then HRP substrate.

The blood sample collection time-points for Study 1541-04138 were pre-dose and .08, .25, .5, .75, 1, 1.5 and 3 hr after dosing on dosing Days 1, 36 and 78 and pre-dose and 5 min. after dosing on the other 10 dosing days. Pre-dose samples from Days 1, 29, 57 and 71 were analysed for anti-SPA antibodies. The sample collection time-points for Study 1541-07115 were pre-dose and 0.08, 0.5, 1, 4, 12, 48 and 72 hr after the first dose and sixth dose, and pre-dose and pre-dose and 5 min. after dosing on the other four dosing days. Blood was collected from all animals prior to dosing on Study Days 1, 28, 50 and 64, and at termination for assessment of anti-SPA antibodies.

Table 1.

Overview of cynomolgus monkey studies.

Study no.	Animals per group main/recovery	Duration of dosing	Recovery group	Doses (μg/kg)	Testing facility
1541-04032	6 male/2 male 6 female/2 female	4 times in 28 days	4 weeks	0, 1, 3, 5 i.v.	Gene Logic Laboratories, Inc.
1541-04138	3 males/2 males 3 females/2 females	12 times in 3 months	4 weeks	0, 1, 5, 25 i.v.	Gene Logic Laboratories, Inc.
1541-07115	5 females/2 females (i.v.) 2 females (s.c. group)	6 times in 6 weeks	4 weeks	0, 5, 25, 100 i.v. 5 s.c.	Gene Logic Laboratories Inc.

i.v., intravenous; s.c., subcutaneous.

Pharmacokinetic parameter calculations. Non-compartmental methods were used to estimate the PK end-points. Semi-log plots were constructed of the individual plasma SPA concentration *versus* time profiles for each subject. Area under the curve (AUC) values were calculated by log-linear trapezoidal integration using the KINETICA™ (Adept Scientific, Ltd, Letchworth, UK) version 2.0.1 validated computer programme. The terminal half-life was calculated as $\ln(2)/\lambda_z$ where λ_z was the calculated slope of the terminal portion of the log plasma concentration *versus* time curve. Extrapolation of the AUC from the last measured plasma concentration to infinity was calculated as Cp_n/λ_z where Cp_n is the last measured analyte plasma concentration. Mean residence time was calculated as $[(AUMC_{0-\infty}/AUC_{0-\infty}) - T_{inf}/2]$, where T_{inf} is the dose infusion duration (0 hr). Plasma clearance (CL) was calculated as $Dose/AUC_{0-\infty}$, and the terminal phase distribution volume (V_z) was calculated as $Dose/(AUC_{0-\infty}*\lambda_z)$. The steady-state distribution volume (V_{ss}) was calculated as $[Dose*AUMC_{0-\infty}/(AUC_{0-\infty}* AUC_{0-\infty})] - [(T_{inf} * Dose)/2*AUC_{0-\infty}]$. The 'apparent' volume of the central compartment (V_c) was estimated as $Dose/Cp_{0extrap}$ where $Cp_{0extrap}$ is the zero time extrapolated plasma SPA concentration value estimated from the initial log-transformed plasma concentration *versus* time curve using the KINETICA™ Version 2.0.1 Software Programme. At least three time-points in the terminal phase and an r^2 value ≥ 0.8 were required to calculate λ_z.

C-Reactive protein assay. C-reactive protein was measured in serum using a commercially available EIA kit (Life Diagnostics, Inc., West Chester, PA, USA). This assay is validated for monkey serum, with an LLQ of 1.2 ng/mL.

Neopterin assay. Neopterin was measured in plasma using a commercially available radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA, USA). This assay is validated for monkey plasma, with a quantification limit of 1 ng/ml.

Troponin I assay. Troponin I was measured in serum using a monkey-specific EIA kit from Life Diagnostics with a quantification limit of 0.15 ng/mL.

Results

Toxicology.

Study 1541-04032 administered four repeated weekly i.v. injections of 0, 1, 3 or 5 μg/kg of SPA. No in-life observations, clinical laboratory, gross organ examination or histopathology showed any meaningful differences between active-treated and control groups. The NOAEL was considered to be 5 μg/kg for four weekly i.v. doses.

