

Histopathology Table for Findings in Kidney:

Male

[H.E. staining]	Group	1 (IL-1Trap Diluted Placebo)			2 (IL-1 Trap)			3 (IL-1 Trap)			4 (IL-1 Trap)		
Organ/Tissue	Dose (mg/kg)	0			5			20.0			50.0		
Findings	Animal No.	2	4	6	12	14	16	18	20	22	24	26	30
Kidney (left)													
Inclusion body, mucosal epithelium, pelvis		+	-	-	-	-	-	-	±	-	-	-	-
Mineralization		-	-	-	-	±	-	-	-	±	-	-	-
Mononuclear cell infiltration, interstitium, focal		-	-	-	-	±	-	±	-	-	±	+	±
Mononuclear cell infiltration, pelvis		-	-	-	±	-	±	-	±	-	-	-	-
Kidney (right)													
Inclusion body, mucosal epithelium, pelvis		+	±	-	-	-	-	-	+	-	-	-	-
Mineralization		-	-	-	-	-	-	±	-	±	-	-	-
Mononuclear cell infiltration, interstitium, focal		-	±	-	-	±	±	±	±	±	-	+	±
Mononuclear cell/eosinophil infiltration, pelvis		-	-	-	-	-	-	-	-	-	±	-	-

Female

[H.E. staining]	Group	1 (IL-1Trap Diluted Placebo)			2 (IL-1 Trap)			3 (IL-1 Trap)			4 (IL-1 Trap)		
Organ/Tissue	Dose (mg/kg)	0			5			20.0			50.0		
Findings	Animal No.	3	5	35	11	13	15	17	19	21	23	25	27
Kidney (left)													
Lymphoid cell infiltration, pelvis		-	-	±	-	±	±	-	-	±	±	-	-
Mononuclear cell infiltration, interstitium, focal		-	±	-	-	±	-	±	-	±	±	±	-
Kidney (right)													
Eosinophil/mononuclear cell infiltration, pelvis		-	-	-	-	-	2+	-	-	-	-	+	-
Inclusion body, mucosal epithelium, pelvis		-	±	-	+	-	-	-	±	-	-	-	-
Mineralization		-	±	-	-	-	-	-	-	-	-	-	-
Mononuclear cell infiltration, interstitium, focal		-	±	±	-	-	-	-	-	±	-	+	-
Protein cast, focal		-	-	-	-	-	-	-	-	-	±	-	-

Histopathology Findings in Liver, Lymph Nodes and Lungs:

The histopathological findings in the liver consisted of increased incidence of the focal mononuclear cell infiltration in the perivascular area in males (1/3 in control vs. 2/3, 2/3 and 1/3 in the low, mid, and high dose respectively) and females compared to controls (1/3 in control vs. 3/3 in high dose). This finding is thus believed to be treatment related. The severity index of such findings was slight to minimal. There was no such finding at the recovery sacrifice.

The only histopathological finding in this study noted by the sponsor and study pathology report was the hyperplasia of the lymphoid follicle in the paracortical areas in the mesenteric and the mandibular lymph node. All males and females in the different treatment group showed such changes, the degree of severity ranged from minimal to moderate. Similar changes were noted in control animals, however, in the number of control animals showing such changes were lower than those of the treated animals. Also, the severities of such findings in the control animals were only slight. Incomplete recovery was noted for this finding after the 6-weeks of the recovery period.

Histopathology Table for Findings in Liver, Lymph Nodes and Lungs:

Male

[H.E. staining] Organ/Tissue Findings	Group Dose (mg/kg) Animal No.	1 (IL-1Trap Diluted Placebo)			2 (IL-1 Trap)			3 (IL-1 Trap)			4 (IL-1 Trap)		
		0			5			20.0			50.0		
		2	4	6	12	14	16	16	20	22	24	26	30
Liver													
Eosinophil/mononuclear cell infiltration, multifocal, with central necrosis		-	-	2+	-	-	-	-	-	-	-	-	-
Mononuclear cell infiltration, multifocal		±	±	-	-	-	-	-	±	-	-	-	-
Mononuclear cell infiltration, perivascular		-	-	-	-	-	-	-	-	±	-	-	-
Liver (left)													
Eosinophil/mononuclear cell infiltration, focal, with central necrosis		-	-	2+	-	-	-	-	-	-	-	-	-
Eosinophil/mononuclear cell infiltration, multifocal		±	-	-	-	-	-	-	-	-	-	-	-
Eosinophil/mononuclear cell infiltration, portal		±	-	-	-	-	-	-	-	-	-	-	-
Mononuclear cell infiltration, perivascular		+	-	-	+	-	±	-	±	-	-	-	-
Mononuclear cell infiltration, focal		-	-	-	-	-	-	-	-	-	-	±	-
Lungs													
Macrophage infiltration, interstitium		-	-	+	-	-	-	-	-	-	-	-	-
Perivascular macrophages containing pigment		2+	-	-	-	-	-	-	-	-	-	-	-
Lymph node (mandibular, left)													
Hyperplasia, lymphoid follicle		-	-	-	±	+	+	±	-	±	-	+	2+
Hyperplasia, paracortical area		±	-	±	+	+	+	+	-	±	±	±	2+
Lymph node (mesenteric)													
Brown pigment, macrophage, sinus		±	+	±	-	±	-	-	±	-	-	-	-
Eosinophil infiltration, sinus		±	±	±	±	-	-	-	±	-	-	±	+
Hyperplasia, lymphoid follicle		-	±	-	-	2+	±	+	±	+	-	-	±
Hyperplasia, paracortical area		-	±	-	±	+	-	±	±	±	±	±	+

Female

[H.E. staining] Organ/Tissue Findings	Group Dose (mg/kg) Animal No.	1 (IL-1Trap Diluted Placebo)			2 (IL-1 Trap)			3 (IL-1 Trap)			4 (IL-1 Trap)		
		0			5			20.0			50.0		
		3	5	35	11	13	15	17	19	21	23	25	27
Liver													
Mononuclear cell infiltration, multifocal		-	-	-	-	-	-	-	-	-	-	±	-
Mononuclear cell infiltration, perivascular		-	-	±	-	-	-	±	-	-	±	-	±
Mononuclear cell infiltration, focal		±	-	-	-	-	-	-	-	±	-	-	-
Liver (left)													
Mononuclear cell infiltration, perivascular		-	-	-	-	-	-	±	-	-	-	-	-
Lungs													
Eosinophil infiltration, peribronchiolar		-	-	-	-	-	-	-	-	±	-	-	-
Lymph node (mandibular, left)													
Extramedullary hematopoiesis		-	-	-	±	-	-	-	-	-	-	-	-
Hyperplasia, lymphoid follicle		+	-	-	-	+	+	-	-	+	-	±	-
Hyperplasia, paracortical area		+	±	-	-	-	+	-	-	+	+	+	-
Neutrophil infiltration, sinus		-	-	-	-	-	-	-	-	-	+	-	±
Lymph node (mesenteric)													
Brown pigment, macrophage, sinus		-	+	±	±	±	-	-	-	-	-	-	-
Eosinophil infiltration, sinus		-	+	±	±	-	-	-	-	-	+	-	±
Hyperplasia, lymphoid follicle		±	-	±	-	±	2+	±	+	2+	-	±	-
Hyperplasia, paracortical area		-	-	-	-	-	2+	-	±	+	-	±	-

Histopathology Findings in Ovary:

Increased incidence of the mineralization of the oocytes were noted in the treated animals, the number of animals showing such changes were 1/3, 2/3, 1/3, and 1/3 in the control, low, mid, and high dose group respectively. The severity of such finding was very slight to moderate in the treated animals experiment, and slight in the control animal. The finding is noted as treatment related by the reviewer. No such changes were noted at recovery.

Histopathology Table for Findings in Ovary:

[H.E. staining] Organ/Tissue	Group Dose (mg/kg)	1 (IL-1 Trap Diluted Placebo)			2 (IL-1 Trap)			3 (IL-1 Trap)			4 (IL-1 Trap)		
		0			5			20.0			50.0		
Findings	Animal No.	3	5	35	11	13	15	17	19	21	23	25	27
Ovaries													
Mineralization, oocyte		+	.	.	±	.	+	.	.	+	2+	.	.

Histopathology Finding in Femur:

The histopathological findings in femur were consisted of closed and decrease growth plate in the knee joints in femur. One male at mid dose and one at high dose showed such change, the severity of this finding was moderate in the male from the mid dose group. There were no such changes in the control males. In females, 2/3 in the control, low, and mid dose and 1/3 at high dose showed such change. The severity of these changes were however, moderate to marked in the low and mid dose group animal and slight in the control animals. Although the sponsor did not mention such changes in their histopathological report, the reviewer consider these changes as treatment related. The biological significance of the findings is not known.

Histopathology Table for the Findings in Femur:

Male

[H.E. staining] Organ/Tissue	Group Dose (mg/kg)	1 (IL-1 Trap Diluted Placebo)			2 (IL-1 Trap)			3 (IL-1 Trap)			4 (IL-1 Trap)		
		0			5			20.0			50.0		
Findings	Animal No.	2	4	6	12	14	16	18	20	22	24	26	30
Femur (bone/knee joint, left)													
Closed growth plate		P
Decrease, growth plate		2+
Femur (bone/knee joint, right)													
Closed growth plate		P
Decrease, growth plate		.	.	±	±	.	.	2+
Disorganization, chondrocyte columns		±
Increased thickening, physal cartilage, focal		±	±

Female

[H.E. staining]	Group	1 (IL-1Trap Diluted Placebo)			2 (IL-1 Trap)			3 (IL-1 Trap)			4 (IL-1 Trap)		
Organ/Tissue	Dose (mg/kg)	0			5			20.0			50.0		
Findings	Animal No.	3	5	35	11	13	15	17	19	21	23	25	27
[H.E. staining]	Group	1 (IL-1Trap Diluted Placebo)			2 (IL-1 Trap)			3 (IL-1 Trap)			4 (IL-1 Trap)		
Organ/Tissue	Dose (mg/kg)	0			5			20.0			50.0		
Findings	Animal No.	3	5	35	11	13	15	17	19	21	23	25	27
Femur (bone/knee joint, left)													
Closed growth plates		.	.	P
Decrease, growth plate		±	±	.	2+	+	.	2+	+
Femur (bone/knee joint, right)													
Decrease, growth plate		+	+	.	3+	2+	.	3+	±	.	.	.	+
Closed growth plates		.	.	P

Although only the changes in the lymph nodes were considered treatment related by the sponsor, some of the changes in the kidney, femur, liver, spleen, heart, thyroid, and ovary are also considered treatment related by the reviewer. The major histological change in the kidney, liver, heart, and thyroid which is considered treatment related is mononuclear cell infiltration. The incidence of such findings is higher in the animals treated with the compound compare to that of controls. The biological significance of such findings is not definitively known, however, the changes might be considered as related to the exaggerated pharmacology the compound associated with the immune system dysfunction. Most of the findings, however, were observed to be recovered after the 6-weeks of treatment free period at high dose. The changes in the femur were most prevalent at the low and mid dose. During recovery, no control animal showed changes in femur, but ½ males and females showed minimal changes in femur, the incidence is similar to what was observed at the terminal sacrifice. There was no animals from the low and the mid dose group for recovery, therefore, it is not known whether animals from those dose groups showed recovery or not.

Toxicokinetics:

The blood samples for the assessment of serum IL-1 Trap concentration were drawn at pre-dose, immediately after dosing (approximately 5 minutes), at 24 and 72 hours post dosing on all dosing days (Days 1, 8, and 15) and on the day of necropsy (Day 22). Samples were also taken at the middle (Day 42) and end (Day 63) of the recovery period.

The blood samples were drawn for anti-IL-1 Trap antibody assays were at one time point per day before each dosing (Days 1, 8, and 15) before necropsy (Day 22), and once during the middle (Day 42) and end (Day 63) of the recovery period.

IL-1 Trap Concentration

- The mean IL-1 Trap plasma concentration increased dose proportionately after the first injection. The concentrations of the IL-1 Trap in plasma 24 hrs post first dose were 82096, 356838, and 900462 ng/mL with 5, 20, and 50 mg/kg respectively. The increase in IL-1 Trap concentration were 4.3 fold and 2.0 fold between low to mid dose (4 fold increase in dose) and mid to high dose (1.5 fold increase in dose) respectively. The less than dose proportional increase might suggest saturation of the compound in the plasma at this time point.
- There was an accumulation of IL-1 Trap in all treated groups as observed by the increased plasma level of the compound in the pre dose samples suggesting long half life of the compound and reduced clearance which might result from the saturation of the elimination mechanism at high drug concentration.
- The mean plasma concentrations of IL-1 Trap decreased in all dose groups at 24 hrs post dose at Day 15 compared to the plasma concentrations that was observed 24 hr post dose on Day 1 indicating increased clearance may be due to the antibody formation.
- The IL-1 Trap serum concentration in the plasma of the animals from the low and mid dose group was negligible after Day 18. The IL-1 Trap plasma concentration was, however, noted at Day 22 in all animals from the high dose groups.

IL-1 Trap Antibody Concentration

- Anti-IL-1 Trap antibody concentration was not observed in any of the animal prior to Day 12. One animal from placebo treated animal had very little antibody titer (2.5) at Day 22.
- Antibody formation was noted in all test article treated animals at Day 16. The range of antibody titer in low, mid, and high dose group were 17-55 RFU, 10-112 RFU, and 5-101 Rheumatoid factor unit RFU (rheumatoid factor unit) respectively, suggesting that no direct dose relationship could be established due to huge individual variation in the antibody formation.
- Measurable amount of the anti product antibody was found in all animals at Day 22. The amount of antibody observed at recovery was negligible.

Mean Plasma Concentration (ng/mL) of IL-1 Trap and IL-1 Trap antibody (RFU/mL) in Cynomolgus Monkeys

Time	Dose Groups					
	IL-1 Trap Concentration			IL-1 Trap Antibody Concentration		
	5 mg/kg	20 mg/kg	50 mg/kg	5 mg/kg	20 mg/kg	50 mg/kg
Day 1 24 hr Post 1 st dose	82096	356838	900462	BLQ*	BLQ	BLQ
Day 8 Pre dose	8497	33825	153370	BLQ	BLQ	BLQ
Day 9 24 hr Post 2 nd dose	155757	357330	1021195	NM*	NM	NM
Day 15 Pre dose	26	99	18166	29	36	55

Day 16 24 hr Post 4 th dose	204458	43184	385295	NM	NM	NM
Day 22 Pre dose	79	31	1890	152	157	209

• NM: IL-1 Trap antibody concentration not measured.

** BLQ: Below level of quantification

Study title: A Six-Week Subcutaneous Exploratory Toxicity Study of IL-1 Trap in Cynomolgus Monkeys (Regeneron Study Number IL1T-TX-02061)

Key study findings:

- This study was done in a non GLP environment; it was conducted as an exploratory study. The doses administered were 0, 15, 30, and 45 mg/kg once a week subcutaneous.
- The only major histological findings from this study were injection site inflammation. According to the sponsor, based on systemic findings, a NOAEL of 45 mg/kg/day was established in this study.

This study was not reviewed in detail because it is a non GLP study. Note that a GLP 6-week toxicity study in monkeys with IL-1Trap was done by the sponsor and submitted with this BLA using a maximum dose of 10 mg/kg administered twice weekly. This study was reviewed in detail below.

Study title: A 6-Week Toxicity Study of IL-1 (Interleukin-1) Trap Administered by Subcutaneous Injection to Cynomolgus Monkeys, with a 4-Week Recovery Period

Key study findings:

- Cynomolgus monkeys (3/sex/group) were administered 0, 1, 3, 10 mg/kg of IL-1 Trap subcutaneously, 2 times a week for 6 weeks. Two animals/sex were observed for recovery from the control and the high dose group.
- The toxicokinetic analysis showed a dose related increase in the serum concentration of the IL-1 Trap. The accumulation of the compound was noted in the serum in all dose groups. Anti product antibody formation was noted within two weeks. At Day 40, only high dose group showed serum concentration of IL-1 Trap indicating clearance of the compound by the antibody.
- No NOAEL could be established in this study due to injection site reaction at all doses. The histopathology of the injection site consisted of mild to moderate infiltration of mononuclear cells. The mononuclear cell infiltrates composed of plasmacytes and lymphocytes with a perivascular distribution. The infiltration was noted primarily in the subcutis, but in some cases the infiltration was noted in the deep dermis. The mononuclear cell infiltration might have resulted from

chronic inflammation or local immune reaction resulted from the treatment. The injection site lesions persisted through recovery.

- Other histopathological changes noted at terminal necropsy were mononuclear cell infiltration in different tissues such as kidney, liver, lung and hyperplasia of the ovary, all these changes were observed to be recovered at high dose.

Study no.: 0949-121

Volume # and page #: eCTD submission Page 1-261

Conducting laboratory and location: _____

Date of study initiation: April 21, 2000

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: IL-1 Trap TP1 F01002 (relative potency ____). Drug Product description: IL-1 Trap recombinant protein, 40 mg/mL in a _____ aqueous vehicle, pH _____, containing _____ histidine, _____ glycine _____, _____ arginine, _____ sucrose, _____ polyethylene glycol 3350, _____

Vehicle lot # PTP1 F0102001TAA; the placebo formulation is similar to the drug product without the IL-1 Trap protein.

Methods

Doses: 0, 1, 3, 10 mg/kg, twice a week for 6 weeks

Species/strain: Cynomolgus monkeys; *Macaca fascicularis*

Number/sex/group or time point (main study): 3/sex/group

Route, formulation, volume, and infusion rate: Subcutaneous, the volume injected was different with different dosages as indicated in the study the design table below.

The injection solution was formulated by the sponsor. The formulation for IL-1 Trap is provided above in the description of the drug lot section.

Satellite groups used for toxicokinetics or recovery: Blood sample for TK analysis were collected from all animal. Two animals/sex in the control and high dose groups were added for the recovery analysis.

Age: Males were 2.5-5.2 years old and females were 2.2-5.0 years old.

Weight: Males weighed 2.6-3.4 kg and females weighed 2.4-3.2 kg

Sampling times: Blood was collected for TK analysis 8 and 16 hrs post dose on Days 1, 8, and 40. Blood was collected on Days 14, 28, and 42 for the evaluation anti product antibody formation.

Unique study design or methodology (if any):

Study Design

Group No.	Number of Males/Females	Dose Level (mg/kg)	Dose Vol. (mL/kg)	Dose Solution Conc. (mg/mL)	Number Sacrificed on:	
					Day 43	Day 71
1	5/5	0 (control)	0.4	0	3/3	2/2
2	3/3	1	0.04	25	3/3	
3	3/3	3	0.12	25	3/3	
4	5/5	10	0.4	25	3/3	2/2

Observations Times and Results:

Mortality and Clinical signs:

Animals were observed twice daily for changes in general appearance and behavior. There was no mortality in this study. The only dose related clinical sign observed was watery stool; one female from high dose had numerous incidences of soft stool formation and was tested positive for *shigella* species infection. The animal was treated with Baytril. The incidence suggests direct treatment related finding of immune suppression associated with increase infection.

Body weights:

The body weights were measure prior to first dose and weekly thereafter. There were no treatment related changes in the body weight gain.

Food consumption:

The food consumption was qualitatively assessed daily for each animal 5 days prior to the first dose and continuing until Day 43 (termination) or Day 71 (recovery). There were no treatment related changes in the food consumption.

Ophthalmoscopy:

Ophthalmic examination was conducted on all animals prior to initiation of dosing, Day 42, and Day 71. There were no ophthalmologic findings related to IL-1 Trap.

EKG:

ECGs (using leads I, II, III, aVR, aVL, and aVF) heart rate and blood pressure were recorded from all animals prior to the initiation of dosing on Days 7, 42, and 70. There was a difference in the systolic, diastolic and MAP. This finding might be related to the treatment. Note that highest plasma concentration of the compound was noted around the first two weeks after which the plasma concentration of the compound is compromised because of the presence of the antibody in the serum. Therefore statistically significant differences in the above mentioned parameter might be directly related to the compound and more relevant to what might be observed in human in equivalent doses. The non statistically significant increase of the parameter at the later days might be due to immune complex formation and is of lesser interest for the safety evaluation.

Hematology:

Blood samples were collected from Day 1 prior to dosing and Days 7, 21, 42, and 70 for hematology and coagulation evaluation. Following parameters were evaluated:

Red blood cell (RBC) counts	Mean cell hemoglobin (MCH)
White blood cells (WBCs) (total and differential*)	Mean corpuscular volume (MCV)
Hemoglobin concentration	Mean corpuscular hemoglobin concentration (MCHC)
Hematocrit	Platelet counts
Reticulocyte counts	Blood cell morphology**

* Includes polysegmented neutrophils, band cells, lymphocytes, monocytes, eosinophils, and basophils

**The blood smear from all animals was examined microscopically at each time point (including prestudy).

Activated partial thromboplastin time (APTT)
Prothrombin time (PT)
Fibrinogen

There was an increase in the WBCs especially leukocytes and monocytes suggesting a chronic inflammatory state possibly associated with a background parasitic infection as indicated by nose bleeding in all dose groups including control. An increase in fibrinogen was noted at Day 7 in high dose group animals; however, this change was not statistically significant.

Clinical chemistry:

Blood samples were collected from Day 1 prior to dosing and Days 7, 21, 42, and 70 for clinical chemistry evaluation. Following parameters were evaluated.

Sodium	Calcium
Potassium	Phosphorus
Chloride	Glucose
Total carbon dioxide (bicarbonate)	Urea nitrogen (BUN)
Total bilirubin	Creatinine
Alkaline phosphatase (AP)	Total protein
Lactate dehydrogenase (LDH)	Albumin
Aspartate aminotransferase (AST)	Globulin
Alanine aminotransferase (ALT)	Albumin/globulin ratio
Gamma-glutamyltransferase (GGT)	Cholesterol
C-Reactive Protein (CRP)*	Triglycerides

*CRP only analyzed on Days 1, 21, 42 and 70.

There were no statistically significant changes in the clinical chemistry parameters. There were no consistent changes in any of the serum chemistry parameters related to the treatment except a slight decrease in the albumin at high dose at Day 7.

Urinalysis:

Urine samples were collected on Day 6, 41, and 69. Following parameters were tested. There were no changes in the urinalysis parameters in this experiment.

Color/Character	Protein
PH	Glucose
Specific gravity	Ketones
Leukocyte esterase	Bilirubin
Nitrite	Occult blood
Urobilinogen	Microscopics*

* Microscopic examination was not performed if the specimen was clear and negative for protein, blood, nitrite and leukocyte esterase.

Gross pathology:

A complete gross necropsy was conducted on all animals. All the gross necropsy findings are reproduced below from the sponsor's table. Most of the lesions are associated with the common parasitic infection and found in the control animals too. At high dose, 2/3 females showed diaphragmatic lobes in lungs and adhesion of lungs to thorax. These changes might be related to the infection. There were no such changes in the control animals. Therefore, the changes are considered as treatment related. The changes might have occurred due to the manifestation of immunosuppression related to the exaggerated pharmacology of the test article. However, no histological changes were noted in lungs in these animals.

