

Heart	x*	x*
Ileum	x	x
Injection site	x	x
Jejunum	x	x
Kidneys	x*	x*
Lachrimal gland	x	x
Larynx		
Liver	x*	x*
Lungs	x*	x*
Lymph nodes, cervical	x	x
Lymph nodes mandibular	x	x
Lymph nodes, mesenteric	x	x
Mammary Gland	x	x
Nasal cavity		
Optic nerves	x	x
Ovaries	x*	x*
Pancreas	x	x
Parathyroid		
Peripheral nerve		
Pharynx		
Pituitary	x*	x*
Prostate	x*	x*
Rectum	x	x
Salivary gland		
Sciatic nerve		
Seminal vesicles	x	x
Skeletal muscle	x	x
Skin	x	x
Spinal cord		
Spleen	x*	x*
Sternum		
Stomach	x	x
Testes	x*	x*
Thymus	x*	x*
Thyroid	x	x
Tongue	x	x
Trachea	x	x
Urinary bladder	x	x
Uterus	x*	x*
Vagina	x	x
Zymbal gland		

X, histopathology performed

*, organ weight obtained

2.6.6.4 Genetic toxicology

There were no genotoxicity studies submitted with this application. Genotoxicity studies are not required for large biologic proteins that do not cross the cell membrane.

2.6.6.5 Carcinogenicity

There was no carcinogenicity studies submitted with this application. IL-1Trap is synthesized to inhibit IL-1 β a vital component of the innate immune system of the body and is predicted to have immunosuppressive property. Immuno suppressant agents including small molecules such as methotrexates, cyclophosphamide as well as monoclonal antibodies and Fc fusion proteins such as enbrel, humira, infliximab, orenicia, orthoclone, amevive are known to increase carcinogenicity in human. The malignancies observed with the above mentioned therapeutics include lymphoma, basal or squamous cell carcinoma. The reviewer believes that IL-1 Trap might cause similar malignancies due to its the immunosuppressive property. The malignancies observed with the marketed human interleukin 1 inhibitor demonstrated an increased incidence of lymphoma formation in the patients treated with IL-1 inhibitors compared to the untreated rheumatoid arthritis patients. An increase in malignancies other than lymphoma such as malignancies of the respiratory and the digestive system, breast and melanoma were also noted in the patients treated with the marketed IL-1 inhibitors. The endogenous nature of the marketed IL-1 inhibitor might have excluded its testing for animal carcinogenicity studies. However, IL-1Trap is a fusion protein constructed by genetic engineering with the extracellular domain of the human IL-1RT1, and IL1 -AcP conjugated with a the Fc receptor of the human IgG to capture IL-1 β to inhibit the inflammation associated with the IL-1 β production. Thus unlike the marketed recombinant proteins created for the inhibition of the IL-1 β mediated inflammation, IL-1 Trap is not produced endogenously. Therefore, it does not fall into the category described in the ICHS1A 'Carcinogenicity studies are not generally needed for endogenous substances given essentially as replacement therapy, particularly where there is a previous clinical experience with similar products'. In this same line ICH S6 stated that 'In those cases where the product is biologically active and non immunogenic in rodents and other studies have not provided sufficient information to allow an assessment of carcinogenic potential then the utility of a single rodent species should be considered'.

All of the above mentioned scientific documentation and regulatory requirement indicate that in light of current findings the applicant might need to evaluate the product for the carcinogenicity assessment in a rodent species. The applicant generated a murine model with surrogate molecules for the IL-1 Trap. It is recommended that the feasibility of the carcinogenicity study in this model be evaluated. Because of the FDA's recommendation in prior meetings that a carcinogenicity waiver for riloncept is appropriate, it is reviewer recommendation that a carcinogenicity study be conducted

_____ for this product due to the new findings with the immunosuppressive agents. In the absence of the carcinogenicity data with the compound the sponsor might need to rationalize why the carcinogenicity data will not be needed for the product for safety assessment for the labeling for the marketing purpose.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Subcutaneous Fertility and Early Embryonic Development to Implantation Study of Murine IL-1 Trap in Mice

Key study findings:

- Murine IL-1 Trap (0, 20, 100, and 200 mg/kg) was administered 3x/week (subcutaneously) to both male and female mice. The males were dosed beginning 8 weeks prior to pairing, throughout the mating period and continuing through 1 or 2 days prior to scheduled euthanasia, following post mortem examination of the mated females on gestation day 15, for a total of 37-38 doses. The females were dosed for 2 weeks prior to pairing and on gestation days 0, 3 and 6, for a total of 9-14 doses. Females with no evidence of mating were dosed through the day prior to euthanasia, following post mortem examination of the mated females on gestation day 15, for a total of 13 doses.
- The TK and the antibody analysis were done in mice following GLP. Plasma concentrations of murine IL -1 Trap increased with dose in male; however, the compound was not detected in females because the sampling time was at least ten days after the administration of the compound. Anti-mIL-1 Trap antibody was found in approximately 90 and 83% of the low dose group males and females respectively. The antibody levels ranged from 1.1-6000, 1.9-328, and 0.9-129 µg/mL in the females from the low, mid, and high dose group respectively. The antibody levels ranged from 2-1335, 1-26, and 1.5 µg/mL in the males from the low, mid, and high dose group respectively.
- The number of unscheduled deaths in the control, low, mid, and high dose group were 0, 2 (females), 1 (male), and 2 (one male, one female) respectively. The necropsy from the high dose females that died at high dose at the GD 7 showed discoloration of the stomach. The necropsy findings from the male that died at high dose had reddened lung, small epididymis, raised preputial area, and darkened injection site. One female from the mid dose died prior to mating at Day 49 of the study. This animal had reddened ears two days prior to death. Two other females from the low dose group were euthanized at GD 11. These animals showed the clinical signs of impaired use of hind limbs and laceration the forelimbs. The cause of death could not be determined from any of these animals by the sponsor.

However, the macroscopic observation of the reddened appearance of the tissue might be associated with inflammation and might be considered treatment related. Also, the impaired movement of the limbs in the low dose animals might also be related to the test article induced injection (the compound was administered at scapula or lumber region in the back) site reaction which had been observed microscopically in all the species treated with IL-1 Trap.

- There was a decrease (14-16%) in the male and female fertility index in the low dose group animals compared to the controls.

- There was an increase (47-51%) in early resorptions in all treatment groups compare to the controls (2.6, 4.9, 4.1, and 5.1 in the 0, 20, 100, and 200 mg/kg group respectively).
- There was approximately 40-45% increase in the post implantation loss in the treatment group compare to that of the control group (3.2, 5.3, 4.1, and 5.8 in 0, 20, 100, 200 mg/kg respectively).
- As apparent from the descriptions mentioned above, the findings are not dose related. However, note that the antibody titer in the males and the females was highest at the low dose which might relate to the higher occurrence of the increased fertility and implantation disorders in the low dose groups. Therefore, although not dose related these above mentioned findings are considered treatment related by the reviewer because of higher plasma concentration of immune complex formation at low dose.
- Because of all these findings mentioned above in the animals from the low dose group, according to the reviewer no NOEL could be established in this study which is unlike the sponsor (sponsor's NOEL is 200 mg/kg).

Study no.: — 460002

Volume # and page #: The final study report is in the electronic document room.

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Conducting laboratory and location:

/ /

Date of study initiation: June 7, 2005

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: Murine IL-Trap; Labeled drug product # B5001K620X11A

Methods

Doses: 20, 100, 200 mg/kg

Species/strain - CD-1(ICR) mice

Number/sex/group: Refer to the study design table below

Route, formulation, volume, and infusion rate: Subcutaneous, formulated in vehicle; 8 mL/kg, bolus administration.

Satellite groups used for toxicokinetics: Blood was collected from each animal at necropsy.

Study design: The compound was administered subcutaneously, 3x/week. The males were dosed beginning 8 weeks prior to pairing, throughout the mating period and continuing through 1 or 2 days prior to scheduled euthanasia, following post mortem examination of the mated females on gestation day 15, for a total of 37-38 doses. The females were dosed for 2 weeks prior to pairing and on gestation days 0, 3, and 6, for a total of 9-14 doses. Females with no evidence of mating were dosed through the day prior to euthanasia, following post mortem examination of the mated females on gestation day 15, for a total of 13 doses.

Group Number	Test Article	Dosage Level ^a (mg/kg/day)	Dosage Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals	
					Males	Females
1	Placebo	0	0	8	30	30
2	Murine IL-1 Trap	20	2.5	8	75	75
3	Murine IL-1 Trap	100	12.5	8	30	30
4	Murine IL-1 Trap	200	25	8	30	30

^a = Dosage levels represent the active moiety.

Parameters and endpoints evaluated: The animals were observed twice daily for clinical signs. Body weights and food consumption was measured twice a week. Vaginal lavage was assessed daily for estrus cycle evaluation and breeding. Mating, fertility and copulation/conception indices were assessed and calculated as follows:

Mating Index (%) = (No. of Males (Females) with Evidence of Mating Male (Female) (or Females Confirmed Pregnant)/Total No. of Males (Females) Used for Mating) x 100

Male Fertility Index (%) = (No. of Males Siring a Litter/ Total No. of Males Used for Mating) x 100

Male Copulation Index (%) = [No. of Males Siring a Litter
No. of Males with Evidence of Mating (or Females
Confirmed Pregnant)] x 100

Female Fertility Index (%) = [No. of Females with Confirmed Pregnancy/Total No. of Females Used for Mating] x 100

Female Conception Index (%) = [No. of Females with Confirmed Pregnancy
/No. of Females with Evidence of Mating (or Females Confirmed Pregnant)
x 100

Male fertility was further evaluated by examining sperm motility and sperm production.

Macroscopic evaluation was made from all animals. Following organ weights were measured: brain, epididymis, testes, ovaries and pituitary gland. Microscopic evaluation of the following tissues were made: cervix, coagulating glands, heart, injection sites, ovaries and oviduct, pituitary glands, prostate glands, seminal vesicles, testes, epididymis and vas deferens, uterus, and vagina and all gross lesions.

Toxicokinetic analyses were done in all animals from the blood collection via vena cava prior to necropsy.

Intrauterine data was collected from GD 15. The number and location of all embryos, early resorptions and the total number of implantation sites were recorded. Viability of the embryos was determined by confirmation of ventricular contraction with the aid of a dissecting stereomicroscope, if necessary. The crown-rump length of each

late resorption was measured; the degree of autolysis was recorded and an external examination was conducted.

Results

Mortality and Clinical Signs:

There was one male from the low dose group that died at Day 16 (following 8 doses). This male had tremor and hypothermia prior to death. One female from the high dose group died at the gestation day (GD) 7. This female had dark red discoloration of the stomach at necropsy, no clinical signs were observed in this female prior to death. One female from the mid dose died prior to mating at Day 49 of the study. This animal had reddened ears two days prior to death. Two other females from the low dose group were euthanized at GD 11. These animals showed the clinical signs of impaired use of hind limbs and laceration the forelimbs. The sponsor believed that the unscheduled death in the low dose group was due to mechanical injury. Doses were administered in the scapular and the lumber region; therefore, the reviewer believed that there was no direct possibility for any mechanical injury associated with impaired use of the hind limb or laceration of the forelimb. However, treatment related injection site reactions were noted in all the species studied with this compound. Therefore it is possible that although not evident from the macroscopic observation, underlying microscopic lesion in the injection site might resulted the deteriorating condition of the health in these two low dose animals causing death.

One female in the mid dose group delivered early at GD 15, all the pups were observed to be normal.

The clinical signs noted in the test article-treated groups in males and females, include hair loss on various body surfaces, abdominal masses, unkempt appearance, swollen and/or red urogenital area, swollen and/or scabbed tail, decreased defecation, tremors, hypoactivity, clear material around the eye and/or reddened, swollen and/or scabbed ear(s). The findings were at similar frequencies in the control group and/or in a manner that was not dose-related. The lesions on the ears were similar to progressive necrosing dermatitis, which sponsored mentioned as common lesion noted in this age and strain of mouse.

Body weight:

Transient differences (statistically significant, $p < 0.05$ or $p < 0.01$) in the mean body weight gains in males were noted in the 100-mg/kg/dose group during study days 0-4 (decrease), in the 20 and 200 mg/kg/dose groups during study days 21-25 (increases) and in the 200 mg/kg/dose group during study days 80-84 (loss). Also, food consumption was found to be increased when the body weight was increased suggesting that body weight loss and gain was associated with treatment. It is not clear from the data whether the changes in the body weight gain were test article related or not.

Sponsor's table showing changes in the Body Weight Gain in Males:

TABLE 5
FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE
SUMMARY OF BODY WEIGHT CHANGES (G)

PROJECT NO. 460002M
SPONSOR: REGENERON PHARM.
SPONSOR NO.: IL1 T-TX-05002

PAGE

GROUP:		0 MG/KG/DOSE	20 MG/KG/DOSE	100 MG/KG/DOSE	200 MG/KG/DOSE
DAY	0- 4				
	MEAN	1.5	1.5	1.1*	1.2
	S.D.	0.67	0.67	0.54	0.64
	N	30	75	30	30
DAY	4- 7				
	MEAN	0.1	0.3	0.2	0.1
	S.D.	0.41	0.47	0.53	0.47
	N	30	75	30	30
DAY	7- 11				
	MEAN	0.6	0.8	0.6	0.7
	S.D.	0.44	0.48	0.60	0.51
	N	30	75	30	30
DAY	11- 14				
	MEAN	0.3	0.3	0.5	0.4
	S.D.	0.57	0.30	0.47	0.37
	N	30	75	30	30
DAY	14- 18				
	MEAN	0.8	1.0	0.7	1.1
	S.D.	0.61	0.47	0.55	0.47
	N	30	74	30	30
DAY	18- 21				
	MEAN	0.3	0.2	0.2	0.3
	S.D.	0.45	0.47	0.43	0.40
	N	30	74	30	30

* = Significantly different from the control group at 0.05 using Dunnett's test
MEAN DIFFERENCES CALCULATED FROM INDIVIDUAL DIFFERENCES

**APPEARS THIS WAY
ON ORIGINAL**

TABLE 5
 PROJECT NO.: 1460002M FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE PAGE 2
 SPONSOR: REGENERON PHARM.
 SUMMARY OF BODY WEIGHT CHANGES [G]
 SPONSOR NO.: ILLI T-TX-05002

GROUP:		MALES			
		0 MG/KG/DOSE	20 MG/KG/DOSE	100 MG/KG/DOSE	200 MG/KG/DOSE
DAY 21-25	MEAN	0.3	0.6*	0.6	0.8**
	S.D.	0.48	0.62	0.49	0.48
	N	30	74	30	30
DAY 25-28	MEAN	0.1	0.1	0.0	0.0
	S.D.	0.55	0.52	1.50	0.56
	N	30	74	30	30
DAY 28-31	MEAN	0.7	0.8	0.9	0.7
	S.D.	0.37	0.69	1.23	0.44
	N	30	74	30	30
DAY 31-35	MEAN	0.2	0.1	0.0	0.0
	S.D.	0.49	0.64	0.37	0.35
	N	30	74	30	30
DAY 35-38	MEAN	0.2	0.3	0.3	0.3
	S.D.	0.39	0.47	0.40	0.45
	N	30	74	30	30
DAY 38-42	MEAN	0.4	0.2	0.4	0.1
	S.D.	0.42	0.56	0.41	0.66
	N	30	74	30	30

* = Significantly different from the control group at 0.05 using Dunnett's test
 ** = Significantly different from the control group at 0.01 using Dunnett's test
 MEAN DIFFERENCES CALCULATED FROM INDIVIDUAL DIFFERENCES

TABLE 5
 PROJECT NO.: 1460002M FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE PAGE 3
 SPONSOR: REGENERON PHARM.
 SUMMARY OF BODY WEIGHT CHANGES [G]
 SPONSOR NO.: ILLI T-TX-05002

GROUP:		MALES			
		0 MG/KG/DOSE	20 MG/KG/DOSE	100 MG/KG/DOSE	200 MG/KG/DOSE
DAY 42-45	MEAN	0.6	0.5	0.5	0.5
	S.D.	0.60	0.48	0.38	0.57
	N	30	74	30	30
DAY 45-49	MEAN	0.3	0.0	0.1	0.1
	S.D.	0.57	0.59	0.30	0.42
	N	30	74	30	30
DAY 49-52	MEAN	0.4	0.6	0.8	0.6
	S.D.	0.49	0.46	0.39	0.47
	N	30	74	30	30
DAY 52-55	MEAN	-0.5	-0.4	-0.5	-0.5
	S.D.	0.53	0.49	0.35	0.29
	N	30	74	30	30
DAY 55-59	MEAN	-0.5	-0.4	-0.5	-0.3
	S.D.	0.68	0.79	0.67	0.80
	N	30	74	30	30
DAY 59-63	MEAN	0.2	0.3	0.2	0.3
	S.D.	0.71	0.68	0.77	0.68
	N	30	74	30	30

None significantly different from control group
 MEAN DIFFERENCES CALCULATED FROM INDIVIDUAL DIFFERENCES

TABLE 5
 FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE
 SUMMARY OF BODY WEIGHT CHANGES (G)
 PROJECT NO.: 460002M
 SPONSOR: REGENERON PHARM.
 SPONSOR NO.: ILL T-TX-05002

DAY	GROUP:	0 MG/KG/DOSE	MALES		100 MG/KG/DOSE	200 MG/KG/DOSE
			20 MG/KG/DOSE	74		
63-	66					
	MEAN	0.6	0.5	0.9	0.8	
	S.D.	0.62	0.89	0.58	0.41	
	N	30	74	30	30	
66-	70					
	MEAN	-0.1	-0.1	-0.5	-0.3	
	S.D.	0.69	0.97	0.48	0.67	
	N	30	74	30	30	
70-	73					
	MEAN	0.7	0.8	1.0	0.7	
	S.D.	0.40	0.48	0.61	0.54	
	N	30	74	30	30	
73-	77					
	MEAN	-0.1	-0.1	0.0	-0.2	
	S.D.	0.58	0.87	0.74	0.48	
	N	30	74	30	30	
77-	80					
	MEAN	0.5	0.6	0.5	0.6	
	S.D.	0.55	0.55	0.58	0.65	
	N	30	74	30	30	
80-	84					
	MEAN	0.3	0.1	0.2	-0.1**	
	S.D.	0.53	0.58	0.51	0.56	
	N	30	74	30	30	

** = Significantly different from the control group at 0.01 using Dunnett's test
 MEAN DIFFERENCES CALCULATED FROM INDIVIDUAL DIFFERENCES

TABLE 5
 FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE
 SUMMARY OF BODY WEIGHT CHANGES (G)
 PROJECT NO.: 460002M
 SPONSOR: REGENERON PHARM.
 SPONSOR NO.: ILL T-TX-05002

DAY	GROUP:	0 MG/KG/DOSE	MALES		100 MG/KG/DOSE	200 MG/KG/DOSE
			20 MG/KG/DOSE	74		
84-	86					
	MEAN	0.6	0.6	0.9	0.4	
	S.D.	0.54	0.49	0.45	1.41	
	N	30	74	30	30	

There was slight decrease in the body weight gain during the experimental Days 32-39, prior to the initiation of the administration of the compound in the females in the treatment group compare to that of the controls (refer to the tables below). Interestingly the food consumption at this period either increased or remained the same in the treatment group during this period. There was no treatment at this period. This might suggest the transient changes in the body weight loss might not be associated with the food consumption.

Sponsor's Table showing Changes in the Body Weight Gain in Females:

TABLE 21
 PROJECT NO.: 460002F FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE PAGE 1
 SPONSOR: RBGENERON PHARM. SUMMARY OF BODY WEIGHT CHANGES (G)
 SPONSOR NO.: ILLI T-TX-05002

GROUP:		0 MG/KG/DOSE	FEMALES 20 MG/KG/DOSE	100 MG/KG/DOSE	200 MG/KG/DOSE
DAY 32-	36				
	MEAN	0.4	0.2	0.2	-0.2**
	S.D.	0.66	0.56	0.59	0.91
	N	30	75	30	30
DAY 36-	39				
	MEAN	0.6	0.6	0.4*	0.7
	S.D.	0.55	0.54	0.58	0.55
	N	30	75	30	30
DAY 39-	42				
	MEAN	0.0	0.2	0.3	0.4
	S.D.	0.59	0.93	0.51	0.75
	N	30	75	30	30
DAY 42-	46-A				
	MEAN	-0.1	0.1	-0.2	-0.2
	S.D.	0.52	0.83	0.66	0.66
	N	30	75	30	30
DAY 46-	50				
	MEAN	0.6	1.0	1.2	1.2
	S.D.	0.66	0.62	0.61	0.69
	N	30	74	30	30
DAY 50-	53				
	MEAN	0.3	0.1	0.5	0.3
	S.D.	0.87	0.65	0.54	0.69
	N	30	74	30	30

* = Significantly different from the control group at 0.05 using Dunnett's test
 ** = Significantly different from the control group at 0.01 using Dunnett's test
 MEAN DIFFERENCES CALCULATED FROM INDIVIDUAL DIFFERENCES
 A = TEST ARTICLE ADMINISTRATION INITIATED ON STUDY DAY 42

TABLE 21
 PROJECT NO.: 460002F FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE PAGE 2
 SPONSOR: RBGENERON PHARM. SUMMARY OF BODY WEIGHT CHANGES (G)
 SPONSOR NO.: ILLI T-TX-05002

GROUP:		0 MG/KG/DOSE	FEMALES 20 MG/KG/DOSE	100 MG/KG/DOSE	200 MG/KG/DOSE
DAY 53-	55				
	MEAN	0.4	0.4	0.5	0.6
	S.D.	0.76	0.72	0.74	0.65
	N	30	74	29	30
DAY 42-	55-B				
	MEAN	1.5	1.6	1.9	1.9
	S.D.	0.95	1.16	1.02	1.35
	N	30	74	29	30

None significantly different from control group
 MEAN DIFFERENCES CALCULATED FROM INDIVIDUAL DIFFERENCES
 A = TEST ARTICLE ADMINISTRATION INITIATED ON STUDY DAY 42

Food consumption:

A statistically significant increase in mean food consumption (g/animal/day) was observed in the 20 mg/kg/dose group males during study days 49-52. The increase was transient and did not occur in a dose-related manner, however, it might be attributed indirectly to the test article since changes in the body weight was associated with the food consumption.