Study 1541-04138 administered 12 weekly injections of 0, 1, 5 or 25 ug/kg of SPA. No mortality or morbidity occurred in any of the animals prior to the scheduled termination. There were no antemortem observations and no SPA-related effects on body-weight, body-weight gain or food consumption and no effects attributable to treatment upon any clinical pathology parameters, gross pathology or organ weights. There were no additional histopathologic changes attributed to SPA in either the original or peer pathology reviews.

Based upon the results of this 3-month study, the NOAEL was considered to be at least 25 μg/kg. Diminishing exposure (plasma drug AUC) was observed over the course of treatment, most markedly at the 25 μg/kg dose level, and this correlated with a positive anti-SPA response.

Study 1541-07115 administered 0, 5, 25 or 100 μg/kg of SPA as six weekly intravenous injections (or in two monkeys, rotated intrascapular subcutaneous injections of 5 μg/kg). Treatment with SPA had no effect on mortality or clinical observations. All animals survived until the scheduled termination. Incidental observations included abrasions, appetite loss, alopecia, vaginal discharge, diarrhoea, discoloured, mucoid or soft faeces and scabbing. These observations were considered incidental because they also occurred in the control animals, and/or occurred in just one animal, and/or are a common findings in laboratory-housed monkeys, and had no relationship to dose. Of interest, treatment of two monkeys with six subcutaneous injections of 5 μg/kg SPA gen2 had no effect on dermal Draize scores at the injection sites, implying minimal cutaneous sensitization over 6 weeks. Treatment with SPA had no effect on body-weight changes.

Treatment with SPA gen2 did have an effect on blood pressure on Study Day 2, when both the diastolic and systolic pressures were significantly higher for the animals dosed at 25 and 100 μg/kg. Although significant, the systolic pressures were comparable to the control animal systolic pressures recorded on Study Day 37. There was no significant increase in blood pressure on subsequent study days.

Treatment with SPA had no effect on the ophthalmologic examinations or electrocardiography. All electrocardiograms remained within normal limits. There was also no effect on gross pathology. Incidental findings at terminal sacrifice included a nodule on the gallbladder, enlarged heart, IV injection site discoloration, lesion on the liver, discoloured liver, lung adhesion, discoloured lung, failure of the lungs to collapse, cyst on the ovary, skin discoloration of the inner thigh, skin abrasion, small thymus, firm thyroid gland, small uterus and vaginal discharge (white). Incidental findings at recovery sacrifice included discoloured ovary, enlarged ovary and crust/scab on the vagina.

There were no biologically or toxicologically relevant changes in the blood chemistry, complete blood count or urinalysis. Treatment also had no effect on histopathology; no test-article-related findings were noted. Inflammatory lesions were noted in vehicle- and active-dosed terminal sacrifice monkeys in the intravenous injection sites, skeletal muscle and the skin of the ventral thigh.

The NOAEL following six weekly intravenous injections of SPA gen2 formulation is considered to be 100 μg/kg.

Biomarkers (Study 1541-07115).

An additional purpose was to study any product-related changes in some of those biomarkers for safety and pharmacodynamic effects which were utilized in single-dose Phase 1 human trials, to evaluate effects after repeated doses of SPA gen 2. These biomarkers included C-reactive protein, serum neopterin and troponin I, all measured prior to and 2 days after the first and fourth dosing with SPA gen2. Troponin I was also assessed serially, as a sensitive marker for any potential myocardial toxicity or inflammation.

C-Reactive protein.

C-reactive protein represents the hepatic acute phase response to pro-inflammatory cytokines such interleukin-6 and interleukin-1. It is typically many times elevated following bacterial infections and can be used to gauge the immune reactogenicity of bacterially derived products. When measured by a specific EIA for monkey, CRP values in healthy Macac Irus are reported to be 0.0–8.4 mg/L with a mean of 2.2 mg/L [17].

