CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

BL 125249/0

PHARMACOLOGY REVIEW(S)

<center>**Memorandum** ·</center>

February 20, 2008

From: David Jacobson-Kram, Ph.D., DABT Office of New Drugs

To: Robert Rappaport, MD

Subject: Review of pharmacology/toxicology section of BLA #125249

I have reviewed Drs. Melon and De's pharmacology/toxicology review of BLA#125249. I agree with the primary and secondary reviewer's conclusions that there are no outstanding nonclinical pharmacology/toxicology issues that would preclude approval. I agree with the proposed pregnancy category C and with the recommended post marketing study request. The suggested wording regarding potential for increased cancer risk associated with immunosuppressive drugs also is appropriate. I have reviewed and agree with the proposed changes to the nonclinical sections of the package insert.

Cc
Leah Ripper
Dan Melon, Ph.D.
Mamata De, Ph.D.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

SUPERVISOR'S SECONDARY REVIEW
PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER:	**125-249**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**05/29/07**
PRODUCT:	**ARCALYST (Rilonacept or IL-1 Trap)**
INTENDED CLINICAL POPULATION:	**Cryopyrin-associated periodic syndromes (CAPS), such as Familial Cold Autoinflammatory Syndrome and Muckle-Wells Syndrome**
SPONSOR:	**Regeneron Pharmaceuticals, Inc.**
DOCUMENTS REVIEWED:	**Primary Pharmacology Toxicology Review referencing ECT Modules 2 and 4**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia and Rheumatology Products (HFD-170)**
PHARM/TOX REVIEWER:	**Mamata De, Ph.D.**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, Ph.D.**
DIVISION DIRECTOR:	**Bob A. Rappaport, M.D.**
PROJECT MANAGER:	**Kathleen Davis**

1-18-08

Date of review:	**January 18, 2008**

Executive Summary

I. Recommendations

A. Recommendation on approvability

I concur with Dr. Mamata De's recommendation that BLA 125-249 is considered a complete response from the nonclinical pharmacology toxicology perspective, pending agreement on the labeling recommendations and proposed phase 4 commitments noted below:

B. Recommendation for nonclinical studies

1. Phase 4 Study: Conduct a juvenile animal study in the cynomolgus monkey that includes specific assessments of sex hormones and bone development.

2. Phase 4 Study: Conduct a study in the cynomolgus monkey examining the effect of IL-1 Trap exposure of the pregnant female during the third trimester of development.

C. Recommendations on labeling

The following recommendations for labeling are based entirely on the nonclinical pharmacology and toxicology data and have not yet been discussed with the entire review team or subject to tertiary review. They should be considered recommendations and not necessarily final labeling.

_4_ Page(s) Withheld

_____ Trade Secret / Confidential

___✓___ Draft Labeling

_____ Deliberative Process

II. Summary of nonclinical findings

A. Pharmacologic activity

Rilonacept (IL-1 Trap) is designed to bind to and neutralize the effects of IL-1β and IL-1α. The IL-1 Trap fusion protein consists of two cytokine receptor extracellular domains fused in tandem to an Fc domain from human IgG1. The first extracellular domain is from the IL-1 Type I Receptor (IL-1RI) and the second domain is from the IL-1 receptor accessory protein (IL-1RAcP). Inclusion of both of these domains mimics the normal IL-1 binding in vivo and allows for high affinity binding to both IL-1α and IL-1β. The figure below depicts the structure of the normal IL-1 receptor binding complex (IL-1RI and IL-1RAcP) and the construction of IL-1 Trap (reproduced from the Sponsor's submission):


IL-1 Receptor Complex
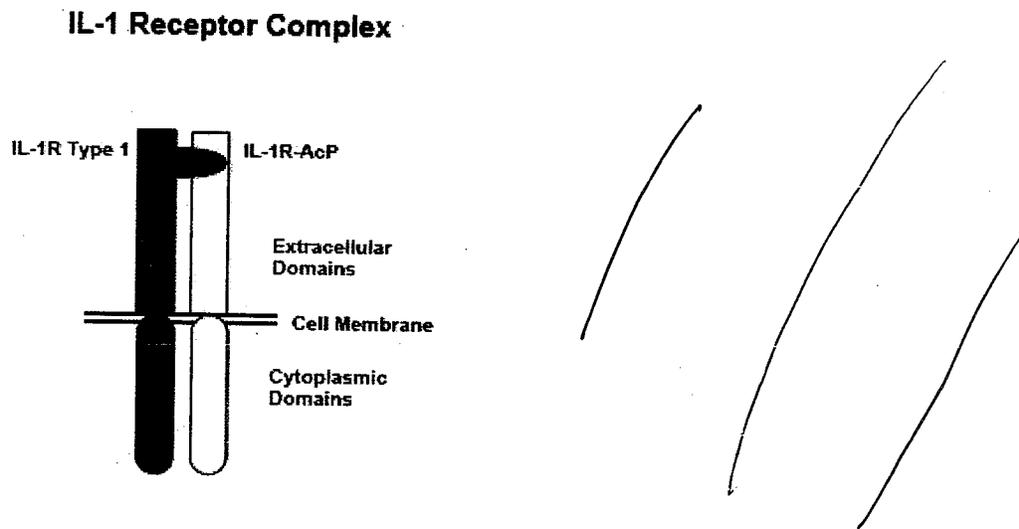
Figure 3.2.S.1.2-1   Schematic of the IL-1 Trap
The IL-1 Trap is created by fusing the sequences encoding the extracellular domains of the AcP, IL-1RI, and Fc inline without any intervening linker sequences.

The Sponsor provided data that suggests that one molecule of IL-1 Trap binds to one IL-1β molecule, as depicted in the diagram below (reproduced from the Sponsor's submission):
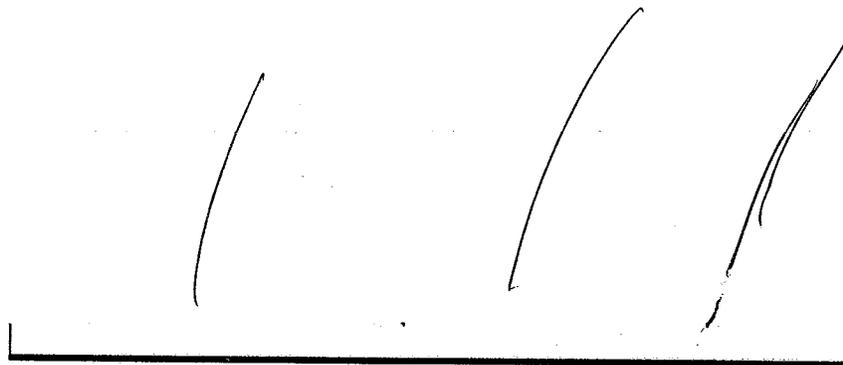
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Figure 4. Model of Potential IL-1β:IL-1 Trap Complex Formation
Schematic diagrams illustrating the possible IL-1β:IL-1 Trap complexes that can form in the presence of either free IL-1 Trap or an IL-1ra:IL-1 Trap complex.

The binding affinity of human IL-1 Trap to human and monkey targets:

	K_D or Equilibrium dissociation constant (pM) Human IL-1 Trap		K_D (pM) Murine IL-1 Trap
	Human	Monkey	Mouse
IL-1β	0.47	0.39	1.4
IL-1α	1.4	3.5	1.0
IL-1ra	6.1	6.8	4.8

As noted in the table above, the fusion protein also has affinity for the endogenous IL-1 receptor antagonist (IL-1ra). Studies suggest that binding of IL-1ra; however, does not block the ability of the protein to bind to IL-1β.

Pharmacodynamic studies suggesting that human IL-1 trap was able to neutralize the effects of human IL-1β in the mouse. In addition, murine IL-1 Trap was effective in the treatment of murine collagen induced arthritis model as well as in the prevention of neointimal hyperplasia induced by ligation of the carotid artery in mice.

These data suggest that the primate is a reasonably appropriate model for the human and that the murine IL-1 Trap protein homolog provides a reasonably alternative model to extrapolate to the human.

B. Brief overview of nonclinical findings

The Sponsor completed chronic repeat-dose toxicology studies for the drug product in the cynomolgus monkey model of a maximum 6-month duration via both the intravenous and subcutaneous routes of administration. The requirements for the standard battery of reproductive and developmental toxicology studies were met via Segment I and III studies using a homologous mouse protein and Segment II studies in

the cynomolgus monkey using the clinical product. No carcinogenicity studies were completed and mutagenicity studies were not required for this biologic product.

1. General Toxicology.

To support the proposed ——— indication, the Sponsor conducted a pivotal 6-month repeat dose toxicology study via the subcutaneous route of administration in the cynomolgus monkey. Both Dr. De and the Sponsor concur that no NOAEL was identified in this study. Monkey's (2-9 years of age) were dosed with 0, 15, 25, 40 and 60 mg/kg, SC three times a week (45, 75, 120, or 180 mg/kg/week). The dose was administered subcutaneously on the back of the animals 3 times per week for a total of 26 weeks. Injects were rotated over four different quadrants.

Summary of Exposure in the 6-Month Subcutaneous Repeat Dose Toxicology Study of IL-1 Trap in the Cynomolgus Monkey:

	Group 1 0 mg/kg	Group 2 15 mg/kg	Group 3 25 mg/kg	Group 4 40 mg/kg	Group 5 60 mg/kg
Toxicokinetic Data[1] Day 6 (Peak: µg/mL) Mean Serum Conc. Range Serum Conc.	BLQ BLQ	323 222-402	623 494-953	1220 562-1978	1646 947-1966
Day 181 (µg/mL) Mean Serum Conc. Range Serum Conc.	BLQ BLQ	119 1-799	202 1-1026	781 1-2622	1616 653-2731
Exposure margin based on mg/kg[2]		5.6	9	15	22
Exposure Margin based on body surface area comparisons (per dose)		1.8	3	4.9	7.3
Exposure margin based on clinical trough serum concentrations[3] (Mean Day 6 – 181)		12-4	23-7	45-29	61-60
Anti-product Antibody Positive Animals	3/14	9/10	14/14	14/14	11/14
Unscheduled Mortality	0/14	0/10	0/14	1/14	1/14
Early Discontinuation of Dosing due to Adverse Reactions	0/14	0/10	0/14	0/14	2/14

[1] Plasma measured 24 hours after final dose (Day 6 and 181)
BLQ = below limit of quantitation (⟨²µg/mL)
[2] Comparison made based on 160 mg maintenance dose given to a 60 kg person (2.7 mg/kg)
[3] Clinical trough serum concentrations in the clinical trials ranged from 20-27 µg/mL following the weekly maintenance dose of 160 mg. This calculation is based on 27 µg/mL.

Two animals were sacrificed early due to moribund condition (mid dose male #46 discontinued dosing on Day 40 and was euthanized on Day 45; high dose male #54 was euthanized on day 108). The cause of death of animal 46 was concluded by the study pathologist to be complications of an immunologic reaction (hypersensitivity) to the test article based on findings suggestive of glomerulonephritis in the kidney, eosinophil infiltration of the kidney, myocardial degeneration and necrosis, and eosinophil infiltration of the heart. This animal also demonstrated a raised mass of skin at the injection site and subcutaneous edema of the face, ventral extremities, and submandibular gland. The cause of death of animal 54 was concluded to be due to perivascular hemorrhage and edema of the lungs suggestive of vascular damage. This

animal also had marked congestion of the heart, kidneys, liver, lungs, and thymus. This animal also showed moderate thickened subcutaneous injection sites, and collapsed shortly after injection on Day 108 and was subsequently euthanized.

Dosing of two other animals was discontinued on Days 39 and 33 (high dose males #56 and 58) based on adverse clinical observations and clinical pathology. According to the Sponsor, the clinical signs and clinical pathology results in these animals were consistent with changes noted in the animals that had to be euthanized early, including serum chemistry changes suggestive of renal dysfunction which resolved after discontinuation of dosing. The histopathological evaluations of these animals were included in the terminal sacrifice data, which must be taken into consideration upon interpretation of the results.

Many of the treatment-related findings in this study were attributed by the Sponsor to post-injection reactions. The findings of myocardial cell degeneration and necrosis, if related to the direct effects of IL-1 Trap, would raise safety concerns. The original pharmacology toxicology reviewers in CBER raised concerns about the myocardial changes noted in the animals following their review of summary data submitted prior to a June 2005 meeting with the Sponsor. At that time, the CBER reviewers requested that the Sponsor submit a final toxicology study report with a signed pathologist's statement to support the Sponsor's conclusion that the findings were attributable to an immunological reaction. The final study report submitted in the BLA contains both a signed pathologist's report as well as a peer pathology review.

The table below reproduces the incidence of heart histopathological findings in cynomolgus monkeys following 6-month treatment via the SC route of administration.

Finding	Males (mg/kg)					Females (mg/kg)				
	0	15	25	40	60[1]	0	15	25	40	60
Heart ventricle										
Mononuclear cell infiltration	1/5	1/5	3/5	1/4	0/2	2/5	2/5	0/5	0/5	0/5
PMN (Eosinophil) infiltration	0/5	1/5	0/5	0/4	1/2	0/5	1/5	0/5	0/5	0/5
Myocardial cell degeneration	0/5	1/5	0/5	0/4	0/2	0/5	0/5	0/5	0/5	0/5
Myocardial cell necrosis	1/5	2/5	0/5	0/4	1/2	0/5	0/5	0/5	0/5	0/5
Vasculitis	1/5	1/5	0/5	0/4	0/2	0/5	0/5	0/5	0/5	0/5
Heart intraventricular septum, left atrium)										
Mononuclear cell infiltration	2/5	1/5	0/5	1/4	1/2	1/5	1/5	1/5	2/5	4/5
PMN (Eosinophil) infiltration	0/5	0/5	0/5	0/4	1/2	0/5	0/5	0/5	0/5	0/5
Myocardial cell degeneration	0/5	0/5	0/5	0/4	0/2	0/5	0/5	0/5	0/5	0/5
Myocardial cell necrosis	0/5	0/5	0/5	0/4	0/2	0/5	0/5	0/5	0/5	0/5
Vasculitis	0/5	0/5	0/5	1/4	0/2	0/5	0/5	0/5	0/5	0/5
Liver										
Mononuclear cell infiltration (perivascular sinusoidal), very slight	1/5	2/5	0/5	2/4	2/2	1/5	2/5	1/5	1/5	3/5

[1]One animal died and two other animals were discontinued prior to scheduled sacrifice. Only data from the animals that were treated throughout the duration of the study is included here.

Of note, one animal (not in the table above) that was sacrificed early due to moribund condition demonstrated evidence of myocardial degeneration and necrosis (animal 46). At the time of scheduled sacrifice, there was one male in the control group with evidence of **myocardial cell necrosis**, and two in the low dose group. Of the two male animals in the low dose group that demonstrated myocardial cell necrosis (animal 16 and 24), only number 24 showed evidence of reduced exposure to IL-1 Trap and the production of anti-IL-1 Trap antibodies. In contrast, no evidence for anti-product antibodies was found for animal #16, and IL-1 Trap levels were elevated throughout the treatment period.

Likewise, there was an increased incidence of **mononuclear cell infiltration** of the intraventricular septum of the heart in high dose females compared to controls. Examination of the anti-IL-1 Trap antibodies in these 4 animals indicates that there was little to no anti-product antibody formed in these animals. As such, the findings are not readily attributable to an immune reaction to the product. A similar finding of increased incidence of mononuclear cell infiltration in liver perivascular sinuses was noted in both mid and high dose males and high dose females compared to control.

Focal **vasculitis in the heart** was noted in only one animal (male, 40 mg/kg group) and not in the control animals. There was evidence for both IL-1 Trap exposure as well as anti-product antibody production in this animal. Therefore it is not possible to determine if the response was due to exposure to IL-1 Trap or to an immune reaction to the product. Vasculitis was not noted in any animal in the high dose groups, and therefore the finding may be incidental.

I concur with Dr. De and the Sponsor that a NOAEL can not be established based on the results of this study. The pathology report attributes these findings to an immune reaction to the product; however, the exposure data and anti-product antibody data obtained do not clearly correlate with such a conclusion. As noted by the Sponsor, it is possible that the presence of high levels of IL-1 Trap may interfere with the detection of anti-IL-1 Trap antibodies and therefore the lack of evidence for anti-product antibodies may not be accurate. Given the relative lack of adverse events noted in the clinical studies conducted to date, the formation of anti-product antibodies in many animals treated with the low dose that produced the most relevant clinical exposure to the product, and the large exposure margin based on mean serum levels in animals compared to trough clinical values, the findings may not have much clinical relevance. Of note, one of the two deaths reported in the open-label clinical trials to date was the sudden death of a young overweight male who died of what (upon preliminary information) appears to be an athlerosclerotic cardiovascular event. Given an otherwise relatively good clinical safety profile (see medical officer review for clinical safety information), the nonclinical findings, although noteworthy, may not have clinical significance.

2. Segment I (Fertility and Early Embryonic Development Study.

Dr. De recommended that ~~_____~~
~~_____~~ study be included in the teratogenicity section of the labeling. Specifically, she recommends the following " ~~_____~~ "

~~_____~~ Although I agree that there is a subtle signal suggesting an increase in the percent of early resorptions, total resorptions and post-implantation loss in rilonacept treated animals compared to controls, these findings are not statistically significant, and the overall fertility indices were not altered in either males or females. Therefore, I do not recommend that ~~_____~~ , be incorporated in the product labeling ~~_____~~ . In addition, the changes do not show a clear dose response; however, it is recognized that due to the formation of anti-product antibodies (particularly in the low dose animals), the utility of evidence for dose-dependency is limited.

Summary of Findings and Exposure in the Segment I Fertility and Early Embryonic Development Study of mIL-1 Trap Homolog

Parameter (% per litter)	Group 1 0 mg/kg	Group 2 20 mg/kg	Group 3 100 mg/kg	Group 4 200 mg/kg
Early resorptions	2.6	4.9	4.1	5.1
Total resorptions	3.2	5.3	4.1	5.8
Post-implantation loss	3.2	5.3	4.1	5.8
Male Fertility Index	30/30 (100%)	68/74 (91.9%)	29/29 (100%)	29/30 (96.7%)
Female Fertility Index	30/30 (100%)	69/74 (93.2%)	29/29 (100%)	30/30 (100%)

Male Fertility Index (%) = # males siring a litter / total # males used for mating x 100
Female Fertility Index (%) = # females with confirmed pregnancy/total # females used for mating x 100

3. Segment II (Embryo-Fetal Development) Study.

The Sponsor has proposed a Pregnancy Category ~~for~~ rilonacept based on their findings from the developmental toxicity study performed in cynomolgus monkeys at high doses of 30 mg/kg given twice a week (30 mg/kg dose corresponds to 360 mg/m^2 which is 3.6 fold higher than the human maintenance dose of 160 mg based on body surface area) with no maternal or embryo-fetal effects observed. The Sponsor concluded that the highest dose administered in this study was the NOAEL for both maternal toxicity and embryo-fetal development.

Summary of Findings and Exposure in the Segment II Embryo-Fetal Development Study of IL-1 Trap in the Cynomolgus Monkey

	Group 1 0 mg/kg/day	Group 2 5 mg/kg/day	Group 3 15 mg/kg/day	Group 4 30 mg/kg/day
Toxicokinetic Data[1] Mean Serum Conc. Range Serum Conc.	BLQ BLQ	0.9 µg/mL 1-1.7 µg/mL	12.1 µg/mL 1.2-32.3 µg/mL	222 µg/mL 4.7-780.3 µg/mL
Exposure margin based on mg/kg^2		1.9	6	11
Exposure Margin based on body surface area comparisons		0.6	1.8	3.7
Exposure margin based on clinical trough serum		0.03	0.4	8.2

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concentrations[3]				
Fetal Abortions/Deaths (total) Late Stage Abortions (GD>25)	2/12 0	3/12 1	1/12 1	2/12 0
Skeletal Variations F1 Presence of lumbar ribs Historical Control Data → ── Control Data	1/10 (10%) 11/87 (12.6%) 6/63 (9.5%)	2/9 (22%)	2/11 (18%)	2/10 (20%)
Skeletal Malformations F1 Abnormal arrangement of ribs and thoracic vertebrae Historical Control Data → ── Control Data	0/10 (1.1%) 1/461 (0.2%)	0/9	1/11 (9%)	0/10

[1] Serum measured 24 hours after final dose (GD49)

[2] Comparison made based on 160 mg maintenance dose given to a 60 kg person (2.7 mg/kg)

[3] Clinical trough serum concentrations in the clinical trials ranged from 20-27 µg/mL following the weekly maintenance dose of 160 mg. This calculation is based on 27 µg/mL.

BLQ = below limit of quantitation ` _ _ _ ` µg/mL)

Dr. De does not agree with the Sponsor's conclusion that there were no adverse effects on embryo-fetal development noted in this study. Specifically, Dr. De notes significant decreases in estrogen concentrations and late abortions in low and mid-dose group females, decreases in organ weights of the spleen, ovary and lung, the presence of lumbar ribs that exceed the historical control range for this species, and the finding of abnormalities in the arrangement of ribs and thoracic vertebrae in one fetus from the mid-dose group.

