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APPLICATION NUMBER:

103950/0

PHARMACOLOGY REVIEW(S)

TOXICOLOGIST'S REVIEW

BLA #: 99-1490 (RMS BLA#103950\0\0)
SPONSOR: AMGEN, INC.
PRODUCT: recombinant, human interleukin-1 receptor antagonist (rhIL-1ra; ANAKINRA™)
FORMULATION: lyophilized powder
RELATED DOCUMENTS: BB IND #3611
PROPOSED INDICATION: rheumatoid arthritis
ABBREVIATIONS: IL-1ra, recombinant, human interleukin-1 receptor antagonist; PBL, peripheral blood leukocytes; $t\frac{1}{2}_{elim}$, elimination half-life; AUC, area under the serum concentrations *vs.* time curve; kD, kilodaltons; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NOAEL, no observable adverse effect level; APTT, activated partial thromboplastin time; GGT, γ-glutamyl transpeptidase; GD, gestational day; PBS, phosphate buffered saline, pH 7.0; LDH, lactic dehydrogenase; LD_{50}, dose which causes lethality in 50% of the animals treated; CPE, cytopathic effect; BUN, blood urea nitrogen; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean cell volume; ELISA, enzyme-linked, immunosorbent assay; RIA, radioimmunoassay

received 12/28/99; assigned 1/14/00; completed 11/13/2000

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ABSTRACT:

The pharmacokinetic, acute and sub-chronic toxicities of IL-1ra were evaluated in rats, cynomolgus and Rhesus monkeys. Pharmacokinetic studies in rats, cynomolgus monkeys and rabbits showed dose- and time-related increases in C_{max} and AUC, and essentially 100% bioavailabiltiy after s/c injection, as compared to i/v administration. The volume of distribution at steady state was equivalent to the extracellular fluid space. Mean plasma clearances were in a range consistent with the rate of glomerular filtration in all three test species, suggesting that renal tubular secretion and degradation is the main route of elimination of IL-1ra. In general, treatment with IL-1ra was well-tolerated at doses of up to 100 mg/kg/d by either s/c or i/v injection for 14 days, and at doses of 2, 20 or 200 mg/kg/d, s/c in rats for 6 months. Toxicities were limited to mild to moderate, gross and histologic evidence of dose-related inflammation, hemorrhage, and fibrosis at the injection site and occurred in all species, regardless of route of administration. These findings were reversible on discontinuation of treatment with IL-1ra. The NOAEL for these effects in the rat was 2 mg/kg/d after daily s/c injection for 6 months, and 30 mg/kg/d following i/v injection for 14 or 28 days. The NOAEL in Rhesus monkeys was 150 mg/kg/24 hours by continuous i/v infusion for 7 days, 10 to 30 mg/kg/d by i/v bolus injection for 14 days, and 5 mg/kg/d by s/c injection for 14 days (as twice daily, divided doses of 2.5 mg/kg/injection). Antibodies to IL-1ra developed in both rats and cynomolgus monkeys by one month of treatment with daily IL-1ra injections, but diminished over time on study and did not appear to be neutralizing. No developmental, embryotoxic, or teratogenic effects of IL-1ra were observed in either Sprague-Dawley rats or New Zealand white rabbits at doses of up to 200 mg/kg/d, administered by s/c injection throughout the critical period of organogenesis. There were no effects of IL-1ra on reproductive capacity of either male or female rats in the F0 or F1 generations, and no effects of IL-1ra on male fertility parameters (sperm counts, morphology, motility, and production rates) were observed following 29 d of treatment with 100 mg/kg IL-1ra. In summary, IL-1ra toxicities are limited mainly to local injection site reactivity. The NOAEL in the rat of 2 mg/kg/d for 6 months is approximately 1.2 to 1.4-fold greater than the dose of 100 mg/d IL-1ra proposed for licensure. These data support the safety of IL-1ra treatment in the clinic, and its licensure for the treatment of rheumatoid arthritis.

INTRODUCTION:

Rheumatoid arthritis is a systemic autoimmune disorder of unknown etiology. The disease is characterized by chronic, symmetric, and erosive inflammation and synovitis of multiple peripheral joints, resulting in pain, stiffness, and loss of function. Histologically, arthritic joints show several distinct features of inflammation, including infiltration by mononuclear cells, and proliferation of fibroblasts and endothelial cells, erosion of cartilage and/or bone, and formation of pannus.

Although the etiology of rheumatoid arthritis is unknown, recent evidence has demonstrated a role of antigen-specific, cytotoxic and helper T lymphocytes and macrophages that produce interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α) and other proinflammatory cytokines in the disease. Interleukin-1 is a key mediator of both immune and inflammatory processes. Among its proinflammatory effects are induction of fever, hypotension, activation of macrophages, stimulation of B and T lymphocyte proliferation, activation, and secretion of other cytokines (*e.g.* IL-2), increased expression of vascular adhesion proteins on endothelial cells, release of histamine and other inflammatory mediators such as thromboxane, and increased

production of prostaglandins and collagenase by fibroblasts[1]. Direct injection of IL-1 into animal joints causes a transient, acute synovitis[2]. Continuous infusion of IL-1 into the knee joints of rabbits is arthritogenic, resulting in both acute and chronic inflammation. On microscopic evaluation, neutrophilic infiltrates, serous and fibrinous exudates, synovial cell proliferation and fibrosis, pannus formation, and erosion of cartilage and bone were prominent features[3]. Systemic administration of IL-1 can exacerbate antigen-induced arthritis in murine models, and can stimulate proliferation and prostaglandin and metalloproteinase production by human synoviocytes and *in vitro*[4]. Additionally, IL-1 is detectable in the plasma and synovial fluid of rheumatoid arthritis patients, and the levels appear to correlate with the activity of the disease.[5]

Interleukin-1 is synthesized by many cell types, including neutrophils, monocytes, and tissue macrophages. There are two forms of the protein, IL-1α and IL-1β, which are biochemically distinct, sharing only 22% sequence homology; however, they bind to and activate the same cell surface receptors. Interleukin-1 binds to two distinct cell surface receptors, IL-1RI and IL-1RII, although intracellular signaling and activation occurs exclusively on binding of IL-1 to IL-1RI. Interleukin-1 receptor I-mediated cell signaling is extremely sensitive, since a level of receptor occupancy as low as 2 to 3% is sufficient to elicit a full response[1].

Under normal conditions, control of IL-1 expression and secretion is tightly regulated, and the biologic activity of IL-1 is controlled at multiple levels. Interleukin-1 synthesis is inhibited by anti-inflammatory cytokines, prostaglandins, and glucocorticoids[1]. In addition, soluble forms of IL-1RII, consisting of the extracellular domain of IL-1RII can inhibit IL-1RI-mediated cell signaling and activation by actively competing with IL-1RI on the cell surface for IL-1 binding, and act to remove excess IL-1 from the circulation[1].

There also exists a specific receptor antagonist for IL-1, the interleukin-1 receptor antagonist, or IL-1ra., which provides another level of control of IL-1 biologic activity. The IL-1ra is a naturally occurring protein that shares approximately 20 to 25% sequence homology with both IL-1α and IL-1β, and when present in excess, can compete for IL-1 binding to both the IL-1RI

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[1] Dinarello, C.A. 1996. Biological basis for interleukin-1 in disease. *Blood*, **87**:2095-2147; Dinarello, C.A. 1991. Interleukin-1 and interleukin-1 antagonism. *Blood*, **77**:1627-1652; Dinarello, C.A. 1998. Interleukin-1, interleukin-1 receptors, and interleukin-1 receptor antagonist. *Internat. Rev. Immunol.*, **16**:457-499.

[2] Henderson, B. and E.R. Pettipher. 1988. Comparison of the *in vivo* inflammatory activities after intra-articular injection of natural and recombinant IL-1 alpha and IL-1 beta in the rabbit. *Biochem. Pharmacol.*, **37**:4171-4176.

[3] Feige, U., A. Karbowski, A.C. Rordorf, and A. Pataki. 1989. Arthritis induced by continuous infusion of interleukin-1 alpha into the rabbit knee joint. *Int. J. Tissue Reactions*, **11**:217-228.

[4] Hom, J.T., A.M. Bendele, and D.G. Carlson. 1988. In vivo administration with IL-1 accelerates the development of collagen-induced arthritis in the mouse. *J. Immunol.*, **141**:834-841; Dayer, J.M., B. deRochemonteix, B. Burrus, S. Demczuk, and C.A. Dinarello. 1986. Human recombinant interleukin-1 stimulates collagenase and prostaglandin E2 production by human synovial cells. *J. Clin. Invest.*, **77**:645-648.

[5] Eastgate, J.A., J.A. Symons, N.C. Wood, F.M. Grinlinton, F.S. diGiovine, and G.W. Duff. 1988. Correlation of plasma interleukin-1 levels with disease activity in rheumatoid arthritis. *Lancet*, **2**:706-709; Miyasaka, N., K. Sato, M. Goto *et al.* 1988. Augmented interleukin-1 production and HLA-DR expression in the synovium of rheumatoid arthritis patients: Possible involvement in joint destruction. *Arthritis & Rheumatism*, **31**:480-486

and IL-1RII receptors[1]. Binding of IL-1ra to the IL-1RI does not initiate IL-1 mediated cell signaling, and therefore effectively competitively inhibits the biologic activity of interleukin-1.

Anakinra (KINERET™, IL-1ra) is the recombinant form of the human IL-1ra. It is identical to the naturally occurring, non-glycosylated form of the protein, with the exception of the addition of an N-terminal methionine residue. *In vivo* studies of IL-1ra in animal models of arthritis have demonstrated reduction in joint swelling and inflammation, and inhibition of bone resorption[6], providing the rationale for rationale for clinical studies. Clinical trials with anakinra showed that IL-1ra treatment is effective improving the clinical signs and symptoms of rheumatoid arthritis in a broad range of patients with varying degrees of disease severity, and may be safely used in combination with other standard medications used in this setting, including methotrexate, gold penicillamine, lefluonimide, and azathrioprine.

Anakinra was tested for safety and efficacy in rheumatoid arthritis in four large, randomized, controlled clinical trials. Efficacy was measured as the percentage improvement in signs and symptoms of rheumatoid arthritis using the American College of Rheumatology (ACR) response criteria. Consistent improvements in the ACR scores were observed in all efficacy studies, with approximately twice as many patients treated with IL-1ra achieving an ACR_{20} response as those treated with placebo. Statistically significant treatment effects on ACR_{20} response rates were seen in these studies as early as week four on treatment, and improvements in ACR_{20} response rates in the IL-1ra treated subjects persisted over the six month duration of the studies. With the exception of swollen joints, all clinical signs and symptoms that are components of the ACR composite score showed statistically significant improvement in patients receiving anakinra as compared to placebo patients ($p \leq 0.05$, based on mean change from baseline).

Radiographic evidence of slowing of disease progression was also observed in patients receiving IL-1ra as compared to those treated with placebo, as demonstrated by evaluation of joint radiograms over the course of the study and an open-label extension phase. Significant reductions in the progression of structural joint damage were seen after one year of IL-1ra treatment as compared t placebo for both the standard total Sharp score ($p = 0.015$) and the erosion score ($p = 0.006$), with a trend approaching statistical significance in the joint space narrowing score ($p = 0.084$). Additionally, placebo patients who were crossed over to open-label treatment with anakinra for 6 months also demonstrated improvement in these three parameters.

Treatment of rheumatoid arthritis patients with IL-1ra was generally well tolerated, with the most common adverse event reported as mild injection site reactions. These were typically characterized by edema, erythema, ecchymosis, inflammation, and pain at the site of administration, and occurred in approximately 70% of the patients on study with no apparent relation in either incidence or severity to the dose of anakinra administered. Upper respiratory infections, sinusitis, influenza-like symptoms, urinary tract infections, and bronchitis were the most frequently reported infections, and occurred at similar rates between patients in the placebo and IL-1ra treated groups. However, the incidence of serious infections, including cellulitis,

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[6] Bendele, A. T, McAbee, M. Woodward *et al.* 1998. Effects of interleukin-1 receptor antagonist in a slow-release hylan vehicle on rat type II collagen arthritis. *Pharaceut. Res.*, **15**:1557-1561; Bendele, A., G. Senello, T. McAbee, J. Frazier, E. Chlipala, and B. Rich. 1999a. Effects of interleukin-1 receptor antagonist alone or in combination with methotrexate in adjuvant arthritic rats. *J. Rheumatol.*, **26**:1225-1229; Bendele, A., T. McAbee, G. Senello, J. Frazier, E. Chipala, and D. McCabe. 1999b. Sustained blood levels of interleukin-1 receptor antagonist in animal models of arthritis: Comparison of efficacy in animal models with human clinical data. *Arthritis and Rheumatism*, **42**:498-506.

pneumonia, and bone and joint infections across all studies was approximately three-fold higher in patients treated with anakinra (1.8%) as compared to those receiving placebo control (0.7%).

Minor reductions in peripheral blood leukocyte, platelet, and absolute neutrophil counts, and increases in the mean eosinophil differential were also observed in patients treated with IL-1ra, as compared to those in the placebo group. Since IL-1 is known to stimulate both neutrophil precursors and release of mature neutrophils form bone marrow, these effects may be directly related to anankinra's ability to suppress the biologic activity of interleukin-1.

Other adverse reactions associated with anakinra treatment at the licensed dose of 100 mg/d included headache, nausea, dyspepsia, abdominal pain and diarrhea, arthralgia and exacerbation of rheumatoid arthritis, and dizziness. In studies 3 and 4 of the pivotal design, serious adverse events occurred in 7.1% of patients treated with anakinra, as compared to 5.6% of patients receiving the placebo control.

PRECLINICAL PHARMACOLOGY AND PHARMACOKINETICS:

Pharmacokinetics Study Summary:

1. A single dose crossover study in male cynomolgus monkeys to compare the pharmacokinetics of r-metHuIL-1ra following intravenous and subcutaneous administration of r-metHuIL-1ra. Study # — 39-40 (Amgen Study #100-420). *Macaca fasicularis*, 2 ♂/group, 6 to 7 years old, weight range 6.16-6.51 kg; 10, 100 mg/kg IL-1ra (lot #25014M8), i/v or s/c; GLP; 4/5 – 11/10/99· —
   — **Volume 4, pp. 4-112.**

2. A single dose crossover study in male Sprague-Dawley rats to compare the pharmacokinetics of r-metHuIL-1ra following intravenous and subcutaneous administration of r-metHuIL-1ra. Study # —.39-43 (Amgen Study #100421). — CD (Sprague-Dawley) IGS rats, 2 %/group, 9 weeks old, weight range 352-378 g; 1, 3, 10, 30, 100 mg/kg IL-1ra (lot #25014M8), i/v or s/c; GLP; 7/13 – 11/11/99; —
   Volume 4, pp. 113-238.

3. A single dose crossover study in male New Zealand White rabbits to compare the pharmacokinetics of r-metHuIL-1ra following intravenous and subcutaneous administration of r-metHuIL-1ra. Study # — 39-42 (Amgen Study #100422). 2 males/group, 9 weeks old, weight range 1.95 – 2.20 kg; 10, 100 mg/kg IL-1ra (lot #25014M8), i/v or s/c; GLP; 6/23 – 11/11/99: — **Volume 4, pp. 239-352.**

4. The effect of multiple-dose administration of PEG-sTNF-RI on the pharmacokinetics of rmetHuIL-1ra in male Sprague-Dawley rats. Study #100564. 4 males/group, 11-12 weeks old, weight range, 369-413 g; 100 mg/kg IL-1ra (lot #25014M8), i/v on days 1 and 27; plus placebo, 1, 25 mg/kg/dose PEG-sTNF-RI (lot #36000D8), s/c, twice weekly for 4 weeks; non-GLP; 5/26 – 6/23/99; Amgen, Inc., Thousand Oaks, CA. **Volume 4, pp. 353-461.**

Pharmacokinetics Study Review:

Study # — 39-40 (Amgen Study #100420). ***A single dose crossover study in male cynomolgus monkeys to compare the pharmacokinetics of r-metHuIL-1ra following intravenous and subcutaneous administration of r-metHuIL-1ra.***

The pharmacokinetic profiles of IL-1ra after a single i/v or s/c administration were compared in male, cynomolgus monkeys. Animals were injected with either 10 mg/kg/ or 100 mg/kg IL-1ra into the cephalic vein or into the subcutis of the back, at a dose volume of 1.0 ml/kg. Samples of peripheral blood for determination of plasma concentration of IL-1ra were obtained immediately prior to dosing, at 10, 20, and 30 minutes after injection, and at 1, 2, 4, 8, 12, 18, 24, 36, and 48 hours after each dose. Animals were also observed for any overt, clinical signs of toxicity, including changes in body weight over the duration of the study. Following a 4 d washout period, the monkeys were retreated with the same dose of IL-1ra, by the opposite route of injection, blood samples collected at the same time points, and evaluated for plasma levels of IL-1ra by ELISA. Individual pharmacokinetic parameters, including C_{max}, T_{max}, $AUC_{(0-\infty)}$, clearance, and elimination half-lives were calculated from the values obtained using noncompartmental analysis and the WinNonlin professional software package (Version 1.5, Scientific Consulting, Inc.).

