PHASE I STUDY OF STAPHYLOCOCCAL PROTEIN A IN PATIENTS WITH ACTIVE RHEUMATOID ARTHRITIS ON METHOTREXATE

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Conclusions

- Weekly infusions of Staphylococcal protein A (SpA) for injection were well tolerated in patients with active rheumatoid arthritis (RA). The most common side effects were a transient flare of RA joint inflammation, headache, and fatigue.
- Approximately 30% of SpA-treated patients and 12% of placebo-treated patients had an ACR50 response by 4 weeks after the last infusion. This response appeared to correlate with RA flares during treatment.
- 76% of active-treated patients developed anti-SpA antibodies. Some apparent RA responders included patients who developed high-titer anti-SpA antibodies.
- Plasma C_{max} showed a roughly linear relationship with dose, but plasma clearance (CL) demonstrated great intra-patient variability within dose groups. CL generally increased 2-4-fold from day 1 to day 22, without increase being clearly correlated to patient's anti-SpA titer.
- SpA-treated patients had a higher categorical response rate for ACR, DAS28-CRP, and clinical disease activity score (CDAI) measures at days 29, 57, 85, and 113 vs placebo.

Introduction

Staphylococcal protein A (SpA) is a bacterial virulence factor which structurally consists of five homologous immunoglobulin (Ig) binding domains placed in tandem (1). Each SpA domain is capable of binding with high affinity to the Fc region of human IgG and also binds to the Fab framework region of Igs in the mammalian VH3 gene family (2). This ability to bind to VH3 Igs results in SPA being one of the best-characterized mammalian B-cell superantigens.

Non-clinical papers suggest that SpA, in the presence of excess serum IgG, may form small complexes of SpA and IgG (3), which in vitro and in vivo can induce "alternatively-activated" or "regulatory" macrophages. These have an anti-inflammatory phenotype, and such complexes may have activity in the mouse collagen-induced arthritis model (4). SpA also interacts directly with Vh3 B-cells and in mice, and at doses of 5 or 50 mg/kg can cause activation followed by apoptosis of VH3-expressing B-cells (5).

The No Observable Adverse Effect level for weekly injections of SpA in cynomolgus monkeys is >100 µg/kg. In patients with active rheumatoid arthritis on methotrexate, SpA was safe and well-tolerated in a prior phase I trial when given as 4 weekly i.v. injections at doses up to 1.5 µg /kg; higher doses of SpA induced delayed, but sustained decreases in RA activity in some patients (6).

Methods*

- Patients with ≥4 swollen and ≥5 tender joints on a stable dose of MTX or leflunomide were enrolled at five rheumatology practice sites. Stable doses of prednisone ≤10 mg/day were allowed. Standard exclusion criteria applied.
- Patients were randomized 3:1 to SpA:placebo. SpA infusions (1.5, 3.0, 6.0, or 12 µg/kg) were administered on days 1, 8,15, 22, and 29. No premedication was required.
- Patients were evaluated on days 57, 85, 113, and 169. The primary endpoint was ACR20/50/70 at day 85. Patients with Clinical Disease Activity Index (CDAI) score >14 could discontinue the study after that visit to receive other treatments.
- Safety assessments included adverse events (AEs), complete blood count, blood chemistry, urinalysis, and electrocardiogram. Pharmacokinetic (PK) profiles were determined after dosing on days 1 and 22.
- Anti-product antibodies were measured on days 1, 22, 57, and 169.
- These biomarkers were assessed: lymphocyte phenotypes by fluorescence-activated cell sorting on days 1, 22, 23, 57 and C3a, C4a, C5a (by cytometric bead array assay of Futhan-preserved plasma) in pre- and post-dose samples on days 1 and 22.
- Disease activity was measured using D (DAS28)-C-reactive protein (CRP), DAS28-erythrocyte sedimentation rate (ESR), American College of Rheumatology (ACR) response, and Clinical Disease Activity Index CDAI (which does not include acute phase reactant).

* This poster contains preliminary analysis of results prior to completion of the clinical study report.

Patient Demographics

	SpA dose					Placebo
	1.5 µg/kg	3.0 µg/kg	6.0 µg/kg	12 µg/kg	All	
No. of patients[a]	6	9	9	5	29	8
Mean age, years (SD)	61.2 (13.3)	57.6 (11.8)	63.3 (9.7)	62.6 (6.8)	61.1 (10.3)	59.9 (9.4)
Age range, years	47–76	30–72	42–71	51–72	30–72	46–71
Male, %	66.7	44.4	33.3	57.1	48.4	30
Caucasian, %	83.3	88.9	77.8	85.7	83.9	80.0
Weight, kg (SD)	74.6 (4.3)	91.4 (20.6)	81.0 (16.2)	86.0 (16.9)	83.9 (16.9)	82.3 (14.0)
Day 1 DAS28-CRP (SD)	4.9 (0.55)	5.3 (0.62)	4.8 (0.67)	4.65 (0.88)	4.93 (0.68)	5.30 (0.87)
Day 1 DAS28 swollen joint count (SD)	10.2 (4.54)	13.0 (6.4)	8.9 (4.23)	13.6 (5.18)	11.2 (5.32)	12.8 (5.50)
Day 1 CDAI (SD)	37.9 (10.7)	44.7 (12.4)	34.7 (11.3)	37.6 (14.0)	39.0 (12.1)	43.6 (13.2)
Day 1 CRP (SD)	0.86 (1.02)	0.29 (0.24)	0.25 (0.18)	0.69 (1.01)	0.49 (0.69	0.74 (0.66)
RA disease duration, years (SD)	10.0 (2.93)	15.9 (11.7)	15.6 (11.1)	8.1 (3.9)	13.0 (9.7)	8.4 (6.8)

[a]Per-protocol population.