Sponsor's Table showing Changes in the Food Consumption in Males:

PROJECT NO.: 460002M FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE PAGE 1
 SPONSOR: REGENERON PHARM. SUMMARY OF FOOD CONSUMPTION (GRAMS/KG/DAY)
 SPONSOR NO.: ILLI T-TX-05002

DAY	GROUP:	0 MG/KG/DOSE	MALES		100 MG/KG/DOSE	200 MG/KG/DOSE
			20 MG/KG/DOSE	75		
0-	4					
	MEAN	196.8	198.7	191.0	201.2	
	S.D.	33.60	25.49	26.91	30.57	
	N	30	75	30	30	
4-	7					
	MEAN	178.8	188.8	188.2	188.2	
	S.D.	20.94	19.58	22.82	23.88	
	N	30	75	30	30	
7-	11					
	MEAN	199.7	196.3	203.2	202.0	
	S.D.	53.81	32.79	52.41	52.75	
	N	30	75	30	30	
11-	14					
	MEAN	173.9	184.0	176.0	179.4	
	S.D.	22.99	24.61	25.19	19.19	
	N	30	75	30	30	
14-	18					
	MEAN	173.6	182.0	182.0	188.2	
	S.D.	13.09	25.35	24.34	18.18	
	N	30	74	30	30	
18-	21					
	MEAN	175.7	174.5	179.6	178.6	
	S.D.	32.87	17.82	18.61	16.83	
	N	30	74	30	30	

None significantly different from control group

PROJECT NO.: 460002M FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE PAGE 2
 SPONSOR: REGENERON PHARM. SUMMARY OF FOOD CONSUMPTION (GRAMS/KG/DAY)
 SPONSOR NO.: ILLI T-TX-05002

DAY	GROUP:	0 MG/KG/DOSE	MALES		100 MG/KG/DOSE	200 MG/KG/DOSE
			20 MG/KG/DOSE	75		
21-	25					
	MEAN	194.2	192.4	194.7	194.8	
	S.D.	50.58	44.42	37.95	38.75	
	N	30	74	30	30	
25-	28					
	MEAN	169.7	172.0	179.9	184.0	
	S.D.	29.63	24.67	32.98	22.48	
	N	30	74	30	30	
28-	31					
	MEAN	181.0	188.0	200.5	193.1	
	S.D.	33.70	40.20	45.05	28.98	
	N	30	74	30	30	
31-	35					
	MEAN	167.0	170.9	173.5	176.6	
	S.D.	21.97	29.67	15.47	18.86	
	N	30	74	30	30	
35-	38					
	MEAN	183.5	185.1	188.5	189.7	
	S.D.	58.11	46.67	38.82	44.76	
	N	30	74	30	30	
38-	42					
	MEAN	168.5	171.8	171.8	176.3	
	S.D.	22.54	20.35	15.64	26.64	
	N	30	72	29	27	

None significantly different from control group

PROJECT NO.: 460002M FERT/EARLY RMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE PAGE 3
 SPONSOR: REGENERON PHARM.
 SPONSOR NO.: ILLI T-TX-05002 SUMMARY OF FOOD CONSUMPTION (GRAMS/KG/DAY)

GROUP:		0 MG/KG/DOSE	MALES		100 MG/KG/DOSE	200 MG/KG/DOSE
			20 MG/KG/DOSE			
DAY 42-	45					
	MEAN	172.0	174.3	174.0	173.7	
	S.D.	22.82	18.26	20.07	16.82	
	N	30	74	28	30	
DAY 45-	49					
	MEAN	161.3	166.5	166.8	171.5	
	S.D.	17.34	22.74	16.45	21.51	
	N	30	70	30	30	
DAY 49-	52					
	MEAN	163.4	173.9	167.2	159.0	
	S.D.	15.84	29.36	18.99	18.13	
	N	30	73	30	30	
DAY 52-	55					
	MEAN	158.9	162.6	166.0	165.7	
	S.D.	20.72	24.31	22.27	14.97	
	N	30	74	30	30	
DAY 70-	73					
	MEAN	163.2	163.8	159.1	162.6	
	S.D.	23.23	22.63	13.52	12.48	
	N	30	73	30	30	
DAY 73-	77					
	MEAN	162.2	168.0	159.4	166.6	
	S.D.	13.17	25.63	13.56	33.35	
	N	30	71	30	30	

None significantly different from control group

PROJECT NO.: 460002M FERT/EARLY RMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE PAGE 4
 SPONSOR: REGENERON PHARM.
 SPONSOR NO.: ILLI T-TX-05002 SUMMARY OF FOOD CONSUMPTION (GRAMS/KG/DAY)

GROUP:		0 MG/KG/DOSE	MALES		100 MG/KG/DOSE	200 MG/KG/DOSE
			20 MG/KG/DOSE			
DAY 77-	80					
	MEAN	189.4	190.1	198.8	190.4	
	S.D.	44.94	34.95	45.17	31.06	
	N	29	67	30	30	
DAY 80-	84					
	MEAN	163.1	165.1	166.3	171.2	
	S.D.	13.98	17.32	15.34	25.99	
	N	30	70	30	30	
DAY 84-	86					
	MEAN	154.9	157.9	157.9	154.1	
	S.D.	12.08	13.60	11.69	25.97	
	N	30	71	30	30	

Food consumption in females, decreased right before mating Days 53-55 (7.5 g in control vs. 6.4 g in the high dose group). A test article related decrease in food consumption was, however, observed. This effect was dose related and statistically significant; therefore, these changes might be attributed to the test article.

Sponsor's Table showing Changes in the Food Consumption in Females:

PROJECT NO.: ~~460002F~~ 460002F FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE PAGE 1
 SPONSOR: REGENERON PHARM. SUMMARY OF FOOD CONSUMPTION (GRAMS/ANIMAL/DAY)
 SPONSOR NO.: ILLI T-TX-050G2

GROUP:		0 MG/KG/DOSE	FEMALES		100 MG/KG/DOSE	200 MG/KG/DOSE
			20 MG/KG/DOSE			
DAY 32-	36					
	MEAN	5.4	5.9	7.7**	6.6	
	S.D.	1.20	1.44	2.72	1.34	
	N	30	75	30	25	
DAY 36-	39					
	MEAN	5.6	5.8	5.7	5.8	
	S.D.	0.78	0.70	0.51	1.13	
	N	30	75	30	30	
DAY 39-	42					
	MEAN	7.0	7.2	7.5	6.7	
	S.D.	2.04	2.39	1.95	2.10	
	N	30	75	30	30	
DAY 42-	46-A					
	MEAN	5.7	5.8	6.2	5.8	
	S.D.	1.05	0.90	1.31	1.12	
	N	30	73	29	30	
DAY 46-	50					
	MEAN	6.6	6.4	6.8	6.3	
	S.D.	2.23	1.10	1.25	1.00	
	N	30	74	29	30	
DAY 50-	53					
	MEAN	5.8	6.3	6.0	6.2	
	S.D.	1.25	1.25	1.35	1.02	
	N	30	74	30	30	

** = Significantly different from the control group at 0.01 using Dunnett's test
 A = TEST ARTICLE ADMINISTRATION INITIATED ON STUDY DAY 42

PROJECT NO.: ~~460002F~~ 460002F FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE PAGE 2
 SPONSOR: REGENERON PHARM. SUMMARY OF FOOD CONSUMPTION (GRAMS/ANIMAL/DAY)
 SPONSOR NO.: ILLI T-TX-050G2

GROUP:		0 MG/KG/DOSE	FEMALES		100 MG/KG/DOSE	200 MG/KG/DOSE
			20 MG/KG/DOSE			
DAY 53-	55					
	MEAN	7.5	6.8*	7.0	5.4**	
	S.D.	1.37	1.07	1.46	1.20	
	N	30	74	29	30	
DAY 42-	55-A					
	MEAN	6.3	6.2	6.4	6.1	
	S.D.	0.95	0.65	0.75	0.73	
	N	30	72	28	30	

* = Significantly different from the control group at 0.05 using Dunnett's test
 ** = Significantly different from the control group at 0.01 using Dunnett's test
 A = TEST ARTICLE ADMINISTRATION INITIATED ON STUDY DAY 42

Toxicokinetics:

The TK and the antibody analysis were done in mice according to GLPs.

IL-1 Trap was detected from the plasma of the male animals. The number of animals with detectable murine IL-1 Trap were 37/74, 29/30 and 30/30 males from low, mid, and high dose group. The blood collection time was only 24 hrs post administration of the compound in males and half life of the compound is approximately 24 hrs which might have counted for the positive findings of murine IL-1 Trap in males. The IL-1 Trap concentration in the plasma increased with the increase in the doses in male. The level of murine IL-1 Trap in the low, mid, and high dose group ranged approximately from 1-78, 5-432, and 29-748 µg /mL respectively indicating that the IL-1 Trap increased with the increase in the dose level. Murine IL-1 Trap antibody was found in 62/74, 7/30 and 2/30 males in the low mid and high dose group respectively. The level of the antibody in males ranged from 2- 13351, 1-26, and 1.5 µg/mL in low, mid, and high dose group respectively.

Murine IL-1 Trap was not detected in any female mice because the plasma collection time was approximately 10 days after the administration of the compound.

Murine IL-1 Trap antibody was detected in almost all females. The number of animals with positive antibody finding was lower with higher dose; this is similar to the observation in males. Also, the levels of the antibodies were higher in the low dose group in males as well as females compare to that of the low dose group. Note that the level of the antibody in the plasma of the males from the low dose group was comparatively higher in the males than in the females. Murine IL-1 Trap antibody was found in approximately 90% of the animals from the low dose group. The antibody levels ranged from 1.1-6000 µg/mL in females. In the mid and high dose group 28/29 and 19/29 females was detected to possess murine IL-1 Trap antibody respectively. The antibody level ranged from 1.9-328 µg/mL in the females from the mid dose group and 0.9 -129 µg/mL in the females from the high dose group.

Summary of the Toxicokinetic Analysis:

Parameters	Dose mg/kg		
	20	100	200
Male			
Number of animals showing IL-1 Trap in Plasma	37/74	29/30	30/30
IL-1 Trap Concentration (µg/mL)	1-78	5-432	29-748
Number of animals showing IL-1 Trap antibody in serum	62/74	7/30	2/30
IL-1 Trap Antibody Concentration (µg/mL)	2-13351	1-26	1.5
Female			
Number of animals showing IL-1 Trap in Plasma	ND	ND	ND
IL-1 Trap Concentration (µg/mL)	ND	ND	ND
Number of animals showing IL-1 Trap antibody in serum	67/73	28/29	19/29
IL-1 Trap Antibody Concentration(µg/mL)	1.1-6000	1.9-328	0.9-129

ND = not detected

1 Page(s) Withheld

Trade Secret / Confidential

Draft Labeling

Deliberative Process

Table 4. Levels of mIL-1 Trap (mcg/mL) and anti-mIL-1 Trap antibodies (mcg/mL) in terminal serum samples from the 200 mg/kg cohort.

Mouse #	Sex	Trap (mcg/mL)	Antibody (mcg/mL)	Mouse #	Sex	Trap (mcg/mL)	Antibody (mcg/mL)
A2937	Female			A1541	Male		
A2939	Female			A1543	Male		
A2950	Female			A1544	Male		
A2937	Female			A1556	Male		
A2938	Female			A1560	Male		
A2939	Female			A1563	Male		
A2940	Female			A1573	Male		
A2945	Female			A1576	Male		
A2952	Female			A1578	Male		
A2954	Female			A1586	Male		
A2964	Female			A1684	Male		
A2967	Female			A1612	Male		
A2973	Female			A1613	Male		
A2986	Female			A1635	Male		
A3002	Female			A1648	Male		
A3026	Female			A1655	Male		
A3007	Female			A1661	Male		
A3010	Female			A1663	Male		
A3016	Female			A1663	Male		
A3020	Female			A1669	Male		
A3033	Female			A1673	Male		
A3043	Female			A1681	Male		
A3044	Female			A1683	Male		
A3048	Female			A1704	Male		
A3072	Female			A1705	Male		
A3085	Female			A1716	Male		
A3095	Female			A1720	Male		
A3101	Female			A1726	Male		
A3109	Female			A1729	Male		
A3110	Female			A1733	Male		

BLQ = Below limit of quantitation (●) mcg/mL in the murine IL1 Trap assay, (●) mcg/mL in the anti-murine IL1 trap assay NS = NO Sample

Necropsy Findings:

The following observations were made at necropsy:

- The schedule necropsy finding from females demonstrated that 1/30 in control and 5/75 in the low dose group had no evidence of mating.
- The necropsy from the high dose females that died at the GD 7 showed discoloration of the stomach. The death might be related to the treatment, however, the cause of death is not known.
- Macroscopic findings from the males showed 1/75 animals had reddened lung, small epididymis, raised preputial area, and darkened injection site. This finding was in a male treated with the low dose; whereas no such findings were noted in control or mid and high dose group males.

Sponsor's table Showing Necropsy Findings:

PROJECT NO.: 46002F
 SPONSOR: REGENERON PHARM.
 SPONSOR NO.: IL1 T-TX-05002

TABLE 29 (NO EVIDENCE OF MATING)
 FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE
 SUMMARY OF MACROSCOPIC FINDINGS

PAGE 1

SCHEDULED NECROPSY

GROUP:	FEMALE			
	1	2	3	4
NUMBER OF ANIMALS IN DOSE GROUP	30	75	30	30
NUMBER OF ANIMALS EXAMINED	1	5	0	0
PITUITARY -SMALL	0	1	0	0
NO SIGNIFICANT CHANGES OBSERVED - ALL EXAMINED TISSUES	1	4	0	0

1- 0 MG/KG/DOSE 2- 20 MG/KG/DOSE 3- 100 MG/KG/DOSE 4- 200 MG/KG/DOSE

PROJECT NO.: ~~125~~-460002F
 SPONSOR: REGENERON PHARM.
 SPONSOR NO.: IL1 T-TX-05002

TABLE 30 (GESTATION DAY 15)
 FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE
 SUMMARY OF MATERNAL MACROSCOPIC FINDINGS

GROUP :	1	2	3	4
NUMBER EXAMINED	29	69	29	30
NO SIGNIFICANT CHANGES OBSERVED	26	58	26	28
NONGRAVID -- AMMONIUM SULFIDE NEGATIVE	0	5	0	0
EUTHANIZED GESTATION DAY 11	0	1	0	0
ORGAN DAMAGED AT NECROPSY	0	4	2	1
OVARIES: CYST(S)	1	1	0	0
KIDNEYS: ABSENT	1	0	0	0
URETERS: ABSENT	1	0	0	0
ABDOMINAL CAVITY: CONTENTS, DARK RED	1	0	0	0
DELIVERED GESTATION DAY 15	0	0	1	0
ORGAN LOST AT NECROPSY	0	1	0	0
BIED GESTATION DAY 7	0	0	0	1
STOMACH: DISCOLORATION, DARK RED	0	0	0	1

1- 0 MG/KG/DOSE 2- 20 MG/KG/DOSE 3- 100 MG/KG/DOSE 4- 200 MG/KG/DOSE

PROJECT NO.: ~~125~~-460002H
 SPONSOR: REGENERON PHARM.
 SPONSOR NO.: IL1 T-TX-05002

TABLE 12 (SCHEDULED NECROPSY)
 FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE
 SUMMARY OF MACROSCOPIC FINDINGS

PAGE 1

SCHEDULED NECROPSY				
GROUP:	1	M A L E	3	4
NUMBER OF ANIMALS IN DOSE GROUP	30	75	30	30
NUMBER OF ANIMALS EXAMINED	30	74	30	30
KIDNEYS				
-AREA(S), DEPRESSED	1	0	0	0
LUNGS				
-DISCOLORATION, DARK RED	0	1	0	0
RT EPIDIDYMIS				
-SMALL	0	1	0	0
SEMINAL VESICLES				
-SMALL	1	0	0	0
EAR				
-SCABBING	3	0	1	1
PREPUTIAL GL				
-AREA(S), RAISED	0	1	0	0
URETERS				
-DISTENDED	1	1	0	1
INJECTION SITE				
-AREA(S), DARK RED	0	1	0	0
TAIL				
-FRACTURED	0	1	0	0
NO SIGNIFICANT CHANGES OBSERVED - ALL EXAMINED TISSUES	25	68	29	28

1- 0 MG/KG/DOSE 2- 20 MG/KG/DOSE 3- 100 MG/KG/DOSE 4- 200 MG/KG/DOSE

PKRS12v4.05
 11/29/2005

Fertility parameters (mating/fertility index, corpora lutea, pre implantation loss, etc.):

- There was an increase (47-51%) in the early resorptions of the embryos in the treatment group compared to the controls (2.6, 4.9, 4.1, and 5.1 in the 0, 20, 100, and 200 mg/kg group respectively).
- There was an increase (37%) in the late resorptions of the embryo in the high dose group compared to that of the controls (0.5% in control vs. 0.8% in 200 mg/kg group).
- There was an increase in the post implantation loss (40-45%) in the test article treated group. The percent of post implantation loss were 3.2, 5.3, 4.1, and 5.8 in 0, 20, 100, 200 mg/kg dose group, respectively.
- In the low dose group, percent of the females w/evidence of mating but non gravid increased 7.3% compared to those of the control animals.
- There was an increase in the percentage of males with evidence of mating that did not sire a litter (16.7%) in the low dose group compared to those of the males from the control dose group. The percentage of males with evidence of mating

but did not sire a litter was also found to increase at the low dose group (7.3%) compared to 0% in control.

- Male fertility index in the low dose group was 84% in the low dose group; in the control 100% fertility and copulation was noted.

The data from the present study when compared to the historical control data from the same laboratory showed that female fertility index is lower than what is observed historically. Historical data for the early resorptions is not comparable with the present study. The post implantation loss is higher than the historical control in ¾ experiments from the historical controls. Interestingly, most of the findings noted in this study are from the low dose group indicating that the abnormalities might have resulted from the antibody formation as the toxicokinetic study indicated higher antibody formation at low dose in the mice in this experiment.

Summary of Findings from the Reproductive Toxicity Study Segment I

Parameters	Dose Group			
	0	20	100	200
Number of Gravid Females	29/30	63/75	28/30	29/30
% Female w/no evidence of mating	3.3	6.8 2-fold↑	0	0
% of Female w/evidence of mating but non gravid	0	7.3%	0	0
Female fertility index	100%	86%; 14%↓	100%	100%
Historical control data ranged from 92-98%				
Historical Control				
% Male w/ no evidence of mating	3.3%	10% 3-fold↑	0	0
% of Male w/evidence of mating that not sired a litter	0 %	8.4 %;	0	0
Male fertility index	97%	84%; 16%↓	100%	100%
Historical control data 76-94%				
Early Resorptions (%/litter)	2.6	4.9; 47%↑	4.1; 37%↑	5.1 50%↑
Historical control data not provided				
Early Resorptions (%/dam)	0.4	0.6	0.5	0.7
Historical control data not provided				
Post Implantation Loss (%/litter)	3.2	5.3 40%↑	4.1; 22%↑	5.8 45%↑
Historical Control/ Post Implantation Loss (%/litter): 4 experiments 6.9, 1.4, 0, 2.5				
Post Implantation Loss (%/dam)	0.4	0.7	0.5	0.8
Historical Control/ Post Implantation Loss (%/litter): 4 experiments 1.3, 0, 0, 0.2				

Sponsor's table Showing Changes in the Early Embryo-fetal Development Period:

TABLE 37
 FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE
 SUMMARY OF EMBRYONIC DATA AT SCHEDULED NECROPSY (% PER LITTER)
 PROJECT NO.: 460002F
 SPONSOR: REGENERON PHARM.
 SPONSOR NO.: IL1 T-TX-05002

GROUP:	0 MG/KG/DOSE	20 MG/KG/DOSE	100 MG/KG/DOSE	200 MG/KG/DOSE
TOTAL RESORPTIONS (%)				
MEAN	3.2	5.3	4.1	5.8
S.D.	4.01	7.61	5.95	9.72
N	29	63	28	29
PRR-IMPLANTATION LOSS (%)				
MEAN	7.6	5.0	6.5	3.3
S.D.	9.03	6.88	11.60	5.74
N	29	63	28	29
POST-IMPLANTATION LOSS (%)				
MEAN	3.2	5.3	4.1	5.8
S.D.	4.01	7.61	5.95	9.72
N	29	63	28	29

PROPORTIONAL (%) DATA COMPARED USING DUNN'S TEST
 CORPORA LUTEA AND IMPLANTATION SITES COMPARED USING DUNNETT'S TEST
 MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
 None significantly different from control group

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TABLE 37
 FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE
 SUMMARY OF EMBRYONIC DATA AT SCHEDULED NECROPSY (% PER LITTER)
 PROJECT NO.: 460002F
 SPONSOR: REGENERON PHARM.
 SPONSOR NO.: IL1 T-TX-05002

GROUP:	0 MG/KG/DOSE	20 MG/KG/DOSE	100 MG/KG/DOSE	200 MG/KG/DOSE
CORPORA LUTEA				
MEAN	14.5	13.9	13.3	14.2
S.D.	1.72	2.07	2.25	1.68
N	29	63	28	29
IMPLANTATION SITES				
MEAN	13.3	13.2	12.5	13.8
S.D.	1.80	1.72	2.80	1.72
N	29	63	28	29
VARIABLE EMBRYOS (%)				
MEAN	96.8	94.7	95.9	94.2
S.D.	4.00	7.61	5.95	9.72
N	29	63	28	29
DEAD EMBRYOS (%)				
MEAN	0.0	0.0	0.0	0.0
S.D.	0.00	0.00	0.00	0.00
N	29	63	28	29
EARLY RESORPTIONS (%)				
MEAN	2.6	4.9	4.1	5.1
S.D.	3.87	6.89	5.95	9.86
N	29	63	28	29
LATE RESORPTIONS (%)				
MEAN	0.5	0.3	0.0	0.8
S.D.	1.99	1.89	0.00	2.26
N	29	63	28	29

PROPORTIONAL (%) DATA COMPARED USING DUNN'S TEST
 CORPORA LUTEA AND IMPLANTATION SITES COMPARED USING DUNNETT'S TEST
 MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
 None significantly different from control group

PROJECT NO. 460002F FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE PAGE 1
 SPONSOR: REGENERON PHARM. SUMMARY OF FEMALE REPRODUCTIVE PERFORMANCE
 SPONSOR NO.: ILLI T-TX05002

DOSE GROUP	1		2		3		4	
	NO.	%	NO.	%	NO.	%	NO.	%
FEMALES ON STUDY	30		75		30		30	
FEMALES THAT DIED OR WERE EUTH. IN EXTREMIS	0		2-A, B		1-C		1-D	
FEMALES PAIRED FOR MATING	30		74-B		29		30-D	
FEMALES WITH EVIDENCE OF MATING	29	96.7	69	93.2	29	100.0	30	100.0
NO. GRAVID	29	100.0	64	92.8	29	100.0	30	100.0
NO. NONGRAVID	0	0.0	5	7.3	0	0.0	0	0.0
FEMALES WITH NO EVIDENCE OF MATING	1	3.3	5	6.8	0	0.0	0	0.0
NO. GRAVID	1	100.0	5	100.0	0	0.0	0	0.0
NO. NONGRAVID	0	0.0	0	0.0	0	0.0	0	0.0
TOTAL FEMALES GRAVID	30	100.0	69	93.2	29	100.0	30	100.0

1- 0 MG/KG/DOSE 2- 20 MG/KG/DOSE 3- 100 MG/KG/DOSE 4- 200 MG/KG/DOSE
 A = FEMALE NO. 2946 WAS EUTHANIZED IN EXTREMIS PRIOR TO PAIRING; NOT INCLUDED IN CALCULATIONS
 B = FEMALE NO. 3073 WAS EUTHANIZED IN EXTREMIS FOLLOWING EVIDENCE OF MATING; INCLUDED IN CALCULATIONS
 C = FEMALE NO. 3004 DIED PRIOR TO PAIRING; NOT INCLUDED IN CALCULATIONS
 D = FEMALE NO. 3110 DIED FOLLOWING EVIDENCE OF MATING; INCLUDED IN CALCULATIONS

PROJECT NO. 460002F FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE PAGE 2
 SPONSOR: REGENERON PHARM. SUMMARY OF FEMALE REPRODUCTIVE PERFORMANCE
 SPONSOR NO.: ILLI T-TX05002

DOSE GROUP	1		2		3		4	
	NO.	%	NO.	%	NO.	%	NO.	%
FEMALE MATING INDEX	30/30	100.0	74/74	100.0	29/29	100.0	30/30	100.0
FEMALE FERTILITY INDEX	30/30	100.0	69/74	93.2	29/29	100.0	30/30	100.0
FEMALE CONCEPTION INDEX	30/30	100.0	69/74	93.2	29/29	100.0	30/30	100.0
MEAN PRE-COITAL INTERVALS								
DAYS	2.8	NA	2.5	NA	2.9	NA	3.1	NA
S.D.	2.09	NA	1.94	NA	1.77	NA	2.43	NA
N	29	NA	69	NA	29	NA	30	NA

FEMALE MATING INDEX (%) = $\frac{\text{NO. OF FEMALES WITH EVIDENCE OF MATING (OR CONFIRMED PREGNANCY)}}{\text{TOTAL NO. OF FEMALES USED FOR MATING}} \times 100$

FEMALE FERTILITY INDEX (%) = $\frac{\text{NO. OF FEMALES WITH CONFIRMED PREGNANCY}}{\text{TOTAL NO. OF FEMALES USED FOR MATING}} \times 100$

FEMALE CONCEPTION INDEX (%) = $\frac{\text{NO. OF FEMALES WITH CONFIRMED PREGNANCY}}{\text{NO. OF FEMALES WITH EVIDENCE OF MATING (OR CONFIRMED PREGNANCY)}} \times 100$

1- 0 MG/KG/DOSE 2- 20 MG/KG/DOSE 3- 100 MG/KG/DOSE 4- 200 MG/KG/DOSE
 PRE-COITAL INTERVALS NOT SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP USING DONNETT'S TEST
 MATING, FERTILITY AND CONCEPTION INDICES NOT SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP USING CHI-SQUARE TEST

MANUAL v1.0
 10/05/2005
 R.11/19/2005

PROJECT NO. 460002F FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE PAGE 1
 SPONSOR: REGENERON PHARM. SUMMARY OF MALE REPRODUCTIVE PERFORMANCE
 SPONSOR NO.: ILLI T-TX05002

