

# PRTX-100 and Methotrexate in Patients With Active Rheumatoid Arthritis: A Phase Ib Randomized, Double-Blind, Placebo-Controlled, Dose-Escalation Study

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## Abstract

PRTX-100 is a highly-purified preparation of staphylococcal protein A (SpA), with immunologic activity in vitro and in animal models of immune-mediated inflammation. Following single-dose healthy volunteer studies of safety and pharmacokinetics (PK), a multicenter, double-blind, placebo-controlled, sequential dose-escalation, repeated-dose phase I trial was conducted in patients with active rheumatoid arthritis (RA) on methotrexate therapy. Patients were randomized to receive either weekly intravenous PRTX-100 (0.15, 0.45, 0.90, or 1.50  $\mu\text{g}/\text{kg}$ ) or placebo for 4 weeks. Safety and disease activity were assessed over 16 weeks. Pharmacokinetic profiles were obtained after the first and fourth doses. The most common treatment-related adverse events were nausea, muscle spasms, dizziness, flushing, fatigue, RA flare, and headache. No serious adverse events were considered related to PRTX-100, and none occurred in the highest dose group. Geometric mean values for plasma  $C_{\text{max}}$  (ng/mL) were 4.1, 15.7, 26.5, and 51.2 for doses of 0.15, 0.45, 0.90, and 1.5  $\mu\text{g}/\text{kg}$ , respectively. Anti-drug antibodies (ADAs) developed in most PRTX-100 patients, but incidence and titer were not dose-dependent. At the two highest doses, data suggest PRTX-100 may have an effect on RA disease activity, even in patients with ADAs.

## Keywords

immunomodulator, staphylococcal protein A, rheumatoid arthritis, phase I, safety

Staphylococcal protein A (SpA) is an immune-modulating virulence protein produced by many strains of *Staphylococcus aureus*. Its notable properties include high affinity binding to the immunoglobulin (Ig)G Fc region, as well as to the heavy chain VH domain of the 20–30% of human immunoglobulins that utilize the VH3 gene for this region.<sup>1,2</sup> Due to this VH3 binding, SpA also binds to the IgM antigen receptor on all Vh3 B-cells,<sup>1,2</sup> resulting in its description as a “B-cell superantigen.” We discovered that in vitro exposure of human macrophages to SpA at concentrations as low as 10–50 ng/mL inhibits both phagocytosis of opsonized platelets and macrophage secretion of tumor necrosis factor-alpha (TNF-alpha), and the up-regulation of CD16 and CD40, after stimulation by bacterial endotoxin (unpublished data). This activity appears to involve inhibitory signaling via Fc receptors and to require the presence of human Ig. Furthermore, low doses of SpA (0.1–0.01  $\mu\text{g}/\text{mouse}$ , I.V.) reduced arthritis severity as measured by joint swelling scores and histopathology scores, to a similar degree as the TNF inhibitor etanercept (unpublished data). When mice form antibodies to SpA, efficacy is not abrogated; this contrasts

with the activity of etanercept, which does not persist after formation of mouse antibodies to this humanized recombinant protein. Recently, similar findings were published with SpA in the mouse CIA model, albeit at higher doses.<sup>3</sup> These investigators also demonstrated that exposure of murine or human macrophages to complexes formed by SpA and immunoglobulin of the same species 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 and decreased STAT-1 phosphorylation in response to IFN-gamma. This work confirms and extends

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our own in vitro findings that SpA forms complexes with IgG that inhibit the activation of human macrophages.

In the 1990s the Food and Drug Administration approved a medical device containing SpA covalently linked to silica beads (PROSORBA<sup>®</sup>) for the plasma-adsorption treatment of patients with immune thrombocytopenia or with refractory RA.<sup>4,5</sup> 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<sup>®</sup> efficacy.<sup>6–8</sup> 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.

Non-human pharmacological studies have emphasized the potential pathologic effects of SpA binding to Vh3 Igs in plasma, as well as to IgM on the surface of B cells and to IgE bound to mast cells.<sup>7,9,10</sup> Usually concentrations and in vivo doses were much higher than utilized in our research. With the exception of the recent publication by MacLellan et al, no prior published data have suggested that SpA might be safely used at appropriate doses to treat certain autoimmune diseases.

After a highly purified form of the native SpA, PRTX-100, (47 kD, 432 amino acids) was prepared from *S. aureus strain* Strain A676, using Good Manufacturing Practices, safety studies in cynomolgus monkeys showed the no observed adverse-effect level for six weekly doses of I.V. SpA to be at least 100 µg/kg (Protalex data, manuscript submitted).

Two single-dose Phase I studies were performed to evaluate the safety, tolerability, PK and pharmacodynamic activity of purified SpA in healthy volunteers, starting at doses as low as 0.03 µg/kg. These studies showed I.V. SpA was well tolerated at doses up to 0.45 µg/kg and to have observable biological activities at doses  $\geq 0.15$  µg/kg.<sup>11,12</sup>

This Phase Ib, double-blind, placebo-controlled, sequential dose-escalation trial was designed to evaluate the safety, PK and antigenicity of four weekly I.V. doses of SpA (PRTX-100) in patients with active RA receiving methotrexate, and to evaluate measures of RA disease activity over 16 weeks.