Table 9A: Individual Animal Gross Necropsy Observations (Day 43)

Study Number: 0949-121

Animal Number	Sex	Organ	Observations
Group 1: Control (0 mg/kg)			
F11730M	M	pancreas	nodule, red, 2 mm, adjacent to pancreas
		colon	nodules, gray, mucosa, fewer than 10, (probable Oesophagostomum)
F13492M	M	cecum and colon	trematode parasites, attached to mucosa, fewer than 5, (probable Gastrodiscoides)
		colon	reddening mucosa, mild, focal
F13490M	M	injection site	subcutis, number 4, focus, red, 0.5 x 1.0 cm, mild
F13417F	F	injection site	reddening subcutaneous, sites numbers 3 and 4 associated with yellow discoloration, skin surface m
F13413F	F	thymus	foci, red, pinpoint to 1 mm, less than 10
		colon	mucosa, nodules, red, 3-5 mm diameter, less than 5, (probable Gastrodiscoides)
		ileum	serosa, nodules, white, 2 mm diameter, less than 5, (probable Mesocostoides)
F13443F	F	injection site number 4	subcutaneous focus, red, 1.0 cm diameter, mild
Group 2: IL-1 Trap (1 mg/kg)			
F13457M	M	thymus	decreased size, moderate
F13464M	M	injection site number 4	subcutis 1.0 cm diameter, red, mild
		inguinal area	subcutis nodule, black, 3 mm x 2 mm
F13146M	M		no visible lesions
F13411F	F	injection site	reddening, subcutaneous, mild, site 4
F13431F	F	injection site	reddening subcutaneous, minimal, site 4
		vagina	white mucoid material in lumen, moderate
FN14042F	F	colon	mucosa, nodule, gray, 5 mm diameter, one, (probable Oesophagostomum)
Group 3: IL-1 Trap (3 mg/kg)			
F13461M	M		no visible lesions
F13454M	M		no visible lesions
F13188M	M		no visible lesions
F13412F	F		no visible lesions
FN14019F	F	skin	yellow discoloration, ventral cranial neck region, associated with reddening edema of underlying musculature, mild
		thymus	decreased size, moderate
		cecum and colon	nodules, gray mucosa, (probable Oesophagostomum), fewer than 10
F13429F	F	left thyroid	cyst, 4 mm diameter
Group 4: IL-1 Trap (10 mg/kg)			
F13491M	M	injection site	reddening subcutaneous, mild, site number 4
F13489M	M	testes	pedunculated nodule, right testicle red and tan, 4x2 mm
F13471M	M		no visible lesions
F13432F	F	lungs	focus, clear, 3 mm, left diaphragmatic lobe
		liver	nodules, tan, 1-5 mm, left medial lobe, fewer than 5
F13428F	F	ovary	right, cyst, 3 mm diameter
		lung	right and left diaphragmatic lobes, adhesions to thoracic wall, mild
		thymus	reduced in size, mild
FS10F	F	left ovary	cyst, 1.0 cm diameter

Organ weights:

The organ weights were measured at the terminal necropsy. Following organs were weighed.

Adrenals	Brain
Epididymides	Heart
Kidneys	Liver
Lungs	Ovaries
Pituitary (post fixation)	Spleen
Testes	Thymus
Thyroid with parathyroids	—

The organ weight analyses were based on the absolute as well as relative brain to organ weight. The increase in the mean ovarian weight was approximately 30% higher at 10 mg/kg compare to those of the control. The increase in testes weight (30%) was highest at the 3 mg /kg. There were histological correlation for the findings in the testes and the ovary which is discussed in the histopathology section below. .

Histopathology:

Adequate Battery: Yes. Peer review: Yes. All tissues from the standard tissue list were processed for the histological evaluation.

The histopathological changes were noted in the injection sites, liver, kidney, lung, testes, thymus, and ovary at the terminal and the recovery sacrifice. The changes in each of the tissues are described below.

Histological Findings from the Injection sites

There was an increase in the mononuclear cell infiltration at the injection site in both males and females. The increase was not always dose related as evident from the sponsor's table below; however, the incidence was higher in the treated group compared to the controls. Therefore, the finding is believed to be treatment related. The mononuclear cell infiltrates composed of plasmacytes and lymphocytes with a perivascular distribution. The infiltration was noted primarily in the subcutis, but in some cases the infiltration was noted in the deep dermis. The mononuclear cell infiltration might have resulted from chronic inflammation or local immune reaction resulted from the treatment. In one male and one female from the high dose group, mononuclear cells infiltrates consisted of a single focus with perivascular mononuclear cells were still apparent indicating incomplete recovery at Day 71.

Histopathology Table for Findings in the Injection Sites:

GROUP:	1M	2M	3M	4M
	(1)	(2)	(3)	(4)
NUMBER OF ANIMALS:	3	3	3	3

	# EX	#	#	#	#
Injection Sites		3	3	3	3
Infiltrate, Mononuclear Cell					
minimal		0	2	3	1
mild		0	0	0	1

GROUP:	1F	2F	3F	4F
	(1)	(2)	(3)	(4)
NUMBER OF ANIMALS:	3	3	3	3

	# EX	#	#	#	#
Injection Sites		3	3	3	3
Infiltrate, Mononuclear Cell					
minimal		0	0	3	1
mild		0	2	0	2

GROUP:	1M	4M
	(1)	(2)
NUMBER OF ANIMALS:	2	2

	# EX	#	#
Injection Sites		2	2
Infiltrate, Mononuclear Cell		0	1

GROUP:	1F	4F
	(1)	(2)
NUMBER OF ANIMALS:	2	2

	# EX	#	#
Injection Sites		2	2
Infiltrate, Mononuclear Cell		0	1

Histopathological findings in Kidney:

There was a dose related increase in the mononuclear cell infiltration in the kidney in both males and females. Increased mineralization was also noted in the kidneys of the test article treated animals compared to those of the controls. All these changes are, therefore, considered test article related. The changes might be associated with the immune complex related protein deposition in the kidney and chronic inflammatory condition associated with test article administration. No such changes were, however, noted at recovery.

Histopathology Table for Findings in Kidney:

GROUP:		1F	2F	3F	4F
NUMBER OF ANIMALS:		(1)	(2)	(3)	(4)
		3	3	3	3
Kidney	# EX	3	3	3	3
Congestion		0	0	0	1
Infiltrate, Mononuclear Cell		1	3	1	2
Inflammation, Chronic, Renal Pelvis		1	0	0	0
Mineralization		0	1	1	1
GROUP:		1M	2M	3M	4M
NUMBER OF ANIMALS:		(1)	(2)	(3)	(4)
		3	3	3	3
Kidney	# EX	3	3	3	3
Infiltrate, Mononuclear Cell		1	1	2	2
Mineralization		0	1	1	0
Regeneration, Tubular Epithelium		0	1	1	0

Histopathological findings in Liver:

There was a dose related increase in the mononuclear cell infiltration in the liver in both males. Increased cytoplasmic vacuolation was also noted in the livers from the females treated with IL-1 Trap. No such changes were noted in control animals. All of these changes are, therefore, considered test article related. The changes might be associated with chronic inflammatory condition associated with test article administration. No such changes were, however, noted at recovery.

Histopathology Table for Findings in Liver:

GROUP:		1F	2F	3F	4F
NUMBER OF ANIMALS:		(1)	(2)	(3)	(4)
		3	3	3	3
Liver	# EX	3	3	3	3
Infiltrate, Mononuclear Cell		0	2	2	2
Inflammation, Pyogranulomatous		0	0	0	1
Necrosis		0	0	0	1
Pigment, Hemosiderin		0	0	1	0
Leukocytosis, Neutrophilic		0	0	1	0
GROUP:		1M	2M	3M	4M
NUMBER OF ANIMALS:		(1)	(2)	(3)	(4)
		3	3	3	3
Liver	# EX	3	3	3	3
Infiltrate, Mononuclear Cell		1	1	0	1
Inflammation, Chronic, Periductal		1	1	0	0
Pigment, Hemosiderin		1	0	1	0
Vacuolation, Cytoplasm, Hepatocyte		0	1	1	0

Histopathological Findings in Lung:

There was an increase in the incidence of the infiltration of the alveolar macrophages in females at high dose (1/3 in control vs. 3/3 at high dose). The changes might be related to the immunosuppression associated with the test article. No such changes were, however, noted at recovery.

Histopathology Table for Findings in Lung:

GROUP:		1F	2F	3F	4F
		(1)	(2)	(3)	(4)
NUMBER OF ANIMALS:		3	3	3	3
Lung	# EX	3	3	3	3
Fibrosis, Pleura		0	2	0	1
Hemorrhage		0	0	0	1
Infiltrate, Macrophage, Alveolus		1	1	1	0
Inflammation, Chronic, Perivascular		1	0	1	0
Pigment, Hemosiderin		1	0	0	0
Pneumoconiosis		3	2	2	3
GROUP:		1M	2M	3M	4M
		(1)	(2)	(3)	(4)
NUMBER OF ANIMALS:		3	3	3	3
Lung	# EX	3	3	3	3
Fibrosis, Pleura		0	1	0	0
Infiltrate, Macrophage, Alveolus		1	1	1	3
Inflammation, Chronic, Perivascular		0	1	0	0
Pneumoconiosis		3	3	1	3

Histopathological Findings in the Reproductive Organs:

Increased hyperplasia of the interstitial cells was noted in ovary in the high dose group females compared to those of the control animals. Increase in cyst formation was also noted at high dose in females. The findings are therefore, considered treatment related. In males, incomplete spermatogenesis and hyperplasia were noted sporadically, in high dose fibrosis was noted in one 1/3 animals incident, no such changes were noted in control.. There was an increase in the testes weight at high dose. It is however, not known clear whether the increase in weight is related to the fibrosis noted because of the rarity of the findings. No such changes were noted in recovery.

Histopathology Table for Findings in Testes and Ovary:

GROUP:		1F	2F	3F	4F
		(1)	(2)	(3)	(4)
NUMBER OF ANIMALS:		3	3	3	3

Ovary	# EX	3	3	3	3
Cyst, Follicular		1	0	1	2
Cyst, Parovarian		0	0	0	1
Hyperplasia, Interstitial Cell		1	1	1	2
Mineralization		2	0	2	1

GROUP:		1H	2H	3H	4H
		(1)	(2)	(3)	(4)
NUMBER OF ANIMALS:		3	3	3	3

Testes	# EX	3	3	3	3
Hemorrhage		0	0	0	1
Incomplete Spermatogenesis		3	3	3	3
Hypoplasia, Germinal Epithelium, Segmental		0	1	0	0
Testes	# EX	3	3	3	3
Fibrosis, Nodular		0	0	0	1

The histopathological changes in the kidney, liver, and lung consisted of increased mononuclear cell infiltration which might be related to the immunosuppressive property of compound. No such changes were noted at recovery. In ovary a dose related increase in the hyperplasia and incidence of cyst was noted. Treatment related changes in the reproductive organs consisted of hyperplasia, fibrosis, and aspermatogenesis in the testes and hyperplasia and cyst formation in the ovary. No such changes were noted at recovery. The treatment related changes in the injection sites were consisted of mild to moderate mononuclear cell infiltration, these changes were only partially recovered after the 4 weeks of the treatment free period.

Toxicokinetics:

The serum from the Days 1, 8, 40, and 71 were used for the evaluation of the IL-1 Trap systemic concentration. There was a dose related increase in the serum IL1-Trap concentrations measured at 16 hr after the first drug injection at Day 1. The average serum concentrations of IL-1 Trap with 1, 3, and 10 mg/kg were 5, 18, and 65 µg/mL respectively at Day 1, 16 hr post dose. Approximately 3.6 folds increase in IL-1 Trap concentration was noted with dose between 1, 3 and 10 mg/kg at this time period. The serum concentrations in all three IL-1 Trap treated cohorts were higher at Day 8 (16 hrs post dose). The average serum concentrations of IL-1 Trap with 1, 3, and 10 mg/kg were 7, 27, and 114 µg/mL respectively at Day 8, 16 hr post dose. The increase was approximately 1.4 to 1.7 fold greater than those measured following the first dose thus indicating that there was accumulation of protein in the circulation. By Day 40, however, serum IL-1 Trap levels dropped dramatically. The number of animals which showed IL-1 Trap concentration in plasma was 0/6, 4/6, and 8/10 in the 1, 3, and 10 mg/kg respectively at Day 40. The average serum concentration of monkeys at Days 1, 8, and 40 with 10 mg/kg group was 65, 114, and 5.5 µg/mL respectively indicating that the IL-1 Trap concentration in the serum is apparently reduced due to anti IL-1 Trap antibody formation.

IL-1 Trap antibodies were not detected in the serum of any of the placebo-treated monkeys. By Day 14, 3/6, 5/10, and 3/10 animals in the 1, 3, and 10 mg/kg groups had detectable serum anti product antibody level. All animals developed anti-IL-1 Trap

antibodies by Day 28, all drug-treated animals were antibody-positive with titers greater than 300 or 6 times the LQ (→g/mL) at Day 28. IL-1 Trap antibody titers were further increased by Day 42 and the level of the antibodies remained elevated at 4 week recovery period. The antibody formation was not related to IL-1 Trap dose or antibody titer.

**Mean Plasma Concentration of IL-1 Trap and IL-1 Trap Antibody in
Cynomolgus Monkey 6-Week SC**

Time	IL-1 Trap (µg/mL)			Time	IL-1 Trap Antibody Titer		
	1 mg/kg	3 mg/kg	10 mg/kg		1 mg/kg	3 mg/kg	10 mg/kg
Day 1, 16 hr post dose	4.9	17.8	64.7	Day 14	850-2250	250-5000	300-12500
Day 8, 16 hr post dose	7	26.6	113.5	Day 28	500-6000	500-9000	800-25000
Day 40 post dose, 16 hr	0.02	0.07	5.5	Day 42	550-9250	1250-9250	2250-6250

Study title: A 3-Month Subcutaneous Toxicity Study of IL-1 Trap in Cynomolgus Monkeys

Key study findings:

- Cynomolgus monkeys (3 animals/sex/group) were administered 0, 1.5, 5, 15 mg/kg of IL-1 Trap, subcutaneously, twice per week for 13 weeks.
- The mean IL-1 Trap plasma concentration increased more than dose proportionately after the first injection. Accumulation was noted with all dosages from Day 1-12. The mean plasma concentrations of IL-1 Trap decreased in all dose groups at 24 hrs post 4th dose on Day 13 compared to the plasma concentrations that was observed 24 hr post dose on Day 1. The IL-1 Trap could not be detected in the plasma of the animals from the low and mid dose group after Day 40.
- Antibody formation was faster in the low dose animals. In the low and mid dose group 2/6 animals had antibody formation at Day 12. By Day 19, 6/6 and 2/6 from the low and mid dose group respectively had detectable antibody.
- No NOAEL could be established in this study because of the histopathology findings at all dosages. The major histopathology findings at the low dose were injection site reaction consisted of the tissue infiltration of the lymphocytes. The sponsor concluded that the IL-1Trap in the 2x/week, sc toxicity study in the monkeys are well tolerated, however, slight injection site reactions were observed histologically even at recovery.
- The other major treatment related histopathological findings consisted of skeletal muscle degeneration at high and mid dose, mononuclear cell infiltration in liver and kidney, microgranuloma in the lung and liver and thymal atrophy. Increased degeneration of seminiferous tubule in the testes and mineralization of the oocytes were also noted at all dosages. There were no recovery group in this study, therefore it is not known whether any of these lesions are recoverable or not. The pathology report provided a general statement that these tissue findings were

either found in the control groups or were within historical control ranges (cites Control Background Data, Vol. 47-2 (2000)). The sponsor did not provide these data.

Study no.: 223.5 (Study-IL1T-TX-02023.pdf)

Volume # and page #: eCTD; Pages 1-310

Conducting laboratory and location:

Date of study initiation: April 11, 2001

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: IL-1 Trap TP1 F01002 (relative potency Drug

Product description: IL-1 Trap recombinant protein, 40 mg/mL in a aqueous vehicle, pH containing histidine, glycine, arginine, sucrose, polyethylene glycol 3350,

Vehicle lot # PTP1 F0102001TAA; the placebo formulation is similar to the drug product without the IL-1 Trap protein.

Methods

Doses: 0, 1.5, 5, 15 mg/kg twice a week for 13 weeks

Species/strain: Cynomolgus monkeys, *Macaca fascicularis*

Number/sex/group or time point (main study): 3/sex/group

Route, formulation: The test article was formulated by sponsor, therefore, CRO need not have to dilute or reformulate the solution; the test article was administered subcutaneously.

Satellite groups used for toxicokinetics or recovery: The recovery was not assessed in this study. Blood samples were analyzed from all animals

Age: 2-7 years

Weight: Male weighed 4.16-6.5 g; Female weighed 3.08-4.44 kg

Sampling times: Blood samples were collected for the toxicokinetics analyses at pre dose and 24 hours post-dose on Days 1, 5, 12, 19, 26, 40, 54, 68, 82, and 92 prior to necropsy. Blood samples were collected ant for the serum anti product body formation pre dose and 24 hours post dose on Days 1, 5, 12, 19, 26, 40, 54, 68, 82, and 92 prior to necropsy.

Unique study design or methodology (if any):

Table A Study Groups and Study Drug Administration

Group	Study Animal Numbers Male and Female ^o	Test or Control Article	Dose Level (mg/kg)	Concentration (mg/mL)	Dose Volume ^a (mL/kg)
1	(1-3) (4-6)	Vehicle Control	0	0	0.60
2	(7-9) (10-12)	IL-1 Trap	1.5	22.9	0.07
3	(13-15) (16-18)	IL-1 Trap	5.0	22.9	0.22
4	(19-21) (22-24)	IL-1 Trap	15	22.9	0.66

a : Individual dose volume (mL) was estimated based on the recent body weight.

b : Note female animals are assigned odd numbers and male animals even numbers.

Observations Times and Results:

Mortality and Clinical Signs:

The animals were observed twice daily for the clinical signs and morbidity. There was no mortality in this study. The test article treated animals had soft stools sporadically. Because this incidence was found only in the test article treated animals and not in control animals, the incidence is believed to be treatment related. Skin irritation and scabs were also noted, however, there was no consistent time or dose dependent trend. Therefore, these signs are not considered as dose related.

Body weights:

The body weights were measured once pre dose and weekly there after throughout the life phase of the experiment. There were no treatment related changes in the body weight gain.

Food consumption:

Daily food consumption was measured for each animal throughout the life phase of the experiment. There were no treatment related changes in the food consumption.

Ophthalmoscopy:

An ophthalmic examination was performed on all animals under ketamine sedation using an indirect ophthalmoscope and slit lamp with appropriate eye drops, once during acclimation and during Week 13. There were no visible lesions in the eyes of any animals in the study.

Blood Pressure:

Blood pressure was measured for all animals under sedation twice prior to dosing and once post dosing on Day 1 and during Weeks 5, 8, and 12. No clinically relevant changes were observed.

EKG:

Electrocardiograms were performed on all animals under sedation using a _____ ECG Analyzer, using four chest leads, twice during acclimation, once after dosing on Day 1 and during Weeks 5, 8 and 12. There were no test article related changes in the ECG parameters.

Body Temperature:

Body temperature was measured on all animals using a _____ Vital Scan Monitor with rectal probe twice during acclimation, once after dosing on Day 1 and during Weeks 5, 8 and 12 under sedation. There were no test article related changes in the body temperatures.

Hematology:

The hematology and coagulation analyses were performed once during acclimation and during Weeks 2 and 13. The parameters tested for the evaluation of the hematology in this study is shown in the sponsor's table below.

Hematology and Coagulation

Parameter	Method	Apparatus
Erythrocyte count		
Leukocyte count		
Hematocrit value		
Hemoglobin concentration		
Platelet count		
Mean corpuscular volume		
Mean corpuscular hemoglobin		
Mean corpuscular hemoglobin Concentration		
Reticulocyte count		
Differential leukocyte count		
Prothrombin time		
Activated partial Thromboplastin time		

There were no treatment related changes in the hematological parameters.

Clinical chemistry:

The serum chemistry analyses were performed on all animals once during the acclimation period and during Weeks 2 and 13. The clinical chemistry parameters tested are reproduced from the sponsor's table below. There were no treatment related changes in the clinical chemistry parameters.

Serum Chemistry

Parameter	Method	Apparatus
Albumin		
Alanine aminotransferase		
Alkaline phosphatase		
Aspartate aminotransferase		
Bilirubin, total		
Blood urea nitrogen		
Calcium		
Chloride		
Cholesterol, total		
Creatinine		
Creatine phosphokinase		
Gamma Glutamyl Transferase		
Glucose		
LDH		
Phosphorus, inorganic		
Potassium		
Protein fraction (including A/G ratio)		
Protein, total		
Sodium		
Triglyceride		
Uric acid		
C-reactive protein		

C-reactive protein analyses:

C-reactive protein analyses were performed on all animals once during the acclimation period and on Days 1, 5, 12, 29 and 85 (approximately 6 hours post-dose). Increase in C reactive proteins were noted in all treated animals, however, the changes on Day 1, 13, and 86 were not consistent within group and even within the individual animals. The changes were not statistically significant in any of the individual animal except animal #17 which showed statistically significant increase of C-reactive protein at Day 1 and Day 86 compared to control. However, since no such changes were noted in controls, the increase in the C-reactive protein is considered treatment related. It is however, not known whether the changes in the C-reactive protein is an indication of tissue inflammation in this study.

Complement analysis:

The serum samples were prepared from blood collected from all animals once during the acclimation period and on Days 1, 5, 12, 29 and 85 (approximately 6 hours post-dose). There were no changes in the complement factors in this study.

Urinalysis:

Urinalysis was performed on all animals. Urine was collected, once during acclimation and once during Weeks 2 and 13. Following parameters were analyzed.

Urinalysis

Parameter	Method	Apparatus
Color	Visual	
pH	Test strips	
Protein	Test strips	
Glucose	Test strips	
Ketones	Test strips	
Bilirubin	Test strips	
Occult Blood	Test strips	
Urobilinogen	Test strips	
Nitrite	Test strips	
Leukocytes	Test strips	
Sediment		
Volume		
Specific gravity		
Sodium		
Potassium		
Chloride		

There were changes in the urine volume and chloride, sodium and the potassium ions in the urine. The changes are higher in the magnitude than that of the controls. Therefore, the changes are considered treatment related, however, the biological significance of the findings are not known. The sponsor did not include urinalysis of these ions in other toxicity studies with IL-1 Trap. Therefore, it is not known whether these changes are isolated findings specific to this study or the changes are related to IL-1 Trap treatment.