DOSE GROUP	1		2		3		4	
	NO.	%	NO.	%	NO.	%	NO.	%
MALES ON STUDY	30		75		30		30	
MALES THAT DIED OR WERE EUTHANIZED	0		1-A		0		0	
MALES PAIRED FOR MATING	30		74-A		29-B		30	
MALES WITH EVIDENCE OF MATING	29	96.7	68	91.9	29	100.0	29	96.7
NO. THAT Sired A LITTER	29	100.0	63	92.6	29	100.0	29	100.0
NO. THAT DID NOT SIRE A LITTER	0	0.0	5	7.4	0	0.0	0	0.0
MALES WITH NO EVIDENCE OF MATING	1	3.3	6	8.1	0	0.0	1	3.3
NO. THAT Sired A LITTER	1	100.0	5	83.3	0	0.0	0	0.0
NO. THAT DID NOT SIRE A LITTER	0	0.0	1	16.7	0	0.0	1	100.0
MALES THAT Sired MORE THAN 1 LITTER	0		1		0		1	

1- 0 MG/KG/DOSE 2- 20 MG/KG/DOSE 3- 100 MG/KG/DOSE 4- 200 MG/KG/DOSE
 A = MALE NO. 1626 DIED PRIOR TO PAIRING; NOT INCLUDED IN CALCULATIONS
 B = MALE NO. 1739 NOT PAIRED DUE TO DEATH OF FEMALE NO. 3004 PRIOR TO PAIRING; NOT INCLUDED IN CALCULATIONS

TABLE 3
FERT/EARLY EMB DEV TO IMPLANTATION OF MURINE IL-1 TRAP IN MICE
SUMMARY OF MALE REPRODUCTIVE PERFORMANCE

PROJECT NO.: 446002F
SPONSOR: REGENERON PHARM.
SPONGE NO.: ILLI T-TX05002

PAGE

DOSE GROUP	1		2		3		4	
	NO.	%	NO.	%	NO.	%	NO.	%
MALE MATING INDEX	30/30	100.0	73/74	98.6	29/29	100.0	29/30	96.7
MALE FERTILITY INDEX	30/30	100.0	58/74	91.9	29/29	100.0	29/30	96.7
MALE COPULATION INDEX	30/30	100.0	58/73	93.2	29/29	100.0	29/29	100.0

MALE MATING INDEX (%) =	NO. OF MALES WITH EVIDENCE OF MATING (OR FEMALES CONFIRMED PREGNANCY)	-----	X 100
	TOTAL NO. OF MALES (FEMALES) USED FOR MATING	-----	
MALE FERTILITY INDEX (%) =	NO. OF MALES Siring A LITTER	-----	X 100
	TOTAL NO. OF MALES USED FOR MATING	-----	
MALE COPULATION INDEX (%) =	NO. OF MALES Siring A LITTER	-----	X 100
	NO. OF MALES WITH EVIDENCE OF MATING (OR FEMALES CONFIRMED PREGNANT)	-----	

1- 0 MG/KG/DOSE 2- 20 MG/KG/DOSE 3- 100 MG/KG/DOSE 4- 200 MG/KG/DOSE

MATING, FERTILITY AND COPULATION INDICES NOT SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP USING CHI-SQUARE TEST

MPNC

Embryo fetal development

Study title: A Study for the Effect of IL-1 Trap on Embryo-Fetal Development in Cynomolgus Monkeys by Subcutaneous Administration

Key study findings:

- Pregnant monkeys were dosed with IL-1 Trap (0, 5, 15, and 30 mg/kg), subcutaneously, 2x/week from gestation days (GD) 20-48; 9 doses were administered/animal (dosed on GD 20, 23, 27, 30, 34, 37, 41, 44, and 48). Pregnancies were terminated by cesarean section on Days 100-103.
- There was dose related increase in the IL-1 Trap plasma concentration up to GD 28. The plasma concentration of the IL-1 Trap at low dose decreased at GD 35, at this time antibody formation in this dose group was found to be in the peak. The plasma concentration of the IL-1 Trap at mid dose decreased between GD 42-50, at this time antibody formation in this dose group was found to be in the peak. The high dose group showed high concentration of the compound until the end of the treatment period. The decrease in the plasma level of the IL-1 Trap is directly related to the antibody formation.
- After GD 28 an increase IL-1 Trap antibody formation was noted. Maximum serum concentration of the antibody was noted at GD 35 and GD55 with low and high dose group respectively. The concentration of the antibody was comparable in the low and mid dose group at the time when peak is reached. The concentration of the antibody in the serum from the high dose group was much less at its peak GD 55 than that of the other two treatment groups. At C-section 10/10, 9/11, 7/10 dams and fetuses from the low, mid, and high dose group was found to be antibody positive. Only one dam from the mid dose group showed IL-Trap plasma concentration at C-section.

- A statistically significant decrease in the estrogen concentration was noted between GD30-GD50. The decrease was higher in the low and mid dose group suggesting that the decrease in the hormone concentration might be related to the IL-1 Trap antibody formation.
- There were two late abortions one at GD38 in the low dose group and one at GD51 with the mid dose group, the timing of the abortion appeared to be related to the maximum antibody formation in these groups.
- There was a decrease in the organ weights in the high dose group compare to the organ weight of the control group. The trend is not always dose related. However, almost all of the organ weights that were measured in the fetuses decrease at high dose. The decrease in the ovary and thymus weights was greater than 10% (ovary 14%, thymus 12%) at high dose. The decrease in the spleen and lung weights was approximately 7% with high dose. The decrease in the thymus, spleen, and ovary was 21, 11, and 24% at low dose respectively; indicating that the effect might be IL-1 Trap antibody related and may be indirectly related to hormone depletion.
- There were two skeletal abnormalities/variations noted in the treatment group. There was abnormal arrangements of the ribs in the thoracic vertebra and absence thoracic vertebral bodies and arches was observed in 1/3 female fetus from the mid dose group. The sponsor reported that in the historical data from — laboratories such changes were noted in one control animal. A skeletal variation was also noted 1 male fetus in the control group, 2 male fetuses in the low and the mid dose group and the 2 female fetuses in the high dose group. The sponsor reported 12.8% changes in the lumbar vertebra are common in the cynomolgus monkeys as observed in the historical data. However, the incidence of variation in this experiment is higher than the historical control value 22.2%, 18.2%, and 20.0% in low, mid, and high dose group indicating a treatment related effect.
- The sponsor mentioned the NOAEL for this study to be 30 mg/kg since the findings are not dose related. However, because of the increase in fetal death in the low and mid dose group and increase in the skeletal abnormalities/ variations in the treatment groups, the reviewer could establish no NOAEL.

Study no.: — 223.15

Volume # and page #: The final study report is in the electronic document room.

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Conducting laboratory and location: —

Date of study initiation: 05-08-2003

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: IL-Trap; Lot #: B02024D600C11A (0.8 mL per vial)

B02024D60012A (2 mL per vial)

B02024D60013A (4 mL per vial)

Methods

Doses: 0, 5, 15, 30 mg/kg, twice/week from gestation days (GD) 20-48, 9 doses were given/animal (dosed on GDs 20, 23, 27, 30, 34, 37, 41, 44, and 48). Pregnancies were terminated by cesarean section on Days 100-103.

Species/strain: Cynomolgus monkey; *Macaca fascicularis*.

Number/sex/group/age /weight: 12/female/group/ 3-12 years old/2.34-6.74 kg.

Route, formulation, and volume: The test article was administered in the intrascapular area by the subcutaneous (bolus) injection. Doses of 5, 15, and 30 mg/kg IL-1 Trap were given undiluted at a concentration of 50 mg/mL. Dose levels in this study were 0 (placebo control), 5, 15, and 30 mg/kg/dose, with a dose volume of 0.6, 0.1, 0.3 and 0.6 mL/kg, respectively.

Satellite groups used for toxic kinetics: All dams and fetuses were subjected to TK blood analysis. Blood collection for maternal TK analysis and the anti-product antibody formation was performed once at pre-dose and 24 hours post-dose on GDs 20, 27, 34, 41 and 48 (the 1st, 3rd, 5th, 7th and 9th dose), once in the morning on GDs 55, 62. Fetal cord blood (approximately 1.0 mL) was collected from the umbilical artery during the scheduled cesarean section.

Study design:

Following is the design for the Reproductive Toxicity Segment II study.

Group/ Color Card	Test or Control Article	Dose Level (mg/kg)*	Dose Conc. (mg/mL)	Dose Volume (mL/kg)	Number of Pregnant Animals (Animal Number)
1 / white	Placebo	0	0	0.60	12 (101 – 103, 105 – 113)
2/ green	IL-1 Trap	5	50	0.10	12 (201 – 212)
3 / blue	IL-1 Trap	15	50	0.30	12 (301, 302, 304 – 313)
4 / red	IL-1 Trap	30	50	0.60	12 (401 – 412)

Parameter and endpoints evaluated:**Dams**

Clinical observations (including pregnancy monitoring data): On dosing days (GDs 20, 23, 27, 30, 34, 37, 41, 44 and 48), full observation was performed on all dams prior to dosing and at approximately 2 to 3 hours after dosing, and mortality was checked a minimum of 4 hours after the initial clinical observations.

On non-dosing days including the day of cesarean section and acclimation period, full observation was performed in the morning and mortality was checked a minimum of 4 hours after the initial clinical observations.

Food consumption: Estimated food consumption of each female was recorded from the first day of acclimation through to the day before scheduled cesarean section except for the 3 day mating session.

Body Weight: Individual dam will be weighed using an electronic balance on GDs 1 (at the end of mating), 19, 26, 33, 40, 47, 54, 61, 68, 75, 82, 89, and 100 (± 1 day) and recorded.

Serum Hormone Concentrations: All dams were subjected to the evaluation of the hormones: Progesterone (P), 17β -estradiol (E2) and prolactin (PL). The frequency of blood collection for the measurement of P and E2: GDs 20, 25, 30, 35, 40, 50, 60, 80, and 100 (± 1 day) and for PL: GDs 20, 40, 60, 80, and 100 (± 1 day)

On GDs 20, 40, 60, 80, and 100, approximately 2.2 mL of blood was drawn to obtain serum (at least 1000 μ L). On GDs 20, 40, 60, 80, and 100: Approximately 3.4 mL of blood was drawn to obtain serum (at least 1600 μ L).

Gross pathology findings:

As there were no maternal deaths or moribund sacrifice in this study, no gross pathological examinations were performed on the dams. Since fetal death was confirmed on GD51 by pregnancy monitoring (ultrasound examination), an emergency cesarean section was performed on one dam (animal no. 311) in the 15 mg/kg group. The dead fetus and placenta from this animal were fixed in 10% neutral buffered formalin.

Spontaneous abortion was confirmed on two dams in the control group (animal no. 108 on GD25 and animal no. 110 on GD24), three dams in the 5 mg/kg group (animal no. 206 on GD24, animal no. 209 on GD25 and animal no. 212 on GD38) and two animals in the 30 mg/kg group (animal no. 407 on GD25 and animal no. 409 on GD24). Because no fetuses could be confirmed by ultrasound, no cesarean section was performed.

Incidence of spontaneous abortions:

	0 mg/kg	5 mg/kg	15 mg/kg	30 mg/kg
Spontaneous abortion	2/12	3/12	0/12	2/12

Fetuses

Viability, sex, body weight, placental weight and external measurements:

After confirmation of fetal viability, all fetuses were euthanized with an over dose intraperitoneal injection of a commercial pentobarbital and phenytoin solution. Each fetus was measured for body weight and placental weight. Each fetus was sexed, and the following measurements, external and placental observations were performed.

Measurements: Head width, distance between the eyes, head circumference, chest circumference, crown-rump length, tail length, right paw and foot length, anogenital distance, amniotic fluid volume, diameters of primary and secondary placenta.

Observations: Body form, symmetry of head, facial form, mandibular formation, eyes and eyelids, hair of head, nipple formation, anus, fingers, toes, finger and toe nails, ears, tail, upper and lower extremities, external genitalia, vertebral column, umbilical cord and palate.

Organ weights (absolute and relative weights) the following organs were weighed and fixed in 10% neutral buffered formalin: adrenal glands, testes, ovaries, heart, lungs, spleen, thymus, liver, kidneys, uterus, and brain. The following organs were fixed in 10% neutral buffered formalin without weighing: eyes, stomach, small and large intestines, the skin of the head, ears, trachea (with thyroids), esophagus and any abnormal organs noted during the gross observation

Skeletal examination (ossification and skeletal length): The carcass of each fetus was fixed in ethyl alcohol, and skeleton was stained by Dawson's method. Each fetus was examined as follows: skeletal abnormalities or variations, skeletal development (number of bones with ossification centers of the vertebral centrum), skeletal length (right side) of ossified parts of the humerus, ulna, radius, femur, tibia, and fibula.

Toxicokinetics in Dams and Fetus:

All dams and fetuses were subjected to the toxicokinetic analysis. The frequency of blood collection was as follows.

Dams (serum): At pre-dose and 24 hours post-dose on selected dosing days (GDs 20, 27, 34, 41, and 48), once in the morning on GDs 55, 62, 69, 83, and once on the day of scheduled cesarean section (before anesthesia).

Fetuses (serum): At cesarean section

Fetuses (amniotic fluid): At cesarean section

Approximately 1.0 mL of blood was collected from a saphenous, femoral, or cephalic vein (dams), or umbilical artery (fetus), and transferred to an SST clot tube. Serum (at least 400 μ L) was obtained by centrifugation (2000x g, 15 minutes at room temperature) after stabilization at room temperature for 40 to 60 minutes.

Amniotic Fluid (fetuses): Amniotic fluid (at least 4 mL) was collected from each fetus (including the control fetuses) at cesarean section using a disposable syringe and needle.

Antibody Measurements in Dams and Fetuses:

Blood collection for maternal antibody measurement was performed once at pre-dose on GDs 20, 27, 34, 41, and 48 (the 1st, 3rd, 5th, 7th and 9th dose), once in the morning on GDs 55, 62, 69, 83, and once on the day of scheduled cesarean section (before anesthesia). Approximately 2.0 mL of blood was collected by venipuncture using a butterfly infusion set or needle and disposable syringe from the saphenous, femoral or cephalic vein from restrained, unsexed dams. Fetal cord blood (approximately 2.0 mL) was collected from the umbilical artery during the scheduled cesarean section. Only serum samples collected on GD20 (pre-dose), GD34 (pre-dose), GD55, and prior to cesarean section, along with a serum sample from fetus were analyzed by Regeneron Pharmaceuticals, Inc.

Results

Mortality (dams) Pre and Post Implantation Loss:

No maternal mortality or moribund sacrifices occurred. There were abortions at GD24 and 25 in 2/12 monkeys in the control, low and high dose group. However, in low dose group there was abortion in one monkey at GD 38 and in the mid dose group fetal death was observed at Day 51. There were no observations of fetal death in the control group in this study. In the mid dose group heartbeats were not heard at GD 25 from 6/12 animal. The heart beats of the fetus from all these animals were heard again on GD30. One of these fetuses died at GD-51. One animal from the low dose group had similar finding at GD 25; embryonic death was noted in this animal at GD38. The significance of such finding is not clearly known, but assumed to be related to the stress in these animals at this time period. These findings of the fetal abortion/death were not dose related. The sponsor's historical control data from 12 studies including the current study indicate that between GD20-35, 3 abortions were noted, between GD 35-46, 3 abortions were noted, and between GD46-55, 2 abortions were noted. The findings (abortion) in the current study was, however, higher in the incidence in the low and the mid dose animals compared to control at later days and coincides with the timing when the antibody formation was highest in the animals from those dose groups. Therefore, the fetal resorptions observed are considered treatment related.

Summary of Major Findings in Segment II Reproductive Toxicity Study:

Parameters	Dosages mg/kg			
	0	5	15	30
Fetal absorption /death	16.7%, 2/12 aborted at GD 25	25%, 2/12 aborted at GD 25, 1/10 aborted at GD 38	8.3%, 1/12 aborted at GD 25; 1/11 aborted at GD 51	16.7%, 2/12 aborted at GD 25
Historical control data from 16 studies including the current study w/181 dams showed that a total of 20 abortions noted, 12/20 occurred between GD20-GD35, 3/20 occurred between GD36-GD45, 2/20 occurred between GD 46-GD55				
Skeletal Abnormalities	0 (similar)	0	1/3 female fetus	0

(thoracic vertebra and arches arranged abnormally)	observation was made in one fetus - historical control)			
Historical control data from 16 studies including the current study w/87 fetus showed that 1/87 that is 1.1 % fetus had skeletal abnormalities in the thoracic vertebra.				
Skeletal Variations(bilateral lumber vertebra)	0 (historical control 12.8%)	22.2%	18.2%	20%
Historical control data from 16 studies including the current study w/87 fetus showed that 11/87 that is 12.6 % fetus had skeletal variations of lumber ribs.				
Single Placenta	3/10	0/92	2/11	4/10
Historical control data from 16 studies including the current study w/108 fetus showed that 20% fetuses had single placenta.				

Clinical signs (dams):

The clinical signs include abnormal stool often very hard or some time very soft in control as well as treated animals during the course of the study. Occurrence of such gastro intestinal disturbances was observed intermittently in all test article treated animals, however, such incidences were found to be recovered during the course of the study. The number of animals suffering from such incidence, however, was higher in treated groups than those of the controls. Bleeding around the external genitalia was also noted in a few animals, which might be associated with dry skin and hard feces. Abrasion in the skin and scab around the injection sites were also noted in higher number of treated animals during the course of the experiment, however, was observed to be recovered at the time of the sacrifice.

Body weight (dams):

The animals were dosed between GD20 and 48. As observed from the following table, there was a dose dependent decrease in the body weight gain during that time period (approximately 10% decrease in body weight gain was noted in the high dose group at Day 40 compared to body weight gain at GD19). The loss in the body weight gain, however, recovered after the treatment was discontinued.

Body weights (kg) in dams:

Dose mg/kg	GD*-19	GD-26	GD-33	GD-40	GD-47	GD-68	GD-82	GD-100
0	4.22	4.31	4.13	4.13	4.21	4.58	4.94	5.23
5	4.35	4.25	4.26	4.27	4.37	4.69	4.88	5.13
15	4.09	3.98	3.90	3.92	4.02	4.49	4.66	5.04
30	4.72	4.52	4.35	4.33	4.38	4.74	4.93	5.33

* GD-gestation days

Food consumption (dams):

The food consumption decreased abruptly during the treatment period as observed by the number of the unconsumed biscuits during the treatment period (refer to the table below).

Loss of appetite was observed in 1/12 control animals and 3/12 animals in each of the low, mid and high dose group. Anorexia was confirmed in the veterinarian's report. Extra fruit and liquid was provided to the animals for overcoming this condition. The reduced food consumption might have been the cause of the reduction in the body weight gain during the treatment period. The loss of appetite might be directly treatment related or might be related to the dryness of the system as demonstrated by the fact that the animals were willing to have fruits but not the biscuits. This might have also been resulted from the treatment related changes in the electrolyte balance and water excretion, which was not explored in this study.

Number of unconsumed biscuits/day:

Dose mg/kg	GD-20	GD-26	GD-33	GD-40	GD-47	GD-68	GD-82	GD-100
0	3.6	6.4	7.3	6.4	6.0	0.8	4.6	1.6
5	3.5	9.1	10.8	6.3	5.1	0.9	4.0	1.1
15	4.0	8.3	10.4	7.2	3.9	0.4	2.2	2.3
30	4.5	10	11.7	9.4	6.8	1.5	2.1	0.7

Serum Hormone Concentrations in Dams:

There was a significant decrease in the estrogen level during the treatment period and immediately after the treatment. Also, the level of estrogen in the treated animals continued to be in a lower level than those of the controls during the rest of the experimental period. There were no difference in the mean progesterone level in control and treated animals during this experimental period. However, there was also a decrease in the prolactin level in the treated animals during the course of the experiment.

In normal pregnancy estrogen and progesterone level continues to rise during pregnancy and remains high until parturition. In the current study, once the administration of the test articles was stopped, the estrogen level increased and the level of estrogen continued to rise until the C-section. The decrease in the estrogen and progesterone level appeared to be directly related to the treatment under this experimental condition.

Effect of IL-1 Trap on the Serum Hormone Concentrations:

Dose mg/kg	GD-20	GD-25	GD-30	GD-35	GD-40	GD-50	GD-80	GD-100
Estrogen (pg/mL)								
0	229	268	215	216	333	336	451	510
5	223	244	201	84*	229	163*	439	477
15	213	227	157*	77*	177*	150*	485	416
30	209	219	150*	115*	168*	163*	416	458
Progesterone (ng/mL)								
Dose mg/kg	GD-20	GD-25	GD-30	GD-35	GD-40	GD-50	GD-80	GD-100

0	2.6	3.9	7.0	6.3	2.0	0.8	2.5	2.8
5	2.9	4.4	8.8	6.8	2.5	1.0	3.5	4.1
15	2.1	7.4	9.6	8.9	4.7	2.02	3.1	3.7
30	2.4	5.0	7.3	6.9	2.4	0.74	2.1	3.2
Prolactin (ng/mL)								
Dose mg/kg	GD-20	GD-25	GD-30	GD-35	GD-40	GD-50	GD-80	GD-100
0	2.3	ND*	ND	ND	13.8	ND	10.9	23.5
5	1.4	ND	ND	ND	9.0	ND	12.2	12.0
15	2.0	ND	ND	ND	8.7	ND	10.8	7.0
30	2.7	ND	ND	ND	14.7	ND	8.5	8.9

ND = not detected

Toxicokinetics:

The plasma concentration of IL-1 Trap GD21 with low mid and high dose was 17.9, 89.3, and 159.1 mg/mL respectively indicating 5-fold increase in the plasma level of the drug from low-mid dose and 2-fold increase in the plasma concentration of the compound from mid to high dose. With low dose at Day 27 (pre dose for 3rd dose) and increase level of the compound (13525 ng/mL) in the plasma was noted. This accumulation was, however, decreased at GD34 (pre dose for 5th dose. Although, the compound was administered up to GD48, a gradual decrease in the plasma level of the compound was observed 24 hr post dosing, and also pre dosing from GD34 onwards, probably due to increase in the antibody formation. Plasma level of the compound was noted in one animal in this group up to GD69. Similar observation was made as regards to the plasma concentration of the compound with mid and high dose. One animal in the mid dose group had plasma concentration of IL-1 Trap in the plasma collected from pre C-section time point. One animal from the high dose group had plasma level of the compound up to GD62.

Anti IL-1 Trap antibody formation was observed in all animals from the treatment group. The antibody formation was detected in the low dose group at GD34; maximum concentration of antibody formation was detected from the animals of this dose group at GD55. All animals were detected to have antibody in the serum at C-section, all fetuses from this group were also positive with the anti IL-1 Trap antibody. The antibody formation with the mid dose maximized around GD55. The serum concentration of the antibody at GD34 with low dose was comparable to that of GD55 at mid dose. All but two dams and fetuses were positive for the presence of anti-IL-1 Trap antibody at C-section. The maximum antibody production with the high dose was detected at GD55; however, the plasma level of the antibody at GD 55 with high dose was much lower than that of the mid dose during that time point. Measurable amount of antibody was noted at C-section from all but 3 dams and fetuses from the high dose group.