As noted in the table above, total fetal abortions did not show an increase in incidence over controls that could be attributed to treatment. The findings of late abortions in the low dose and mid dose animal were not evident in the high dose animals which demonstrated the greatest exposure to IL-1 Trap. Given the high background rate for spontaneous abortions in the monkey model (~20%), these nonclinical findings do not appear to be treatment-related.

The effect of IL-1 Trap on **17-beta-estradiol levels** in the monkey are reproduced from Dr. De's review below (emphasis added):

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

NOTE: IL-1 Trap administered twice weekly from GD-20 to GD-48.

Although the Sponsor does not dismiss the estradiol changes as unrelated to treatment, they did consider them to be toxicologically insignificant because they interpreted the results of the study to show no adverse effects on the maintenance of pregnancy or fetal development. I agree with Dr. De's conclusion that the findings appear to be treatment-related and should not be dismissed without further investigations into the potential clinical significance of the hormonal changes. I am particularly concerned about this finding, since the impact of these hormone changes on the developing fetus may not be apparent in the nonclinical studies conducted to date. Specifically, a large body of data exists regarding the effects of compounds that alter hormone levels on human health (examples of such compounds are referred to as endocrine disruptors; for example PCBs, which have both estrogenic and antiestrogenic properties, and bisphenol A, which has estrogenic properties). The FDA approved label for tamoxifen, an estrogen receptor antagonist, includes the statement "effects on reproductive functions are expected from the antiestrogenic properties of the drug." Tamoxifen is also known to alter bone development (Gallagher, et al., 1993; Perry, et al., 2005; Chagin, et al., 2007). Although the effects noted with IL-1 Trap on estradiol levels in the monkey may not result in as profound effects as PCBs, bisphenol A or tamoxifen, the potential impact of the estradiol changes noted in the primate following IL-1 Trap administration during gestation are not known. Alterations in hormone levels during sensitive periods of development may have adverse effects on growth and functional integrity of the organism, particularly the brain (Carrer and Cambiasso, 2002; Jacobs, et al., 2003). It is possible that some effects would be noted in a Segment III toxicology study; however, we do not have Segment III data in the cynomolgus monkey. Estrogen levels were not obtained in the homologous mouse model to provide any further support for the Sponsor's conclusion the changes did not result in adverse effects. Although reduction in estradiol also would not be considered a direct teratogenic effect, reductions in estradiol during development could theoretically have a significant impact on the developing offspring and·

The increased incidence of **skeletal variations** (presence of unilateral or bilateral lumbar ribs) compared to the control incidence, although not dose-dependent, exceed the historical control incidence reported by the Sponsor for the laboratory and as reported by ̃ and therefore should also not be dismissed as incidental based on the information provided to date. Although not unusual in standard developmental toxicology studies in rodents, the biological significance of supernumerary or accessory ribs is of some debate. According to a recent review, supernumerary ribs are believed to signify basic alterations in the architecture of the axial skeleton. They can be induced by xenobiotics (valproic acid) and/or maternal stress, but are permanent structures that can be associated with adverse health effects (Chernoff and Rogers, 2004).

The finding of **skeletal malformations** in one fetus from the mid-dose group was described by the Sponsor as "abnormal arrangement of the ribs and the thoracic vertebrae (multiple fusion and absence of the ribs and thoracic vertebral bodies and

arches). Specifically, the animal line listing notes that there was fusion of the right $3^{rd}/4^{th}$, $6^{th}/7^{th}/8^{th}$, and $10^{th}/11^{th}$ and left $3^{rd}/4^{th}/5^{th}$, and $8^{th}/9^{th}$ ribs and fusion and/or absence of multiple thoracic vertebral bodies and arches. The Sponsor concluded that this finding was judged to be incidental because the there were no skeletal malformations observed in the high dose group and since there have been similar changes reported in control fetuses at an incident of 1.1% (the number of animals that were examined to obtain this percentage was not provided). The historical control data reference provided by the Sponsor of the BLA is _____ control background data from 2003. Historical control data from _____ suggests an incidence of 1/461 of fused ribs at GD100 and 1/461 vertebral column fusion and 2/461 vertebral column absent observations (it is not known if these are mutually exclusive or not), giving an incidence of 0.2-0.4% _____.

Evaluation of the estradiol, progesterone, or prolactin data from the pregnant female that carried this fetus (#306) does not reveal any unique hormone responses that could clearly tied to the finding. However, examination of the IL-1 Trap levels in this female indicates that this female was the **only** female that had prolonged exposure to measurable IL-1 Trap from Gestation Day 55 to pre C-section (GD 103). No IL-1 Trap was detected in the amniotic fluid nor in the fetal plasma. Plasma levels for the mid dose group are depicted in the table below, reproduced from the Sponsor's submission.

Appendix A (Data Tables) continued:

Table 5: Monkey Plasma and Amniotic fluid Concentrations (ng/mL) of IL-1 Trap in the 15 mg/kg Cohort

Dose	Gestation Day	Draw Time	301	302	303[b]*	304	305	306	307	308	309	310	311[d]	312	313
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 (— ng/mL)   NS = No sample available for analysis   CS= cesarean section
*False positive diagnosis of pregnancy, animal excluded from evaluation of study
[b]: Monkey aborted, last sample collected on gestational day 26
[d]: Monkey aborted, last sample collected on gestational day 52

There is a low background incidence of fused or absent vertebrae or fused ribs in this species, and the finding was not found in the high dose group; however, the prolonged exposure is unique to this animal and therefore the finding may be related to the IL-1 Trap exposure during the later portion of gestation. Given the known role of IL-1 in

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bone metabolism (Swolin-Eide, et al., 2004; Nakamura and Jimi, 2006), it is biologically possible that neutralization of endogenously released IL-1 may have an effect on developing bone. Therefore, until further studies are conducted to clarify this finding, the ⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯ and the Pregnancy Category should be a C. The Sponsor should also conduct further studies to confirm if the exposure to IL-1 Trap during the later stages of gestation produces skeletal malformations or alters bone development.

Unless more specific information is provided to demonstrate that the effects on estrogen, the potential skeletal malformations, and the skeletal variations do not represent a treatment-related finding, and given the fact that the primate produces antibodies against the therapeutic product making the study difficult to interpret, I agree with Dr. De's recommendation for a Pregnancy Category C. I would recommend the proposed labeling language be changed to comply with the CFR requirements for labeling as noted above.

4. Segment III (Prenatal and Postnatal Developmental Toxicology) Study.

Dr. De has recommended that the Sponsor complete further evaluations of the cause of fetal deaths noted in the Segment III study. The Sponsor submitted a prenatal and postnatal developmental toxicology study in the mouse model using a murine IL-1 Trap homologous protein. Animals were dosed with 20, 100 or 200 mg/kg/day (60, 300, 600 mg/m^2). The proposed clinical adult maintenance dose of 160 mg/60 kg person would be 2.67 mg/kg or 98.67 mg/m^2; therefore, the high dose of the mouse homologous protein would be approximately 6-fold higher than the clinical maintenance dose based on body surface area. The Sponsor reports that the NOEL (No Effect Dose) for maternal toxicity, F_1 embryofetal survival, F_1 offspring growth and development through maturation, F_1 offspring behavior (general activity, learning and memory) and reproductive parameters (through parturition of F_2 generation) was the high dose of 200 mg/kg. The Sponsor reports no treatment-related effects in this study, although there are several findings from the study that deserve further discussion. Dr. De notes that there are apparent treatment-related findings, including an increase in gestational abortions, total litter deaths and unscheduled deaths of F_1 offspring during maturation, as noted in the tables below:

Summary of Findings and Exposure in the Segment III Prenatal and Postnatal Development Study of mIL-1 Trap Homolog

Key Potential Treatment-Related Findings	0 mg/kg	20 mg/kg	100 mg/kg	200 mg/kg
Toxicokinetic Data[1]				
Mean Serum Conc.	BLQ	0.39 µg/mL	37.8 µg/mL	222 µg/mL
Range Serum Conc.	BLQ	0.9-9.6 µg/mL	1-269 µg/mL	4.7-780.3 µg/mL

Exposure margin based on mg/kg[2]		7	37	74
Exposure Margin based on body surface area comparisons		0.6	3	6
Exposure margin based on clinical trough serum concentrations[3]		0.01	1.4	8.2
Findings in F_1 Offspring				
F_1 Pups Delivered				
Liveborn	367	873	338	350
Stillborn	1 (0.3%)	3 (0.3%)	0 (0%)	3 (0.86%)
Uncertain	0	1	0	3 (0.86%)
Total Dead/Missing	1 (0.3%)	4 (0.5%)	0	6 (1.7%)
Livebirth Index[2] (Mean %)	99	100	100	98
F_0 total litter deaths during lactation	1/30	1/68	1/27	2/29
Unscheduled deaths of F_1 offspring during maturation	0	6	2	1
F_1 gestational abortions	0	0	0	1

[1] Serum measured 24 hours after final dose
[2] Comparison made based on 160 mg maintenance dose given to a 60 kg person (2.7 mg/kg)
[3] Clinical trough serum concentrations in the clinical trials ranged from 20-27 µg/mL following the weekly maintenance dose of 160 mg. This calculation is based on 27 µg/mL.
BLQ = below limit of quantitation ⎯ µg/mL)

Dr. De's review indicates that there was an increase in **stillborn F_1 pups** born to F_0 dams. Dr. De used the conservative approach and assumed the uncertain animals were stillborn for her assessment (1.7% stillborn in the high dose group). All three of the stillborn pups in the high dose group were from the same litter (Dam # A87305; page 287 of 761). This dam had serum mIL-1 Trap levels of only 62.2 µg/mL and anti-mIL-1 Trap Antibody levels of 5.2 µg/mL (page 205 of 761); therefore, there is no correlation with exposure that could be used to explain the finding. The Sponsor states that the increase in stillborn pups in the high dose group was not statistically significant. Although requested, historical control data for the laboratory ⎩ ⎯ were not provided by the Sponsor to put the data into a larger context; therefore, I must assume that this finding may be real. The ability to compare the doses tested with the clinical dose is difficult due to the fact that this is not the clinical drug, rather a murine homolog. The Sponsor proposes that the dose of 200 mg/kg is approximately 10-fold higher than the clinically effective dose in a mouse model of arthritis, which would presumably provide a safety margin of about 5-fold for a mouse with arthritis. As there is no obvious way to put these data into perspective for the product labeling, I concur with Dr. De that although the effect is not statistically significant, it should not be dismissed in the absence of historical control data to justify that the increase is not biologically significant. As such, the finding should be included in the Pregnancy portion of the label.

There were two F_0 females with **total litter loss during lactation**. The female treated with 200 mg/kg displayed clinical signs of thin, hunched, and pale appearance, hypoactivity, ataxia, squinted eyes and few to no feces. This high dose female was found dead on lactation day 4 (LD4). Total litter death occurred between LD3 and LD4. The total litter loss was likely due to maternal toxicity, possibly related to the murine IL-1 Trap protein exposure. In contrast, the female treated with the 20 mg/kg dose had total litter loss on LD1. The total litter loss can not be clearly explained; however, there does not appear to be a dose-related effect and the exposure at the low dose was low due to the production of anti-product antibody. Although the litter loss in the low dose animal could be dismissed as unrelated to treatment, the litter loss in the high dose animal should not be dismissed without further data to support the conclusion that the effect is not real.

As illustrated in the table above, there were a total of 9 unscheduled deaths of F_1 offspring during maturation. The increase in **unscheduled deaths of F_1 offspring** during maturation shows reverse-dose dependency. This reverse dependency suggests that the unscheduled deaths are more likely due to the formation of anti-product antibody rather than exposure to IL-1 Trap itself. The Sponsor has provided their interpretation of these deaths and doenot believe that they are treatment-related. Two of the low-dose and one of the mid-dose deaths were attributed by the Sponsor to be due to urinary obstruction that they state is common in this strain of mouse. Interestingly, there were no such findings in the control animals, suggesting that the treatment may have contributed to the urinary obstruction. I do not think that asking them to evaluate the cause of the fetal deaths in this study would provide useful information due to the low and unpredictable frequency of the findings. Nonetheless, there were no losses in the control group, and until there is an explanation of the findings that suggest the effect is not related to the murine IL-1 protein, the recommendation of a Pregnancy Category C is further justified based on potential effects on the offspring of animals treated with IL-1 Trap.

During gestation, a 200 mg/kg/day F_1 dam aborted on GD17. The **gestational abortion** in the high dose group could be due to previous exposure to the murine IL-1 Trap homolog, as the high dose group had the least anti-product antibody formation. Although we can not rule out this finding as being unrelated to the therapeutic protein, the high dose administered in this study is approximately 6-fold greater than the proposed clinical maintenance dose based on body surface area (3-fold exposure margin at the no effect dose for this endpoint). A pharmacokinetic comparison is difficult, due to the inconsistent development of antibodies to the product in the animal study, as illustrated by the ranges in the table above and the use of a homologous protein rather than the clinical formulation. With those caveats in mind, the human trough serum levels at the proposed dose ranged from 20-27 μg/mL, suggesting that the body surface area approach may be a more conservative estimate of exposure than mean serum levels. Therefore, although it can not be ruled out as unrelated to the treatment, the safety margin suggests that the effect is not likely to have clinical significance if used as labeled. However, the data do suggest further that a Pregnancy

Category C is warranted, unless further data can be provided to demonstrate that the finding is not real. Due to the variability of the exposure data, exposure margins for the product labeling should be ⎯⎯⎯⎯⎯

The data regarding the increase in death of the F_2 pups are shown in the table below:

Parameter	Group 1 0 mg/kg	Group 2 20 mg/kg	Group 3 100 mg/kg	Group 4 200 mg/kg
F_2 Pups Delivered	240	713	252	216
Liveborn	238	704	250	216
Stillborn	2	8	2	0
Uncertain	0	1	0	0
Livebirth Index (Mean %)	99	99	99	100
Viability Index (Mean %)	96	96	99	91
F_2 Pup Disposition				
Killed	0	0	0	0
Died	2 (0.8%)	2 (0.3%)	1 (0.4%)	9 (4%)
Cannibalized	0	0	0	0
Missing	7 (2.9%)	25 (3.6%)	2 (0.8%)	7 (3.2%)
Total	9	27	3	16

Livebirth Index (number born alive/number born)
Viability Index (number alive day 4 precull/number liveborn)

Although not statistically significant, there was a slight decrease in viability index in the F_2 pups from the high dose group, which is apparently due to the increase in death of pups between postnatal days 1 and 4. Again, historical control data would be useful to put this possible treatment-related finding into perspective; however, the Sponsor did not submit the requested historical control data. As there is no logical explanation for such a treatment-related effect, and the only explanation would have to be an indirect effect such as a change in maternal behavior, the clinical significance of the findings appears to be remote. Given the lack of statistical significance, the existence of a small safety margin, and the lack of any logical explanation of such a finding, I do not believe the finding impacts the potential safety of the clinical use of this therapeutic protein.

5. Carcinogenesis.

Long-term studies in animals have not been performed to evaluate the carcinogenic potential of rilonacept. The Sponsor was granted a waiver for rodent carcinogenicity studies, as rodents are not an appropriate species for toxicity testing due to the specificity of this product. However, the BLA does not contain any form of carcinogenicity assessment. Although traditional 2-year bioassays are not feasible for this product, alternative strategies could be considered to determine if this product has the potential to alter carcinogenicity. As discussed in ICHS6 titled Guidance for Industry: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals, "product-specific assessment of carcinogenic potential may still be needed depending upon duration of clinical dosing, patient population, and/or biological activity of the

product (e.g., growth factors, immunosuppressive agents, etc.). When there is a concern about carcinogenic potential, a variety of approaches may be considered to evaluate risk." As rilonacept is intended to block the effects of overexpressed IL-1, it must be considered an immunosuppressive agent. Therefore, based on current international guidance, carcinogenic potential should still be evaluated.

In the case of rilonacept, there are limited options to assess the carcinogenic potential of this product. One option would be to test the already developed mouse homologous product in long-term studies to see if there is any evidence for immunosuppression-related tumors. Although there was exposure to murine IL-1 Trap in the mouse fertility study (particularly the male mice), animals were developing anti-product antibodies within the duration of treatment and there was no detectable exposure in the females. Therefore, since the mice developed antibodies against this homologous protein in the reproduction and developmental toxicology studies, long-term studies using the mouse homologous product do not appear to be a viable option.

Exposure Data from Male Mice Treated with Murine IL-1 Trap in the Segment I Study

Treatment Groups	0 mg/kg	20 mg/kg	100 mg/kg	200 mg/kg
Toxicokinetic Data[1]				
Mean Serum Conc.	BLQ	6.2 µg/mL	140.7 µg/mL	371.8 µg/mL
Range Serum Conc.	BLQ	0.9-78.3 µg/mL	0.9-431.8 µg/mL	29.1-748.3 µg/mL
Anti-product Antibody Positive Animals				
Females	5/29	61/68	28/29	19/29
Males	7/30	63/74	7/29	2/29

[1] Serum measured 24 hours after final dose

BLQ = below limit of quantitation (— µg/mL)

NOTE: mIL-1 Trap levels in females were all below the limit of quantitation in the fertility study.

A second option that is described in ICHS6 would be to incorporate sensitive indices of cellular proliferation in long-term repeated-dose toxicity studies, in order to provide potentially useful information to detect a signal of potential concern. However, although exposure to IL-1 Trap was maintained in the long-term repeat-dose toxicity studies in the primate model; most animals produced anti-product antibodies within weeks that diminish the utility of longer term studies in this species.

Summary of Exposure in the 6-Month Subcutaneous Repeat Dose Toxicology Study of IL-1 Trap in the Cynomolgus Monkey:

	Group 1 0 mg/kg	Group 2 15 mg/kg	Group 3 25 mg/kg	Group 4 40 mg/kg	Group 5 60 mg/kg
Toxicokinetic Data[1]					
Day 6 (Peak; µg/mL)					
Mean Serum Conc.	BLQ	323	623	1220	1646
Range Serum Conc.	BLQ	222-402	494-953	562-1978	947-1966
Day 181 (Peak; µg/mL)					
Mean Serum Conc.	BLQ	119	202	781	1616
Range Serum Conc.	BLQ	1-799	1-1026	1-2622	653-2731
Exposure Margin based on body surface		1.8	3	4.9	7.3

area comparisons					
Anti-product Antibody Positive Animals	3/14	9/10	14/14	14/14	11/14

[1] Plasma measured 24 hours after final dose (Day 6 and 181)
BLQ = below limit of quantitation (—— µg/mL)

Examination of the published literature regarding the potential role of IL-1 on tumor formation provides several useful studies that help to evaluate the potential impact of IL-1 neutralization that would occur with rilonacept clinical use. Specifically, IL-1 has been shown to play a role in tumor cell metastasis and growth and blocking IL-1 activity has been reported to reduce angiogenesis, solid tumor cell metastasis, and solid tumor cell growth (Vidal-Vanaclocha, et al., 1996; Voronov, et al., 2003; Bar, et al., 2004; Elaraj, et al., 2006; Lewis, et al., 2006; Apte, et al., 2006a; Apte, et al., 2006b; Lavi, et al., 2007; Krelin, et al., 2007). The potential impact of IL-1 neutralization on immunosuppression-related tumors, such as lymphomas; however, is less well characterized.

In light of the above discussion, Dr. De has recommended that the Sponsor do one of the following:

1. Label the product as — promote tumor growth via immunosuppression
2. Conduct further studies (if feasible) to address the carcinogenic potential of rilonacept, as standard carcinogenicity studies are not feasible, or,
3. Provide a risk assessment based on the available information they have collected via your own studies as well as those published in the literature regarding the potential impact of rilonacept (blockade of IL-1 effects) on tumor surveillance and tumor development.

Since the Sponsor did not address the potential carcinogenicity of their product in the original submission in any manner, this request is very reasonable and consistent with the approach the Division is taking to address the challenge of assessing the carcinogenic potential of biologic products. In the case of rilonacept, I would propose that the label include information indicating that the product has the potential to increase the risk of immunosuppression-related tumors, unless the Sponsor can provide data or scientific information to allow the division to conclude that such a risk is not present for this product.

6. Juvenile Animal Study.

The disorder that the Sponsor is proposing to treat with rilonacept is a genetic disorder and therefore pediatric patients are also presumably likely to be treated with this therapy. The Sponsor is currently seeking both an adult indication as well as pediatric indication for ages — to 17. Given the orphan status of this disorder and the limited adult patient population that exists to enroll in clinical trials, the ability to assess the potential impact of rilonacept on normal human development is limited. To date, very

few pediatric patients have been studied, and the Division has allowed these patients to be enrolled based in part on the lack of any approved treatment for this disorder, the familiar nature of the disorder, and the clinical experience to date with similar products that block the effects of IL-1. At the pre-BLA meeting, the Division provided the following comment to the Sponsor:

> A juvenile animal study may be required to support a pediatric indication; however, final determination of the need for such a study can only be made upon review of the final reproductive toxicology study reports.

Typically, products intended for chronic use in pediatric patients are evaluated in adults prior to their use in pediatric populations. Obviously, given the orphan status of this indication, the clinical experience in adults prior to approval is relatively limited. I agree with Dr. De's recommendation that in such a situation, a juvenile animal study should provide useful information to identify any developmental effects that may be unique to pediatric patients.