Summary of Changes Urinalysis Parameters

Parameters	Dose Groups							
	Male				Female			
	0 mg/kg	1.5 mg/kg	5 mg/kg	25 mg/kg	0 mg/kg	1.5 mg/kg	5 mg/kg	25 mg/kg
Urine Volume (mL)								
Days -2	27±3	55±46	37±15	36±43	10±2	46±58	19±7	159±144
Day 9	14±6	65±64	66±43	180±213	13±9	50±42	31±13	154±192
Day 89	34±6	91±58	70±29	37±27	14±3	22±8	62±66	64±9
Sodium ion (mEq/L)								
Days -2	34±12	48±25	26±5	13±6	44±31	38±45	29±12	7±0.3
Day 9	62±18	27±22	26±14	27±12	87±100	19±10	49±43	15±10
Day 89	66±12	31±9	27±8	26±11	78±51	42±15	35±32	29±17
Potassium ion (mEq/L)								
Days -2	110±63	109±69	96±20	69±19	108±70	63±60	103±68	28±24

Day 9	144±31	76±30	59±14	87±67	104±39	62±14	87±80	51±8
Day 89	83±8	50±19	55±17	56±48	82±41	68±18	65±51	33±11
Chloride ion (mEq/L)								
Days -2	68±29	83±65	41±6	25±13	57±41	39±35	52±25	14±5
Day 9	67±6	22±7	42±6	52±33	47±31	35±19	61±68	21±7
Day 89	37±11	19±6	27±5	17±8	22±9	33±10	31±20	25±8

Gross pathology:

The external surface of the body, all orifices, the cranial, thoracic and abdominal cavities, and all organs and tissues were examined grossly from all animals at necropsy. The gross pathological observations consisted of the following.

1. Marked fibrous adhesion in the parietal pleura of the lung in one male (5 mg/kg dose group). This was associated histologically with focal fibrosis in pleura, sponsored provided reference showing that such changes were noted in control animals historically.
2. Congestion in spleen in one male (5 mg/kg dose group), sponsor believes that this was caused by an insufficient exsanguinations during euthanasia.
3. Small size spleen in 2/6 (females), 3/6 (one male, two females) and 1/6 (female) animals in control, low and mid dose group animals respectively.
4. Parasitic infestation in cecum in one male from the low dose was also observed.

Organ weights:

The following organs of all animals were weighed at necropsy: adrenals, brain (cerebellum, cerebrum and brain stem), epididymes, heart, kidneys, liver, lungs (including bronchi), ovaries, pituitary, prostate/seminal vesicles, spleen, sub mandibular glands, testes, thymus, thyroids (with parathyroids) and uterus. Absolute organ weight data as well as relative organ weight data based on body weight and brain weights were provided by the sponsor. The organ weights were assessed by the reviewer based on the relative brain weights.

There was a dose related increase in the lung, pituitary, and ovarian weight in females. The increase in pituitary, ovary, and lung were 20, 22, and 24 % respectively at high dose compared to control. In addition, an increase in adrenal (20%) and thymus (37%) weight were noted in females with 1.5 mg/kg dose compared to those of the controls. The thymus weight decreased 40% compared to that of the control.

There was a dose related increase in the prostate, testes, spleen, and adrenal in males. The increase in the prostate, testes, and adrenal were 41, 48, and 11%. There was a dose related decrease in the lung (12%) and liver (23%) and thyroid/parathyroid (37%) weight in males compared to those of the controls.

Histopathology: Adequate Battery: Yes; peer review: Yes.

All tissue from the standard tissue list were collected and fixed in 10% neutral formalin. Eyes with optic nerves were fixed in a mixture solution of 3% gluteraldehyde and 2.5% neutral-buffered formalin; the testes were fixed in Bouin’s solution. The histopathology findings were noted in the injection site, kidney liver, lung, skeletal muscle, ovary, and uterus. The detail of the changes in each tissue is described below.

Histopathological changes were graded according to the following scale:

- Grade**
- : No abnormal changes
 - ± : Very slight
 - + : Slight
 - 2+ : Moderate
 - 3+ : Marked
 - P : Non-graded change
 - U : Unexamined

Histopathology Findings in the Injection Sites:

An increase in the eosinophil infiltration was noted in the perivascular region of the subcutis in males and females in the injection sites in the treated animals graded as very slight by the pathologist. No such changes were noted in control. In addition, an increase in the lymphoid cell infiltration in the perivascular region was noted in males and females in dermis and subcutaneous region. All these indicate chronic inflammatory changes in the injection sites which might be associated with local immune reaction related. The infiltration of lymphoid cells in the dermis (very slight to slight) suggesting that the intensity of the lesion might be high, however, in the absence of the recovery data it is unknown whether the lesion was recoverable or not. This finding is described by the sponsor as treatment-related, the reviewer agrees with this conclusion.

Histopathology Table for Injection Site Findings:

[H.E.staining] Organs/Tissues	Group Dose (mg/kg)	1 (Vehicle) 0.0			2 (IL-1 Trap) 1.5			3 (IL-1 Trap) 5.0			4 (IL-1 Trap) 15.0		
Findings	Animal No.	2	4	6	8	10	12	14	16	18	20	22	24
Injection site (back skin)													
Eosinophil infiltration, perivascular, subcutaneous		-	-	-	-	-	-	-	-	-	±	-	-
Hemorrhage, subcutaneous		-	-	±	-	-	-	-	-	-	±	-	-
Lymphoid cell infiltration, perivascular, dermis/subcutaneous		-	-	-	±	±	-	±	±	±	+	±	±
Macrophage infiltration, subcutaneous, focal		-	±	-	-	-	-	-	-	-	-	-	-

[H.E.staining] Organs/Tissues	Group Dose (mg/kg)	1 (Vehicle) 0.0			2 (IL-1 Trap) 1.5			3 (IL-1 Trap) 5.0			4 (IL-1 Trap) 15.0		
Findings	Animal No.	1	3	5	7	9	11	13	15	17	19	21	23
Injection site (back skin)													
Hemorrhage, subcutaneous		-	-	-	-	-	-	-	±	-	-	-	-
Lymphoid cell infiltration, perivascular, dermis/subcutaneous		-	-	-	+	±	±	+	±	±	+	+	+
Eosinophil infiltration, perivascular, subcutaneous		-	-	-	-	-	-	-	-	-	+	-	-
Microgranuloma, subcutaneous		-	-	-	-	-	±	-	-	-	-	-	-

Histopathology Findings in Kidney:

The histopathological changes in the kidney consisted of focal lymphoid cell infiltration in the pelvic mucosa and focal mononuclear cell infiltration in the interstitial. These changes were seen in both males and females. The number of animals showing mononuclear cells and lymphoid infiltration in males and females were higher in treated

animals compare to control. Another interesting kidney finding was inclusion body in pelvis which consisted of mucosal epithelium. Animal #6 (control male), #9 (low dose female), #s 16 and 18 (mid dose males), and #s 21 and 22 (high dose male and female) showed this finding. This finding is considered treatment related, however, what caused this change is unknown.

Histopathology Table for Findings in Kidney

[H.E.staining]	Group	1 (Vehicle)			2 (IL-1 Trap)			3 (IL-1 Trap)			4 (IL-1 Trap)		
Organs/Tissues	Dose (mg/kg)	0.0			1.5			5.0			15.0		
Findings	Animal No.	2	4	6	8	10	12	14	16	18	20	22	24
Kidney (left)													
Brown pigment, tubular epithelium, focal		±	-	-	-	-	-	-	-	-	-	-	-
Fibrosis, interstitium, focal		-	-	±	-	-	-	-	-	-	-	-	-
Glomerulosclerosis, focal		±	-	-	-	-	-	-	-	-	-	-	-
Inclusion body, mucosal epithelium, pelvis		-	-	±	-	-	-	-	±	±	-	±	-
Lymphoid cell infiltration, focal		-	-	-	±	-	±	-	-	-	-	-	-
Lymphoid cell infiltration, pelvic mucosa		-	-	±	-	-	±	±	-	-	-	-	-
Mononuclear cell infiltration, interstitium, focal		-	-	-	-	±	-	±	-	-	±	-	-
Mineralization		-	±	±	-	-	-	-	-	-	-	±	±
Kidney (right)													
Brown pigment, tubular epithelium, focal		-	-	-	-	-	-	-	±	-	-	-	-
Glomerulosclerosis, focal		±	-	-	-	-	-	-	-	-	-	-	-
Inclusion body, mucosal epithelium, pelvis		-	-	±	-	-	-	-	±	±	-	-	-
Inflammatory cell infiltration, pelvic mucosa, focal		-	-	-	-	-	-	-	-	-	-	-	±
Lymphoid cell infiltration, pelvic mucosa		-	-	-	-	-	±	-	-	-	-	-	±
Mononuclear cell infiltration, interstitium, focal		±	±	±	-	-	±	±	-	±	-	-	±
Mineralization		-	±	±	±	±	-	±	-	-	±	-	±
Protein cast, focal		±	-	-	-	-	-	-	-	-	-	-	-

[H.E.staining]	Group	1 (Vehicle)			2 (IL-1 Trap)			3 (IL-1 Trap)			4 (IL-1 Trap)		
Organs/Tissues	Dose (mg/kg)	0.0			1.5			5.0			15.0		
Findings	Animal No.	1	3	5	7	9	11	13	15	17	19	21	23
Kidney (left)													
Brown pigment, tubular epithelium, focal		±	-	-	-	±	-	±	±	-	-	-	±
Cyst		-	-	-	-	-	-	-	-	-	p	-	-
Fibrosis, interstitium, focal		-	-	-	-	-	-	-	±	-	-	-	-
Glomerulosclerosis, focal		-	-	-	-	-	±	-	-	-	-	-	±
Inclusion body, mucosal epithelium, pelvis		-	-	-	-	±	-	-	-	-	-	±	-
Mononuclear cell infiltration, interstitium, focal		±	-	-	±	-	±	±	±	±	±	±	-
Mineralization		±	-	±	-	±	-	±	-	-	±	-	±
Syncytial epithelial cell, pelvic mucosa		-	-	-	-	-	-	-	±	-	-	-	-
Thickening, B's capsule, focal		-	-	-	-	-	-	-	-	-	-	±	-
Kidney (right)													
Brown pigment, tubular epithelium, focal		±	-	-	-	±	-	-	±	-	-	-	-
Glomerulosclerosis, focal		-	±	-	±	-	-	-	-	-	-	-	-
Inclusion body, mucosal epithelium, pelvis		-	-	-	-	±	-	-	-	-	-	±	-
Lymphoid cell infiltration, focal		-	±	-	-	-	-	-	-	-	-	-	-
Mononuclear cell infiltration, interstitium, focal		±	-	-	±	-	±	±	-	±	±	±	-
Mineralization		-	±	-	±	±	-	±	-	-	±	-	-
Thickening, B's capsule, focal		-	-	-	-	-	±	-	-	-	-	-	-

Histopathology Findings in Lungs

One male and one female from the high dose group showed microgranuloma in lung (graded as very slight by the pathologist), the finding is considered treatment related. The sponsor did not mention the cause of microgranuloma formation. The sponsor did not mention this finding as treatment related.

Histopathology Table for Findings in Lung

[H.E.staining]	Group	1 (Vehicle)			2 (IL-1 Trap)			3 (IL-1 Trap)			4 (IL-1 Trap)		
Organs/Tissues	Dose (mg/kg)	0.0			1.5			5.0			15.0		
Findings	Animal No.	2	4	6	8	10	12	14	16	18	20	22	24
Lung													
Fibrosis, pleura, focal		±	±	±	-	±	±	±	±	±	±	±	±
Microgranuloma		-	-	-	-	-	-	-	-	-	-	±	-

[H.E.staining]	Group	1 (Vehicle)			2 (IL-1 Trap)			3 (IL-1 Trap)			4 (IL-1 Trap)		
Organs/Tissues	Dose (mg/kg)	0.0			1.5			5.0			15.0		
Findings	Animal No.	1	3	5	7	9	11	13	15	17	19	21	23
Lung													
Fibrosis, pleura, focal		±	±	±	±	±	±	±	±	-	±	±	-
Microgranuloma		-	-	-	-	-	-	-	-	-	±	-	-

Histopathology Findings in Liver

In the liver microgranuloma was observed in the animal #8 (low dose male) and #s 14 and 15 (mid dose male and female). No such findings are noted in control. Increase in the mononuclear cell infiltration was also noted in the treated animals compare to control. The above mentioned findings are considered treatment related by the reviewer. The sponsor did not mention this finding as treatment related.

Histopathology Table for Findings in Liver

[H.E.staining] Organs/Tissues	Group Dose (mg/kg)	1 (Vehicle) 0.0			2 (IL-1 Trap) 1.5			3 (IL-1 Trap) 5.0			4 (IL-1 Trap) 15.0		
Findings	Animal No.	2	4	6	8	10	12	14	16	18	20	22	24
Liver													
Mononuclear cell infiltration, focal		-	-	±	±	±	-	±	±	-	±	±	±
Liver (left)													
Microgranuloma		-	-	-	±	-	-	±	-	-	-	-	-
Mononuclear cell infiltration, focal		-	±	±	-	-	-	±	±	-	±	±	±
Necrosis, hepatocyte, focal		-	-	±	-	-	-	-	-	-	-	-	-

[H.E.staining] Organs/Tissues	Group Dose (mg/kg)	1 (Vehicle) 0.0			2 (IL-1 Trap) 1.5			3 (IL-1 Trap) 5.0			4 (IL-1 Trap) 15.0		
Findings	Animal No.	1	3	5	7	9	11	13	15	17	19	21	23
Liver													
Microgranuloma		-	-	-	-	-	-	-	-	-	-	±	-
Mononuclear cell infiltration, focal		±	±	±	-	±	±	±	±	±	±	±	-
Liver (left)													
Brown pigment, macrophage, focal		-	-	-	-	-	-	-	-	-	±	-	-
Microgranuloma		-	-	-	-	-	-	-	±	-	-	-	-
Mononuclear cell infiltration, focal		-	±	-	±	-	±	±	-	±	±	±	-

Histopathology Findings in Skeletal Muscle

The degeneration of the skeletal muscle was noted in two animals, #9 and 12 (low dose male and female) and three animals #19 (high dose female), #s 20 and 22 (high dose males). No such findings are noted in control animals. Therefore, these findings are considered treatment related. The sponsor did not conclude that this finding as treatment related as the study pathologist concluded that the focal nature of the finding would not be consistent with a treatment-related toxicity to the skeletal muscle.

Histopathology Table for Findings in Skeletal Muscle

[H.E.staining] Organs/Tissues	Group Dose (mg/kg)	1 (Vehicle) 0.0			2 (IL-1 Trap) 1.5			3 (IL-1 Trap) 5.0			4 (IL-1 Trap) 15.0		
Findings	Animal No.	2	4	6	8	10	12	14	16	18	20	22	24
Skeletal muscle (quadriceps femoris)													
Degeneration/regeneration, muscle fiber, focal		-	-	-	-	-	±	-	-	-	±	2*	-

[H.E.staining] Organs/Tissues	Group Dose (mg/kg)	1 (Vehicle) 0.0			2 (IL-1 Trap) 1.5			3 (IL-1 Trap) 5.0			4 (IL-1 Trap) 15.0		
Findings	Animal No.	1	3	5	7	9	11	13	15	17	19	21	23
Skeletal muscle (quadriceps femoris)													
Degeneration/regeneration, muscle fiber, focal		-	-	-	-	±	-	-	-	-	±	-	-

Histopathology Findings in Ovary

The histopathological changes in the ovary consisted of either increase in number of animals with mineralization of oocytes in the treatment group or increase in the severity of mineralization in the oocytes of the treated animals compared to control. The

biological significance of these findings is not known. The sponsor did not mention this finding as treatment related.

Histopathology Table for Findings in Ovary

[H.E.staining] Organs/Tissues	Group Dose (mg/kg)	1 (Vehicle) 0.0			2 (IL-1 Trap) 1.5			3 (IL-1 Trap) 5.0			4 (IL-1 Trap) 15.0		
Findings	Animal No.	1	3	5	7	9	11	13	15	17	19	21	23
Ovary													
Mineralization, oocyte		-	±	-	±	-	±	2+	-	-	-	-	±

Histopathology Findings in Thymus

As indicated below increase cyst formation and atrophy was noted in the thymus in all test article treated groups compared to those of the controls. The significance of the findings is not known.

Histopathology Table for Findings in Prostrate

[H.E.staining] Organs/Tissues	Group Dose (mg/kg)	1 (Vehicle) 0.0			2 (IL-1 Trap) 1.5			3 (IL-1 Trap) 5.0			4 (IL-1 Trap) 15.0		
Findings	Animal No.	2	4	6	8	10	12	14	16	18	20	22	24

Thymus													
Atrophy		+	-	-	2+	2+	±	±	+	+	3+	±	±
Cyst		P	-	P	P	P	P	-	-	-	-	-	-

[H.E.staining] Organs/Tissues	Group Dose (mg/kg)	1 (Vehicle) 0.0			2 (IL-1 Trap) 1.5			3 (IL-1 Trap) 5.0			4 (IL-1 Trap) 15.0		
Findings	Animal No.	1	3	5	7	9	11	13	15	17	19	21	23

Thymus													
Atrophy		±	-	-	±	±	-	±	±	-	±	-	+
Cyst		P	-	P	-	P	-	-	-	-	-	-	-

Histopathology Findings in Testes

As indicated in the table below moderate focal, bilateral degeneration of the seminiferous tubules was noted in one male in the 5 mg/kg dose group compare to those of the controls, the significance of the finding is not known.

Histopathology Table for Findings in Testes

[H.E.staining] Organs/Tissues	Group Dose (mg/kg)	1 (Vehicle) 0.0			2 (IL-1 Trap) 1.5			3 (IL-1 Trap) 5.0			4 (IL-1 Trap) 15.0		
Findings	Animal No.	2	4	6	8	10	12	14	16	18	20	22	24

Testis													
Degeneration, seminiferous tubule, focal, bilateral immature		-	-	-	-	-	-	-	2+	-	-	-	-
		-	P	P	-	-	-	-	-	P	-	-	-

The histopathological changes in the liver and the kidney consisted of mononuclear cell infiltration which might indicate tissue response to immunosuppression related to the exaggerated pharmacology of the compound. Increase in the mononuclear cell infiltration was also noted in the prostate, although the finding did not appear to be dose-related. Degeneration of the seminiferous tubules was noted in one mid dose male.

Increased in the incidence of the mineralization was noted in the oocytes in the ovary. The biological significance of these findings is not known.

Increase in the microgranuloma formation was noted in the liver and lungs; lymphocyte infiltration was noted in the urinary bladder. These findings might be indicative of the increase infection related to the immunosuppressive nature of the compound.

Interestingly, the degeneration of the skeletal muscles were noted at low (2/6) and mid dose (3/6), the severity index for such findings were higher at high dose, the significance of such finding is not known. An inflammatory condition was noted at the injection site with lymphocyte infiltration deep within the tissue. The recoveries of the histological changes were not studied under this experimental protocol. Therefore, it is not known whether the tissue changes are recoverable after the 3-months of the treatment period or not.

Toxicokinetics:

Blood samples were collected pre dose and 24 hours post-dose on Days 1, 5, 12, 19, 26, 40, 54, 68, 82, and 92 prior to necropsy.

Blood samples were also collected from all animals for the analyses of antibody to IL-1 Trap in conjunction with TK sample collection. Blood samples were collected pre dose and 24 hours post dose on Days 1, 5, 12, 19, 26, 40, 54, 68, 82, and 92 prior to necropsy.

Toxicokinetics: Major Findings

IL-1 Trap Concentration

- The mean IL-1 Trap plasma concentration increased more than dose proportionately after the first injection. The concentrations of the IL-1 Trap in plasma 24 hrs post first dose were 8880, 43744, and 106279 with 1.5, 5, and 25 mg/kg respectively. A more than dose proportional increase in IL-1 Trap concentration (4.9 fold) was noted for a 3.3-fold dose increase in dose (low to mid dose). However, only a 2-fold increase in plasma concentration was noted for a 5-fold dose increase in dose (mid to high dose) indicating saturation of the compound in plasma at this time point.
- There was an accumulation of IL-1 Trap in all dose groups as observed by the increased plasma level of the compound in the pre dose samples suggesting long half life of the compound. The accumulation of the compound decreased with the increase in the duration of the experiment.
- The mean plasma concentrations of IL-1 Trap decreased in all dose groups at 24 hrs post 4th dose on Day 13 compared to the plasma concentrations that was observed 24 hr post dose on Day 1.
- The IL-1 Trap could not be detected in the plasma of the animals from the low and mid dose group after Day 40. The IL-1 Trap plasma concentration was, however, noted at Day 92 in four animals (#s 19, 20, 21, and 22) from high dose groups. The concentrations of the IL-1 Trap in the plasma of these animals were, however, much lower at Day 92 except animal #22. In all of these four animals; the pre dose level

of the IL-1 Trap was low indicating enhanced clearing and, therefore, less accumulation.

IL-1 Trap Antibody Concentration

- IL-1 Trap antibody concentration was not observed in any of the animals prior to Day 12.
- Anti-IL-1 Trap antibody formation was faster in the low dose animals. In the low and mid dose group 2/6 animals had antibody formation at Day 12. By Day 19, 6/6 and 2/6 from the low and mid dose group respectively had detectable antibody. The number of animals showing serum anti product antibody level were 6/6, 5/6, and 4/6 animals from the low, mid and high dose group respectively at Day 26.
- Measurable IL-1 Trap antibody was detected from all of the animals from all treatment groups except one animal (#22) in the high dose group. In this animal pre dose level of the IL-1 Trap concentration decreased from Day 26 compare to the pre dose IL-1 Trap concentration at Day 19. Also, the level of the post dose IL-1 Trap concentration decreased from Day 41 through Day 92 compare to the IL-1 Trap concentration at Day 27. The decreased levels of the test article in the pre dose level and post dose level compared to the earlier time points suggest that the antibody had been formed in this animal; however, due to the substantial level of IL-1 Trap in the serum, the antibody could not be detected.
- Measurable amount of the anti product antibody was found in all animal at Day 92, except 1/6 animal in the low dose group and 2/6 animals at high dose group.