All animals survived the study duration, and were returned to the colony following completion of the study. There were no adverse effects on the overall health or body weights of the treated monkeys, at either dose of IL-1ra. A summary of the pharmacokinetic profiles obtained by the two routes of administration is presented in Table I, below:

Table I – Pharmacokinetic Profile of IL-1ra in Male Cynomolgus Monkeys

P/K Parameter	Intravenous Injection		Subcutaneous Injection	
	10 mg/kg	**100 mg/kg**	**10 mg/kg**	**100 mg/kg**
n	4	4	4	4
C_{max} (µg/ml)	157 ± 28[a]	2220 ± 570	8.48 ± 0.93	34.5 ± 6.3
T_{max} (h)	0 ± 0	0 ± 0	2.0 ± 0	4.5 ± 2.52
$T_{1/2elim}$ (h)	3.72 ± 1.35	5.55 ± 2.84	2.51 ± 0.53	3.44 ± 0.99
$AUC_{(0-\infty)}$ (µg+h/ml)	61.6 ± 8.5	723 ± 117	57.1 ± 9.6	596 ± 76
Cl or Cl/F (ml/min/kg)	2.75 ± 0.39	2.35 ± 0.38	2.99 ± 0.57	2.83 ± 0.4
V_c (ml/kg)	65.3 ± 12.1	47.1 ± 10.3	n.a.[b]	n.a.
V_{dss} (ml/kg)	176 ± 86	166 ± 76	n.a.	n.a.
MRT (h)	1.07 ± 0.51	1.23 ± 0.74	5.26 ± 0.94	11.4 ± 2.1
F (%)	n.a.	n.a.	92.4 ± 5.2	84.3 ± 19.0

[a] mean, ± S.D.
[b] n.a. = not applicable

Maximal plasma concentrations and total exposure, as defined by $AUC_{(0-\infty)}$ after i/v injection were approximately linearly related to the dose of IL-1ra injected. Similar findings for total exposure were observed after s/c injection; however, the C_{max} for the 100 mg/kg dose group was

approximately 2-fold lower than predicted. This finding was due to an apparent prolongation of absorption in this dose group after s/c administration (below).

Following i/v injection, plasma concentration-time profiles for IL-1ra best fit a three-compartmental model, with an initial, rapid distribution phase into the plasma volume (V_c, Table I, above), followed by subsequent distribution into approximately the extracellular fluid space at steady state (V_{dss}, table I, above). Elimination half-lives and clearances were not significantly different between the two doses and the two routes of administration, suggesting that the elimination of IL-1ra occurred via a non-saturable mechanism. Clearance rates were in a range consistent with the published glomerular filtration rate of 2.08 ml/min/kg in the monkey[7], suggesting that elimination of IL-1ra occurred predominantly by tubular secretion and degradation.

Maximal plasma concentrations of IL-1ra were obtained at approximately 2 hours following s/c injection of 10 mg/kg. At the higher dose of 100 mg/kg, absorption was more variable and delayed; mean T_{max} values for the 4 monkeys in this dose group were 4.5 $\pm$ 2.52 hours, and ranged between 2 and 8 hours after dosing. Mean residence times (MRT) were also increased in the monkeys treated with 100 mg/kg IL-1ra as compared to those injected with 10 mg/kg by the same route of administration, resulting in both longer, circulating concentrations of IL-1ra in the plasma, and a slower (although not statistically significant) elimination half-life. Mean residence time values for the 10 mg/kg and 100 mg/kg groups were 5.26 and 11.4 hours following s/c injection, respectively, with mean $T_{1/2elim}$ of 2.61 and 3.44 hours for the two respective dose groups.

Mean values for the bioavailability of IL-1ra after s/c injection of 10 or 100 mg/kg were 92% and 84%, respectively, as determined by the ratio of $AUC_{(0-\infty)}$ between the i/v and s/c doses.

In summary, s/c administration of either 10 or 100 mg/kg IL-1ra to adult, male cynomolgus monkeys resulted in serum concentration levels and exposures approximately equivalent to those achieved following i/v administration. Elimination half-lives were approximately 3 hours by either route of administration, and the volume of distribution at steady state was equivalent to the extracellular fluid space. Mean plasma clearances were in a range consistent with the rate of glomerular filtration in the monkey, suggesting that renal tubular secretion and degradation is the main route of elimination of IL-1ra in this species.

Study [illegible] 39-43 (Amgen Study #100421). ***A single dose crossover study in male Sprague-Dawley rats to compare the pharmacokinetics of r-metHuIL-1ra following intravenous and subcutaneous administration of r-metHuIL-1ra.***

The pharmacokinetic profiles of IL-1ra after a single i/v or s/c administration were compared in male, outbred Sprague-Dawley rats. Two animals per group were injected with 1, 3, 10, 30, or 100 mg/kg IL-1ra via either the intravenous or s/c route of injection, at a volume of 1.0 ml/kg. Samples of peripheral blood for determination of plasma concentrations of IL-1ra were obtained from the cervical veins immediately prior to dosing, at 10, 20, and 30 minutes after injection, and at 1, 2, 4, 8, 12, and 18 hours after each dose. Animals were also observed for any overt, clinical signs of toxicity, including changes in body weight over the duration of the study. Following a 5

[7] Davies, B. and T. Morris. 1993. Physiological parameters in laboratory animals and humans. *Pharmaceutical Res.*, **10**:1093-1095.

day washout period, the rats were retreated with the same dose of IL-1ra by the alternate route of injection, blood samples collected at the same time points, and evaluated for plasma levels of IL-1ra by ELISA. Individual pharmacokinetic parameters, including C_{max}, T_{max}, $AUC_{(0-\infty)}$, clearance, and elimination half-lives were calculated from the values obtained using noncompartmental analysis and the WinNonlin professional software package (Version 1.5, Scientific Consulting, Inc.).

All animals survived the study duration, and were euthanized by carbon dioxide inhalation at the end of the study. There were no adverse effects on the overall health or body weights of the treated rats at any dose of IL-1ra. A summary of the pharmacokinetic profiles obtained by the two routes of administration, as analyzed using noncompartmental modeling is presented in Tables II, A and B, below:

Table II – Pharmacokinetic Profile of IL-1ra in Male Rats

A. I/V Injection

P/K Parameter	Dose of IL-1ra				
	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
n	4	4	4	4	4
C_{max} (ng/ml)	7740 ± 2320[a]	30300 ± 14300	78400 ± 21300	237000 ± 135000	729000 ± 199000
$T_{1/2elim}$ (h)	1.15 ± 0.5	1.25 ± 0.6	0.96 ± 0.2	1.42 ± 0.4	1.40 ± 0.3
$AUC_{(0-\infty)}$ ng+h/ml	1700 ± 100	5890 ± 1370	17400 ± 2200	52400 ± 11200	18100 ± 63000
Cl (ml/min/kg)	9.85 ± 0.6	9.0 ± 2.7	9.7 ± 1.2	9.9 ± 2.01.10	10.2 ± 3.9
V_c (ml/kg)	139 ± 42	129 ± 90	135 ± 38	168 ± 107	145 ± 37
V_{dss} (ml/kg)	252 ± 37	238 ± 123	235 ± 49	270 ± 135	284 ± 90
MRT (h)	0.43 ± 0.09	0.43 ± 0.12	0.40 ± 0.06	0.45 ± 0.17	0.47 ± 0.07

[a] mean value ± standard deviation

B. S/C Injection

P/K Parameter	Dose of IL-1ra				
	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
n	4	4	4	4	4
C_{max} (ng/ml)	437 ± 60[a]	1860 ± 440	4400 ± 900	13100 ± 1500	30900 ± 1200
T_{max} (h)	0.71 ± 0.4	0.71 ± 0.4	1.17 ± 1.0	1.17 ± 1.0	1.33 ± 0.8
$T_{1/2elim}$ (h)	0.85 ± 0.08	0.82 ± 0.09	0.81 ± 0.14	0.84 ± 0.06	1.11 ± 0.07
$AUC_{(0-\infty)}$ ng+h/ml	1280 ± 160	5570 ± 1500	16300 ± 2400	51300 ± 5900	158000 ± 15000
MRT (h)	1.83 ± 0.1	1.95 ± 0.1	2.3 ± 0.2	2.41 ± 0.3	3.25 ± 0.3
F (%)	75.3 ± 9.7	95.0 ± 14.0	94.0 ± 11.0	100 ± 15.0	97.8 ± 43.1

[a] mean, ± S.D.

Maximal plasma concentrations and total exposure, as defined by $AUC_{(0-\infty)}$ were approximately linearly related to the dose of IL-1ra injected, by either the i/v or s/c route of injection.

Elimination half-lives by either route of exposure were approximately 1 hour. Following s/c injection of IL-1ra in rats, bioavailability, as calculated by the ratio of $AUC_{(0-\infty)}$ for the i/v and s/c doses ranged between 75 and 100% of the same dose administered by i/v bolus injection. Both the time to peak plasma concentration and the MRT after s/c administration appeared to be increased in proportion to the dose of IL-1ra injected, suggesting an apparent prolongation of absorption in the higher dose groups after s/c administration.

Following i/v injection, plasma concentration-time profiles for IL-1ra best fit a three-compartmental model. Recalculation of the parameters using this model showed an initial, rapid distribution phase into a central compartment with a mean volume between the dose groups of 46.0 ml/kg (V_c), followed by subsequent distribution into approximately the extracellular fluid space at steady state (V_{dss}, Table I, above). Elimination half-lives and clearances were not significantly different between the different doses and the two routes of administration, suggesting that the elimination of IL-1ra occurred via a non-saturable mechanism. Clearance rates were in a range consistent with the published glomerular filtration rate of 5.24 ml/min/kg in the rat[8], suggesting that elimination of IL-1ra occurs predominantly by tubular secretion and degradation.

Maximal plasma concentrations of IL-1ra were obtained at approximately 1-2 hours following s/c injection of 1- 100 mg/kg. At the higher dose of 100 mg/kg, absorption was more variable and delayed; mean T_{max} values for the rats in this dose group were 1.3 ± 1.1 hours, and ranged from between 0.3 and 2 hours after dosing. Mean residence times were also increased in a dose-related fashion after s/c administration resulting in longer circulating levels of IL-1ra in the plasma. However, there was no apparent decrease in clearance after s/c injection of the different doses, nor increase in elimination half-life.

Mean values for the bioavailability of IL-1ra after s/c injection of 10 or 100 mg/kg were 100% and 97.8%, respectively, as determined by the ratio of $AUC_{(0-\infty)}$ between the i/v and s/c doses.

In summary, i/v or s/c administration of 1-100 mg/kg IL-1ra to adult, male Sprague-Dawley rats resulted in dose-related, linear increases in maximal serum concentration levels and total exposures, as defined by $AUC_{(0-\infty)}$. Total exposures at each dose level after s/c injection were approximately equivalent to those achieved following i/v administration, with mean bioavailability ranging from 75 to 100%. Elimination half-lives were approximately 1 hour by either route of administration, and the volume of distribution at steady state was equivalent to the extracellular fluid space. Mean plasma clearances were in a range consistent with the rate of glomerular filtration in the rat, suggesting that renal tubular secretion and degradation is the main route of elimination of IL-1ra in this species.

Study # __ 39-42 (Amgen Study #100422). ***A single dose crossover study in male New Zealand White rabbits to compare the pharmacokinetics of rmetHuIL-1ra following intravenous and subcutaneous administration of rmetHuIL-1ra.***

Male, New Zealand white rabbits were used to evaluate the pharmacokinetic profiles of IL-1ra after a single i/v or s/c administration. Animals were injected with either 10 mg/kg/ or 100

[8] Kim, D.-C., B. Reitz, D.F. Carmichael, and D.C. Bloedow. 1995. Kidney as a major clearance organ for recombinant human interleukin-1 receptor antagonist. *J. Pharmaceutical Sci.*, **84**:575-580.

mg/kg IL-1ra into the auricular vein (i/v) or into the subcutis of the back (s/c), at a dose volume of 1.0 ml/kg. Samples of peripheral blood for determination of plasma concentration of IL-1ra were obtained immediately prior to dosing, at 15 and 30 minutes after injection, and at 1, 2, 4, 8, 12, 18, 24, 36, and 48 hours after each dose. The IL-1ra treated rabbits were also observed for any overt, clinical signs of toxicity, including changes in body weight over the duration of the study. Following a 5 d washout period, the rabbits were crossed over to treatment with the same dose of IL-1ra by the alternate route of injection, blood samples collected at the same time points, and evaluated for plasma levels of IL-1ra by ELISA. Individual pharmacokinetic parameters, including C_{max}, T_{max}, $AUC_{(0-\infty)}$, clearance, and elimination half-lives were calculated from the values obtained using noncompartmental analysis and the WinNonlin professional software package (Version 1.5, Scientific Consulting, Inc.). Additional plasma samples were analyzed for the development of anti-IL1RA antibody activity, using an EIA specific for anti-IL1RA.

All animals survived the study duration, and were euthanized by CO_2 inhalation following the final blood sampling on d 7 of the study. Twenty-four and 48 hours after injection of the second, i/v dose, rabbits who had previously received 100 mg/kg IL-1ra by the s/c dose developed decreased appetite and stool volumes, and hematuria. These findings were not observed in the two animals at this dose who received the i/v injection for the first administration, and the s/c injection for the second administration. There were no adverse effects on the overall health or body weights of any rabbits treated with 10 mg/kg IL-1ra, at any time point on study.

A summary of the pharmacokinetic profiles obtained by the two routes of administration, as calculated using nonparametric methods is presented in Table III, below:

Table III – Pharmacokinetic Profile of IL-1ra in Male New Zealand White Rabbits

P/K Parameter	Intravenous Injection		Subcutaneous Injection	
	10 mg/kg	100 mg/kg	10 mg/kg	100 mg/kg
n	4	2	4	4
C_{max} (ng/ml)	85700 ± 33900[a]	744000[b]	7750 ± 940	40900 ± 4700
T_{max} (h)	0 ± 0	0	1.02 ± 0.01	1.13 ± 0.63
$T_{1/2elim}$ (h)	0.96 ± 0.18	2.30	1.10 ± 0.19	1.36 ± 0.06
$AUC_{(0-\infty)}$ (ng+h/ml)	30600 ± 9700	335000	30300 ± 2200	223000 ± 35000
Cl or Cl/F (ml/min/kg)	5.89 ± 1.88	5.63	5.53 ± 0.41	7.61 ± 1.06
V_c (ml/kg)	133 ± 58	178	n.a.[c]	n.a.
V_{dss} (ml/kg)	185 ± 62	231	n.a.	n.a.
MRT (h)	0.53 ± 0.08	0.67	2.73 ± 0.27	3.85 ± 0.21
F (%)	n.a.	n.a.	107 ± 35	79.2 ± 15.9

[a] mean, ± S.D.