At pre-dose baseline, all monkeys had values of <8.4 mg/L. In contrast to the vehicle group, four of 21 (19%) of monkeys in the 5.0, 25.0 and 100 μg/kg SPA gen2 dose groups showed a transient elevation of CRP after the first dose to >8.4 mg/L. This was not noted after repeated doses. The monkeys who had CRP elevations after the first dose did not demonstrate sustained elevations and had a decreased or absent CRP response to the last dose. Thus, SPA did not seem more reactogenic with repeated dosing than with a single dose. Table 2 below compares the mean CRP increases, by treatment, at 48 hr after the first and fourth dose of SPA.

Neopterin.

Neopterin is released by activated macrophages and is typically elevated after live-virus vaccines or viral infections. Serum neopterin reflects the actions of gamma-interferon, and to a lesser extent, type-1 interferons, on macrophages. These responses are similar in man and non-human primates. A normal range of 0–10 nM/L (0–2.53 ng/mL) is generally accepted for both human beings and monkeys [18,19]. While one monkey in the vehicle group and one monkey in the 5 μg/kg group had neopterin values prior to the first SPA gen2 dosing which were elevated (40.1 and 6.3 ng/mL, respectively), and these two had declining, but still elevated values throughout the study, no other monkeys had any elevated neopterin values on Study Days 1, 3, 22 or 24.

Troponin I.

Troponin I is a sensitive indicator of cardiac muscle damage and is released into serum during cardiac damage (e.g. post-ischaemic) or inflammation (e.g. myocarditis). No elevated levels of troponin I were detected in any monkeys. Mean values in the control group and active-treated groups were not significantly different at any time-point.

Absolute lymphocyte count.

Dose-related changes in the ALC showed a trend to decrease from baseline at 24 hr after the first dose; however, these changes compared with vehicle group did not reach statistical significance. This change was most marked in the nine monkeys dosed with the 5 μg/kg dose, either i.v. or s.c., and did not show a dose response across the 25 and 100 μg/kg doses. These values are shown in table 3 below.

Figure. 1 below examines the 24-hr after dosing lymphopenic response after the first and fourth dose of SPA gen2 in the group of monkeys treated with 5 μg/kg of drug, i.v. It can be seen from both the mean and the maximum increases, that with 5 μg/kg of SPA gen2, there is attenuation of the response after four doses, and at the higher doses, there is no increase in this response with repeated doses.

Pharmacokinetics and anti-SPA antibody results.

Study 1541-04138 provided pharmacokinetic profiles for i.v. SPA, as well as assessment of anti-SPA antibodies across study time-points. The initial decline in plasma SPA concentration was rapid after i.v. administration of the five and 25 μg/kg dose. The plasma SPA concentrations following multiple-dose i.v. administration of the 1 μg/kg dose group were below the LLQ, and the plasma SPA concentrations after the 5 μg/kg dose declined below the LLQ within approximately 3 hr. Plasma SPA concentrations and $AUC_{0-\infty}$ were roughly proportional to dose on Study Day 1 after i.v. administration of five and 25 μg/kg of SPA. Mean plasma C_{max} on Study Day 1 was 104 (SD 9.64) ng/mL and 464 (SD 92.2) ng/mL in the five and 25 μg/kg dose groups, and the mean plasma $AUC_{0-\infty}$ was 483 (SD 591) hr*ng/mL and 2184 (SD 1365) hr*ng/mL on Study Day 1 after administration of the

Table 2.

Mean after dosing change from baseline in C-reactive protein for monkeys receiving 5, 25 or 100 μg of SPA (study 1541-07115).

	Change over 48 hr, pre-/post-first dose[1]	Change over 48 hr, pre-/post-fourth dose[1]
Vehicle		
Mean (S.D.)	0.86 (2.63)	−0.04 (0.08)
Range (min;max)	−1.5 to 6.0	−0.15 to 0.04
5 μg/kg liquid (original drug substance)		
Mean	0.37 (1.20)	−0.07 (0.14)
Range (min;max)	−0.54 to 0.47	−0.30 to 0.06
5 μg/kg (SPA gen2)		
Mean	3.55 (5.92)	0.06 (0.25)
Range (min;max)	−0.83 to 15.10	−0.18 to 0.54
25 μg/kg (SPA gen2)		
Mean	1.26 (3.18)	1.09 (2.97)
Range (min;max)	−0.21 to 8.45	−0.17 to 7.82
100 μg/kg (SPA gen2)		
Mean	3.64 (4.32)	−0.13 (0.27)
Range (min;max)	0.04–10.55	−0.64 to 0.17

S.D., standard deviation; min, minimum; max, maximum.