## Subjects and Methods

This study (South African Clinical Trials Register number DOH-27-0209-2558) was a multicenter, double-blind, placebo-controlled, sequential dose-escalation, repeated-dose study in patients with active RA and an inadequate response to methotrexate. The study was performed between July 2010 and March 2012 at five centers in South Africa, and was conducted in accordance with the Declaration of Helsinki and ICH

Guidelines for Good Clinical Practice. The protocol was approved by the Medicines Control Council of South Africa and by the applicable Institutional Review Boards (IRBs) (see Supplementary Table S1 for sites and IRBs). All patients provided written informed consent.

## Patients

Men and women aged  $\geq 18$  years were eligible if they had: a diagnosis of RA by revised ACR criteria<sup>11</sup>; a disease duration of  $\geq 6$  months; received methotrexate for  $\geq 12$  weeks (stable dose of 7.5–25 mg/week for  $\geq 4$  weeks);  $\geq 6$  swollen and  $\geq 6$  tender joints at study entry (screening and baseline); and high-sensitivity C-reactive protein (hs-CRP)  $\geq 0.5$  mg/dL. Patients may have been taking oral prednisone at a dose of  $\leq 10$  mg/day and non-steroidal anti-inflammatory drugs, providing that doses were stable for  $\geq 2$  weeks before baseline.

Exclusion criteria included any form of arthritis other than RA, current or recent serious infection, history of malignancy, or use of biologic agents for RA within 8 weeks before screening (6 months for rituximab). Patients were screened to exclude those with human immunodeficiency virus, hepatitis C or active hepatitis B virus infection.

## Study Design

Patients in each of four sequential dose cohorts (0.15, 0.45, 0.90, or 1.50 µg/kg) were randomized to receive either PRTX-100 or placebo administered I.V. weekly for 4 weeks, with eight active-dosed and two placebo-dosed patients per cohort. For operational reasons, the last cohort was truncated and enrolled five active-dosed and two placebo-dosed patients. A Safety Monitoring Committee reviewed the data from each dose cohort before dose escalations. On study Days 0, 7, 14, and 28, PRTX-100 was administered by injection over 10–15 seconds (later amended to 60–90 seconds) into the port of a rapidly flowing (250 mL/h) saline I.V. line.

## Outcome Measures

Safety was assessed by monitoring adverse events (AEs) and serious AEs (SAEs), hematology, blood chemistry and vital signs, from randomization through to study Week 16. AEs were graded by severity and as either related (probably or possibly) or unrelated to study drug. Complete blood count, blood chemistry and urinalysis results were evaluated before the first, second, third, and fourth weekly doses and on study Days 28, 42, 84, and 112. Triplicate electrocardiograms were performed before and 2–3 hours after each dose, and a single electrocardiogram was performed on Day 112 or the end of study visit. Urine pregnancy tests were performed before each administration in all women of reproductive potential.

Heparinized plasma samples for analysis of PRTX-100 concentrations were obtained pre-dose and at 2, 15, 30, 60, 120, and 180 minutes, and at 24 and 48 hours after the first and last doses of PRTX-100. On other dosing days, a trough PK sample was obtained 15–30 minutes pre-dose and a peak sample was obtained at 2 minutes post-dose.

An assay to measure PRTX-100 in plasma was developed and validated (the sandwich ELISA utilized a chicken anti-SpA capture antibody and biotinylated mouse anti-SpA detection antibody, see Supplementary Table S2). The assay limit of quantitation was 0.78 ng/mL.

### Pharmacokinetic Analyses

PRTX-100 PK parameters were calculated from non-compartmental analyses using SAS<sup>®</sup> for Windows<sup>®</sup> (Version 9.3; SAS Institute, Inc., Cary, NC) under Windows XP Professional. Actual sampling times were used for all pharmacokinetic (PK) analyses. Only plasma concentrations above the limit of quantitation (LOQ) for the assay (0.78 ng/mL) were used in the PK analysis.