[b] n = 2; animals #7 and #8M excluded from analysis due to development of anti-IL1RA antibody titers

[c] n.a. = not applicable

Following i/v injection of IL-1ra, maximal plasma concentrations and total exposure, as defined by $AUC_{(0-\infty)}$ were approximately linearly related to the dose injected. Similar findings for total

exposure were observed after s/c injection; however, the C_{max} for the 100 mg/kg dose group was approximately 2-fold lower than predicted. This finding could not be explained by an apparent prolongation of absorption in this dose group after s/c administration, since values for T_{max} for the two doses were approximately equivalent (below).

Compartmental analysis of the plasma concentration-time profiles for IL-1ra after i/v injection showed that the data best fit a three-compartmental model, with an initial, rapid distribution phase into the plasma volume (V_c, Table III, above), followed by subsequent distribution into approximately the extracellular fluid space at steady state (V_{dss}, Table III, above). An approximate 4-fold increase in elimination half-life, and two-fold increases in both V_{dss} and MRT were detected for rabbits treated with 100 mg/kg IL-1ra as compared to animals treated with 10 mg/kg by the i/v route. However, the calculated clearances were not significantly different between the two doses and the two routes of administration. Further analysis of the data demonstrated that two animals, #7M and #8M, had developed significant anti-IL1ra antibody titers by 48 h after the second dose of 1:12800 and 1:51200, respectively, suggesting that the increased volume of distribution, as well as the prolonged half-life and MRT of IL-1ra were likely related to circulating, antibody-bound IL-1ra, which was not distinguishable from free IL-1ra by the ELISA assay used. These two animals were excluded from the final calculations presented in Table III, above. However, mean values for MRT and $T_{1/2_{elim}}$ in the two remaining animals in this dose group were still increased approximately 2-fold compared to the rabbits treated with 10 mg/kg IL-1ra by i/v injection.

Maximal plasma concentrations of IL-1ra were obtained at approximately 1 h following s/c injection of 10 mg/kg. At the higher dose of 100 mg/kg, absorption was more variable and delayed; mean T_{max} values for the 4 rabbits in this dose group were 1.13 ± 0.63 hours, and ranged between 0.5 and 2 hours after dosing. Mean residence times were also increased by 40% in the rabbits treated with 100 mg/kg IL-1ra, as compared to those injected with 10 mg/kg by s/c injection, resulting in both longer, circulating concentrations of IL-1ra in the plasma, and slower (although not statistically significant) clearance and elimination half-life. Mean residence time values for the 10 mg/kg and 100 mg/kg groups were 2.73 and 3.85 hours following s/c injection, respectively, with mean $T_{1/2_{elim}}$ of 1.10 and 1.36 hours for the two respective dose groups.

Mean values for the bioavailability of IL-1ra after s/c injection of 10 or 100 mg/kg were $107 \pm 35\%$ and $79.2 \pm 15.9\%$, respectively, as determined by the ratio of $AUC_{(0-\infty)}$ between the i/v and s/c doses.

In summary, s/c administration of either 10 or 100 mg/kg IL-1ra to male New Zealand White rabbits resulted in serum concentration levels and exposures approximately equivalent to those achieved following i/v administration. Elimination half-lives were approximately 1-2 hours by either route of administration, and the volume of distribution at steady state was equivalent to the extracellular fluid space. Development of significant antibody titers against IL-1ra was observed in the two rabbits initially injected with 100 mg/kg by the s/c route, resulting in prolonged MRT and elimination half-life of IL-1ra following a second, i/v dose. Anti-IL1ra antibody titers were undetectable in animals treated with either 100 mg/kg IL-1ra by the i/v route followed by the s/c exposure, or in all rabbits treated with the 10 mg/kg dose regardless of sequence.

Study #100564. ***The effect of multiple-dose administration of PEG-sTNF-RI on the pharmacokinetics of r-metHuIL-1ra in male Sprague-Dawley rats.***

Comment: This study is not relevant to the current application for IL-1ra monotherapy in rheumatoid arthritis. A complete review of the data from this study will be conducted at the time of application for the combination therapy. At the present time, a brief summary of the findings will be presented here.

A total of 12 Sprague-Dawley rats received a single i/v dose of 100 mg/kg IL-1ra on day 1. Beginning 24 h later, four rats per group received s/c injections of placebo, 1, or 25 mg/kg PEG-sTNF-RI twice weekly for a total of 8 doses. On study d 27, following the final dose of PEG-sTNF-RI all rats received a second injection of 100 mg/kg IL-1ra. Plasma samples were collected on study days 1 and 27 prior to IL-1ra dosing and at selected time points up to 48 h later, and analyzed by ELISA for both IL-1ra and PEG-sTNF-RI levels. Development of both anti-IL1ra and anti-PEG-sTNF-RI antibody activity was monitored on serum samples obtained prior to each dose of test article, for seroconversion measurements by EIA analysis.

There were no statistically significant differences in IL-1ra pharmacokinetic parameters following three weeks of dosing with either placebo, 1, or 25 mg/kg PEG-sTNF-RI. After the second injection of IL-1ra, both the C_{max} and the $AUC_{(0-\infty)}$ were increased by 16 to 53%, as compared to following the first injection. However, similar increases were observed in animals receiving the placebo control as were observed in rats injected with either 1 or 25 mg/kg/dose PEG-sTNF-RI. The results suggest that co-administration of PEG-sTNF-RI had no effects on single dose pharmacokinetics of i/v IL-1ra.

At the end of the treatment period, all rats treated with PEG-sTNF-RI developed positive seroreactivity, as detected by EIA. The presence of antibodies increased the clearance of PEG-sTNF-RI in the 1 mg/kg/dose group, but not in the 25 mg/kg/dose group. Two of 8 rats tested showed positive antibody development against IL-1ra at low titers (1:100) following the second i/v injection. Histologic evaluation of the kidneys demonstrated renal tubule vacuolization in animals treated with IL-1ra and 25 mg/kg/dose PEG-sTNF-RI, but not in the rats injected with IL-1ra alone, or in combination with 1 mg/kg PEG-sTNF-RI. Other histopathologic findings in the kidney included cortical infarcts in 2 rats in the IL-1ra alone group, pyelonephritis in one rat each in the IL-1ra alone and the high-dose, combination treatment group, and interstitial nephritis in one rat in the group treated with IL-1ra and 25 mg/kg/dose PEG-sTNF-RI. However, these findings were minimal in severity, and were considered incidental to treatment with either or the test articles.

Safety Pharmacology Study Summary:

1. A general pharmacology study of anakinra. Study #SY05-T94-03 (—>Study ' —, 66-80). —.CD-1(ICR) mice, 6 males/group, 6 weeks old, weight range 24.0-32.4 g; 20, 70, 200, 720 mg/kg IL-1ra, lot #, i/v or s/c; — Wistar rats, 6 males/group, 8 weeks old, weight range 267-304 g, 20, 70, 200, 720 mg/kg IL-1ra, lot #, i/v· — Hartley guinea pigs, 20 males, 4 weeks old, weight range 261-294 g (isolated ileum used for *in vitro* testing); 3 male beagles, 9-10 month old; weight range 10.4-11.8 kg, sequential dosing with 10, 30, 90 mg/kg IL-1ra, lot #, i/v infusion; GLP; ' —

Safety Pharmacology Study Review:

Study #SY05-T94-03 — Study [1] — 66-80). *A general pharmacology study of anakinra.*

Safety pharmacology parameters were evaluated in male mice and rats, and in male beagle dogs following injection with IL-1ra, and in an *in vitro* assay using isolated guinea pig ileum. For ease of review, each study and its findings is presented individually, below.

a). Effects of IL-1ra on general activity and behavior in mice.

This study was conducted to determine the behavioral toxicities potentially associated with IL-1ra treatment. Groups of six male mice each were treated with either vehicle or 20, 70, or 200 mg/kg IL-1ra by a single, i/v injection and monitored according to the methods of Irwin[9] for acute behavioral changes prior to dosing, and at 15, 30, 60, and 120 minutes following administration of the control or test articles.

No evidence of mortality, clinical signs of toxicity, nor effects on general activity or behavioral changes were observed in any of the mice on study. In summary, there were no remarkable behavioral changes or clinical toxicities noted in mice after a single, i/v administration of 20, 70, or 200 mg/kg IL-1ra.

b). Effects of IL-1ra on the locomotor activity in mice.

The effects of IL-1ra on spontaneous locomotor activity were determined in male mice after treatment with vehicle, 20, 70, or 200 mg/kg IL-1ra by a single, i/v injection. Mobility was measured every 15 min for 2 h after injection, using a standard activity meter consisting of a photo cell counter equipped with three infrared photo cells. Each interception of an infrared beam lasting longer than 0.1 sec was counted as one locomotor activity.

IL-1ra at doses of up to 200 mg/kg had no apparent effects on spontaneous locomotor activity in mice, as compared to animals injected with the placebo control. In summary, a single i/v administration of IL-1ra to mice at doses of 20, 70, or 200 mg/kg had no significant effects on spontaneous locomotor activity.

c). Effects of IL-1ra on thiopental-induced sleeping time in the mouse.

The objective of this study was to assess the effects of intravenous administration of IL-1ra on thiopental-induced sleeping time in mice. Six male mice per group were treated with vehicle, 20, 70, or 200 mg/kg IL-1ra by a single, i/v injection. Fifteen minutes later, each animal received a single i/p injection of 455 ng/kg sodium thiopental. Time to onset of sleep (as defined by loss of righting reflex), and duration of sleeping time of each mouse was recorded. A loss of righting reflex for more than 15 sec was considered sleep.

No significant effects of IL-1ra, as compared to the vehicle control were noted on thiopental-induced sleeping time in mice treated with 20, 70, or 200 mg/kg by a single, i/v injection.

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[9] Irwin, S. 1964. Drug screening and evaluation of new drugs in animals. *In*: Animal and Clinical Pharmacologic Techniques in Drug Evaluation, J.M. Nodine and P.I. Siegler, eds., Year Book Medical Publishers, Inc., Chicago, IL; pp. 36-64.

d). Effects of IL-1ra on acetic acid-induced writhing in mice.

Four groups of six mice each were injected i/v with placebo control, 20, 70, or 200 mg/kgIL-1ra. Fifteen minutes later, 10 ml/kg of a 0.6% solution of acetic acid in water was injected i/p to induce writhing. The frequency of writhing over a 10 minute period was monitored, beginning 10 min after injection of the acid challenge.

Following a single i/v injection of 20, 70, or 200 mg/kg IL-1ra, there were no significant effects of the treatment on the frequency of acetic-acid induced writhing in male, ICR mice.

e). Effects of IL-1ra on convulsions in mice.

The potential anti-convulsant activity of IL-1ra was evaluated in male, ICR mouse using the minimal pentetrazol and maximal electroshock tests. Six mice per group were treated with placebo control, 20, 70, or 200 mg/kg IL-1ra by a single i/v injection. For the groups in the maximal test, 50 mA electroshock was applied to the cornea of both eyes 15 min later, using an electronic stimulator to induce convulsions, and the incidence of tonic and/or clonic convulsions in the mice treated with IL-1ra compared with that in the control group.

For the groups in the minimal pentetrazol test, animals received 150 mg/kg pentetrazol by i/p injection 15 min after treatment with a single i/v injection of placebo, 20, 70, or 200 mg/kg IL-1ra. The time until onset of convulsions and the duration of tonic convulsions, and the number and time to death(s) was recorded, and compared to that observed in the control group. In a second group of animals, the proconvulsant activity of IL-1ra to a sub-threshold dose (50 mg/kg) of pentetrazol was evaluated. Mice were injected i/p with pentetrazol 15 min following a single, i/v injection of placebo or 20, 70, or 200 mg/kg IL-1ra, and the incidence of tonic and/or clonic convulsions observed for 120 min.

In the proconvulsant test, IL-1ra had no effect on the incidence of clonic convulsions, and did not induce tonic convulsions in mice treated with the sub-threshold (50 mg/kg) dose of pentetrazol. In the minimal pentetrazol test, IL-1ra had no effect on the time until onset of tonic convulsion, the duration of tonic convulsions, or the time until death in mice treated with 150 mg/kg pentetrazol. Similarly, treatment of mice with 20, 70, or 200 mg/kg IL-1ra had no effect on the incidence of tonic convulsions or mortality, as compared to the control group, in mice given the maximal electroshock test.

In summary, IL-1ra at doses of 20, 70, and 200 mg/kg had no pro- or anti-convulsant activity in mice under the conditions of the present assays.

f). Effects of IL-1ra on rectal body temperature in rats.

Changes in body temperature in male Wistar rats were evaluated after treatment with IL-1ra. Six rats per group were treated with placebo, 20, 70, or 200 mg/kg IL-1ra by a single, i/v injection. Rectal body temperature of each rat was measured 15, 30, 60, and 120 minutes later, and the effects of treatment with the test article compared to the control group.

There were no effects of treatment with IL-1ra at 20, 70, or 200 mg/kg on the rectal body temperature in rats at any time point after treatment, as compared to animals injected with the

placebo control article. In conclusion, intravenous administration of IL-1ra at doses of 20, 70, or 200 mg/kg resulted in no significant elevations or decreases of body temperature in rats.

g). Effect of IL-1ra on autonomic nervous system and smooth muscle.

The effects of IL-1ra on the pharmacologically induced contraction of isolated guinea pig ileum in response to challenge with acetylcholine (1 µg/ml), histamine (1 µg/ml), and barium chloride (100 µg/ml) were evaluated in an *in vitro* test. Approximately 2 cm segments of the ileum from male Hartley guinea pigs (weight range 261-294 g, 4 weeks old) were removed and suspended in Tyrode's solution at 37°C, aerated with 95% O_2 and 5% CO_2. Separate sections of ileum were used for each agonist, and the changes in tension of the isolated sections were recorded using a thermal array recorder and an amplifier system. The effects of the vehicle control or IL-1ra at 0.1, 0.3, and 0.9 mg/ml on both spontaneous and agonist-induced contractions were examined either as single agents added to the bath fluid, or when added together with each of the three agonists at doses, which induced submaximal contractions.

No significant effects on either the resting tone of guinea pig ileal muscle, nor in the amplitude of the contractions induced by submaximal concentrations of acetylcholine, histamine, or barium chloride were noted after inclusion of IL-1ra at concentrations of 0.1, 0.3, or 0.9 mg/ml in the bath fluid. Therefore, it was concluded that IL-1ra at these dose levels had no direct agonist activity (modification of resting tone), nor caused any significant changes in the amplitude of the contractions induced by a standard panel of agonists in this assay.

h). Effects of IL-1ra on the cardiovascular and respiratory systems in anesthetized beagle dogs.

The objective of this study was to evaluate the effects of IL-1ra on the cardiovascular and respiratory systems in the anesthetized beagle dog. Three male beagle dogs (weight range 10.4-11.8 kg, aged approximately 9-10 months) were anesthetized by an i/v injection of 20 mg/kg sodium pentobarbital. Anesthesia was maintained by continuous, i/v infusion of 5-10 mg/kg/hour sodium pentobarbital solution. Blood pressure was monitored using a catheter inserted into the femoral artery and connected to a pressure transducer; heart rate was determined electronically using a heart rate meter, with systolic blood pressure as the trigger. Femoral blood flow information was collected using an electromagnetic blood flow meter. Respiration rate was measured by calculating the differences in temperature between exhaled and inhaled air, using a coupler amplifier connected to a tracheal cannula. All parameters were measured prior to IL-1ra administration, then continuously for a total of 30 min after injection of placebo, or 10, 30, or 90 mg/kg IL-1ra.

Electrocardiogram recordings were obtained using electrodes placed on the limbs and monitored using a [redacted] electrocardiograph, and were obtained prior to and immediately after dosing, and at 5, 10, 15, and 30 min after administration of IL-1ra.

Administration of IL-1ra at the doses specified above had no remarkable effects on the systolic, diastolic, or mean blood pressure, and did adversely affect either the heart or respiratory rates. A transient increase in heart rate was observed in one dog (animal #J3M) at the start of the 30 mg/kg IL-1ra administration, and 5 min after beginning infusion of the 90 mg/kg test dose.