Safety

Treatment-emergent AE (TEAE), % of patients	SpA dose					Placebo (n=10)
	1.5 µg/kg (n=6)	3.0 µg/kg (n=9)	6.0 µg/kg (n=9)	12.0 µg/kg (n=7)	All (n=31)	
Patients with ≥1 TEAE	66.7	33.3	66.7	85.7	61.3	60
Cardiac disorders	0	11.1	0	0	3.2	10
Gastrointestinal disorders	0	11.1	0	0	3.2	20
General disorders (fatigue, flu-like symptoms, infusion reaction)	16.7	11.1	11.1	14.3	12.9	20
Infection	0	0	11.1	14.3	6.5	0
Infusion-related reaction	16.7	0	0	14.3	6.5	0
Investigations – QTc	0	11.1	0	0	3.2	0
Musculoskeletal, RA symptom flare	50	22.2	33.3	57.1	38.7	20
Nervous system (dizziness or headache)	0	11.1	22.2	28.6	16.1	20
Respiratory (asthma)	0	0	0	0	0	10
Skin disorders (rash)	16.7	0	0	0	3.2	0
Moderate or severe TEAE	50	11.1	22.2	71.4	38.7	30
Moderate arthralgia or fatigue	**50**	**11.1**	**22.2**	**57.1**	**32.2**	**20**

- The most common AEs in SpA-treated patients were transient RA flares (39%), headache (16%), and fatigue (13%).
- Of five severe AEs, only one was treatment-related (dyspnea, tachypnea, chest pain with onset 1 h after the first infusion [12 µg/kg dose group]). The patient was not treated further.
  - No increase in plasma tryptase or complement activation occurred; event resolved without sequelae. Patient had pre-existing atherosclerotic cardiovascular disease and stent.
- Related RA flares of moderate severity were more common with SpA 1.5 µg/kg i.v. push and 12 µg/kg (1 hr infusion) than in placebo patients.
- No laboratory abnormalities were associated with SpA other than transient reductions in absolute lymphocyte count 24 hr post infusion.
- Circulating CD19+ B-cell counts were not decreased at day 57 vs patients' baseline.
- 37 of 41 randomized patients completed day 85; 2 of 31 SpA-treated and 2 of 10 placebo-treated patients withdrew because of AEs.

Pharmacokinetics/Immunogenicity

Dose (µg/kg) /study day	Infusion time (min)	PK parameters for first and fourth dose, mean (SD)					
		C_{max} (ng/mL)	C_{max} /dose	AUC_{inf} (h·ng/mL)	$T_{½}$ (hr)	CL (mL/hr/kg)	Vz (L)
1.5 / 1	2	48 (9.3)	32	1065 *	19.5*	115*	3.23*
1.5 / 22	2	45 (16.4)	30	984 (1213)	16.4 (15.5)	1586 (2534)	4.04 (1.6)
3.0 / 1	30	67 (40.7)	22.3	2419 (4069)	18.1 (24.4)	3080 (4007)	6.0 (3.1)
3.0 / 22	30	58 (46.2)	19.3	647 (1178)	10.0 (11.7)	5973 (7325)	11.2 (8.9)
6.0 / 1	30	158 (22.3)	26.3	2931 (2549)	19.0 (9.2)	228 (124)	5.2 (1.7)
6.0 / 22	30	109 (42.1)	18.2	4199 (5399)	30.4 (32.1)	407 (381)	7.8 (4.5)
12 / 1	60-120	378 (93.8)	31.5	9057 (3078)	20.4 (6.5)	128 (43)	3.47 (0.7)
12 / 22	60-120	259 (77)**	21.5	1777 (956)	8.43 (4.0)	737 (330)	8.0 (0.3)

AUC, area under the curve; C_{max}, maximum concentration; CL, clearance; $T_{½}$, half-life; Vz, volume of distribution during terminal phase. * = N of 1 for parameter calculation. ** = outlier (over-rapid infusion) omitted

Incidence of anti-SpA antibodies and lack of relationship to decreased day 22 plasma AUC or to Disease Activity Responses				
Anti-SpA dilution titer day 22	0	<400	≥400	All active-dosed
Placebo-dosed	8	0	0	
SpA-dosed	7	10	12	29
Ratio day 22:day 1 AUC_{0-N}[a]	0.36 (301)	0.35 (50)	0.75 (55)	0.45 (130)
Ratio day 22:day 1 C_{max}[a]	0.83 (30)	0.70 (29)	1.01 (22)	0.84 (30)
No. active dosed with CDAI ≤14 x 3	1	5	4	10