### Anti-Drug Antibodies

The level of anti-PRTX-100 antibodies (anti-drug antibodies [ADAs]) was quantified by ELISA at baseline and at Days 21, 42, and 112 or end of study. PRTX-100 (SPA) was adsorbed on 96-well ELISA plates and non-specific binding of human immunoglobulin (Ig)G by protein A was blocked by pre-incubation with porcine IgG. Bound anti-product antibodies were detected with pig anti-human immunoglobulin. Evaluation of samples proceeded in three phases. First, all samples were evaluated in a screening enzyme immunoassay. A cut-point of 1.25 times the Day 0 pre-dose optical density read-out for each patient was used as an *individual patient-specific cut-point* for the Days 21, 42, and 112 screening assays, since pooled drug-naïve human sera had a significant ADA titer and resulted in a very high cut-point. Second, screen-

positive samples were further evaluated by immunodepletion with re-assay after the addition of PRTX-100 at 10 µg/mL. Any sample with a decrease of >50.0% in ADA screen optical density after immunodepletion was considered to be a true positive ADA result. Finally, for ADA-positive samples, serial dilutions were performed to determine the ADA titer. This assay, by definition, detected antibodies to SpA, in individuals whose anti-SpA screening ELISA showed a 25% or greater increase OD compared to their baseline titer. Thus the assay was optimized to detect any post-dose increase in ADAs. In other phase I studies, utilizing a fixed assay cut point, ADAs were detectable in about 10% of subjects *prior to dosing*.<sup>13</sup> The rapid increase in ADA's after a single dose indicates memory B-cells specific for SpA might have been present in other subjects, but their pre-dose ADAs were below the detection cut-point of this assay. Thus the ADA assay is quite specific but of limited sensitivity. It easily detects increased titers after dosing, but does not reliably detect very low pre-dose titers. Furthermore (see Table 2, below), ADA titer at Day 21 correlated with change in product clearance between Day 1 and 21 doses.

Potential IgE anti-PRTX-100 antibodies were evaluated in samples positive for ADA by a sensitive enzyme immunoassay. Protein A was coated on ELISA plates and porcine IgG was used to block its binding of antibodies or reagents via their Fc or Vh3 regions. A unique feature of the assay was its heterologous calibration curve. As there were no positive human IgE anti-protein A sera available, a surrogate reference curve was developed by using the anti-human IgE Fc drug omalizumab (Genentech, Inc.). This human IgG1 antibody was bound by the protein A coated on the plate (without addition of porcine IgG) and was then free to bind human IgE from a calibration serum that was analyzed at multiple dilutions. This allowed for the construction of a multipoint IgE calibration curve. The assay cut-off that defines the optical density above which a specimen is identified as containing IgE anti-

**Table 1.** Summary of PRTX-100 Pharmacokinetic Parameters Following Day 0 Injection, by Dose

PRTX-100 dose (µg/kg)	0.15	0.45	0.90	1.50
No. of patients	6–8	7–8	7–8	5
C <sub>max</sub> (ng/mL)				
Arithmetic mean (SD)	4.25 (1.11)	16.06 (2.80)	27.97(10.72)	52.84 (11.15)
Range	2.74–6.20	11.99–19.58	16.00–51.68	41.67–66.12
C <sub>max</sub> /dose	28	36	31	35
AUC <sub>(0–168)</sub> (h ng/mL)				
Arithmetic mean	40.8 (55.8)	268.2(255.0)	210.9 (124.2)	1132.8 (1143.1)
Range	2.4–164.5	4.2–871.6	18.3–388.4	67.1–3274.0
AUC/dose	272	596	234	755
Mean clearance (mL/h/kg) (range)	10.1 (0.7–47.0)	16.3 (0.5–6.8)	4.86 (1.94–41.57)	2.06 (0.85–16.8)
Mean Vz (mL/kg) (range)	41.67 (30.2–50.1)	39.7 (32.2–51.3)	57.74 39.5–68.2)	44.99 (30.1–61.8)
Mean half-life (hours) (range)	16.5 (0.7–31.2)	21.9 (0.5–47.0)	8.39 (1.55–19.3)	15.11

protein A was determined statistically based on the 95% upper confidence limit by analyzing the sera from 20 non-protein A exposed volunteers in 10 assays. While the heterologous calibration curve using omalizumab had a lower limit of detection (LLD) of 0.5 ng/mL for detection of omalizumab-bound IgE in human serum, it is possible that due to steric hindrance of SpA epitopes by molecules of porcine IgG blocker, that the LLD for direct detection of actual IgE anti-SpA antibodies in serum would be higher.

### Disease Activity Assessments

Exploratory clinical efficacy assessments included the modified Disease Activity Score (28 joints) (DAS28) using the DAS28-CRP and its core components (tender and swollen joint counts, physician's global assessment of disease activity, patient's assessment of disease activity, patient's assessment of pain, hs-CRP),<sup>14,15</sup> and the erythrocyte sedimentation rate (ESR). These were assessed at Days 0 (baseline), 14, 28, 42, 56, 70, 84, and 112 or end of study. Composite summaries of disease activity measures at various time points included the American College of Rheumatology (ACR) 20/50/70 response rates<sup>14</sup>; percentage of patients attaining DAS28-CRP low activity (<3.2)<sup>15-17</sup>; and change over time in Clinical Disease Activity Index (CDAI, which does not incorporate measures of acute phase reactants).<sup>18</sup>

### Statistical Analyses

All data were summarized by PRTX-100 dose group; placebo patients were pooled for analyses. For continuous variables, such as DAS28-CRP components, change from baseline was calculated. Descriptive statistics were used to summarize both categorical and continuous variables. An exploratory analysis was conducted using the CDAI scores, when it became apparent the CRP was poorly correlated with changes in the clinical assessments.