Mean Plasma Concentration (ng/mL) of IL-1 Trap and IL-1 Trap antibody (RFU/mL) in Cynomolgus Monkeys

Time	Dose Groups					
	IL-1Trap Concentration			IL-1 Trap Antibody Concentration		
	1.5 mg/kg	5 mg/kg	25 mg/kg	1.5 mg/kg	5 mg/kg	25 mg/kg
Day 1 24 hr Post 1 st dose	8880	43744	106279	BLQ	BLQ	BLQ
Day 5 Pre dose	5568	15304	99361	BLQ	BLQ	BLQ
Day 6 24 hr Post 3 rd dose	17269	40067	208089	BLQ	BLQ	BLQ
Day 12 Pre dose	10451	17603	32638	11.3	22	5.7
Day 13 24 hr Post 4 th dose	15155	19420	36236	NM*	NM	NM
Day 26 Pre dose	56	17	4871	33.6	53	50
Day 27 24 hr Post 8 th dose	303	1488	29158	NM	NM	NM
Day 40 24 hr Post 1 st dose	BLQ**	BLQ	2236	NM	NM	NM

Day 41 24 hr Post 8 th dose	BLQ	BLQ	19753	NM	NM	NM
Day 57 Pre dose	BLQ	BLQ	1298	154	164	477
Day 57 24 hr Post dose	BLQ	BLQ	19903	NM	NM	NM
Day 92 Pre dose	BLQ	BLQ	10503	95	206	356

- NM: IL-1 Trap antibody concentration not measured.
- ** BLQ: Below level of quantification

TABLE 2 GROUP 2

3 MONTH MULTIPLE DOSE MONKEY TOX STUDY
 Dose Level (mg/kg) 1.5
 Dose Conc. (mg/mL) 25
 Dose Vol. (mL/kg) 0.06

*ND = No Data
 STUDY # ~~223.5~~ 223.5 (~~223.5~~ 223.5)
 BLQ (below limit of quantitation) <4 ng/mL
 Statistics were calculated using zero for BLQ
 Data in ng/mL
 Mean of Means = 5583 ng/mL

DAY	TIME PT.	Monkey No.						Mean
		7F	8M	9F	10M	11F	12M	
1	Pre							
2	24 hr							8880
5	Pre							5566
6	24 hr							17289
12	Pre							10451
13	24 hr							15155
19	Pre							254
20	24 hr							486
26	Pre							56
27	24 hr							303
40	Pre							
41	24 hr							
57	Pre							45
58	24 hr							
68	Pre							
69	24 hr							
82	Pre							
83	24 hr							45
92	Pre							

TABLE 3 GROUP3

3 MONTH MULTIPLE DOSE MONKEY TOX STUDY
 Dose Level (mg/kg) 5
 Dose Conc. (mg/mL) 25
 Dose Vol. (mL/kg) 0.2

STUDY # ~~223.5~~ (223.5)
 BLQ (below limit of quantitation) < ~~4000~~ ng/mL
 Statistics were calculated using zero for BLQ
 Data in ng/mL Mean of Means = 25444 ng/mL

DAY	TIME PT.	Monkey No.						Mean
		13F	14M	15F	16M	17F	18M	
1	Pre							
2	24 hr							43744
5	Pre							15304
6	24 hr							40067
12	Pre							17603
13	24 hr							19420
19	Pre							537
20	24 hr							5503
26	Pre							17
27	24 hr							1488
40	Pre							
41	24 hr							43
57	Pre							
58	24 hr							
68	Pre							
69	24 hr							
82	Pre							
83	24 hr							
92	Pre							

TABLE 4 GROUP 4

3 MONTH MULTIPLE DOSE MONKEY TOX STUDY
 Dose Level (mg/kg) 15
 Dose Conc. (mg/mL) 25
 Dose Vol. (mL/kg) 0.6

STUDY # ~~223.5~~ (223.5)
 BLQ (below limit of quantitation) < ~~4000~~ ng/mL
 Statistics were calculated using zero for BLQ
 Data in ng/mL Mean of Means = 90989 ng/mL

DAY	TIME PT.	Monkey No.						Mean
		19F	20M	21F	22M	23F	24M	
1	Pre							79
2	24 hr							106279
5	Pre							99361
6	24 hr							208099
12	Pre							32638
13	24 hr							36236
19	Pre							7887
20	24 hr							34562
26	Pre							4871
27	24 hr							29158
40	Pre							2236
41	24 hr							19753
57	Pre							1298
58	24 hr							19903
68	Pre							1101
69	24 hr							11049
82	Pre							2224
83	24 hr							12032
92	Pre							10503

14. Appendix Table No.1: Analysis of Interleukin-1 Trap Antibodies in Monkey Serum

Group	Monkey No.	Sex	Level mg/kg	Conc. mg/ml	Days Volume mL/kg	Days						
						1	12	18	28	57	82	92
						Pre (RFU/mL)						
1	1	F	0	0	0.60							
1	2	M	0	0	0.60							
1	3	F	0	0	0.60							
1	4	M	0	0	0.60							
1	5	F	0	0	0.60							
1	8	M	0	0	0.60							
2	7	F	1.5	25	0.06							
2	8	M	1.5	25	0.06							
2	9	F	1.5	25	0.06							
2	10	M	1.5	25	0.06							
2	11	F	1.5	25	0.06							
2	12	M	1.5	25	0.06							
						Mean	11.3400	33.6892	68.6934	150.4278	173.2080	94.6530
3	13	F	5.0	25	0.20							
3	14	M	5.0	25	0.20							
3	15	F	5.0	25	0.20							
3	16	M	5.0	25	0.20							
3	17	F	5.0	25	0.20							
3	18	M	5.0	25	0.20							
						Mean	14.2040	22.6491	53.3648	104.6172	254.2261	208.6392
4	19	F	15	25	0.80							
4	20	M	15	25	0.80							
4	21	F	15	25	0.80							
4	22	M	15	25	0.80							
4	23	F	15	25	0.80							
4	24	M	15	25	0.80							
						Mean	5.7850	47.9118	148.6254	477.8283	573.0741	356.6732

Study title: A 26-Week Subcutaneous Toxicity Study of IL-1 Trap in Cynomolgus Monkeys Followed by an 8-Week Recovery Period

Key study findings:

- Cynomolgus monkeys (7/sex/group) were treated subcutaneously, 3x/week for 26 weeks with 0, 15, 25, 40, or 60 mg/kg. Two animals/sex /group (except the 15 mg/kg dose group) were followed for an additional 8-weeks recovery period.
- No NOAEL could be established in this study due to the occurrence of adverse findings at low dose. The animal #24 (15 mg/kg dose group) showed respiratory distress on Day 180, and myocarditis which was believed to be due to the immune related hypersensitivity by the sponsor’s pathologist and reviewer. *The risk of these events to be repeated in human might be less because the protein is designed based on human protein structure. The sponsor mentioned that in the clinical development 3% of the patients showed similar reaction. But the intensity of the reaction was much less than what was observed in the animals.*
- There were two unscheduled deaths in this study; one male (#46) from 40 mg/kg showed clinical signs of discomfort at Day 40, dosing was discontinued, the animals continue to show discomfort, therefore, sacrificed at Day 45, histopathology showed myocarditis; another male (#54) died after dosing on Day 108, histopathology showed congestion in several organs like kidney etc., this

animals also showed congestion in lung due to granuloma as indicated by edema and perivascular lung blockage. Both of these animals did show high antibody titer, and the sponsor believes that the cause of deaths resulted from immune mediated hypersensitivity reaction. The reviewer agrees with sponsor analysis of data from these unscheduled deaths.

- Other adverse events consists of the following: one male from low dose showed adverse events consisting of breathing discomfort at day 180 (recovered by itself) and signs of myocarditis; one female treated with 40 mg/kg showed clinical signs of hypersensitivity, histopathology in this animal was associated with increased foci at GI tract; and two other males at high dose showed clinical sign of discomfort, hunched posture etc. around Day 40, dosing was discontinued, these animals was sacrificed at the end of Week 26, the histopathology showed congestion in different organs, mononuclear cell infiltration in heart and kidney. The clinical signs and histopathological consequences associated with these adverse events appeared to be coincided with the time when the antibody titer was high in these animals. Therefore, the reviewer concurs with the Sponsor's analysis of the data that these events are immune complex mediated hypersensitivity reaction.
- The hematology changes were associated with statistically significant decrease in erythrocytes and hemoglobin in males, and non-statistically significant increase in eosinophils in males and females which might be associated with the immune complex-related hypersensitivity reaction observed in few animals.
- The serum chemistry changes were dose related increase in the number of animals showing low albumin and low calcium. The significance of these findings is not known. However, low calcium might be associated with treatment-induced decrease estrogen production and might further be related to the decrease in the width of the bones in the knee joints observed histopathologically.
- There was a dose related increase in urinary total protein and blood cells in urine; this might be associated with clearance of the high load of the treatment protein as well as immune complex.
- Histopathological findings show increased incidence of myocardial degeneration which was mostly associated with the anti-product antibody formation except in one animal #16, in which no antibody formation was observed, the animal showed high IL-1 Trap at 26 weeks, terminal necropsy showed myocardial degeneration, no adverse events, however, were seen in these animals.
- Histopathological findings also showed glomerulonephritis in one female at 40 mg/kg dose and one male at 60 mg/kg dose. Anti-product antibody formation was high in these animals. However, no eosinophil in filtration was noted. Mononuclear cell infiltration was noted in kidney.
- There was a decrease in lung weight, in animals from terminal necropsy as well as necropsy; however, no histological correlation was noted. There was an increase in the weight of the pituitary (75% increases) and adrenal (30-42% increase) from the terminal necropsy, no histological changes were noted. However, IL-1 is known to effect the adreno-hypophysis axis, therefore, inhibition of IL-1 might result different hormone secretion without histological changes. Incomplete recovery of these parameters was noted after the recovery period.

- There was a dose related decrease in the thymus cellularity with incomplete recovery noted at the end of the experimental period; this effect may be related to the immunosuppressive activity of the compound.
- There was a decrease in the width of the femur growth plate at the knee joint and closer of the knee plate, the number of animals with such findings increase with dose indicating that the finding was treatment related.

Study no.: IL-1T-TX03021

Volume # and page #: eCTD submission; Volume 1, Page 1-1418

Conducting laboratory and location: _____

Date of study initiation: April 16, 2004

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: IL-1 Trap, Lot # B03015VD600E21A (relative potency _____ and Lot # B03015 D600E22A (relative potency _____ Drug Product description: IL-1 Trap recombinant protein, 40 mg/mL in a _____ aqueous vehicle, pH _____ containing _____ histidine. _____ o glycine _____ arginine, _____ sucrose, _____ polyethylene glycol 3350, _____

The placebo has all the components of the drug product except the IL-1 Trap placebo Lot # B04002V700F11A

Methods

Doses: 0, 15, 25, 40, and 60 mg/kg administered 3x/ week, subcutaneously for 26 weeks followed by an 8-week recovery period.

Species/strain: Cynomolgus monkey

Number/sex/group or time point (main study): 7/sex/group in all groups except the low dose group (see the study design table below).

Route, formulation, volume, and infusion rate: Subcutaneous. The compound was supplied by the Sponsor as drug product with a concentration of 40 mg/mL. The volume administered was different in different treatment group (see the study design table below); the treatment was administered as bolus.

Satellite groups used for toxicokinetics or recovery: Toxicokinetic analysis were done from all animals, 2 animals/sex/group from control, low mid, high mid and high dose group was followed for additional 8-weeks after the treatment was stopped for recovery.

Age: 2-9 years old

Weight: Males weighed 3.14-6.72 kg; females weighed 2.62-4.01 kg

Sampling times:

Unique study design or methodology (if any):

Table A: Study Design

Treatment Group/ Color Code	Test and Control Articles	Dose Level (mg/kg)	Dose Concentration (mg/mL) ^a	Dose Volume (mL/kg) ^b	Number of animals		Study Specific Animal Numbers	
					Females	Males	Females	Males
1 / white	IL-1 Placebo	0	0	1.50	7	7	1 ^d , 3 ^d , 5, 7, 9, 11 ^{c,d} , 13 ^{c,d}	2 ^d , 4 ^d , 6, 8, 10, 12 ^{c,d} , 14 ^{c,d}
2 / green	IL-1 Trap	15	40	0.38	5	5	15 ^d , 17 ^d , 19, 21, 23	16 ^c , 18 ^d , 20, 22, 24
3 / blue	IL-1 Trap	25	40	0.63	7	7	25 ^d , 27 ^d , 29, 31, 33, 35 ^{c,d} , 37 ^{c,d}	26 ^d , 28 ^d , 30, 32, 34, 36 ^{c,d} , 38 ^{c,d}
4 / red	IL-1 Trap	40	40	1.00	7	7	39 ^d , 41 ^d , 43, 45, 47, 49 ^{c,d} , 51 ^{c,d}	40 ^d , 42 ^d , 44, 46 ^e , 48, 50 ^{c,d} , 52 ^{c,d}
5 / orange	IL-1 Trap	60	40	1.50	7	7	53 ^d , 55 ^d , 57, 59, 61, 63 ^{c,d} , 65 ^{c,d}	54 ^d , 56 ^{d,e} , 58 ^e , 60, 62, 64 ^{c,d} , 66 ^{c,d}

^a Nominal dose concentration. Actual concentration of each lot of test article was recorded in the raw data and included in the Final Report.

^b Individual animal dosing volume (mL) was calculated based on the most recent body weight. Dose volumes were rounded up to the next readable syringe increment.

^c Two animals per sex in Groups 1, 3, 4, and 5 were designated as recovery animals and were euthanized following the 8-week recovery period.

^d Two terminal animals per sex in all groups were designated for Natural Killer (NK) Cell Activity and Cell Proliferation (CP) analysis. In addition, the 2 recovery animals per sex in Group 1, 3, 4, and 5 were also be tested for NK/CP analysis.

^e Dosing was stopped on animals # 58, # 56, and # 46 on Days 33, 39, and 40, respectively.

Observations Times and Results:

Mortality and Adverse event Findings:

The animals were monitored twice daily for the mortality or morbidity findings. There were six serious adverse event findings from this study (see table below) including two unscheduled deaths. Two males one each from 40 (#46) and 60 (#54) mg/kg died prior to scheduled sacrifice. The male #46 (40 mg/kg, dose group) showed signs of discomfort on Day 40, the dose was discontinued but the animal was sacrificed on Day 45 due to persistent decrease in the body weight and food consumption and swelling of face and neck. At necropsy slightly enlarged pale kidney, thickening of the injection site, raised skin in the midline near the injection site, and an organized thrombus in a large arteriole of the lung was also found. The male # 54 (60 mg/kg dose group), was unconscious slightly after dosing, congestion was noted in kidney, lungs, liver, adrenal, and thymus. Disturbance in circulatory and respiratory system appeared to be the cause of death. There were two other males in the high dose group which showed clinical signs of discomfort at Days 35-40, the dosing was discontinued in these animals and the animals were sacrificed at the end of the 26 weeks treatment period, the major histopathological changes from these animals showed mono and polymorphonuclear cell infiltration in kidney and heart. One male animal (#24) from the low dose group showed breathing problem just after dosing at Day 180, the animal, however, recovered spontaneously. The histopathological findings in this animal include myocardial cell degeneration. One

other female (#49) from the 40 mg/kg dose group showed clinical signs of breathing problem, dilated pupil, and loss of consciousness, the animals was treated with epinephrine periodically, the histopathology at the end of 26-week period showed changes in GI tract, raised foci in jejunum and ceca.

All the above mentioned clinical signs and histopathological findings are believed to be the result of immune mediated hypersensitivity reaction by the sponsor. Reviewer agrees with this conclusion. In general, the adverse event coincided with high serum titer in animals indicating that immune complex formation which might be related to these adverse findings.

Summary of Findings from Animals with Adverse Events:

Animal # & Dose	IL-1 Trap mcg/mL	TK of IL-1 Trap Ab	Clinical Signs	Clinical Pathology/ urinalysis	Histopathology
#46, male 40 mg/kg, dosing discontinued on Day 40, sacrificed on Day 45	NS	1386 mcg/mL	Swelling on neck and face, hunched position.	Increase urine protein and blood, in urine, increase in BUN, C reactive protein, serum creatinine, potassium, fibrinogen, decrease in serum albumin, calcium	Myocarditis; Myocardial cell necrosis, mono and polymorphonuclear cell infiltration in heart and kidney, granuloma on a large arteriole of lung, pale kidney, thickening of the skin at mid dorsal line
#56 male 60 mg/kg, dosing discontinued on Day 39, sacrificed on Day 182	132 at Day 40	128 mcg/mL at Day 40	Hunched position, liquid feces, irritation up to 39 days, then again around Days 80 - 120	Increase urine protein and blood, in urine, increase in BUN, C reactive protein, serum creatinine, potassium, fibrinogen, decrease in serum albumin, calcium, resolved at Day 182	Chronic inflammation of heart (mild), mono and polymorphonuclear cell infiltration in kidney
#58 male 60 mg/kg, dosing discontinued on Day 33, sacrificed on Day 182	322 at Day 40	843 mcg/mL at Day 40	Hunched position, liquid feces, irritation up to 39 days, then again around Days 80 - 120	Increase urine protein and blood, in urine, increase in BUN, C reactive protein, serum creatinine, potassium, fibrinogen, decrease in serum albumin, calcium, resolved at Day 182	Mono and polymorphonuclear cell infiltration in kidney, and liver, hemosiderin in perivascular lung
#54 male, 60 mg/kg, animals collapsed and sacrificed	NS	1500 mcg/mL at Day 80, no antibody assessme	No clinical signs	Clinical pathology not done	Moderate congestion of kidney, lung, adrenal, liver and thymus, edema in lung, vascular

on Day 108		nt was done after Day 80			damage in lung
#24 male, 15 mg/kg, animals showed adverse reaction post injection at Day 180	NS	2195 mcg/mL	One minute post dose, animal face become reddish, and breathing become abnormal, the animal recovered spontaneously	Increased fibrinogen	Myocarditis. Myocardial cell necrosis, Increase urine protein and blood, in urine, increase in BUN, C reactive protein, serum creatinine, potassium, fibrinogen, decrease in serum albumin, calcium
#49 female, animals showed adverse reaction post injection on Day 110, 115, 152	NS	4055-563 mcg/mL from Days 80 - 234	Dilated pupil, falling in and out of consciousness, cold to touch, labored breathing, the animal vomited food on Day 152	No major changes	Raised nodules on cecal mucosa, widely scattered white raised firm foci on serosa of the jejunum

Clinical signs:

The clinical signs in the animals were monitored twice daily throughout the experimental period. There were no dose related changes in the clinical signs. However, the treated animals showed more clinical signs than control animals. Following are the major changes observed in males and females from the different dose groups.

Changes in males: In low dose group males (15 mg/kg), the clinical signs observed were minimal, only soft stools were noted occasionally. There was an increase in the number of males from the high, and high mid dose groups (40 and 60 mg/kg) with loss of fur, increased irritation, and hunched position at the end of the experimental period compared to those of the control animals. The clinical signs in the males were most profound in the low mid dose group (25 mg/kg). Two out seven males (#36 and #38) showed abnormal clinical signs like head trauma, swelling in hind limb between 30 - 120 day, these animals also showed skin abnormalities (like scales and occasional scabs) and loss of fur all over the body around Month 4. These conditions persisted through the end of the study indicating that the treatment was not well tolerated in the animals. Another male from this dose group (#26) continue to show discharge from the nose and irritation for a long time almost up to 6 months, this animals also had hunched position occasionally.

Changes in females: In females from the high and high mid dose group, the clinical signs observed were loss of fur, and irritation etc. These findings are similar to those observed in males from the high dose groups. The number of females showing these clinical signs

was, however, higher (4-5 out of 7) compared to those of the males (2/7). Three females from each group (40 and 60 mg/kg) had foamy emesis between Day 87 - 90. The clinical signs of irritation and fur loss were seen up to the end of the experimental period indicating that these two doses were not very well tolerated in the female. In the low dose group bleeding in genitalia was noted in one female for the first few days after the administration of the drug. At least one animal from the 15 and 25 mg/kg showed emesis around Day 124, there was an increase in number of females with loss of fur and hunched position during 4 - 10 weeks, after which the animals from the two low dose groups did not show any clinical signs.

The skin abnormalities observed at high dosages might be directly related to the compound, the plasma concentration of the compound at high dosages was high and the number of animals producing antibody at high dosages were low. The other clinical signs like loss of fur, hunched position etc. were seen at low as well as high dosages. These clinical signs might be related to the general discomfort of the body associated either with immune complex formation or the high plasma concentration of the compound.

Body weights:

There were no treatment related changes in body weight of the animals under this experimental condition.

Food consumption:

The food consumption was measured twice daily.

There was a decrease in food consumption in 2/7, 1/7 and 1/7 females from 25, 40, and 60 mg/kg dose group and 3/7 males from the high dose groups (see sponsor's table below). The changes occurred transiently. The decrease in the food consumption appeared to be directly or indirectly related to the treatment. The IL-1 Trap plasma concentration in animal #27 and #37 at the time of decrease food consumption were 1046 µg/mL and BLQ respectively. At the same time the serum concentration of the antibody in the animal #27 and #37 were BLQ and 1549 µg/mL respectively. The IL-1 Trap exposure and the anti-product antibody exposure between Days 30 - 40 in the animal #46 was 3.8 and 1386 µg/mL respectively. The IL-1 Trap exposure and the anti-product antibody exposure between Days 30 - 40 in the animal #54 was 1.5 and 587 µg/mL respectively. The IL-1 Trap exposure and the anti-product antibody exposure between Days 4 - 43 in the animal #56 was 132 and 128 µg/mL respectively. There was no anti-product antibody formation in animal #66 and #61; however, the IL-1 Trap concentration around Day 97 - 122 in animal #61 and #66 and 1534 was 2492 µg/mL respectively. The IL-1 Trap exposure and the anti-product antibody exposure between Days 97 - 100 in the animal #61 was BLQ. The exposure data mentioned above indicate that the decrease in food consumption may be directly related to IL-1 Trap concentration (#27, #61 and #66) or indirectly due to immune complex formation (#37, #46, #54), or may be due to exposure of IL-1 Trap and the immune complex together in the body (#56).

Decrease in Food Consumption:

Group	Animal #	Sex	Days Showing Decrease
3	27	Female	Days 76- 80
	37	Female	Days 78-83
4	46	Male	Days 30-33, 36-40
5	54	Male	Days 34-37
	56	Male	Days 40-43
	66	Male	Days 119-122
	61	Female	Days 97-100

Ophthalmoscopy:

Ophthalmologic examinations were conducted in all animals during acclimation, and Weeks 13, 26, and 35 prior to necropsy. No ophthalmological changes were noted in this experiment.

EKG:

All animals were examined for ECG using leads, QT changes, and heart rate during acclimation, and Weeks 13, 26, and 35 prior to necropsy. There were no treatment-related changes in the ECG parameters.

Hematology:

The hematology and coagulation assessment was performed on all animals twice during acclimation and during Weeks 2, 4, 7, 10, 13, and 26 and at the end of the recovery period at Week 34.

The hematology and the coagulation parameters evaluated and the methodology followed is reproduced from the Sponsor’s table below.

Parameter	Method	Apparatus	Units
Erythrocyte count (RBC)			X10e6/ μ L
Leukocyte count (WBC)			X10e3/ μ L
Hematocrit value (HCT)	Calculation		%
Hemoglobin concentration (Hgb)			dL
Platelet count (PLT)			X10e3/ μ L
Mean platelet volume (MPV)	Calculation		fL
Mean corpuscular volume (MCV)	Calculation		fL
Mean corpuscular hemoglobin (MCH)	Calculation		pg
Mean corpuscular hemoglobin concentration (MCHC)	Calculation		g/dL
Reticulocyte count (Retic)			%
Differential leukocyte count			X10e3/ μ L

Parameter	Method	Apparatus	Units
Prothrombin time (PT)			seconds
Activate Partial Thromboplastin Time (APTT)			seconds
Fibrinogen (Fib)			mg/dL

Summary of Changes in the Hematology Parameters: 6-Month SC

Parameters	Dose Groups							
	Male				Female			
	0 mg/kg	15 mg/kg	40 mg/kg	60 mg/kg	0 mg/kg	15 mg/kg	40 mg/kg	60 mg/kg
Erythrocyte x10E3/μL								
Days -12	5.7 \pm 0.2	5.1 \pm 0.4	5.4 \pm 0.3	5.6 \pm 0.1	5.3 \pm 0.2	5.4 \pm 0.1	5.2 \pm 0.4	5.5 \pm 0.5
Week 13	5.7 \pm 0.1	5.0 \pm 0.4	5.1 \pm 0.1	5.2 \pm 0.6	5.1 \pm 0.2	5.3 \pm 0.2	5.1 \pm 0.4	5.3 \pm 0.3
Week 26	5.6 \pm 0.1	5.1 \pm 0.4	5.3 \pm 0.2	5.2 \pm 0.4 8% \downarrow	5.2 \pm 0.2	5.2 \pm 0.1	5.1 \pm 0.2	5.3 \pm 0.3 3% \downarrow
Week 34	6.04		5.9	5.6	5.3		5.2	5.3
Eosinophil x10E3/μL								
Days -12	0.1 \pm 0.01	0.09 \pm 0.08	0.2 \pm 0.1	0.09 \pm 0.04	0.13 \pm 0.07	0.19 \pm 0.1	0.18 \pm 0.07	0.26 \pm 0.02
Week 13	0.16 \pm 0.08	0.18 \pm 0.05	0.37 \pm 0.03	0.49 \pm 0.07	0.15 \pm 0.1	0.21 \pm 0.1	0.22 \pm 0.1	0.27 \pm 0.03
Week 26	0.15 \pm 0.07 1.5-fold \uparrow	0.25 \pm 0.1	0.4 \pm 0.03	0.29 \pm 0.04 3-fold \uparrow	0.19 \pm 0.1	0.22 \pm 0.1	0.43 \pm 0.07	0.4 \pm 0.03
Week 34	0.1		0.8	0.13	0.19		0.14	0.36

1. The major hematological changes noted at Week 26 were a 3-fold increase in the eosinophil count at high dose compared to 1.3 fold increase in the eosinophil count in the control males. There were no such changes in females.
2. There was a very slight decrease in the erythrocyte counts in males and females.