IL-1 Trap Concentration and Anti-product Antibody Concentration in the Plasma 24 hr Pre and Post Treatment in Different Gestation Days (GD)

Dose mg/kg	GD-21	GD-27	GD-28	GD-34	GD-35	GD-41	GD-42	GD-48	GD-49	GD-69	GD-100
IL-1 Trap Plasma Concentration											
5	17964	13525	29408	615	2015	330	767	272	905	144	ND
15	89320	91169	144470	8736	28044	5232	22602	1815	12071	256	163
30	159118	237104	340904	29693	87386	10779	54731	19434	51774	4132	109
IL-1 Trap Antibody (RFU/mL)											
	GD 34			GD 55			Pre C Section			Fetus	
5	9-152			90-1024			8-57			2-25	
15	1-91			106-4444			10-325			4-102	
30	9-276			1-922			3-44			7-30	

REGENERON

Sample Analysis Report No. ILIT_TX_03010_SA_01V1

Appendix A (Data Tables) continued:

Table 3: Monkey Plasma and Amniotic Fluid Concentrations (ng/mL) of IL-1 Trap in the 5 mg/kg Cohort

Dose	Gestation Day	Draw Time	Animal #											
			201	202	203	204	205	206*	207	208	209*	210	211	212*
1	20	Pre Dose												
	21	24h Post Dose												
3	27	Pre Dose												
	28	24h Post Dose												
5	34	Pre Dose												
	35	24h Post Dose												
7	41	Pre Dose												
	42	24h Post Dose												
9	48	Pre Dose												
	49	24h Post Dose												
	55	Plasma TK												
	62	Plasma TK												
	69	Plasma TK												
	83	Plasma TK												
	Pre CS	Plasma TK												
	CS	Amniotic fluid												
	Fetus	Plasma TK												

BLQ = Below limit of quantitation (< 100 ng/mL) NS = No sample available for analysis CS = cesarean section

*: Monkey aborted, last sample collected on gestational day 25

†: Monkey aborted, last sample collected on gestational day 26

‡: Monkey aborted, last sample collected on gestational day 39

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Table 4: Mean Monkey Plasma and Amniotic Fluid Concentrations (ng/ml) of IL-1 Trap in the 5mg/kg Cohort

Dose	Gestation Day	Draw Time	Mean*	SD	N**	Increase***
1	20	Pre Dose	270	935	12	NA
	21	24h Post Dose	17964	9368	12	17964
3	27	Pre Dose	13525	4931	10	NA
	28	24h Post Dose	29408	9439	10	15883
5	34	Pre Dose	615	822	10	NA
	35	24h Post Dose	2015	2040	10	1400
7	41	Pre Dose	330	655	9	NA
	42	24h Post Dose	767	955	9	437
9	48	Pre Dose	272	540	9	NA
	49	24h Post Dose	905	891	9	633
	55	Plasma TK	145	435	9	NA
	62	Plasma TK	154	463	9	NA
	69	Plasma TK	144	433	9	NA
	83	Plasma TK	0	0	9	NA
	Pre CS	Plasma TK	0	0	9	NA
	CS	Amniotic fluid	0	0	9	NA
	Fetus	Plasma TK	0	0	9	NA

*Mean values were calculated using 0 for BLQ, where applicable (Refer to Table 3)

**Only monkeys who had a blood collection on that given day; animals who aborted were not included

***[24h Post Dose] - [Pre Dose]

NA: Not applicable

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Table 6: Mean Monkey Plasma and Amniotic Fluid Concentrations (ng/ml) of IL-1 Trap in the 15mg/kg Cohort

Dose	Gestation Day	Draw Time	Mean*	SD	N**	Increase***
1	20	Pre Dose	0	0	12	NA
	21	24h Post Dose	89320	36290	12	89320
3	27	Pre Dose	91169	26349	12	NA
	28	24h Post Dose	144470	45881	12	53301
5	34	Pre Dose	8736	10999	12	NA
	35	24h Post Dose	28044	19507	12	19308
7	41	Pre Dose	5232	10071	11	NA
	42	24h Post Dose	22602	23282	11	17370
9	48	Pre Dose	1815	2078	11	NA
	49	24h Post Dose	12071	12249	11	10256
	55	Plasma TK	860	2438	11	NA
	62	Plasma TK	449	1490	11	NA
	69	Plasma TK	256	848	11	NA
	83	Plasma TK	127	422	11	NA
		Pre CS	Plasma TK	163	542	11
	CS	Amniotic fluid	0	0	11	NA
	Fetus	Plasma TK	0	0	11	NA

*Mean values were calculated using 0 for BLQ, where applicable (Refer to Table 5)

**Only monkeys who had a blood collection on that given day; animals who aborted were not included

***[24h Post Dose] - [Pre Dose]

NA: Not applicable

Table 7: Monkey Plasma and Amniotic Fluid Concentrations of IL-1 Trap (ng/mL) in the 30 mg/kg Cohort

Dose	Gestation Day	Draw Time	Animal #													
			401	402	403	404	405	406	407 ^b	408	409 ^a	410	411	412		
1	20	Pre dose														
	21	24h post Dose														
3	27	Pre dose														
	28	24h post Dose														
5	34	Pre dose														
	35	24h post Dose														
7	41	Pre dose														
	42	24h post Dose														
9	48	Pre dose														
	49	24h post Dose														
	55	Plasma TK														
	62	Plasma TK														
	69	Plasma TK														
	83	Plasma TK														
	Pre CS	Plasma TK														
	CS	Amniotic fluid														
	Fetus	Plasma TK														

BLQ = Below limit of quantitation (<math>≤ 1000</math> ng/mL)

NS = No sample available for analysis CS = cesarean section

^a: Monkey aborted, last sample collected on gestational day 25

^b: Monkey aborted, last sample collected on gestational day 26

Table 10: Anti-IL-1 Trap Antibody Titer (RFU/mL) in Monkeys Administered 5 mg/kg IL-1 Trap

Dose	Gestation Day	Draw Time	Animal #											
			201	202	203	204	205	206 ^a	207	208	209 ^b	210	211	212 ^c
1	20	Pre AB												
5	34	Pre AB												
	55	Serum AB												
	Pre CS	Serum AB												
	Fetus	Serum AB												

BLQ = Below limit of quantitation (< 100 RFU/mL) NS = No sample available for analysis CS= cesarean section
^a: Monkey aborted, last sample collected on gestational day 25
^b: Monkey aborted, last sample collected on gestational day 26
^c: Monkey aborted, last sample collected on gestational day 39

Appendix A (Data Tables) continued:

Table 11: Anti-IL-1 Trap Antibody Titer (RFU/mL) in Monkeys Administered 15 mg/kg IL-1 Trap

Dose	Gestation day	Draw Time	Animal #											
			301	302	303 ^a	304	305	306	307	308	309	310	311 ^d	312
1	20	Pre AB												
5	34	Pre AB												
	55	Serum AB												
	Pre CS	Serum AB												
	Fetus	Serum AB												

BLQ = Below limit of quantitation (< 100 RFU/mL) NS = No sample available for analysis CS= cesarean section
^a: False positive diagnosis of pregnancy, animal excluded from evaluation of study
^b: Monkey aborted, last sample collected on gestational day 26
^c: Monkey aborted, last sample collected on gestational day 52

Table 12: Anti-IL-1 Trap Antibody Titer (RFU/mL) in Monkeys Administered 30 mg/kg IL-1 Trap

Dose	Gestation day	Draw Time	Animal #											
			401	402	403	404	405	406	407 ^a	408	409 ^b	410	411	412
1	20	Pre AB												
5	34	Pre AB												
	55	Serum AB												
	Pre CS	Serum AB												
	Fetus	Serum AB												

BLQ = Below limit of quantitation (< 100 RFU/mL) AB+ = Antibody positive NS = No sample available for analysis
 CS= cesarean section
^a: Monkey aborted, last sample collected on gestational day 25
^b: Monkey aborted, last sample collected on gestational day 26

Terminal and necropsic evaluations: C-section data:

All dams were subjected to C-section between days 100-103. Male: female ratio of the fetuses in the control, low, mid, and high dose group was 7:3, 4:5, 8:3, and 4:6 respectively. A single placenta was observed in 3/12 control animals and 4/12, high dose group animal. This findings were observed at high dose, thus might be considered as treatment related. However, the sponsor cited reference indicating that it might be a common occurrence for this species.

Offspring (malformations, variations, etc.):

No visceral anomalies were observed in any of the fetuses.

Following table shows the organ weights of the fetuses. There was a decrease in the organ weights in the high dose group compare to the organ weight of the control group. The trend is not always dose related. However, almost all of the organs weights that were measured in the fetuses decrease at high dose. The decrease in the ovary and thymus weights was greater then 10% (ovary 14%, thymus 12%). The decrease in the spleen and

lung weights was approximately 7%. Since IL-1 is known to have effects on the above mentioned organs, the inhibition of IL-1 might have an effect on these organs. Therefore, these effects might be considered as exaggerated pharmacological consequences of the inhibition of the target molecule.

Relative Organ Weights to Fetal Body Weights in the Fetus:

Dose mg/mL	Adrenal	Heart	Liver	Lung	Kidney	Spleen	Thymus	Ovary	Testes
0	0.436	4.06	25.6	16.3	5.20	1.59	1.88	0.185	0.169
5	0.361	4.06	25.9	16.5	5.10	1.43	1.50	0.141	0.181
15	0.367	4.10	23.9	16.6	5.43	1.43	1.46	0.217	0.154
30	0.428	3.80	25.2	15.3	5.06	1.48	1.66	0.157	0.161

There were two skeletal abnormalities/variations noted in the treatment group. There was abnormal arrangements of the ribs in the thoracic vertebra and absence thoracic vertebral bodies and arches was observed in 1/3 female fetus from the mid dose group. The sponsor reported that in the historical data from _____ such changes were noted in one control animal. A skeletal variation, bilateral lumbar vertebra was also noted 1 male fetus in the control group, 2 male fetuses in the low and the mid dose group and the 2 female fetuses in the high dose group. The sponsor reported 12.8% changes in the lumbar vertebra are common in the cynomolgus monkeys as observed in the historical data. However, the incidence of variation in this experiment is higher than the historical control value 22.2%, 18.2%, and 20.0% in low, mid, and high dose group indicating a treatment related effect. Considering that there was a significant difference in the levels of estrogen and prolactin between the control and treated animal and a direct relationship of such hormones with the bone ossification and density exist, the skeletal variation might be considered as indirect pharmacological consequence of the product. Also, there was an increased incidence of single placenta in the IL-1 Trap treated animals. The number of dams with single placenta were 3/10, 0/9, 2/11, and 4/10 in control, low, mid, and high dose.

Sponsor's Table Showing Skeletal Variations in the Cynomolgus Monkeys

Table 9-1 Summary of Morphological Examinations in Cynomolgus Monkey Fetuses

Group/Dose		1 IL-1 Trap Placebo (0 mg/kg)	2 IL-1 Trap (5 mg/kg)	3 IL-1 Trap (15 mg/kg)	4 IL-1 Trap (30 mg/kg)
No. of viable fetuses		10	9	11	10
External abnormalities	n (%)	0 0.0	0 0.0	0 0.0	0 0.0
Placental abnormalities	n (%)	3 30.0	0 0.0	2 18.2	4 40.0
Single placentas	n (%)	3 30.0	0 0.0	2 18.2	4 40.0
Visceral abnormalities	n (%)	0 0.0	0 0.0	0 0.0	0 0.0
Visceral variations	n (%)	0 0.0	0 0.0	0 0.0	0 0.0
Skeletal abnormalities	n (%)	0 0.0	0 0.0	1 9.1	0 0.0
Abnormal arrangement of the ribs and thoracic vertebrae	n (%)	0 0.0	0 0.0	1 9.1	0 0.0
Skeletal variations	n (%)	1 10.0	2 22.2	2 18.2	2 20.0
Lumbar ribs	n (%)	1 10.0	2 22.2	2 18.2	2 20.0

n : Number of fetuses with abnormalities or variations.
Not significantly different from control.

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Table 9-2 Individual Morphological Findings in Cynomolgus Monkey Fetuses

Group/Dose	Study Animal No. (Taboo No.)	Sex	External findings	Placental findings	Visceral findings		Skeletal findings	
					Abnormalities	Variations	Abnormalities	Variations
1 IL-1 Trap Placebo (0 mg/kg)	101 (020417)	Male	N	N	N	N	N	Lumbar ribs (bilaterally)
	102 (020586)	Male	N	N	N	N	N	N
	103 (020900)	Male	N	N	N	N	N	N
	105 (020925)	Male	N	N	N	N	N	N
	106 (020416)	Male	N	N	N	N	N	N
	107 (020936)	Male	N	Single placenta	N	N	N	N
	108 (020913) ^a	NA	NA	NA	NA	NA	NA	NA
	109 (020912)	Male	N	N	N	N	N	N
	110 (020654) ^b	NA	NA	NA	NA	NA	NA	NA
	111 (020924)	Female	N	Fused placenta	N	N	N	N
	112 (020934)	Female	N	Single placenta	N	N	N	N
	113 (020931)	Female	N	Single placenta	N	N	N	N
	2 IL-1 Trap (5 mg/kg)	201 (020919)	Female	N	N	N	N	N
202 (020930)		Female	N	N	N	N	N	N
203 (020916)		Female	N	N	N	N	N	N
204 (020906)		Male	N	N	N	N	N	N
205 (020409)		Male	N	N	N	N	N	N
206 (020409) ^c		NA	NA	NA	NA	NA	NA	NA
207 (020418)		Female	N	N	N	N	N	N
208 (020914)		Male	N	N	N	N	N	N
209 (020973) ^d		NA	NA	NA	NA	NA	NA	Lumbar ribs (bilaterally)
210 (020509)		Female	N	N	N	N	N	Lumbar rib (rudimentary, right)
211 (020922)	Male	N	N	N	N	N	N	
212 (011080) ^e	NA	NA	NA	NA	NA	NA	NA	

a) No. 108: Abortion was confirmed on Day 25 of gestation.
b) No. 110: Abortion was confirmed on Day 24 of gestation.
c) No. 206: Abortion was confirmed on Day 24 of gestation.
d) No. 209: Abortion was confirmed on Day 25 of gestation.
e) No. 212: Abortion was confirmed on Day 36 of gestation.
N: Normal
NA: Not applicable

Table 9-3 Individual Morphological Findings in Cynomolgus Monkey Fetuses

Dose (mg/kg)	Study Animal No. (Tattoo No.)	Sex	External findings	Placental findings	Visceral findings		Skeletal findings	
					Abnormalities	Yield/loss	Abnormalities	Variations
3	301 (020920)	Male	N	N	N	N	N	N
	302 (011075)	Male	N	N	N	N	N	N
	304 (020905)	Female	N	N	N	N	N	N
	305 (020385)	Male	N	N	N	N	N	N
IL-1 Trap (15 mg/kg)	306 (020921)	Male	N	Single placenta	N	N	Abnormal arrangement of the ribs and thoracic vertebrae (A)	N
	307 (020996)	Male	N	N	N	N	N	Lumbar ribs (bilateral)
	308 (020392)	Female	N	N	N	N	N	N
	309 (020414)	Male	N	Single placenta	N	N	N	N
	310 (011095)	Male	N	N	N	N	N	Lumbar ribs (bilateral)
	311 (011107) ^h	NA	NA	NA	NA	NA	NA	NA
	312 (020411)	Female	N	N	N	N	N	N
	313 (010341)	Male	N	N	N	N	N	N
	401 (020416)	Female	N	Single placenta	N	N	N	Lumbar ribs (bilateral)
	402 (020497)	Male	N	Single placenta	N	N	N	N
IL-1 Trap (30 mg/kg)	403 (020402)	Female	N	N	N	N	N	Lumbar ribs (bilateral)
	404 (020918)	Male	N	N	N	N	N	N
	405 (011171)	Male	N	N	N	N	N	N
	406 (020467)	Female	N	Single placenta	N	N	N	N
	407 (020905) ^g	NA	NA	NA	NA	NA	NA	NA
	408 (020907)	Female	N	N	N	N	N	N
	409 (020923) ^h	NA	NA	NA	NA	NA	NA	NA
	410 (020412)	Female	N	N	N	N	N	N
	411 (020501)	Male	N	N	N	N	N	N
	412 (020911)	Female	N	Single placenta	N	N	N	N

^h No. 311: Fetal death was confirmed on Day 51 of gestation.
^g No. 407: Fetal death was confirmed on Day 25 of gestation.
ⁱ No. 409: Fetal death was confirmed on Day 24 of gestation.
 N: Normal
 NA: Not applicable
 (A) Fusion of ribs: 3rd/4th, 6th/7th/8th and 10th/11th; 1st: 3rd/4th/5th and 6th/7th
 Fusion and/or absence of multiple thoracic vertebral bodies and arches.

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Prenatal and postnatal development

Study title: Subcutaneous Injection Study for Effects on Pre- and Post-natal Development, Including Maternal Function and Toxicokinetic with Murine IL-1 Trap in Mice

Key study findings:

- Pregnant mice were dosed with murine IL-1 Trap (0, 20, 100, 200 mg/mL) subcutaneously, 3x/week at gestation day (GD) 6, 8, 13, 15, 17 and lactation day (LD) 1, 3, 5, 8, 10, 12, 17, and 20. There were 24 mice each in the control, low, mid, and high dose toxicokinetic group; there were 30, 75, 30, and 30 animals in the main study in the control, low, mid, and high dose group respectively.
- Toxicokinetic analysis was done for IL-1 Trap and its antibody after 24 hrs of last dosing from the animals in the main cohort and after 24 hrs of dosing at GD6, GD17, and LD20. There was a dose related increase in the serum concentration of the IL-1 Trap at the end of the lactation period. The number of animals with detectable IL-1 Trap was higher in the high dose animals compare to those of the low dose group animals. The concentration of the IL-1 Trap antibody was much higher in the low dose group animals compare to those of the high dose group animals. Similarly, the number of animals in which the antibody was detected was much higher with low dose compare to that of the high dose group. The clearance of IL-1 Trap by its antibody might be the cause for the detection of the lower serum level of the IL-1 Trap in the low dose animals. *Most of the mortality findings in the study as described below showed higher incidence at low dose compare to those of the high dose group, the reviewer believes that IL-1 Trap antibody formation might have been the cause for such findings.*
- The number of F₀ dams mortality at 0, low, mid, and high dose was 2, 7, 5, 4 respectively. There was no mortality of control dams during gestation period. One dams each from low and mid dose group was found dead around GD17. At

LD1 mortality of F₀ females from 0, low, mid, and high dose was 2, 1, 1, 2 respectively. The number of dams found dead or the number of dams with total litter death between LD2-18 at 0, low, mid, and high dose group was 0, 5, 3, and 2 respectively.

- The percentage of still born F₀ pups from control, low, mid, and high dose group were 0.3, 0.5, 0 and 1.7% respectively. *This finding might be directly related to IL-1 Trap concentration in serum.*
- The mortality of F₁ pups between LD1-4 was 47, 56, 22, 68 in control, low, mid and high dose group respectively; similarly pup mortality between LD5-21 was 2, 16, 16 and 0 in control, low, mid and high dose group respectively. *This reverse dose relation of the increase death of the pups might be correlated to the increased antibody formation in low dose group.*
- The entire litter death between LD1-4 was 2, 3, 1, 4 in control, low, mid and high dose group respectively; entire litter death between LD5-21 was 0, 2, 2, and 0 in control, low, mid and high dose group respectively. *This reverse dose relation of the increase death of the litter in the later stage of the lactation might be correlated to the increased antibody formation in low dose group.*
- There were 4 deaths of the low dose F₀ offspring during maturation. Between Day 24-93, 3 low dose F₁ males died. There was macroscopic and microscopic correlation of the necropsy findings in these animals renal pelvic dilation and protein cast in the tubules were observed, one low dose female died enlarged spleen was observed in this animal. One male at mid dose was also found dead, similar necropsy findings of kidney obstruction was noted. In addition, 2 low dose males and one mid dose male was removed from the study due to urinary obstruction at Day 84 of maturation. *Protein deposition in the kidney in the low and mid dose group animals might be resulted from the antibody formation in these groups. Urinary obstruction also might have been resulted from the protein deposit. The sponsor mentioned increased incidence of pelvic dilation in males in CD1 mice, however, the absence of such findings in control and high dose animals where no antibody formation was noted indicate that there might be a relationship of immune complex formation with the protein cast deposition and renal pelvis dilation in the low and high dose males.*
- The mortality of F₁ dams during gestation and lactation period was 2, one each at GD17 and LD4 with high dose. The IL-1 Trap related decrease in estrogen production was noted in cynomolgus monkey. Hormones were not measured in mice during the late gestation period. However, these deaths might be indirectly related to the higher exposure of the animals with the IL-1 Trap. Note that the highest concentration of the serum IL-1 trap was found in F₀ dams from the high dose group. In addition, total litter death was observed in one F₁ dam from the low dose group at LD1. Similar observation of increased total litter death was also noted in the F₀ dams and may well be related to the treatment.
- The mortality of F₂ pups between LD1-4 was 9, 27, 3, 16 in control, low, mid, and high dose group respectively; the entire litter death between LD1-4 was 0, 1, 0, 1 in control, low, mid and high dose group, respectively.
- The pseudopregnancy was observed in F₀ females 0, 6, 3, and 1 in control, low, mid and high dose group respectively. The findings might be related to IL-1 Trap

antibody formation. IL-1 related increase in the pseudopregnancy is reported in the literature.

- There was a decrease in the body weight gain in F₀ dams during lactation which might have resulted in decrease in the body weight gain in F₁ pups during weaning. A decrease in the body weight gain was noted in the compound treated F₁ males and females when compared to those of the controls during maturation. There was decrease in the body weight in the F₂ pups also from the high dose group. *The reason for such decrease in body weight gain is unknown, however, the findings are considered treatment related. Similar findings were noted in treatment with other pro inflammatory cytokines. Serum concentration of IL-1 Trap or its antibody was not measured in these animals. However, increase in IL-1 Trap antibody was noted in animals with low dose of IL-1 Trap treatment. In high dose F₁ pups and weaned F₁ animals there might be low circulating IL-1 Trap which might have generated the antibody causing the decrease in the body weight gain.*
- There was an increase in number of F₁ and F₂ pups with thin, hunched appearance from the treatment group compare to control. Sores, hypoactivity, few feces etc was also noted in F₁ and F₂ pups from the treatment group. *Similar observation was noted with IL-1 inhibition in adults from previous studies, therefore, these findings are believed to be treatment related.*
- There was a decrease in the locomotor activity and learning behavior in F₁ pups from the high dose group than those in the control group. The increase in the locomotor activity in males and females in control from day 22 to week 5 was 23 and 13% respectively. The increase in the locomotor activity in males and females in the high dose group from day 22 to week 5 was 15 and 7% respectively. There was a slight decrease in the learning behavior in the high dose males (14%) and females (7%) compare to control group. *The reason for such decrease in the behavioral test is unknown. Since the findings are noted in the high dose, therefore the findings are considered treatment related. Hypoactivity, thinness etc was noted in the pups from the high dose group which might have a reflection in the behavioral test .which might be related to the lethargy observed in the clinical observation.*
- There was a slight decrease (7%) of the implantation sites in the high dose animals from the F₁ dams compare to those of the controls. This finding is considered treatment related due to its occurrence at the high dose. *The cause of such findings is not known, however, increased IL-1 level is noted during the perimplantation time in mice, any modulation of IL-1 level by IL-1 Trap might have some implication in the loss of implantation site, therefore the finding might be related to the physiological effect of IL-1 during pregnancy.*
- Another noteworthy finding was prolonged diestrus in F₁ females, the occurrence of such finding in the control, low, mid, and high dose were 14, 21, 17, and 28% respectively, indicating modulation of estrogen level. The compound was shown to decrease estrogen level in the monkeys. Since the serum hormonal effect of the compound was not measured in the mice, the cause of the prolonged diestrus can not be confirmed. However, it can be assumed that the

compound has a prolonged effect in the modulation of the hormones regulating estrus cycle, which might have an impact in the fertility in the F₁ generation.

- No NOAEL could be determined in this study because of the findings in the low dose group which is unlike the sponsor who determined 200 mg/kg as NOEL due to the lack of dose response for the findings.

Study no.: 7369-111

Volume # and page #: Volume 1&2; Page #1-761, submission in EDR

Conducting laboratory and location: _____

Date of study initiation: May 16, 2005

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: Murine IL-1 Trap, B0500 1K620X11A, and _____

Methods

Species/strain: CD-1 (ICR) BR, 12 weeks

Study design: Refer to the table for dose, route, and number of animals used in the main study and satellite group for toxicokinetic analysis.

Group	No. of Animals		Dose Level (mg/kg/day)	Dose Concentration (mg/mL)	Dosing Schedule ^a Days
	Female				
Main Study Animals					
1 (Control)	30		0	0	GD6-LD20 ^a
2 (Low)	75		20	2.5	GD6-LD20 ^a
3 (Mid)	30		100	12.5	GD6-LD20 ^a
4 (High)	30		200	25	GD6-LD20 ^a
Toxicokinetic Animals					
5 (Control)	24		0	0	GD6-LD20 ^a
6 (Low)	24		20	2.5	GD6-LD20 ^a
7 (Mid)	24		100	12.5	GD6-LD20 ^a
8 (High)	24		200	25	GD6-LD20 ^a

^a Subcutaneous injection given once daily on GD 6, 8, 10, 13, 15, and 17 per animal and LD 1, 3, 5, 8, 10, 12, 15, 17, and 20 per animal to F₀ females only.