## Results

### Patients

Overall, 37 patients were randomized to treatment (intent-to-treat population); 29 received PRTX-100 and 8 placebo. All 37 patients received at least one dose of study drug; 27 patients (93.1%) in the PRTX-100 group and eight (100%) in the placebo group received all four doses. For active-treated and placebo-treated patients, respectively, the mean baseline values (28 joints) for tender joint count (TJC) were 16.3 and 16.0, for SJC were 10.6 and 8.5, and for DAS28-CRP were 5.78 and 5.77. The mean values for ESR were 27.5 and 22.5 mm/h, respectively, and for hs-CRP were 1.43 and 1.67 mg/dL, respectively. This population had moderate to severe disease activity (see Supplementary Table S3).

Five patients withdrew from the study; three patients because of an AE (two patients in the PRTX-100 0.45 µg/kg group: one with acute colitis, and one with worsening nausea; one patient in the placebo group: worsening RA); one patient withdrew consent (PRTX-100 0.90 µg/kg group) following a peri-dosing reaction after the third dose; and one patient (PRTX-100 0.15 µg/kg group) required a knee replacement and stopped taking methotrexate.

### Safety and Tolerability

Over the 4-month study period, all patients reported at least one AE. Treatment-emergent AEs (TEAEs) reported by >5% of the combined PRTX-100 groups included: lymphopenia, colitis, diarrhea, nausea, toothache, vomiting, fatigue, pyrexia, nasopharyngitis, sinusitis, urinary tract infection, animal bite, hypokalemia, hyponatremia, muscle spasms, musculoskeletal stiffness, myalgia, RA (verbatim terms: worsening of RA, aggravation of RA, and RA flare-up), dizziness, headache, rash, flushing, and hypertension. The incidence of these TEAEs was higher in the PRTX-100 groups than in the placebo group with the exception of nasopharyngitis, which was higher in the placebo group. Generally, there were no dose-dependent trends in the rate of these TEAEs.

Ten of 29 active-treated patients (34.5%) reported a severe TEAE versus one of eight placebo-treated patients (12.5%). For PRTX-100 0.15, 0.45, 0.9, and 1.5 µg/kg the rate of severe TEAEs was 25%, 50%, 37.5%, and 20%, respectively, with no apparent dose response noted. Nineteen patients (65.5%) in the PRTX-100 group and one (12.5%) in the placebo group experienced at least one treatment-related AE. There was no dose-dependent increase in the incidence or severity of these AEs. The most common treatment-related AEs (incidence >5%) are shown in Supplementary Table S4. Many were associated with dosing reactions, which occurred in three patients in the 0.45 and 0.9 µg/kg PRTX-100 groups.

In the 0.15 µg/kg group, three patients had a moderate RA flare during the study; none was considered related to PRTX-100. One patient each in the 0.45 and 0.9 µg/kg groups had a moderate RA flare considered possibly or probably treatment-related. In the 1.5 µg/kg group, one patient had a severe RA flare considered treatment-related, and one patient had a moderate flare considered treatment related. In the placebo group, one patient had severe worsening of RA considered unrelated to treatment.

There were no significant findings or trends over time, either by dose or in comparison to the placebo group, for clinical laboratory parameters, vital signs, physical examination, electrocardiograms or chest X-rays. The proportion of patients with clinically significant post-treatment laboratory abnormalities was comparable

between the PRTX-100 and placebo groups. Laboratory findings remained within the expected level of variation over the course of the study. Some laboratory abnormalities were graded as severe, although the majority of these were considered not related to study drug. Individual clinically significant laboratory changes included anemia, hyponatremia, hypokalemia, and lymphopenia, but none was considered to be study drug related. TEAEs associated with vital signs were mild or moderate and were generally not considered related to study drug. Except for joint counts, physical examinations remained unchanged with the exception of one patient (0.45  $\mu\text{g}/\text{kg}$  PRTX-100) who developed a mild maculo-papular rash lasting 5 days. Clinically significant electrocardiogram abnormalities were observed in both the PRTX-100 and placebo groups but none was reported as an AE and no systematic changes were observed. No significant QT prolongation was noted between pre-dose and 2–3 hours post-dose on dosing days and no incidence of pathologic QT prolongation was observed.

Three patients (0.45  $\mu\text{g}/\text{kg}$ :  $n = 1$ ; 0.90  $\mu\text{g}/\text{kg}$ :  $n = 2$ ) experienced mild to moderate infusion reactions to PRTX-100 within 5 minutes of dosing, all after the third dose. These reactions were characterized by dizziness and at least two of following symptoms: flushing, muscle spasms, nausea, palpitations, or dyspnea. No patient had received premedication; all reactions resolved without treatment within 5 minutes of onset and no hallmarks of anaphylaxis such as hypotension or wheezing was associated with these reactions. One of the three patients withdrew consent after this infusion reaction; the others received a fourth dose without a further reaction. No other dosing reactions occurred in the two highest dose groups after the infusion time was lengthened from 10–20 to 90 seconds, suggesting reactions were dose-rate dependent rather than dose dependent.