[a]Geometric mean (geometric % CV)

- C_{max} was similar for SpA 1.5 and 3.0 µg/kg doses because 1.5 µg/kg dose was infused more rapidly.
- Even on Day 1, a large variability in SpA plasma clearance was noted within dose groups.
- Overall, mean CL increased and mean $AUC_{(0-N)}$ decreased on day 22 vs day 1.
  - However this decrease did not have a positive correlation with presence or titer of anti-drug antibodies (ADAs). In fact the mean Day 22/Day 1 AUC(0-N) ratio for 4 pts with ADA titer >800 was 0.86, (-14% decrease) and for 10 pts with ADA titer <200 was 0.24 (-76% decrease) (t test, p < 0.01)
- ADAs were observed by day 22 (fourth dose) in **76%** of SpA-dosed patients but ADAs did not preclude a CDAI response (≤14 on 3 consecutive visits).

Biomarkers — Complement Activation

Mean change in anaphylatoxin, % (SD)	Day 1 (post-dose/pre-dose)			Day 22 (post-dose/pre-dose)		
	C3a	C4a	C5a	C3a	C4a	C5a
Placebo	-19 (13.6)	-19 (21.2)	-2 (13.7)	2 (14.7)	5 (31.2)	- 6 (23.7)
1.5 µg/kg	-5 (30.3)	9.4 (41.5)	2.7 (19.0)	54 (89.4)	-14.4 (20.1)	-13 (10.6)
3.0 µg/kg	83 (137.3)	0 (28.9)	7.3 (22.2)	266 (319)	48 (72.5)	-5 (16.4)
6.0 µg/kg	59.6 (103)	-12 (30.6)	18 (71.5)	238 (418)	-13 (48.5)	6 (27.6)
12 µg/kg	9 (14.8)	-9.8 (28.4)	-15 (37.1)	259 (166)	116 (239)	31 (51.2)

- SpA-treated patients had a greater mean post-dose increase in C3a values on day 22 vs day 1 (225% vs 35%; p<0.03).
- Spa-treated patients had greater post-dose C3a increases on both day 1 (p=0.005) and day 22 (p=0.01) vs placebo-treated patients.
- These increases in C3a post-infusion showed no correlation with any post-dosing AEs or with RA response to treatment, perhaps because they were modest in absolute terms.

RA Disease Activity

Disease activity endpoint,[a] % of patients	SpA dose					Placebo (n=8)
	1.5 µg/kg (n=6)	3.0 µg/kg (n=9)	6.0 µg/kg (n=9)	12.0 µg/kg (n=5)	All (n=29)	
ACR20 CRP day 29	33.3	33.3	22.2	40.0	31.0	12.5
ACR20 CRP day 57	50.0	50.0	33.3	80	50.0	37.5
ACR20 CRP day 85	33.3	33.3	66.7	48.3	48.3	42.9
ACR50 CRP day 29	16.7	11.1	11.1	20	**13.8**	**0**
ACR50 CRP day 57	33.3	37.5	22.2	40	**32.1**	**12.5**
ACR50 CRP day 85	16.7	22.2	25.0	20	**21.4**	**14.3**
ACR70 CRP day 57	16.7	12.5	11.1	0	10.7	0
DAS28-CRP <3.2 day 57 and day 85	16.7	37.5	22.2	60	**32.1**	**12.5**
DAS28-CRP <3.2 day 85	33.3	37.5	33.3	80	**42.9**	**14.3**
DAS28-CRP < 3.2 day 113	40	12.5	42.9	66.7	**34.8**	**16.7**
CDAI ≤14 at 3 consecutive visits	50	11.1	22.2	60	**34.4**	**12.5**
Patients with RA flare ≥ moderate as related AE and CDAI ≤14 at 3 visits	3/3	1/1	2/4	2/2	80 (8 of 10)	NA

[a]Per-protocol population.
DAS28-CRP <3.2 = low disease activity; CDAI ≤10 = low disease activity; CDAI ≤22 = moderate activity.

RA DISEASE ACTIVITY RESPONSES

- SpA-treated patients had a higher categorical response rate for ACR, CDAI, and DAS28-CRP measures at days 29, 57, 85, and 113 vs placebo.
- With a mean pre-treatment DAS28-CRP of 4.93, 42.9% of active-treated evaluable patients achieved DAS28-CRP of <3.2. With a mean pre-treatment CDAI of 39.0, 34% of active-treated evaluable patients achieved a CDAI of ≤14 on 3 consecutive visits.
- ACR responses appeared less sensitive to SpA than CDAI categorical analysis:
  - Most patients had baseline high-sensitivity-CRP <1 mg/dL
  - Unlike anti-cytokine treatment, SpA does not rapidly reduce CRP or ESR.
- Unexpectedly, patients with moderately severe transient RA flares during SpA treatment had a higher RA response rate (80% of 10 patients with RA flare vs 34% of all SpA-treated patients).
- At the two highest doses, more treatment responses were maintained at day 85 and later time points.
- The small number of patients in the study makes assessment of a dose–response relationship difficult.

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