The primary target of rilonacept is the immune system, which is largely developed by the age of ⁓in humans. Therefore, the need for a developmental immunotoxicology study does not appear to be needed for the current proposed patient population. However, this should be reevaluated if the Sponsor wishes to treat younger patients.

Dr. De notes that there are signals in the reproductive toxicology studies that would suggest that rilonacept may have an impact on skeletal development, possibly via alterations in hormonal levels. She specifically notes the findings of skeletal variations and reduced estradiol levels noted in the Segment II study in primates. The presence of lumbar vertebrae ribs may be considered a variation that does not necessarily reflect delayed development nor teratogenicity; however, should it occur clinically may have adverse effects and is consistent with a potential effect of this product on bone. The estradiol changes noted in the Segment II monkey study have potential implications regarding embryonic and/or fetal development. If such changes were to occur in pediatric patients, there may be effects on development. Unfortunately, estrogen was not measured during the 6-month toxicology studies. A juvenile animal study that specifically employs measurements of hormones should provide more definitive characterization of the potential effects on development that may not be reflected in the minimal pediatric clinical data obtained to date.

It should be noted that the existing Segment III study data do not raise significant concerns regarding development. Specifically, that study found no significant differences across treatment groups in reflexes and development (preputial separation, vaginal opening, eye opening, hair growth, incisor eruption, pinna unfolding, auditory reflex, surface righting reflex), motor activity or learning and memory tests. Although slight differences were occasionally noted, there do not appear to be any clear treatment-related differences that would suggest potential developmental consequences of murine IL-1 Trap on the offspring of dams treated with the

therapeutic protein. It should be noted that Segment III studies do not address the potential impact of rilonacept following direct administered to the juvenile animals.

Finally, it should be noted that there are known developmental delays associated with excessive IL-1 expression in humans, and since a juvenile animal studies would be conducted in healthy animals rather than a disease model, any changes noted in a juvenile animal model may not be relevant to the clinical use of the drug for this indication. Nonetheless, given the limited clinical adult and pediatric experience with this drug, the suggestion of altered estradiol levels, studies of juvenile animals could provide reassurance for appropriate monitoring of pediatric patients treated with this product. From a nonclinical pharmacology and toxicology perspective, based on the existing findings in animals suggesting IL-1 Trap-induced changes in estradiol levels of pregnant females during gestation, the lack of estradiol levels in the 6-month monkey repeat-dose toxicology studies, and the potential effects of reduced estradiol and/or IL-1 Trap on bone metabolism, from a nonclinical pharmacology toxicology perspective, a juvenile animal study is recommended prior to a pediatric indication being granted.

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BLA# 125-249
Arcalyst (Rilonacept, IL-1 Trap)
Regeneron

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND
RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER:	**125-249**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**05/02/07**
PRODUCT:	**Rilonacept, Interleukin-1 Trap (IL-1 Trap)**
INTENDED CLINICAL POPULATION:	**Treatment of Cryopyrin-associated periodic syndromes (CAPS)**
SPONSOR:	**Regeneron Pharmaceuticals, Inc**
DOCUMENTS REVIEWED:	**eCTD Module 2 and 4**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia, and Rheumatology Drug Products (HFD-170)**
PHARM/TOX REVIEWER:	**Mamata De, Ph.D.**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, Ph.D.**
DIVISION DIRECTOR:	**Bob A. Rappaport, M. D.**
PROJECT MANAGER:	**Kathleen Davies**

Date of review finalization: 12-19-2007

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EXECUTIVE SUMMARY

I.   Recommendations

     A.   Recommendation on approvability:

        From the pharmacology toxicology perspective, BLA 125-249 may be
        APPROVED, pending agreement on the labeling and Phase 4 commitments
        (under discussion) outlined below.

Pregnancy Labeling

     B.   Recommendation for non clinical studies:

1. Evaluate the cause of fetal death in the Segment III study (whether it is due to maternal effect or non teratogenic effect, if it is a non teratogenic effect a mechanistic study to demonstrate the mechanism for such effect need to be known that is whether it is due to developmental immunotoxicity or any thing else).

2. Labeling for carcinogenicity, feasible studies or risk assessment because increase lymphoma and skin cancer noted in IL-1 inhibitors and numerous other immunosuppressive compounds

3. Juvenile animal study to assess the safety in pediatrics population because the product is found to modulate estrogen (statistically significantly) and other hormones like progesterone and prolactin and skeletal variation were noted in primates.

II. Summary of non clinical findings

A. Pharmacologic activity

The IL-1 Trap is a recombinant fusion protein designed to capture IL-1β to moderate the underlying common pathophysiological mechanism associated with IL-1 β associated inflammation in patients with cryopyrin associated periodic syndrome (CAPS). IL-1 Trap is created by fusing the sequences encoding the extracellular domains of the two receptors of the IL-1 namely IL-1AcP (interleukin 1 accessory protein) and IL-1RI (IL-1 receptor type I). IL-1 Trap is a dimeric glycoprotein with a total molecular weight of ~251 kDa, ⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯. The dimer is ⎯ linked ⎯⎯⎯⎯ in the Fc region of the human IgG1. A schematic diagram of IL-1 Trap is provided below.

IL-1 Receptor Complex   **IL-1 Trap**

The applicant characterized the primary and secondary pharmacology of the compound in several *in vitro* assays. The binding experiments were conducted to evaluate the affinity of IL-1 Trap to human, monkey and murine IL-1 cytokines. These experiments demonstrated that IL-1 Trap binds human IL-1β, IL-1α, and IL-1ra (endogenous receptor antagonist of IL-1) with an equilibrium binding constant (K$_D$) of approximately 0.5, 1.4 and 6 pM respectively. The binding affinities for monkey cytokines were determined to be similar to the human ligands. Therefore, cynomolgus monkey is appeared to be an appropriate animal model for the general toxicity testing for IL-1 Trap. The applicant developed a surrogate IL-1 Trap fusion protein for mice. The binding affinity of the murine IL-1 Trap to murine IL-1α, IL-1β, and IL-1ra were 1.4, 1.0, and 4.8 pM respectively. The sponsor conducted the reproductive and developmental toxicity studies (Segment I and Segment III) in the mice using this surrogate model.

The applicant evaluated the indirect/nonspecific activity of IL-1 Trap mediated by Fc receptor binding activities which include ADCC, CDC, and Fcγ receptor interactions. The results from these studies showed that while IL-1 Trap by itself does not produce any Fc receptor mediated effects, IL-1Trap:IL-1Trap antibody complex interacts with a number of low affinities the Fcγ receptor. The binding of the IL-1Trap: IL-1 Trap antibody complex may lead to the clustering of *low affinity Fcγ* receptors on the surface of cells can lead to a wide range of events including activation of B cells, endocytosis of antibody/antigen complexes, phagocytosis of antibody-coated particles or cells, and antibody dependent cellular cytotoxicity. It is known that the functional effect of the binding of the Fc domain to the low affinity Fcγ receptor is unique for human and not be predicted from even the non human primate model of toxicity. And therefore, the effect of IL-1 Trap:IL-1 Trap antibody complex formation should be carefully monitored in the clinical settings. The immune complex binding to the *high affinity Fcγ* receptors would be anticipated to promote release of inflammation mediators, B cell activation, endocytosis, phagocytosis, and cytotoxic attack, while high affinity binding to the inhibitory Fcγ receptors would be expected to decrease cell based proinflammatory responses as well as B cell activation, endocytosis. Non human primates such as monkey are, however, a good model for high affinity FcγRIII binding; therefore nonspecific proinflammatory or immunosuppressive effect of the compound mediated through FcγRIII binding might be predicted in the toxicity studies.

In a stoichiometric assay, the applicant showed that the dimeric IL-1 Trap binds a single molecule of IL-1β with high affinity, which suggests that binding of a second molecule of IL-1 is sterically unfavorable. Because all IL-1R1 sites act independently and with equal affinity, there is a normal distribution of IL-1ra bound throughout all IL-1R1 sites in solution. Thus the nature of IL-1ra-IL-1Trap binding will depend on their molar ratio in a solution and therefore endogenous IL-1ra might block binding of IL-1β to IL-1 Trap at higher concentration.

The applicant developed an ELISA method to evaluate IL-1 Trap:IL-1β complex in human plasma. The data showed that IL-1Trap:IL-1β complex deposition increased with the duration of time. The immune complex deposition, in general, activates a proinflammatory reaction to initiate phagocytosis for the removal of the immune complex

from the tissue. Therefore, a proinflammatory condition is expected in a situation where immune complex deposition is evident such as in chronic exposure with the drug.

In short, the applicant provided an in depth evaluation of the binding and deposition of IL-1 Trap to its ligands and antibody forming complex. The result suggests that the cynomolgus monkey is an appropriate model for the evaluation of the toxicity of IL-1 Trap. However, some of the indirect toxicities which might result from the binding of the Fc domain to the low affinity Fcγ receptor interaction could not be assessed in any of the non human species. The pharmacology studies also showed increased immune complex deposition over time which might initiate a proinflammatory condition in the body. Also, the stoichiometric results showed due to the structure of the compound two IL-1ra molecules might bind to one molecule of IL-1 Trap at higher concentration while only one IL-1β could bind to one molecule of the IL-1 Trap at any one time. The binding of IL-1ra to IL-1Trap is concentration dependent.

B. Brief Overview of Nonclinical Findings and Clinical Implication:

Nonclinical studies include general toxicity studies in cynomolgus monkeys. The study compound, IL-1 Trap was administered to the monkeys either via subcutaneous or via intravenous route for the evaluation of the toxicity for the short term administration as well as chronic administration. The primates were dosed more frequently in the toxicity studies and the dosing regimens were in no way comparable to the clinical dosing regimen.

The toxicokinetic data shows that anti-product antibody formation occurred in monkey administered the human IL-1Trap and also in the mice administered the murine surrogate IL-1 Trap within 2 weeks of the IL-1 Trap administration. The serum circulating anti-product antibody production could be detected for a long period of time and even during recovery. The methods of the detection of biologic therapeutics are known to be hampered by the presence of the anti-product antibody and the immune complex in the same compartment. With the current product several immune complexes such as IL-1 Trap:IL-1α, IL-1Trap:IL-1β, 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 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 mono/polymorphonuclear cell tissue infiltration, arteritis, cellular degeneration, granuloma formation, and mineralization. The major target organ of toxicity was identified as injection site, 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-1β/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. 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 polymorphonuclear cell (eosinophil) infiltration. Both of these might result in multifocal lesion and tissue degeneration. Another one is increased infection associated with immunosuppression. Lastly, there was depletion of the thymus and changes in the ovary and testes which may be related to exaggerated pharmacology of the compound.

There were two unscheduled deaths in the pivotal 6-month subcutaneous toxicity study with the cynomolgus monkeys, dosed 3x/week; one male each from the mid and high dose died. The histological finding from the mid dose (40 mg/kg HED=12.9 mg/kg) animal that was sacrificed at Day 45, showed myocarditis associated with mononuclear cell infiltration; the high dose (60 mg/kg, HED=19.3 mg/kg) male died after dosing on Day 108, histopathology showed congestion in several organs including the kidney and lung. This animal 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, and this reviewer agrees with the sponsor's that this is the most likely explanation for these unscheduled deaths. 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 granuloma formation in the high dose male might be due to infection associated with immune suppression. In another chronic toxicity study, 6-month IV toxicity study, dosed 1x/week there was one unscheduled death of a high dose male was euthanized in extremis on Day 176 due to bacterial infection. This is likely due to 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 (15 mg/kg, HED=4.8 mg/kg) showed adverse events consisting of breathing discomfort at day 180, recovered by itself, showed signs of myocarditis, one female from 40 mg/kg (HED=12.9 mg/kg) showed clinical signs of hypersensitivity, histopathology in this animal was associated with increased foci at GI tract. Two other males at high dose showed clinical signs of discomfort, such as hunched posture around Day 40. Dosing was discontinued, the animals were sacrificed at the end of Week 26, and 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 coincide with high anti-product antibody titer 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 reactions, however, the possibility of the proinflammatory reaction due to increase immune complex deposition can not be eliminated.

The major adverse effects associated with the IL-1 Trap were clinical signs of lethargy, emesis, and cold to touch 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.

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 studies (6-week and 3-month subcutaneous administration of the compound). In the subchronic studies, the cell 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/polymorphnuclear 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 and the condition persisted during recovery. There was no NOAEL for injection site findings from any of the toxicity study reviewed under this application. The finding although not related to the IL-1 Trap concentration by itself but appear to be treatment related. The infiltration of mono- and polymorphonuclear cell might suggest proinflammatory condition. IL-1β is a known chemo attractant for these types of cells. The immune complex IL-1 Trap:IL-1β or the captured IL-1β aggregates in the injection sites might be associated with the local immune reaction in the injection site. It might be predicted that in clinical condition with the increase in duration complication associated with inflammation of small blood vessels might appear.

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. 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.

The treatment related histological changes in the <u>kidney</u> 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 of 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 polymorphnuclear cell infiltration. There was, however, an increase in the mono/polymorphnuclear 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. This suggests that the pathological changes observed in the kidney in the arteriole might be predictive of what might be found in human if similar immune complex is generated in human due to chronic exposure.

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 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 non human primates are known to have latent infections 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 was also noted at a higher incidence in the test article treated animal. Pulmonary fibrosis is a common sequel of chronic lower respiratory tract inflammation. The increased

fibrosis noted in the lungs of the treatment group might have resulted from the increase in the immune complex deposition in the lung.

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 organisms. 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 unbridle.

Clinical Implication of the General Toxicity Findings

The proposed human clinical dose is 160 mg/week. In the pivotal clinical trial, in CAPS patient for 24 weeks with this dose, mean steady state trough levels of total IL-1 Trap ranged from 20-27 μg/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 μg/mL and IL-1 Trap antibody concentration ranged from 18-235 RFU (rheumatoid factor unit). 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 (rheumatoid factor unit). The histopathological findings in the low dose were injection site reactions. No NOAEL could be determined because of this finding.

The other toxicity findings such as myocardial degeneration, congestion in kidney, and granuloma formation in the lungs which was found in dosages 40 and 60 mg/kg are approximately 5x and 7x higher than the proposed clinical dosing. The toxicity findings in the kidney and heart are believed to be due to the hypersensitivity reaction due to antibody formation and might not be relevant in the clinical situation, however, one can not out rule the possibility of proinflammatory reaction mediated mono/polymorphonuclear cell infiltration in tissue causing congestion or tissue degeneration.

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, II, and III studies are as follows:

- Decrease in fertility index in male and female @ 20 mg/ kg (HED= 6.6mg/kg, proposed human dosing is 2.6 mg/kg; 2.5 fold safety margin) 14-16%. ↓ lower than control
- Incidence in females with evidence of mating but not gravid @ low dose; 7- fold ↑ higher compare to control.
- Early resorptions: 47-50% increase at all dosages tested (20, 100, and 200 mg/kg), safety margin with the proposed human dose compare to low dose is 2.5 –fold.
- Post Implantation loss: 40=45% at all dosages tested (20, 100, and 200 mg/kg), safety margin with the proposed human dose compare to low dose is 2.5 –fold.
- IL-1 Trap concentration in male @ 20, 100, and 200 mg/kg ranged between 1-78, 5-432, and 29-748 μg/mL in Segment I study
- IL-1 Trap antibody concentration in male @ 20, 100, and 200 mg/kg ranged between μg/mL 2-13332, 1-26, and 1-1.5 μg/mL in Segment I study
- IL-1 Trap antibody concentration in male @ 20, 100, and 200 mg/kg ranged between μg/mL 1-6000, 2-328, and 1-129 μg/mL in Segment I study
- 2- 4 fold increase in the abortion in the F_0 dams @ all dosages in Segment III study
- 1.5 and 5.5 –fold increase in still borne pup @ low and high dose in Segment III study
- 1.5, 2, 2-fold increase in the number of pups with empty stomach at low, mid and high dose in Segment III study
- Number of F_0 dams with pseudo pregnancy 0, 6, 3, and 1 in control, low, mid, and high dose in the Segment III study.
- 8-fold increase in the death of the F1 pups between 5-21 days of lactation at low and mid dose
- 2-fold increase in the F_2 litter death at all dosages in the Segment III study
- IL-1 Trap concentration in dams @ all dosages ranged between 7-26 μg/mL IL-1 Trap antibody concentration in dams @ all dosages ranged between 35-12 μg/mL in Segment III study I during parturition.
- Changes in estrus cycle (prolonged) were noted in F_1 females.
- In segment II study in monkey the spontaneous abortions number of spontaneous abortion were 2, 3, 2, 2 in control, low, mid, and high dose, in low and mid dose fetal death was observed at day 38, 51 when the IL-1 Trap and its antibody was at very high concentration. Mean estrogen level in the animal group was lowest during the time when late abortions were noted in the treated animals.

- In segment II study in monkey There was an increase in the percent of skeletal variation of lumber vertebra 12, 22, 18, and 20 in 0, 5, 15, and 30 mg/kg

Following conclusions can be drawn from the reproductive toxicity data

a) No dose response in the findings for the early and post implantation loss, therefore no NOAEL could be determined in any of the reproductive studies.
b) The findings could not be related to the exposure of IL-1 Trap or its antibody by itself.
c) Literature data showed that miscarriage is common in the woman with malfunctioning Th1 cells.
d) Proinflammatory cytokines such a sIL-1, IL-6 gene expression increase at pre and post implantation time when estrogen and progesterone level are high decrease in IL-1 level decrease estrogen & progesterone levels suggesting lowering the incidence of successful pregnancy.
e) Increase in abortion in Fo dams, still borne pups, later day deaths of the F1 pups and F2 pups compare to control. There was no dose response; therefore no NOAEL could be determined in Segment III study. The exposure of IL-1 Trap and its antibody was not was no directly related to these finding.
f) There was a dose related finding in the F1 pups with empty stomach. The sponsor did not evaluate the cause for this finding. Therefore, it is not know whether the compound is inhibition lactation or the empty stomach was caused by the behavioral changes in the nesting behavior in females.
g) Due to spontaneous abortions and skeletal variation in the primates no NOAEL could be determined.
h) The findings might be related to the IL-1 mediated estrogen effects caused by the inhibition of IL-1 by IL-1 Trap. However, the sponsor did not explore the findings to provide an appropriate explanation to why these findings are not of any concerns in human.
i) The antibody was detected in the fetus in the primates indicating that it can cross the placental barrier, it is not known, however, whether , IL-1 Trap or its antibody could be secreted in milk.

The definitive **clinical implications** of the reproductive toxicity findings are not yet known. However, the late pregnancy spontaneous abortion in the *primates* were noted in an IL-1Trap 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 20-27μg/mL. The skeletal variation observed might be related to the estrogen depletion and its effect in bone formation. Literature data shows IL-1 and estrogen are intimately related to the bone formation. The applicant did not analyze the immune cell development and immune system development in the F1 and F2 generation although interleukin 1 is a pivotal candidate responsible for innate immunity and suppression of it might induce developmental immunotoxicity.

Currently, the labeling from the applicant indicated Pregnancy Category – for the IL-1 Trap, however, due to the presence of reproductive findings in the primates with unexplained reason, the reviewer believes that the compound should be labeled as Pregnancy Category C.

There were no carcinogenicity studies submitted with this application. IL-1Trap is synthesized to inhibit IL-1β a vital component of the innate immune system of the body and is predicted to have immunosuppressive property. Immunosuppressant agents including small molecules such as methotrexates, cyclophosamide as well as monoclonal antibodies and Fc fusion proteins such as enbrel, humira, infliximab, orencia, 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-1β to inhibit the inflammation associated with the IL-1β 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. It is recommended that the <u>feasibility</u> of the carcinogenicity assessment in animal models be evaluated. Because of the FDA's recommendation in prior meetings that a carcinogenicity waiver for rilonacept is appropriate, it is reviewer recommendation that a carcinogenicity assessment be conducted   _____

_____   . for this product due to the new findings with the immunosuppressive agents. There are different methods to evaluate the potential of IL-1 Trap in the tumor formation such as long term toxicity studies , tumor promotion assays etc.

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.

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

BLA number: 125249
Review number: 1
Sequence number/date/type of submission: 000/original BLA
Information to sponsor: No (x)
Sponsor and/or agent: Regeneron Pharmaceuticals, Inc.
Manufacturer for drug substance: Regeneron Pharmaceuticals, Inc.
Reviewer name: Mamata De, Ph. D.
Division name: Division of Anesthesia, Analgesia, and Rheumatology Drug Products
HFD #: 170
Review completion date: September 28, 2007

Drug:

    Trade name: ARCALYST (proposed)
    Generic name: Rilonacept
    Code name: IL-1 Trap
    CAS registry number: 501081-76-1

Relevant BB-INDs: — 11781,

IND	Indication	Division	Status	Status Date
11781	Autoimmune Inflammatory Disease (CAPS)	DAARP	Active	10-6-2004

Drug class: Anti-inflammatory; IL-1Trap is a fusion protein, selectively inhibits IL-1.