Three patients (10.3%) in the PRTX-100 group and one (12.5%) in the placebo group experienced an SAE. One patient in the 0.45  $\mu\text{g}/\text{kg}$  group had a fracture of the left humerus; one patient in the 0.45  $\mu\text{g}/\text{kg}$  group had acute gastritis and colitis, which resolved after discontinuation of meloxicam and treatment with a proton-pump inhibitor and 5-aminosalicylic acid (no acute colonoscopy or biopsy was performed), the patient withdrew during study follow-up; one patient in the 0.9  $\mu\text{g}/\text{kg}$  group experienced diverticulitis or infectious colitis, which was treated with antibiotics. One patient in the placebo group had a worsening of RA requiring hospitalization (onset 6 weeks after last dose); the patient withdrew during study follow-up.

No SAEs were considered related to study drug and none occurred in the highest dose group.

### Immunogenicity

The methods used to analyze ADAs quantitate an increase relative to the individual's pre-dose baseline. By defini-

tion at baseline, all 37 patients were negative for ADAs, and thus the possibility of pre-existing antibodies to SpA cannot be excluded, and indeed has been demonstrated to occur in prior phase I studies.<sup>13</sup> In this study, 20 of the 29 active-dosed patients (69.0%) had measurable increases in ADAs on Day 21. Serial dilutions were performed to titer positive samples. Six active-dosed patients had a peak titer of  $<1:400$  and 14 had titers of  $\geq 1:400$ ; no placebo-dosed patients had a detectable increase in ADAs. ADAs, when detected, peaked between Days 21 and 42, and declined by Day 112. The incidence and titer of ADA antibodies on Days 21 and 42 was similar across all four PRTX-100 dose cohorts.

Because development of IgE antibodies to SpA could predispose patients to immediate hypersensitivity reactions upon repeat dosing, the SpA-specific IgE titer was also evaluated (in all patients with positive ADAs) using a the ELISA described above. Using this methodology, as was seen in prior single-dose clinical trials,<sup>12</sup> no samples showed detectable IgE antibodies binding to SpA.

### Pharmacokinetics

The relatively constant  $C_{\text{max}}$ /dose ratios across groups (Table 1) suggests a linear relationship between dose and maximal plasma concentration ( $C_{\text{max}}$ ). The plasma concentration–time area under the curve ( $\text{AUC}_{(0-168)}$ ) after dosing on Day 0 is also shown in Table 1. The relationship between dose and AUC (exposure) is difficult to determine with this small sample due to the variability in AUC, the limited sampling time points employed, and the considerable variability within dose groups. In some patients, the AUC or terminal elimination rate constant could not be reliably derived from the PK data.

For doses of 0.15–1.5  $\mu\text{g}/\text{kg}$ , the mean volume of distribution ( $V_z$ ) ranged from 40 to 58 mL/kg, or approximately human blood volume (Table 1). For the 0.9 and 1.5  $\mu\text{g}/\text{kg}$  doses, the mean half-life of PRTX-100 was 8.4 hours (range 1.6–19.3 hours) and 15.1 hours (range 2.4–50.5 hours), respectively (see Table 1).

Table 2 shows the ratio of the Day 21 to the Day 0 value for selected PRTX-100 PK parameters, grouped by the value of Day 21 ADAs. As these ratios are ratios of sequential values for each individual patient, all PRTX-100 dose groups were pooled and then tabulated by ADA status. The titer of 400 was an arbitrary cut-point. For patients with no ADAs, the AUC, clearance and half-life showed a  $<2$ -fold change (increase or decrease) between the first and last doses. However, for AUC and half-life, these ratios were lower for patients with ADAs, and lower still for those patients with ADAs at higher titers ( $>400$ ). Figure 1 shows illustrative examples of time–concentration curves after Day 0 and 21 dosing for patients with low and with high ADA titers on Day 21.

**Table 2.** PRTX-100 Pharmacokinetic Parameter Ratios Between first and fourth dosing (Day 21/Day 0 Value), by Day 21 Antibody Titer

Parameter <sup>a</sup>	Day 21 antibody titer		
	0	<400	>400
C <sub>max</sub> , ng/mL	0.92 (16.7) [6]	0.86 (44.3) [6]	0.63 (29.7) [12]
AUC <sub>(0-t)</sub> , h ng/mL	0.66 (57.9) [6]	0.17 (65.5) [6]	0.03 (76.6) [12]
AUC <sub>(0-168)</sub> , h ng/mL	0.68 (59.2) [3]	0.42 (83.0) [2]	0.04 (56.2) [6]
Clearance, mL/h/kg	1.77 (51.2) [3]	2.95 (99.2) [2]	31.2 (57.1) [6]
V <sub>z</sub> , mL/kg	1.52 (42.2) [3]	1.47 (5.54) [2]	1.64 (43.5) [6]
Half-life, hours	0.86 (44.3) [3]	0.50 (109) [2]	0.05 (47.6) [6]

<sup>a</sup>Geometric mean (geometric % coefficient of variation) [No. of patients].