The increase in the eosinophil counts in males might be related to the histopathological changes observed in these animals. One reason for such increase might be hypersensitivity; however, why males would more susceptible to such changes is not known.

Clinical chemistry:

The serum chemistry assessment was performed on all animals once during acclimation and during Weeks 4, 7, 10, 13, and 26 and at the end of the recovery period at Week 34.

The serum chemistry parameters evaluated and the methodology followed is reproduced from the sponsor's table below. There were no treatment related changes in the serum chemistry parameters.

Parameter	Method	Apparatus	Units
Albumin (Alb)			g/dL
Alanine Aminotransferase (ALT)			U/L
Alkaline Phosphatase (ALP)			U/L
Aspartate Aminotransferase (AST)			U/L
Bilirubin, Total (Tbili)			mg/dL
Blood Urea Nitrogen (BUN)			mg/dL
Calcium (Ca)			mg/dL
Chloride (Cl)			mEq/L
Cholesterol, Total (TChol)			mg/dL
Creatinine (CRN)			mg/dL
Creatine Phosphokinase (CK)			U/L
Gamma Glutamyl Transferase (GGT)			U/L
Glucose (Glu)			mg/dL
Lactate Dehydrogenase (LDH)			U/L
Phosphorus, inorganic (IP)			mg/dL
Potassium (K)			mEq/L
Protein, total (TP)			g/dL
Sodium (Na)			mEq/L
Triglyceride (Trig)			mg/dL

Urinalysis:

The serum chemistry assessment was performed on all animals once during acclimation and during Weeks 4, 7, 10, 13, and 26 and at the end of the recovery period at Week 34.

The sponsor measured following urinalysis parameters using the equipments and methods listed below.

Parameter	Method	Apparatus	Units
Color	Visual	Not Applicable	NA
Volume	Manual	Volumetric Cylinder	mL
Specific gravity	Refractometer		NA
pH	Test strips		NA
Protein	Test strips		mg/dL
Glucose	Test strips		mg/dL
Ketones	Test strips		mg/dL
Bilirubin	Test strips		NA
Occult Blood	Test strips		NA
Urobilinogen	Test strips		mg/dL
Nitrites	Test strips		NA
Leukocytes	Test strips		NA
Urine Total Protein			mg/dL

The statistically significant changes in the urinalysis are listed below

1. There was a dose related increase in the urinary total protein, 2/7 males each from dose ages 25, 40, and 60 mg/kg at Week 4. One other male from the high dose also had high urinary protein at Week 10.
2. At least one male out of 2 from each group with high total urinary protein had abnormal protein.
3. All of the animals which showed high total protein level at 40 and 60 mg/kg showed high incidence of blood cells in urine, at similar time points. In addition, one other male at high dose also showed similar findings.

All the above mentioned findings in males are considered test article related by the sponsor and the reviewer. However, no such changes were noted at week 26 and at recovery.

Gross pathology:

The lesions from the terminal as well as recovery necropsy and their correlation with the microscopic lesion are reproduced below from the sponsor's table:

There were two treatment related findings of macroscopic lesions associated with the microscopic lesions in the low and low mid dose. One is thymic atrophy and the other is foci on the surface of the lung. Interestingly such changes were not noted in the high dose groups which is suggestive of indirect effect of the compound related to immune complex formation. The finding of enlarged lymph node associated with hyperplasia observed at high dose might be directly related to the IL-1 Trap exposure. During the recovery, adhesion of pericardium of heart and adhesion of lungs with the thoracic wall (associated with fibroplasia and mononuclear cell infiltration) was noted in animal #63 and 51 respectively (both are females from the high dose group). These are new findings not noted during the treatment period.

SSAN	Group	Gross lesion	Microscopic lesion
3	Terminal 1	Small cysts on pleural surface of lungs	Black/brown pigment, perivascular, peribronchiolar, very slight
20	Terminal 2	Marked thymic atrophy	Decreased cellularity, moderate
21	Terminal 2	Multiple non-raised foci on dorsal pleural surface of lungs	Black/brown pigment, perivascular, peribronchiolar, very slight
30	Terminal 3	Marked thymic atrophy	No thymus in trimmed tissue
25	Terminal 3	Marked thymic atrophy	Decreased cellularity, moderate
33	Terminal 3	Few clear foci on surface of lungs	Black/brown pigment, perivascular, peribronchiolar, very slight
42	Terminal 4	Red nodules on mucosa of cecum	None
42	Terminal 4	Slight adhesion of lung pleura to thoracic wall	None
47	Terminal 4	Slight bilateral enlargement of axillary and inguinal lymph nodes	Slight hyperplasia, paracortex of axillary and none for inguinal lymph nodes.
49	Recovery 4	Slightly raised red nodules on the cecal mucosa	None
49	Recovery 4	Widely scattered white foci on jejunum, raised, firm, serosa	None
51	Recovery 4	Left side of lung adhered to the thoracic wall, aorta and pleura	Fibroplasia, mononuclear cell infiltration, pleura, slight
63	Recovery 5	Slight adhesion of pericardium to heart	None

Organ weights:

The major weight changes are reported in this section as follows and will be discussed in the histopathology section.

1. There was an increase in the adrenal weight (28%) in males and 42% in females at low dose. The increase in adrenal weight was also seen in the 25, and 40 mg/kg. No such changes were noted at recovery.
2. The weight of the pituitary gland increased significantly in females, higher increase (75%) was seen at low dose; increase at high dose was 37%. No such changes were seen in males. However, during recovery the weight of the pituitary increased (66%) in males. The weight of the pituitary in females decreased compare to control at this time with all different dosages.
3. There was a marked decrease (60 - 70%) in the thymus weight in male and females at all dose during terminal necropsy and the recovery period.

Summary of Changes in Relative Organ Weights:

Organ weights Relative to Brain (%)	Male					Female				
	Dose (mg/kg)									
	0	15	25	40	60	0	15	25	40	60
Terminal sacrifice										
Adrenal	0.68	0.78	0.72	0.78	0.68	0.7	1.0	0.8	0.72	0.8
Pituitary	0.1	0.1	0.1	0.11	0.09	0.08	0.14	0.1	0.1	0.11

Thymus	4	2.8	2.3	4.4	4.2	5.1	3.3	3.9	3.5	4.2
Recovery										
Adrenal	0.8		0.8	0.6	0.7	0.5		0.45	0.6	0.4
Pituitary	0.06		0.09	0.08	0.1	0.12		0.11	0.10	0.06
Thymus	8		5	4	6	9.5		7	5	6

Histopathology: Adequate Battery: Yes. Peer review: Yes.

The major histopathological changes in the animals from the **unscheduled deaths** are described above in the mortality section of the result. The detail finding from these animals is reproduced in the sponsor's table below. Note that myocardial degeneration, congestion of the kidneys is the major findings from the animal #46 (40 mg/kg dose group, male, sacrificed on Day 45). And the major findings from the animal #54 were congestion of the kidneys and congestion of the lungs due to perivascular hemorrhage and edema, autolysis in ileum and jejunum. Following gradations were used to analyse the severity of the histology findings.

Grade

- : No abnormal changes
- ± : Very slight
- + : Slight
- 2+ : Moderate
- 3+ : Marked
- P : Non-graded change
- U : Unexamined

Table 17 - 19 Histopathology Findings in Male Cynomolgus Monkeys (Moribund Sacrifice)

Study No. ~~306~~ 223

[H.E. staining]	Group Control & Test Articles Dose (mg/kg) SSAN	Group				
		1	2	3	4	5
		IL-1 Placebo	IL-1 Trap	IL-1 Trap	IL-1 Trap	IL-1 Trap
Organs/Tissues		0	15	25	40	60
Findings		NA	NA	NA	46(a)	54(b)
Adrenals						
Congestion					-	+
Aorta (thoracic)					-	U
Brain stem (medulla oblongata)					-	-
Cecum					-	-
Autolysis					-	2+
Cerebellum					-	-
Cerebrum (thalamus)					-	-
Colon					-	-
Autolysis					-	+
Duodenum					-	-
Autolysis					-	2+
Epididymides					-	UN
Immature but normal					P	-
Esophagus (thoracic)					-	-
Eye ball/Optic nerve, left					-	-
Eye ball/Optic nerve, right					-	-
Femur with knee joint, left (bone/bone marrow)					-	-
Closed growth plate					-	P
Femur with knee joint, right (bone/bone marrow)					-	-
Closed growth plate					-	P
Gall bladder					-	-
Heart (left ventricle, right ventricle)					-	-
Mononuclear/polymorphonuclear cell (eosinophil) infiltration					2+	U
Myocardial cell degeneration/mineralization, focal					2+	U
Necrosis					2+	U
Heart (interventricular septum, left atrium)					-	-
Mononuclear cell infiltration					±	U

Table 17 - 20 Histopathology Findings in Male Cynomolgus Monkeys (Moribund Sacrifice) Study No. 223.17

(H.E. staining)	Group	Control & Test Articles				
		1	2	3	4	5
Organs/Tissues	Dose (mg/kg)	IL-1 Placebo	IL-1 Trap	IL-1 Trap	IL-1 Trap	IL-1 Trap
Findings	SSAN	NA	NA	NA	45(a)	54(b)
Ileum						
Autolysis					-	+
Jejunum						
Autolysis					-	+
Injection sites. (skin, back, thorax, upper)						
Fibroplasia, subcutis					-	2+
Macrophage/mononuclear cell infiltration, subcutis					+	-
Mononuclear/polymorphonuclear cell (eosinophil) infiltration, perivascular, subcutis					-	+
Injection sites. (skin, back thorax, lower)						
Fibroplasia, subcutis					-	2+
Macrophage/mononuclear cell infiltration, subcutis					+	-
Mononuclear/polymorphonuclear cell (eosinophil) infiltration, perivascular, subcutis					-	+
Kidney, left						
Congestion					-	2+
Increase, mesangial matrix, glomeruli					2+	-
Mineralization					+	-
Mononuclear/polymorphonuclear cell (eosinophil) infiltration, interstitium/perivascular					+	-
Tubular casts/degeneration					+	-
Kidney, right						
Congestion					-	2+
Increase, mesangial matrix, glomeruli					2+	-
Mineralization					+	-
Mononuclear/polymorphonuclear cell (eosinophil) infiltration, interstitium/perivascular					+	-
Tubular casts/degeneration					±	-
Lacrimal gland (left)						
Mononuclear cell infiltration					-	±
Liver, left lobe						
Congestion					-	+
Mononuclear cell infiltration, perivascular, sinusoidal					±	-
Liver						
Congestion					-	+
Mononuclear cell infiltration, perivascular, sinusoidal					±	-
Lungs/Bronchi						
Brown/black pigment, perivascular/peribronchiolar					±	±
Congestion, perivascular hemorrhage and edema					-	2+
Organized thrombus, large arteriole					p	-
Lymph node, cervical						
Black pigment					+	-

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The major histopathological changes from the **schedule necropsy** are listed below.

1. There was an increase in the incidence of the **injection site reaction** in the treatment group (76% male and 91% female) compared to the controls (5% in male, and 17% in female). The changes were slight to moderate. The severity of the changes was higher at low dose. These changes are consisted of mononuclear and polymorphonuclear cell infiltration related to inflammation and considered as treatment related. In the recovery group no animals from the high dose group showed injection site reaction, however, 4/4 and 3/4 animals from the 25, and 40 mg/kg group showed similar effect compared to that of the terminal necropsy indicating incomplete recovery in these dose groups. Detailed of the histopathological findings related to changes in the injection sites are shown from the sponsors table below.

Injection sites

Table 17 - 1 Histopathology Findings in Male Cynomolgus Monkeys (Terminal Sacrifice) Study No. 223.17

(H.E. staining)	Group	Control & Test Articles																						
		1		2		3		4		5														
Organs/Tissues	Dose (mg/kg)	IL-1 Placebo		IL-1 Trap		IL-1 Trap		IL-1 Trap		IL-1 Trap														
Findings	SSAN	2	4	6	8	10	16	18	20	22	24	28	28	30	32	34	40	42	44	48	58	58	60	62

Males: Terminal Sacrifice

severity in the glomerulonephritis in these animals. The increase in the kidney weight in the treated males might be related to the histopathological changes associated with the increased mono and polymorphonuclear cell infiltration in the animals from the treatment group. Increase in mononuclear cell infiltration was noted in higher incidence in the high dose animals (see histopathology summary table). At recovery, mononuclear cell infiltration was still noted indicating partial recovery, however, changes in the mesangial matrix of the kidney was not evident at this time. Detail of the kidney changes are reproduced below from the sponsors' table.

Kidney

Table 17 - 1 Histopathology Findings in Male Cynomolgus Monkeys (Terminal Sacrifice) Study No. ~~125-249~~ 223.17

[H.E. staining]	Group Control & Test Articles	Group																							
		1 IL-1 Placebo		2 IL-1 Trap		3 IL-1 Trap	4 IL-1 Trap		5 IL-1 Trap																
Organs/Tissues	Dose (mg/kg)	0		15		25		40		80															
Findings	SSAN	2	4	6	8	10	16	18	20	22	24	26	28	30	32	34	40	42	44	48	58	58	60	62	
Male: Terminal Sacrifice																									
Kidney, left																									
Increase, mesangial matrix, glomeruli		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Medial cell proliferation, arterioles		-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Vasculitis, arterioles		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Mineralization		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mononuclear cell infiltration, interstitium/perivascular		-	-	±	±	-	-	-	-	-	-	±	±	-	-	-	-	±	±	-	-	±	±	-	-
Mononuclear/polymorphonuclear cell (eosinophil) infiltration, interstitium/perivascular		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tubular casts/degeneration		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±
Kidney, right																									
Increase, mesangial matrix, glomeruli		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Medial cell proliferation, arterioles		-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Vasculitis, arterioles		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Mineralization		±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±
Mononuclear cell infiltration, interstitium/perivascular		±	-	±	±	±	-	-	-	-	-	±	±	-	-	-	±	±	-	-	±	±	-	-	±
Mononuclear/polymorphonuclear cell (eosinophil) infiltration, interstitium/perivascular		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±
Tubular casts/degeneration		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

Male Recovery

[H.E. staining]	Group Control & Test Articles	Group							
		1 IL-1 Placebo		3 IL-1 Trap		4 IL-1 Trap		5 IL-1 Trap	
Organs/Tissues	Dose (mg/kg)	0		25		40		80	
Findings	SSAN	12	14	30	38	50	52	64	68
Male Recovery									
Kidney, left									
Mineralization		±	±	-	-	-	-	-	-
Mononuclear cell infiltration, interstitium/perivascular		±	±	±	-	±	±	±	±
Kidney, right									
Mineralization		-	-	-	±	-	-	-	-
Mononuclear cell infiltration, capsule		-	-	-	-	-	-	-	-
Mononuclear cell infiltration, interstitium/perivascular		-	±	±	±	±	±	-	±

Females: Terminal Necropsy

[H.E. staining]	Group Control & Test Articles	Group																									
		1 IL-1 Placebo		2 IL-1 Trap		3 IL-1 Trap	4 IL-1 Trap		5 IL-1 Trap																		
Organs/Tissues	Dose (mg/kg)	0		15		25		40		80																	
Findings	SSAN	1	3	5	7	9	15	17	19	21	23	25	27	29	31	33	39	41	43	45	47	53	55	57	59	61	
Females: Terminal Necropsy																											
Kidney, left																											
Increase, mesangial matrix, glomeruli		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Mineralization		±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Mononuclear cell infiltration, interstitium/perivascular		±	±	±	-	-	±	±	-	-	-	±	±	-	-	-	±	±	-	-	±	±	-	-	±	±	
Subcapsular cysts		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Kidney, right																											
Increase, mesangial matrix		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Mineralization		±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Mononuclear cell infiltration, interstitium/perivascular		±	±	±	±	±	-	-	±	-	-	±	±	-	-	-	±	±	-	-	±	±	-	-	±	±	
Lacrimal gland (left)																											
Mononuclear cell infiltration		U	-	U	-	-	-	U	-	-	-	±	-	-	-	-	-	-	-	-	-	-	-	U	-	-	

Female Recovery

[H.E. staining]	Group Control & Test Articles	Group							
		1 IL-1 Placebo		3 IL-1 Trap		4 IL-1 Trap		5 IL-1 Trap	
Organs/Tissues	Dose (mg/kg)	0		25		40		80	
Findings	SSAN	11	13	35	37	49	51	63	65
Female Recovery									
Kidney, left									
Mineralization		±	-	±	+	-	-	-	-
Mononuclear cell infiltration, interstitium/perivascular		±	±	±	±	±	±	±	±
Kidney, right									
Mineralization		-	-	-	±	-	-	-	-
Mononuclear cell infiltration, interstitium/perivascular		±	-	-	±	±	-	-	-

3. The histological changes in the **heart** were not associated with gross lesion or weight changes. Myocardial cell degeneration and necrosis was observed in one male (#24) in 25 mg/kg, the changes in this animal were similar to the animal #46, the animal that died during the experiment. The peer review pathologist suggested that the necrosis in the heart may be associated with hypersensitivity reaction as eosinophil was observed in the tissue. The catecholamine released by the stressed adrenals might also result such changes, an increase in the adrenal weight is also noted in the animals from the treatment group indicating increased stress condition. One out of fourteen control animals showed myocardial necrosis. The incidence of the occurrence of the myocardial necrosis in the 15, 25, 40, and 60 mg/kg dose groups were 2/10 (animals #15 and #16), 3/14 (#24, #31, and #36), 1/14 (#46), and 1/14 (#62). The antibody titer in the animals #15, 16, 24, 31 and, 36, 46, and 62 at Day 180 was 2354, BLQ, 2195, 2223, 2139, BLQ, and 197 mcg/mL respectively. The IL-1 Trap plasma concentration in the animals #15, 16, 24, 31 and, 36, 46, and 62 at Day 180 was 41, 800, not significant (ns), (BLQ), (ns), and (ns) µg/mL respectively. Note that the animal #16 is the only animal which showed apparent discomfort like respiratory problem at the end of the treatment period at Day 180, this animal recovered spontaneously and immediately. However, it is noted that there was no antibody formation in this animal during the treatment period, also, IL-1 Trap plasma concentration in this animal at the time of the adverse event was approximately 800 µg/mL indicating that this finding is directly related to the IL-1 Trap treatment. IL-1 is known to have profound effect on adrenal both cortex and medulla, therefore, inhibition of IL-1 might relate to adrenomedullary hormonal secretion which are known to produce myocardial cell degeneration in different concentrations. The necrosis in all of these animals was associated with mononuclear cell infiltration. The pathologist suggested that myocarditis induced by catecholamine and hypersensitivity although differ in morphology, may not be mutually exclusive and both might have occurred in #24 and #46. Myocardial necrosis was not observed in the recovery animals. A detail histopathology finding from the sponsor is reproduced from the sponsor's table below.

Heart

Table 17 - 1 Histopathology Findings in Male Cynomolgus Monkeys (Terminal Sacrifice) Study No. [REDACTED] 223.17

[H.E. staining]	Group	Control & Test Articles				
		1	2	3	4	5
Organs/Tissues	Dose (mg/kg)	IL-1 Placebo	IL-1 Trap	IL-1 Trap	IL-1 Trap	IL-1 Trap
		0	15	25	40	60
Findings	SSAN	2 4 6 8 10	16 18 20 22 24	26 28 30 32 34	40 42 44 46	50 56 60 62

Male: Terminal Necropsy

Heart (left ventricle, right ventricle)		1		3		4		5	
Control & Test Articles		IL-1 Placebo		IL-1 Trap		IL-1 Trap		IL-1 Trap	
Dose (mg/kg)		0		25		40		60	
SSAN		12	14	36	38	50	52	64	66
Mononuclear cell infiltration		-	-	±	-	±	±	-	-
Mononuclear/polymorphonuclear cell infiltration		-	-	-	-	-	-	-	-
Mononuclear/polymorphonuclear cell (eosinophil) infiltration		-	-	-	+	-	-	-	-
Myocardial cell degeneration		-	-	-	-	-	-	-	-
Myocardial cell necrosis		-	-	±	-	-	-	-	-
Vasculitis		-	+	-	-	-	-	-	-
Heart (interventricular septum, left atrium)									
Chronic inflammation, epicardium, left atrium		-	-	-	-	-	-	-	-
Mononuclear cell infiltration		-	-	±	-	-	-	±	-
Mononuclear/polymorphonuclear cell infiltration, perivascular, pericardium, septum		-	-	-	-	-	-	-	-
Mononuclear cell infiltration, subendocardium, atrium		±	-	-	-	-	-	-	-
Vasculitis		-	+	-	-	-	-	-	-
Vasculitis, focal		-	-	-	-	-	-	+	-

Male: Recovery

[H.E. staining]		Group		1		3		4		5	
Control & Test Articles		IL-1 Placebo		IL-1 Trap		IL-1 Trap		IL-1 Trap		IL-1 Trap	
Dose (mg/kg)		0		25		40		60			
SSAN		12	14	36	38	50	52	64	66		
Heart (left ventricle, right ventricle)											
Mononuclear cell infiltration		-	-	±	±	±	±	±	-	-	-
Myocardial cell necrosis		-	-	±	-	±	-	-	-	-	-
Heart (interventricular septum, left atrium)											
Mononuclear cell infiltration, endocardium, left atrium		-	±	-	-	-	-	-	-	-	-
Mononuclear cell infiltration, left atrium		-	-	±	-	-	-	-	-	-	-

Female: Terminal Necropsy

[H.E. staining]		Group		1		2		3		4		5															
Control & Test Articles		IL-1 Placebo		IL-1 Trap		IL-1 Trap		IL-1 Trap		IL-1 Trap		IL-1 Trap															
Dose (mg/kg)		0		15		25		40		60																	
SSAN		1	3	5	7	9	15	17	19	21	23	25	27	29	31	33	39	41	43	45	47	53	55	57	59	61	
Heart (left ventricle, right ventricle)																											
Mononuclear cell infiltration		±	±	-	-	-	±	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Myocardial cell necrosis		-	-	-	-	-	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Heart (interventricular septum, left atrium)																											
Atherosclerosis, aorta		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Atherosclerotic plaque, aorta		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Mononuclear cell infiltration		-	-	±	±	-	-	-	-	-	±	±	±	-	-	-	-	-	-	-	-	±	±	±	±	-	
Myocardial degeneration		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

Female Recovery

[H.E. staining]		Group		1		3		4		5	
Control & Test Articles		IL-1 Placebo		IL-1 Trap		IL-1 Trap		IL-1 Trap		IL-1 Trap	
Dose (mg/kg)		0		25		40		60			
SSAN		11	13	35	37	49	51	63	65		
Heart (interventricular septum, left atrium)											
Mononuclear cell infiltration, left atrium		-	-	-	-	-	-	-	-	-	±
Mononuclear cell infiltration, septum		-	-	-	-	±	-	-	-	-	±

4. The decrease in the weight of the thymus tissue and atrophy of the thymus during gross necropsy observation was associated with the histopathological observation of the decreased cellularity and might be directly related to the pharmacology of the compound. This finding might be related to the expected immunosuppressive activity of the compound.