Transient increases in respiratory rate, and decreases in heart rate, systolic, diastolic, and mean blood pressure were observed in all animals after the first infusion of the vehicle control solution. However, these changes were not observed following the second infusion of the control article, and were considered incidental to treatment.

An increase in mean femoral blood flow, which was neither significant nor dose-related was noted in one dog (animal #J3M) at the start of the 30 mg/kg IL-1ra administration. This finding did not recur on treatment with the 90 mg/kg dose, and was therefore considered incidental to the test article. In the other two dogs at doses of 10, 30, or 90 mg/kg IL-1ra, there were no significant effects of treatment on femoral artery blood flow.

All three dogs exhibited transient decreases, followed by increases in dogs #J1M and #J3M in femoral blood flow during the first administration of the vehicle, as compared to baseline measurements. However, these findings were not observed during the second infusion of the vehicle control article, and were considered incidental to treatment.

Electrocardiogram recordings revealed no significant effects of treatment with 10, 30, or 90 mg/kg IL-1ra on the P-R intervals or the QRS complex. A slight decrease in the R-wave amplitude, and the appearance of a negative T wave were observed in all three dogs during the first, but not the second infusion of the control article. The clinical significance of this finding is unknown.

Comment: In the discussion section of the report, it was noted that the vehicle (placebo) for IL-1ra contains polysorbate 80, which has previously been reported to cause cardiovascular effects, including hypotension due to negative inotropic effects and vasodilation secondary to endogenous histamine release[10]. However, the cardiovascular effects were noted only on first administration of the vehicle control, not after the second infusion, and not during infusions of the test article at the different doses, which would be expected to also contain polysorbate 80.

In summary, i/v infusion of either vehicle, or IL-1ra at doses of 10, 30, or 90 mg/kg at 30 minute intervals had no remarkable effects on blood pressure, respiratory or heart rates, electrocardiogram profiles, or mean femoral artery blood flow. Transient changes, including increases in heart rate, femoral blood flow, and slight changes in ECG profiles were observed in one or more dogs following the first, but not the second infusion of the vehicle control article. A transient, although not consistent or dose related increase in femoral blood flow was noted in one dog immediately following infusion of the 30 mg/kg dose, but was not present after treatment with the highest dose. The significance of these findings in relationship to the cardiovascular pharmacology of IL-1ra in humans is unknown.

i). Effects of IL-1ra on intestinal motility using the charcoal propulsion test in the mouse.

The effects of intravenous administration of IL-1ra on gastrointestinal motility in the mouse were assessed in a charcoal propulsion assay. Six mice per group were fasted overnight, then injected i/v with vehicle, 20, 70, or 200 mg/kg IL-1ra. Fifteen minutes after treatment with control or test article, each mouse received 0.1 ml of a 7.5% suspension of activated charcoal powder in 5%

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[10] Masini, E., J. Planchenault, F. Pezziardi, P. Gautier, and J.P. Gagnol. 1985. Histamine-releasing properties of polysorbate 80 *in vitro* and *in vivo*: Correlation with its hypotensive action in the dog. *Agents Actions*, **16**:470-477; Gough, W.B., R.H. Zeiler, P. Barreca, and N. El-Sharif. 1982. Hypotensive action of commercial intravenous aminodarone and polysorbate 80 in dogs. *J. Cardiovasc. Pharmacol.*, **4**:375-380.

aqueous, arabic gum solution by oral gavage. Thirty minutes after charcoal treatment, each mouse was sacrificed and the intestinal tracts removed. The total length of the small intestine, as well as the distance at which the charcoal traveled from the pyloric sphincter was determined, and expressed as a ratio of the total gut length.

No remarkable effects of IL-1ra treatment were noted on gastrointestinal tract motility in mice, after a single, i/v injection of 20, 70, or 200 mg/kg as compared to mice injected with the placebo control. It can be concluded from these data that in the mouse, IL-1ra has no apparent adverse effects on intestinal motility.

j). Effects of IL-1ra on urine volume and electrolyte excretion in the rat.

The effects of IL-1ra on urinary volume, electrolyte excretion, creatinine clearance, and excretion or urinary N-acetyl-β-D-glucosaminidase (NAG) were examined in male Wistar rats. Six rats per group were treated twice with 15 ml/kg physiologic saline by oral gavage, at an interval of 30 min. Thirty minutes later, the rats were treated with a single i/v dose of placebo control, 20, 70, or 200 mg/kg IL-1ra, and the rats were immediately placed in individual metabolic cages. Urine was collected over the next 24 h at 0-3, 3-6, and 6-24 h intervals after treatment, and the volume and creatinine levels were determined for each animal. Blood samples were also drawn at the end of each collection interval for the determination of plasma creatinine levels. Urine samples were analyzed using an automatic electrolyte analyzer for Na^+, K^+, Cl^-, and for NAG using a commercially available test kit.

No remarkable alterations in urine volume, electrolyte excretion, creatinine clearance, or urinary NAG levels were noted in rats treated with 20, 70, or 200 mg/kg IL-1AR, i/v as compared to animals injected with the placebo control article.

In conclusion, i/v injection of IL-1ra in rats, mice, and beagle dogs as doses from 10 to 200 mg/kg had no significant effects on central or autonomic nervous system function, behavior, cardiovascular, digestive system, or renal physiology, as compared to animals treated with the vehicle control.

PRECLINICAL TOXICOLOGY:

Mutagenicity Study Summary:

1. *Salmonella* /mammalian-microsome plate incorporation mutagenicity assay (Ames test) and *Escherichia coli* WP2uvrA reverse mutation assay with a confirmatory assay – interleukin-1 receptor antagonist (IL-1ra). Study #SY05-T92-13. IL-1ra (lot #L93-009), 0.10, 0.33,1.0, 3.3, 10, 33, 100, 333, 1000, or 5000 µg/plate, or vehicle control (citrate-buffered saline plus 0.1% Tween, pH 6.5), with or without Aroclor™-activated, rat liver microsomes (S-9 fraction); GLP; 2/9 – 7/13/93· —— **Volume 27, pp. 5-71.**

2. L5178Y TK +/- mouse lymphoma mutagenesis assay with a confirmatory assay – interleukin-1 receptor antagonist (IL-1ra). Study #SY05-T92-14. IL-1ra (lot #(lot #L93-009), 0.5, 1.0, 5.0, 10, 50, 100, 500, 1000, or 5000 µg/ml, or vehicle control (citrate-buffered saline plus 0.1% Tween, pH 6.5, lots #L93-008 and #L93-012), with or without Aroclor™-

activated, rat liver microsomes (S-9 fraction); GLP; 2/3 – 8/2/93: [redacted]
[redacted] **Volume 27, pp. 5-107.**

3. *In vitro* cytogenetics test of interlekin-1 receptor antagonist (IL-1ra). Study #SY05-T92-15. IL-1ra (lot #L93-009), 0.2. 0.6, 2.0, 6.0, 20, 60, 200, 250, 500, 600, 1000, or 2000 µg/ml, or vehicle control (citrate-buffered saline plus 0.1% Tween, pH 6.5), with or without Aroclor™-activated, rat liver microsomes (S-9 fraction); GLP; 2/4 – 8/4/93; Microbiological **Volume 27, pp. 109-146.**

4. Micronucleus cytogenetic assay in mice – interleukin-1 receptor antagonist (IL-1ra). Study #SY05-T92-16. ICR mice; 5/sex/group, weight range 29.5-36.5 g (male), 22.8-27.7 g (female); 500, 1000, 2000 mg/kg IL-1ra (lot #L93-009), or vehicle control (citrate-buffered saline plus 0.1% Tween, pH 6.5, lots #L93-008 and #L93-012); GLP; 2/11 – 8/2/93; **Volume 27, pp. 147-173.**

Mutagenicity Study Review:

Study #SY05-T92-13. ***Salmonella /mammalian-microsome plate incorporation mutagenicity assay (Ames test) and Escherichia coli WP2uvrA reverse mutation assay with a confirmatory assay – interleukin-1 receptor antagonist (IL-1ra).***

Cultures of TA98, TA 100, TA 1535, TA97a, and TA102 test strains of *Salmonella typhimurium* and *Escherichia coli* tester strain WP2*uvr*A were incubated in triplicate at 37°C for 72 hrs with IL-1ra at multiple concentrations ranging from 0.1 to 5000 µg/plate. The IL-1ra preparation was diluted in citrate-buffered saline plus 0.1% Tween 80, which was also used as the vehicle control for the experiment. The highest concentration of IL-1ra tested (5000 µg/plate) was the highest level that could be feasibly tested in this system, based on reconstitution vial concentrations. Positive control chemicals used were 2-aminoanthracene (1.0 µg/plate), 2-nitrofluorene (1.0 µg/plate), sodium azide (1.0 µg/plate), 9-aminoacridine (75 µg/plate, tester strain TA1537 only), and methyl methanesulfonate (1000 µg/plate, *E. coli* WP2*uvr*A test strain only).

Range-finding studies were initially conducted to determine the cytotoxic (growth-inhibitory) effect of IL-1ra on the test system. *Salmonella typhimurium* tester strain TA 100 and *E. coli* tester strain WP2*uvr*A were incubated in increasing concentrations of IL-1ra (0.1 to 5000 µg/plate, by half-log dilutions), both in the presence and the absence of metabolic activation by rat liver microsomes. No cytotoxicity of IL-1ra to either of the tester strains was observed in the range-finding assay, at concentrations of up to 5000 µg/plate.

The mutagenicity assay was performed using the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA97a, and TA102, and the *E. coli* test strain WP2*uvr*A. Five doses of IL-1ra, ranging from 33 to 5000 µg/plate were tested in the assay, in the presence or absence of Aroclor™ activated rat liver microsomes. IL-1ra exhibited no toxicity to any of the test strains at doses as high as 5000 µg/plate. All of the test strains treated with IL-1ra exhibited mean reversion frequencies (number of histidine$^+$ revertant colonies above the control incidence) similar to the vehicle controls, with or without S9 activation. There was no evidence of dose-related effects on reverse mutation up to the highest concentration of IL-1ra tested. Each positive control produced a marked mutagenic response in the appropriate tester strain. These results

were confirmed in three additional assays, using the same range of concentrations of IL-1ra and positive and negative controls, with or without metabolic activation.

In summary, IL-1ra exhibited no evidence of mutagenic potential in five tester strains of *Salmonella typhimurium*, and in *E. coli* strain WP2*uvr*A, using the standard Ames microbial mutagenicity plate incorporation tests.

Study #SY05-T92-14. ***L5178Y TK +/- mouse lymphoma mutagenesis assay with a confirmatory assay – interleukin-1 receptor antagonist (IL-1ra).***

The ability of IL-1ra to induce mutations in mammalian cells was evaluated in the L5178Y TK+/- mouse lymphoma mutagenesis assay, in the presence and absence of Aroclor™-induced rat liver microsomes as an exogenous source of metabolic activation. In the initial, range-finding assay, duplicate cultures of L5178Y lymphoma cells were exposed to either vehicle control or nine concentrations of IL-1ra, ranging from 0.5 to 5000 µg/ml, in the presence or absence of rat liver microsomes. The highest concentration of 5000 µg/ml IL-1ra was the maximally feasible test concentration, based on the dilution of the test vial. After a 4 h incubation, the cultures were washed in complete culture medium, resuspended to a 20 ml volume, and cultured at 37°C for an additional 24 to 48 h. Ethyl methanesulfonate (EMS; 0.25 or 0.5 µl/ml) and 7, 12 dimethylbenzanthracene (DMBA; 2.5, 5.0 µg/ml) were used as the positive controls for the non-activated, and metabolic activation phases of the study, respectively.

At the end of the incubation period, cells were recovered by centrifugation, plated in 0.23% agar, and cloned in medium containing trifluorthymidine, to select for TK -/- revertants. The number of revertant colonies was counted manually, following incubation at 37°C 10 to 12 days later.

The preliminary range-finding and cytotoxicity test did not indicate any toxicity related to IL-1ra at concentrations of up to 5000 mg/ml, in either the presence or absence of exogenous metabolic activation. The first trial of the non-activated portion of the range-finding test failed; data from that assay were recorded, but not reported in the final report. The positive controls EMS and DMBA produced the expected number of revertants in the absence or presence of rat liver S9, respectively.

After a 48 h incubation following a 4 h exposure to IL-1ra, cloned cells from the non-activated and S9-activated cultures produced a range of suspension growth of 90% to 102% of vehicle control. The S-9 activated cultures that were cloned produced a range of suspension growth of 90% to 99% of control. None of the cloned cultures from either the S-9 activated or the non-activated groups exhibited a mutation frequency that was at least two times that of the vehicle controls. Total growth of these cultures ranged from 93% to 117% of vehicle control in the non-activated cultures, and 86% to 112% of controls in the cultures incubated with IL-1ra in the presence of metabolic activation. There was no relationship in either suspension or total growth levels to the dose of IL-1ra used to treat the L5178Y cells.

Similar findings were observed in the confirmatory assay. Ten doses of IL-1ra, ranging from 375 to 5000 µg/ml were used for the exposure period, either in the presence or absence of exogenous metabolic activation by rat liver microsomes. No increases in mutation frequency of greater than or equal to two times background (vehicle control) levels were observed in any of the IL-1ra treatment groups, either in the presence or the absence of S9 microsomes. Total growth of the

cloned cells ranged from 78% to 141% of control in the non-activated group, and from 75% to 131% for L5178Y cells exposed to IL-1ra in the presence of exogenous metabolic activation.

In summary, under the conditions of this assay, treatment of mouse L5178Y TK+/- cells with IL-1ra at doses of up to 5000 µg/ml did not adversely affect cell growth or viability, or increase the frequency of TK -/- revertants, both in the absence or presence of exogenous metabolic activation.

***Study #SY05-T92-15. In vitro* cytogenetics test of interleukin-1 receptor antagonist (IL-1ra).**

The clastogenic potential of IL-1ra was assessed in an *in vitro* assay using mitogen-stimulated, Chinese hamster ovary (CHO) cells in the presence and absence of Aroclor™-induced rat liver microsomes as an exogenous source of metabolic activation. Duplicate cultures of CHO cells were treated with Il-1ra at dose levels of 0.2, 0.6, 2, 6, 20, 60, 200, 600, or 2000 µg/ml, both in the absence and presence of rat liver S-9 microsomes. Metaphase cells were collected 20 h after initiation of IL-1ra treatment, incubated for 2 h in colchicine and stained with Giemsa stain, and evaluated microscopically for evidence of chromosomal breaks or translocations, polyploidy, and reduction in mitotic indices. Triethylenemelamine (0.5 µg/ml) and 50 µg/ml cyclophosphamide were used as positive control agents in non-activated and activated phases of the study, respectively.

In the absence of metabolic activation, no reduction in mitotic indices, nor increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed in CHO cells at any concentration of IL-1ra tested, after a 20 h exposure. No statistically significant increases in chromosome aberrations or mitotic inhibition were observed, as compared to the vehicle control in CHO cells treated for 20 h with up to 2000 µg/ml IL-1ra in the presence of exogenous metabolic activation.

A second, confirmatory assay was conducted in the absence and presence of rat liver S-9 at dose levels of 250, 500, 1000, and 2000 µg/ml IL-1ra. Metaphase cells were collected for microscopic evaluation as described above, either 20 or 44 hours after continuous IL-1ra exposure in the absence of metabolic activation, or at 20 and 44 hours after initiation of a four hour pulse treatment with IL-1ra in the presence of rat liver S-9. There were no dose-related effects of IL-1ra on cell growth, mitotic inhibition, or reduction of monolayer confluency at either time point, in the presence or absence of rat liver microsomal activation. So statistically significant increases in chromosomal translocations, breaks, gaps, or other aberrations were observed in either the activated or non-activated IL-1ra dose groups, as compared to their respective vehicle control groups, at either the 20 or 44 hour harvest time points. The positive controls cyclophosphamide and triethylenemelamine produced the expected clastogenic responses in Chinese hamster ovary cells in both assays, with the total percentage of aberrant cells in both of the positive control groups ranging from 52 to 81% of the metaphase cells examined. These results are in the range of values expected for triethylenemelamine and cyclophosphamide under the conditions of this assay, confirming the sensitivity of the test system.