Intended clinical population: Treatment of Cryopyrin-associated periodic syndromes (CAPS), such as Familial Cold Autoinflammatory Syndrome and Muckle-Wells Syndrome.

Clinical formulation: Rilonacept is provided as a lyophilized drug product for reconstitution with sterile water for injection. The product formulation contains rilonacept (80 mg/mL), histidine ———— , polyethylene glycol 3350, ——— —, glycine, arginine, and sucrose at pH 6.5.

Route of administration: Subcutaneous Injection

The proposed dosage and administration is as follows:

- **Adult patients 18 yrs and older:** Treatment should be initiated with a loading dose of 320 mg delivered as two, 2-mL, subcutaneous injections of 160 mg each. Dosing should be continued with a once-weekly injection of 160 mg administered as a single, 2-mL, subcutaneous injection. ARCALYST should not be given more often than once weekly.

- **Pediatric patients ages — to 17:** Treatment should be initiated with a loading dose of 4.4 mg/kg, up to a maximum of 320 mg, delivered as one or two subcutaneous injections with a maximum single-injection volume of 2 mL. Dosing should be continued with a once-weekly injection of 2.2 mg/kg, up to a maximum of 160 mg, administered as a single subcutaneous injection, up to 2 mL. ARCALYST should not be given more often than once weekly.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

Study Number: IL1T-MX-6018

Pharmacology	
Study Number	**Study Title**
Study Number: IL1T-MX-6018	Study Title: Determination of Equilibrium Binding Constants for the Interaction of Ligands with IL-1 Trap Protein
IL1T-MX-06019	Determination of Equilibrium Binding Constants for the Interaction of Mouse and Monkey IL-1 Receptor Binding Ligands with IL-1 Trap Protein
IL1T-MX-06020	Determination of Equilibrium Binding Constants for the Interaction of Ligands with Murine IL-1 Trap
IL1T-MX-06021	Determination of IL-1 Trap Binding Stoichiometry to both IL-1 Beta and IL-1ra Using a Functional IL-1 Trap ELISA

IL1T-MX-06022	Determination of IL-1 Beta:IL-1 Trap Complex Levels in Samples from Monkey Toxicology Study IL1T-TX-03021
IL1T-MX-06023	Evaluation of Fc-Effector Function for IL-1Trap
IL1T-MX-06026	Description of Cell Line and Process for Production of Murine IL-1 Trap for Toxicology Studies
IL1T-MX-06027	Evaluation of Murine IL-1 Trap to Suppress Neointimal Hyperplasia in an Injury-Induced Murine Animal Model
IL-1T-MX-1T#41	Immunogenicity of Human IL1-Trap in Rodents
ADME	
IL-1T-PK-00013.0	A Pharmacokinetic study of IL-1 (Interleukin 1) Trap as a Single Subcutaneous Injection and Bioavailability of IL-1 Trap after Subcutaneous and Bolus Intravenous Injection to Cynomolgus Monkeys.
IL-1T-PK-00010	Analysis of Serum Samples from —— ⅃ Study N0 0948-121, A Pharmacokinetic Study of IL-1 (Interleukin 1) Trap Administered as a Single Subcutaneous Injection and Bioavailability of IL-1 Trap after Subcutaneous and Bolus Intravenous Injection to Cynomolgus Monkeys
IL-1T-PK-00011	Pharmacokinetic and Bioavailability of I Toxicology Lot (Lot # 00408) ISC823 Following Intravenous and Subcutaneous Administration to Sprague Dawley Rat
IL-1T-PK-00009.0	Bio distribution of ISC823 in Normal Sprague Dawley Female Rats
IL-1T-PK-0006	Pharmacokinetic and Bioavailability of ISC823 Following Administered as a Single Subcutaneous injection to Sprague Dawley Rat
IL-1T-PK-0005.1	Pharmacokinetic and Bioavailability of ISC823 Following Intravenous and Subcutaneous administration to Sprague Dawley Rat
IL-1T-MX-0628	Pharmacokinetics of Il-1Trap following Subcutaneous Administration to Sprague Dawley Rat: Correlation Between Pharmacokinetic Parameters and ———
IL-1T-PK-0007.0	Pharmacokinetic and Bioavailability of ISC823 Following Intravenous and Subcutaneous Administration to C57BL/6 Mice
Toxicology	
	A Two-Month Subcutaneous Exploratory Study of Murine IL-1 Trap in CD-1 Mice
—— 223.13	A Six-Week Subcutaneous Exploratory Toxicity Study of IL-1 Trap in Cynomolgus Monkeys
—— ‚223.12	A 3-Week IV Toxicity Study of IL-1 Trap with a 6-Week Recovery Period in Cynomolgus Monkeys
—— 0949-121	A 6-Week Toxicity Study of IL-1 (Interleukin-1) Trap Administered by Subcutaneous Injection to Cynomolgus Monkeys, with a 4-Week Recovery Period
—— 223.5	A 3-Month Subcutaneous Toxicity Study of IL-1 Trap in

	Cynomolgus Monkeys
⎯ 223.17	A 26-Week Subcutaneous Toxicity Study of IL-1 Trap in Cynomolgus Monkeys Followed by an 8-Week Recovery Period
⎯ 223.19	Six-Month Intravenous Toxicity Study of IL-1 Trap in Cynomolgus Monkeys
⎯ ł60002	Subcutaneous Fertility and Early Embryonic Development to Implantation Study of Murine IL-1 Trap in Mice
⎯ ¿23.11	A Study for the Effect of IL-1 Trap on Embryo-Fetal Development in Cynomolgus Monkeys by Subcutaneous Administration
⎯ /369-111	Subcutaneous Fertility and Early Embryonic Development to Implantation Study of Murine IL-1 Trap in Mice
IL1T-TX-05004	Cross-Reactivity of Biotinylated IL-1 Trap with Human and Cynomolgus Monkey Tissues Ex Vivo

Studies not reviewed within this submission: All submitted studies were reviewed.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

The IL-1 Trap is a recombinant fusion protein indicated for the ⎯   treatment of a rare inflammatory disorder known as Cryopyrin Associated Periodic Syndrome (CAPS). CAPS is an autoinflammatory syndrome characterized commonly by rash, arthralgia, and fever and is associated with three distinct clinical conditions namely familial cold autoinflammatory syndrome (FCAS), Muckle-Well syndrome (MWS), and neonatal onset of multiinflammatory syndrome (NOMID). The clinical signs observed are inherited. FCAS-MWS-NOMID conditions are rare (only 200-300 patients) in the United States. Currently there is no approved therapy for CAPS. At present the symptoms are only partially treated with IL-1 receptor antagonist. Therefore, IL-1Trap has received orphan drug status.

The severe inflammatory conditions in CAPS are related to over production of the proinflammatory cytokines from the Interleukin-1 (IL-1) family such as IL-1β and IL-18. The genetic disorder is known to be due to mutations in the CIAS1 gene in located in 1q44 region of the human chromosome. The CIAS1 gene is expressed in the peripheral leukocytes, the mutation in this gene results in the formation of a dysfunctional cryopyrin protein. Cryopyrin resides in the cytosolic protein complex, it forms inflammasome that regulates the synthesis of pro inflammatory cytokine IL-1β from its precursor protein pro IL-1β by activating enzymes such as caspase 1 and adaptor proteins. Mutated cryopyrin increases the production of IL-1β, and thus induces chronic inflammatory condition and autoimmunity which is revealed in the CAPS patients.

IL-1 Trap is designed to capture IL-1β to moderate the underlying common pathophysiological mechanism associated with inflammation in the patients with CAPS. IL-1 Trap is created by fusing the sequences encoding the extracellular domains of the

two receptors of the IL-1 namely IL-1AcP (interleukin 1 accessory protein) and IL-1RI
(IL-1 receptor type I). IL-1 Trap is a dimeric glycoprotein with a total molecular weight
of ~251 kDa, ~ ————————————————————————————— ———— The
dimer is ——— linked ———— ~s in the Fc region. It is hypothesized that IL-1
is trapped between the two chains of the IL-1AcP receptor in this genetically engineered
fusion protein. A schematic diagram of IL-1 Trap is provided below.

IL-1 Receptor Complex   **IL-1 Trap**

IL-1R Type 1   IL-1R-AcP

                 Extracellular
                 Domains

                 Cell Membrane

                 Cytoplasmic
                 Domains
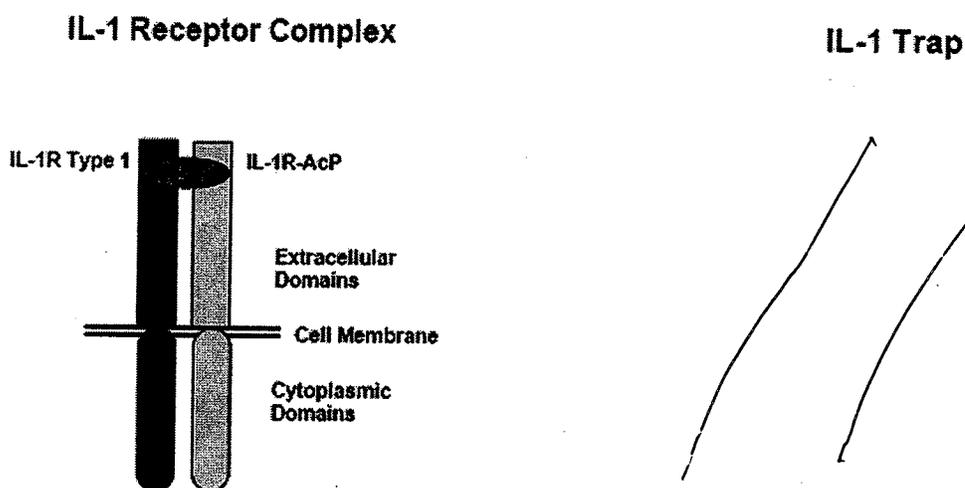
The sponsor provided pharmacology data to assess the specific and nonspecific binding
of IL-1 Trap to evaluate the primary and secondary pharmacological effects and to
rationalize the choice of species for the toxicity studies. The sponsor also provided data
for the assessment of the IL-Trap, ligand, and the antibody complex formation on the
immunogenicity.

The product, human IL-1 Trap binds with its ligands IL-1β, IL-1α, and IL-1ra with
picomolar concentration in the in vitro binding assays. This suggests that it should
effectively bind with the ligands in vivo. The binding affinity of the IL-1 Trap for the
human IL-1β was approximately 4.6×10^{-13} M which is 2.9-fold greater than the affinity
for human IL-1α and 13-fold greater than the affinity for human IL-1ra. The binding
affinity of IL-1 Trap for the IL-1β ligand from the cynomolgus monkey was
approximately 4.9×10^{-13} M; 8.8- and 17-fold higher than that of IL-1α and IL-1ra
respectively. The binding affinity of the IL-1 Trap is thus considered more or less similar
in the human and the monkey. Therefore, cynomolgus monkey is appeared to be an
appropriate animal model for the toxicity testing in the IL-1 Trap.

The sponsor also developed a surrogate IL-1 Trap fusion protein for mice. The binding
affinity of the murine IL-1 Trap to murine IL-1α is 9.6×10^{-12}; 3- and 8-fold higher than

that of murine IL-1β and IL-1ra respectively. IL-1α is known as the major cytokine from the IL-1 family in mice. Also, sponsor evaluated the efficacy of murine IL-1 Trap in an animal model of surgery induced vascular injury in which the murine IL-1 Trap was found to prevent the development of neointimal hyperplasia in a dose dependent manner indicating a functional effect of the compound in vivo. All these suggest that the surrogate molecule, murine IL-1 Trap could be effectively used in the mice to evaluate toxicity.

The sponsor evaluated the indirect/nonspecific activity of IL-1 Trap mediated by Fc receptor binding activities which include ADCC, CDC, and Fcγ receptor interactions. The results from these studies showed that while IL-1 Trap by itself does not produce any Fc receptor mediated effect, IL-1Trap:IL-1Trap antibody complex interacts with a number of low affinity the Fcγ receptor. There are four different types of Fcγ receptors. *In vivo* these receptors display varying functions, with the FcγRIIa and FcγRIIIa receptors playing an activating role in cell mediated proinflammatory processes and the FcγRIIb and FcγRIIIb receptors being inhibitory. The binding of the IL-1Trap: antibody complex may lead to the clustering of low affinity Fcγ receptors on the surface of cells can lead to a wide range of events including activation of B cells, endocytosis of antibody/antigen complexes, phagocytosis of antibody-coated particles or cells, and antibody dependent cellular cytotoxicity (Fridman et al., 1992). As a result of the high affinity binding of an Fc domain to the activation receptors would be anticipated to promote release of inflammation mediators, B cell activation, endocytosis, phagocytosis, and cytotoxic attack, while high affinity binding to the inhibitory Fcγ receptors would be expected to decrease cell based proinflammatory responses as well as B cell activation, endocytosis, and phagocytosis (Nimmerjahn and Ravetch 2006). A table showing characteristics of Fcγ receptors is reproduced below. All these information suggests that IL-1 Trap: antibody complex formation might increase immunogenicity. It is known that the functional effect of the binding of the Fc domain to the low affinity Fcγ receptor is unique for human and not be predicted from even the non human primate model of toxicity. And therefore, the effect of IL-1 Trap:IL-1 Trap antibody complex formation should be carefully monitored in the clinical settings. Non human primates such as monkey are, however, a good model for high affinity FcγRIII binding, therefore nonspecific proinflammatory or immunosuppressive effect of the compound mediated through FcγRIII binding might be predicted in the toxicity studies. A summary of the probable effect of the Fc domain-Fcγ receptor interaction is provided below.

Table 1 Summary of Fcγ Receptor Characteristics

Receptor	FcγRI (CD16)	FcγRIIa (CD32a)	FcγRIIb (CD32b)	FcγRIII (CD64)
Binding Order of affinity	IgG1 $10^{-9}M$ - $10^{-8}M$ 1) IgG1=IgG3 2) IgG4 3) IgG2	IgG1 ~2 x $10^{-6}M$* 1) IgG1 2) IgG3=IgG2 3) IgG4	IgG1 ~2 x $10^{-6}M$ 1) IgG1=IgG3 2) IgG4 3) IgG2	IgG1 ~2 x $10^{-6}M$* IgG1=IgG3
Cell type	Macrophages Neutrophils Eosinophils Dendritic cells	Macrophages Neutrophils Eosinophils Platlets Langerhans cells	Macrophages Neutrophils Eosinophils B cells Mast cells	NK cells Eosinophils Macrophages Neutrophils Mast cells
Effect of ligation	Stimulation	Stimulation	Inhibition	Stimulation
Number of receptors		FcγRIIa, FcγRIIb, FcγRIIc		FcγRIIIa, FcγRIIIb

Table modified from *Immunobiology* Charles A. Janeway ed. 2001(Gessner et al., 1998; Maenaka et al., 2001.

* Receptor polymorphisms can effect binding affinity.

In a stoichiometric assay, the sponsor showed that the dimeric IL-1 Trap binds a single molecule of IL-1β with high affinity, which suggests that binding of a second molecule of IL-1 is sterically unfavorable. Because all IL-1R1 sites act independently and with equal affinity, there is a normal distribution of IL-1ra bound throughout all IL-1R1 sites in solution. Thus the nature of IL-1ra-IL-1Trap binding will depend on their molar ratio in a solution and therefore endogenous IL-1ra might block binding of IL-1β to IL-1 Trap at higher concentration.

The sponsor developed a validated ELISA method to evaluate IL-1 Trap - IL-1β complex in human plasma. A similar method, although not validated, was used to estimate immune complex formation in the 6-month SC monkey study (Study # IL_1T-TX 03021). The result showed that both IL-1 Trap and IL-1β:IL-1 Trap complex was detected in Day 5 samples and the levels of the complex increased with increasing dose. Mean IL-1 Trap levels were generally highest on Day 5 because the emergence of anti-IL-1 Trap antibodies had a major impact on IL-1 Trap plasma levels in these animals. By Day 12, anti-IL-1 Trap antibodies started to appear in most of the animals, reducing plasma levels of IL-1 Trap in these animals. By Day 19, anti-IL-1 Trap antibody levels were high enough to drastically reduce IL-1 Trap levels in the animals. Mean IL-1β complex levels increased with dose towards saturation at early time points but then continued to increase further in the Day 80 samples. The data showed that IL-1Trap:IL-1β complex deposition increased with the duration of time may be due to the increase in the IL-1 Trap: Anti-IL-1 Trap antibody formation resulting in deposition of this immune complex. This might generate a proinflammatory reaction.

The sponsor provided an in depth evaluation of the consequences of the binding and deposition of the IL-1 Trap to its ligands and antibody forming complex. The result suggests that cynomolgus monkey is an appropriate model for the evaluation of the toxicity of IL-1Trap. However, some of the indirect toxicities which might result from

the binding of the Fc domain to the low affinity Fcγ receptor interaction could not be assessed in any of the non human species. The pharmacology studies also showed increased immune complex deposition over time which might initiate proinflammatory condition in the body. Also, the stoichiometric results showed due to the structure of the compound two IL-1ra molecules might bind to one molecule of IL-1 Trap at higher concentration while only one IL-1β could bind to one molecule of the IL-1 Trap at any one time. The binding of IL-1ra to IL-1 Trap is concentration dependent.

2.6.2.2 Primary pharmacodynamics

Following pharmacodynamics studies were performed to determine the specificity of the compound and to show consistency of the drug substance from the different manufacturing lots.

Study Number: IL1T-MX-6018
Study Title: Determination of Equilibrium Binding Constants for the Interaction of Ligands with IL-1 Trap Protein

Objective of the study: To show consistency between drug substance lots and overall high affinity interaction for each of the ligands.

Results:

The binding of IL-1 Trap to human IL-1β, IL-1α, and IL-1ra was examined for four drug substance lots namely B 5003, B 5009, B 5008, B 50010 and the following results were noted.
- IL-1 Trap association constant (ka) for IL-1β, IL-1α, and IL-1ra ranged from 1.89×10^7 to 2.33×10^7, 6.43×10^7 to 7.78×10^7, and 2.56×10^7 to 3.65×10^7 $M^{-1}s^{-1}$ respectively.
- IL-1 Trap t½ data for IL-1β, IL-1α, and IL-1ra ranged from 18.39 - 21.16, 19.8 - 21.4, and 8.5 - 12.7 hrs respectively.

Table 1 – Binding Parameters for the Interaction of IL-1 Trap and IL-1β

IL-1 Trap-823(lot#)	Ligand	$k_a (M^{-1}s^{-1})$	$k_d (s^{-1})$	$K_D (M)$	Rmax (RU)	$t_{1/2}$
B05003M410	hIL-1beta	2.26E+07	1.05E-05	4.64E-13	15.3	18.39h
B05008M410	hIL-1beta	2.11E+07	1.01E-05	4.79E-13	13	19.01h
B05009M410	hIL-1beta	1.92E+07	9.10E-06	4.73E-13	14.6	21.16h
B05010M410	hIL-1beta	1.89E+07	9.20E-06	4.88E-13	14.2	20.90h
B05010M410	hIL-1beta	2.33E+07	9.77E-06	4.19E-13	13.7	19.70h

Table 2 – Summary of Binding Parameters for the Interaction of IL-1 Trap and hIL-1α

IL-1 Trap-823(lot#)	Ligand	k_a (M^{-1}s^{-1})	k_d (s^{-1})	K_D (M)	Rmax (RU)	$t_{1/2}$
B05003M410	hIL-1alpha	6.68E+06	9.96E-06	1.49E-12	14.4	19.8 hr
B05008M410	hIL-1alpha	6.43E+06	9.09E-06	1.41E-12	13.7	21.2 hr
B05009M410	hIL-1alpha	7.78E+06	9.20E-06	1.18E-12	14.5	20.9 hr
B05010M410	hIL-1alpha	6.67E+06	9.00E-06	1.35E-12	14.4	21.4 hr

Table 3 – Summary of Binding Parameters for the Interaction of IL-1 Trap and hIL-1ra

IL-1 Trap-823(lot#)	Ligand	k_a (M^{-1}s^{-1})	k_d (s^{-1})	K_D (M)	Rmax (RU)	$t_{1/2}$
B05003M410	hIL-1ra	2.56E+06	1.51E-05	5.88E-12	21.4	12.7 hr
B05008M410	hIL-1ra	3.05E+06	1.94E-05	6.36E-12	20.2	9.92 hr
B05009M410	hIL-1ra	3.65E+06	2.26E-05	6.21E-12	20.9	8.52 hr
B05010M410	hIL-1ra	3.42E+06	2.00E-05	5.85E-12	20	9.63 hr

Reviewer's Comment: The similarity in the IL-1 Trap association constant and t½ for IL-1 β, IL-1α, and IL-1ra indicates that all of the drug substance lots possess equivalent ka and kd. The equilibrium dissociation constants (K_D) calculated from the ratio of dissociation rate constant to the association rate constant (kd/ka) indicates that all drug substance lots tested are equivalent in their ability to bind to human IL-1β. The calculated affinity of the IL-1 Trap for hIL-1β was approximately 4.6 x 10^{-13} M; 2.9-fold tighter than for hIL-1α and 13-fold tighter than for hIL-1ra.