Based on the Day 21:0 ratio, increasing ADA titer was associated with an increase in the apparent plasma clearance (Table 2) by the day of the fourth dose. There was minimal change in V<sub>z</sub> (Table 2), suggesting that ADA titer did not affect the distribution volume of PRTX-100.

### Measures of Disease Activity

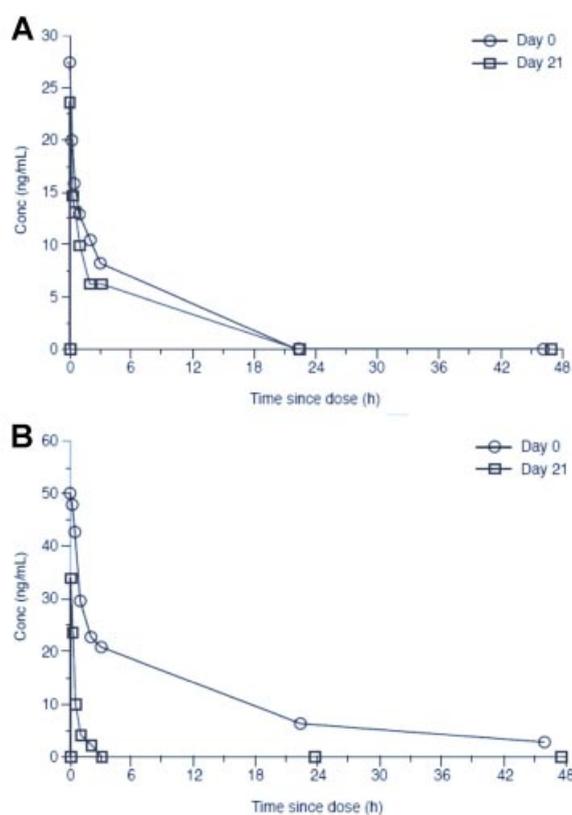
The proportion of patients in each cohort who met the ACR20, ACR50, and ACR70 criteria for improvement at 12 weeks (Day 84) was similar between groups (Table 3), although for ACR50 and ACR70 the 1.5 µg/kg group had the most responders (60% and 20%, respectively). The placebo ACR20 response rate was unusually high (62.5% at Day 84).

The pre-specified efficacy endpoint for categorical analysis was low disease activity (DAS28-CRP ≤3.2) at Day 42; this was achieved by three patients (10.3%) receiving PRTX-100 (Table 3). However at Day 70, 6 of 29 active-treated patients (20.7%) met this endpoint (Table 3) versus none of the placebo-treated patients. This difference was not statistically significant by Chi-square analysis. Of the 7 patients who met this response criterion at either Day 42 or 70, three had no ADA titer, three had Day 29 titers >1,080, and one had a titer of 540. This suggests PRTX-100 may affect disease activity despite development of ADAs.

From Day 70 through the final Day 112 visit, the largest mean change from baseline in DAS28-CRP was in the 1.5 µg/kg treatment group, although this finding cannot be statistically evaluated, given the small sample sizes (Table 3). Maximal decreases in disease activity scores were not seen until Weeks 6–10 (3–7 weeks after the last treatment with study drug).

The 1.50 µg/kg PRTX-100 group also showed the highest mean decrease in swollen joint count (SJC) compared with the other groups (Table 3), and this was observed at all post-baseline time points.

A greater percentage of patients in the PRTX-100 0.90 and 1.50 µg/kg groups attained a CDAI score of ≤10 (indicative of low disease activity) at time points from Day 70 through end of study (Table 3). Two of eight patients (25%) in the 0.90 µg/kg group, met this criteria, as did 2/5 patients (40.0%) in the 1.50 µg/kg group. In contrast, only 1/8 patients (12.5%) achieved this result in the placebo group. Overall, these data indicate a possible effect on disease activity in the PRTX-100 0.9 and 1.5 µg/kg groups, although group sizes are too small to evaluate statistical



**Figure 1.** First and fourth dose plasma concentrations of PRTX-100 in individual patients with low or high values for Day 21 ADAs. **A:** PRTX-100 0.9 µg/kg, patient with ADA titer of 146. **B:** PRTX-100 1.5 µg/kg dose, patient with ADA titer of 1,080.