Thymus:

Male: Terminal Necropsy

Table 17 - 1 Histopathology Findings in Male Cynomolgus Monkeys (Terminal Sacrifice) Study No. 223.17

[H.E. staining]		Group		1		2		3		4		5											
Control & Test Articles		IL-1 Placebo		IL-1 Trap		IL-1 Trap		IL-1 Trap		IL-1 Trap		IL-1 Trap											
Dose (mg/kg)		0		15		25		40		60													
SSAN		2	4	8	10	16	18	20	22	24	26	28	30	32	34	40	42	44	48	60	66	68	
Thymus																							
Decreased cellularity		3+	+	-	-	U	-	2+	±	-	-	-	2+	-	2+	+	+	-	-	3+	-	-	
No thymus in trimmed tissue		-	-	P	-	U	P	-	-	-	P	P	-	-	-	-	-	-	-	-	-	-	

Male: Recovery

[H.E. staining]		Group		1		3		4		5	
Control & Test Articles		IL-1 Placebo		IL-1 Trap		IL-1 Trap		IL-1 Trap		IL-1 Trap	
Dose (mg/kg)		0		25		40		60			
SSAN		12	14	36	38	50	52	64	66		
Thymus											
Decreased cellularity		-	-	±	+	-	±	+	±	-	-

Female: Terminal Necropsy

[H.E. staining]	Group	1					2					3					4					5				
Organs/Tissues	Control & Test Articles	IL-1 Placebo					IL-1 Trap					IL-1 Trap					IL-1 Trap					IL-1 Trap				
Findings	Dose (mg/kg)	0					15					25					40					60				
Thymus	SSAN	1	3	5	7	9	15	17	19	21	23	25	27	29	31	33	39	41	43	45	47	53	55	57	59	61

Female Recovery

[H.E. staining]	Group	1				3				4				5			
Organs/Tissues	Control & Test Articles	IL-1 Placebo				IL-1 Trap				IL-1 Trap				IL-1 Trap			
Findings	Dose (mg/kg)	0				25				40				60			
Thymus	SSAN	11	13	35	37	49	51	63	65								

- The increase in weight of the **testes** may be associated with water retention; however, aspermatogenesis was noted in one animal at 40 mg/kg, mineralization of the seminal vesicle was also noted in this animal. Although the weight of the testes remained higher than the control in the recovery group, there were no histological changes associated with the weight changes of the testes during recovery. Two females at terminal necropsy had mononuclear cell infiltration and had decreased weight of the **ovary**, because the finding is at high dose, it appears to be related to the test article. Increase in weight of the ovaries during terminal necropsy was found to be associated with mineralization of the follicles; the incidence was higher in 15, and 25 mg/kg. Interestingly, no such incidence was noted at 40 mg/kg during terminal necropsy in ovary, however 2/2 females with this dose group from the recovery animals showed similar finding indicating late occurrence of toxicity at higher dose.
- The significant decrease in the **lung** weight in the animals during terminal and recovery was not associated with histological changes; however, immune complex mediated decrease in the lung weight might be one explanation for such changes. The histopathology findings from the sponsor's table are reproduced below.

Lung

Male: Terminal Necropsy

Table 17 - 1 Histopathology Findings in Male Cynomolgus Monkeys (Terminal Sacrifice) Study No. 223.17

[H.E. staining]	Group	1					2					3					4					5				
Organs/Tissues	Control & Test Articles	IL-1 Placebo					IL-1 Trap					IL-1 Trap					IL-1 Trap					IL-1 Trap				
Findings	Dose (mg/kg)	0					15					25					40					60				
Lungs/Bronchi	SSAN	2	4	6	8	10	16	18	20	22	24	26	28	30	32	34	40	42	44	46	48	54	56	58	60	62

Male: Recovery

[H.E. staining]	Group	1				3				4				5			
Organs/Tissues	Control & Test Articles	IL-1 Placebo				IL-1 Trap				IL-1 Trap				IL-1 Trap			
Findings	Dose (mg/kg)	0				25				40				60			
Lungs/Bronchi	SSAN	12	14	36	38	50	52	64	66								

Female: Terminal Necropsy

[H.E. staining] Organs/Tissues Findings	Group Control & Test Articles Dose (mg/kg) SSAN	1				2				3				4				5							
		IL-1 Placebo				IL-1 Trap																			
		0	15	25	60	15	19	21	23	25	27	29	31	33	39	41	43	45	47	53	55	57	59	61	
Lungs/Bronchi		±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
Brown/black pigment, perivascular/peribronchiolar		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hyperplasia, smooth muscle, terminal bronchioles		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mononuclear cell infiltration, interstitium, arteriolar walls		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Female: Recovery

[H.E. staining] Organs/Tissues Findings	Group Control & Test Articles Dose (mg/kg) SSAN	1		3		4		5					
		IL-1 Placebo		IL-1 Trap		IL-1 Trap		IL-1 Trap					
		0	25	40	60	11	13	35	37	42	51	63	65
Lungs/Bronchi													
Brown/black pigment, perivascular/peribronchiolar				±	±	±	±	±	±	±	±	±	±
Fibroplasia, mononuclear cell infiltration, pleura				-	-	-	-	-	-	-	-	-	-

- There was an increase in the organ weight of the pituitary gland compared to controls; the compound is expected to modulate different hormones, however, there were no histological changes associated with the weight change. The changes in the pituitary may be related to the over or under activation of hormone secretion due to the exaggerated pharmacology of the compound; however, in the absence of such data, this observation could not be explained.
- A unique finding with this compound was changes in the knee joints (closed growth plate) and decrease width of the growth plate in males and females with this compound is increase in the knee joints which is believed to be treatment related by the reviewer. In males increased incidence and severity of the findings were noted at 15 and 25 mg/kg group, no such changes were seen in recovery animals. In females, increase in the number of animal with similar incidence increased with dose. The severity index was higher at 40 mg/kg compare to the animals from other dose group. One out of two (1/ 2) females at recovery showed similar finding indicating incomplete recovery. The IL-1 related modulation of the bones are well known, the findings might be related to the exaggerated pharmacology of the compound. Changes in femur/knee joints are tabulated in sponsor's table as shown below.

Femur

Male: Terminal Necropsy

Table 17 - 1 Histopathology Findings in Male Cynomolgus Monkeys (Terminal Sacrifice) Study No. ~~125-249~~-223.17

[H.E. staining] Organs/Tissues Findings	Group Control & Test Articles Dose (mg/kg) SSAN	1				2				3				4				5							
		IL-1 Placebo				IL-1 Trap																			
		0	15	25	60	15	19	21	23	25	27	29	31	33	39	41	43	45	47	53	55	57	59	61	
Femur with knee joint, left (bone/bone marrow)																									
Closed growth plate		P	P	-	-	P	P	P	-	P	-	-	-	P	-	-	-	P	-	-	-	-	-	-	-
Decreased width, growth plate		-	-	-	-	-	-	-	-	-	-	-	-	2+	2+	2+	-	-	-	-	-	-	-	-	-
Femur with knee joint, right (bone/bone marrow)																									
Closed growth plate		P	-	-	-	P	P	P	-	P	-	-	-	P	-	-	-	P	-	-	-	-	-	-	-
Decreased width, growth plate		-	2+	-	-	-	-	-	-	-	-	-	-	2+	2+	2+	-	-	-	-	-	-	-	-	-

Male: Recovery

[H.E. staining] Organs/Tissues Findings	Group Control & Test Articles Dose (mg/kg) SSAN	1		3		4		5					
		IL-1 Placebo		IL-1 Trap		IL-1 Trap		IL-1 Trap					
		0	25	40	60	12	14	30	38	50	52	64	66
Femur with knee joint, left (bone/bone marrow)													
Decreased width, growth plate			+	-	-	-	-	-	-	-	-	-	-
Femur with knee joint, right (bone/bone marrow)													
Decreased width, growth plate			±	-	-	-	-	-	-	-	-	-	±

Uterus/ mononuclear cell infiltration						-	-	-	-	2/5; all±
Immune System Tissues										
Thymus-decrease cellularity (one unexamined tissue in low dose group)	2/5; 3+,+	¾; 2+,+, P	3/5; 2+,P, P	¾; 2+,+, +	¼; 3+	2/5; all ±	5/5; +,+, ±	4/5; ±;2+,2 +,+	5/5; ±,2+ 2+,+ +	5/5; ±,2+ 2+,+ +
Submandibular gland	1/5; all ±	2/5; all ±	1/5; all ±	2/4; all ±	2/4; all ±	2/5; all ±	4/5; all ±	3/5; all ±	3/5; all ±	2/5; all ±
Cervical lymph node	1/5; ±	1/5; ±	1/5; ±	1/5; ±	1/5; ±	1/5; ±	1/5; ±	1/5; ±	3/5; all ±	1/5; ±
GI Tract Tissue										
Stomach/decrease in parietal cell in pylorus and increase in mononuclear cell infiltration	-	-	1/5; 3+	-	¼;2+	1/5, 3+	-	-	1/5; +	-
Duodenum/hyperplasia	-	-	-	-	-	1/5, ±	-	-	1/5; 2+	-
Skeletal system										
Femur/decrease in growth plate	-	-	-	-	-	1/5, ±,P	3/5;P, P,3+, U	5/5; 2+,+, P,P,P	4/5; 3+, P,P, P	5/5; +,P, P,P, P

Note: ±: very slight, +: Slight, 2+:moderate; 3+ marked
 -: no changes noted
 P: non graded change
 U: unexamined

C - Reactive protein Analysis:

Blood was collected from all animals once during acclimation and 6 hours post dosing on Day 1, 5 and Weeks, 4, 13, and 26, and at the end of the recovery period at Week 34.

The major findings with the C-reactive protein are as follows:

1. 25 mg/kg dose group: high C-reactive protein in one male animal #30 at week 4 and 26
2. 40 mg/kg dose group: high C-reactive protein in two male animals #44 at week 4 and #48 at week 4 and 13.
3. 60 mg/kg dose group: high C-reactive protein in one male animal #62 at week 4 and one female at week 26.

Complement Analysis:

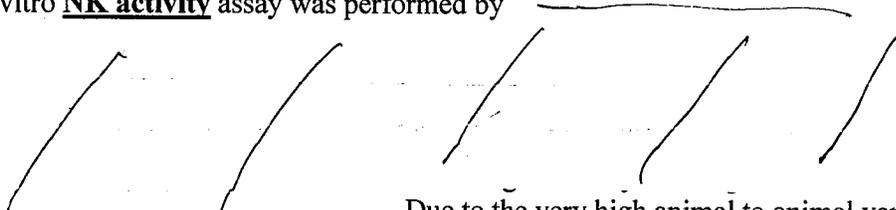
Complement was analyzed from the plasma of the blood that was collected from all animals once during acclimation and 6 hours post dosing on Day 1, 5 and Weeks, 4, 13, and 26 , and at the end of the recovery period at Week 34. The sponsor measured the Complement C3a (which is generated via classical as well as alternative pathway), C4d (which is unique to classical pathway), and sC5b-9 which is generated by the assembly of C5 through C9 as a consequence of activation). There was a dose dependent increase in

the C3a level in males between Weeks 4-13. The C3a level in control, 15, 25, 40, and 60 mg/kg dose ranged from 153-164 ng/mL, 159-492 ng/mL, 211-1010 ng/mL, 284-1099 ng/mL, and 161-1762 ng/mL respectively. No such changes in females were observed. There were no changes in the other complement factor tested. The activation of the C3a in males is clearly treatment related as concluded by the sponsor. This indicates an increase in alternative pathway of complement activation as C4d was not activated. Although the biological relevance of the finding is not clear, this result might be an indication of increased immune complex formation in males which resulted in complement activation.

Immune Function Assays

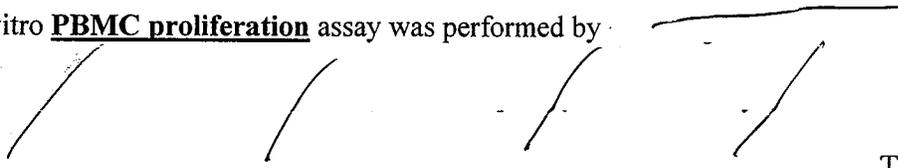
Samples were collected from all animals once during the acclimation period, from two terminal necropsy animals per sex per group, and all recovery animals during Weeks 2, 4, 13, and 26 of the dosing period, and from all recovery animals during the last week of the recovery period. Samples were collected at least 24 hours after dosing.

An in-vitro **NK activity** assay was performed by



Due to the very high animal to animal variation with the increase in the IL-2 stimulated NK cell activity, appropriate interpretation of the data is not possible.

An in vitro **PBMC proliferation** assay was performed by



There were no treatment related changes in the T-cell proliferation under this experimental protocol.

Toxicokinetics:

Blood samples was collected on pre dose and 24 hours post dose on days 1, 5, 12, 19, 26, 40, 54, and every 4 weeks thereafter for the remainder of the dosing period. Samples were also collected on weeks 28, 30, and 34 of the recovery period. Plasma sample from the above mentioned time period was prepared to conduct IL-1 Trap toxicokinetics. Similarly serum was prepared from the sample to conduct the quantization of the anti product antibody.

Assessment of IL-1 Trap Plasma Concentrations:

There were no major differences in the males and females in the toxicokinetic profile. The following tables show the plasma concentration at different days of treatment. There was a dose dependent increase in the plasma level in Day 1 (130, 260, 354, and 553

mcg/mL with 15, 25, 40, and 60 mg/kg respectively. Similar dose dependent increase in the plasma level was noted up to Day 12 with all doses. Accumulation was noted with all doses, higher the dose resulted in greater accumulation. The terminal phase half life of the compound was demonstrated to be 60 hr; therefore, accumulation is expected with 3x administration/week. There was a decrease in the mean peak and trough drug concentrations compared to the first dose after the 5th dose which was maintained with the 15 and 25 mg/kg dose through the end of the study. However, with 40 and 60 mg/kg another increase in the mean peak and trough was noted at Day 40. In the animals from the 40 mg/kg the mean peak and trough concentration the IL-1 Trap continued to increase in the plasma in the subsequent days and exceeded that of the first dose around Day 180. However, in the animals from the 60 mg/kg treatment group the mean peak and trough plasma concentration continued to increase in the subsequent days and exceeded that of the first dose around Day 80.

The sponsor's plasma concentration data for the IL-1 Trap from the individual animals showed that most of the animals (9/10) in the low dose group (15 mg/kg) showed decrease in the IL-1 Trap level between Day 20 - Day 27. One male (#16) continue to have increased plasma concentration of the therapeutic protein throughout the experimental period. Interestingly, two females in this group started showing increase in the IL-1 Trap concentration from around Day 80 of the experimental period.

In the low mid dose group (25 mg/kg), the individual plasma concentration data from the animals showed that 11/14 animals showed a decrease in the peak and trough level. However, one male (#26) and two females (#25, and #27) continue to show increase in the IL-1 Trap plasma concentration throughout the experiment. Two females at this dose group started to have an increase in the plasma level again from Day 80 of the experiment (#33 and #29).

In the high mid dose group (40 mg/kg), the individual plasma concentration data from the animals showed that 10/14 animals showed a decrease in the peak and trough level. However, two males (#50, and #52) and one female (#39) continue to show increase in the IL-1 Trap plasma concentration throughout the experiment. Two females (#41, #45, #51) and one male (#44) from this dose group started to have increase plasma level between Days 40-81 and their plasma IL-1 Trap level at the end of the experimental period was close to their peak IL-1 Trap level that was observed after the 5th dose. In the high dose group (60 mg/kg), the individual plasma concentration data from the animals showed that 5/14 animals showed a decrease in the peak and trough level between days 20-27. Most of the animals continue to show increase in the IL-1 Trap plasma concentration throughout the experiment. The animals (#s 54, 56, 58, 60, 62, all males) which showed a decrease in the plasma level of IL-1 Trap between Days 20 - 26 did not show an increase in the plasma for the rest of the experimental period.

Mean Plasma Concentration of IL-1 Trap and IL-1 Trap Antibody in Cynomolgus Monkey

Time	IL-1 Trap (µg/mL)	Time	IL-1 Trap Antibody (RFU/mL)
	Dosages mg/kg		Dosages mg/kg

	15	25	40	60		15	25	40	60
Day 1	130	260	354	535	Day 1	BLQ	BLQ	BLQ	BLQ
Day 13	124	251	583	1079	Day 12	3-77	5-16	3.2-18	4-11
Day 27	29	116	255	915	Day 26	21-574	7-330	2-1207	3-186
Day 41	20	129	278	1125	Day 40	15-1448	9-1346	7-2546	3-843
Day 85	43	188	231	1260	Day 80	74-3406	15-2644	40-4055	84-1500
Day 181	78	202	781	1616	Day 180	18-2354	2.5-2224	3-1463	2-198
Day 234	118	0	1.77	1.43	Day 234	NR	72-1653	16-2907	35-76

Mean Plasma Concentration (µg/mL) 15 mg/kg and 25 mg/kg

Day	Time	All Monkeys				All Monkeys			
		Mean*	SEM*	Increase**	# of Animals	Mean*	SEM*	Increase**	# of Animals
1	0min	0.00	0.00	NA	10	0.14	0.14	NA	14
2	24h	130.69	11.07	130.69	10	260.06	15.84	259.92	14
5	0min	224.52	11.68	NA	10	448.59	18.49	NA	14
6	24h	322.71	20.17	98.19	10	622.52	35.91	173.93	14
12	0min	124.42	57.22	NA	10	307.13	51.52	NA	14
13	24h	93.45	43.87	-30.97	10	251.63	49.12	-55.50	14
19	0min	44.46	25.80	NA	10	102.04	38.52	NA	14
20	24h	67.46	27.58	23.00	10	173.90	39.45	71.86	14
26	0min	29.93	19.02	NA	10	77.28	35.41	NA	14
27	24h	33.03	15.16	3.10	10	116.02	35.60	38.74	14
40	0min	20.38	16.36	NA	10	78.99	47.33	NA	14
41	24h	43.10	31.38	22.72	10	129.70	71.59	50.71	14
80	0min	52.72	51.69	NA	10	116.94	73.46	NA	14
81	24h	89.09	58.29	36.37	10	188.13	104.34	71.19	14
136	0min	60.70	54.68	NA	10	168.54	96.81	NA	14
137	24h	78.60	69.03	17.90	10	183.06	88.16	14.52	14
180	0min	83.22	64.17	NA	10	160.09	78.56	NA	14
181	24h	118.93	79.73	35.71	10	202.31	92.14	42.22	14
192	Rec					0.68	0.68	NA	4
234	Rec					0.00	0.00	NA	4

Mean Plasma Concentration ($\mu\text{g/mL}$) 40 mg/kg and 60 mg/kg

Day	Time	All Monkeys				All Monkeys			
		Mean*	SEM*	Increase**	# of Animals	Mean*	SEM*	Increase**	# of Animals
1	0 min	0.00	0.00	NA	14	0.23	0.16	NA	14
2	24h	354.42	30.88	354.42	14	553.36	36.09	553.13	14
5	0 min	717.58	50.37	NA	14	1256.74	96.58	NA	14
6	24h	1219.85	119.78	502.27	14	1645.82	177.96	389.08	14
12	0 min	628.84	131.24	NA	14	1180.09	143.64	NA	14
13	24h	583.83	133.18	-45.01	14	1079.84	125.02	-100.25	14
19	0 min	337.89	92.77	NA	14	749.73	170.40	NA	14
20	24h	406.82	88.92	68.93	14	886.24	175.75	136.51	14
26	0 min	193.61	67.46	NA	14	738.29	174.57	NA	14
27	24h	253.81	77.16	62.20	14	915.67	178.53	177.38	14
40	0 min	166.23	74.74	NA	14	823.32	201.63	NA	14
41	24h	278.01	94.15	111.78	13	1125.25	251.61	301.93	12
80	0 min	174.56	106.35	NA	13	1020.81	224.63	NA	13
81	24h	231.54	109.93	56.98	13	1260.47	247.37	239.66	12
136	0 min	430.89	146.47	NA	13	1432.06	293.07	NA	12
137	24h	566.04	186.30	135.15	13	1709.48	283.65	277.42	11
180	0 min	621.54	187.49	NA	13	1246.61	279.10	NA	12
181	24h	781.10	244.32	159.56	13	1616.06	285.05	369.45	11
192	Rec.	144.99	104.23	NA	4	226.57	115.61	NA	4
234	Rec.	1.77	1.77	NA	4	1.43	0.83	NA	4

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Table 9. Monkey Plasma Concentrations (mcg/mL) of IL-1 Trap in the 60 mg/kg Cohort

Day	Time	Monkey # (Sex)													
		53 (F)	54 (M)	55 (F)	56 (M)	57 (F)	58 (M)	59 (F)	60 (M)	61 (F)	62 (M)	63 (F)	64 (M)	65 (F)	66 (M)
1	0min														
2	24h														
5	0min														
6	24h														
12	0min														
13	24h														
19	0min														
20	24h														
26	0min														
27	24h														
40	0min														
41	24h														
80	0min														
81	24h														
136	0min														
137	24h														
180	0min														
181	24h														
192	Rec														
234	Rec														

Rec = Recovery Sample NS = No Sample NR = Not Recovery Animal BLQ = Below Limit of Quantitation (<math>0.05</math> mcg/mL)

Assessment of IL-1 Trap antibody Concentrations:

The animals which had a decrease in IL-1 Trap concentration demonstrated an increase in anti-product antibody formation.

The anti-product antibody formation in the low dose group (15 mg/kg) was observed in 9/10 animals between Days 20 - 26. The animal #16 which continued to show increase in the plasma concentration of IL-1 Trap did not produce any antibody. The range of antibody formation, however, varied widely. The antibody formation ranged between --- mcg/mL) in 3/9 animals (all females). The range of antibody formation in the rest of the animals from this group varied between --- mcg/mL). It was noted that the timing of antibody formation in the animals coincides with the time at which the IL-1 Trap plasma concentration decreased in these animals.