In summary, IL-1ra showed no evidence of clastogenic potential in this *in vitro* chromosomal aberration assay in Chinese hamster ovary cells, at up to the highest feasible concentration (2000 µg/ml) which could be evaluated.

Study #SY05-T92-16. ***Micronucleus cytogenetic assay in mice – interleukin-1 receptor antagonist (IL-1ra).***

The potential of IL-1ra to induce chromosomal damage was evaluated in vivo using the mouse bone marrow micronucleus assay. This test is used to screen agents that cause chromosomal damage, manifested by acentric chromatids and chromosome fragments, which are retained by the daughter cells during mitosis as secondary nuclei. The presence of micronuclei in the cell cytoplasm constitutes evidence that the DNA has undergone some type of damage, in response to the test article.

An initial dose-ranging study was conducted in mice to determine the toxicity of the test article. Fifteeen mice per sex were treated by s/c injection with the vehicle control (0.1% Tween 80 in citrate-buffered sterile saline, pH 6.5), or 500, 1000, or 2000 mg/kg IL-1ra on study d 1. Five mice per sex were sacrificed at 24, 48, or 72 hours after treatment. Five mice per sex were also treated with 40 mg/kg cyclophosphamide by i/p injection and sacrificed 24 h later, as a positive control for the study. Bone marrow erythrocyte smears were prepared from femoral bone marrow samples, fixed and stained with acridine orange for evaluation of chromosomal abnormalities by fluorescent microscopy. No clinical signs of toxicity, nor evidence of increased nucleated cells in bone marrow smears was observed at any dose of IL-1ra tested, as compared to the vehicle control group.

Five mice per sex per group were used for the definitive assay, and dosed with a single, s/c injection of either vehicle control, 500, 1000, or 2000 mg/kg/d IL-1ra. An additional group of five mice per sex was dosed with 40 mg/kg cyclophosphamide, i/p as a positive control. Mice were sacrificed 24, 48, and 72 hours after dosing with the test articles. Bone marrow smears were prepared as described above for the dose-ranging assay. Micronucleated erythrocytes were scored by fluorescent microscopy; for each mouse, approximately 1000 polychromatic nucleated erythrocytes (PCE) were evaluated for the presence of micronuclei. Micronucleated, normochromatic erythrocytes (NCE) were estimated based on the number of micronucleated NCE counted and an estimated total number of NCE, according to the methods of Hart and Engbert-Pedersen[11], to determine the PCE/NCE ratio for each dose group.

The number of micronuclei present in the vehicle control cells ranged from 0/1000 to 2/2000, and from 0/1000 to 3/1000 in cells from the IL-1ra treated mice at all time points after injection. There were no statistically significant increases in the incidence of micronuclei formation as compared to the vehicle control group for any of the groups of mice treated with IL-1ra. By contrast, the number of micronucleated cells at 24 hours after dosing in the animals treated with 50 mg/kg cyclophosphamide ranged from 6 to 21 per 1000 PCE evaluated, confirming the sensitivity of the assay. There were no remarkable differences in the mean values for the PCE/NCE ratios in any of the groups of IL-1ra treated animals, as compared to cells obtained from mice treated with the vehicle control.

In summary, the results of this assay demonstrate that under the conditions employed, IL-1ra at doses of up to 2000 mg/kg, s/c did not induce any statistically significant changes in the incidence of micronucleated bone marrow cells, suggesting that it is not clastogenic after in vivo exposure.

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[11] Hart, J.W. and H. Engbert-Pedersen. 1983. Statistics of mouse bone-marrow micronucleus test: Counting, distribution, and evaluation of results. *Mutat. Res.*, **111**:195-207.

Comment: The studies conducted using the Salmonella typhimurium and E. coli bacterial mutagenesis assays (Ames test) are inappropriate for protein biotherapeutics. Similarly, the in vitro assay using cultured Chinese hamster ovary cells and the *in vivo* micronucleus assay in mice will not provide relevant information regarding the clastogenic potential of IL-1ra. These assays are designed to detect mutagenic effects of small molecule drugs, chemicals, and environmental agents that cause direct damage to DNA molecules.

Toxicology Study Summary:

1. Acute intravenous toxicity study of interleukin-1 receptor antagonist (IL-1ra) in rats. Study #SY05-T94-01. — .CD® BR VAF/Plus rats, 8 weeks old, weight range 244-261 g (♂), 193-235 g (♀); vehicle (10 mM sodium citrate, 140 mM NaCl, 0.1% polysorbate 80; lot A#L94-008), 1.5, 15, 150, 720 mg/kg IL-1ra, i/v (lots #L94-009, L94-010, L94-011, and L94-012); GLP; 1/18 – 2/2/94: — **Volume 5, pp. 27-99.**

2. Acute intravenous toxicity study of interleukin-1 receptor antagonist (IL-1ra) in cynomolgus monkeys. *Macaca fasicularis*, 2 young, adult ♀, weight 2.2 kg each; vehicle (citrate buffered saline plus 0.1% polysorbate 80, lot #L94-008), 1.5, 15, 150 mg/kg IL-1ra, i/v (lots #L94-009, L94-010, L94-023); GLP; 1/18 – 1/26/94· —
— **Volume 5, pp. 100-237.**

3. Subchronic, subcutaneous (b.i.d.) toxicity study of interleukin-1 receptor antagonist in Sprague-Dawley rats. Study #SY05-T89-10. — CD® (SD)BR rats, 10/sex/group, 7-9 weeks old, weight range 180-279 g (♂), 157-206 g (♀); vehicle (citrate buffered saline plus 0.5 mM EDTA, pH 6.5; lot #005-90), 5, 20, 80 mg/kg/d IL-1ra (lots #006-90, 007-90, 008-90), as divided doses twice daily for 14 d; GLP; 1/18 – 2/2/90· —
— **Volume 6, pp. 1-306.**

4. Subchronic high-dose (q.i.d.) toxicity study of interleukin-1 receptor antagonist (IL1ra) in Sprague-Dawley rats. Study #SY05-T89-12. — .CD® (SD)BR rats, 20/sex/group, 46-53 days old, weight range 207-249 g (♂), 162-202 g (♀); vehicle (phosphate buffered saline, pH 7.0, lot #072-89); 120 mg/kg/d IL-1ra, lot #073-89, as divided doses 4 times daily for 7 or 14 d; GLP; 12/6 – 12/20/89· —
— **Volume 7, pp. 1-268.**

5. Subcutaneous 6-month toxicity study in rats treated with anakinra, including 1-month interim and 1-month recovery groups. Study #SY05-T94-04. — CD® (SD)BR rats, 4 weeks old, weight range 129.5-155.3 g (♂), 112.9-132.3 g (♀); 20/sex/group; vehicle control (citrate-buffered saline plus 0.1% w/v polysorbate 80, lot #L94-104), 2, 20, 200 mg/kg/d IL-1ra, s/c, lots #L94-105, #L94-106, and #L94-107, as divided doses 2 times daily for 6 months; GLP; 7/22/94 – 2/22/95 — **Volumes 8-10.**

6. Intravenous (14-day) systemic toxicity study of interleukin-1 receptor antagonist (IL1ra) in Sprague-Dawley rats. Study #SY05-T89-01. — CD® (SD)BR rats, 3/sex/group, weight range 205.8-255.2 g; vehicle control (PBS, pH 7.0; lot #0001-89), 0.047, 0.231, 1.17 mg/kg/d

IL-1ra, lots #0002-89, #0003-89, #0004-89, i/v; GLP; 2/9 – 2/23/89; —
— . **Volume 11, pp. 4-109.**

7. A subchronic intravenous toxicity study of interleukin-1 receptor antagonist (IL1ra) in Sprague-Dawley rats. Study #SY05-T89-02. — .CD® (SD)BR rats, 5/sex/group, weight range 180-210 g; vehicle (PBS, pH 7.0, lots #0009-89 and #0013-89), 2, 5, 20 mg/kg/d IL-1ra, lots #0010-89, #0011-89, #0012-89, and 30014-89, i/v; GLP; 4/19/ - 5/3/89, —
— **Volume 11, pp. 110-265.**

8. Subchronic intravenous toxicity study of interleukin-1 receptor antagonist (IL1ra) in Sprague-Dawley rats. Study #SY05-T89-05. — CD® (SD)BR rats, 10-20/sex/group, 7 to 8 weeks old, weight range 266-336 g (♂), 180-227 g (♀); vehicle control (phosphate buffered saline plus 0.1 mM EDTA, pH 7.0, lot #0031-89); 3, 10, 30 mg/kg/d IL-1ra, lots #0032-89, #0033-89, #0034-89, i/v GLP; 7/13 – 8/24/89; —
— **Volume 12, pp. 4-371.**

9. Subchronic intravenous toxicity study of interleukin-1 receptor antagonist (IL1ra) in Sprague-Dawley rats. Study #SY05-T89-09. — CD® (SD)BR rats, 10/sex/group, 5 to 7 weeks old, weight range 207-293 g (♂), 1450190 g (♀); vehicle control (sterile PBS plus 0.1 mM EDTA, pH 7.0, lot #009-90), 30 mg/kg/d IL-1ra, lot #010-90, i/v; GLP; 1/17 – 1/31/90; — **Volume 13, pp. 4-214.**

10. A two-week nose-only inhalation toxicity study of recombinant human interleukin-1 receptor antagonist (IL-1ra) in the rat. Study #SY05-T92-01. — .CD® BR rats, 10/sex/group, 7-8 weeks old, weight range 220.5-254.8 g (♂), 193.2-232.5 g (♀); vehicle control (citrate buffered saline plus 0.5 mM EDTA, 0.1% Tween 80, pH 6.5; lot #L92-024), 0 (air only), 7.5, 75 mg/m^2 (cumulative, gravimetric exposure of 0, 0,10, or 99 mg/m^2) IL-1ra (lots #L92-025, #L92-029); GLP; 4/1 – 4/15/92: — **Volume 14, pp. 4-317, and Volume 15, pp. 4-146.**

11. A subchronic (2 week) systemic toxicity study of interleukin-1 receptor antagonist in male and female Rhesus monkeys following repeated subcutaneous injections. Study #SY05-T89-11. *Macaca mulatta*, 3/sex/group, weight range 2.83-4.15 kg; vehicle control (citrate buffered saline plus 0.5 mM EDTA, pH 6.5, lot #001-90), 5, 20, 80 mg/kg/d IL-1ra, lots #002-90, #003-90, #004-90, by s/c injection as twice daily divided doses for 14 d; GLP; 2/13 – 2/28/90; —
Volume 15, pp. 147-359.

12. A 4-week toxicity study of IL-1ra administered by subcutaneous injection to Rhesus monkeys, with a 4-week recovery period. Study #960040. *Macaca mulatta*, 3-5/sex/group, prepubertal to young adults (2 years, 6 months to 6 years of age), weight range 2.9-4.2 kg; control (IL-1ra placebo, lot #1105166E6), 10, 100, 200 mg/kg/d IL-1ra, lot #001F05; GLP; 5/30 – 7/26/96; — **Volume 16, pp. 4-378.**

13. A one week, intravenous infusion study of interleukin-1 receptor antagonist (IL1ra) in male Rhesus monkeys. Study #SY05-T90-02. *Macaca mulatta*, 2 control, 3 test ♂/group, weight range 3.46-5.24 kg; placebo (composition not specified, lot #090-90), 150 mg/kg/d IL-1ra

(lot #091-90), CIVI at flow rate of 5 ml/hr; GLP; 12/11 – 12/21/90 —
— . . . . . **Volume 17, pp. 4-106.**

14. A range-finding toxicity study of interleukin-1 receptor antagonist (IL1ra) in male cynomolgus monkeys following repeated intravenous injections. Study #SY05-T89-03. *Macaca fasicularis*, 3 males/group, weight range 4.23-8.47 kg; vehicle control (sterile PBS + 0.1 mM EDTA, pH 7.0, lot #0015-98); 0.1, 1. 10 mg/kg/d IL-1ra (lots #0016-89, 0017-89, 0018-89), i/v, x 14 d; GLP; 6/12 – 6/27/89; —
— **Volume 17, pp. 107-228.**

15. Subchronic systemic toxicity study with recovery period of interleukin-1 receptor antagonist (IL1ra) in Rhesus monkeys. Study #SY05-T89-08. *Macaca mulatta*, 3-6/sex/group, weight range 3.41-6.75 kg; vehicle control (PBS plus 0.1 mM EDTA, pH 7.0, lot #0064-89); 3, 10, 30 mg/kg/d IL-1ra (lots #0065-89, #0066-89, #0067-89); GLP; 10/30 – 12/12/89; —
— **Volume 18, pp. 4-298.**

16. An antigenicity study if interleukin-1 receptor antagonist (IL1ra) in male Rhesus monkeys following repeated intravenous administrations. Study #SY05-T89-07. *Macaca mulatta*, 4 males, weight range 5.77-7.17 kg; 10 mg/kg/d IL-1ra (lot #0059-89), i/v x 19 d; GLP; 9/14 – 10/2/89; —
Volume 18, pp. 301-329.

17. Fertility and general reproduction (Segment I) study of interleukin-1 receptor antagonist (IL-1ra) administered by subcutaneous injection to rats. Study #SY05-T93-01 — CD®BR VAF/Plus® rats (Sprague-Dawley, specific pathogen-free); 40/sex/group, weight range 232-345 g (males), 182-249 g (females); vehicle control (citrate-buffered saline plus 0.1% polysorbate 80, pH 6.5, lots #L93-034, #L-93-081); 12.5, 50, 200 mg/kg/d IL-1ra, s/c (lots #L93-035, L#93-082, L#93-036, #L93-083, #L93-037, and #L93-084); GLP; 5/17 – 9/19/93; —
— **Volume 19, pp. 4-305 and Volume 20, pp. 9-293.**

18. A study of fertility and early embryonic development to implantation of recombinant-methionyl human interleukin-receptor antagonist (r-met-HuIL-1ra) administered subcutaneously in rats. Study #960044 — CD®BR VAF/Plus® rats (Sprague-Dawley, specific pathogen-free); 25/sex/group, weight range 268-405 g (males), 193-282 g (females); vehicle control (IL-1ra excipient, composition not specified, lot #1105306F6); 10, 100, 200 mg/kg/d IL-1ra, s/c (lot #001F05); GLP; 6/18/96 – 4/30/97; — —
— **Volume 20, pp. 295-406, and Volume 21.**

19. Developmental toxicity study in rats with interleukin-1 receptor antagonist (IL-1ra). Study #SY05-T92-03. — CD®BR VAF/Plus® rats (Sprague-Dawley, specific pathogen-free); 40 females/group, weight range 229-287 g; vehicle control (citrate-buffered saline plus 0.1% polysorbate 80, pH 6.5, lot #L92-023); 12.5, 50, 200 mg/kg/d IL-1ra, s/c (lots #L92-026, L#92-028, L#92-030, #L92-062, #L92-063, and #L92-064); GLP; 4/21/92 – 8/19/93; —
— **Volume 22, pp. 4-332 and Volume 23, pp. 4-384.**

20. Developmental toxicity study in rabbits with interleukin-1 receptor antagonist (IL-1ra). Study #SY05-T92-04. New Zealand white rabbits, 20 pregnant females/group, weight range 2.8–3.5 kg; vehicle control (citrate-buffered saline plus 0.1% polysorbate 80, pH 6.5, lot #L92-023); 12.5, 50, 200 mg/kg/d IL-1ra, s/c (lots #L92-027, L#92-029, and L#92-031); GLP; 5/26 – 12/15/92; — **Volume 24, pp. 4-270.**

Toxicology Review:

Study #SY05-T94-01. ***Acute intravenous toxicity study of interleukin-1 receptor antagonist (IL-1ra) in rats.***

The acute toxicity of IL-1ra was studied after a single, i/v dose in outbred, Sprague-Dawley rats. On study day 1, rats received a single injection into the tail vein with vehicle control (citrate buffered saline plus 0.1% polysorbate 80), or 1.5, 15, 150, or 720 mg/kg IL-1ra. Animals were observed immediately after dosing of day 1, and then daily for 14 d for clinical signs of toxicity or mortality. Body weights were determined immediately prior to dosing, and on study days 5, 9, 13, and 15 at study termination. Animals were euthanized on d 15 by CO_2 inhalation, and complete necropsies performed. Organs with any grossly visible lesions at necropsy were removed and processed for histologic evaluation following staining with hematoxylin and eosin. Additionally, samples of peripheral blood were collected by retro-orbital puncture at time of sacrifice, for determination of anti-IL-1ra antibody profiles by EIA.