Parameters and endpoints evaluated:

All F₀ animals were observed twice daily for mortality, abnormalities and signs of pain and distress. Body weights were taken in 3-day intervals until autopsy. Food consumption was measured at the same time. The date of delivery, litter size (number born live and dead), and sex, weight, and observations of individual offspring was recorded after birth. All dead pups were examined for cervical, thoracic, and abdominal viscera abnormalities and preserved in alcohol. During lactation, the general growth/development and functionality of pups were monitored as follows (days testing starts): pinna unfolding (Day 1), surface righting reflex (Day 4), hair

growth (Day 7), incisor eruption (Day 7), eye opening (Day 11), and auditory startle (Day 21). Pups were weaned on LD21, and 20 pups/sex/litter were randomly selected for the F₁ maturation phase (7 weeks in duration). At Lactation Day 4 litter size and the sex, weight, and observations of individual offspring were recorded prior to culling. Litters with more than eight pups were reduced (culled, via random card draw) to that number to produce, as nearly as possible, litters containing four males and four females to reduce possible confounding effects resulting from different litter sizes. Culled pups were euthanized, examined for thoracic, cervical, or abdominal viscera abnormalities, and discarded.

At LD 7, 14, and 21 (weaning) litter size and the sex, weight, and observations (by exception) of individual offspring were recorded. At the completion of weaning for each litter, one pup/sex/litter was randomly selected and individually identified. Records of the derivation of each animal were maintained so that sibling matings were avoided. An additional five pups/sex/group were randomly selected to serve as a replacement pool. These animals were maintained until all selected F₁ animals began the maturation phase. If not used, these animals were euthanized and discarded without necropsy. All F₁ pups not selected were euthanized and discarded without necropsy. Postweaning maturation phase, week 0 maturation data collection began on Day 28 ± 3 days postpartum.

F₁ females were weighed on GD 0, 7, 14, and 17 and LD 0 and 4. After litters were killed, females were returned to the pre-mating weekly body weight recording schedule. At each body weight interval, each animal was examined (animal removed from cage); abnormal or normal responses were recorded for each animal. Food consumption record was collected for all F₁ females on GD 0, 7, 14, and 17 and LD 0 and 4. For 2 weeks prior to mating, daily vaginal lavage was assessed for stage of estrus at approximately the same time every day. Estrous cycle determination continued until confirmation of mating occurred or the mating period ended. After the F₁ animals completed the 7-week post weaning maturation phase, each female was cohabited with a male from the same group. Sibling matings were avoided. Once mating occurred, the males and females were separated. Each pair had a maximum of 14 days to achieve mating. Females for which no evidence of mating was detected were placed in nesting boxes after the mating period. F₁ females were allowed to deliver naturally. The date of delivery, litter size (number born live and dead) and sex, weight, and observations of individual offspring was recorded as soon as possible after birth. All dead pups were examined for cervical, thoracic, and abdominal viscera abnormalities and then preserved in alcohol. Lactation Day 4 clinical observations, body weights, and confirmation of sex were conducted for F₂ pups.

Results

Summary Table Showing Major Findings:

Parameters	Dose Group (mg/kg)			
	0	20	100	200
Number of F0 Dams found Aborted	0	3	4	2
Number of F0 Dams showing TLD	2	4	2	3
Number of F0 Dams showing gravid but not pregnant	0	6	3	1
Increase % of still borne pups	0.3	0.5	0	1.7
Total pup necropsy findings/empty stomach	4	6	8	8
F1 Pups dying/killed/missing 0-4	47	56	22	68
F1 Pups dying/killed/missing 5-21	2	16	16	0
Entire litter died/killed/missing 0-4	2	3	1	4
F1 Pups dying/killed/missing 5-21	0	2	2	0
% of F1 animals showing prolonged diestrus	14	21	17	28
F2 Total Litter Death	0	2	0	2

F₀ mortality:

There was an increase in the number of deaths in the treated F₀ dams compared to those of the controls. The number of dams found dead or aborted was 0 in the control group but 3, 4, and 2 in the low mid and high dose group. Among these one low dose dam was found dead at GD-17 another mid dose animals was found to be aborted at GD-16. All other dams died in the later stage of the lactation period, since no blood samples could be collected from these animals the quantitative analysis of IL-1 Trap and/or its antibody in the serum could not be conducted. The data suggest that although not dose related, the death of the dams in the later period of the gestation or lactation period appeared to be treatment related.

The number of dams showing total litter death (TLD) was 2, 4, 2, and 3 in the control, low, mid, and high dose group respectively. Note that both of the TLDs in control occurred at LD 1. However, in low dose treated animals, 2/4 TLDs were at LD 2, when the serum level of the IL-1 Trap antibody was 73 and 43 mcg/mL, and 1/4 TLD occurred at LD7 when the serum level of the IL-1 Trap antibody was approximately 8 mcg/mL. In mid dose, there were 2 TLDs but both occurred in the LD1, no measurable serum levels

of the drug or related antibody were detected in these animals. In the high dose group, there was 2/3 TLDs at LD-1, no measurable serum levels of the drug or related antibody were detected in these animals. However, 1/3 TLD at this dose occurred at LD4, when the serum level of IL-1 Trap was approximately 250 mcg/mL. All these data suggest that the TLD in the later period of the lactation might be treatment related. There were 0, 6, 3, and 1 dam in control, low, mid, and high dose group respectively, where no delivery was observed, therefore, these animals were sacrificed at GD26. Necropsy findings in these animals confirmed that these animals were not pregnant. Although, no toxicokinetic analysis was done from these animals an IL-1 Trap or its antibody related effect in these animal might be ruled out. Induction of pseudo pregnancy by the modulation of IL-1 is reported in the literature. Therefore, this might be considered as a treatment related effect. The applicant mentioned that the contract research organization (CRO) for this study the _____, did not have any historical control data for th Segment III study, similar historical findings from another CRO the Charles River Lab and suggested that this finding is not treatment related.

Unscheduled Deaths of TK F₀ Animals

Dose Level	Animal ID	Death Category	Day	Levels of murine IL-1 Trap (mcg/mL)	Levels of anti-murine IL-1 Trap antibodies (mcg/mL)
20 mg/kg/day	A87364	TLD	LD 7	BLQ	7.4
100 mg/kg/day	A87386	Found dead	LD 16	NS	NS
100 mg/kg/day	A87390	Aborted, Sacrificed	GD 16	46.7	BLQ
200 mg/kg/day	A87412	Found dead	LD 16	NS	NS

BLQ Below limit of quantitation

NS No Sample

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Unscheduled Deaths of Main Study F₀ Animals

Dose Level	Animal ID	Death Category	Day	Remarkable Clinical Observations	Remarkable Necropsy Observations	Levels of murine IL-1 Trap (mcg/mL)	Levels of anti-murine IL-1 Trap antibodies (mcg/mL)
0 mg/kg/day	A87174	Total litter death, sacrificed	LD 1	None	None	NS	NS
	A87183	Total litter death Sacrificed	LD 1 LD 3	None	None	BLQ	8.0
20 mg/kg/day	A87195	Found dead	LD 18	Hypoactivity	None	NS	NS
	A87200	Found dead	LD 13	None	None	NS	NS
	A87201	Total litter death, sacrificed	LD 1	Sore/scab(s)	None	NS	NS
	A87204	Found dead	GD 17	None	None. Confirmed pregnant	NS	NS
	A87230	Total litter death, sacrificed	LD 2	None	None	BLQ	73.8
	A87232	Total litter death, sacrificed	LD 2	None	None	BLQ	43.5
100 mg/kg/day	A87263	Found dead	LD 11	None	None	NS	NS
	A87265	Total litter death, sacrificed	LD 1	None	None	BLQ	BLQ
	A87287	Found dead	LD 9	None	None	NS	NS

Dose Level	Animal ID	Death Category	Day	Remarkable Clinical Observations	Remarkable Necropsy Observations	Levels of murine IL-1 Trap (mcg/mL)	Levels of anti-murine IL-1 Trap antibodies (mcg/mL)
200 mg/kg/day	A87311	Total litter death, sacrificed	LD 4	None	None	219.7	BLQ
	A87318	Found dead/total litter death	LD 1	None	Gelatinous skin in the dorsal cervical and dorsal thoracic area	NS	NS
	A87320	Total litter death sacrificed	LD 1 LD 3	None	None	1.0	BLQ

BLQ Below limit of quantitation

NS No Sample

F₀ reproduction:

Natural delivery data from the dams showed that the percentages of still born pups were 0.3, 0.5, 0, and 1.7% in control, low, mid, and high dose group respectively. There was an increase in the number of pups dying between LD1-21 compare to that of the controls. Number of pups died between LD1 and LD21 in control, low, mid, and high dose were 49, 72, 38, and 68 respectively. Similarly, the number of entire litter died between LD1

and 21 in control, low, mid, and high dose were 2, 5, 3, and 4 respectively. The death of pups by litter was 2, 5, 3, and 4 for control (pups died at LD1), low (one pup died at LD1, one pup died at LD2, one pup each died at LD 8, 13, and 18), mid (one pup died at LD1, one pup died at LD8, one pup died at LD9), and high (3 pups died at LD1 and one pup died at LD3). The deaths of the pups from the late lactation day period might be related to the drug related antibody formation within the serum of the pups as observed from the reverse dose response.

NATURAL DELIVERY DATA AND LITTER DATA SUMMARY - F ₀ GENERATION					
DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 20 MG/KG/DAY	GROUP 3 100 MG/KG/DAY	GROUP 4 200 MG/KG/DAY
Pup Survival Indices					
Livebirth Index (Number born alive/number born)	MEANS	99	100	100	96
Viability Index (Number alive Day 4 precull/ number liveborn)	MEANS	88	93	93	82
Weaning Index (Number alive at weaning/ number alive at Day 4 postcull)	MEANS	99	97	92	100
Pup Disposition					
Culled day 4	TOTAL	108	298	133	90
Killed		0	17	16	12
Died		6	13	2	5
Cannibalized		10	33	18	17
Missing		33	9	2	34
Pups Surviving at 21 days	TOTAL	208	503	187	192
Pups Dying, Killed, Missing, and/or Cannibalized					
days 0-4		47	56	22	68
days 5-21		2	16	16	0
Entire Litter Died, Killed, Missing, and/or Cannibalized					
days 0-4	N	3	3	1	4
days 5-21	N	0	2	2	0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * - P<0.05 ** - P<0.01.
 N = Number of Litters.
 TOTAL = Number of Pups or Implants.

F₀ dams clinical signs:

The clinical observation in F₀ dams consisted of swollen abdomen in 3, 7, 2, and 1 dams from the control, low, mid, and high dose group, respectively; discolored abdomen in 1, 6, 3, and 1 dams from the control, low, mid, and high dose group, respectively. The reverse dose response curve for such findings could not be explained, however, the effect of IL-1 Trap antibody in these animals might be a possibility.

F₀ pups clinical signs:

The clinical observation of the pups from in the treatment group were limited to cyanotic appearance pale appearance in at least one litter from the high dose group, missing tails were also observed from two animals at the low dose group and one animal from the high dose group. No such observation was noted in the pups from the control animals.

F₀ body weight:

During the lactation period there was a decrease in the body weight gain in all groups. However, the changes body weight gain between the LD 17-21 in low, mid, and high dose group was 16, 28, and 19% lower than that of the controls respectively. Similarly, the changes body weight gain between the LD 10-14 in low, mid, and high dose group was 24, 36, and 12% lower than that of the controls respectively. There were a higher number of deaths of the F₀ dams and TLDs during this time period. The significance of

this finding is unknown; however, an indirect hormone related effect of the compound might not be ruled out.

F₀ dams necropsy:

One dam at high dose group showed gelatinous skin and one animal each from low and high dose group showed gravid uterus indicating pseudo pregnancy. These effects might be considered as treatment related since skin effects are common with anti-IL-1 therapy.

F₀ pups necropsy:

There was an increase in post mortem autolysis in litters and empty stomach in treated animals compare to those of the controls (refer to attach table). The total pup necropsy observation was 4, 6.1, 8.0, and 7.7% in the pups from the control; low, mid, and high dose animals.

Summary of Pup Necropsy Observations - F₀ Generation

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 20 MG/KG/DAY	GROUP 3 100 MG/KG/DAY	GROUP 4 200 MG/KG/DAY
Litters Evaluated	N	25	66	25	26
Pups Evaluated	N	116	316	114	109
Liveborn	N	115	313	114	106
Stillborn	N	1	3	0	3
POST MORTEM AUTOLYSIS					
Pup Incidence	N ‡	2 1.7	3 0.9	2 1.8	2 1.8
Litter Incidence	N ‡	1 4.0	2 3.0	2 8.0	2 7.7
STOMACH-EMPTY					
Pup Incidence	N ‡	0 0.0	2 0.6	0 0.0	0 0.0
Litter Incidence	N ‡	0 0.0	2 3.0	0 0.0	0 0.0
TOTAL PUP NECROPSY OBSERVATIONS					
Pup Incidence	N ‡	2 1.7	5 1.6	2 1.8	2 1.8
Litter Incidence	N ‡	1 4.0	4 6.1	2 8.0	2 7.7

N = Number

F₁ mortality:

Three 20 mg/kg/day males were found dead, one each on day 18, day 49, and day 50. One 100 mg/kg/day male was found dead on day 93. One 20 mg/kg/day female was found moribund and subsequently sacrificed on day 24, and one 200 mg/kg/day female was found dead on day 52. Clinical observations included findings of thin and hunched appearance, ataxia, hypoactivity, squinted eyes, few or no feces, and/or yellow/rough haircoat, which were primarily noted in the 20 mg/kg/day male and 20 mg/kg/day female. Remarkable necropsy findings were observed (dilated pelvis(es) and aplasia of the kidneys) in the 100 mg/kg/day male and (enlarged spleen) in the 200 mg/kg/day female. Two 20 mg/kg/day males and one 100 mg/kg/day male were removed from study on Day 84 per veterinarian request due to signs that included sores on genital region, swollen penis, sores on ears, swollen perineal region, rough hair coat, and yellow hair coat in the urogenital region. The latter three deaths were attributed to urinary obstruction that was mentioned in the published literature with this strain of mice. During gestation, a 200 mg/kg/day F₁ dam aborted on GD17 with findings of red/black vaginal discharge. During lactation, one 200 mg/kg/day F₁ dam had a total litter death on LD 3-4, had

findings of thin, hunched, and pale appearance, hypoactivity, ataxia, squinted eyes, and/or few or no feces, and was subsequently found dead on LD 4. Pups from this litter were cold to touch. On LD1, one 20 mg/kg/day F₁ dam had a total litter death. Note that no mortality occurred from the control group of F₁ generation during development or gestation or during the lactation period suggesting the mortality in the F₁ generation are related to the treatment.

F₁ physical development:

There was a slight decrease in the mean age in days for eye opening, hair growth, and incisor opening in treated group compare to that of the control indicating earlier maturation in the treated groups. These findings although not always statistically significant are dose-related. The significance of these findings is unknown.

Reflex and Development - Mean Age in Days Pups Reaching Criterion - F₀ Generation

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 20 MG/KG/DAY	GROUP 3 100 MG/KG/DAY	GROUP 4 200 MG/KG/DAY
PREPUTIAL SEPARATION	MEAN	35.81	36.43	36.79	36.25
	S.D.	1.18	1.46	1.25	1.15
	N	27	43	24	24
	COVARIATE ADJUSTED MEAN	35.82	36.41	36.79	36.28
VAGINAL OPENING	MEAN	30.59	30.40	30.21	30.24
	S.D.	1.05	0.77	0.66	0.52
	N	27	63	24	25
	COVARIATE ADJUSTED MEAN	30.59	30.39	30.21	30.25
EYE OPENING	MEAN	15.54	15.33	15.13	14.92
	S.D.	1.14	1.22	0.74	0.91
	N	28	64	24	25
	COVARIATE ADJUSTED MEAN	15.56	15.30	15.13	14.97
HAIR GROWTH	MEAN	10.96	10.64	10.04**	10.64
	S.D.	1.10	1.07	1.14	0.95
	N	28	64	25	25
	COVARIATE ADJUSTED MEAN	11.00	10.60	10.04	10.71
INCISOR ERUPTION	MEAN	12.14	12.05	11.67	11.92
	S.D.	0.89	0.79	0.70	1.00
	N	28	64	24	25
	COVARIATE ADJUSTED MEAN	12.15	12.04	11.67	11.93

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * - P<0.05 ** - P<0.01.
N = Number of Litters

F₁ body weights:

There was a decrease in the body weight gain in F₁ females noted at LD 7-20. Note that there was a decrease in the body weight of F₀ lactating females at this time period. Although there was a partial recovery of the body weight gain between days 21-42, a decrease in the body weight gain was noted again between days 56-70 (50-43% decrease at high dose compare to controls). There were no apparent changes in the body weight gain from control and treated animals in gestating females from F₁ generation. However, lactating females from the treated groups of the F₁ females were found to gain 3-5% less weight compare to those of the controls during the lactating days 0-4.

MEAN BODY WEIGHT CHANGES (g) - F₁ GENERATION

SEX: FEMALES		DOSE LEVEL	GROUP 1 0 MG/KG/DAY	GROUP 2 20 MG/KG/DAY	GROUP 3 100 MG/KG/DAY	GROUP 4 200 MG/KG/DAY
DAY	63 TO 70	MEAN	-1.9	10.8	19.2	-3.2
		S.D.	-	14.4	-	1.7
		N	1	4	1	2

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

Mean Maternal Body Weight Changes During Lactation (g) - F₁ Generation

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 20 MG/KG/DAY	GROUP 3 100 MG/KG/DAY	GROUP 4 200 MG/KG/DAY	
DAYS	0 TO 4	MEAN	7.52	7.11	7.45	7.42
		S.D.	1.71	2.38	2.85	1.87
		N	12	85	20	16

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

The mean body weight gain in F₁ females during the rest phase between days 70-112 decreased in treated groups compare to those of the controls as shown in the table below. The decrease in body weight gain although not statistically significant appeared to be dose related. The decrease in the body weight gain in high dose group vs. control was 1.1 vs. 0.5 between days 70-77 and 0.1-0.7 between days 105-111.

Mean Body Weight Changes (g) - F₁ Female Rest Phase

SEX: FEMALES		DOSE LEVEL	GROUP 1 0 MG/KG/DAY	GROUP 2 20 MG/KG/DAY	GROUP 3 100 MG/KG/DAY	GROUP 4 200 MG/KG/DAY
DAY	70 TO 77	MEAN	0.5	1.0	-	-1.1
		S.D.	-	-	-	0.6
		N	1	1	-	2
DAY	77 TO 84	MEAN	-3.0	-2.9	-3.0	-3.6
		S.D.	4.0	3.1	4.2	4.4
		N	26	57	23	22
DAY	84 TO 91	MEAN	0.3	0.2	0.3	0.6
		S.D.	2.5	1.3	1.6	1.2
		N	28	61	23	22
DAY	91 TO 98	MEAN	0.1	0.0	-0.3	0.0
		S.D.	1.0	1.8	1.7	1.4
		N	28	62	24	22
DAY	98 TO 104	MEAN	-0.2	-0.4	0.4	-0.4
		S.D.	1.2	1.5	0.1	2.3
		N	5	35	4	4
DAY	105 TO 111	MEAN	0.7	0.4	0.5	0.1
		S.D.	1.5	1.5	1.0	1.4
		N	23	27	20	18

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

There was a decrease in the body weight gain in the F₁ males between days 49 - 112. The decrease was consistent throughout this time period except for a slight rebound during the days 70-77 and days 98-105. The decrease in the body weight gain was dose related although statistically significant (P<0.01) only at day 84. The decrease in the body weight gain in the high dose vs. control males were -0.4 vs. -0.8 between day 49-56; 1.0 vs. 1.6 between days 56-63; -0.6 vs. 0.3 between days 77-84, and 0 vs. 0.6 between days 105-112.

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MEAN BODY WEIGHT CHANGES (G) - F1 GENERATION

SEX: MALES		DOSE LEVEL	GROUP 1 0 MG/KG/DAY	GROUP 2 20 MG/KG/DAY	GROUP 3 100 MG/KG/DAY	GROUP 4 200 MG/KG/DAY
DAY	63 TO 70	MEAN	0.6	0.1*	0.0*	0.4
		S.D.	0.9	1.0	0.7	0.7
		N	28	60	24	25
DAY	70 TO 77	MEAN	0.3	0.3	0.3	0.4
		S.D.	0.8	0.9	0.9	1.0
		N	28	60	24	25
DAY	77 TO 84	MEAN	0.3	0.1	-0.3	-0.6**
		S.D.	1.1	0.9	0.9	0.8
		N	28	60	24	25
DAY	84 TO 91	MEAN	0.9	0.6	0.5	0.8
		S.D.	1.0	0.8	1.2	0.9
		N	28	58	23	25
DAY	91 TO 98	MEAN	0.5	0.4	0.4	0.0
		S.D.	1.0	0.9	0.7	0.9
		N	28	58	22	25
DAY	98 TO 105	MEAN	-0.3	0.0	0.2	0.4*
		S.D.	0.9	0.9	0.9	0.9
		N	28	58	22	25
DAY	105 TO 112	MEAN	0.6	0.1	0.5	0.0
		S.D.	0.7	0.8	0.5	0.9
		N	23	25	18	21

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * - P<0.05 ** - P<0.01.

F₁ clinical signs:

During the maturation phase yellow hair coat was observed in approximately 20% of the low dose males around Day 42 which was recovered largely at day 49. At day 49 an increase in the number of mid and high dose males showing similar clinical signs compare to those of the low dose groups were noted. No such findings were noted in control animals. Other clinical signs in males consisted of rough hair coats during day 50 in low dose males. Higher incidence of no or little feces, pale appearance in mid and high dose animals were also noted in males from mid and high dose group. In females sores were noted at day 42, number of females in the low dose group showing such incidence was 3/67. At day 43, 3/67 females also had red/black discharge. At day 49, sores were noted in 1/30, high dose group females. Rough hair coat and a few or no feces were also noted in a few animals from the treatment group.

The clinical signs during gestation period of F₁ females were similar in control and treatment group and consisted of rough hair coat and sores. However, one pregnant female from the high dose group had red and black discharge at day 17, and one other female from the same group aborted at the same day. No such findings were noted in females from the control group.

The F₁ males and females were observed up to 70-112 days for clinical signs during the resting period. In females, the incidence of clinical findings such as sores, alopecia, squinted eye, and rough hair coat were high in treated animals compare to that of the controls in the later period of observation that is between 3-4 months.

F₁ behavioral evaluation:

The locomotor activity was performed on day 22 and week 5 with the pups from the F₁ generation. There was an increase in the locomotor activity in all treated animals compare to control at day 22. It was noted that at week 5 the increase in the locomotor activity from day 22 in the control males and females were 23 and 13% respectively. At the same time period the locomotor activity of the male pups from the low, mid, and high

dose group was approximately 15% respectively and those of the female pups from different dose groups were 7%. The decrease in the motor activity in the treated group might be related to the pharmacological activity of the compound.