Table 12. Anti-IL-1 Trap Antibody Titer (RFU/mL) in Monkeys Administered 15 mg/kg IL-1 Trap.

Day	Time	Monkey # (Sex)									
		15 (F)	16 (M)	17 (F)	18 (M)	19 (F)	20 (M)	21 (F)	22 (M)	23 (F)	24 (M)
1	Predose										
12	Predose										
26	Predose										
40	Predose										
80	Predose										
136	Predose										
180	Predose										

BLQ = Below Limit of Quantitation (<math>0.05</math> RFU/mL)

The anti-product antibody formation in the low mid dose group (25 mg/kg) was observed in 11/14 animals between Days 20-26. The timing of the antibody production is similar to the time when decrease in the IL-1 Trap concentration was noted in these animals.

The animal #25, #26 and #27 which did not show a decrease in the IL-1 Trap concentration did not show antibody production. The range of antibody formation, however, varied widely. The antibody formation ranged between was low in 1/5 (mcg/mL), in rest of the animals the amount of antibody varied (— mcg/mL). All the recovery animals (2 females and 2 males) showed antibody in their serum at the end of the recovery period (the level ranged from — mcg/mL at week 234).

Table 13. Anti-IL-1 Trap Antibody Titer (RFU/mL) in Monkeys Administered 25 mg/kg IL-1 Trap

Day	Time	Monkey # (Sex)													
		25 (F)	26 (M)	27 (F)	28 (M)	29 (F)	30 (M)	31 (F)	32 (M)	33 (F)	34 (M)	35 (F)	36 (M)	37 (F)	38 (M)
1	Predose														
12	Predose														
26	Predose														
40	Predose														
80	Predose														
136	Predose														
180	Predose														
192	Rec														
206	Rec														
234	Rec														

BLQ = Below Limit of Quantitation (< RFU/mL) NR = Not Recovery Animal

The anti-product antibody formation in the high mid dose group 40 mg/kg was observed in 11/14 animals between Days 20-26. The timing of the antibody production is similar to the time when decrease in the IL-1 Trap concentration was noted in these animals. The animal #39, #50 and #52 who did not show a decrease in the IL-1 Trap plasma concentration did not show antibody production). The animals (#s 41, 44, 45, and 51) whose plasma concentration of the IL-1 Trap was close to the peak (maximum concentration observed at 5th week) at the end of the experimental period showed very little antibody production at this time. One out of two females showed significant amount of the antibody at the end of the recovery period. The other female (#51) which were sacrificed at recovery showed less antibody formation compare to the females in the group which were sacrificed terminally. The two males in the recovery group did not show any antibody production. Therefore, recovery of the antibody production from this dose group can not be ascertained.

Table 14. Anti-IL-1 Trap Antibody Titer (RFU/mL) in Monkeys Administered 40 mg/kg IL-1 Trap

Day	Time	Monkey # (Sex)													
		39 (F)	40 (M)	41 (F)	42 (M)	43 (F)	44 (M)	45 (F)	46 (M)	47 (F)	48 (M)	49 (F)	50 (M)	51 (F)	52 (M)
1	Predose														
12	Predose														
26	Predose														
40	Predose														
80	Predose														
136	Predose														
180	Predose														
192	Rec														
206	Rec														
234	Rec														

BLQ = Below Limit of Quantitation (< RFU/mL) NS = No Sample NR = Not Recovery Animal

The anti-product antibody formation in the high dose group 60 mg/kg was observed in 5/14 animals between Days 20-26. The timing of the antibody production is similar to the time when decrease in the IL-1 Trap concentration was noted in these animals. The

animals that did not show a decrease in the IL-1 Trap plasma concentration did not show antibody production. The maximum amount of antibody formation in these animals were seen between Days 40-80, however, 1/4 animals (male) showed detectable antibody level at recovery.

Table 15. Anti-IL-1 Trap Antibody Titer (RFU/mL) in Monkeys Administered 60 mg/kg IL-1 Trap

Day	Time	Monkey # (Sex)													
		53 (F)	54 (M)	55 (F)	56 (M)	57 (F)	58 (M)	59 (F)	60 (M)	61 (F)	62 (M)	63 (F)	64 (M)	65 (F)	66 (M)
1	Predose														
12	Predose														
26	Predose														
40	Predose														
80	Predose														
136	Predose														
180	Predose														
192	Rec														
206	Rec														
234	Rec														

BLQ = Below Limit of Quantitation (< 0.5 RFU/mL) NS = No Sample NR = Not Recovery Animal

Conclusion from the Toxicokinetics Assessment of the IL-1 Trap and its Antibody:

Following major conclusions may be drawn from the toxicokinetic analysis of the therapeutic protein:

1. Administration of the IL-1 Trap protein resulted in the formation of anti-product antibodies in the animals from all dose group, between 3-4 weeks.
2. The increase in the antibody production was directly related to the decrease in the plasma concentration of IL-1 Trap suggesting increased clearance of the IL-1 Trap by the antibody.
3. The number of animals producing the antibody is higher at the low dose.
4. More animals from the low dose group showed antibody in the serum at the end of the recovery period compare to the control.
5. Some animals from all dose groups either do not produce anti-product antibody or the clearance of the anti-product antibody was very high in these animals. These animals showed high accumulation of the compound throughout the experimental period, consistent with conclusion that the anti-product antibody formation was not occurring.
6. Between weeks 40 - 80, several animals in the 25, 40, and 60 mg/kg dose group showed anti-product antibody as well as IL-1 Trap levels indicating that the animals are exposed to the immune complex as well as the proteins at this time period.

Study title: Six-Month Intravenous Toxicity Study of IL-1 Trap in Cynomolgus Monkeys

Key study findings:

- Cynomolgus monkey 6/sex/group were administered intravenously (infusion) with 0, 3, 10, 30, 100 mg/kg for 26 weeks (injection was given every two weeks, a total of 14 doses were give for 26 weeks). Three animals/sex from groups with 0, 30, and 100 mg/kg dosing were followed for 8 weeks post treatment for recovery.
- The toxicokinetic data demonstrated that IL-1 Trap plasma levels increased dose proportionately. The plasma concentrations of IL-1 Trap decreased at Day 29, and subsequent time periods compared to that of the Day 1 with 3 and 10 mg/kg dose groups suggesting elimination of the compound by anti product antibody formation. The antibody formation was noted in almost all animals (except 3 animals at high doe). A high titer of antibody was noted in the recovery animals from these dose groups from the 30 and 100 mg/kg.
- There was one unscheduled death in this study; one high dose male was sacrificed at Day 176. Bacterial infection was noted in this animal.
- No NOAEL could be established in this study because of the histopathological findings at the low dose. The histological findings at low dose consisted of myocardial inflammation, and inflammation in the kidney associated with infiltration of the multinucleated cells in the epithelium. These histological changes were considered treatment related due to the increase of such incidence in the treated animals compared to those of the controls.
- The other treatment related histological changes consisted of vacuolation in the liver and increase granuloma in lung. Aspermatogenesis in the testes and mineralization in the ovary was also noted in the treated animals but not in the control animals.

Study no.: — toxicology study number 223.19 (Regeneron study number IL1T-TX-03050)

Volume # and page #: eCTD submission; Page: 1-1419

Conducting laboratory and location: / /

Date of study initiation: June 28, 2002

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: Drug Lot # B03015M600E1; 40 mg/mL IL-1 Trap prepared in vehicle was prepared by the sponsor in the vehicle. The CRO diluted it with 0.9% saline for injection.

Placebo Lot # B04001P700F0, pH 6.5 (containing — histidine, — glycine, — arginine, — sucrose, — polyethylene glycol 3350, and —) was the control article used for this study. This was used as vehicle.

Methods

Doses: 0, 3, 10, 30, and 100 mg/kg

Species/strain: Cynomolgus monkey; *Macaca fascicularis*

Number/sex/group or time point (main study): 6/sex/group

Route, formulation, volume, and infusion rate: Intravenous infusion, once every two weeks for 26 weeks, total of 14 doses were used.

Satellite groups used for toxicokinetics or recovery: Toxicokinetic analysis were done from all animals, 3 animals/sex/groups (0, 30, and 100 mg/kg) were euthanized following 8-weeks of recovery.

Age: 3-6 years old

Weight: Females weighed 2.4-4.09 kg; Males weighed 2.73-4.25 kg

Sampling times: Blood samples were collected pre-dose, and then at 5 minutes, 24, 72, and 168 hours post dose at each dosing through week 13, at every other dosing thereafter, and on the last day of dosing. Samples were also collected prior to the terminal necropsy and from the recovery animals during weeks 30 and 34.

Unique study design or methodology (if any):

Study Design

Treatment Group/ Color Code	Test and Control Articles	Dose Level (mg/kg)	Dose Concentration (mg/mL) ^a	Dose Volume (mL/kg) ^b	Number of animals		Study Specific Animal Numbers	
					Females	Males	Females	Males
1 / white	IL-1 Placebo	0	0	5.0	6 + 3 ^c	6 + 3 ^c	1 ^d , 3 ^d , 5 ^d , 7 ^d , 9 ^d , 11 ^d , 13 ^{d,e} , 15 ^{d,e} , 17 ^{d,e}	2 ^d , 4 ^d , 6 ^d , 8 ^d , 10 ^d , 12 ^d , 14 ^{d,e} , 16 ^{d,e} , 18 ^{d,e}
2 / green	IL-1 Trap	3	0.6	5.0	6	6	19 ^d , 21 ^d , 23 ^d , 25 ^d , 27 ^d , 29 ^d	20 ^d , 22 ^d , 24 ^d , 26 ^d , 28 ^d , 30 ^d
3 / blue	IL-1 Trap	10	2.0	5.0	6	6	31 ^d , 33 ^d , 35 ^d , 37 ^d , 39 ^d , 41 ^d	32 ^d , 34 ^d , 36 ^d , 38 ^d , 40 ^d , 42 ^d
4 / red	IL-1 Trap	30	6.0	5.0	6 + 3 ^c	6 + 3 ^c	43 ^d , 45 ^d , 47 ^d , 49 ^d , 51 ^d , 53 ^d , 55 ^{d,e} , 57 ^{d,e} , 59 ^{d,e}	44 ^d , 46 ^d , 48 ^d , 50 ^d , 52 ^d , 54 ^d , 56 ^{d,e} , 58 ^{d,e} , 60 ^{d,e}
5 / orange	IL-1 Trap	100	20	5.0	6 + 3 ^c	6 + 3 ^c	61 ^d , 63 ^d , 65 ^d , 67 ^d , 69 ^d , 71 ^d , 73 ^{d,e} , 75 ^{d,e} , 77 ^{d,e}	62 ^d , 64 ^d , 80 ^d , 82 ^d , 70 ^d , 72 ^d , 74 ^{d,e} , 76 ^{d,e} , 78 ^{d,e}

^aNominal dose concentration. Actual concentration of each lot of test article was recorded in the raw data and in the final report (Appendix 9).

^bIndividual animal dosing volume (mL) was calculated based on the most recent body weight. Dose volumes were rounded up to the next readable syringe increment.

^cThree animals per sex in Groups 1, 4, and 5 were designated as recovery animals and were euthanized following the 8-week recovery period.

^dAnimals in Subset A: #s 1, 2, 3, 4, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 31, 32, 33, 34, 35, 36, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 65, 80, 67, 82, 69, 70, 71, 72

^eAnimals in Subset B: #s 5, 6, 7, 8, 9, 10, 11, 12, 23, 24, 25, 26, 27, 28, 29, 30, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 57, 58, 59, 60, 61, 62, 63, 64, 73, 74, 75, 76, 77, 78

Observations Times and Results:**Mortality:**

The animals were monitored twice daily for the mortality or morbidity findings.

There was one unscheduled death in this study. One high dose male (#76) was found dehydrated, recumbent on the cage floor, and in hunched position on day 174 of the study period, the animal was sacrificed on day 176. There was a decrease in the hemoglobin concentration (12.4 g/dL at acclimation vs. 11.0 g/dL at day 85; $P < 0.01$), erythrocyte count ($5.14 \times 10^6/\mu\text{L}$ at acclimation vs. $4.64 \times 10^6/\mu\text{L}$ at day 85; $P < 0.01$), fibrinogen level (269 mg/dL at acclimation vs. 90 mg/dL at day 85) and slight increase in the APTT in this animal (26 msec at acclimation vs. 33 msec at day 85).

Clinical signs:

The animals were monitored twice daily on the dosing days and once daily for clinical signs. There was an increased incidence of emesis/retching in the treatment group with the increase in the duration of the study indicating poor tolerance of the compound after IV administration. The number of animals showing emesis were 4/12 (3 females and one male), 4/12 (2 males and 2 females), and 5/12 (2 males and 3 females) at 10, 30, and 100 mg/kg dose. The emesis occurred approximately after one hour post dosing. The emesis was mostly foamy in nature. One female (# 43) in the 30 mg/kg dose group was observed to assume recumbent position at approximately one hour after the infusion of the 5th dosing, the animal recovered spontaneously within 7-12 mins. Another male from the high dose group become non responsive during the drug infusion of the 12th dose, the animal had dorsiflexed head position, with constricted pupils and pale gums. The animal, however, recovered within 10 mins post infusion. Following is a summary table for the clinical signs as reported by the sponsor.

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Clinical Signs Observed after (during or soon) Test Article Administration:

Dose	Group	Animal #	Observations
1st			No abnormal findings
2nd	4	47	Foamy emesis about 17 minutes after start of dosing
	5	70	Foamy and bile like emesis about 10 minutes after start of dosing
3rd			No abnormal findings
4th	4	50	Mucous like emesis (~ 1 mL) about 65 minutes after completion of dosing
5th	3	37	Retching during dosing
		40	Emesis during dosing
	4	43	The animal lay down about 30-45 minutes after completion of dosing
		47	Emesis about 18 minutes after start of dosing
	5	70	Emesis about 20 minutes after start of dosing
		72	Emesis about 19 minutes after start of dosing
6th	4	45	Food like emesis (~ 10 mL) about 25 minutes after start of dosing
		47	Food like and liquid emesis (~ 10 mL) about 29 minutes after start of dosing
	5	70	Food like and liquid emesis (~ 15 mL) about 32 or 33 minutes after start of dosing
7th	3	38	Retching and white foamy emesis approximately 4.5 hrs after completion of dosing
	4	47	Emesis about 15 minutes after start of dosing. Bile-like emesis 20 minutes after start of dosing
	5	77	Emesis about 16 minutes after start of dosing
8th	4	47	Food and foamy emesis about 15 minutes after start of dosing. Food like emesis about 22 minutes after start of dosing

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Dose	Group	Animal #	Observations
9th	3	34	Food like emesis (~ 10 mL) about 18 min after start of dosing
	4	47	Food like emesis about 13(~60 mL), 16 (~ 60 mL) and 24 (~ 5 mL) minutes after start of dosing
		59	Blood observed coming from right nostril during dosing.
	5	70	Foamy and food like emesis about 14 minutes after start of dosing
10th	3	34	Food like emesis (about 10 mL) about 23 min after start of dosing
	4	47	Food like emesis about 11 minutes after start of dosing, Food like emesis about 13-15 minutes after start of dosing
	5	67	Food like emesis about 10 minutes after start of dosing
		69	Food like emesis about 10 minutes after start of dosing
11th	4	43	Emesis about 16 minutes after start of dosing
		47	Food like emesis about 13 minutes after start of dosing
	5	62	The animal became unresponsive, head was back, and eyes rolled back about 14 minutes after start of dosing. Three minutes later, dose was suspended. The animal returned to be bright, alert and responsive. Dose re-started 7 minutes after dose suspension and the animal appeared normal.
12th	4	43	Emesis about 17 minutes after start of dosing
		47	Food like emesis (~ 15 mL) about 11 minutes after start of dosing
	5	62	The animal became unresponsive about 12 minutes after start of dosing and dose was suspended at once. Its head leaned back, gums were pale, and the pupils were constricted. The animal became responsive and dosing was re-started 12 minutes after dose suspension. Animal appeared normal after re-start of dosing.
		69	Foamy emesis (~ 2 mL) about 10 minutes after start of dosing.
13th	4	47	Liquid and mucous like emesis (about 5 mL) about 10 minutes after start of dosing, Foamy emesis (~ 3mL) about 18 minutes after start of dosing
14th	4	44	Foamy emesis (~ 3 mL) about 12 minutes after start of dosing
		47	Emesis (~ 10 mL) about 13 minutes after start of dosing

There were increases in the number of treated animals compare to that of the control with hair loss, skin rash, hunched position, and soft feces at the end of dosing period indicating that these signs might be directly related to the compound administration.

Body weights:

All animals were weighed once during the second week of acclimation, once weekly throughout the dosing and recovery periods, and once the respective days of necropsy.

There were no test article related changes in the body weight during this study period.

Food consumption:

The food consumption was recorded twice daily.

There were no test article related changes in the food consumption during this study period.

Ophthalmoscopy:

All animals were examined for ophthalmology once during acclimation and once prior to necropsy.

There were no test article related changes in the ophthalmology during this study period.

Blood pressure:

All animals were assessed twice during acclimation, during Weeks 2, 12 and 26 and during the last week of the recovery period. Blood pressure was measured using Vital Scan Monitor with Rectal Probe.

There were no test article related changes in the blood pressure during this study period.

EKG:

All animals were assessed twice during acclimation, during Weeks 2, 12 and 26 and during the last week of the recovery period. A qualitative analysis of data from leads I, II, aVR, aVL, and aVF and quantitative analysis of heart rate in beats/min was performed by the study cardiologist.

There were no test article related changes in the rectal temperature during this study period.

Rectal temperature:

All animals were assessed twice during acclimation, during Weeks 2, 12 and 26 and during the last week of the recovery period. Rectal Temperature was measured using Vital Scan Monitor with Rectal Probe.

There were no test article related changes in the rectal temperature during this study period.

Hematology:

Hematology and coagulation analyses were performed on all animals once during acclimation, during Weeks 5, 13, and 28 and during the last week of the recovery period. Following hematology and coagulation parameters were examined.

Table C: Hematology Parameters

Parameter	Method	Apparatus	Units
Erythrocyte count (RBC)			X10e6/ μ L
Leukocyte count (WBC)			X10e3/ μ L
Hematocrit value (HCT)	Calculation		%
Hemoglobin concentration (Hgb)			g/dL
Platelet count (PLT)			X10e3/ μ L
Mean platelet volume (MPV)	Calculation		fL
Mean corpuscular volume (MCV)	Calculation		fL
Mean corpuscular hemoglobin (MCH)	Calculation		pg
Mean corpuscular hemoglobin concentration (MCHC)	Calculation		g/dL
Reticulocyte count (Retic)			%
Differential leukocyte count			X10e3/ μ L

Parameter	Method	Apparatus	Units
Prothrombin time (PT)			second
Activate Partial Thromboplastin Time (APTT)			second
Fibrinogen (Fib)			mg/dL

- 1) There was a decrease (10%) in the erythrocyte counts in males at Day 191, the decrease was, however, not statistically significant. There was a statistically significant ($P < 0.01$) decrease (9%) in the hemoglobin concentration in males with high dose at Day 191. At recovery the decrease in the hemoglobin content increased (12%, $P < 0.01$) in this group of animals. Similar changes were seen in the other dose groups in males; however, the changes were not statistically significant. In female no changes in the erythrocytes and hemoglobin were noted.
- 2) There was no significant change in the platelet counts in males. In females, a dose related increase in the platelet counts was noted at week 26 (15, 20, 50% increase with 10, 30, and 100 mg/kg dose respectively). At recovery, platelet counts were still high (40% increase) compare to control in the high dose group females.
- 3) There was a significant increase in the eosinophil counts in the high dose animals. At Days -4, 29, 85, 191, 233 the eosinophil counts were 0.049, 0.084, 0.099, 0.16, $0.1 \times 10^3/\mu\text{L}$ respectively in the high dose group, maximum increase noted at Week 26 was 226%. Similar patterns of increase in the eosinophil was seen in other dose groups in males, however, the changes were much lower in the intensity. In females pattern of increase in the eosinophil counts were similar to that noted in males, the maximum increase was noted in the high dose females. The increase in eosinophil counts in this group at Weeks 26 and 34 were 2.8 and 3.2 fold respectively compared to that of the pre dose level in the same animals.

Clinical chemistry:

Serum chemistry analysis was performed on all animals once during acclimation, in Weeks 5, 13 and 28, and during the last week of the recovery period. Following serum chemistry parameters were examined.

There was no meaningful difference in the serum chemistry parameters in any of the treatment groups is this study.

Parameter	Method	Apparatus	Units
Albumin (Alb)			g/dL
Alanine Aminotransferase (ALT)			U/L
Alkaline Phosphatase (ALP)			U/L
Aspartate Aminotransferase (AST)			U/L
Bilirubin, Total (Tbili)			mg/dL
Blood Urea Nitrogen (BUN)			mg/dL
Calcium (Ca)			mg/dL
Chloride (Cl)			mEq/L
Cholesterol, Total (TChol)			mg/dL
Creatinine (CRN)			mg/dL
Creatine Phosphokinase (CK)			U/L
Gamma Glutamyl Transferase (GGT)			U/L
Glucose (Glu)			mg/dL
Lactate Dehydrogenase (LDH)			U/L
Phosphorus, inorganic (IP)			mg/dL
Potassium (K)			mEq/L
Parameter			Units
Protein, total (TP)			g/dL
Sodium (Na)			mEq/L
Triglyceride (Trig)			mg/dL

There was no meaningful difference in the serum chemistry parameters in this study.

Urinalysis:

Urinalysis was conducted from all animals once during acclimation, in Weeks 5, 6, 13 and 26, and during the last week of the recovery period. Following parameters were measured.

Table B: Urinalysis Parameters

Parameter	Method	Apparatus	Units
Color	Visual	Not Applicable	NA
Volume	Manual	Volumetric Cylinder	mL
Specific gravity	Refractometer		NA
pH	Test strips		NA
Protein	Test strips		mg/dL
Glucose	Test strips		mg/dL
Ketones	Test strips		mg/dL
Bilirubin	Test strips		NA
Oculta Blood	Test strips		NA
Urobilinogen	Test strips		mg/dL
Nitrites	Test strips		NA
Leukocytes	Test strips		NA
Urine Total Protein			Mg/dL

There was an increase in the urinary protein level in all animals at all dose groups; the finding was not statistically significant (except in Group 3 males at Week 6). High urinary blood level was noted in two males one each from 3 mg/kg group (#20) at Week 26 and 10 mg/kg group (#32) at Week 6. Occasional findings of blood in the urine of females may be due to menstruation.

Serum C-Reactive Protein:

Blood was collected from all animals once during acclimation, 6 hours post-dosing ($\pm 5\%$) on Days 1, 5, 15, and in Weeks 5, 13 and 27 of the dosing period, and in the last week of the recovery period. Slight increase in the C-reactive protein was noted at high dose.

Plasma C3a, C4d, and Soluble C5b-9 Complement Analysis:

Blood was collected from all animals once during acclimation, 6 hours post-dosing ($\pm 5\%$) on Days 1, 5, 15, and in Weeks 5, 13 and 27 of the dosing period, and in the last week of the recovery period. No changes in complement activation were noted.