**Table 3.** ACR 20/50/70, DAS-28-CRP, and CDAI Responses and Change in Swollen Joint Count After Treatment With Either PRTX-100 or Placebo

PRTX-100 dose ( $\mu\text{g}/\text{kg}$ )	0.15	0.45	0.90	1.50	Placebo
Efficacy measure	n = 8	n = 8	n = 8	n = 5	n = 8
ACR20 responders					
Day 84, n (%)	3 (37.5)	5 (62.5)	4 (50)	3 (60)	5 (62.5)
ACR50 responders					
Day 84, n (%)	1 (12.5)	3 (37.5)	3 (37.5)	3 (60)	2 (25)
ACR70 responders					
Day 84, n (%)	0	0	1 (12.5)	1 (20)	1 (12.5)
DAS 28 CRP $\leq 3.2$					
Day 42	1 (12.5)	1 (14.3)	1 (14.3)	0	0
Day 70	1 (12.5)	0	3 (37.5)	2 (40)	0
CDAI $\leq 10$ , n (%)					
Day 70	1 (12.5)	0	2 (25.0)	2 (40.0)	2 (25.0)
Day 84	0	2 (25.0)	3 (37.5)	2 (40.0)	2 (25.0)
Day 112	1 (12.5)	3 (37.5)	2 (25.0)	2 (40.0)	1 (12.5)
Both Day 84 and 112	0	1 (12.5)	2 (25.0)	2 (40.0)	1 (12.5)
Mean (SD) change from baseline in DAS28 swollen joint count					
Day 70	-3.1 (6.2)	-3.7 (2.02)	-2.9 (3.7)	-10.4 (9.6)	-4.4 (4.1)

SD, standard deviation.

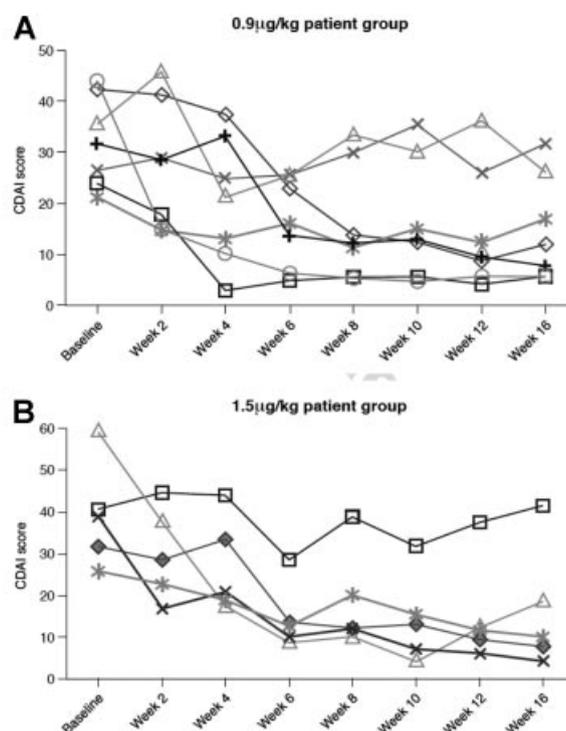
significance or to predict a true effect size. Figure 2 shows disease activity over time for these two groups and illustrates the delay between the last treatment dose (Day 21) and changes in the CDAI.

No treatment had a marked effect on the change from baseline in either CRP (see Supplemental Table S5) or ESR (data not shown) at any time point. Even patients who showed a decrease in CDAI to  $\leq 10$  had, as a group, little change in acute-phase reactants. In this small study, PRTX-100 treatment did not appear to affect those measures over a 16-week study period.

## Discussion

PRTX-100 is a highly purified form of SpA. SpA has a variety of postulated immunomodulatory and anti-inflammatory activities.<sup>3</sup> PRTX-100 was well tolerated overall by patients with active RA on stable doses of methotrexate, when weekly doses of 0.15–1.5  $\mu\text{g}/\text{kg}$  PRTX-100 were administered by I.V. infusion.

Three patients had mild to moderate infusion reactions, which did not recur after slowing the administration rate from 5–30 to 90 seconds. Given the biochemical characteristics of PRTX-100, the apparent dosing rate dependence of the infusion reactions and their clinical characteristics, these reactions may be related to complement activation and anaphylatoxin production following over-rapid infusion. Complement activation-related pseudo-anaphylaxis reactions have been reported after infusions of liposomal



**Figure 2.** CDAI scores by study visit for individual patients dosed with PRTX-100 at (A) 0.9  $\mu\text{g}/\text{kg}$  and (B) 1.5  $\mu\text{g}/\text{kg}$ , illustrating the frequent delayed onset of disease activity responses.

doxorubicin and amphotericin or after high-dose I.V. Igs and are caused by activation of complement leading to increases in anaphylatoxins and thromboxane.<sup>19–21</sup> At the higher doses of 0.9 or 1.5  $\mu\text{g}/\text{kg}$  for which the infusion time was lengthened from 5–20 to 90 seconds, no infusion reactions occurred. Measures of complement activation after dosing may be worthwhile studies to incorporate in future clinical trials with PRTX-100.