No deaths occurred on study, and individual body weights and body weight gains did not differ significantly between the control and treatment groups. On clinical observations, findings were limited the presence of slight red material around the nose of control female #73107F on study days 8-12 and male #73142 (720 mg/kg dose group) on study days 7-10. Other incidental clinical findings included a small lesion on the dorsal surface of the neck of rat #73113 (male, 1.5 mg/kg dose group), and a medium laceration on the ear of female #73128 (15 mg/kg) on study days 11-15.

At necropsy, macroscopic lesions were noted in 4/50 rats on study, and included mild pelvic dilatation of the right kidney in control male #73101 and female #73137 in the 150 mg/kg dose group, an abrasion of the ear in female #73128 (15 mg/kg, above), and a scab on the skin of the neck in male #73113 (1.5 mg/kg, above). Histologic evaluation of the skin lesions showed the presence of an ulcer with chronic, active inflammatory infiltrates and necrosis present in the ear of female #73128, and subacute inflammation in the skin of the neck of male rat #73113. The kidney lesions were confirmed microscopically as hydronephrosis with tubular degeneration and/or regeneration in rats #73101 (control, male) and #73137 (150 mg/kg, female). Since these findings were not present in any other animals treated with IL-1ra and were present in one animal each in the control and treated groups, they were considered by the reviewing pathologist to be incidental to treatment with IL-1ra.

In summary, a single, i/v treatment of outbred, Sprague-Dawley rats with IL-1ra at doses up to and including 720 mg/kg was well-tolerated, and not associated with any significant adverse effects. The NOAEL for IL-1ra by this route of administration, is therefore 720 mg/kg in rats.

Comment: The protocol stated that samples of peripheral blood for determination of anti-IL-1ra antibody development were obtained at terminal sacrifice, however, no analysis of these samples appears to have been conducted, and no data regarding the results are included in the final report.

Study #SY05-T94-02. ***Acute intravenous toxicity of interleukin-1 receptor antagonist (IL-1ra) in cynomolgus monkeys.***

The acute toxicity of IL-1ra was studied after i/v exposure in cynomolgus monkeys. Two female macaques were treated with i/v injections of vehicle, 1.5, 15, or 150 mg/kg IL-1ra, i/v. Each dose was separated by a 48 h washout period, and the animals were observed for an additional 48 h following the final dose. On study day 9, both monkeys were euthanized, and examined at necropsy for evidence of gross pathologic changes. Target organs were removed, weighed, and any lesions were preserved in fixative and evaluated histologically for microscopic pathologies.

No deaths were observed, and no treatment-related clinical signs of toxicity were noted on study. A red, vaginal discharge was noted in one monkey on study d 6. There were no significant changes from baseline in regard to body weights, body weight gain, nor food consumption.

At necropsy, there were no gross lesions observed on evaluation of the two animals on study. No microscopic analysis of the tissues was performed.

In summary, sequential treatment of female, cynomolgus monkeys with 1.5, 15, and 150 mg/kg IL-1ra by i/v bolus injection was not associated with any remarkable toxicities, including changes in clinical signs, body weights and body weight gains, food consumption, or gross pathologic findings. The NOAEL in this study was 150 mg/kg, i/v, for female macaques.

Study #SY05-T89-10. ***Subchronic, subcutaneous (b.i.d.) toxicity study of interleukin-1 receptor antagonist in Sprague-Dawley rats.***

Repeat-dose toxicity studies were conducted with IL-1ra in Sprague-Dawley rats. Ten animals/sex were treated twice daily for 14 days with vehicle control (citrate buffered saline plus 0.5 mM EDTA), 5, 10, or 20 mg/kg/d IL-1ra, as divided doses by s/c injection. Animals were observed twice daily for overt signs of toxicity, changes in behavioral patterns, and general appearance. Body weights were determined at baseline (study days -8, -4, and -1), on day 1 immediately prior to dosing, and on study days 4, 7, 10, 14, and on d 15 immediately prior to sacrifice. Body temperatures were recorded at the same time intervals as body weights, using a tympanic membrane thermometer. Food consumption was measured at 3 to 5-day intervals on study. Ophthalmologic examinations were performed on all rats at baseline (Study week –1), and on study d 12 or 13, by both slit lamp microscopy and examination of the fundus with a binocular indirect ophthalmoscope. Samples of peripheral blood for evaluation of clinical chemistry profiles were obtained from all animals following an overnight fast, by retro-orbital puncture on study at study days –7, -1, and at sacrifice (please see below). Samples for evaluation of coagulation factor parameters were also obtained on study day –1 and at sacrifice.

At terminal sacrifice, blood was collected by retro-orbital puncture and hematologic and serum biochemistry profiles evaluated. Samples of serum were also obtained for analysis of anti-IL1RA activity from all control and treated animals, and from five untreated, sentinel male rats.

Full necropsies were performed on each rat, and target organs were evaluated both macroscopically and histologically for evidence of toxicity.

All rats survived the 14 day treatment period. Clinically, all animals appeared normal, with the exception of opacities and other lesions in the right eye of several rats in all treatment groups, including the controls. These lesions were attributed to trauma associated with the retro-orbital puncture used to obtain blood samples, and were not considered related to treatment with IL-1ra. Ophthalmologic examination revealed no treatment related changes in the eyes of any rats during the course of the study.

There were no significant differences between the control and the IL-1ra treated rats with regard to total body weights, body weight gain, or food consumption over the duration of the study. Slight, but statistically not significant increases in mean body temperature were observed in some groups of IL-1ra treated rats as compared to the control animals at various time points on study. However, magnitude of the increases was < 1oC and the changes in body temperature were not related to the dose of IL-1ra administered; therefore, these findings were considered incidental to treatment with the test article.

There were no remarkable alterations, either from baseline or as compared to the vehicle control group, in serum biochemistry profiles of any of the animal treated with any dose level of IL-1ra. Findings of unknown clinical significance included minor, sporadic increases or decreases in ALT, GGT, OCT, and α1-globulin in individual male rats in all treatment groups, as compared to the mean values for the control groups, and magnesium levels and gamma globulin serum fraction for female animals. However, these changes were not considered related to treatment with the test article.

Similarly, no treatment-related alterations in hematology profiles were observed in any of the groups of rats injected with IL-1ra, as compared to either the group mean values at baseline, or to the mean value for the control animals. Increases in the number of reticulocytes above the normal ranges were observed for both male and female rats in the control and the IL-1ra treated groups, and were attributed to a compensatory response to repeated bleeding for clinical pathology samples. There were no effects of IL-1ra treatment on coagulation factor (PTT, APTT, and fibrinogen) parameters as compared to either baseline values or the group mean value for the control animals, at study d 15.

Urinalysis showed no statistically significant, nor clinically meaningful changes in specific gravity, pH, nitrite, glucose, bilirubin, or urobilinogen levels in the rats treated with IL-1ra, as compared to either their own baseline values at study days –7 and –1, or to animals in the vehicle control group at d 15. Several male animals did have evidence of hematuria (slight to mild in severity), trace proteinuria, and slight ketone bodies in the urine. However, these findings were present at approximately equal incidence and severity in all groups of male rats including those treated with the vehicle control, and were present at all time points tested, including study baseline. Female rats in all dose groups had some evidence of slight to moderate hematuria and ketone bodies at all time points on study, but in general, the incidence and severity were less than that observed in the male rats.

At necropsy, the only lesion present on gross pathologic evaluation was a mild lens opacity in the right eye of animal #237F, in the vehicle control group. Microscopic evaluation of this lesion

revealed a moderate, unilateral phthisis bulbi present in the eye; the clinical significance of this finding was unknown, and it was not considered related to treatment with the test article.

Statistically significant decreases in the relative brain and testes weights were observed in male rats treated with 5 or 20 mg/kg/day IL-1ra as compared to the vehicle control group. However, no significant decreases in the mean absolute organ weights were observed for male rats in these groups, as compared to the control animals. There were no histologic findings suggestive of organ toxicity in either of these organs, from any animal in these two dose groups. There were no significant differences in either absolute or relative organ weights for female rats in any treatment group, as compared to the vehicle controls.

Treatment-related lesions on histologic evaluation were limited to trace to mild, subacute inflammatory infiltrates and hemorrhage present in the subcutis at the site of injection of the test article. These lesions were present in 1 male and 1 female rat each in the vehicle control group, in 0/20 rats treated with 5 mg/kg/d IL-1ra, in one male and one female rat each in the mid-dose group, and in 2/10 male and female rats each, after injection of 80 mg/kg IL-1ra/day for 14 days. Although the incidence of these lesions was increased in the highest dose group as compared to rats in the vehicle control group, the differences were not statistically significant.

Trace to mild, interstitial pneumonia was present in the lungs of both male and female rats in all dose groups, including the vehicle control. This finding occurred at approximately equal incidence and severity in all groups, and was considered incidental to treatment with IL-1ra. Evaluation of bone marrow smears for myeloid:erythroid ratios revealed no differences between control and IL-1ra treated animals.

In summary, treatment of outbred, Sprague-Dawley rats by twice daily. s/c injection with 5, 20, or 80 mg/kg IL-1ra was not associated with any overt signs of toxicity, gross or histopathologic lesions, or alterations in clinical chemistry or urinalysis profiles after 14 consecutive days of treatment, as compared to animals receiving vehicle control. Histologic findings were limited to trace to mild inflammatory infiltrations and hemorrhage at the injection site, and were observed in rats in the control, as well as in the mid- and high-dose IL-1ra groups. The NOAEL for IL-1ra under the conditions of this study was greater than or equal to 80 mg/kg/d.

Comment: Although samples of serum were obtained for analysis of anti-IL1ra antibody development, there were no data included in the submission that would confirm that these assays were ever conducted.

Study #SY05-T89-12. ***Subchronic high-dose subcutaneous (q.i.d.) toxicity study of interleukin-1 receptor antagonist (IL1ra) in Sprague-Dawley rats.***

The objective of this study was to determine the toxicologic profile of high doses of IL-1ra in Sprague-Dawley rats after 7 and 14 days of cumulative exposure. Rats were injected 4 times daily with vehicle control (PBS, pH 7.0) or 30 mg/kg IL-1ra, for a total daily exposure of 0 or 120 mg/kg IL-1ra. All animals were observed twice daily for clinical signs of toxicity and behavioral changes, general appearance, and mortality. Body weights and body temperatures were determined 5 times prior to IL-1ra administration, at days –15, -11, -8, -7, -4, and –1, and just prior to dosing to establish baseline measurements and confirm animal health. Following initiation of treatment with IL-1ra, body weights were determined on study days 4, 7, 11, 14, and

at terminal sacrifice on study days 8 or 15. Food consumption was recorded at 2 to 4 day intervals over the duration of the study period. All rats underwent ophthalmologic examinations on study day –1, day 5, and surviving animals on study d 12.

Ten rats/sex/group were sacrificed after 7 d of treatment, as an interim group to determine subchronic toxicities associated with IL-1ra. At the end of the 14 d treatment period, the remaining, animals were euthanized. Full necropsies were performed on all animals at terminal sacrifice, and peripheral blood samples were collected for clinical pathology and determination of anti-IL1ra antibody titers.

One rat (animal #183, vehicle control group) died on study d 8 are a result of anesthesia overdose while collecting the peripheral blood specimens. There were no overt, clinical signs of toxicity in the rats receiving 120 mg/kg/d IL-1ra, as compared to the remaining animals treated with the vehicle control group. No remarkable differences in body temperature, total body weights, body weight gains, or food consumption between the control and the IL-1ra treated animals were noted at any time point over the duration of the study.

Clinical observations included red, crusted material around the nose, and alopecia of the front paws in rats in both the treated and the vehicle control group. These findings occurred at various time points on study, and at approximately equal incidence in both the control group and the rats treated with 120 mg/kg/d IL-1ra. Several animals had bloody looking, right eyes at various times on study, and occasional appearance of an opaque right eye. Ophthalmologic evaluation of the animals at study d -1 revealed lesions, including mild, periocular discharge in one rat each in the control and IL-1ra treatment groups, subconjunctival hemorrhage in 7/20 rats in the control group, and attenuation of retinal blood vessels on rats #174M and #194F in the IL-1ra treatment groups. Severe ocular damage was noted in animal #157 in the control group, who had a partial ocular proptosis (bulging eye), with sever conjunctival hemorrhage, an immature cataract, and peripapillary retinal hemorrhage in the right eye. Rat #187F in the IL-1ra treatment group exhibited dyscoria, pallor of the vasculature in the iris, and retinal edema and evidence of detachment in the right eye on slit lamp evaluation.

In addition to the reddish material around the eyes, other lesions were noted on ophthalmologic examination at d 5 on study. These included corneal opacification in rat #160M in the vehicle control group and in animals #172M, 174M, and 193M in the IL-1ra treated group, attenuation of retinal blood vessels in rat #174M (present at baseline exam as well) and in rat #184F in the control group, and severe corneal xerosis, scarring, and vascularization in rat #157M, in the control group. Similar lesions were noted at the d 12 examination in the surviving animals, and were not dose-related. In all instances, the ocular findings were attributed to the trauma induced following the sequential, blood collections from the retro-orbital sinus.

There were no significant changes in serum biochemistry or urinalysis profiles in rats treated with 120 mg/kg/d IL-1ra, as compared to the animals injected with the vehicle control. Hematologic profiles showed slight, but statistically not significant decreases in red cell counts, hemoglobin, and hematocrit in male and female rats in both dose groups at days –1 and 8 on study, as compared to baseline values at study d –7. A 2-fold increase in reticulocyte counts was observed in all groups at study d –1, as compared to study d –7 (baseline), consistent with an increased production of red cells by the marrow in response to the repeated bleeding. These values had returned to baseline by study termination at d 15. There were no significant changes in total white blood cell, neutrophil, or lymphocyte counts, or coagulation parameters (PT,

APTT, and fibrinogen) between the control and the IL-1ra treatment groups at any time point during the study period.

At interim sacrifice on study day 8, both mean absolute and relative spleen and pituitary weights were increased in both male and female rats treated with 120 mg/kg/d IL-1ra, as compared to animals injected with the vehicle control. These changes were statistically significant only for the spleen:body weight ratio in the male rats and for the relative pituitary weight in rats of both sexes ($p \leq 0.05$, ANOVA). At terminal sacrifice on study d 15, both absolute and relative spleen weights remained increased in the IL-1ra treated groups, but were no longer statistically different. However, relative thyroid weights were significantly lower in the male rats treated with IL-1ra than in the control group ($p \leq 0.05$, ANOVA). This finding was not considered related to treatment, since the mean value for the females in this dose group were no different from the controls.

There were no remarkable findings on gross evaluation at necropsy in the interim sacrificed groups of rats on study d 8. At terminal sacrifice on study d 15, two female rats in the control group (animals #180F and #181F) had enlarged, mandibular lymph nodes present on macroscopic evaluation; however, histologically these tissues were within normal limits. One male rat (animal #170) in the group treated with IL-1ra had a ruptured right eye at necropsy, with an opaque cornea, which was not confirmed microscopically. Female rat #182F in this dose group had a small (3mm) brown focus present on the left lungs on gross evaluation; microscopically, this lesion was correlated with areas of acute, multi-focal, mild, interstitial pneumonia. Animal #193F in the IL-1ra treated rats also had enlarged mandibular lymph nodes on macroscopic evaluation, with no histologic findings on microscopic evaluation.