Study Number: IL1T-MX-06019
Study Title: Determination of Equilibrium Binding Constants for the Interaction of Mouse and Monkey IL-1 Receptor Binding Ligands with IL-1 Trap Protein

Objective of the study: To determine high affinity interaction for each of the murine and monkey ligands for binding to IL-1 Trap to show consistency between drug substance lots.
Results:

The binding of IL-1 Trap to monkey IL-1β, IL-1α, and IL-1ra was examined for four drug substance lots namely B 5003, B 5009, B 5008, B 50010 and the results are noted below.

- IL-1 Trap association constant (ka) for IL-1β, IL-1α, and IL-1ra ranged from 2.4 x 10^7 to 4.2 x 10^7, 3.4 x 10^7 to 3.9 x10^7, and 2.1 x 10^7 to 2.7 x 10^7 M^{-1}s^{-1} respectively.
- IL-1 Trap t½ data for IL-1β, IL-1α, and IL-1ra ranged from 14.9 - 17.1, 15.4 to 16.8, and 11.4 to 12.7 respectively.

Monkey Samples:

Table 4 – Summary of Binding Parameters for the Interaction of IL-1 Trap and monkey IL-1β

IL-1 Trap-823(lot#)	Ligand	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	Rmax (RU)	$t_{1/2}$
B05003M410	mk IL-1beta (REGN)	2.62E+07	1.29E-05	4.93E-13	13.3	14.9 hr
B05008M410	mk IL-1beta (REGN)	4.09E+07	1.17E-05	2.86E-13	14.1	16.4 hr
B05009M410	mk IL-1beta (REGN)	4.17E+07	1.24E-05	2.97E-13	15.1	15.5 hr
B05009M410	mk IL-1beta (REGN)	2.37E+07	1.20E-05	5.06E-13	13.6	16.1 hr
B05010M410	mk IL-1beta (REGN)	4.20E+07	1.31E-05	3.12E-13	14.5	14.7 hr
B05010M410	mk IL-1beta (REGN)	2.40E+07	1.13E-05	4.71E-13	14	17.1 hr
	Average	3.31E+07	1.22E-05	3.94E-13		

Table 5 – Summary of Binding Parameters for the Interaction of IL-1 Trap and monkey IL-1α

IL-1 Trap-823(lot#)	Ligand	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	Rmax (RU)	$t_{1/2}$
B05003M410	mk IL-1alpha (REGN)	3.36E+06	1.22E-05	3.64E-12	14	15.8 hr
B05008M410	mk IL-1alpha (REGN)	3.62E+06	1.26E-05	3.48E-12	13	15.3 hr
B05009M410	mk IL-1alpha (REGN)	3.35E+06	1.16E-05	3.47E-12	15	16.6 hr
B05010M410	mk IL-1alpha (REGN)	3.91E+06	1.25E-05	3.20E-12	15	15.4 hr
B05010M410	mk IL-1alpha (REGN)	3.54E+06	1.22E-05	3.45E-12	15	15.8 hr
	Average	3.56E+06	1.22E-05	3.45E-12		

Table 6 – Summary of Binding Parameters for the Interaction of IL-1 Trap and monkey IL-1ra

IL-1 Trap-823(lot#)	Ligand	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	Rmax (RU)	$t_{1/2}$
B05003M410	mk IL-1ra (REGN)	2.53E+06	1.69E-05	6.70E-12	19	11.4 hr
B05008M410	mk IL-1ra (REGN)	2.28E+06	1.81E-05	7.96E-12	22	10.6 hr
B05009M410	mk IL-1ra (REGN)	2.76E+06	1.54E-05	5.58E-12	19	12.5 hr
B05009M410	mk IL-1ra (REGN)	2.42E+06	1.55E-05	6.41E-12	20	12.4 hr
B05010M410	mk IL-1ra (REGN)	2.31E+06	1.67E-05	7.23E-12	20	11.5 hr
B05010M410	mk IL-1ra (REGN)	2.12E+06	1.51E-05	7.10E-12	19	12.7 hr
	Average	2.40E+06	1.63E-05	6.83E-12		

Murine Sample:

The binding of IL-1 Trap to murine IL-1β, IL-1α, and IL-1ra was examined for four drug substance lots namely B 5003, B 5009, B 5008, B 50010. The following are the results:

- IL-1 Trap association constant (ka) for IL-1β, IL-1α, and IL-1ra ranged from 1.32×10^7 to 1.36×10^7, 4.1×10^7 to 5.4×10^7, and 7.9×10^7 to 9.2×10^7 $M^{-1}s^{-1}$ respectively.
- IL-1 Trap t½ data for IL-1β, IL-1α, and IL-1ra ranged from 4.0 to 4.5, 3.6 to 3.8, and 2.3 to 3.0 respectively.

Table 1 – Summary of Binding Parameters for the Interaction of IL-1 Trap and murine IL-1β

IL-1 Trap-823(lot#)	Ligand	k_a (M^{-1}s^{-1})	k_d (s^{-1})	K_D (M)	Rmax (RU)	$t_{1/2}$
B05003M410	mIL-1beta (R&D)	1.36E+06	4.75E-05	3.49E-11	14.2	4.05 hr
B05008M410	mIL-1beta (R&D)	1.32E+06	4.43E-05	3.35E-11	20	4.34 hr
B05009M410	mIL-1beta (R&D)	1.34E+06	4.45E-05	3.31E-11	20	4.32 hr
B05010M410	mIL-1beta (R&D)	1.35E+06	4.25E-05	3.16E-11	18.8	4.53 hr
	Average	1.34E+06	4.47E-05	3.33E-11		

Table 2 – Summary of Binding Parameters for the Interaction of IL-1 Trap and murine IL-1α

IL-1 Trap-823(lot#)	Ligand	k_a (M^{-1}s^{-1})	k_d (s^{-1})	K_D (M)	Rmax (RU)	$t_{1/2}$
B05003M410	mIL-1 alpha (R&D)	5.46E+06	5.26E-05	9.63E-12	17.3	3.66 hr
B05008M410	mIL-1 alpha (R&D)	4.78E+06	4.98E-05	1.04E-11	20.1	3.87 hr
B05009M410	mIL-1 alpha (R&D)	4.18E+06	4.97E-05	1.19E-11	20.5	3.87 hr
B05010M410	mIL-1 alpha (R&D)	4.65E+06	5.05E-05	1.08E-11	19.5	3.81 hr
	Average	4.77E+06	5.07E-05	1.07E-11		

Table 3 – Summary of Binding Parameters for the Interaction of IL-1 Trap and murine IL-1ra

IL-1 Trap-823(lot#)	Ligand	k_a (M^{-1}s^{-1})	k_d (s^{-1})	K_D (M)	Rmax (RU)	$t_{1/2}$
B05003M410	mIL-1ra(R&D)	7.98E+05	7.52E-05	9.42E-11	26.3	2.56 hr
B05008M410	mIL-1ra(R&D)	8.29E+05	8.35E-05	1.01E-10	23.3	2.31 hr
B05009M410	mIL-1ra(R&D)	8.56E+05	7.21E-05	8.42E-11	23.9	2.67 hr
B05010M410	mIL-1ra(R&D)	9.23E+05	6.40E-05	6.93E-11	21.9	3.01 hr
	Average	8.52E+05	7.37E-05	8.72E-11		

Reviewer's Comment: The IL-1 Trap ka and kd showed minimal variation between the batches for IL-1β, IL-1α, and IL-1ra binding in the monkey. Similar results were observed in mice. The result indicates consistency between the lots for the affinity biding of IL-1 Trap to the different ligands. The K_D of the IL-1 Trap, in monkey, for IL-1β was approximately 8.8- and 17-fold higher than IL-1 α and IL-1ra respectively. Similarly, the K_D for IL-1α was 2-fold higher that IL-1ra in monkey. Interestingly, in mice, the K_D of the IL-1 Trap for IL-1α was approximately 3- and 8-fold higher than IL-1β and IL-1ra respectively.

Study Number: IL1T-MX-06020
Study Title: Determination of Equilibrium Binding Constants for the Interaction of Ligands with Murine IL-1 Trap

Objective of the study: To determine the kinetics and the equilibrium binding parameters of the murine IL-1 Trap with murine IL-1β, murine IL-1α, and murine IL1ra.

Results: The interaction between murine IL-1 Trap and murine IL-1β, mIL-1α, and mIL-1ra was monitored by surface plasmon resonance technologies using Biacore instrument. The results showed that the ka for murine IL-1 Trap for the mIL-1α, mIL-1β and mIL-1ra was 6.58×10^{-6}, 1.37×10^{-7}, 1.08×10^{-6} respectively. The t½ for murine IL-1 Trap for the mIL-1β, IL-1α, and mIL-1ra 28, 10, and 37.2 hrs respectively.
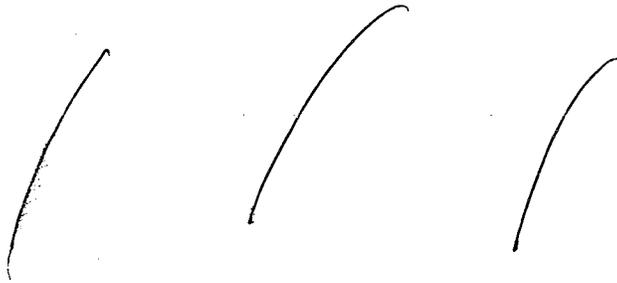
Figure 1. BiaCore sensorgrams measuring the association of varying concentrations of murine IL-1β to murine IL-1 Trap Lot# 030425 and subsequent dissociation reactions. The concentrations of murine IL-1β tested were ⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯

Objective of the study: To determine the stoichiometry of IL-1β and IL-1ra binding to IL-1 Trap.

Results:

The concentration of functional IL-1 Trap is determined by the level of IL-1β inhibition observed in an ELISA. Initial experiments suggested that the assay could detect approximately ⎯ ng/mL of free IL-1 Trap in the sample.

The results from 5 sets of experiments examining the effect of complexes with 0:1, 0.5:1, 1:1, 1.5:1, 2:1, 3:1 and 4:1 ratios of IL-1ra to IL-1 Trap consistently suggested that at least two moles of IL-1ra are required to block most of the IL-1 Trap binding to IL-1β (Figure 2).

Complexes of 1:1 IL-1ra and IL-1 Trap bound about 45% of the IL-1β (Figure 3). Even complexes formed from a 1.5:1 ratio of IL-1ra to IL-1 Trap bound about 40% of the IL-1β. It wasn't until the ratio of IL-1ra to Trap was at 2:1 that little binding (<20%) to IL-1β was observed. Increasing the ratio of IL-1ra to IL-1 Trap, up to 3:1, slightly reduced IL-1 Trap binding of IL-1β beyond the 2:1 complex; however, increasing the ratio of IL-1ra to Trap beyond 3:1 did not further decrease the drug binding IL-1β.
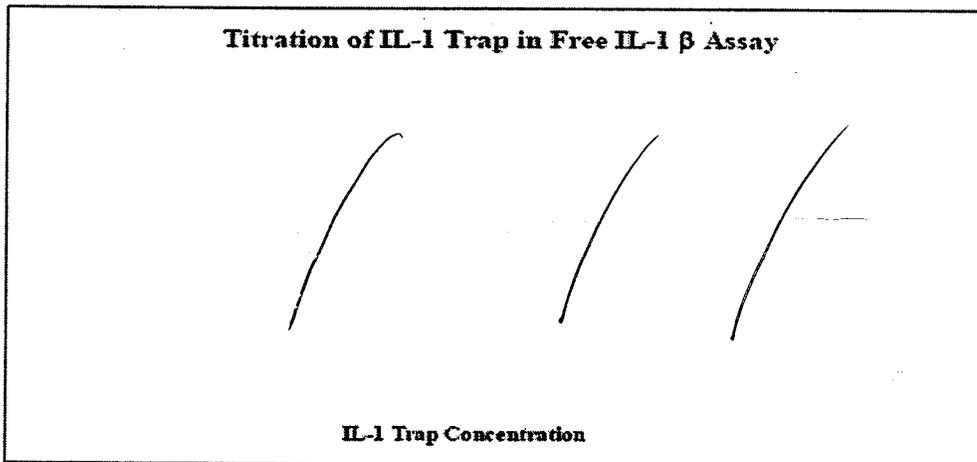
Figure 1. Titration of IL-1 Trap in Free IL-1β Assay
Increasing concentrations of IL-1 Trap were incubated in the presence of 8500 pg/mL of IL-1β for 1 hr and then the levels of free IL-1β where determined in the free IL-1β assay in a 0.2% serum matrix. The graph indicates the percentage of IL-1β recovered in each sample.

Effect of Various RA:Trap Complexes on the Detection of IL-1beta
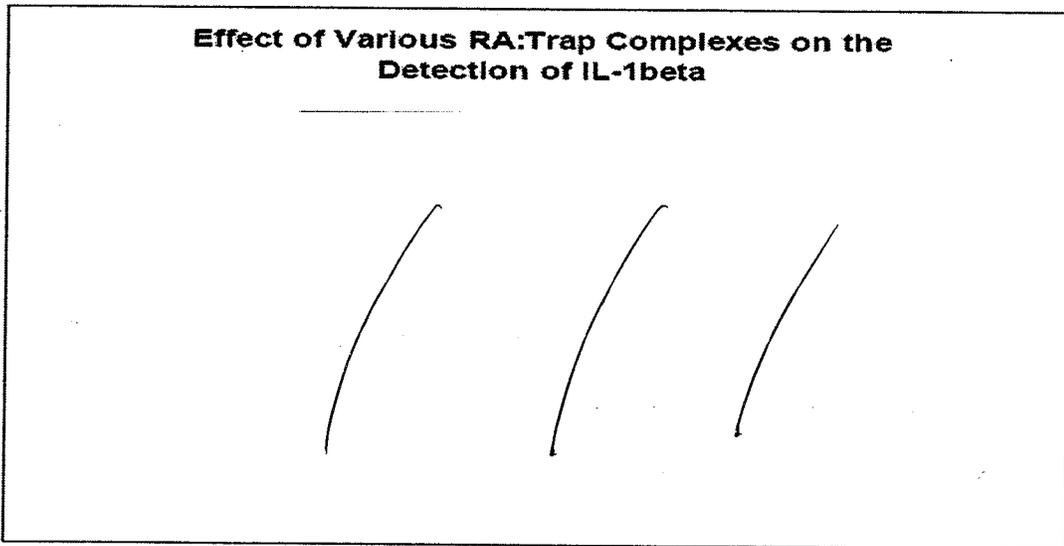
Figure 2. Effect of Various IL-1ra:IL-1 Trap Complexes on the Detection of IL-1β
In five independent experiments, IL-1 Trap and IL-1 Trap complexes (CX), with ratios of IL-1ra:IL-1 Trap of 0.5:1, 1:1, 1.5:1, 2:1, 3:1 and 4:1, were examined in the functional IL-1 Trap assay in a 0.1% serum matrix. The graph presents the individual results from each of the five experiments (Set 1-5) and the average results from all five (Avg). All samples contained a final concentration of 85 pg/mL IL-1β. A Trap and a No Trap sample was included in each run as a control for maximum binding and full recovery of IL-1β, respectively, in each of the runs.

Adjusted Recovery of IL-1 Beta in Presence of Various IL-1ra Complexes
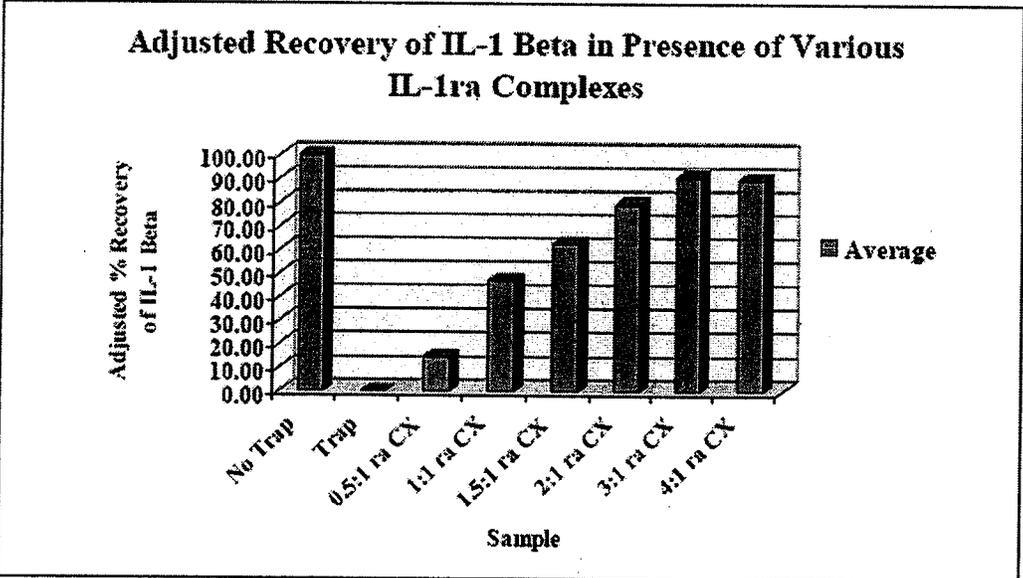
Figure 3. Adjusted % Recovery of IL-1β in Presence of Various IL-1ra:IL-1 Trap Complexes
Average results of experiments from Figure 2 were re-calculated to provide an adjusted % recovery where the un-complexed Trap has 0% recovery of IL-1β and the No Trap control has 100% recovery.

Reviewer's Comment: The reviewer agreed with the sponsor's conclusion that the result suggests formation of a ternary complex with IL-1 Trap, IL-1ra, and IL-1β. Two molecules of IL-1ra are necessary to prevent binding of IL-1 Trap to IL-1β. Note that IL-1β can bind to IL-1RT1 and IL-1 RAcP. However, only one molecule of IL-1β can bind to both domains of IL-1 Trap at one time. IL-1ra only binds to IL-1RT1, so 2 molecules of IL-1ra can bind at one time to the IL-1 Trap with equal affinity. Therefore, the binding of one ligand to an IL-1R1 site competitively blocks access of a second ligand to that site, but does not interfere with the binding to unoccupied sites on the drug. In

other words, all IL-1R1 sites of the IL-1 Trap act independently. Because all IL-1R1 sites act independently and with equal affinity, there is a normal distribution of IL-1ra bound throughout all IL-1R1 sites in solution. Thus the nature of IL-1ra:IL-1Trap binding will depend on their molar ratio in a solution.
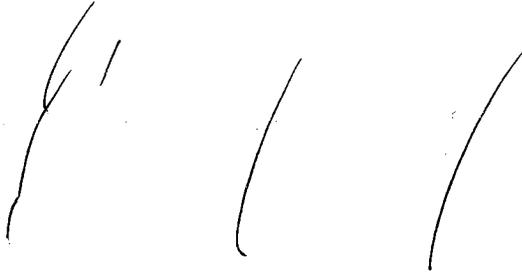
Figure 4. Model of Potential IL-1β:IL-1 Trap Complex Formation
Schematic diagrams illustrating the possible IL-1β:IL-1 Trap complexes that can form in the presence of either free IL-1 Trap or an IL-1ra:IL-1 Trap complex.
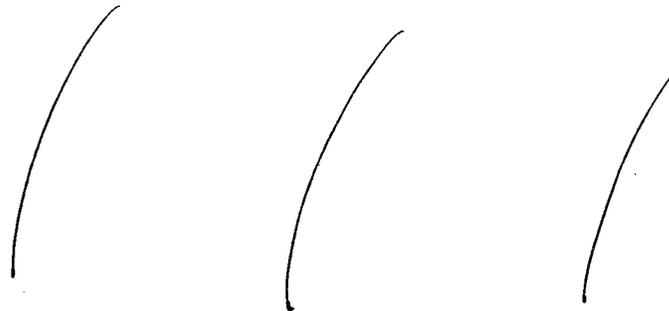
Figure 5. Distribution of IL-1ra:IL-1 Trap Complexes at Equal Molar Ratios of IL-1ra and Trap
At equal molar concentrations of IL-1ra and IL-1 Trap half of the IL-1R1 sites would be bound with IL-1ra. The resulting distribution would be 1:2:1 of un-complexed, single complexed and double complexed IL-1 Trap, respectively.

Study Number: IL1T-MX-06022
Study Title: Determination of IL-1 Beta:IL-1 Trap Complex Levels in Samples from Monkey Toxicology Study IL1T-TX-03021

Objective of the study: The objective of this analysis was to examine how increasing levels of IL-1 Trap affect IL-1β:IL-1 Trap complex levels in plasma.

Results:

The sponsor developed a validated ELISA method to evaluate IL-1Trap:IL-1β complex in human plasma. A similar method, although not validated, was used to estimate the immune complex formation in the 6-month SC monkey study (Study # IL_1T-TX 03021). The result showed that
- Both IL-1 Trap and IL-1β:IL-1 Trap complex was detected in Day 5 samples and the levels of the complex increased with increasing dose. Mean IL-1 Trap levels were generally

highest on Day 5 because the emergence of anti-IL-1 Trap antibodies had a major impact on IL-1 Trap plasma levels in these animals.