[1]High-sensitivity C-reactive protein in mg/L.

Table 3.
Mean (S.D.) per cent change in ALC compared pre-dose baseline (Study 1541-07115).

Dose	N	24 hr after first dose	24 hr after fourth dose	Day 38, after sixth dose
Vehicle	6	−2.2 (38.1)	26.3 (64.9)	−11.2 (34.5)
5 μg/kg SPA (original drug substance)	5	−16.5 (15.6)	−3.5 (11.8)	−22.0 (10.2)
5 μg/kg SPA gen2	7	−34.3 (13.3)	−19.9 (13.3)	−25.6 (18.0)
5 μg/kg SPA gen2 (s.c.)	2	−33.8 (6.2)	−11.1 (32.9)	27.4 (6.1)
25 μg/kg SPA gen2	7	−19.12 (15.9)	−8.34 (13.4)	−35.1 (5.8)
100 μg/kg SPA gen2	7	−14.9 (30.9)	−5.4 (45.8)	−25.3 (14.6)

ALC, absolute lymphocyte count; S.D., standard deviation; s.c., subcutaneous.

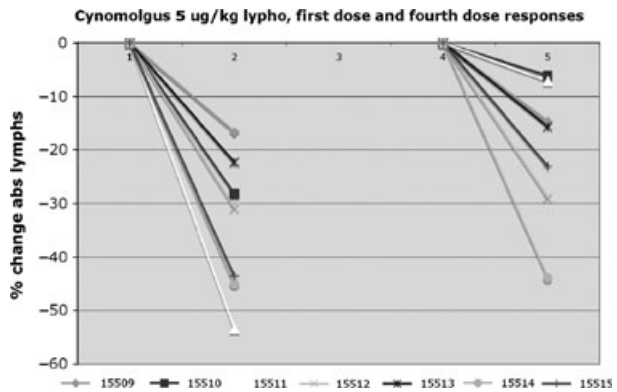

Fig. 1. Changes in the absolute lymphocyte count at 24 hr following the first and fourth dose 5 μg/kg intravenous staphylococcal protein A (SPA). The per cent decrease in the absolute lymphocyte count (ALC) observed consequent to SPA dosing was identified during a Phase 1 single-dose clinical study as a pharmacodynamic marker of SPA activity. The ALC per cent change below was derived from complete blood counts in cynomolgus monkeys obtained prior to and 24 hr after each dose of SPA.

five and 25 μg/kg doses, respectively (see tables 4 and 5). On Study Day 78, decreased (relative to Day 1) values for both C_{max} and $AUC_{0-\infty}$ were observed in both dose groups.

Clearance (CL, L/hr/kg) increased between Day 1 and Day 78, and this increase was statistically significant for the 25 μg/kg dose group (two-tailed $p < 0.005$ by paired t-test). Volume of distribution after the first dose was 0.046 L/kg (5 μg/kg group) and 0.052 L/kg (25 μg/kg group), representing approximately plasma volume.

Approximately 61% (11/18) of active-treated animals in the main study group developed detectable antibodies against SPA. During the treatment phase of the study (Days 29, 57 and 71), 0/6 (controls), 1/6 (1 μg/kg), 4/6 (5 μg/kg), and 6/6 (25 μg/kg) monkeys tested positive for anti-SPA antibodies. All four animals in the 25 μg/kg recovery group also developed antibodies to SPA. Positive antibody responses in high-dose monkeys were first recorded on Days 8 or 15, and toxicokinetic results revealed exposure were lower after Day 36 injections in this subgroup and that a > 10 times reduction in mean $AUC_{0-\infty}$ was noted following the final (12th) dose relative to the first dose. Following the 12th dose, the mean $AUC_{0-\infty}$ in this 25 μg/kg subgroup, was less than that observed in the 5 μg/kg dose group. Plasma SPA levels following the administration of 1 μg/kg were below the level of quantitation.