Other microscopic findings that were not evident on gross evaluation included eosinophilic infiltrates of trace severity in the uterus and cervix in 3/5 female rats treated with the vehicle control (animals #176F, #178F, and #179F), and in 2/5 female rats in the group treated with 120 mg/kg/d IL-1ra (animals #188F and #189F) at the interim sacrifice on study d 8. The eosinophilic infiltrates were also present in all 5 female rats in both the control and the IL-1ra treated groups at terminal sacrifice on d 15. Hydronephrosis, congestion, and tubular regeneration were noted in the kidneys of one male rat in the control group and one female rat treated with IL-1ra at the d 8 interim sacrifice, and in one male and one female rat each in the group treated with IL-1ra at terminal sacrifice on d 15. These findings were considered to be spontaneous and not unexpected for Sprague-Dawley rats of this age group, and were deemed incidental to treatment with IL-1ra.

Animals in both the vehicle control and the IL-1ra injected groups had evidence of hemorrhage at the injection site(s) on the tail on both gross and microscopic evaluation. These lesions were trace to mild in severity, and were present at approximately equal incidence in both control and IL-1ra treated animals at days 8 and 15 sacrifices. However, at the d 15 sacrifice, chronic inflammatory changes were present at the injection sites in 3/5 male and 4/5 female rats in the 120 mg/kg/d IL-1ra dose group only. These changes were of trace severity in 6/7 of the affected animals and mild in severity in one male rat (animal #170M), and were associated predominantly with focal lymphocytic infiltration.

In summary, daily, i/v injection of 120 mg/kg IL-1ra as four, divided doses was well tolerated in Sprague-Dawley rats, with no evidence of overt or systemic toxicities. Clinical findings were limited to ophthalmologic trauma associated with repetitive blood sampling from the retro-orbital sinus, and hemorrhage at the injection sites in both control and IL-1ra treated rats. Chronic,

inflammatory infiltrates of lymphoid cells at the injection site, of trace to mild severity were the only findings in this study related to test article treatment, and were only present at the d 15 sacrifice. Because of this local toxicity, no NOAEL for IL-1ra can be determined for this study.

Comment: The protocol states on p.7 that serum samples were collected for immunologic evaluation and shipped to the sponsor for analysis. The results of this analysis were not included in the final report for this study, or in the BLA submission from Amgen for IL-1ra in rheumatoid arthritis.

Study #SY05-T94-04. ***Subcutaneous 6-month toxicity study in rats treated with anakinra, including 1-month interim and 1-month recovery groups.***

Repeat dose, chronic toxicity studies were conducted with IL-1ra in Sprague-Dawley rats. Four groups of 20 rats/sex were treated by twice daily, s/c injection for 4 or 26 weeks, at doses of 1, 10, or 100 mg/kg/injection for a total daily dose of 2, 20, or 200 mg/kg IL-1ra. Vehicle control rats were injected twice daily with sterile, citrate-buffered saline with 0.1% w/w polysorbate 80. Individual animals dosing volumes were based on the most recently recorded body weight. At the end of 4 weeks on study, 5 rats/sex/dose group were sacrificed for an interim evaluation of toxicity of IL-1ra. After 26 weeks of treatment, 10 rats/sex were sacrificed for each dose group, and the remaining animals held for a 4 week, treatment-free recovery period.

All animals were examined twice daily for mortality and once daily for clinical signs of overt toxicity. Weekly physical examinations were also conducted to determine the overall health of the animals. Body weights and food consumption were recorded weekly, beginning at study week –1. At terminal sacrifice, all rats were fasted overnight and fasting body weight recorded at time of sacrifice.

Ophthalmologic examinations (both fundoscopic and slit-lamp biomicroscopic) were performed on all animals prior to initiation of the study, and at weeks 4 and 26 on just the groups scheduled for necropsy at the end of the treatment period. Ophthalmologic examinations were not performed on animals in the interim sacrifice or recovery groups.

Whole blood samples for clinical chemistry and hematology evaluations were collected at sacrifice only, at weeks 27 (end of treatment) or 31 (end of recovery), from ether-anesthetized rats via the abdominal aorta. Additional blood samples for determination of plasma IL-1ra levels and anti-IL-1ra neutralizing activity were obtained from the tail vein of unanesthetized rats from all 20 rats/group prior to study initiation, and from 5 rats/sex/group at days 4, 8, and 15 and weeks 8, 13, and 26 on study. The same 5 animals/sex/group were used for each time point. Samples from animals in the treatment-free recovery group were obtained prior to terminal sacrifice.

Comment: Plasma samples for IL-1ra and neutralizing antibody levels were shipped to the sponsor for analysis by ELISA assays. The results were included in the final report submitted to the BLA, as Appendix 27. Results of these analyses are presented in Tables IV and V, below.

Urinalysis was performed on samples collected during weeks 26 and 30 on study, from the 10 rats/sex/group in the main and recovery portions of the study. Individual animals were placed in metabolic cages and deprived of food and water overnight, and 24 h urine samples collected for

analysis. Additional urine samples were collected on days 2 and during weeks 4 and 26 from 5 rats/sex/dose group for determination of urinary IL-1ra levels. The same 5 animals/sex/group were used for each time point.

At their scheduled terminations, all animals were exsanguinated from the abdominal aorta under ether anesthesia, and subjected to a complete gross necropsy. Organ weights were obtained at this time, along with numerous tissues collected and processed for histopathologic evaluation.

One female rat (#1504F, control group) was found dead during week 18 on study. Clinical signs in this animal prior to death included severe swelling and limited usage of the right hindleg, and a firm mass detected in the abdomen. Macroscopic and histologic evaluation of tissues from this animal revealed a widespread, disseminated lymphosarcoma, which was considered unrelated to treatment. One female rat in the 2 mg/kg/d IL-1ra dose group (animal #2509F) was sacrificed during week 14. This animal had developed progressive, severe swelling and limited usage of both hindlimbs during weeks 9 to 11 on study, weight loss, and severe swelling in the left forepaw prior to sacrifice. Histologic findings included lesions in the spleen and sternal bone marrow suggestive of possible septicemia, which was considered by the reviewing pathologist to be unrelated to treatment with IL-1ra.

There were no additional deaths or overt, clinical signs of toxicity in the remainder of the animals on study. Clinical findings were limited to the presence of reddish-brown staining on the forepaws and/or forelimbs of both control and IL-1ra treated animals, and alopecia or thinning of the fur of the cervical or thoracic regions, forelimbs, forepaws, or hindpaws. No grossly evident signs of irritation or inflammation were present at the site of injection in any animal during the treatment period.

In the 4 week, interim sacrifice groups, there were no remarkable clinical observations; differences in body weights, body weight gains, or food consumption noted between the control and the IL-1ra treated groups of rats at any dose level. A statistically significant, 2-fold increase in total leukocyte counts, without a shift in differential cell populations was noted in the male rats treated with 200 mg/kg/d IL-1ra, as compared to both the control and the 2 and 20 mg/kg/d dose groups at the interim sacrifice ($p \leq 0.05$, ANOVA). A significant increase in mean cell hemoglobin concentration (MCHC), without remarkable increases in other erythrocyte parameters was also seen in this dose group as compared to control at the 4 week time point ($p \leq 0.01$, Dunnett's test). Mean platelet volumes were significantly decreased, as compared to control in male rats treated with either 2 or 200 mg/kg/d IL-1ra for 4 weeks ($p \leq 0.05$, Dunnett's test), without evidence of other significant changes in platelet counts. Hematologic profiles in the female rats treated with IL-1ra were not significantly different from vehicle control treated rats at the 4 week sacrifice.

Significant differences in serum biochemistry profiles, as compared to the control group were only observed in male rats treated with 200 mg/kg/d IL-1ra at the interim sacrifice. The findings included a sight (approximately 10%) increase in both serum phosphorous and total protein, and a 16% increase in total globulin with a concomitant, 17% decrease in A:G ratio ($p \leq 0.01$, Dunnett's test). In the female rats, a significant, 75% increase in the group mean AST level was noted for the 200 mg/kg/d dose group, and was significantly different from both the control and the low- and mid-dose groups ($p \leq 0.05$, ANOVA). Other significant differences from control were a slight (3%) decrease in serum chloride and a 16% decrease in the A:G ratio, without a concomitant increase in globulin levels or decrease in albumin in the female rats treated with 20 mg/kg/d IL-1ra for 4 weeks.

At necropsy, there were no remarkable differences in terminal body weights, absolute, or relative organ weights between the male rats in the control and IL-1ra treated groups, with the exception of a slight (0.1%) decrease in the mean value for pituitary weights relative to body weight in the 20 mg/kg/d dose group. This change was not evident, however, when the pituitary weights relative to brain weight were compared. In the female rats, absolute and relative spleen weights were increased in all groups of IL-1ra treated animals as compared to control; however, this difference was significant only for absolute spleen weights in the group treated with 20 mg/kg/d Il-1ra for 4 weeks ($p \leq 0.05$, Dunnett's test). Absolute, but not relative left adrenal gland weights were also significantly increased over control in this group at the interim sacrifice time point.

Treatment-related findings on gross pathologic evaluation at interim sacrifice included dark areas and ulceration at the injection site, which were present in all groups of rats, with approximately equal incidence in either the control or the IL-1ra treated animals. Histologically, these findings were correlated with areas of hemorrhage, and a granuloma was present at the injection site in one male control rat (animal #1017M). Other gross and histologic findings, including depressed and/or dilated areas on the kidneys, lung nodules, and enlargement of lymph nodes with corresponding lymphoid hyperplasia occurred sporadically (1-2 animals per group) and were of minimal severity and considered incidental to treatment with IL-1ra.

At the week 27 sacrifice (end of treatment), statistically significant, 11-12% increases in group mean body weights were noted for male rats in the two groups treated with 2 or 200 mg/kg/d IL-1ra, as compared to the vehicle control group ($p \leq 0.05$, Dunnett's test). Increases in body weights were noted beginning at weeks 7 and weeks 4, respectively, for rats in the 2 and 200 mg/kg/d groups, and persisted throughout the duration of the study. Significant ($p \leq 0.05$, Dunnett's test) increases in food consumption were also observed in these two groups of rats as compared to males in the vehicle control group, beginning at week 3 for the high-dose and week 4 for the rats treated with 2 mg/kg/d IL-1ra. However, the increased food consumption was not consistent over the duration of the study. Increased food consumption, without a significant increase in body weight over control groups was also observed sporadically in male rats in the 20 mg/kg/d dose group, and in female rats in all three dose groups.

There were no statistically significant changes in hematologic profiles between the male rats in the vehicle control group, and the groups treated with 2, 20, or 200 mg/kg/d at either the week 27 terminal or week 31 recovery sacrifices. Female rats treated with 200 mg/kg/d IL-1ra had a 2-fold decrease in absolute neutrophil counts, with a concomitant 21% increase in lymphocytes on differential evaluation at the week 27 time point. These changes were statistically different from the control group ($p \leq 0.01$, Dunnett's test). By the end of the recovery period, there were no differences in any leukocyte total or differential counts between the control and the IL-1ra treated, female rats. There were no remarkable changes at either time point in erythrocyte parameters, platelet counts, or mean platelet volumes for female rats in either the control or the IL-1ra treated groups.

Several differences were noted in serum biochemistry profiles between control and IL-1ra treated rats. At the week 27 terminal sacrifice, a significant, 36% increase in serum cholesterol and a 10% increase in serum globulin were noted in the male rats treated with 200 mg/kg/d IL-1ra ($p \leq 0.05$ as compared to vehicle control, Dunnett's test). A 61% increase in serum cholesterol and a 2.3-fold increase in mean triglyceride values were also observed in male rats in the 2 mg/kg dose group at the week 27 sacrifice, but were not statistically different from the control group. These findings were attributed to an acute elevation in both cholesterol (491 mg/dL) and triglycerides

(684 mg/dL) in a single male rat (animal #2011M) in this dose group. At the end of the 4 week recovery period, there were no remarkable differences in serum biochemistry profiles between control and IL-1ra treated male rats, with the exception of serum globulin levels, which were significantly increased ($p \leq 0.05$, Dunnett's test) as compared to the vehicle control group in the male rats treated with 20 or 200 mg/kg/d IL-1ra. A concomitant, significant decrease in A:G ratios was also observed in all three IL-1ra treated groups of male rats, as compared to the control animals.

In the female rats at the week 27 sacrifice, the only significant findings were a slight (6.9%) increase in the mean value for total serum protein in the 20 mg/kg/d dose group, and significant increases in mean globulin levels in both the 20 and 200 mg/kg/d groups, as compared to the vehicle control group ($p \leq 0.05$, Dunnett's test). At the week 31 recovery sacrifice, mean values for cholesterol were significantly decreased from control in the female rats treated with 20 mg/kg/d IL-1ra ($p \leq 0.01$, Dunnett's test). However, globulin levels had decreased at this time point in all three groups of female rats treated with IL-1ra, as compared to the control and were not significantly different at this time point.

Comment: The contracting laboratory has provided a list of reference ranges for the clinical biochemistry and hematology parameters. Although significant differences were found in some parameters in the IL-1ra treated rats as compared to the control group, the mean values for the most part were within the normal limits for Sprague-Dawley rats of this age group. The exceptions were the increase in serum globulin in the female rats treated with 20 mg/kg/d at the 27 week time point, and the decreased A:G ratio in all groups of IL-1ra treated, male rats at the week 31 recovery time point, which were both outside of the normal limits for this strain. However, the contracting laboratory did not feel that these changes were biologically significant.

Urinalysis data showed increases in total urine protein in all groups of male rats treated with IL-1ra, regardless of dose at week 26 on study. The incidence of animals with urine protein levels $\geq$ 3.0 g/L were 3/10, 8/10, 6/10, and 9/10 for male rats treated with vehicle control, 2, 20, or 200 mg/kg/d IL-1ra, respectively. In the female rats at week 26, the incidence of animals with urine protein levels $\geq$ 3.0 g/L was 0/10 for the control and 2 mg/kg/d dose groups, and 4/10 and 6/10 for rats in the 20 and 200 mg/kg/d dose groups, respectively. By week 30 on study, the proteinuria had resolved in the recovery animals, and no significant differences were noted between the control and IL-1ra treated groups.

Comment: Histologic analysis revealed chronic, progressive nephropathy in the kidneys of both male and female rats treated with IL-1ra, which was apparently related to treatment with the test article. Additionally, IL-1ra protein was detected in the urine of the sampled rats at weeks 13 and 26 o n study, although at low (ng/ml) levels. These findings, together with the proteinuria observed on urinalysis suggest some low level of renal damage, or impairment of tubular resorption and degradation of IL-1ra following chronic administration at high dose levels.

At terminal sacrifice after 26 weeks of treatment, male rats treated with 200 mg/kg/d IL-1ra had statistically significant increases in absolute kidney weights (right and left, 19% and 20% increases, respectively), and a 34% increase in group mean liver weights, as compared to the control group. Female rats in the 20 mg/kg/d and 200 mg/kg/d dose groups also had significant, 36% and 23%, respective increases in absolute liver weight at the 27 week sacrifice ($p \leq 0.05$, Dunnett's test). Absolute pituitary weights were also increased as compared to the control group in the 20 mg/kg/d dose group at this time point ($p \leq 0.05$, Dunnett's test).

Relative brain weights (to body weight) were decreased in male rats in the 2 and 200 mg/kg/d dose groups, as compared to the mean value for the vehicle control group ($p \leq 0.05$, Dunnett's test). In the female animals, the only significant finding was a 29% increase in liver weight relative to body weight in the 20 mg/kg/d dose group, as compared to the vehicle control group ($p \leq 0.001$, Dunn's statistic). Neither the absolute or relative increases in liver weights were present in the Il-1ra treated groups after the 4 week, treatment-free recovery period.

Gross pathologic findings at the 27 week terminal sacrifice included areas of either pale color or discoloration on the surface of the liver in animals in all dose groups, including the vehicle control, with no relationship to the dose of the test article. Enlarged mandibular lymph nodes were also present in 2/10 control male and 1/10 control female rats, 5/5 male rats each in the 2 and 200 mg/kg/d IL-1ra dose groups, and 3/10 male and 1/10 female rats in the 20 mg/kg/d dose group. At the injection site, there were dark areas present in the subcutis on gross evaluation, with microscopic evidence of hemorrhage in virtually all animals in all dose groups. Both the liver and the injection site findings were considered related to the treatment with the test article, although there were no apparent dose-relationships present. At the end of the 4 week recovery period, the findings in the liver were still present in several animals in all dose groups, however, the injection site changes had resolved in all but one female rat each in the control and low-dose groups.