Other common TEAEs were headache, fatigue, and RA flare (verbatim terms: worsening of RA, aggravation of RA, and RA flare-up). Most cases of product-related flares in RA activity were followed by prolonged decreases in RA activity, reflected by decreased tender/swollen joint counts and CDAI scores.

No deaths occurred during the study. The proportions of patients who experienced an SAE were comparable between the combined PRTX-100 group (10.3%) and the placebo group (12.5%). None of the SAEs was considered related to study drug and no SAEs occurred in the highest dose group (1.50  $\mu\text{g}/\text{kg}$ ).

PRTX-100 is an antigenic bacterial protein and elicited ADAs in the majority of patients. No dose response was evident in either the incidence or mean titer of ADAs. Evaluation of patient samples with high ADA titers showed no detectable IgE antibodies against PRTX-100. This is consistent with prior clinical trials with PRTX-100<sup>11,12</sup> and suggests that this bacterial protein, while antigenic, shows no evidence to date of being allergenic following single or repeated dosing.

The relationship of PRTX-100 dose to  $C_{\text{max}}$  was linear, but clearance and AUC were extremely variable between patients, even after the first dose. It is possible that clearance of these very low doses of drug might be affected by pre-existing antibodies to staphylococcal protein A which are too low to quantitate. The plasma half-life for PRTX-100 ranged from 1.5 to 50.5 hours after the first dose with considerable inter-patient variability within dose groups. However the plasma assay does not measure PRTX-100 that might be bound, after association with IgGs, to circulating mononuclear cells via Fc receptors. Vz appeared to approximate blood volume, but further investigation is required before imputing physiologic significance to this finding. Development of an assay to measure PRTX-100 concentrations in both plasma and whole blood samples will be helpful to measure cell-bound drug in circulation.

Development of ADAs after repeated doses appeared to increase plasma clearance of PRTX-100 but ADAs were not associated with any AEs. Development of ADAs did not appear to preclude an effect of PRTX-100 on measures of disease activity; however, this relationship requires further evaluation in future studies. PRTX-100 binds with very high affinity to all Vh3 Igs.<sup>1</sup> Thus, in the presence or absence of ADAs, PRTX-100 may interact with its target as a multimeric complex bound with

antibody molecules. MacLellan et al<sup>3</sup> suggested that the inhibitory signaling by this complex is mediated by engagement of Fc $\gamma$ RI. Human Fc $\gamma$  receptors display extensive polymorphisms, which may influence the response to therapies such as high-dose IVIG.<sup>22</sup> Whether such polymorphisms could affect the response to PRTX-100 remains to be investigated.

There was a substantial placebo effect, which is not clearly explained by any baseline characteristics. This is not uncommon in phase I studies where the spontaneous variability in disease activity can generate spurious signals in small samples. It is also possible that study enrollment and frequent visits increased compliance with weekly MTX therapy in some patients. While the two lowest doses of PRTX-100 showed little effect on disease activity measures, this study suggested some potentially therapeutic responses at the 0.9 and 1.50  $\mu\text{g}/\text{kg}$  dose levels.

Of note, the average time to reach the maximum change in the CDAI, DAS28-CRP and SJC was 6–10 weeks for the apparent PRTX-100 responders, suggesting that maximum response to treatment may occur as long as 7 weeks after the last dose, and that it might not be temporally correlated with the drug presence in the plasma compartment.

In marked contrast to RA studies of anti-cytokine therapies, improvement in the CDAI was not associated with any rapid or significant decrease in acute-phase reactants over the doses studied. Thus, treated patients showed less change in the DAS28-CRP measure than in the CDAI. This study enrolled methotrexate-treated patients who had moderate to severe disease activity based mainly on their joint counts and on patient and physician global assessments, with few patients showing highly elevated CRP or ESR. Any effect of PRTX-100 on CRP in those patients who started with a CRP  $>20$  mg/L may be masked by the contribution of patients with lower CRPs to the group means. In future larger studies, the change in CRP for patients who enter with CRPs in the higher range should be subject to a subgroup analysis.

This study failed to determine the highest dose of PRTX-100 that could be administered weekly to RA patients with acceptable toxicities. There is a suggestion that, at the two higher doses, PRTX-100 has an effect on disease activity, even in patients with ADAs. These findings warranted further studies in patients with active RA, using PRTX-100 at doses of 1.5  $\mu\text{g}/\text{kg}$  and higher. A rheumatoid arthritis study (NCT # 01749787) which is administering weekly PRTX-100 treatments of up to 12  $\mu\text{g}/\text{kg}$  is currently in progress and has completed enrollment and dosing.

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### Declaration of Conflicting Interests

Dr. William Gannon, Dr. Edward Bernton, and Dr. William Kramer are paid consultants for Protalex, Inc. Dr. Eduard Krantz is a paid employee at Parexel Clinical Pharmacology, a contractor for Protalex, Inc., during the conduct of this study. Fellows of the American College of Clinical Pharmacology: none. Members of the American College of Clinical Pharmacology: Drs. Bernton and Kramer.

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