Study 1541-07115 evaluated the pharmacokinetic profile of SPA gen2 administered at 5, 25 or 100 μg/kg once weekly for 6 weeks to adult female cynomolgus monkeys (seven per group). A pilot group of two monkeys received 5 μg/kg doses subcutaneously (s.c.) at rotated intrascapular sites, and five females received the earlier liquid dose formulation.

The decline in plasma SPA gen2 concentration was mono-exponential in most animals after an intravenous dose, except for animals in the 100 μg/kg dose group that exhibited a bi-exponential decline. The plasma SPA gen2 exposure parameters are summarized in table 6.

Table 4.
Plasma SPA pharmacokinetic parameters measured on study Day 1 and study Day 78 following weekly administration of 5 μg/kg doses (Study 1541-04138).

	Study Day 1			Study Day 78			
PK parameter	Mean	S.D.	n	Mean	S.D.	n	*p*-value*
AUC_{0-n} hr*ng/mL	131.9	105.3	6	47.2	43.7	6	0.05
$AUC_{0-\infty}$ hr*ng/mL	482.6	591.3	6	313.4	656.6	6	NS
C_{max}, ng/mL	103.6	9.64	6	86.7	15.2	6	0.05
CL, L/hr/kg	0.0429	0.0411	6	0.1076	0.0763	6	NS
V_c, L/kg	0.04461	0.0051	6	0.0481	0.0076	6	NS
V_z, L/kg	0.04486	0.0056	6	0.0492	0.0083	6	NS
Half-Life (hr)[1]	1.3825	3.5624	6	0.5251	5.3357	6	NS

S.D., standard deviation; n, number; NS, not significant.
*Paired t-test, 2 tailed *p*-value.
[1]Geometric mean half-life.

Table 5.
Plasma SPA pharmacokinetic parameters measured on study Day 1 and study Day 78 following weekly administration of 25 μg/kg doses (Study 1541-04138).

	Study Day 1			Study Day 78			
PK parameter	Mean	S.D.	n	Mean	S.D.	n	*p*-value*
AUC_{0-n} hr*ng/mL	839.2	304.6	6	124.4	59.6	6	0.005
$AUC_{0-\infty}$ hr*ng/mL	2147.8	1365.1	6	146.1	71.5	6	0.02
C_{max}, ng/mL	463.7	92.2	6	264.8	61.1	6	0.01
CL, L/hr/kg	0.01750	0.01323	6	0.2043	0.0846	6	0.005
V_c, L/kg	0.05239	0.00832	6	0.08526	0.01026	6	0.005
V_z, L/kg	0.07594	0.02199	6	0.09335	0.00513	6	NS
Half-Life (hr)[1]	3.5949	2.3819	6	0.3441	0.2086	6	0.001

S.D., standard deviation, n, number, NS, not significant.
*Paired t-test, 2 tailed *p*-value.
[1]Geometric mean half-life.

Table 6.
Plasma SPA exposure in cynomolgus monkeys after IV dose administration of liquid formulation SPA or of SPA gen2 every 7 days (Study 1541-07115).

Week	Dose, μg/kg	C_{max}, ng/mL[1]	AUC_{0-n}, hr*ng/mL[1]	n
1	5 (original drug substance)	99.9 (16.3)	49.9 (31.0)	5
6	5 (original drug substance)	103 (14.0)	35.0 (39.4)	5
1	5 (SPA gen2)	87.1 (19.0)	278 (624)	7
6	5 (SPA gen2)	94.0 (17.4)	67.4 (90.6)	7
1	25 (SPA gen2)	330 (83.7)	824 (1145)	7
6	25 (SPA gen2)	285 (65.2)	379 (649)	7
1	100 (SPA gen2)	1191 (208)	6173 (3587)	7
6	100 (SPA gen2)	1336 (338)	2111 (2810)	7

S.D., standard deviation; n, number.
[1]Mean (S.D.).