Summary of Motor Activity Counts – F₁ Generation

DAY 22 POSTPARTUM											
MINUTES:	1-5	6-10	11-15	16-20	TOTAL	1-5	6-10	11-15	16-20	TOTAL	
GROUP: 1 Male (0 MG/KG/DAY)						GROUP: 1 Female (0 MG/KG/DAY)					
N	33	33	33	33	33	N	33	33	33	33	
MEAN	302	240	208	180	931	MEAN	305	225	192	185	
S.D.	60.2	41.7	60.7	58.1	212.6	S.D.	70.7	62.4	59.9	69.6	
GROUP: 2 Male (20 MG/KG/DAY)						GROUP: 2 Female (20 MG/KG/DAY)					
N	68	68	68	68	68	N	68	68	68	68	
MEAN	341	256	223	188	1008	MEAN	319	238	189	180	
S.D.	94.8	66.5	55.9	50.8	223.2	S.D.	70.4	53.9	58.2	56.1	
GROUP: 3 Male (100 MG/KG/DAY)						GROUP: 3 Female (100 MG/KG/DAY)					
N	29	29	29	29	29	N	29	29	29	29	
MEAN	316	238	195	182	930	MEAN	305	239	198	185	
S.D.	68.1	56.9	58.5	55.5	199.6	S.D.	69.9	52.8	46.0	48.1	
GROUP: 4 Male (200 MG/KG/DAY)						GROUP: 4 Female (200 MG/KG/DAY)					
N	30	30	30	30	30	N	30	30	30	30	
MEAN	340	246	202	180	968	MEAN	318	230	196	185	
S.D.	86.0	63.8	42.0	49.0	200.6	S.D.	67.6	61.3	60.1	71.1	

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

SUMMARY OF MOTOR ACTIVITY COUNTS - F₁ GENERATION

WEEK 5											
MINUTES:	1-5	6-10	11-15	16-20	TOTAL	1-5	6-10	11-15	16-20	TOTAL	
GROUP: 1 Male (0 MG/KG/DAY)						GROUP: 1 Female (0 MG/KG/DAY)					
N	33	33	33	33	33	N	33	33	33	33	
MEAN	360	283	237	217	1067	MEAN	345	297	268	1159	
S.D.	86.2	66.6	58.2	61.9	238.9	S.D.	62.8	72.6	71.7	72.7	
GROUP: 2 Male (20 MG/KG/DAY)						GROUP: 2 Female (20 MG/KG/DAY)					
N	67	67	67	67	67	N	67	67	67	67	
MEAN	351	272	239	218	1080	MEAN	339	275	250	1106	
S.D.	79.3	66.7	53.9	49.9	220.1	S.D.	77.4	69.3	68.2	78.8	
GROUP: 3 Male (100 MG/KG/DAY)						GROUP: 3 Female (100 MG/KG/DAY)					
N	29	29	29	29	29	N	29	29	29	29	
MEAN	354	269	225	215	1062	MEAN	350	280	259	1132	
S.D.	79.5	63.3	65.8	56.6	225.1	S.D.	79.2	65.2	75.6	69.2	
GROUP: 4 Male (200 MG/KG/DAY)						GROUP: 4 Female (200 MG/KG/DAY)					
N	30	30	30	30	30	N	30	30	30	30	
MEAN	345	258	225	211	1039	MEAN	334	274	252	1100	
S.D.	74.7	58.0	49.0	48.1	202.4	S.D.	58.4	50.8	60.6	58.3	

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

There was a slight decrease in the learning high dose males (14% decreases) and females (7% decrease) compare to those of the controls as observed in the Maize test.

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Water "M" Maze Test 1: Learning - F₁ Generation

Trial #:	Males						Females					
	1	2	3	4	5	6	1	2	3	4	5	6
	Group 1 - 0 mg/kg/day											
Number of animals tested	33	33	33	33	33	33	33	33	33	33	33	33
Number of animals with a positive response	5	12	23	26	27	24	3	12	25	28	24	30
Percent of animals with a positive response	15	36	70	79	82	73	9	36	76	85	73	91
	Group 2 - 20 mg/kg/day											
Number of animals tested	68	68	68	68	68	68	68	68	68	68	68	68
Number of animals with a positive response	16	36	49	54	53	57	9	31	51	55	52	53
Percent of animals with a positive response	24	53	72	79	78	84	13	46	75	81	76	78
	Group 3 - 100 mg/kg/day											
Number of animals tested	29	29	29	29	29	29	29	29	29	29	29	29
Number of animals with a positive response	2	12	19	19	23	25	5	15	17	24	23	27
Percent of animals with a positive response	7	41	66	66	79	86	17	52	59	83	79	93
	Group 4 - 200 mg/kg/day											
Number of animals tested	30	30	30	30	30	30	30	30	30	30	30	30
Number of animals with a positive response	5	17	21	26	25	19	3	15	23	21	23	25
Percent of animals with a positive response	17	57	70	87	83	63	10	50	77	70	77	83

Note: All trials were conducted with the escape ramp positioned in the right-hand compartment.

F₁ reproduction:

There was a slight decrease (7%) in the implantation sites in high dose females compare to controls. Slight decrease in the gestation index was also noted in high dose group (71%) compare to those of the controls (79%).

Natural Delivery Data and Litter Data Summary - F₁ Generation

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 20 MG/KG/DAY	GROUP 3 100 MG/KG/DAY	GROUP 4 200 MG/KG/DAY
Females:	Mated	N 31	66	29	28
	Pregnant	N 24	63	25	24
	Delivering	N 19	56	20	17
		N 61	85	49	41
Duration of Gestation:	MEAN	19.0	19.0	18.9	18.9
	S.D.	0.0	0.0	0.5	0.4
	N	19	54	19	17
Females with Liveborn Pups	N	19	56	20	17
Gestation Index	%	79	89	80	71
With Stillborn Pups	N	2	6	2	0
	%	11	11	10	0.0
Females with no Liveborn Pups	N	0	0	0	0
Females with no Pups Delivered	N	0.0	0.0	0.0	0.0
	%	0	0	0	0
Pups Delivered	MEAN	240	713	252	216
	S.D.	12.63	12.73	12.60	11.71
	N	1.77	2.30	2.87	1.86
	N	19	56	20	17
Liveborn		238	704	250	216
Stillborn		2	8	2	0
Uncertain		0	1	0	0
Implantation Sites	MEAN	252	753	256	210
	S.D.	13.26	13.45	13.30	12.35
	N	1.45	2.12	2.92	3.71
	N	19	56	20	17

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.
N = Number of Females or Litters.
TOTAL = Number of Pups or Implants.

Natural delivery data from the F₁ females also showed that the viability index from high dose F₁ females for pups was slightly lower (91/100) than that of the controls (96/99) at Day 4. Also, the number of pups died at high dose was 16/200 (8%) compared to 9/229 in controls (4%). Between Days 0-4 entire litter death was observed in two animals one each from low and high dose respectively. No such incidence was noted in controls.

LITTERS 41
NATURAL DELIVERY DATA AND LITTER DATA SUMMARY - F1 GENERATION

DOSE LEVEL	GROUP			
	1 0 MG/KG/DAY	2 20 MG/KG/DAY	3 100 MG/KG/DAY	4 200 MG/KG/DAY
Pup Survival Indices				
Livebirth Index (Number born alive/number born)	MEAN% 99	99	99	100
Viability Index (Number alive Day 4 precull/ number liveborn)	MEAN% 96	96	99	91
Pup Disposition				
Killed	0	0	0	0
Died	2	2	1	9
Cannibalized	0	0	0	0
Missing	7	25	2	7
Pups Surviving at 4 days	TOTAL 229	677	247	200
Pups Dying, Killed, Missing, and/or Cannibalized				
days 0-4	9	27	3	16
Entire Litter Died, Killed, Missing, and/or Cannibalized				
days 0-4	N 0	1	0	1

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.
N = Number of Litters.
TOTAL = Number of Pups or Implants.

The clinical observations were limited to the high dose animals during the lactating period were pale appearance, hypoactivity, squinted eyes, sores, few or no feces, and rough hair coat.

Summary of Clinical Observations During Lactation - F1 Generation

		LACTATION DAY : 0 1 2 3 4 TOTAL				
NUMBER OF ANIMALS EXAMINED						
GROUP	1 0 MG/KG/DAY	19	0	0	0	19
GROUP	2 20 MG/KG/DAY	56	1	0	0	57
GROUP	3 100 MG/KG/DAY	20	0	0	0	20
GROUP	4 200 MG/KG/DAY	17	0	1	1	19
NORMAL						
NO SIGNIFICANT CLINICAL OBSERVATIONS						
		1	18	0	0	19
		2	55	0	0	57
		3	18	0	0	19
		4	15	0	0	15
DEAD						
FOUND DEAD						
		1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
		4	0	0	0	1
TOTAL LITTER DEATH, SACRIFICED						
		1	0	0	0	0
		2	0	0	0	1
		3	0	0	0	0
		4	0	0	0	0
APPEARANCE						
CSO - THIN						
		1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
		4	0	0	1	1
CSO - HUNCHED						
		1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
		4	0	0	1	1

Another noteworthy finding was prolonged diestrous in F₁ females, the occurrence of such finding in the control, low, mid, and high dose were 14, 21, 17, and 28% respectively, indicating low circulating estrogen level. The compound was shown to decrease estrogen level in the monkeys. Since the serum hormonal effect of the compound was not measured in the mice, the cause of the prolonged diestrus can not be

confirmed. However, it can be assumed that the compound has a modulatory effect on the hormones regulating estrus cycle which is continued to be observed in the F₁ generation and might have an impact in the fertility.

F₁ necropsy findings:

Macroscopic observation of the F₁ animals consisted of increased incidence of dilated renal pelvis in males and females. The percentages of animals showing pelvic dilation were 6, 18, 14, and 10 in the control, low, mid, and high dose respectively. An increase level of the antibody formation was noted at low dose compare to that of the high dose with this compound. Therefore, this reverse dose related findings might well be correlated with the immunogenicity of the compound. The incidence of pelvic dilation was higher in males than in females; the number of males showing pelvis dilation was 2, 12, 4, and 3 with control, low, mid, and high dose group respectively. One female at high dose group also showed pelvis dilation. The sponsor mentioned that historical control from CD1 mice showed that the incidence of pelvis dilation is higher in males than in females. Most of the macroscopic observation of pelvis dilation was confirmed microscopically. Microscopic examination of the kidney was associated with the higher incidence of the microconcretion of the tubule, and protein cast in treated animals compare to those of the controls. One of the males, which were found dead, had an aplastic kidney in addition to renal pelvis dilation. One female, which was found dead from high dose group showed, enlarged spleen. Due to the fact that the finding was in the high dose group, the finding might be considered as treatment related

Summary of Parental Necropsy Observations - F₁ Generation

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 20 MG/KG/DAY	GROUP 3 100 MG/KG/DAY	GROUP 4 200 MG/KG/DAY
MALES	N	33	62	29	30
KIDNEY(S)-DILATED PELVIS (ES)	N	2	12	4	3
KIDNEY(S)-APLASTIA	N	0	0	1	0
KIDNEY(S)-ENLARGED	N	0	0	1	0

N - Number

**APPEARS THIS WAY
ON ORIGINAL**

Summary of Microscopic Observations - Unscheduled Death - F₁ Generation

TABLE INCLUDES: SEX=ALL; GROUP=ALL; WEEKS=1-24 DEATH=UNSCHED; FIND=ALL; SUBSET=T		-- NUMBER OF ANIMALS AFFECTED --							
		SEX: -----MALE-----				-----FEMALE-----			
ORGAN AND FINDING DESCRIPTION	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
	*** TOP OF LIST ***	NUMBER:	0	0	1	0	0	0	0
KIDNEY (KD)	NUMBER EXAMINED:	0	0	1	0	0	0	0	0
	NOT REMARKABLE:	0	0	0	0	0	0	0	0
--INFLAMMATION, CHRONIC		0	0	1	0	0	0	0	0
--BASOPHILIC TUBULES		0	0	1	0	0	0	0	0
--PELVIC, DILATATION		0	0	1	0	0	0	0	0
--PROTEIN CAST		0	0	1	0	0	0	0	0
--TUBULE, CYST		0	0	1	0	0	0	0	0
--TUBULE, DILATATION		0	0	1	0	0	0	0	0
--UNILATERALLY EXAMINED/APLASIA		0	0	1	0	0	0	0	0
--GLOMERULUS, FIBROSIS		0	0	1	0	0	0	0	0
--RENAL FIBROSIS		0	0	1	0	0	0	0	0
*** END OF LIST ***									

Summary of Microscopic Observations - Terminal Sacrifice - F₁ Generation

TABLE INCLUDES: SEX=ALL; GROUP=ALL; WEEKS=1-24 DEATH=T; FIND=ALL; SUBSET=T		-- NUMBER OF ANIMALS AFFECTED --							
		SEX: -----MALE-----				-----FEMALE-----			
ORGAN AND FINDING DESCRIPTION	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
	*** TOP OF LIST ***	NUMBER:	5	15	6	6	3	3	3
KIDNEY (KD)	NUMBER EXAMINED:	5	15	6	6	3	3	3	4
	NOT REMARKABLE:	0	0	0	1	0	0	0	0
--INFLAMMATION, CHRONIC		5	11	5	5	3	2	2	3
--BASOPHILIC TUBULES		4	4	3	4	3	2	0	2
--PELVIC, DILATATION		2	9	3	4	0	0	0	1
--TUBULE, MICROCONCRETION		0	3	1	0	0	0	0	0
--PROTEIN CAST		1	10	3	3	2	1	2	3
--TUBULE, CYST		2	1	0	1	1	2	0	1
--TUBULE, DILATATION		2	2	1	0	2	1	1	3
--TUBULE, BROWN PIGMENT		0	0	0	2	2	0	0	0
--CHR INFLAM PERIRENAL FAT		0	0	1	0	0	0	0	0
*** END OF LIST ***									

F₂ findings:

The clinical observation of the F₂ pups limited to the high dose animals during Day 0-4. Few pups from all of the treatment groups were found pale, thin, weak, and cold to touch, no such findings were observed in control animals (refer to the tables below).

SUMMARY OF CLINICAL OBSERVATIONS DURING LACTATION - F1 GENERATION

		LACTATION DAY : 0 1 2 3 4 TOTAL					
NUMBER OF ANIMALS EXAMINED							
GROUP	1	0 MG/KG/DAY	19	0	0	0	19
GROUP	2	20 MG/KG/DAY	56	1	0	0	56
GROUP	3	100 MG/KG/DAY	20	0	0	0	20
GROUP	4	200 MG/KG/DAY	17	0	1	1	17
CSC - BROWN HAIRCOAT							
	1		0	0	0	0	0
	2		0	0	0	0	0
	3		0	0	0	0	0
	4		0	0	1	1	1
EXCRETION							
CSC - FEW OR NO FECES							
	1		0	0	0	0	0
	2		0	0	0	0	0
	3		0	0	0	0	0
	4		0	0	0	0	1
DAM / LITTER							
TOTAL LITTER DEATH							
	1		0	0	0	0	0
	2		0	1	0	0	1
	3		0	0	0	0	0
	4		0	0	0	1	1
PUP(S) - BY LITTER							
PUP(S) - PALE							
	1		0	0	0	0	0
	2		1	0	0	0	1
	3		2	0	0	0	2
	4		0	0	0	0	0
PUP(S) - COLD TO TOUCH							
	1		0	0	0	0	0
	2		0	0	0	0	0
	3		0	0	0	0	0
	4		1	0	0	0	1

SUMMARY OF CLINICAL OBSERVATIONS DURING LACTATION - F1 GENERATION

		LACTATION DAY : 0 1 2 3 4 TOTAL					
NUMBER OF ANIMALS EXAMINED							
GROUP	1	0 MG/KG/DAY	19	0	0	0	19
GROUP	2	20 MG/KG/DAY	56	1	0	0	56
GROUP	3	100 MG/KG/DAY	20	0	0	0	20
GROUP	4	200 MG/KG/DAY	17	0	1	1	17
PUP(S) - WEAK							
	1		0	0	0	0	0
	2		0	0	0	0	2
	3		0	0	0	0	1
	4		0	0	0	0	0
PUP(S) - THIN							
	1		0	0	0	0	0
	2		0	0	0	0	0
	3		0	0	0	0	1
	4		0	0	0	0	0
PUP(S) - MISSING TAIL, PARTIAL							
	1		0	0	0	0	0
	2		1	0	0	0	1
	3		0	0	0	0	0
	4		0	0	0	0	0

There was a slight decrease in the mean body weights gains of the F₂ pups from the treatment group compare to those of the control (refer to the table below). A decrease of approximately 3-7% was observed in male pups at Day 4.

NATURAL DELIVERY DATA AND LITTER DATA SUMMARY - F1 GENERATION					
DOSE LEVEL		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0 MG/KG/DAY	20 MG/KG/DAY	100 MG/KG/DAY	200 MG/KG/DAY
Pup Weight/Litter (grams)					
day 0 MALES - Postcull	MEAN	1.59	1.60	1.63	1.58
	S.D.	0.13	0.14	0.17	0.08
	N	19	56	20	17
	Covariate Adjusted MEAN	1.59	1.60	1.63	1.58
day 0 FEMALES - Postcull	MEAN	1.54	1.51	1.55	1.51
	S.D.	0.12	0.12	0.15	0.11
	N	19	55	20	17
	Covariate Adjusted MEAN	1.54	1.51	1.55	1.51
day 4 MALES	MEAN	2.66	2.63	2.65	2.57
	S.D.	0.33	0.39	0.27	0.36
	N	19	55	20	16
	Covariate Adjusted MEAN	2.65	2.63	2.65	2.58
day 4 FEMALES	MEAN	2.57	2.53	2.49	2.48
	S.D.	0.34	0.36	0.35	0.37
	N	19	54	20	16
	Covariate Adjusted MEAN	2.54	2.54	2.49	2.49

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.
N = Number of Litters.

Toxicokinetics:

Toxicokinetic analyses were performed from all of the animals in the main cohort after 24 hrs of the final dosing. Murine IL-1 Trap was detected from 11/63, 22/23, and 25/25 animals in the low, mid, and high dose group respectively after 24 hrs of the final dosing. The levels of IL-1 Trap in the serum ranged from 0.9-9.6 µg/mL in the low dose group, 1.0-269 µg/mL in the mid dose group, and 4.7-780 µg/mL in the high dose group. The results showed that although the serum level of the compound varied widely within the individuals of the same group, there is clearly a dose related increase in the serum level of the compound. The LOQ of murine IL-1 Trap in this assay was approximately — ng/mL. At the end of the study period all animals in the high dose group had detectable IL-1 Trap level which is unlike the animals in the low dose group where only 17% of the animals had detectable IL-1 Trap level. This indicates that increase IL-1 Trap antibody in the low dose group might be responsible for hastening the clearance of IL-1 Trap from the low dose group animals.

Murine IL-1 Trap antibody was detected in the serum of 6/28, 59/63, 16/23, and 6/24 mice from the placebo, low, mid, and high dose group a respectively after 24 hrs of final dosing. The levels of IL-1 Trap antibody in the serum ranged from 2.7-32.4 µg/mL in the placebo group; 1.4-4868 µg/mL in the low dose group, 1.4-404.4 µg/mL in the mid dose group, and 5.2-18.5 µg/mL in the high dose group. The cause of antibody positively in the placebo group is unknown. The increase in the antibody positive mice in low dose group is a general phenomenon with the administration of the biological proteins. In addition, the subcutaneous administration of the protein might have enhanced the antibody production.

The murine IL-1 Trap and the antibody to the murine IL-1 Trap was analyzed from the serum of all animals in the toxicokinetic group after 24 hrs of dosing at GD6, GD17, and LD23. In the animals from the placebo group no IL-1 Trap was determined at days GD6, 17, and LD23, however, IL-1 Trap antibody was detected in 1/8 animals in the LD23 from the placebo group. The number of animals with circulating IL-1 Trap at GD6 was 7/8 at low dose, the level of the IL-1 Trap in this dose group at this time point was approximately 2.4 µg/mL. IL-1 Trap was not detected from the serum of the low dose group animals at GD 17 and LD23. However, IL-1 Trap antibody was detected in 2

animals at GD17 and all animal at LD23 in the serum of the low dose group animals, the range of the concentrations of the antibody was 20-466.6 µg/mL. All animals in the mid and high dose group had circulating IL-1 Trap at GD6, the level of the IL-1 Trap in these dose groups at this time point ranged from 7-23.4 µg/mL. The number of mice at mid and high dose group with detectable IL-1 Trap at GD17 was 2/8 and 4/8 respectively.

Similarly, the number of mice at mid and high dose group with detectable IL-1 Trap at LD23 was 0/5 and 4/7 respectively. The number of mice at mid and high dose group with detectable IL-1 Trap antibody at GD17 was 3 and the number of animals with detectable IL-1 Trap antibody at LD23 was 5. Note that although the number of animals showing antibody positivity in these two dose groups is similar the level of antibody production was higher in the mid dose group (1.6- 150.8 µg/mL) than that of the high dose group (5.9-54.6 µg/mL).

Summary of the Toxicokinetic Analysis

Analyses of Data from the Toxicokinetic Cohorts			
Dose Groups	GD6 (2 hr post dose)	GD17 (48 hr post dose)	LD20-23 (72 hr post dose)
IL-1 Trap Concentration (µg/mL)/# of Animals Showing IL-1 Trap Concentration			
20	0.8-1.7; 7/8	1.7; 1/8	BLQ; 0/8
100	1.1-17; 8/8	4.9-26; 2/8	46.7; 1/8
200	7.8-60; 8/8	1-26; 3/8	19-81; 4/8
IL-1 Trap Antibody Concentration (µg/mL) /# of Animals Showing Antibody Concentration			
20	BLQ; 0/8	8-35; 2/8	7.4-466; 8/8
100	2.5; 1/8	5.3-25.4; 3/8	1.6-151; 5/8
200	BLQ; 0/8	7.1-12.8; 2/8	5.9-54; 5/8
Dose Groups	GD6 (2 hr post dose)	GD17 (48 hr post dose)	LD20-23 (72 hr post dose)
Analyses of Data from the Main Study Cohorts			
Dose Groups	24 hrs post LD20		
IL-1 Trap Concentration (µg/mL)/# of Animals Showing IL-1 Trap Concentration			
20	1.5-3.1; 11/63		
100	7.9-269; 22/23		
200	1-402; 25/25		
IL-1 Trap Anti body Concentration (µg/mL) /# of Animals Showing Antibody Concentration			
20	1.4-4868; 59/63		
100	3.1-47.2; 16/23		
200	5.2-108; 6/24		

3 Page(s) Withheld

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 Draft Labeling

 Deliberative Process

Table 5: Levels of mL-1-1 Trap (mcg/mL) and anti-mL-1-1 Trap antibodies (mcg/mL) in placebo-treated mice in the toxicokinetic study cohort.

Gestation Day 6 (2 h after 1st dose)			Gestation Day 17 (48 h after previous dose)			Lactation Day 20/23 (72 hours after previous dose)		
Mouse#	mIL-1 Trap (mcg/mL)	Antibody (mcg/mL)	Mouse#	mIL-1 Trap (mcg/mL)	Antibody (mcg/mL)	Mouse#	mIL-1 Trap (mcg/mL)	Antibody (mcg/mL)
A87322			A87330			A87338†		
A87323			A87331			A87339†		
A87324			A87332			A87340†		
A87325			A87333			A87341†		
A87326			A87334			A87342*		
A87327			A87335			A87343*		
A87328			A87336			A87344		
A87329			A87337			A87345†		
Mean**	0.0		Mean**	0.0		Mean**	0.0	
SD**	0.0		SD**	0.0		SD**	0.0	
SEM**	0.0		SEM**	0.0		SEM**	0.0	

* Animal underwent an unscheduled sacrifice or was found dead; data not used

**0 used to calculate mean, SD, SEM if value is BLQ

†Sample collected on LD23

BLQ = Below limit of quantitation (< 0.05 mcg/mL in the murine IL1 Trap assay, < 0.05 mcg/mL in the anti-murine IL1 trap assay)

NS = No Sample

AB+ = Antibody Positive

Table 6: Levels of mL-1-1 Trap (mcg/mL) and anti-mL-1-1 Trap antibodies (mcg/mL) in the 20 mg/kg toxicokinetic study cohort.

Gestation Day 6 (2 h after 1st dose)			Gestation Day 17 (48 h after previous dose)			Lactation Day 20/23 (72 h after previous dose)		
Mouse#	mIL-1 Trap (mcg/mL)	Antibody (mcg/mL)	Mouse#	mIL-1 Trap (mcg/mL)	Antibody (mcg/mL)	Mouse#	mIL-1 Trap (mcg/mL)	Antibody (mcg/mL)
A87346			A87354			A87362†		
A87347			A87355			A87363†		
A87348			A87356			A87364*		
A87349			A87357			A87365†		
A87350			A87358			A87366†		
A87351			A87359			A87367		
A87352			A87360			A87368		
A87353			A87361			A87369		
Mean**	2.4		Mean**	0.2		Mean**	0.0	
SD**	2.3		SD**	0.6		SD**	0.0	
SEM**	0.8		SEM**	0.2		SEM**	0.0	

* Animal underwent an unscheduled sacrifice or was found dead; data not used

**0 used to calculate mean, SD, SEM if value is BLQ

†Sample collected on LD23

BLQ = Below limit of quantitation (< 0.05 mcg/mL in the murine IL1 Trap assay, < 0.05 mcg/mL in the anti-murine IL1 trap assay)

NS = No Sample

Table 7: Levels of mL-1-1 Trap (mcg/mL) and anti-mL-1-1 Trap antibodies (mcg/mL) in the 100 mg/kg toxicokinetic study cohort.