Gross pathology:

At necropsy, the external surfaces of the body, all orifices, and the cranial, thoracic, and abdominal cavities and their contents were examined.

In the moribund sacrificed male (#76) of Group 5, marked hemorrhage was observed in the urinary bladder, prostate, and urethra. In addition, the prostate was markedly enlarged, spleen was slightly small and kidneys were pale. Marked atrophy of the thymus and slightly enlarged adrenals were also observed. No food contents were found in the stomach and small intestine. Another animal (#70) showed bleeding in the colon. In a low dose female (#31) watery content was noted in the large intestine, in this animal enlargement of spleen was also noted. Parasites were noted in the cecum (#72, #11, and #47); thyroid cyst was noted in animal #40.

Organ weights:

The organs weights reported are shown in the histopathology inventory table. There was a decrease in the prostate and the testes weight; aspermatogenesis were noted in the histopathological analyses in one animal. A decrease in the pituitary weight at the terminal necropsy was noted, the only histological finding correlated with such finding was cyst formation.

Summary of Organ Weight Findings:

Organ weights Relative to Brain (%)	Male					Female				
	Dose (mg/kg)									
	0	3	10	30	100	0	3	10	30	100
<i>Terminal sacrifice</i>										
Adrenal	0.87	0.92	0.71	0.76	0.77	0.84	0.73	0.74	0.82	0.78
Pituitary	0.1	0.08	0.07	0.1	0.09	0.85	0.79	0.95	0.1	0.1
<i>Recovery</i>										
Adrenal	0.79	-	-	0.56	0.65	0.09	-	-	0.78	0.65
Pituitary	0.09	-	-	0.07	0.07	0.09	-	-	0.09	0.1

Histopathology: Adequate Battery: Yes; peer review: Yes

The histopathology findings from animal #76 which was sacrificed on Day 176 showed severe inflammation and hemorrhage of urethra, prostate, and urinary bladder; bacterial colonies were present in the urinary bladder. Dilation of stomach associated with mild, glandular multifocal mucosa was apparent in this animal. Moderate hypocellularity of bone marrow was also observed microscopically. Other observations were atrophy of testis (mild) and thymus (severe), cyst was observed in multiple sites in thymus. All these observations are correlated with increase IL-1 Trap concentration in plasma since no anti-product antibody formation was noted in this animal.

There were two other animals (#78, #80) which did not show any anti-product antibody formation suggesting that the histopathological finding is directly related to the IL-1 Trap plasma protein concentration. The major histopathological findings in the animal #80 were chronic, multifocal inflammation in kidney, necrosis in the mucosa of stomach (multifocal), lymphocyte infiltration in the urinary bladder, cyst on multiple sites in thymus, and subacute inflammation in the liver, associated with vacuolar cell in sinusoids of liver. The major histopathological findings in the animal #78 were myocardial inflammation, inflammation in pancreas (focal) with pigmented macrophages, cyst on multiple sites in thymus, inflammation in the prostate, degeneration of testes.

In the males from the high dose group #s 82, 70, 62, 64, and 72 which were sacrificed in Day 191, inflammation of myocardium, infiltration of lymphocytes in urinary bladder, vacuolation in the sinusoidal cells of liver, inflammation of prostate, chronic inflammation of kidney, inflammation in eye, atrophy of thymus, multiple cyst in thymus, testicular degeneration was also noted. In some of these animals parasites (protozoa/trematode) were noted in intestine. In the females from the high dose group, similar changes such as inflammation and/or infiltration of lymphocytes were also noted in heart (#s 63, 65, 67, 69,71), kidney (#s 61, 63, 65, 67, 69, 71), liver (#61, 63, 65, 67, 69, 71) eye (#61), urinary bladder (#s 63, 65, 67, 71). Mineralization of ovary (#s 63, 65, 67) was also seen in 3/6 animals in these group. In the recovery animals from the high dose group inflammation in kidney, heart, and vacuolation in liver were still noted. Another common finding in females was intestinal inflammation which still persisted at recovery. In this group of animals IL-1 Trap as well as the anti-product antibody level was high around the time of necropsy suggesting that the effect might be due to the combination of therapeutic protein as well as the antibody produced as a result of the compound administration.

The inflammation in heart, kidney, and vacuolation of the cells in the liver was also apparent in the 30, 10, and 3 mg/kg dose group. However the incidence was lower. The recovery was better in 30 mg/kg dose group compare to those of the high dose group.

Summary of the Histopathological Findings:

Tissue Findings	Male					Female				
	Dose (mg/kg)									
	0	3	10	30	100	0	3	10	30	100
Brain										
Mononuclear cell	-	-	-	2/6	-		1/6	3/6	2/6	-

infiltration										
Heart	-	-	-		-		-	-	-	-
Myocardial degeneration	-	-	-	1/6	-		-	-	1/6	-
Myocardial necrosis (multifocal)	-	-	-	-	1/6	-	-	-	2/6	-
Inflammation /myocardial	1/6	4/6	3/6	0/6	4/6	0	3/6	3/6	4/6	2/6
Injection sites(skin, back, thorax, upper										
Degeneration				1/6	-	-	-	-	1/6	-
Necrosis	-	-	-	-	1/6	-	-	-	1/6	-
Kidney										
Inflammation/tubular changes/multinucleated cell min epithelium	1/6	-	3/6	3/6	5/6	-	-	-	3/6	5/6
Liver										
Vacuolation in the sinusoidal cells	-	-	-	3/6	5/6	-	2/6	-	4/6	5/6
Pancreas										
Inflammation	-	1/6	-	-	-	-	-	1/6	2/6	-
Pituitary	-	-	-	-	-	-	-	-	-	-
Cyst	0	1/6	1/6	0		1/6	0	2/6	2/6	1/6
Stomach										
Dilation/ inflammation	1/6		2/6	2/6	2/6	-	-	-	-	-
Necrosis	-	1/6	-	-	1/6	-	1/6	-	-	-
Eye										
Chronic inflammation/ conjunctiva	-	1/6	1/6	2/6	3/6	-	1/6	0/6	2/6	1/6
Lungs										
Alveolitis	-	-	-	-	1/6	-	-	-	-	-
Granulomatous	-	-	-	-	-	-	-	-	-	1/6
Urinary bladder										
Infiltration of lymphocytes	-	2/6	2/6	5/6	5/6	-	2/6	2/6	5/6	5/6
Male/Female Reproductive organ										
Testes, aspermatogenesis	-	-	-	-	1/6	-	-	-	-	-
Ovaries /mineralization of follicles	-	-	-	-	-	-	-	-	3/6	3/5

Note: -: no change

Toxicokinetics:

Blood samples were collected pre-dose, and then at 5 minutes, 24, 72, and 168 hours post dose at each dosing through week 13, at every other dosing thereafter, and on the last day of dosing. Samples were also collected prior to the terminal necropsy and from the recovery animals during weeks 30 and 34.

Analyses of IL-1 Trap Plasma concentration:

- The toxicokinetic data demonstrated that IL-1 Trap plasma level increased dose proportionately (plasma concentrations of IL-1 Trap at 5 mins after 3, 10, 30, and 100 mg/kg administration of the compound were 83, 377, 865, and 2890 mg/mL respectively).
- The C_{max} was observed at 5 mins with all dose groups after the first dosing in Day 1 complete clearance of the IL-1 Trap protein was noted at the 168 hrs (5 days after the first dosing) in all dose groups except the 100 mg/kg cohort.
- The plasma concentration of IL-1 Trap at 5 mins after the second dosing in Day 15 was lower (with 3, 10, and 30 mg/kg) or equivalent (100 mg/kg) to that of the first dose indicating the following: firstly, there is no accumulation of protein with IV administration up to 30 mg/kg dosing at this time point; secondly, the formation of the clearing antibody might have generated with low dose group at this time points.
- The plasma concentrations of IL-1 Trap decreased at day 29, and subsequent time periods compared to that of the day 1 with 3 and 10 mg/kg dose groups suggesting elimination of the compound by anti-product antibody formation.
- The plasma concentrations of IL-1 Trap was either close to similar or increased up to day 57, and subsequent time periods compared to that of the day 1 with 30 and 100 mg/kg dose groups suggesting accumulation or delayed clearance of the immune complex with these dosing.
- Significant amount of IL-1 Trap was noted at mid and high dose group even at the end of the dosing period Day 183, indicating that the concentration of the neutralizing anti product antibody formation is minimal in these dose groups or the clearance was high. Three animals (#s 76, 78, and 80) showed no decrease in the IL-1 Trap, indicating no antibody formation. Two of these animals (#s 78 and 80) showed significant IL-1 Trap plasma level 3-weeks after the dosing was terminated. No IL-1 Trap plasma concentrations were noted at 8 weeks after the termination of the dosing.

Analyses of IL-1 Trap Antibody concentration in serum:

- The antibody formation was noted in all animals in the two low dose groups (3 and 10 mg/kg). The antibody was detected in most of the animals from these dose groups at Day 15. The peak antibody formation was noted around Week 13 in most of the animals with all dose groups. The serum antibody concentration decreased in the animals from the low dose group (3 mg/kg) at Week 26 compare to that of Week 13. However, the serum antibody concentration increased in the animals from the low mid dose group at Week 26 compare to that of Week 13. No animals from these two dose groups were used for recovery; therefore, it is unknown how long these sustaining antibodies might stay in the circulation.
- The anti-product antibody formation was noted in all animals in the high mid dose group (30 mg/kg). A high titer of antibody was noted in the recovery animals from this dose group.

- The anti-product antibody formation was noted in all but 3 animals (#s 76, 78, and 80) animals in the high dose group (100 mg/kg). A high titer of antibody was noted in the recovery animals from this dose group also.

Mean Plasma Concentration of IL-1 Trap and IL-1 Trap Antibody in Cynomolgus Monkey

Time	IL-1 Trap (µg/mL)				IL-1 Trap Antibody (RFU/mL)			
	3	10	30	100	3	10	30	100
Day 1	83	377	865	2890	BLQ	BLQ	BLQ	BLQ
Day 15	78	359	843	3070	7-58	4-106	8-172	3-45
Day 29	72	334	868	3112	7-68	15-370	56-239	3-437
Day 57	40	209	707	3190	95-164	109-434	103-334	4-806
Day 85	20	157	605	2790	71-180	133-400	170-850	262-1312
Day 183	10	46	558	2531	24-68	132-542	179-612	56-1795
Day 234	NR	NR	1.02	1.2	NR	NR	68-357	21-367

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ON ORIGINAL**

Table 11. Mean Monkey Plasma Concentrations (mcg/mL) of IL-1 Trap in IL-1 Trap Treated Monkeys

Day*	Time*	3 mg/kg Cohort		10 mg/kg Cohort		30 mg/kg Cohort		100 mg/kg Cohort	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
D1	0min	0.00	0.00	0.88	0.88	0.00	0.00	0.00	0.00
D1	5min	83.07	4.40	377.50	19.21	865.67	26.39	2890.69	176.21
D2	24h	34.11	2.59	158.11	18.23	457.94	22.39	1455.24	91.45
D4	72h	10.98	0.98	63.87	7.29	243.10	12.24	1015.32	43.00
D8	168h	1.78	0.31	14.62	2.97	64.66	4.38	407.60	20.91
D15	0min	0.00	0.00	1.16	0.95	0.36	0.16	11.57	7.88
D15	5min	78.22	6.08	359.58	25.34	843.98	29.25	3070.56	217.41
D162	24h	24.25	4.27	97.69	15.63	288.73	32.16	1634.72	68.52
D18	72h	2.98	1.36	17.53	5.08	69.91	17.71	886.69	52.93
D22	168h	0.48	0.34	0.56	0.53	0.56	0.56	78.18	36.70
D29	0min	0.00	0.00	0.54	0.54	0.00	0.00	2.03	1.15
D29	5min	72.45	4.58	334.33	26.08	868.06	25.95	3112.98	238.96
D30	24h	13.38	3.99	66.27	14.11	222.59	30.08	1467.44	123.75
D32	72h	1.39	0.78	8.43	3.92	48.45	14.92	778.42	80.39
D36	168h	0.00	0.00	0.87	0.87	0.08	0.08	68.58	36.45
D57	0min	0.00	0.00	0.72	0.72	0.08	0.08	14.47	9.32
D57	5min	40.94	5.76	209.35	24.58	707.96	41.95	3190.08	228.01
D58	24h	0.10	0.10	9.20	5.64	66.05	21.23	973.08	145.75
D60	72h	0.00	0.00	1.25	0.85	5.35	3.16	495.46	117.96
D64	168h	0.00	0.00	0.82	0.73	0.32	0.15	91.66	49.56
D85	0min	0.10	0.10	0.63	0.51	0.28	0.16	17.20	10.85
D85	5min	20.30	3.55	157.08	17.02	605.97	44.01	2790.43	200.43
D86	24h	0.00	0.00	1.21	0.87	24.70	11.22	815.16	133.61
D88	72h	0.00	0.00	0.95	0.95	0.41	0.33	306.52	107.79
D92	168h	0.00	0.00	0.63	0.63	0.30	0.21	84.97	46.10
D169	0min	0.00	0.00	0.71	0.58	0.70	0.53	26.22	14.67
D169	5min	6.69	1.84	41.45	8.83	320.31	34.88	2590.07	201.63
D170	24h	0.00	0.00	0.65	0.65	0.73	0.34	594.09	163.47
D172	72h	0.00	0.00	0.63	0.63	0.17	0.12	245.51	116.00
D176	168h	0.00	0.00	0.65	0.50	0.54	0.33	84.59	45.64
D183	0min	0.00	0.00	0.44	0.31	0.43	0.43	19.25	13.32
D183	5min	10.34	5.51	46.90	8.52	558.23	65.85	2531.55	226.03
D184	24h	0.00	0.00	0.62	0.62	0.75	0.43	493.80	163.23
D186	72h	0.00	0.00	0.80	0.80	0.07	0.07	180.95	99.27
D190	168h	0.00	0.00	0.92	0.79	0.21	0.21	74.31	50.80
D191	NecRec	0.00	0.00	0.79	0.63	0.26	0.26	64.27	43.87
D204/205	Rec	NR	NR	NR	NR	3.02	1.66	16.32	15.58
D233/234	Rec	NR	NR	NR	NR	1.02	1.02	1.20	1.20

*: Day refers to Study Specific Day and Time refers to time post infusion that the sample was collected

Nec=Necropsy Rec=RecoverySample

NR = No Recovery Animals

Plasma Concentration of IL-1 Trap from Individual Animals of 3 mg/kg Cohort

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Table 15. Anti-IL-1 Trap Antibody Titer (RFU/mL) in Monkeys Administered 30 mg/kg IL-1 Trap

Study Day	Time	Monkey # (Sex)																	
		43 (F)	44 (M)	45 (F)	46 (M)	47 (F)	48 (M)	49 (F)	50 (M)	51 (F)	52 (M)	53 (F)	54 (M)	55 (F)	56 (M)	57 (F)	58 (M)	59 (F)	60 (M)
D1	Pre																		
D15	Pre																		
D29	Pre																		
D57	Pre																		
D85	Pre																		
D169	Pre																		
D191	Nec/Rec																		
D204/205	Rec																		
D233/234	Rec																		

BLQ = Below Limit of Quantitation (< 100 RFU/mL) NR = Not Recovery Animal M = Male F = Female
 Pre = Predose Nec/Rec = Before Necropsy or Start of Recovery Period Rec = Recovery Period
 NA = Not Analyzed

Table 16. Anti-IL-1 Trap Antibody Titer (RFU/mL) in Monkeys Administered 100 mg/kg IL-1 Trap

Study Day	Time	Monkey # (Sex)																	
		61 (F)	62 (M)	63 (F)	64 (M)	65 (F)	67 (F)	68 (F)	70 (M)	71 (F)	72 (M)	73 (F)	74 (M)	75 (F)	76 (M)	77 (F)	78 (M)	80 (M)	82 (M)
D1	Pre																		
D15	Pre																		
D29	Pre																		
D57	Pre																		
D85	Pre																		
D169	Pre																		
D191	Nec/Rec																		
D204/205	Rec																		
D233/234	Rec																		

BLQ = Below Limit of Quantitation (< 100 RFU/mL) NR = Not Recovery Animal M = Male F = Female
 Pre = Predose Nec/Rec = Before Necropsy or Start of Recovery Period Rec = Recovery Period
 NS = No Sample

Study title: A Two-Month Subcutaneous Exploratory Study of Murine IL-1 Trap in CD-1 Mice

This study was conducted as a pilot non GLP study. The study was designed to evaluate the potential toxicity and immunogenicity of mIL-1 Trap construct when administered 3x/week by subcutaneous injection for 8 weeks at dose levels of 0, 20, 100, 200 mg/kg. This study compared the safety and tolerability of IL-1 Trap in mice using two different commercial sources (C... [CD1®] and ... [ICR(CD-1®)]). Following is the study design:

5.4.4 Dose Levels and Group Identification

Main study

Group No.	Strain of Mouse	Total daily dose (mg/kg/day)	Concentration (mg/mL)	Volume per dose (mL/kg)	*No. of Mice	Male Animal Numbers	Female Animal Numbers
CFE1	CD-1®	0	0	8	10	1-5	41-45
CFE2	CD-1®	20	2.5	8	10	6-10	46-50
CFE3	CD-1®	100	12.5	8	10	11-15	51-55
CFE4	CD-1®	200	25.0	8	10	16-20	56-60
CFE5	ICR (CD-1®)	0	0	8	10	21-25	61-65
CFE6	ICR (CD-1®)	20	2.5	8	10	26-30	66-70
CFE7	ICR (CD-1®)	100	12.5	8	10	31-35	71-75
CFE8	ICR (CD-1®)	200	25.0	8	10	36-40	76-80

The first 3 main study mice/sex/group were used for clinical chemistry sample collection while blood samples collected from the remaining 2 main study mice/sex/group were evaluated for hematology parameters.

There were three deaths at the mid-dose group in the main study from the CD1 mice (2 males and one female); mice were found dead on Day 10. There was one death at the low-dose group in the main study from the ICR/CD1 mice (male). The clinical signs noted for these mice were unkempt appearance. There were no consistent or biological meaningful changes in body weight, body weight gain, or food consumption, clinical chemistry, and hematology parameters with the mIL-1 Trap administration in this study.

The toxicokinetic analyses from these animals demonstrated that the test article concentrations increased with dose at the two time points examined but was typically higher at the Week 8 time point compared with the Week 4, though the increase was not always linear. This apparent accumulation of test article was most substantial in the 20 mg/kg cohorts where the mean serum murine IL-1 Trap levels at Week 8 were 5- to 19-fold greater than those at Week 4. Also, 54% of the animals who received IL-1 Trap developed generated IL-1 Trap antibody while 27% of the 100 mg/kg cohort, and 8% of the 200 mg/kg cohort group had detectable levels of the anti-product antibody formation which might be related to the increase lethality in the mice at the low dose group. The PK studies in the mice showed gender difference, higher exposure noted in the males, the increase in the lethality in males in this study might be due to the higher exposure of the IL-1 Trap at the pharmacological dosages and related anti-product antibody production. There was too much individual variation in the plasma concentration of IL-1 Trap in the individual animals as shown in the sponsor's toxicokinetic table below in this study. Therefore, no definitive conclusion might be made out of these results.

Table 3: Serum Concentrations (ng/ml) of Murine IL-1 Trap in the 20 mg/kg Cohort

Mouse #	Strain	Gender	Time Point	Serum Concentration	Mean*	SEM*	Median*		
84	CD-1	Male	4 weeks	762	356	325			
85	CD-1	Male	4 weeks						
86	CD-1	Male	4 weeks						
87	CD-1	Male	4 weeks						
144	CD-1	Female	4 weeks						
145	CD-1	Female	4 weeks						
146	CD-1	Female	4 weeks						
147	CD-1	Female	4 weeks						
114	ICR	Male	4 weeks	230	83	215			
115	ICR	Male	4 weeks						
116	ICR	Male	4 weeks						
117	ICR	Male	4 weeks						
174	ICR	Female	4 weeks						
175	ICR	Female	4 weeks						
176	ICR	Female	4 weeks						
177	ICR	Female	4 weeks						
88	CD-1	Male	8 weeks	3890	920	4046			
89	CD-1	Male	8 weeks						
90	CD-1	Male	8 weeks						
91	CD-1	Male	8 weeks						
92	CD-1	Male	8 weeks						
148	CD-1	Female	8 weeks						
149	CD-1	Female	8 weeks						
150	CD-1	Female	8 weeks						
151	CD-1	Female	8 weeks						
152	CD-1	Female	8 weeks						
118	ICR	Male	8 weeks				4323	1242	4098
119	ICR	Male	8 weeks						
120	ICR	Male	8 weeks						
121	ICR	Male	8 weeks						
178	ICR	Female	8 weeks						
179	ICR	Female	8 weeks						
180	ICR	Female	8 weeks						
181	ICR	Female	8 weeks						
182	ICR	Female	8 weeks						

BLQ = Below limit of quantitation

*A value of 0 was used to calculate these parameters if sample was BLQ

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Table 4: Anti-Murine IL-1 Trap Antibody Levels (ng/mL) in the 20 mg/kg Cohort.

Mouse #	Strain	Gender	Time Point	Antibody Concentration
84	CD-1	Male	4 weeks	
85	CD-1	Male	4 weeks	
86	CD-1	Male	4 weeks	
87	CD-1	Male	4 weeks	
144	CD-1	Female	4 weeks	
145	CD-1	Female	4 weeks	
146	CD-1	Female	4 weeks	
147	CD-1	Female	4 weeks	
114	ICR	Male	4 weeks	
115	ICR	Male	4 weeks	
116	ICR	Male	4 weeks	
117	ICR	Male	4 weeks	
174	ICR	Female	4 weeks	
175	ICR	Female	4 weeks	
176	ICR	Female	4 weeks	
177	ICR	Female	4 weeks	
88	CD-1	Male	8 weeks	
89	CD-1	Male	8 weeks	
90	CD-1	Male	8 weeks	
91	CD-1	Male	8 weeks	
92	CD-1	Male	8 weeks	
148	CD-1	Female	8 weeks	
149	CD-1	Female	8 weeks	
150	CD-1	Female	8 weeks	
151	CD-1	Female	8 weeks	
152	CD-1	Female	8 weeks	
118	ICR	Male	8 weeks	
119	ICR	Male	8 weeks	
120	ICR	Male	8 weeks	
121	ICR	Male	8 weeks	
178	ICR	Female	8 weeks	
179	ICR	Female	8 weeks	
180	ICR	Female	8 weeks	
181	ICR	Female	8 weeks	
182	ICR	Female	8 weeks	

BLQ = Below limit of quantitation

Histopathology inventory (optional)

Study	223.19	223.17
Species		
Adrenals	x*	x*
Aorta	x	x
Bone Marrow smear	x	x
Bone (femur)	x	x
Brain	x*	x*
Cecum	x	x
Cervix	x	x
Colon		
Duodenum		
Epididymis	x*	x*
Esophagus	x	x
Eye	x	x
Fallopian tube		
Gall bladder	x	x
Gross lesions	x	x
Harderian gland		