Pre-dose plasma values showed minimal plasma accumulation of SPA following once a week i.v. dose administration for up to 6 weeks. Most active-treated animals tested positive for anti-SPA antibody (ADA) formation during the study including: 4/5 animals receiving 5 μg/kg (original drug substance), 4/7 animals in receiving 5 μg/kg (SPA gen2), 2/2 animals receiving 5 μg/kg s.c., 6/7 animals receiving 25 μg/kg (SPA gen2) and 5/7 animals receiving 100 μg/kg (SPA gen2). None of the animals in the vehicle control group tested positive for ADA formation. Using this sensitive assay, there were 13 animals in the active-dose groups that tested positive for ADA prior to Day 1 dosing, suggesting that many animals may have had pre-existing antibodies to staphylococcal protein A. Animals in the 100 μg/kg dose group that tested positive for ADA formation tended to have lower mean SPA AUC_{0-n} exposure than those animals that tested negative. For the 100 μg/kg dose group, the mean (S.D.) AUC_{0-n} exposure was 9196 (1757) hr*ng/mL and 5424 (4025) hr*ng/mL on Week 1 and Week 6 in the two animals that tested negative for ADA formation and was 4964 (3484) hr*ng/mL and 786 (333) hr*ng/mL in the five ADA-positive animals on Week 1 and Week 6, respectively. The mean (S.D.) plasma SPA concentration *versus* time for the ADA-negative and ADA-positive animals in this dose group is provided in fig. 2.

Mean volume of distribution ranged from 0.052 L/kg for 5 μg/kg doses to 0.072 L/kg for 100 μg/kg doses. No accumulation of SPA was observed in plasma after multiple-dose i.v. administration of 5, 25 or 100 μg/kg doses of SPA gen2 once per week for up to 6 weeks. After s.c. dosing of the SPA gen2 formulation, plasma SPA concentrations were undetectable at all time-points.

Discussion

Review of the pharmacology and toxicology literature reveals limited information on chronic effects of SPA. SPA at bi-weekly doses of 60 μg/kg or less protected rats from myelotoxicity after benzene exposure, from carbon tetrachloride-induced hepatotoxicity and from leukopenia following cyclophosphamide treatment. SPA treatment was associated with increases in glutathione-S-transferase. [20–22] One prior safety study of intravenous SPA in the monkey was found in the published literature [14]. However, this non-GLP pilot study did not characterize the origin or purity of the SPA utilized for dosing and contained no toxicokinetic information on the plasma exposures attained. This study dosed groups of two male *Macaca fascicularis* monkeys daily for 28 days with 160, 400 or 1000 μg/kg of SPA. All dose groups showed elevations of alkaline phosphatase without changes in transaminase values. Liver histopathology was benign. The authors concluded that 'toxic changes were not seen' with doses up to 1000 μg/kg of daily intravenous SPA.

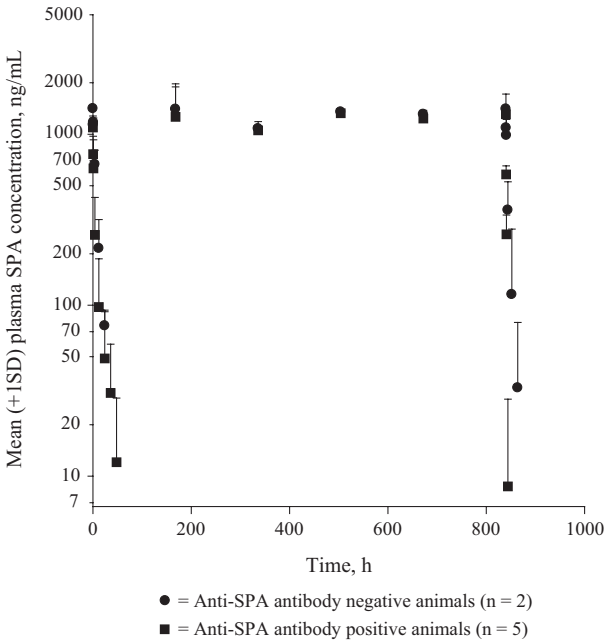

Fig. 2. Mean (+1 S.D.) plasma SPA concentrations following weekly i.v. injections of 100 μg/kg lyophilized of SPA for 6 weeks. Plasma concentrations were measured with a qualified ELISA method with a lower limit of quantification (LLQ) of 2 ng/mL. Rabbit anti-SPA was used for positive controls and calibration samples. No accumulation of plasma SPA was observed over time (pre-dose values not shown). Note that plasma clearance is more rapid in rabbits with anti-SPA antibodies even after the first dose, consistent with low levels of pre-existing antibodies.