Gestation Day 6 (2 h after 1st dose)			Gestation Day 17 (48 h after previous dose)			Lactation Day 20/23 (72 h after previous dose)		
Mouse#	mIL-1 Trap (mcg/mL)	Antibody (mcg/mL)	Mouse#	mIL-1 Trap (mcg/mL)	Antibody (mcg/mL)	Mouse#	mIL-1 Trap (mcg/mL)	Antibody (mcg/mL)
A87370			A87378			A87386*		
A87371			A87379			A87387†		
A87372			A87380			A87388†		
A87373			A87381			A87389†		
A87374			A87382			A87390*		
A87375			A87383			A87391*		
A87376			A87384			A87392		
A87377			A87385			A87393		
Mean**	7.0		Mean**	3.9		Mean**	0.0	
SD**	4.3		SD**	9.1		SD**	0.0	
SEM**	1.6		SEM**	3.2		SEM**	0.0	

* Animal underwent an unscheduled sacrifice or was found dead; data not used

**0 used to calculate mean, SD, SEM if value is BLQ

† Sample collected on LD23

NS = No Sample

AB+ = Antibody Positive

BLQ = Below limit of quantitation (< 0.05 mcg/mL in the murine IL1 Trap assay, < 0.05 mcg/mL in the anti-murine IL1 trap assay)

Table 8: Levels of mL-1 Trap (mcg/mL) and anti-mL-1 Trap antibodies (mcg/mL) in the 200 mg/kg toxicokinetic study cohort.

Gestation Day 6 (2 h after 1st dose)			Gestation Day 17 (48 h after previous dose)			Lactation Day 20 (72 h after previous dose)		
Mouse#	mIL-1 Trap (mcg/mL)	Antibody (mcg/mL)	Mouse#	mIL-1 Trap (mcg/mL)	Antibody (mcg/mL)	Mouse#	mIL-1 Trap (mcg/mL)	Antibody (mcg/mL)
A87394			A87402			A87410		
A87395			A87403			A87411		
A87396			A87404			A87412*		
A87397			A87405			A87413		
A87398			A87406			A87414		
A87399			A87407			A87415		
A87400			A87408			A87416		
A87401			A87409			A87417		
Mean**	23.4		Mean**	13.8		Mean**	22.1	
SD**	18.4		SD**	22.0		SD**	29.0	
SEM**	6.3		SEM**	7.8		SEM**	11.0	

* Animal underwent an unscheduled sacrifice or was found dead; data not used

**0 used to calculate mean, SD, SEM if value is BLQ

BLQ = Below limit of quantitation (< 0.05 mcg/mL in the murine IL1 Trap assay, < 0.05 mcg/mL in the anti-murine IL1 trap assay)

NS = No Sample

2.6.6.7 Local tolerance

There was no local toxicity study submitted with this application. Local tissue reactions were characterized via the repeat-dose toxicology studies.

2.6.6.8 Special toxicology studies

Study title:**Cross-Reactivity of Biotinylated IL-1 Trap with Human and Cynomolgus Monkey Tissues Ex Vivo**

Methods: All tissues from the standard tissue list from human and Cynomolgus monkeys were stained for specific binding using IL-1 Trap following immunohistochemical procedure.

Results: There was no specific binding in any of the tissue for IL-1 Trap in human or monkeys.

2.6.6.9 Discussion and Conclusions

The toxicity findings associated with IL-1 Trap administration consisted of treatment related mortality in primates, clinical signs of lethargy, emesis, cold to touch and histopathological observations including tissue infiltration mono/polymorphonuclear cells, arteritis, cellular degeneration, granuloma formation, and mineralization. The major target organ of toxicity was identified as injection sites, heart, kidney, lung, reproductive organs, and immune system. The toxicity findings are not always related to IL-1 Trap plasma concentration but might be related to a combination of IL-1 Trap, IL-1 Trap:IL-1 β /IL-1ra complex, IL-1 Trap:IL-1 Trap antibody, and combination of all of the above which are present in the serum/plasma at the same time. This might also be related to the poor method of detection which in turn might also be related to the presence of different immune complex in the body.

Interpretation of the Toxicokinetics Findings:

IL-1 Trap is a dimerized fusion protein consisting of two human IL-1 receptors namely IL-1 receptor Type I (IL-1R1) and IL-1 receptor accessory protein (AcP) and Fc portion of the human IgG1. The Sponsor's method of detection of the IL-1 Trap is an ELISA based assay,

For determining the anti-product antibody formation in the plasma, the assays were done according to GLP conditions. The huge variation was noted in the IL-1 Trap serum concentrations in different experiments. For example the mean serum concentration of IL-1 Trap after the subcutaneous administration of the compound in Day 1 at doses between 10-15 mg/kg and after 16-24 hrs were 65, 10627, and 100 μ g/mL in the 6-week, 3-month, and 6-month SC administration respectively. Similar differences were observed in the IL-1 Trap serum concentrations after the IV administration in the 3-week and 6-month toxicity studies. Although this huge discrepancy might be partially attributed to the individual variation in the primates for IL-1 Trap binding, the differences in the IL-1 Trap serum concentration might also be due to the method of detection. The binding of the product might be disturbed in the presence of the immune complex such as IL-1 β :IL-1 Trap complex, or IL-1 Trap:IL-1 Trap antibody complex or IL-1 β :IL-1 Trap-IL-1 ra complex, and/or combination of the entire immune complex mentioned above. Similarly, the estimation of the antibody formation and kinetics of it might not be

properly assessed due to the same reason mentioned earlier. This is a common problem for the detection of the biologics and the anti-product antibody generated with the biologics. Because of the fact that this particular compound (IL-1 Trap) is observed to be highly immunogenic in the animal models, the appropriate detection of the product and its antibody might only be consider as estimation and not an accurate measure of the product or its antibody in the serum and the plasma. In general, however, the toxicokinetics were observed to be dose related for the IL-1 Trap in all of the toxicity studies conducted and no gender differences were observed in the primates. Antibody formation was noted within two weeks in all of the toxicity studies conducted with IL-1 Trap. The quantity of the antibody was highest at low dose at the 2 weeks period and most of the animals at low dose showed antibody formation at earlier time points. During the scheduled necropsy with all studies the product as well as the anti-product antibody was present in a measurable amount indicating that the histological changes might be related to the combination of the product alone as well as due to the immunogenicity of the compound.

In addition, the sponsor developed an ELISA method of detection for the IL-1 Trap:IL-1 β complex in monkey; the method is not validated for the non clinical studies, and was tested only in the pivotal toxicity study (6-month SC toxicity study). In this study it was observed that the mean IL-1 β complex levels increased with dose towards saturation at early time points and then continued to increase further with the duration of the study. This suggests that once this immune complex is formed unlike the product itself, this immune complex is not neutralized or cleared by the anti-product antibody and might deposit in the tissue which might have added to the histopathological findings of the compound. Rate of formation of this entire immune complex might also be responsible for the nonlinear findings of the toxicokinetic parameters.

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

Time	IL-1 Trap ($\mu\text{g/mL}$)			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

Mean Plasma Concentration (ng/mL) of IL-1 Trap and IL-1 Trap Antibody (RFU/mL) in Cynomolgus Monkeys in 3-Month SC Administration

Time	Dose Groups					
	IL-1 Trap Concentration			IL-1 Trap Antibody Concentration		
	1.5 mg/kg	5 mg/kg	15 mg/kg	1.5 mg/kg	5 mg/kg	15 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	14.6	5.7
Day 13 24 hr Post 4 th dose	15155	19420	36236	NM*	NM	NM
Day 26 Pre dose	56	17	4871	70	53	149
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	150	165	478
Day 57 24 hr Post dose	BLQ	BLQ	19903	NM	NM	NM
Day 92 Pre dose	BLQ	BLQ	10503	95	207	356

NM: IL-1 Trap antibody concentration not measured.

** BLQ: Below level of quantification

Mean Plasma Concentration of IL-1 Trap and IL-1 Trap Antibody in Cynomolgus Monkey in 6-Month SC Administration

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 (ng/mL) of IL-1 Trap and IL-1 Trap Antibody (RFU/mL) in Cynomolgus Monkeys in 3-Week IV Administration

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

Mean Plasma Concentration of IL-1 Trap and IL-1 Trap Antibody in Cynomolgus Monkey in 6-Month IV Administration

Time	IL-1 Trap ($\mu\text{g/mL}$)				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

Summary of Adverse Event Findings:

There were two unscheduled deaths in the pivotal 6-month subcutaneous toxicity study; one male each from the mid and high dose died. The histological finding from the mid dose animal that was sacrificed at Day 45, showed myocarditis associated with mononuclear cell infiltration; the high dose male 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, the sponsor believes that the cause of deaths resulted from immune mediated hypersensitivity reaction, reviewer agrees with sponsor analysis of data from these unscheduled deaths. However, it is known that the detection of the biological products becomes difficult in the presence of the anti-product antibody in the same compartment. Therefore, it is recognized that one can not definitively out rule the possibility of a drug product mediated effect, however the toxicological findings are consistent with an immune complex pathology.. The sponsor IL-1 Trap: IL-1 β immune complex assay showed the immune complex formation

increases with increase in the duration of the experiment which might add to the complication associated with tissue congestion and edema related to the proinflammatory reaction that might have generated due to the immune complex deposition in the body. The granuloma formation in the high dose male might also be due to infection associated with immune suppression. It was noted from the pharmacology of the compound that IL-1 Trap is capable of binding to IL-1ra which is an anti-inflammatory compound. The fact that the granuloma formation was seen at the high dose and IL-1ra bind to the IL-1 Trap at higher concentration adds to the fact that more immune suppression might be seen at higher concentration with the compound. This might increase the immune suppressive property of the compound. In another chronic toxicity study, 6-month IV toxicity study there was one unscheduled death in this study, one high dose male was sacrificed at day 176, bacterial infection was noted in this animal, this might also be due to the increase immunosuppression associated with exaggerated pharmacology of the compound.

The other adverse events observed in the 6-month subcutaneous toxicity studies consists of the following: one male from low dose showed adverse events consisting of breathing discomfort at day 180, recovered by itself, showed signs of myocarditis, one female from 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 is in concurrence with the sponsor's analysis of the data that these events are immune complex mediated hypersensitivity reaction; however, the possibility of the proinflammatory reaction due to increase immune complex deposition can not be eliminated. At the same time the method of detection of IL-1 Trap might not be adequate for detecting the product in the presence of the anti-product antibody, therefore, the reason for the adverse events can not be determined conclusively.

The other major adverse effects associated with the IL-1 Trap was clinical signs of lethargy, emesis, and cold to touch etc which was observed around the second week in the 3-week IV study. There was no dose relation with the findings. Thus it appeared to be associated with IL-1 Trap as well as its antibody since the serum concentrations of both IL-1 Trap and its antibody was high at this time.

There were three distinctly different treatment related histopathological changes noted in the toxicity studies conducted with IL-1 Trap. One is tissue congestion/degeneration which might be associated with either proinflammatory reaction due to immune complex deposition or hypersensitivity vasculitis associated with mono and polymorpho (eosinophil) nuclear cell infiltration. Both of these might result in multifocal lesion and tissue degeneration. Another one is increase infection associated with immunosuppression. Lastly, there was exaggerated pharmacology associated with depletion of the thymus and changes in the ovary and testes. The major histopathological changes associated in the monkeys consisted of injection site lesions, tissue changes in

heart, kidney, lung, and liver. The analyses of the pathological changes are discussed below.

Comparison of the Toxicity Findings from Different Toxicity Studies:

The histological changes in the *injection sites* were observed within 3-weeks, after the intravenous administration. The histological lesion in the injection sites were consisted of mononuclear cell infiltration in the perivascular region of the subcutis. The mononuclear cell infiltrates composed of macrophages, eosinophil, plasmocytes, and lymphocytes with a perivascular distribution. Similar histological changes were noted in the subchronic (6-week and 3-month) subcutaneous toxicology studies. In the subchronic studies, the infiltration was noted primarily in the subcutis, but in some cases the infiltration was noted in the deep dermis which might have resulted from chronic inflammation or local immune reaction associated with the treatment. In both of the chronic toxicity studies (6-month IV and SC), a substantial increase in mononuclear/polymorphonuclear cell (eosinophil) infiltration occurred in all treatment groups by comparison with control animals; these changes are considered to be test article-related. The incidence was further characterized in the 6-month SC toxicity study to evaluate the clinical implication. Injection site lesions were noted in 1/20 (5%) in untreated males and 55/72 (76%) in treated males. In females this change was observed in 3/18 (17%) sites examined in untreated animals and 69/76 (91%) in treated animals. Primary tissue changes included hemorrhage, edema, and/or inflammation in the subcutis and dermis and degeneration, regeneration, and interstitial inflammation of the underlying muscle. This change was slight and severity appeared to decrease with increasing dose. Microscopic examination of the injection site skin samples revealed tissue alterations associated with perivascular cell infiltration and inflammation indicating inflammation of the small blood vessels. One monkey each from the 6-month IV toxicity study had acute phlebitis and moderate venous thrombosis. All these pathological alterations of the tissue indicate that the injection site lesions increased in severity and incidence with the duration of the treatment. The finding although not clearly related to the IL-1 Trap concentration by itself but obviously treatment related. It might be predicted that in clinical condition with the increase in duration complication associated with inflammation of small blood vessels might appear. Following gradation were used for the assessment of the severity in all of the following tables.

- : no change
- ± Minimal
- + Slight
- 2+Mild
- 3+Moderate

Tissue Findings	Male					Female				
Injection sites(skin, back, thorax, upper); 6 -Month SC; 3x/week										
Dosages	0	15	25	40	60	0	15	25	40	60
Mononuclear cell infiltration	1/5;±	5/5; ±,± +,2+,2+	5/5; ±, ±,±,+,+	4/4; ±, ±, +,2+	2/5; ±,+	2/5;U all±	4/5;U ±,±, 2+,2+	5/5; ±,±,±,±,2+	5/5; all±,	5/5; all±,
Injection sites(skin, back, thorax, upper); 3 -Month SC; 2x/week										
Dosages	0	1.5	5	15		0	1.5	5	15	

Lymphoid cell infiltration, perivascular, dermis	0/3	3/3;±	3/3;±	3/3;+		0/3	2/3;±	3/3;±	3/3;+	
Microgranuloma	0/3	0/3	1/3±	0/3		0/3	0/3	0/3	0/3	
Injection sites(skin, back, thorax, upper); 6-Week SC; 2x/week										
Dosages	0	1	3	10		0	1	3	10	
Infiltration of mononuclear cell	0/3	2/3; ±	0/3	2/3; 2+		0/3	2/3; ±	3/3; ±	3/3; 2+	
Injection sites(skin, back, thorax, upper); 6-Month IV; 1x/2weeks										
	0	3	10	30	100	0	3	10	30	100
Degeneration	0/6	0/6	0/6; ±	1/6; ±	1/6;±	0/6	0/6	0/6	1/6; ±	1/6; ±
Inflammation	2/6; ±	2/6; ±	4/6;±	4/6; 2+	4/6;2+	1/6; ±	2/6; ±	1/6; ±	2/6; ±	2/6; 2+
Phlebitis, acute		1/6;+			1/6;+					
Thrombosis, venous					1/6;2+					
Injection sites(skin, back, thorax, upper); 3-Week IV; 1x/week										
Dosages	0	5	20	50		0	5	20	50	
Mononuclear cell /perivascular	0/3	1/3	2/3	0/3		0/3	0/3	0/3	0/3	

The treatment related histological changes in the *kidney* were observed in all of the studies reviewed under this application. The histological lesion in the kidney was consisted of lymphocytes and the mononuclear cell infiltration indicating tissue inflammation. In the chronic 6-month, SC, toxicity study, there was an increase in the mesangial matrix of slight severity in the glomeruli in one 40 mg/kg female and one 60 mg/kg male. In the 60 mg/kg male, the kidney changes were accompanied by slight tubular casts/degeneration, slight medial cell proliferation, and vasculitis of arteriole and slight mononuclear/eosinophil infiltration in the interstitium or perivascular regions. In the chronic 6-month, IV toxicity study one male from the 10 mg/kg group monkey had slight subacute arteritis in the hialar region with multifocal lesion, the morphology was not associated with increase in the polymorphonuclear cell infiltration. There was, however, an increase in the mono/polymorphonuclear cell in other males and females from this study. The histological findings revealed that with chronic duration different manifestation of the pathology might be observed in the kidney which is clearly different from what is known about the protein deposition and associated kidney disease. The compound or the immune complex generated in the presence of the compound appeared to target the arterioles.

Tissue Findings	Male					Female				
Kidney, 6-Month SC; 3x/week										
Dosages	0	15	25	40	60	0	15	25	40	60
Mononuclear /polymorphonuclear cell infiltration	4/5;all±	2/5; all±	3/5; all±	4/4; all±	4/4; ± +,±,±	2/5; all±	3/5; all±	3/5; all±	5/5; all±	5/5; all±
Tubular cast degeneration/perivascular	0/3	0/3	0/3	0/3	¼; +	0/3	0/3	0/3	0/3	1/5;

eosinophil infiltration/increase mesangial matrix/vasculitis of arteriole											±
Kidney; 3-Month SC; 2x/week											
Dosages	0	1.5	5	15		0	1.5	5	15		
Mononuclear & lymphoid cell infiltration	1/3±	3/3;+, ±,±	1/3±	1/3±		1/3±	2/3;+, ±,±	1/3±	1/3±		
Kidney; 6-Week SC; 2x/week											
Dosages	0	1	3	10		0	1	3	10		
Mononuclear cell infiltration	1/3	1/3	2/3	2/3		1/3	3/3	1/3	1/3		
Mineralization	0/3	3/3	1/3	0/3		0/3	1/3	1/3	1/3		
Regeneration of tubular epithelium	0/3	3/3	1/3	0/3		0/3	0/3	0/3	0/3		
Kidney; 6-Month IV; 1x/2 weeks											
Dosages	0	3	10	30	100	0	3	10	30	100	
Inflammation/tubular changes/multinucleated cell in epithelium	1/6	-	3/6	3/6	5/6	-	-	-	3/6	5/6	
Chronic nephropathy	0/6	0/6	1/6 +	0/6	0/6	0/6	0/6	0/6	0/6	0/6	1/6+
Kidney; 3-Week IV; 1x/week											
Dosages	0	5	20	50		0	5	20	50		
Lymphoid cell infiltration	1/3;±	3/3;+, ±,±	3/3; ±	3/3; ;+, ±,±		2/3;±	3/3;±,±,2+	2/3;±	2/3;±,+		

The histopathological changes consisting of mono and polymorphonuclear cell administration in the *heart* occurred early, after only three IV injections in males. Similar changes were observed in males and females in the subchronic studies.

In the 6-month, SC, pivotal toxicity study, the morphologic findings in the heart consisted of slight myocardial cell degeneration and necrosis at 15 mg/kg, mononuclear and polymorphonuclear cell (eosinophil) infiltration at 15 mg/kg and 60 mg/kg and injection sites (very slight mononuclear/polymorphonuclear cell infiltration at 15 mg/kg and higher). One 15 mg/kg dosed male with morphologic changes in the heart and injection site had a post-injection reaction; a relationship between these findings was suspected. Similar vascular changes were also noted in the monkeys from all of the test article treated animals in the 6-month IV chronic toxicity study. As noted in the table below myocardial degeneration and necrosis were noted in the monkeys from the mid high, high dose group. All these lesions appeared to be associated with immune complex deposition induced vasculitis. In human, most drug/immune complex induced vasculitis appears to be non necrotizing, hypersensitivity type. This form is characterized by mono/polymorphonuclear cells and eosinophil in the walls of arterioles and capillaries, venules and small veins with sparing of large veins (Mullick et al, 1979).

Tissue Findings	Male	Female
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	Dose (mg/kg)									
	0	15	25	40	60	0	15	25	40	60
Heart; 6- Month SC; 3x/week										
Mononuclear cell infiltration	1/5;±	3/5;+	3/5;±	0/5	2/4;±	1/5;±	1/5;±	1/5;±	3/5;±,±,±	4/5; all ±
Myocardial cell degeneration/necrosis	1/5;±	1/5;±,±	2/5±	1/5±	1/5;±	0/5	1/5;±	1/5;±	0/5	0/5
Heart; 6-Week SC; 2x/week										
	0	1	3	10		0	1	3	10	
Mononuclear cell infiltration	0/3	0/3	0/3	0/3		0/3	0/3	2/3	1/3	
Heart; 6- Month IV										
	0	3	10	30	100	0	3	10	30	100
Myocardial degeneration	0/3	0/3	0/3	1/6	0/3	0/3	0/3	0/3	1/6	1/6
Myocardial necrosis (multifocal)	0/3	0/3	0/3	0/3	1/6;+	0/3	0/3	0/3	2/6;+	3/6;±
Inflammation /myocardial	1/6;±	4/6; +	3/6;+	0/6	4/6;±	0	3/6;±	3/6;±	4/6;±	2/6;±
Heart; 3- Week IV										
	0	5	20	50		0	5	20	50	
Mononuclear cell infiltration	1/3;±	1/3;±	2/3;±	3/3;±,±,±		0/3	0/3	0/3	0/3	
Degeneration of myocardial fiber	0/3	0/3	0/3	1/3±		0/3	0/3	0/3	0/3	

The changes in the *lungs* were associated with the mononuclear and alveolar cell infiltration and granuloma or microgranuloma formation which might be associated with inflammation related to infection. However, there was no infectious agent that was observed in the lungs. One animal from the high mid dose group from 6-month chronic toxicity study which underwent unscheduled necropsy was found to have large organized thrombus in one of the main arteriole of the lung with edema and perivascular inflammation. The granuloma/microgranuloma formation is believed to be more relevant to immunosuppressive property of the compound; it might also be due to the tissue reaction resulting from the immune complex deposition in lung. The primates are known to have latent infection such as tuberculosis and infestation of different pulmonary larvae. This kind of bacterial and parasitic infection is associated with granuloma formation in lungs. It is therefore possible that at higher dosages, the test article is suppressing the immune system of the primates to a certain extent at which latent infections are exposed and granuloma is formed as a consequence of that. Fibrosis is also noted at a higher incidence in the test article treated animal. Pulmonary fibrosis is a common sequel of chronic lower respiratory tract inflammation. It is known that in the non human primate focal pulmonary fibrosis occurred as a response to chronic infestation by parasites (Greaves 2003). The increased fibrosis noted in the lungs of the treatment group might have resulted from the increase in the parasite infection caused by the immune suppression initiated by the test article.

Another instance of inflammatory conditions associated with infection was noted in the monkeys in the stomach with both of the 6-month chronic studies (IV and SC). Although inflammatory conditions due to microorganisms are rare in stomach, monkeys are known to have a variety of spiral organism in the gastric mucosa. Similar conditions are also

noted in monkeys in the presence of *Helicobacter*-like organism (Reed 1988). The increase incidence of inflammatory condition in the stomach might also be associated with the test article related immune suppression in monkey resulting in the increase infection of the monkey with *H. pylori* or other microorganism which might have been already present but was latent under the normal conditions in the monkey.

Interestingly, the inflammatory condition in the stomach and the granuloma formation in the stomach were found in a higher incidence in the 3-month and 6-month toxicity studies compared to the short term toxicity studies. This might indicate that with the increase in the duration of the immunosuppression, there is a possibility that more latent bacterial or parasite infection would appear unbridled.

Another significant finding in the 6-month IV chronic toxicity study was the presence of prominent stellate (Ito) cells in the liver of some monkeys from the 30 mg/kg (3 males and 1 female), and 100 mg/kg (5 males and 1 female) treatment groups. The change, graded minimal to mild and characterized by the presence within the hepatic sinusoids of cells with a large cytoplasmic vacuole and a peripheral small dark nucleus, was considered to be a background finding. The sponsor mentioned that because this change was present in both control and test article-treated monkeys from the recovery phase, and because no other dose-related alterations were observed in the liver and other tissues, a cause and effect relationship with IL-1 Trap treatment is uncertain. Ito cells are, however, associated with the formation of fibrosis and hyperplasia. Ito cells acts as an antigen presenting cell. It is however, not known whether such cell causes fibrosis or helps prevent the formation of the fibrosis. In the subchronic studies cytoplasmic vacuolation and mononuclear cell infiltration was noted indicating inflammatory condition in the liver tissue; however, no infection was reported. The appearance of Ito cells is a new finding in the 6-month IV toxicity study and the biological significance is not known.