- By Day 12, anti-IL-1 Trap antibodies started to appear in most of the animals, reducing plasma levels of IL-1 Trap in these animals.
- By Day 19, anti-IL-1 Trap antibody levels were high enough to drastically reduce IL-1 Trap levels in the animals.
- Mean IL-1β complex levels increased with dose towards saturation at early time points but then continued to increase further in the Day 80 samples.

Mean IL-1 Trap and IL-1β: IL-1 Trap Complex level for all dose groups

Dose	Day 5		Day 12		Day 19		Day 80	
	IL-1 Trap	Complex	IL-1 Trap	Complex	IL-1 Trap	Complex	IL-1 Trap	Complex
mg/kg	µg/ml	pg/ml	µg/ml	pg/ml	µg/ml	pg/ml	µg/ml	pg/ml
15	223	0	151	0	53	2233	66	0
25	441	1061	337	1273	115	227	136	2207
40	718	4220	629	5001	338	5228	175	3920
60	1257	5132	1180	6942	750	4775	1021	11575

Table 6. Mean IL-1 Trap and IL-1β:IL-1 Trap Complex levels for all dose groups.
Mean IL-1 Trap levels (µg/mL) and mean IL-1β:IL-1 Trap complex levels (Complex: pg/mL) are presented for the Day 5, 12, 19 and 80 samples from each dose cohort (15, 25, 40 and 60 mg/kg). For the calculation of means, all BLQ values were set at 0.

Reviewer's Comment: The data showed that IL-1 Trap:IL-1β complex deposition increased with the duration of time may be due to the increase in the IL-1 Trap:IL-1 Trap antibody formation led to the deposition of this immune complex. This might generate a pro inflammatory reaction eventually as mentioned by the sponsor.

Study Number: IL1T-MX-06023
Study Title: IL1T-MX-06023: Evaluation of Fc-Effector Function for IL-1Trap

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Objective of the Study: Evaluation of the ability of the IL-1 Trap to exert its mechanism of action *via* Fc-mediated activities including ADCC, CDC, and FcγR interactions.

Results: The result shows the following:

- IL-1 Trap was unable to mediate the complement activation and effector cell mediated cytotoxicity. In addition, FACS analysis of LPS stimulated THP-1 cells and human monocytes showed no binding of the IL-1 Trap to either cell surface, a result consistent with the observed lack of effector mediated activity demonstrated in the CDC and ADCC assays.
- The binding of IL-1 Trap to the activation and inhibitory low affinity Fcγ receptors was also studied. IL-1 Trap, in the absence of IL-1 ligands or ___) antibody, exhibited micromolar binding affinities for all of the Fcγ receptors studied in either binding format. Similar affinities were measured for bevacizumab and rituximab, suggesting that experimental conditions were suitable for the calculation of equilibrium dissociation constants.
- The anti-IL-1 Trap antibody U10E9, however, was observed to increase the calculated equilibrium dissociation constants for the IL-1 Trap interaction with several of the low affinity receptors. FcγRIIa (His131), FcγRIIa (R131), and FcγRIIb/c were each observed to bind to the antibody bound IL-1 Trap with comparable increased affinity, suggesting that

antibody bound to IL-1 Trap can lead to avidity driven changes in Fcγ receptor occupancy. Small increases in binding affinity were also observed for the interaction of clustered IL-1 Trap for FcγRIIIa (F176), FcγRIIIa (V176), and FcγRIIIb (see table below).
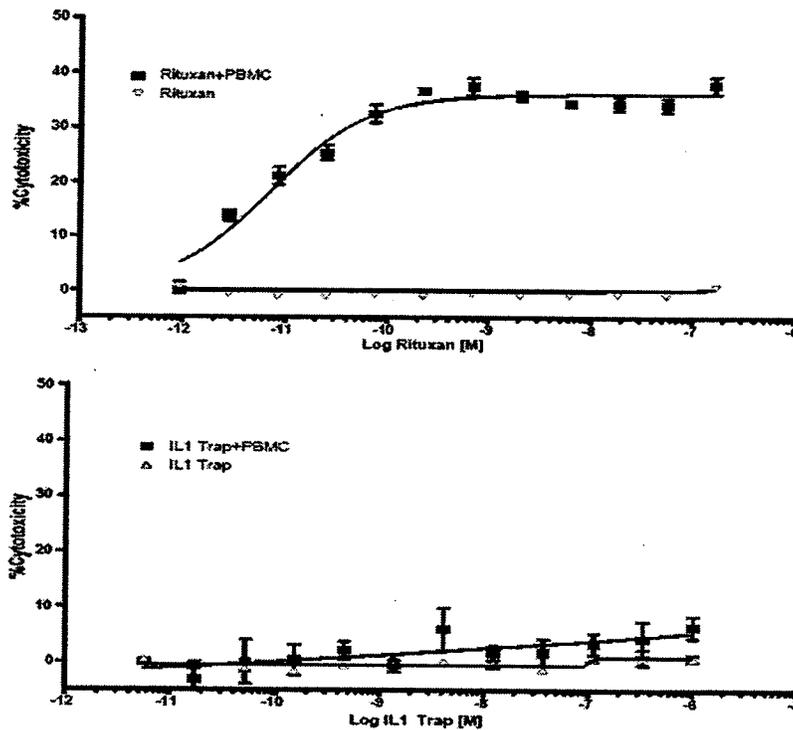

Figure 1 Effect of IL-1 Trap in the ADCC assay

(Top) Daudi cells incubated with rituximab were used as a positive control to show the activity of the PBMCs in the assay. (Bottom) THP-1 cells were incubated with human PBMCs and various concentrations of IL-1 Trap. The THP-1 cells in this graph were incubated overnight with LPS; cells with no LPS treatment gave similar results. No cell lysis was seen. The lowest concentration point on either graph shows the % cytotoxicity measured for a buffer control (no IL-1 Trap) in the presence of all other reaction components.
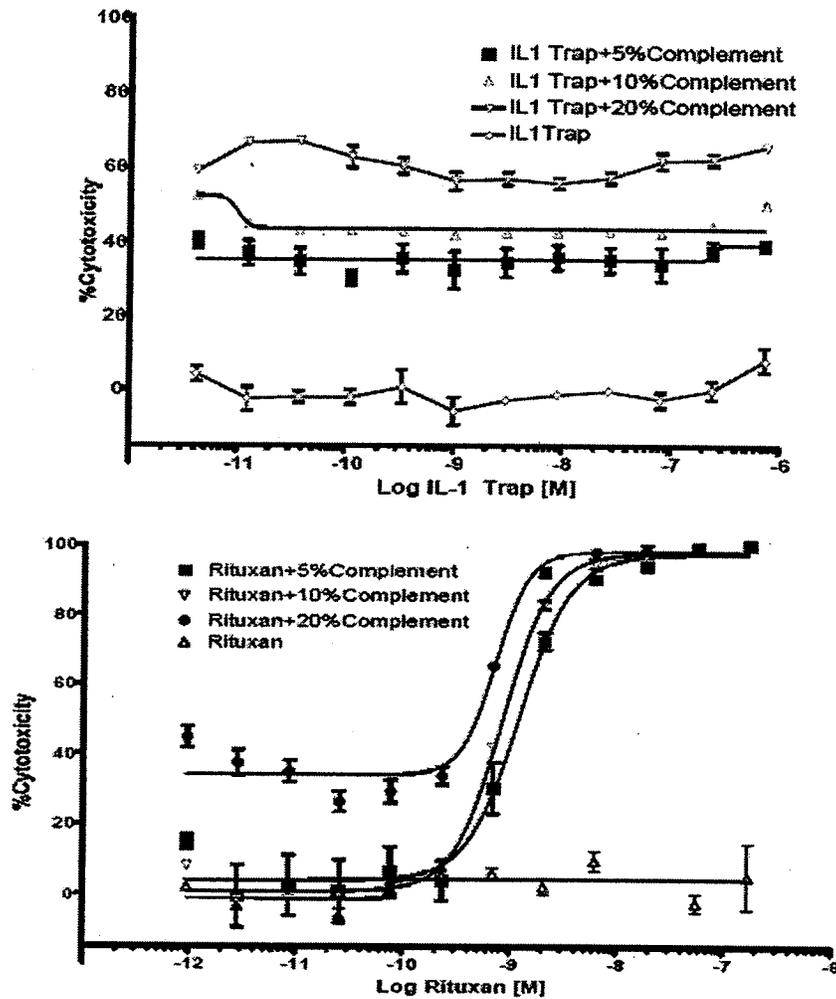
Figure 3 Effect of IL-1 Trap in the CDC assay.

(Top) IL-1 Trap and THP-1 cells (LPS treated) incubated with 5%, 10%, or 20% human serum complement. Cells incubated with IL-1 Trap alone showed no cytotoxicity. (Bottom) Rituximab and Daudi cells incubated with 5%, 10%, or 20% human serum complement. The lowest concentration point on either graph shows the % cytotoxicity measured for a buffer control (no IL-1 Trap) in the presence of all other reaction components.

Table 3 Summary of Equilibrium Dissociation Constants Calculated for IL-1 Trap and Comparator Antibody Interaction with C1q and Fcγ Receptors

Surface	Bevacizu-mab K_D (M)	Rituxi-mab K_D (M)	IL-1 Trap K_D (M)	IL-1 Trap + IL-1β K_D (M)	IL-1 Trap + IL-1ra K_D (M)	IL-1 Trap + K_D (M)	IL-1 Trap + K_D (M)	IL-1 Trap + K_D (M)	mAb K_D (M)
FcγR1	4.0×10^{-9}	2.8×10^{-9}	3.9×10^{-9}	2.9×10^{-9}	2.9×10^{-9}	1.4×10^{-9}	1.4×10^{-9}	1.6×10^{-9}	NB[a]
FcγRIIa (R131)	1.2×10^{-6}	1.2×10^{-6}	1.1×10^{-6}	2.3×10^{-6}	1.4×10^{-6}	0.1×10^{-6}	0.1×10^{-6}	0.1×10^{-6}	1.6×10^{-6}
FcγRIIa (H131)	0.6×10^{-6}	0.4×10^{-6}	1.0×10^{-6}	1.2×10^{-6}	0.9×10^{-6}	0.1×10^{-6}	0.1×10^{-6}	0.1×10^{-6}	6.0×10^{-6}
FcγRIIb	2.2×10^{-5}	3.9×10^{-6}	3.8×10^{-6}	11×10^{-6}	5.6×10^{-6}	0.3×10^{-6}	0.2×10^{-6}	0.3×10^{-6}	$30. \times 10^{-6}$
FcγRIIIa (V176)	0.7×10^{-6}	0.3×10^{-6}	0.7×10^{-6}	0.7×10^{-6}	0.7×10^{-6}	0.2×10^{-6}	0.3×10^{-6}	0.3×10^{-6}	NB
FcγRIIIa (F176)	0.9×10^{-6}	1.4×10^{-6}	1.5×10^{-6}	1.7×10^{-6}	1.5×10^{-6}	0.5×10^{-6}	0.6×10^{-6}	0.5×10^{-6}	NB
FcγRIIIb	2.8×10^{-6}	3.9×10^{-6}	3.3×10^{-6}	7.2×10^{-6}	4.7×10^{-6}	2.0×10^{-6}	1.9×10^{-6}	1.5×10^{-6}	NB

Equilibrium dissociation constants obtained for the interaction of captured Fcγ receptors and solution phase IL-1 Trap and comparator antibodies.

[a]NB No specific equilibrium dissociation constant could be determined.

Reviewer's Comment: The CDC and ADCC are important effector domain-based mechanisms employed in the eradication of pathogens and tumor cells. These activities are mediated through the Fc domain of some antibody isotypes, and potentially also through Fc containing receptor ectodomain of fusion proteins including the IL-1 Trap. This nonspecific activity of the compound might result from the IL-1Trap:IL-1Trap antibody complex as observed for the interaction of clustered IL-1 Trap for FcγRIIIa (F176), FcγRIIIa (V176), and FcγRIIIb (see Table 3 above, U10E9 is an antibody for IL-1Trap). The question is whether the increased binding of the immune complex for the low affinity receptors Trap could lead to increase ADCC-related activities. It could not be assessed in the nonhuman primates since the low affinity receptors in humans are unique. Therefore, the part of the immunogenicity toxicity of the IL-1 Trap: antibody complex is unique to human and could only be monitored clinically.

Study Number: IL1T-MX-06026
Study Title: Description of Cell Line and Process for Production of Murine IL-1 Trap for Toxicology Studies

Procedure:
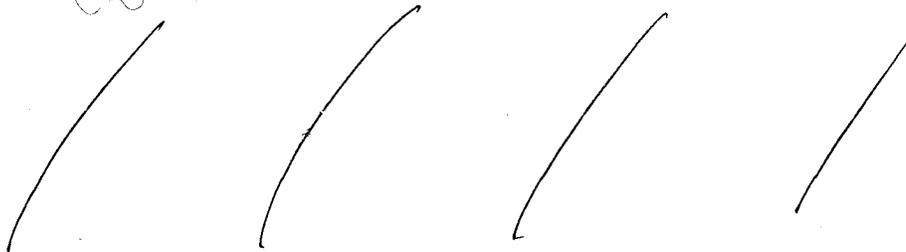
_____1_____ Page(s) Withheld

_____✓_____ Trade Secret / Confidential

_____ Draft Labeling

_____ Deliberative Process

Study Number: IL1T-MX-06027
Study Title: Evaluation of Murine IL-1 Trap to Suppress Neointimal Hyperplasia in an Injury-Induced Murine Animal Model
Objective of the Study: The objective of the study is to determine the whether mIL-1 Trap reduce vascular injury in mice ⸺ strain). Dose and Route of administration: SC, 3x/week, 0, 0.02, 1, 10 and 30 mg/kg/sex/animal

Result: Murine IL-1 Trap prevented the development of neointimal hyperplasia in a surgery induced vascular injury animal model in a dose dependent manner, 50% reduction of lesion area was observed at 1 mg/kg and 85% reduction of lesion area was achieved with the highest dose group at 30 mg/kg.

Figure 1   Histology of common carotid artery segments demonstrates the development of neointimal hyperplasia in injured arteries

Placebo   mIL-1 Trap (10mg/kg)
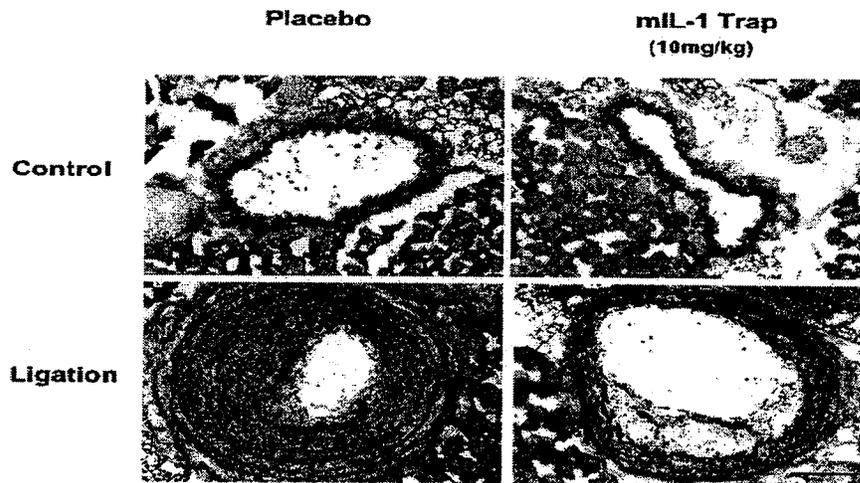
Control

Ligation

Legend: Representative photographs of control and injured (ligation) carotid arteries from placebo injected and mIL-1 Trap injected mice are shown. Common carotid ligation injury was performed using 6-0 silk suture before bifurcation. The animals were sacrificed 28 days after surgery and the whole neck sections were collected for histological analysis. Cryosections were stained using hematoxylin and eosin method. The lumen and internal elastic lamina (IEL) at the ligated vessel are highlighted with green and red dashed lines, respectively. Scale bar, 100 μm.

Figure 2   Quantitative analysis of neointimal lesion area in mice treated with murine IL-1 Trap

Reviewer's Comment: The reviewer agrees with the sponsor's conclusion that murine IL-1 Trap is biologically active in this animal model.
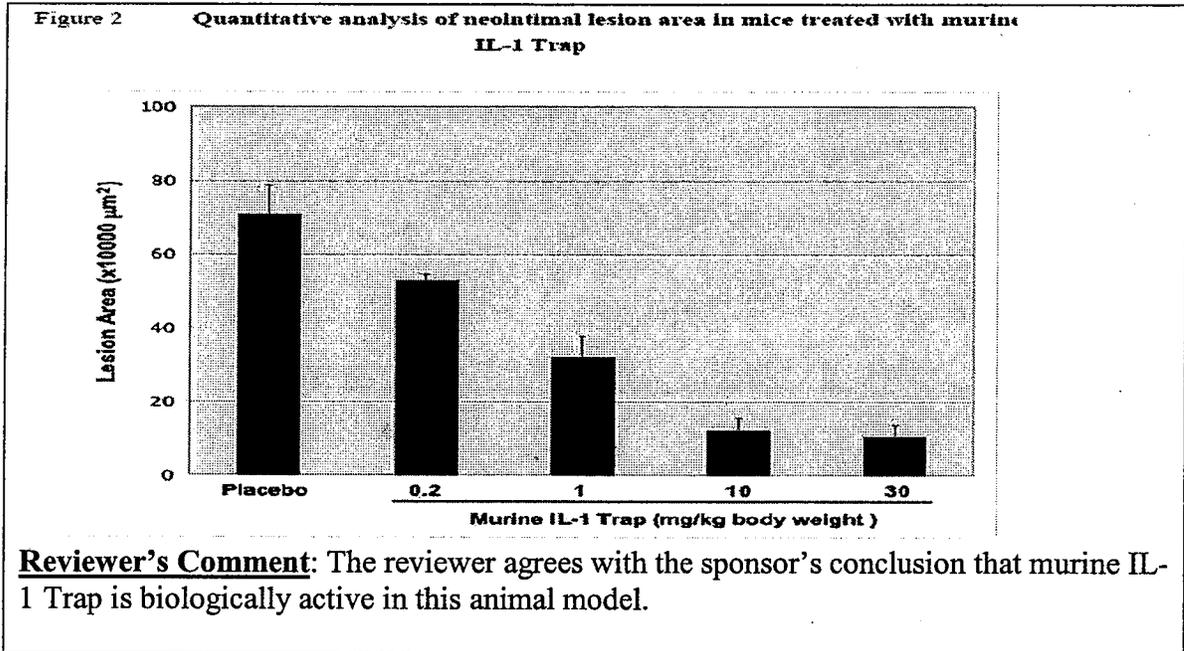
2.6.2.3 Secondary pharmacodynamics

Study Number IL-1T-MX-1T#41
Study Title : Immunogenicity of Human IL1-Trap in Rodents
Objective of the study: To determine the timing and magnitude of the antibody response against IL-1 Trap in rats and two different strains of mice. **Study Design:** • All animals were administered with 2, and 20 mg/kg hIL-1Trap 3x week for 24-39 days **Result:** • CD-1 mice and Sprague-Dawley rats began to mount a detectable immune response to IL-1 Trap between Days 6 and 15 such that animals who received the recombinant protein had substantial levels of antibody after 2 weeks of dosing. There was no apparent correlation between doses of IL-1 Trap and the rate or magnitude of antibody formation in these animals. C57BL/6 mice also began to exhibit a detectable immune response in the same time frame but there was a lower frequency of animals who were antibody positive and a lower magnitude of maximal response than that observed for the other two species.

2.6.2.4 Safety pharmacology

There was no safety pharmacology study submitted for this BLA. Safety pharmacology studies are not need for biologics according ICH S6 Guidance.

2.6.2.5 Pharmacodynamics drug interactions

There was no non clinical pharmacodynamics drug interaction studies conducted for this application.

2.6.3 PHARMACOLOGY TABULATED SUMMARY: NA

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The sponsor studied IL-1 Trap plasma concentrations following administration to the cynomolgus monkey (several different sources), rat, and different strains of mice after single subcutaneous or intravenous administration of the compound. The plasma concentration of the compound increased dose proportionally. The relative bioavailability of the mIL-1 Trap in mice was 78%, and hIL-1 Trap in monkey was 70%. Accumulation was noted after the single SC administration of the compound in both species. Slight difference in the exposure was noted between the males and females in mice, the significance of these findings is not known.

2.6.4.2 Methods of Analysis: The pharmacokinetics analysis were conducted by ELISA, the validation of the assays are reviewed by the product reviewers.