Results from the 6- and 13-week monkey toxicology studies presented here indicate that SPA is well-tolerated and essentially non-toxic following weekly intravenous doses of up to 100 μg/kg. The monkey is considered the most predictive animal model based upon similarity to human being in terms of IgG, B-cell and monocyte/macrophage binding, and results in this species are generally consistent with those obtained to date in human beings. Not unexpected, given that SPA is a bacterial protein antibody responses to this foreign protein develop in this species with repeat dosing. Importantly, no evidence of a hypersensitivity response has been observed in any of these toxicology studies. Associated with the antibody response, however, marked dose-dependent reductions in plasma exposure have occurred a few weeks into the treatment period, associated with accelerated plasma clearance.

At a dose of 5 μg/kg (approximately 15 μg per animal), the pharmacodynamic response [lymphopenia 24 hr after dosing, from the complete blood count (CBC)] was similar to that seen in the two human Phase 1 single-dose studies [15], confirming that the monkey was a pharmacodynamically responsive test species for SPA. The lymphopenic response shows SPA at these doses has pharmacodynamics activity, but appears to be acute and self-limited.

The majority of active-treated monkeys in the second two studies developed antibodies against SPA. As study 1541-07115 demonstrates, C_{max} is not affected by development of ADAs, and after the first dose was 87 (SD 19) ng/mL, 330 (SD 84) ng/mL and 1191 (SD 208) ng/mL for 5, 25 and 100 μg/kg doses, respectively. However, the development of ADAs clearly increased plasma clearance of SPA, and by the sixth weekly dose, the AUC was decreased by 76%, 54% and 66% for the 5, 25 and 100 μg/kg dose groups, respectively. This suggests that further escalation of dose in subchronic safety studies, to define dose-limiting toxicities at greater plasma exposures, may be impractical. Due to antiproduct antibodies elicited by the clearly immunogenic test article, higher plasma exposures may not be possible with higher doses of product. More importantly, the dose-limiting toxicities with chronic dosing at higher doses (which might lead to conditions of antigen excess) might also include immune complex disease. However, these three toxicology studies have supported human repeated-dose Phase 1b studies in patients with active rheumatoid arthritis, where five weekly intravenous doses of 1.5–12 μg/kg have been well tolerated [E. Bernton, Unpublished Observations, PRTX-100-104 Investigator's Brochure] with no immune-mediated hypersensitivity responses observed, despite development of anti-SPA antibodies in many patients.

Conflict of Interest

Edward Bernton functioned as a paid consultant and Medical Director for Protalex Inc. David Haughey was an employee of Prevalere Biosciences which was a contractor for Protalex Inc. paid to conduct PK and immunogenicity assays and pharmacokinetic analyses. Funding for all studies was provided by Protalex Inc.

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References

1 Graille M, Stura E, Corper L, Sutton B, Taussig M, Charbonnier J *et al.* Crystal structure of a *Staphylococcus aureus* protein A domain complexed with the Fab fragment of a human IgM antibody: structural basis for recognition of B-cell receptors and superantigen activity. Proc Natl Acad Sci USA 2000;**97**:5399–404.

2 Sasso E, Silverman G, Mannik M. Human IgA and IgG F(ab′)2 that bind to staphylococcal protein A belong to the VHIII subgroup. J Immunol 1991;**147**:1877–83.

3 MacLellan L, Montgomery J, Sugiyama F, Kistson S, Thummler K, Silverman G *et al.* Co-opting endogenous immunoglobulin for the regulation of inflammation and osteoclastogenesis in humans and mice. Arthritis Rheum 2011;**63**:3897–907.