Tissue Findings	Male					Female				
Lung: 6-Month SC; 3x/week										
	0	15	25	40	60	0	15	25	40	60
Mononuclear cell infiltration	0/5	0/5	0/5	0/5	¼; +	0/5	0/5	¼; +	¼; +	0/5
Lung: 3-Month SC; 2x/week										
	0	1.5	5	15		0	1.5	5	15	
Microgranuloma	0/5	0/5	0/5	1/3; ±		0/5	0/5	0/5	1/3; ±	
Lung: 6-week SC; 2x/week										
	0	1	3	10		0	1	3	10	
Infiltration of alveolar macrophages	1/3; ±	1/3; ±	1/3; ±	3/3; ±		1/3; ±	1/3; ±	1/3; ±	0/3	
Fibrosis	0/3	1/3; ±	0/3	0/3		0/3	1/3; ±	0/3	1/3; ±	
Lungs: 6-Month IV										
	0	3	10	30	100	0	3	10	30	100
Alveolitis	0/5	0/5	0/5	0/5	1/6	0/5	0/5	0/5	0/5	1/5+
Granulomatous	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/6+

Tissue Findings	Male					Female				
GI Tract Tissue: 6-Month SC										

Dosages	0	15	25	40	60	0	15	25	40	60
Stomach/decrease in parietal cell in pylorus and increase in mononuclear cell infiltration	-	-	1/5; 3+	-	¼;2+	1/5, 3+	-	-	1/5;+	-
Dudoneum/hyperplasia	-	-	-	-	-	1/5, ±	-	-	1/5; 2+	-
Stomach; 6-Month IV										
Dosages	0	3	10	30	100	0	3	10	30	100
Dilation/inflammation	1/6		2/6	2/6	2/6	-	-	-	-	-
Necrosis	-	1/6	-	-	1/6	-	1/6	-	-	-

Tissue Findings	Male					Female				
Liver: 6-Month SC										
Dosages	0	15	25	40	60	0	15	25	40	60
Mononuclear cell infiltration	2/5; all ±	3/5; all ±	3/5; all ±	2/4; all ±	¼; all ±	2/5; all ±	3/5; all±	1/5; ±	3/5; ±	3/5; all±
Hepatocyte degeneration	-		1/5; ±	-	-	-	-	-	-	-
Liver: 6-Month IV										
Dosages	0	3	10	30	100	0	3	10	30	100
Vacuolation in the sinusoidal cells	-	-	-	3/6	5/6	-	2/6	-	4/6	5/6

Consistent treatment related changes in the reproductive tissues were noted in all of the subchronic and chronic toxicity studies with this test article. The changes in the females reproductive tissue consisted of mineralization of the oocytes in the ovary and mononuclear cell infiltration nuclear in the ovary and the uterus. The incidence increases with the duration of the studies. The compound was shown to have an inhibitory effect on the estrogen production which might be related to the changes in the oocytes formation and thus might be considered as an indirect effect of the compound. IL-1 has been shown to have a profound effect on the maintenance of the estrus cycle, implantation, and pregnancy in mice. Inhibitions of IL-1 by IL-1 Trap have been shown to disrupt estrus cycle in the reproductive toxicity studies. The histological changes in the male reproductive organ are associated with aspermatogenesis in the testes and mineralization in the seminal vesicle. The significance of are not clear.

Male/Female Reproductive organ										
6-Month SC										
	Males					Females				
Dosages	0	15	25	40	60	0	15	25	40	60
Testes, aspermatogenesis	-	-	1/5, 2+	-	-					
Seminal vesicle/mineralization of lumina	2/5, all+	3/5; ±,+	4/5; ±,2+ ,+	2/5; ±, ±	2/5; ±,+					
Dosages	0	3	10	30	100	0	3	10	30	100
6-Month IV										
Testes, aspermatogenesis	-	-	-	-	1/6	-	-	-	-	-
6-Month SC										
Dosages	0	15	25	40	60	0	15	25	40	60
Ovaries						1/5;	3/5;	3/5;	-	1/5

/mineralization of follicles						±	all±	±,±, 2+		;+
Uterus/ mononuclear cell infiltration						-	-	-	-	2/5 ; all ±
6-Month IV										
Dosages	0	3	10	30	100	0	3	10	30	100
Ovaries /mineralization of follicles	-	-	-	-	-	-	-	-	3/6	3/5

There was an interesting finding of decreased ossification of the growth plate in the skeletal system which was noted mainly in the 3-week IV toxicity study and 6-month SC chronic toxicity study. There was an increase in the incidence and the severity with the increase in dose in the 6-month study. In the 3-week IV study, the incidence was similar; however, the severity increases with the increase in dose. The decrease in the ossified growth plate might be related to the estrogen depletion noted with IL-1 Trap. The clinical significance of the finding is not clear.

Skeletal system; 6-Month SC										
Dosages	0	15	25	40	60	0	15	25	40	60
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
Skeletal system; 3-Week IV; 1x/week										
Dosages	0	5	20	50		0	5	20	50	
Femur/decrease in growth plate	1/3,±	1/3;±	1/3;2±	-		2/3; ±	2/3; 2+	2/3; 3+	-	

The histopathological changes of the immune system were consisted of hypocellularity or atrophy of thymus, hypo or hyperplasia of the mesangial, submandibular and mandibular lymph node. These changes are considered as exaggerated pharmacology of the compound.

Pharmacological Implication of the Toxicity Findings:

The general toxicity findings described above was not always dose related may be due to the combined effect of the product as well as its antibody and immune complex. No NOAEL could be determined in the toxicity studies as described in the text for individual studies. The toxicity findings were noted in the presence of the IL-1 Trap, anti-product antibody, and the immune complex associated with the IL-1 Trap binding to the targets (IL-1β and IL-1ra) and its antibody. Therefore, the toxicity findings might be related to all of the above. As mentioned above the exaggerated pharmacology of the compound predicted it to be immunosuppressive, however, the plausible binding of the compound to IL-1ra which is anti-inflammatory might increase the immunosuppressive property of the compound. The immune complex formed due to the binding of IL-1 Trap to IL-1β/IL-

IL-1ra was detected to sustain for a long period of time indicating that a proinflammatory condition might arise due to the continuous deposition of the immune complex in the tissue. In the pharmacology studies, it was also noted that the IL-1 Trap:anti-product antibody formation modulated the binding of the Fc receptor part of the IL-1 Trap to the high and low affinity Fc γ receptor which might also add to the indirect effect or the secondary pharmacodynamics of IL-1 Trap. Although low affinity bindings of the Fc γ receptor in human can not be predicted even from the primate studies, the modulation of the high affinity binding might result in increase stimulatory or inhibitory effect of the compound which might add to the toxicity related to the proinflammatory or immunosuppressive findings associated with the compound. One of the major findings was injection site reaction which might have resulted from local immune reaction; the increase eosinophil infiltration along with mononuclear cell infiltration was noted in the injection sites. This might be due to a host defense mechanism. However, IL-1 β is known to be chemotactic to eosinophil, it might be possible that aggregation of IL-1 β captured by IL-1 Trap locally resulted in increase mono/polymorphonuclear cell infiltration and tissue degeneration. The clinical implication of the findings is not clear. However, the compound is known to be immunogenic approximately 40% of the clinical population has been shown to produce antibody. The immunogenicity section of the label shows that a

..... This suggests that the clinical population might encounter the similar situation as in primates where different immune complexes as mentioned above are present in the serum simultaneously with product and its antibody. Therefore, it is possible that clinical findings like injection site reaction and macrophage activation might be related to the similar pathological changes that have been observed in the primates. In addition, the pharmacological mechanism such as proinflammatory changes, hypersensitivity due to protein complex deposition etc might also be similar if not identical in human and non human primates.

Clinical Implication:

The proposed human clinical dose is 160 mg/week. In the pivotal clinical trial, in CAPS patients for 24 weeks with this dose, mean steady state trough levels of total IL-1 Trap ranged from 20-27 μ g/mL. The pivotal toxicity study, 6-month subcutaneous study, used 15 mg/kg (HED =4.8 mg/kg) as the lowest dose, the serum concentration of IL-1 Trap concentration at 6 month was found to be 78 μ g/mL and IL-1 Trap antibody concentration ranged from 18-235 RFU. At this dose hypersensitivity reaction described as breathing difficulty and loss of consciousness was observed in one out of five monkeys. The histopathological findings include myocardial degeneration in the monkey which showed hypersensitivity reaction. The rest of the animals from the low dose group showed mononuclear cell infiltration in the heart and kidney. All of the animals from this dose group showed injection site reactions. Because of these findings no NOAEL could be established. The toxicity above the lowest dose in this study consisted of increase in the severity and incidence of the histological findings already noted and increase in immunosuppression related infection and inflammatory reaction. Another 6-month toxicity study in monkey with intravenous drug administration used 3 mg/kg (HED= 0.96 mg/kg) as the lowest dose. The serum concentration of IL-1 Trap concentration at 6

month was found to be 10 µg/mL and IL-1 Trap antibody concentration ranged from 132-542 RFU. The histopathological findings in the low dose were injection site reactions. No NOAEL could be determined because of this finding. In the higher doses in this study increase in heart and kidney findings associated with myocardial degeneration, tubular necrosis etc was noted. In both of these toxicity studies other than injection site lesions the incidence of the histopathological findings were observed to be decreased during the recovery period. All these observations are predictive of the major injection site reactions in human. The histological findings observed in the non human primates are also predictive in human.

Reproductive Toxicity Assessment:

The sponsor submitted Segment I and III studies from mice where they used the surrogate molecule. The Segment II study was conducted in the primates with human IL-1 Trap.

The major reproductive toxicity findings in the Segment I and Segment III reproductive toxicity studies included early and post implantation resorptions of embryos, fetal death, increased still borne pups and empty stomachs (lack of milk) in the fetuses. The findings are not always dose related, therefore, it is not clear whether the findings are directly associated with IL-1 Trap or its antibody or the immune complex formation associated with IL-1 Trap binding. However, IL-1 is known to be associated with increase estrogen production maintenance of pregnancy. Modulation of IL-1 might result in estrogen modulation and therefore causes gravid uterus without pregnancy or early resorptions which is being observed in these studies. The effects observed might thus be due to the exaggerated pharmacology of the compound associated with the hormonal modulation. In addition, anti-inflammatory nature of the compound might cause depletion of the nitric oxide (NO) production. NO is essential for the increase vasodilatation in the placenta for the maintenance of the fetus. Depletion of the NO which might result from IL-1 Trap might also cause fetal death. In the Segment II study reduction in levels of estrogen were noted at low dose. The finding was related to plasma level of IL-1 Trap. In the same experiment higher rate of spontaneous abortion were noted compared to the controls from the same experiments. The findings are not dose related. Another major finding in the Segment II study is the increase skeletal variation (compared to the controls in the same experiment and historical control) in the lumbar region and increase in skeletal abnormalities in the thoracic region. The effect of IL-1 and estrogen in the bone morphogenesis is well known. It is possible that depletion of IL-1 β might have some contribution to the skeletal anomalies. However, due to the lack of dose response it is not clear whether the findings are directly or indirectly related to the compound. The lack of milk as observed in some of the fetuses in the Segment III study might be related to the negligence of mother or due to the lack of milk formation in the mother due to the IL-1 Trap or immunogenicity related to IL-1 Trap. The anti-product antibody was found in fetus in the primates in the Segment II studies at Day 100, indicating that the antibody from mother is long lasting and cross the placental barrier. The effect of the antibody in the F₁ generation in the monkey on the immune system development was not studied. In the mice although the antibody was not measured in the F₁ and F₂ fetuses, in F₁ mother the antibody was found in later stages of lactation indicating that it might have some

effect in lactation. The reproductive studies resulted in exaggerated pharmacology related findings; however there is no dose response. The causes of the findings are not known.

The clinical implication of the findings is not yet known. However, the late pregnancy spontaneous abortion in the primates were noted in a concentration (2015 ng/mL at GD 35 and 12,071 ng/mL at Day 49 which is less than the concentrations of IL-1 Trap noted in clinical studies with clinical dosing of 160 mg which resulted in human exposure of 20-27 µg/mL. Also, note that the mean estrogen level in the animals from the samples just prior to the abortion was the lowest indicating that estrogen levels along with the individual animal variation might be related to the spontaneous abortion.

Currently, the labeling from the sponsor propose a Pregnancy Category – for the IL-1 Trap, however, due to the presence of reproductive findings with unexplained cause, the reviewer believes that the compound should be labeled as Pregnancy Category C.

Summary of the Toxicokinetic Analysis:

Parameters	Dose mg/kg		
	20	100	200
Male			
Number of animals showing IL-1 Trap in Plasma	37/74	29/30	30/30
IL-1 Trap Concentration (µg/mL)	1-78	5-432	29-748
Number of animals showing IL-1 Trap antibody in serum	62/74	7/30	2/30
IL-1 Trap Antibody Concentration (µg/mL)	2-13351	1-26	1.5
Female			
Number of animals showing IL-1 Trap in Plasma	ND	ND	ND
IL-1 Trap Concentration (µg/mL)	ND	ND	ND
Number of animals showing IL-1 Trap antibody in serum	67/73	28/29	19/29
IL-1 Trap Antibody Concentration (µg/mL)	1.1-6000	1.9-328	0.9-129

Summary of Findings from the Reproductive Toxicity Study Segment I

Parameters	Dose Group			
	0	20	100	200
Number of Gravid Females	29/30	63/75	28/30	29/30
% Female w/no evidence of mating	3.3	6.8 2-fold↑	0	0
% of Female w/evidence of mating but non gravid	0	7.3%	0	0
Female fertility index	100%	86%; 14%↓	100%	100%
Historical control data ranged from 92-98%				
Historical Control				
% Male w/ no evidence of mating	3.3%	10% 3-fold↑	0	0
% of Male w/evidence of mating that not sired a litter	0 %	8.4 %;	0	0
Male fertility index	97%	84%; 16%↓	100%	100%

Historical control data 76-94%				
Early Resorptions (%/litter)	2.6	4.9; 47%↑	4.1; 37%↑	5.1 50%↑
Historical control data not provided				
Early Resorptions (%/dam)	0.4	0.6	0.5	0.7
Historical control data not provided				
Post Implantation Loss (%/litter)	3.2	5.3 40%↑	4.1; 22%↑	5.8 45%↑
Historical Control/ Post Implantation Loss (%/litter): 4 experiments 6.9, 1.4, 0, 2.51				
Post Implantation Loss (%/dam)	0.4	0.7	0.5	0.8
Historical Control/ Post Implantation Loss (%/litter): 4 experiments 1.3, 0, 0, 0.2				

IL-1 Trap Concentration and Anti-product Antibody Concentration in the Plasma 24 hr Pre and Post Treatment in Different Gestation Days (GD)

Dose mg/kg	GD-21	GD-27	GD-28	GD-34	GD-35	GD-41	GD-42	GD-48	GD-49	GD-69	GD-100
IL-1 Trap Plasma Concentration (ng/mL)											
5	17964	13525	29408	615	2015	330	767	272	905	144	ND
15	89320	91169	144470	8736	28044	5232	22602	1815	12071	256	163
30	159118	237104	340904	29693	87386	10779	54731	19434	51774	4132	109
IL-1 Trap Antibody (RFU/mL)											
	GD 34			GD 55			Pre C Section			Fetus	
5	9-152			90-1024			8-57			2-25	
15	1-91			106-4444			10-325			4-102	
30	9-276			1-922			3-44			7-30	

Effect of IL-1 Trap on the Serum Hormone Concentrations in Segment II Study:

Dose mg/kg	GD-20	GD-25	GD-30	GD-35	GD-40	GD-50	GD-80	GD-100
Estrogen (pg/mL)								
0	229	268	215	216	333	336	451	510
5	223	244	201	84*	229	163*	439	477
15	213	227	157*	77*	177*	150*	485	416
30	209	219	150*	115*	168*	163*	416	458

* Statistically significant difference from control P < 0.001

Summary of Major Findings in Segment II Reproductive Toxicity Study:

Parameters	Dosages mg/kg			
	0	5	15	30
Fetal absorption /death	16.7%, 2/12 aborted at GD 25	25%, 2/12 aborted at GD 25, 1/10 aborted at GD 38	8.3%, 1/12 aborted at GD 25; 1/11 aborted at GD 51	16.7%, 2/12 aborted at GD 25
Historical control data from 16 studies including the current study w/181 dams showed that a total of 20 abortions noted, 12/20 occurred between GD20-GD35, 3/20 occurred between GD36-GD45, 2/20 occurred between GD 46-GD55				
Skeletal Abnormalities (thoracic vertebra and arches arranged abnormally)	0 (similar observation was made in	0	1/3 female fetus	0

	one fetus - historical control)			
Historical control data from 16 studies including the current study w/87 fetus showed that 1/87 that is 1.1 % fetus had skeletal abnormalities in the thoracic vertebra.				
Skeletal Variations(bilateral lumber vertebra)	0 (historical control 12.8%)	22.2%	18.2%	20%
Historical control data from 16 studies including the current study w/87 fetus showed that 11/87 that is 12.6 % fetus had skeletal variations of lumber ribs.				
Single Placenta	3/10	0/92	2/11	4/10
Historical control data from 16 studies including the current study w/108 fetus showed that 20% fetuses had single placenta.				

Summary of the Toxicokinetic Analysis in Segment III Study

Analyses of Data from the Toxicokinetic Cohorts				
Dose Groups	GD6 (2 hr post dose)	GD17 (48 hr post dose)	LD20-23 (72 hr post dose)	
IL-1 Trap Concentration (µg/mL)/# of Animals Showing IL-1 Trap Concentration				
20	0.8-1.7; 7/8	1.7; 1/8	BLQ; 0/8	
100	1.1-17; 8/8	4.9-26; 2/8	46.7; 1/8	
200	7.8-60; 8/8	1-26; 3/8	19-81; 4/8	
IL-1 Trap Antibody Concentration (µg/mL) /# of Animals Showing Antibody Concentration				
20	BLQ; 0/8	8-35; 2/8	7.4-466; 8/8	
100	2.5; 1/8	5.3-25.4; 3/8	1.6-151; 5/8	
200	BLQ; 0/8	7.1-12.8; 2/8	5.9-54; 5/8	
Dose Groups	GD6 (2 hr post dose)	GD17 (48 hr post dose)	LD20-23 (72 hr post dose)	
Analyses of Data from the Main Study Cohorts				
Dose Groups	24 hrs post LD20			
IL-1 Trap Concentration (µg/mL)/# of Animals Showing IL-1 Trap Concentration				
20	1.5-3.1; 11/63			
100	7.9-269; 22/23			
200	1-402; 25/25			
IL-1 Trap Anti body Concentration (µg/mL) /# of Animals Showing Antibody Concentration				
20	1.4-4868; 59/63			
100	3.1-47.2; 16/23			
200	5.2-108; 6/24			

Summary Table Showing Major Findings from the Segment III Reproductive Toxicity Study:

Parameters	Dose Group (mg/kg)			
	0	20	100	200
Number of F0 Dams found Aborted	0	3	4	2
Number of F0 Dams showing TLD	2	4	2	3
Number of F0 Dams	0	6	3	1

showing gravid but not pregnant				
Increase % of still borne pups	0.3	0.5	0	1.7
Total pup necropsy findings/empty stomach	4	6	8	8
F1 Pups dying/killed/missing 0-4	47	56	22	68
F1 Pups dying/killed/missing 5-21	2	16	16	0
Entire litter died/killed/missing 0-4	2	3	1	4
F1 Pups dying/killed/missing 5-21	0	2	2	0
% of F1 animals showing prolonged diestrous	14	21	17	28
F2 Total Litter Death	0	2	0	2

2.6.6.10 Tables and Figures NA

2.6.7 TOXICOLOGY TABULATED SUMMARY

OVERALL conclusions and recommendations

Conclusions:

IL-1 Trap is a recombinant fusion protein designed to inhibit IL-1 β . The compound is predicted to suppress the innate immunity. The pharmacological data shows that IL-1 Trap binds to IL-1 α , β , and IL-1 ra in a picomolar concentration. The compound was found to be highly immunogenic.

The toxicokinetic data shows that antibody formation occurred in monkey with the human IL-1Trap and the mice with the murine surrogate IL-1 Trap within 2 weeks of the IL-1 Trap administration. The serum circulating antibody production could be detected for a long period of time and even during recovery. The methods of the detection of the biologics are known to be hampered by the presence of the antiproduct antibody and the immune complex in the same compartment. With the current product several immune complexes such as IL-1Trap-IL-1 antibody, IL-1Trap-IL-1 β , IL-1 Trap -IL-1ra, and a combination of all of these complexes might be present in the serum and in the plasma at the same time. This makes the detection of the product difficult. Therefore, it is difficult to correlate the toxicity findings with the exposure of the compound or immune complex generated by the treatment with the compound. The compound is evaluated for the general toxicity in the cynomolgus monkeys which is an appropriate animal model for

toxicity indicated by the high affinity binding of the human IL-1 Trap with the IL-1 β . The toxicity findings associated with IL-1 Trap administration consisted of treatment related mortality in primates, clinical signs of lethargy, emesis, and cold to touch and histopathological observations including tissue infiltration mono/polymorphonuclear cells, arteritis, cellular degeneration, granuloma formation, and mineralization. The major target organ of toxicity was identified as injection sites, heart, kidney, lung, reproductive organs, and immune system. As mentioned above, the toxicity findings are not always related to IL-1 Trap plasma concentration but might be related to a combination of IL-1 Trap, IL-1 Trap:IL-1 β /IL-1ra complex, IL-1Trap:IL-1 Trap antibody, and combination of all of the above which are present in the serum/plasma at the same time. This might also be related to the poor method of detection which in turn might also be related to the presence of different immune complex in the body. There were three distinctly different treatment related histopathological changes noted in the toxicity studies conducted with IL-1 Trap. One is tissue congestion/degeneration which might be associated with either proinflammatory reaction due to immune complex deposition or hypersensitivity vasculitis associated with mono and polymorpho (eosinophil) nuclear cell infiltration. Both of these might result in multifocal lesion and tissue degeneration. Another one is increase infection associated with immunosuppression. Lastly, there was exaggerated pharmacology associated with depletion of the thymus and changes in the ovary and testes.

The reproductive toxicity studies for fertility and postnatal development were evaluated in the mice with the IL-1 Trap surrogate molecule and teratogenicity was conducted in the cynomolgus monkeys with the human IL-1Trap. The findings from the reproductive toxicity studies include spontaneous abortions, post implantation loss, fetal resorptions, and feta death. IL-1 Trap related decrease in the estrogen level was noted in the dams in the teratogenicity study and moderation of the estrus cycle was noted in the fertility and the postnatal studies. In the teratogenicity study skeletal variation associated with lumber bone formation was noted. The antibody was found to cross the placental barrier in the monkey and was detected in the fetus. In addition, the fetal death associated with empty stomach in the fetus was noted in the post natal study in the mice. The applicant did not determine whether the empty stomach was due to the negligence of mother that is changes in the nesting behavior or due to the lack of milk formation in the dams. The applicant did not study the immune system in the F₁ and F₂ generation. The development of immune system is more sensitive in the fetus and juvenile animals and immunosuppressive agents might have an effect on the development of immune system through indirect exposure via lactation or placental transfer of the antibody .and or product.

Also, it is not clear whether the changes are related to the IL-1 Trap exposure or the exposure of the different immune complex associated with it.

Unresolved toxicology issues (if any): There were a few outstanding issues with IL-1 Trap safety evaluation

1. The carcinogenicity potential of the IL-1 Trap was not evaluated.
2. The applicant likes to market this compound in the pediatric population; however, no juvenile studies in the animals were conducted.

3. The effect of the compound on lactation might need to be evaluated in a multigeneration reproductive toxicity study if the compound is administered in the pregnant mother. In that same line development of the immune system in the fetuses might need to be characterized.

Recommendations: Refer to executive summary section.

Suggested labeling: Refer to executive summary section.