2.6.4.3 Absorption

The results from the pharmacokinetic studies are summarized below:

Study No: IL-1T-PK-00013.0	
Study title: A Pharmacokinetic study of IL-1 (Interleukin 1) Trap as a Single Subcutaneous Injection and Bioavailability of IL-1 Trap after Subcutaneous and Bolus Intravenous Injection to Cynomolgus Monkeys.	
Objective of the Study: (1) to determine the PK parameter estimates of IL-1 Trap after a single (SC) dose, (2) to assess dose proportionality, (3) to determine if gender specific	**IL-Trap Plasma Concentration following SC Administration:** The reviewer agrees with the sponsor's conclusion that the mean PK parameter estimates after SC administrations were found to be similar across the dose range tested
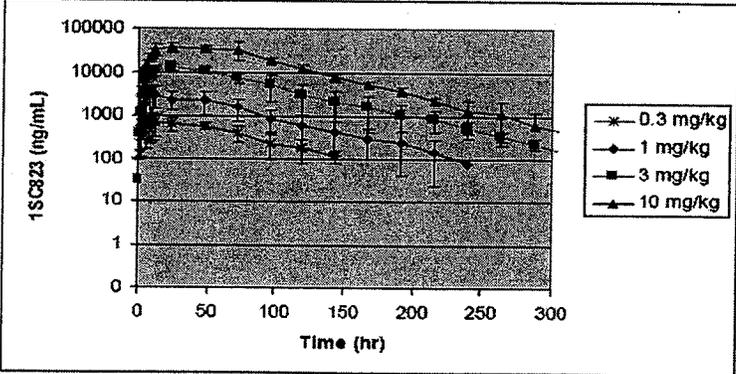
differences in PK parameter estimates exist, (4) to determine bioavailability of IL-1Trap when administered as a SC or a bolus IV injection in a crossover study design in cynomolgus monkeys.

Species:

Cynomolgus monkeys (Source

(1) 3/sex/group for SC
(2) 2 males and 1 female/sex for IV

Study Design:

Dose and Route of administration:
-Single, SC, 0.3, 1, 3,10 mg/kg/ sex /animal
- Single, SC or IV 3 mg/kg/ sex/animal

Following PK parameters were noted.
1) The mean elimination half-life of IL-Trap found to be 34, 58, 67, and 57 hours for the 0.3, 1, 3, and 10 mg/kg dose groups, respectively.
2) The mean ± standard deviation (SD) clearance/bioavailability (CL/F) of 1SC823 w: found to be 5.39 ± 1.32mL/hr/kg, 3.56 ± 0.81 mL/hr/kg, 2.65 ± 0.64, and 2.51 ± 0.28 mL/hr/kg for the 0.3, 1, 3, and 10 mg/kg dose groups, respectively.
3) The mean ± SD of the volume of distribution was found to be 250 ± 56, 298 ± 114, 256 ± 62 mL/kg, and 205 ± 35 mL/kg for the 0.3, 1, 3, and 10 mg/kg dose groups, respectively.

IL- Trap Plasma Concentration following IV and SC Administration:
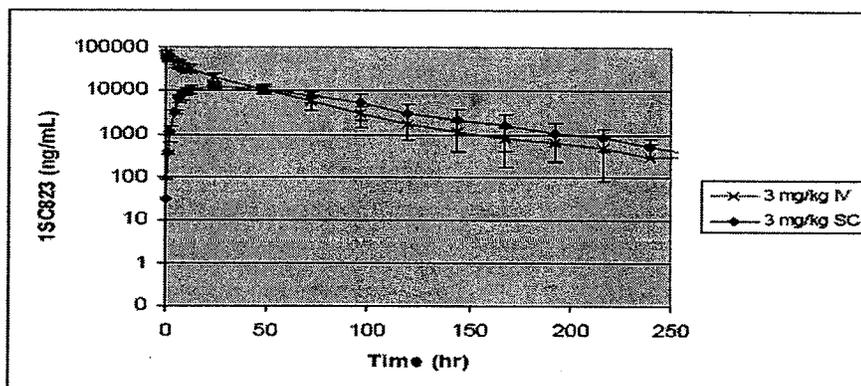
The bioavailability of IL-1 Trap was determined from the ratios of SC/IV of both the AUC_{0-t} and $AUC_{0-\infty}$ that were determined from the first injection only of the cross-ovei monkeys administered with 3 mg/kg by either IV or SC administration. The absolute bioavailability of IL-1 Trap after SC administration was determined to be 70%.

The Mean Cmax after 0.3, 1, 3, and 10 mg/kg Dose
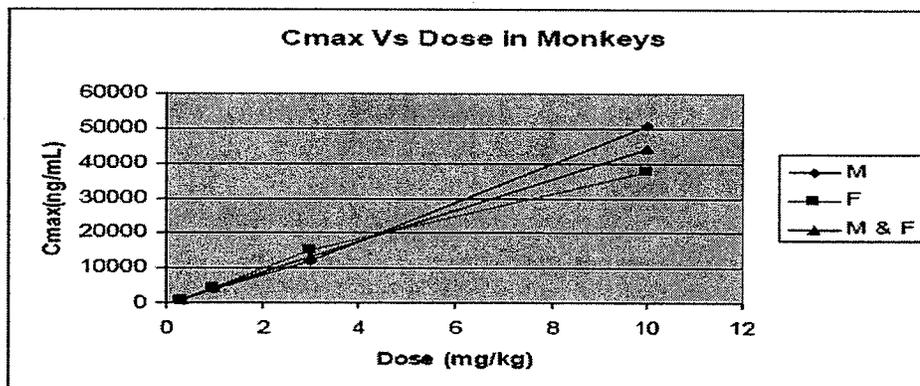
The mean Cmax values after subcutaneous administration of 1SC823 were 800, 4168, 13072 and 44184 ng/mL in the 0.3, 1, 3, and 10 mg/kg dose groups, respectively. The: values were approximately proportional to dose in the dose range of 1 to 10 mg/kg however, between 0.3 and 1 mg/kg, the increase in Cmax was slightly more than proportional with dose.

The Mean AUC(0-∞) after 0.3, 1, 3, and 10 mg/kg Dose

AUC$_{inf}$ Vs Dose in Monkeys

The peak plasma IL-1 Trap concentrations ranged from 554 to 1107 ng/mL, 3264 to 5489 ng/mL, 12069 to 14967 ng/mL, and 34930 to 55367ng/mL after the SC administration of 0.3 mg/kg, 1 mg/kg, 3 mg/kg and 10 mg/kg in cynomolgus monkeys respectively.

Reviewer's comment: The Cmax, Tmax, AUC (0-∞) showed dose linearity after singl SC administration. There were no apparent gender differences in the PK parameters. The bioavailability data could not be obtained from the same individuals due to the antibody formation in the cross over studies; therefore, the bioavailability determined for the SC studies is an estimation of the relative bioavailability.

Study No: IL-1T-PK-00010

Study title: Analysis of Serum Samples from ——— Study N0 0948-121, A Pharmacokineti Study of IL-1 (Interleukin 1) Trap Administered as a Single Subcutaneous Injection and Bioavailability c IL-1 Trap after Subcutaneous and Bolus Intravenous Injection to Cynomolgus Monkeys.

Study Design: Same as Study No: IL-1T-PK-00013.0.	**Reviewer's Comment:** In this study the source from where the monkeys were obtained was ` ——— ....n contrast to the study #00013.0 in which the source c the monkeys were ——→ it was noted that the result obtained from this study was similar to the result obtained from the study #00013.0 which is documented above

Study No: IL-1T-PK-00011.

Study title: Pharmacokinetic and Bioavailability of I Toxicology Lot (Lot # 00408) ISC823 Following Intravenous and Subcutaneous Administration to Sprague Dawley Rat

Objective of the Study: (1) PK profile of IL-1 Trap in rat after SC and IV administration (2) Bioavailability after single (SC) dose **Species:**	**Reviewer's Comment:** The pharmacokinetic profile (Tmax, Cmax and AUC) of the compound was observed to be similar in rat compared to the monkey after single administration. The bioavailability of the compound in rat following subcutaneous administration was 54%. However, there were no toxicity studies that were done with this compound in rat probably due to immunogenicity. **IL- Trap Plasma Concentration following SC Administration:**

Female Sprague Dawley Rat **Study Design:** <u>Dose and Route of administration:</u> Single, SC and IV, 1 mg/kg/ /animal	**IL- Trap Plasma Concentration following IV and SC Administration:** The following is a comparison of PK parameters from rat after SC and IV administration:
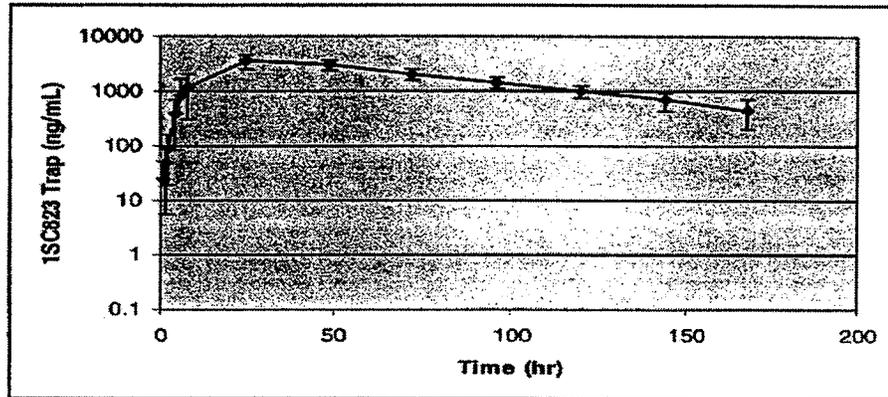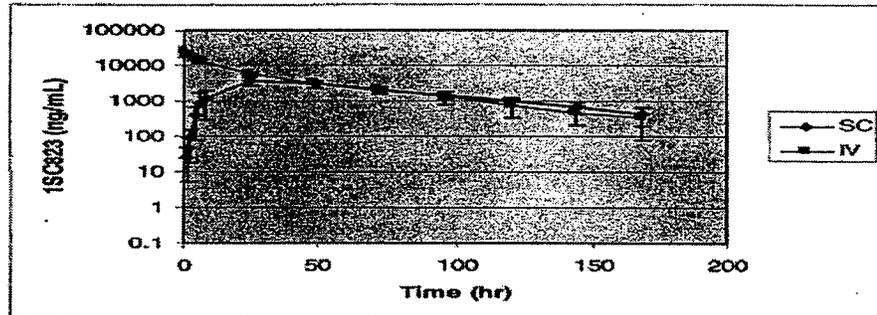
**APPEARS THIS WAY
ON ORIGINAL**

The Mean PK Parameter Estimates of 1SC823 in Rats after Intravenous and Subcutaneous Administration of 1 mg/kg of 1SC823

PK Parameters Estimates	SC	IV
Body Weight (grams)	261.8 ± 12.5	269.4 ± 16.7
Tmax (hour)	24 ± 0.0 [a]	0.043 ± 0.022 [b]
Cmax (ng/mL) [c]	3615 ± 971	26040 ± 2117
AUC$_{0-t}$ (ng X hours/mL) [d]	288876 ± 72245	534466 ± 61069
AUC$_{0-∞}$ (ng X hours/mL) [e]	319214 ± 90655	555170 ± 71035
AUC % Extrapolate (%) [f]	9 ± 5	4 ± 3
t$_{1/2el}$ (hours) [g]	42 ± 13	38 ± 7
CL (mL/hours/kg) [h]	3.35 ± 0.95 [i]	1.83 ± 0.27
Vz (mL/kg) [j]	194 ± 50 [k]	100 ± 22
Vss (m/kg) [l]		74 ± 10
MRT (hours) [m]	64 ± 4	35 ± 4
Bioavailability AUC$_{0-t}$ (%) [n]	54	
Bioavailability AUC$_{0-∞}$ (%) [o]	57	

*Values are (mean ± standard deviation [SD])
[a]Tmax = Time at maximum observed concentration
[b]Tmax = Time at 1st sample
[c]Cmax = Maximum observed concentration
[d]AUC$_{0-t}$ = Area under the concentration-time curve from time 0 to t hours
[e]AUC$_{0-∞}$ = Area under the concentration-time curve from time 0 to infinity
[f]AUC % Extrapolate (%) = Percent of AUC$_{0-∞}$ that was extrapolated from AUC$_{0-t}$
[g]t$_{1/2el}$ = Elimination half-life
[h]CL = Clearance
[i]CL/F = Clearance /Bioavailability
[j]Vz = Volume of distribution
[k]Vd/F = Volume of distribution/Bioavailability
[l]Vss = Volume of distribution at steady state
[m]MRT = Mean residence time
[n]Bioavailability AUC$_{0-t}$ = (AUC$_{0-t sc}$/ AUC$_{0-t iv}$) X 100%
[o]Bioavailability AUC$_{0-∞}$ = (AUC$_{0-∞ sc}$/ AUC$_{0-∞ iv}$) X 100%

Study No: IL-1T-PK-0006

Study title: Pharmacokinetic and Bioavailability of IL-1Trap Following Administered as a Single Subcutaneous Injection to Sprague Dawley Rat

Objective of the Study: (1) to determine the PK parameter estimates of IL-1 Trap after a single (SC) dose, (2) to assess dose proportionality, (3) to determine if gender specific differences in	**IL- Trap Plasma Concentration following SC Administration:** APPEARS THIS WAY ON ORIGINAL

/animal	
	APPEARS THIS WAY **ON ORIGINAL**

Study No: IL-1T-PK-0007.0

Study title: Pharmacokinetic and Bioavailability of ISC823 Following Intravenous and Subcutaneous Administration to C57BL/6 Mice

Objective of the Study:	IL- Trap Plasma Concentration following SC Administration:
(1) PK profile of IL-1 Trap after SC and IV administration (2) Bioavailability after single (SC) dose **Species:** Mice **Study Design:** Dose and Route of administration: Single, SC and IV, 1 mg/kg//animal	
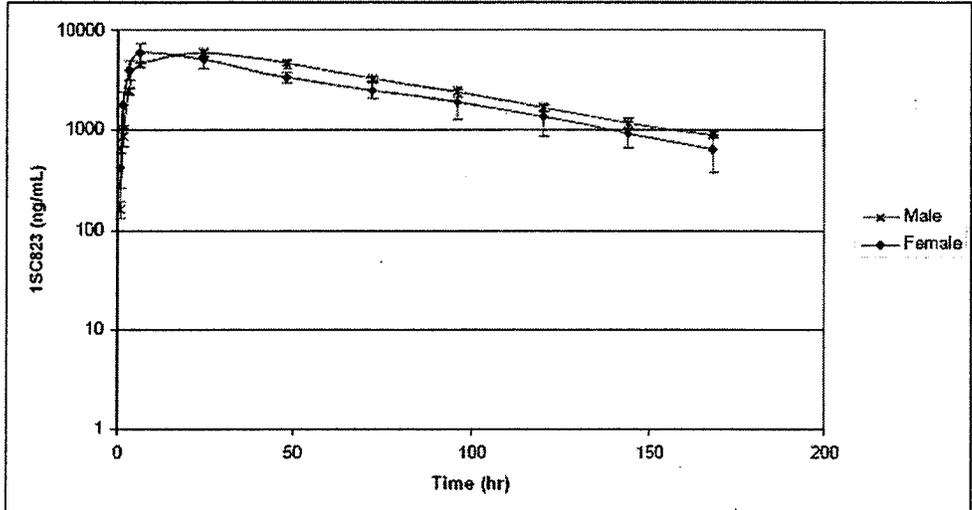
The Mean PK Parameter Estimates of ISC823 in Mice after Subcutaneous Administration of 1 mg /kg of ISC823

	Male (n=4)	Female (n=5)	Male and Female
Body weight(grams)	26.4 ± 0.7	21.4 ± 1	23.6 ± 2.6
Tmax (hours)	24 ± 0	6 ± 0	14 ± 9
Cmax (ng X hrs/mL)	5769 ± 585	6061 ± 1415	5931 ± 1074
AUC_{0-t} (ng X hours/mL)	503747 ± 26776	433975 ± 93730	464985 ± 77548
$AUC_{0-\infty}$ (ng X hours/mL)	569803 ± 24494	475030 ± 111144	517151 ± 94321
AUC % Extrapolate (%)	12 ± 2	8 ± 3	10 ± 3
$t_{1/2el}$ (hours)	51 ± 7	46 ± 8	48 ± 7
CL/F (mL/hours/kg)	1.76 ± 0. 07	2.21 ± 0.56	2.01 ± 0.46
Vz/F (mL/kg)	128 ± 20	147 ± 46	139 ± 36
MRT (hours)	61 ± 1	55 ± 3	57 ± 4
Bioavailability AUC_{0-t} (%)	73	84	78
Bioavailability $AUC_{0-\infty}$(%)	70	85	77

Values are mean ± standard deviation [SD]

Tmax = Time of Maximum Concentration

Cmax = Maximum Concentration

AUC_{0-t} = Area under the concentration-time curve from 0 to t (hours)

$AUC_{0-\infty}$ = Area under the concentration-time curve from time 0 to infinity

AUC% Extrapolate (%) = Percent of $AUC_{0-\infty}$ that was extrapolated from AUC_{0-t}

$t_{1/2el}$ = elimination half-life

F = Bioavailability

CL/F = Clearance/bioavailability

Vz/F = Volume of distribution at terminal phase/bioavailability

MRT = Mean Residence time

Bioavailability AUC_{0-t} = ($AUC_{0-t}sc/AUC_{0-t}$ iv) X 100%

Bioavailability $AUC_{0-\infty}$ = ($AUC_{0-\infty}sc./AUC_{0-\infty}$iv) X 100%

Reviewer's Comment: The mouse was used for the segment I and segment III reproductive toxicity studies. In this study the bioavailability of the compound was approximate 78%. It was noted from the above table that the Tmax and mean resident

| | time of the compound in male and female mice were different after they single administration of the compound. The PK profile of the compound was found to be similar to what observed in rat and monkeys after single administration. |

2.6.4.4 Distribution:

A tissue distribution study using radiolabeled IL-1 Trap was completed in the rat model. The study results indicated that the radiolabel rapidly distributed to highly perfused organs, such as liver, kidney, lung, and spleen. However, due to differential clearance of the iodinated protein compared to the unlabeled protein, the study is not very informative.

Study No: IL-1T-PK-00009.0	
Study title: Biodistribution of ISC823 in Normal Sprague Dawley Female Rats	
Objective of the Study: To determine the biodistribution of ^{125}I- in rats. **Study Design:** Dose and Route of administration: -Single, IV, 250 mg/female rats, the compound was tagged with ^{125}I and competed w/untagged cold compound	**Reviewer's Comment:** The sponsor noted that there was a large discrepancy between serum concentrations of the labeled and unlabeled IL-1 Trap as indicated by the radioactivity and ELISA quantization, respectively. Because the iodinated IL-1 Trap is cleared much faster than native IL-1 Trap, it is unlikely that the tissue distribution observed for the ^{125}I-labeled-1SC823 is relevant to the intact protein. The reviewer agrees with the sponsor's observation. The biodistribution studies with biologics are challenging due to the fact that the proteins are very sensitive to labeling methods with ————— s which is used in this study and most likely ^{125}I-labeled portion which is measure is not tagged with the original protein. APPEARS THIS WAY ON ORIGINAL

2.6.4.5 Metabolism:

There was no metabolism study submitted with this BLA. The metabolism study is not needed for this fusion protein as the proteins are expected to metabolize by catabolism.

2.6.4.6 Excretion

The effect of ⸻ on clearance of the compound was evaluated in the rat model.

Study No: IL-1T-MX-0628
Study title: Pharmacokinetics of IL-1 Trap following Subcutaneous Administration to Sprague Dawley Rat: Correlation Between Pharmacokinetic Parameters and ⸻

| **Objective of the Study:** To determine the relationship between ⸻ and PK profile of IL-1 Trap in rodent. **Species:** Sprague Dawley Rat **Study Design:** <u>Dose and Route of administration:</u> Single, SC, 1 mg/kg; *serum concentration of IL-1 Trap from different manufacturing batches were compared* | |
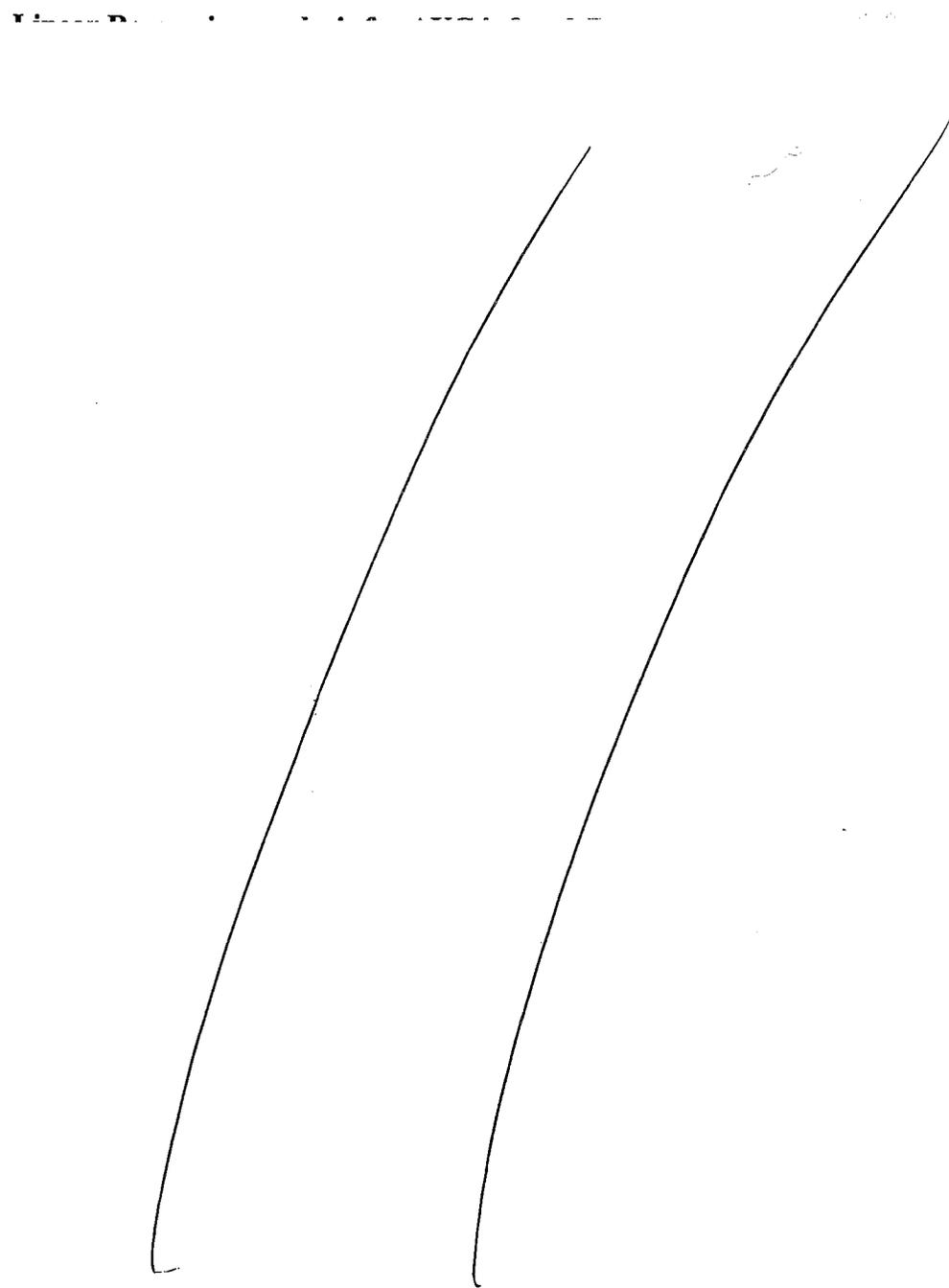
2.6.4.7 Pharmacokinetic drug interactions:

There were no non clinical pharmacokinetic drug interaction studies completed for this BLA submission.