4 Synder H, Cochran S, Balint J, Bertram J, Mittelman A, Guthrie T *et al.* Experience with protein A-immunoadsorption in treatment-resistant adult immune thrombocytopenic purpura. Blood 1992;**79**:2237–45.

5 Felson D, LaValley M, Baldassare A, Block J, Caldwell J, Cannon G *et al.* The Prosorba column for treatment of refractory rheumatoid arthritis: a randomized, double-blind, sham-controlled trial. Arthritis Rheum 1999;**42**:2153–9.

6 Balint J, Jones F. Evidence for proteolytic cleavage of covalently bound protein A from a silica based extracorporeal immunoadsorbent and lack of relationship to treatment effects. Transfus Sci 1995;**16**:85–94.

7 Goodyear C, Sugiyama F, Silverman G. Temporal and dose-dependent relationships between *in vivo* B cell receptor-targeted proliferation and deletion-induced by a microbial B cell toxin. J Immunol 2006;**176**:2262–71.
8 Silverman G, Goodyear C, Siegel D. On the mechanism of staphylococcal protein A immunomodulation. Transfusion 2005;**45**:274–80.
9 Marone G, Tamburini M, Giudizi M, Biagiotti R, Almerigogna F, Romagnani S. Mechanism of activation of human basophils by *staphylococcus aureus* Cowan. Infect Immunol 1987;**55**:803–9.
10 White M, Noble W. The cutaneous reaction to staphylococcal protein A in normal subjects and patients with atopic dermatitis or psoriasis. Br J Dermatol 1985;**113**:179–83.
11 Anderson A, Sporici R, Lambris J, Larosa D, Levinson A. Pathogenesis of B-cell superantigen-induced immune complex-mediated inflammation. Infect Immun 2006;**74**:1196–203.
12 Ainsworth S, Pilia P, Pepkowitz S, O'Brien P. Toxicity following protein A treatment of metastatic breast adenocarcinoma. Cancer 1988;**61**:1495–500.
13 Arbiser J, Dzieczkowski J, Harmon J, Duncan L. Leukocytoclastic vasculitis following staphylococcal protein A column immunoadsorption therapy. Arch Dermatol 1995;**131**:707–9.
14 Yanagimoto Y, Yamamoto H, Nishida M, Matsuo A, Matsuoka K, Sato H. Four week intravenous toxicity study of staphylococcal protein A in monkeys. Oyo Yakuri 1989;**37**:517–27.
15 Ballow C, Leh A, Slentz-Kesler K, Yeh J, Haughey D, Bernton E. Safety, pharmacokinetics, immunogenicity, and pharmacodynamic responses in healthy volunteers following a single intravenous injection of purified Staphylococcal protein A. J Clin Pharmacol 2013;**53**:909–18.
16 Draize J, Woodard G, Calvery H. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1944;**82**:377–90.
17 Hart B, Bank R, De Roos J, Brok H, Jonker M, Theuns H *et al.* Collagen-induced arthritis in rhesus monkeys: evaluation of markers for inflammation and joint degradation. Br J Rheumatol 1998;**37**:314–23.
18 Mager D, Neuteboom B, Jusko W. Pharmacokinetics and pharmacodynamics of PEGylated IFN-beta 1a following subcutaneous administration in monkeys. Pharm Res 2005;**22**:58–61.
19 Murr C, Widner B, Wirleitner B, Fuchs D. Neopterin as a marker for immune system activation. Curr Drug Metab 2002;**3**:175–87.
20 Shankar U, Kumar A, Rao G, Dwivedi P, Pandya K, Ray P. Modulation of benzene induced toxicity by protein A. Biochem Pharmacol 1993;**46**:517–24.
21 Singh K, Saxena A, Zaidi S, Dwivedi P, Srivastava S, Seth P *et al.* Protection against carbon tetrachloride-induced hepatotoxicity by protein A. J Appl Toxicol 1988;**8**:407–10.
22 Zaidi S, Singh K, Saxena A, Ray P, Protein A. induced abrogation of cyclophosphamide toxicity is associated with concomitant potentiation of immune function of host. Immunopharmacol Immunotoxicol 1990;**12**:479–512.