2.6.4.8 Other Pharmacokinetic Studies:

None.

2.6.4.9 Discussion and Conclusions

The multiple dose toxicokinetic studies were done in monkeys after SC and IV administration. The results of these studies are discussed in the conclusion section.

2.6.4.10   Tables and figures to include comparative TK summary

2.6.5   PHARMACOKINETICS TABULATED SUMMARY

2.6.6   TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

The sponsor conducted a 3-week IV and a 6-month IV toxicity studies in cynomolgus monkey. The sponsor also conducted subcutaneous toxicity studies in monkey with different duration such as 6-weeks, 3-months, and 6-months. There were unscheduled deaths and adverse events related to congestion of heart and lung in the 6-month chronic toxicity studies. The toxicity findings from the scheduled autopsy included injection site lesions at all time points. Myocardial degeneration and mono/polymorphonuclear cell administration were also noted at almost all time points with IV and SC administration; inflammatory changes in the kidney were also noted; the changes in the lung was associated with granuloma formation and inflammatory cell infiltration. In the 6-month IV toxicity study, stellate cells were noted in liver in the treatment group, the significance of this finding is not known. The changes in the testes and ovary were noted in animals from all dose groups administered with the test article.

The toxicokinetic analyses revealed dose related increase of the plasma concentration of IL-1 Trap in first two weeks. IL-1 Trap antibody formation; however, was also noted within two weeks. In the later time period in the study clearing of IL-1 Trap by the antibody was noted in general, at the low dosages. The high dose group showed IL-1 Trap in the plasma for a longer period of time. IL-1 Trap antibody was found in the

serum even at recovery at high concentration. There was no direct correlation between the toxicokinetics of IL-1 Trap and its antibody with the toxicity findings.

Genetic toxicology: There were no genetic toxicology studies submitted with this BLA. Such studies are not needed with this product.

Carcinogenicity: There were no carcinogenicity studies submitted with this BLA. Such studies are not needed with this product.

Reproductive toxicology:

The sponsor conducted a Segment II, embryo fetal development study in the monkey with the human IL-Trap. In this study the pregnant monkeys were injected subcutaneously from the gestation days 20-50, the animals were C-sectioned at Day 100. The toxicokinetics analyses showed that the antibody formation in the low and mid dose groups occurred around Day 35 and 50 respectively. There were abortions in the monkeys at this time period. Spontaneous abortions were also noted at earlier gestation days (23-27) in all dose groups including controls. There was an increase in the variation of the lumber vertebra formation in the monkeys in the test article treated group. There was a decrease in the estrogen level in the monkeys between Day 30-Day 60 in all of the test article treated groups. The antibody was found to be present in the fetuses at C-section indication placental transfer of the antibody from the mother to fetus.

The sponsor also conducted Segment I and Segment III reproductive toxicity studies in the mice with the mIL-1Trap. The findings in the fertility studies consisted of decrease in the female fertility index, and increase in the early resorptions and post implantation resorptions of the embryos. The major findings from the pre and post natal reproductive toxicity studies include increase fetal death (both F0 and F1) and increase in the early and post implantation resorptions in both generations.

Special toxicology: A series of studies were completed to evaluate immune function and potential for serum complement activation. Immune function tests included white blood cell profiles via flow cytometry, natural killer cell activity; lymphocyte proliferation and C4d complement activation.

2.6.6.2 Single-dose toxicity:

There was no single dose toxicity study submitted with this BLA.

2.6.6.3   Repeat-dose toxicity

Study title: A 3-Week IV Toxicity Study of IL-1 Trap with a 6-Week Recovery Period in Cynomolgus Monkeys

Key study findings:

- Cynomolgus monkeys (3/sex/group) were administered 0, 5, 20, and 50 mg/kg IL-1 Trap, IV, once per week for 3 weeks; 2 animals/sex were observed for recovery from the control and the high dose group.
- Toxicokinetic analysis of the IL-1 Trap and the anti product antibody formation showed a dose related increase of the serum concentration of the compound. Incomplete clearance was noted after 7 days of the compound administration indicating accumulation, detectable antibody formation was noted at Day 15.
- No NOAEL could be established in this study because of the adverse events findings such as emesis and lethargy at low dose. This might be due to the increase in the immune complex formation at the low dose. The sponsor had the same conclusion.
- The histopathological findings in the treated animals were consisted of mononuclear cell infiltration in kidney, liver, heart, hyperplasia of the mesenteric lymph node, mandibular lymph nodes, and decrease and closed growth plates in the knee joints. Recovery was noted at high dose for most of the findings. No animals were observed for recovery from the low and mid dose group. Therefore, it is not known whether recovery was achieved in these groups.

Study no.: 223.12
Volume # and page #: eCTD submission; Pages 1-416 (file study-il1t-tx-02045.pdf)
Conducting laboratory and location: ꞌ

Date of study initiation: September 10, 2002
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: IL-1 Trap TP1 F01002 (relative potency ⸺ . Drug Product description: IL-1 Trap recombinant protein, 40 mg/mL in a ˙ ⸺ aqueous vehicle, pH , containing ⸺ . histidine, ⸺ ꞌ ⸺⸺⸺ ⸗ glycine, ⸺ ⸺ . arginine, ⸺ ꞌ sucrose, ⸺ ꞌ polyethylene glycol 3350, and ⸺⸺⸺⸺

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

Methods:

Doses: 0, 5, 20, 50 mg/kg, once/week for 3 consecutive weeks
Species/strain: Cynomolgus monkeys; *Macaca fascicularis*
Number/sex/group or time point (main study): 3 animals/sex/group
Route, formulation, volume, and infusion rate: IV, the test article was diluted in water for injection, the test article was administered by slow (within 30 mins) infusion in the cephalic vein, and the volume administered was 10 mL
Satellite groups used for toxicokinetics or recovery: Two animals/sex/group was designated as recovery animals. Blood sampling for TK analysis was done in all animals.

Age: 2-7 years

Weight: 2-4.2 kg
Sampling times: Blood samples for serum IL-1 Trap concentration were taken at pre-dose, immediately after dosing (approximately 5 minutes), at 24 and 72 hours post dosing on all dosing days (Days 1, 8, and 15) and on the day of necropsy (Day 22). Samples were also taken at the middle (Day 42) and end (Day 63) of the recovery period.
Unique study design or methodology (if any): Following is the study design.

Table A: Study Design

Group	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)[a]	Number of Animals		Study Specific Animal Numbers	
				F	M	F	M
1	0	0	12.5	3 + 2[b]	3 + 2[b]	35,3,5, **7,33**	2,4,6, **8,10**
2	5	1	5	3	3	11,13,15	12,14,16
3	20	4	5	3	3	17,19,21	18,20,22
4	50	4	12.5	3 + 2[b]	3 + 2[b]	23,25,27, **29,31**	24,26,**28, 30,34**

[a] For total dose solutions per animal that exceed 10mL the dose volume (mL/kg) was calculated based on the most recent body weight and dose volumes were rounded up to the next syringe graduation (if dose volume fell in the range of 0.6mL to 0.9 mL) or half way between graduations (if dose volume fell in the range of 0.1mL to 0.5mL) according to ⎯⎯ SOP.

[b] Two males and two females (numbers in bold) from the control and high dose group were designated as recovery group animals.

Observations Times and Results

Mortality & Clinical signs:

The clinical observations were recorded twice daily for the mortality morbidity and the clinical signs.

The sponsor reported that adverse events consisting of emesis, lethargy, and cold to touch was observed in several animals during Day 15 and 22. Following clinical observations were made:

- There were three animals, one female and two males (#s11, 14, and 16) from the low dose group who had adverse reactions. Animal #11 was observed to be lethargic after dosing but recovered by Day 16. The animal #14 had emesis during dosing at Day 15, and was pale and cold to the touch, this animal was observed to be in hunched and curled position at Day 16, and it also showed emesis at Day 22. The animal #16 had emesis several times during dosing. No emesis and/or lethargy was observed in this animal at Day 16, however, less food consumption was noted in this animal at Day 17.
- There were three animals in the mid dose group who were lethargic after dosing at Day 15. There were no signs of lethargy in these animals at Day 16. The animal #s 18 and 19 from this dose group had emesis on Day 22.
- There were two high dose animals (#s 29 and 30) that had emesis during dosing at Day 15. The animal #30 was also observed to be lethargic. In the afternoon of Day 16, animal #29 had emesis and animal #30 exhibited lethargy/exhaustion. These observations were not present for either animal on Day 17 or thereafter.

The emesis and the lethargy were not noted in the control animals. The observed adverse events are, therefore, considered treatment related and might be due to the immune complex formation. The timing for the adverse reaction observed in the above mentioned animals correlated with formation of the anti product antibody after the IV administration of IL-1 Trap. Interestingly, the number animals showing such incidence were higher in the low dose group. This coincides with the observation that the increase in the quantity of the anti product antibody formation was higher in the animals at the low dose group. The intolerance of the immune complex formation in the animals might have been revealed by the clinical signs of lethargy and emesis.

Table G: Animals with adverse reactions on Day 15

Group	SSAN	Sex
2 (5 mg/kg)	11 14,16	F M
3 (20 mg/kg)	17, 19 22	F M
4 (50 mg/kg)	29 30	F M

Body weights:

The body weight was measured from all animals once pre dose and weekly thereafter. There were no test article related changes in the body weight.

Food consumption:

The food consumption per animal per day was calculated from the number of biscuits supplied to each animal and the number of biscuits remaining at the time new food was offered. There were no consistent test article related changes in the food consumption.

Ophthalmoscopy:

Eyes were evaluated once during acclimation (Day –5) and prior to the day of necropsy at the end of the dosing period (Week 3, Day 21), and at the end of the recovery period (Week 9, Day 63), using an indirect ophthalmoscope and slit lamp microscope under ketamine sedation. There were no ophthalmologic changes in this study.

EKG, Blood Pressure, and Rectal Temperature:

The electrocardiography was performed using a   ————   ECG Analyzer and ——
·—— Monitor (Standard lead method) twice during acclimation (Days –12 and – 6), once during Week 1 (Day 1, approximately 2 hours after the end of dose infusion), Day 16 (approximately 24 hours ± 4 hours after the end of dose infusion), and at the end of the recovery period (Week 9, Day 63). The blood pressure and the rectal body temperature was measured at the same time as ECGs, using a   ———   ECG Analyzer and ——
—. Monitor (with Rectal Probe). The ECGs blood pressure and rectal temperatures were measured on all animals under ketamine sedation. There were no test article related changes in the EKG parameters in this study.

Hematology:

The blood samples were drawn for the hematology and blood coagulation analyses from all animals during acclimation (Day –8) for baseline, and during Week 2 (Day 11), Week 3 (Day 21) and at the end of the recovery period (Week 9, Day 60). Following parameters were assayed for the evaluation of changes in hematology and coagulation. There were no statistically significant changes in the hematology parameters in this study. However, there was a significant increase in the eosinophil and the platelet counts in the males, such changes were noted in females. The biological significance of this finding is not known.

Table C: Hematology and blood coagulation parameters

Parameter	Method	Apparatus	Units
Erythrocyte count (RBC)			$X10e^6/\mu L$
Leukocyte count (WBC)	method		$X10e^3/\mu L$
Hematocrit value (HCT)	Calculation		%
Hemoglobin concentration (Hgb)			g/dL
Platelet count (PLT)			$X10e^3/\mu L$
Mean platelet volume (MPV)	Calculation		fL
Mean corpuscular volume (MCV)	Calculation		fL
Mean corpuscular hemoglobin (MCH)	Calculation		pg
Mean corpuscular hemoglobin concentration (MCHC)	Calculation		g/dL
Reticulocyte count (Retic)			%
Differential leukocyte count			$X10e^3/\mu L$
Prothrombin time (PT)			Sec
Activated partial thromboplastin time (APTT)			Sec
Fibrinogen (Fib)			mg/dL

Clinical chemistry:

The blood samples were drawn for the clinical pathology analyses from all animals during acclimation (Day –8) for baseline, and during Week 2 (Day 11), Week 3 (Day 21) and at the end of the recovery period (Week 9, Day 60). Following parameters were assayed for the evaluation of changes in clinical pathology. A dose related decrease in the creatinine phosphokinase, triglycerides, and an increase in inorganic phosphate were noted. However, the standard deviation was too high to deduce any meaningful biological relevance to these changes.

Table D: Serum chemistry parameters

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

C - Reactive Protein and Complement Analyses

The blood was drawn for C-reactive protein analysis and complement analysis from all animals on Day 1 (pre-dose and approximately 6 hours after completion of dose infusion), on Day 5, once during Week 2 (Day 8, approximately 6 hours after completion of dose infusion), on Day 16, on Day 22 (prior to anesthesia at terminal necropsy), and at the end of the recovery period (Day 63).

There was an increase in the C-reactive protein at Day 16 after the 3rd dosing in the treatment groups compared to control. The concentration of the C-reactive protein in the control, low, mid, and high dose group was 0, 3.7, 3.3, and 5.7 mg/dL respectively. The increase was considered test article related, suggesting increase in acute phase protein in the serum which might be indicative of the tissue inflammation.. There were no changes in the complement factors in this study.

Summary Table (from sponsor) Showing C - reactive protein Formation in the Serum.

Group/Dose	Study Animal No.	C-Reactive Protein (mg/dL)						
		Week 1 Day 1/Pre	Week 1 Day 1/6hr	Week 1 Day 5	Week 2 Day 8/6hr	Week 3 Day 16	Week 4 Day 22	Recovery Day 63
1 IL-1 Trap Placebo (0 mg/kg)	2 4 6 8 10							
N Mean SD		0	2 0.85	0	1	1	0	0
2 IL-1 Trap (5 mg/kg)	12 14 16							
N Mean SD		0	1	0	0	3 3.70 0.85	0	0
3 IL-1 Trap (20 mg/kg)	18 20 22							
N Mean SD		0	1	0	0	3 3.30 0.61	0	0
4 IL-1 Trap (50 mg/kg)	24 26 28 30 34							
N Mean SD		0	2 1.00	0	2 0.70	5 5.84 3.03	1	0

CRP = C-Reactive Protein levels below linear are noted in bold and are not included in calculation of mean
NA = Not applicable, animals necropsied

Urinalysis:

The urine analyses were performed on all animals once during acclimation (Day –13) for baseline, during Week 2 (Day 10), Week 3 (Day 20) and the end of the recovery period (Week 9, Day 59). Following parameters were assayed for the evaluation of changes in urinalysis. There were no test article related changes in the urinalysis parameters.

Table B: Urinalysis parameters

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

Gross pathology:

At necropsy during terminal sacrifice (Day 22) and recovery (Day 64) gross lesion were evaluated from all animals. There were no treatment related changes in the gross necropsy findings.

Organ weights:

Following organs were weighed from all animals

Table E: Organs weighed

Adrenals	Pituitary
Brain (cerebrum, cerebellum and brain stem)	Prostate/Seminal vesicles
Epididymides	Spleen
Heart	Submandibular glands
Kidneys	Thyroids[1] (including parathyroids)
Liver	Testes
Lungs (including bronchi)	Thymus
Ovaries	Uterus (body and cervix)

[1] Weighed individually, combined in report.

The organ weights were evaluated based on the absolute body weight and relative brain weights for safety assessment by the reviewer. There were no treatment related changes in the organ weights.

Histopathology:

Adequate Battery: Yes; peer review: No

The histopathological examination was done from the following organ by fixing all the tissues in formaldehyde except testes (which was fixed in Bouin's) and eyes (which was fixed in a mixture of gluteraldehyde and formalin).

Table F: Organs for examination

Adrenals**	Large Intestine	Small Intestine
Aorta (thoracic)	-cecum	-duodenum
Bone*	-colon	-ileum
-femur / knee joint ***	-rectum	-jejunum
Bone Marrow*	Liver	Spinal Cord (thoracic)
-sternum	Lungs**	Spleen
Brain	(with bronchi**)	Stomach
-brain stem	Lymph Nodes	-fundus
-cerebellum	-mesenteric	-pylorus
-cerebrum	-mandibular***	Submandibular glands***
Epididymides**	Mammary Glands***	Testes**
Esophagus (thoracic)	Ovaries**	Thymus
Eye Balls / Optic Nerves**	Pancreas	Thyroids with
Gall Bladder	Pituitary	Parathyroids(if possible)**
Gross Lesions	Prostate	Tongue
Heart	Sciatic Nerve***	Trachea
Injection sites (vein)	Seminal Vesicle	Urinary Bladder

Kidneys**	Skeletal Muscle*** (quadriceps femoris)	Uterus
Lacrimal glands***	Skin*** (gluteal area)	Vagina

* Bone and bone marrow were observed as decalcified specimens. Bone marrow smears (sternum) were also prepared. The bone marrow smears were not examined microscopically.

** Both left and right organs were examined.

*** Both left and right organs were collected. If there were no gross lesions, only the left was examined.

The histopathological changes were noted in the lymph nodes, liver, kidney, heart, spleen, thyroids and ovary at the terminal and the recovery sacrifice. The changes in each of the tissues are described below. Following gradation were used to mark the severity of the toxicity findings.

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

Note:
- NA : Not applicable

Histopathology Findings in Heart:

Increase incidence of mononuclear cell infiltration was noted in heart in males. The number of animals with mononuclear cell infiltration was 1/3, 2/3, 2/3, and 3/3 in 0, 5, 20, and 50 mg/kg dose group. The severity index for such findings was described as very slight in all animals exept one high dose male that was graded as slight. However, due to increased occurrence of the infiltration in the test article treated animals, the finding is considered treatment related by the reviewer. No such changes were noted at recovery in males. There was no mononuclear cell infiltration in heart in females in this experiment.

Histopathology Table for Findings in Heart:

[H.E. staining] Organ/Tissue Findings	Group Dose (mg/kg) Animal No.	1 (IL-1Trap Diluted Placebo) 0			2 (IL-1 Trap) 5			3 (IL-1 Trap) 20.0			4 (IL-1 Trap) 50.0		
		2	4	6	12	14	16	18	20	22	24	26	30
Heart (interventricular septum, left atrium)													
Mononuclear cell infiltration, focal		±	.	.	±	.	.	±	.	.	.	.	.
Heart (left & right ventricle)													
Degeneration, myocardial fiber, focal		.	.	.	.	.	.	.	.	.	±	.	.
Mononuclear cell infiltration, focal		±	.	.	.	±	.	±	±	.	+	±	±

Histopathology Findings in Kidney:

Treatment related increase in the incidence of the mononuclear cell infiltration was noted in the kidney in males and females in this experiment. Three of three (3/3) animals (both males and females) in the low, mid, and high dose group showed focal mononuclear cell infiltration in pelvis and interstitium compare to 1/3 females in control. The severity index for such finding was slight in all animals. In addition, one animal (female) each from the low and high dose group showed increased eosinophil infiltration, the severity index for this finding was moderate to minimal. No control animal had eosinophil infiltration. All these kidney findings are considered to be treatment related by the reviewer. Incomplete recovery was noted at the end the experimental period.