Other findings on gross pathologic evaluation included areas of depression, discoloration, dilatation, or dark foci in the kidneys, dark areas in the lung in 2 control male rats and one male rats in the high-dose group, lymph node enlargement or discoloration in one animal each in all dose groups, adhesions, cysts, pale areas in the spleen in male rats and in one female in the 2 mg/kg/d dose group, and pale areas in the prostate in 1 or 2 male animals in each dose group, including the vehicle control. The kidney findings were not present in any male rats in the recovery group; renal dilatation was only present in 1 female rat each in the 2 and 200 mg/kg/d dose groups, while the depressed areas were present only in 2 females in the 20 mg/kg/d IL-1ra dose group.

Histologically, the macroscopic findings in the livers of the male and female rats at the 27 week sacrifice time point were correlated with hepatocellular vacuolization, mixed, mononuclear and polymorphonuclear cell inflammatory infiltrates, hepatocellular necrosis, and areas of fibrosis. These findings occurred at approximately equal incidence and severity (slight to mild) in the vehicle control and IL-1ra treated groups. The dark areas present at the injection site on gross pathologic evaluation were correlated microscopically with hemorrhage and granuloma formation. Mixed cell infiltrates, consisting of monocytes, eosinophils, polymorphonuclear leukocytes and plasma cells were also present at the injection site in animals in all dose groups, without an apparent relationship in either incidence or severity to the dose of IL-1ra injected. At the end of the 4 week recovery period, the findings in the liver had completely resolved in all dose groups. The majority of the injection site findings had also resolved by the end of the recovery period, with the exception of an area of hemorrhage on one female control rat, granulomas in one male rat each in the control and 2 mg/kg/d dose groups, and a small inflammatory, mixed cell infiltrate in one high-dose male rat.

Microscopic changes in the kidneys were present in animals of both sexes in all dose groups, including the control and included a cyst in one male, mid-dose rat, dilatation of the renal pelvis and/or tubules, pyelitis, and mineralization at the corticomedullary junction. These findings were sporadic, occurring in 1 to 2 animals per group, of slight to mild severity, and had no apparent

relationship to the dose of IL-1ra administered. Of more concern was the presence of mononuclear cell infiltrates, and chronic, progressive nephropathy, which were found to have an apparent relationship to the dose of the test article. Mononuclear cell infiltrates were found in 1/10 each control and low-dose male rats, 4/10 male and 2/10 female rats at the 20 mg/kg/d dose group, and 2/10 male and 3/10 female rats treated with 200 mg/kg/d IL-1ra for 26 weeks. Chronic progressive nephropathy was similarly increased in incidence with relation to the dose of the test article, and was present in 2/10 control males, 3/10 male rats in the 2 mg/kg/d dose group, 1/10 male and 3/10 female rats in the mid-dose group, and 6/10 male and 1/10 female rats in the 200 mg/kg/d dose group. The severity of the lesions in the male rats in the highest dose group was increased in relation to that observed in the control and lower dose animals. Two male rats in this group had findings that were considered slight in severity, 3 rats had lesions of mild severity, and one male rat each had either moderate or severe, chronic progressive nephropathy present. Severe, chronic progressive nephropathy was also present in one female rat each in the groups treated with 20 or 200 mg/kg/d IL-1ra for 26 weeks.

After the 4 week recovery, chronic progressive nephropathy was still present in 1/5, 2/5, and 2/5 male rats in the 2, 20, and 200 mg/kg/d dose groups, respectively, and in 1/5 control female rats. The majority of the findings were slight in severity; however, the lesions present in both male rats in the 20 mg/kg/d IL-1ra dose group were considered mild in severity. These data suggest that the chronic progressive nephropathies observed were related to treatment with IL-1ra, but were reversible on discontinuation of the biologic. The relevance of these findings to humans treated with IL-1ra is unknown.

Comment: Proteinuria was noted in all male rats and most of the female animals in the 200 mg/kg/d IL-1ra dose group, and may have been related to the chronic, progressive nephropathy detected on microscopic evaluation.

Toxicokinetic evaluations of IL-1ra in plasma were conducted prior to initiation of dosing, and at days 4, 8, and 15 and weeks 8, 13, and 26 on study. Plasma IL-1ra concentrations 2 h after dosing were found to increase linearly in a dose-related fashion at days 4 and 8 on study. However, significant accumulation of IL-1ra was detected beginning on day 15 in rats in the 20 and 200 mg/kg/d dose groups. The data for the mean values, ± S.D. for each time point are presented in Table IV, below:

Table IV – Toxicokinetic Evaluation of IL-1ra Plasma Levels in Sprague-Dawley Rats

A. Males

Dose (mg/kg/d)	Plasma Concentration of IL-1ra (ng/ml), ± S.D.						
	Pre-dose	Day 4	Day 8	Day 15	Week 8	Week 13	Week 26
Control	0.0 ± 0.0	5.1 ± 7.2	0.9 ± 2.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2	0.0 ± 0.0	189 ± 31	193 ± 22	2074 ± 1966	592 ± 533	1013 ± 1533	601 ± 587
20	0.0 ± 0.0	2274 ± 452	2567 ± 1863	7494 ± 3004	58620 ± 48810	35740 ± 22590	17640 ± 8423
200	0.0 ± 0.0	21090 ± 4493	20390 ± 1602	48470 ± 21470	257200 ± 145000	203100 ± 113600	221000 ± 81470

B. Females

Dose (mg/kg/d)	Plasma Concentration of IL-1ra (ng/ml), ± S.D.						
	Pre-dose	Day 4	Day 8	Day 15	Week 8	Week 13	Week 26
Control	0.0 ± 0.0	0.0 ± 0.0	1.6 ± 2.4	0.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2	0.0 ± 0.0	154 ± 55	178 ± 30	1570 ± 1311	3419 ± 7116	1411 ± 2333	901 ± 1259
20	0.0 ± 0.0	1830 ± 201	6125 ± 7568	17840 ± 12200	92920 ± 31370	30900 ± 21160	11990 ± 5098
200	0.0 ± 0.0	19760 ± 4423	16120 ± 10440	82190 ± 27180	452600 ± 156000	317000 ± 69530	295400 ± 105300

In general, plasma concentrations increased following day 8 in all groups of IL-1ra treated animals, reaching peak concentrations at study week 8 and declining slightly at weeks 13 and 26. Plasma IL-1ra levels were highly variable between individual animals on study, as evidenced by the large standard deviations. In several cases, the values for the coefficient of variation (C.V.) were in excess of 200%. Normalization of the individual concentration data by gender revealed that there were no apparent effects of gender on the systemic exposure to IL-1ra.

Detectable levels of IL-1ra were present in the urine of rats from all treatment groups, including the controls, at d 2 and weeks 4 and 26 on study. Mean urine IL-1ra concentrations in the control and 2 mg/kg/d dose groups were not significantly different; however, there were positive samples in the control group detected, which suggest an interference of some component in urine with the assay being used. Concentrations of IL-1ra in urine were approximately linearly related to the dose of IL-1ra injected, and ranged from 0.8 to 13.3 ng/ml in the rats treated with 2 mg/kg/d, to 7099 to 93380 ng/ml in the rats treated with 200 mg/kg/d IL-1ra. The mean IL-1ra levels in the urine samples from the male rats in each treatment group were approximately 2 to 10-fold greater than the mean values for the female rats in the same dose groups. At all time points on study, and in all dose groups tested, less than 1% of the administered dose of IL-1ra was recovered in the urine.

Animals in all groups, including the vehicle control, developed anti-IL-1ra antibody activity by week 8 on study. In the rats treated with the test article, antibodies were first detectable in the plasma samples obtained from rats treated with 2 or 20 mg/kg/d IL-1ra at d 15 on study, and tended to decline in titer over the duration of the study. No antibody was detected in the rats treated with 200 mg/kg/d IL-1ra, with the exception of 3/5 female rats on study day 15, who had low titers present which then disappeared by the week 4 time point. By week 27 on study, all animals were negative for plasma anti-IL-1ra antibodies. The data are presented in Table V, A and B, below.

Table V – Anti-IL-1ra Antibody Evaluation in Plasma from Sprague-Dawley Rats

A. Males

Dose (mg/kg/d)	Mean Anti-IL-1ra Antibody Titer (Reciprocal Dilution), ± S.D.						
	Pre-dose	Day 4	Day 8	Day 15	Week 8	Week 13	Week 27
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4624 ± 8886	1600 ± 1319	0.0 ± 0.0
2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	800 ± 1025	2176 ± 2004	1568 ± 2075	0.0 ± 0.0
20	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	32 ± 44	64 ± 88	32 ± 72	0.0 ± 0.0
200	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

B. Females

Dose (mg/kg/d)	Mean Anti-IL-1ra Antibody Titer (Reciprocal Dilution), ± S.D.						
	Pre-dose	Day 4	Day 8	Day 15	Week 8	Week 13	Week 27
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	288 ± 237	416 ± 312	0.0 ± 0.0
2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4288 ± 9056	2208 ± 2669	320 ± 370	0.0 ± 0.0
20	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	160 ± 160	32 ± 72	16 ± 36	0.0 ± 0.0
200	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	32 ± 34	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Comment: The sponsor states that the development of anti-IL1ra antibody activity at week 8 in the rats in the vehicle control group may have been due to inadvertent injection of some animals in this group with the test article. However, review of the final protocol, and its amendments (Volume 9, Appendix 25; pp. 215-261) did not reveal any documentation that the control groups were mistakenly treated with the test article.

Comment: The reasons for the apparent, inverse relationship in antibody development to the dose of IL-1ra administered were not clearly explained by the sponsor. Several possibilities exist, the first being that inhibition of antibody formation is an extension of the pharmacologic effects of IL-1ra, which acts to selectively suppress T lymphocyte function, including Th_1 sub-populations involved in regulating the immune response. Although unlikely, down-regulation of Th_1 function may inhibit production of antibody in response to the foreign protein. Alternatively, the assay used to detect anti-IL-1ra antibody activity is a sandwich ELISA technique, in which IL-1ra coated plates are exposed to serum samples from treated rats. Anti-IL-1ra antibody present in the samples from the treated animals will bind to the IL-1ra on the plate, and is then detected with a secondary anti-rat antibody conjugated to a chromogenic agent. However, the binding of anti-IL-1ra antibody is competitive, and any free IL-1ra in the sample can inhibit the interaction of the antibodies with the IL-1ra coated to the plates, thereby giving a falsely negative readout. To minimize this possibility, the samples for antibody determination were obtained prior to dosing with the next injection of IL-1ra. However, the toxicokinetic data at the trough levels (11 h following the last administration of IL-1ra) still show very high levels of free IL-1ra present in the plasma samples from rats in the 20 and 200 mg/kg/d dose groups. Both the peak and trough IL-1ra plasma levels were found to increase over time on study, and may have interfered with the detection of the antibody responses. To date, the sponsor has not developed

another assay to evaluate the anti-IL-1ra antibody response following chronic treatment with the test article.

In summary, treatment of Sprague-Dawley rats by twice daily, s/c injection with 1, 10, or 100 mg/kg IL-1ra (total daily injection of 2, 20, or 200 mg/kg/d) was not associated with any overt, clinical signs of toxicity. Increases in food consumption, body weights, and body weight gains were noted in male rats treated with 2 or 200 mg/kg/d IL-1ra beginning at 4 to 6 weeks after study initiation, and continuing throughout the treatment period. There were no remarkable hematologic changes, or alterations in serum biochemistry profiles that were related to treatment with the test article. Increases in proteinuria were observed in both male and female rats after IL-1ra treatment, and were related both in incidence and severity to the dose of IL-1ra administered. The increased proteinuria, as well as increased urinary excretion of IL-1ra were correlated with microscopic findings of chronic progressive nephropathies, which were also dose-related in both incidence and severity. After 26 weeks of treatment, other findings included both gross and microscopic evidence of inflammation and mixed leukocytic cell infiltrates at the injection site, liver enlargement, and histologic evidence of hepatocellular hypertrophy, vacuolization, necrosis, and leukocytic infiltration. The macroscopic and microscopic findings in the liver were resolved by the end of a 4 week, treatment-free recovery period. The injection site inflammatory changes, as well as the chronic progressive nephropathies in the kidney were resolved in the majority of the recovery group animals by the end of the treatment-free period, suggesting that these changes are reversible. Because of the treatment-related, microscopic findings in the liver and kidneys, no NOAEL for this study could be determined.

Study #SY05-T89-01. ***Intravenous (14-day) systemic toxicity study of interleukin-1 receptor antagonist (IL1ra) in Sprague-Dawley rats.***

A two week, intravenous dose toxicity study of IL-1ra was conducted in Sprague-Dawley rats. Three rats/sex/group were injected daily in the caudal vein with vehicle (sterile PBS, pH 7.0), 0.047, 0.231, or 1.17 mg/kg/d IL-1ra, at a volume of 1.0 ml/kg for 14 consecutive days. Animals were observed twice daily for mortality and for clinical and behavioral signs of toxicity. Body weights were recorded at and on Study Day 1, prior to dosing, then at 3 to 4 day intervals until terminal sacrifice on d 15. Food consumption was measured at 3 to 4 day intervals on study. Samples of peripheral blood for determination of hematology, clinical chemistry, and immunologic evaluation, and overnight collection of urine samples for urinalysis were collected only at terminal sacrifice on d 15. At necropsy, complete gross pathologic evaluations were conduced on each animal to determine any target organs of toxicity. Samples of representative target tissues were preserved in neutral buffered formalin, processed and stained with hematoxylin and eosin, and evaluated microscopically for evidence of organ pathology.

All animals survived the 14 d treatment period. There were no overt, clinical signs of toxicity noted in any of the groups of rats on study. In general, body weights and body weight gains were not affected by treatment with IL-1ra, with the exception of the male rats treated with 0.047 mg/kg/d IL-1ra on study d 11. The mean value for body weight at this time point in these animals was statistically significantly less than control ($p \leq 0.05$, ANOVA). However, this finding was considered incidental to treatment with IL-1ra, since it was not observed in male rats at the mid- and highest dose levels, and was not present in the female animals at this time point. Slight, although statistically not significant decreases in total body weight gains were noted in female rats treated with 0.047 mg/kg/d IL-1ra at study termination. However, female rats in all

other groups, including those treated at the highest dose level of 1.17 mg/kg/d IL-1ra had gained weight by the end of the treatment period. The clinical significance of this finding is unknown.

There were no statistically significant differences in food consumption between the control and the IL-1ra treated rats at any time point on study, although one rat (animal #2566F, 0.231 mg/kg/d) had a sharp, approximately 50 g increase in food intake between days 8 and 11 on study. This finding was not observed in any other rats in either the control or the IL-1ra treated groups, and is of unknown clinical significance.

There were no remarkable alterations in either serum biochemistry, hematology, or urinalysis profiles in rats treated for 14 d with IL-1ra, as compared to animals injected for 14 d with the vehicle control. There were no macroscopic signs of organ toxicity on gross evaluation at necropsy. Histologic evaluation of the various organs did not reveal any treatment-related evidence of pathology. Incidental findings included mild to moderate, hydronephrosis and trace to mild, chronic inflammation in the kidneys, and chronic inflammatory lesions with the presence of infiltrating alveolar macrophages in the lungs. The kidney lesions were present in 2/3 control male rats, and 1/3 male rats each in the 0.047 and 1.17 mg/kg/d dose groups. The inflammatory findings in the lungs were present in 6/6 control, mid- and high-dose rats, and in 1/3 male rats and 2/3 female rats in the group treated with 0.231 mg/kg/d IL-1ra. These changes have previously been described in rat lungs, and were considered incidental to treatment with IL-1ra. Other miscellaneous findings included a pituitary cyst (animal #2554F, control group), and edema and inflammation in the skeletal muscle in one control rat (animal #2553F).

In summary, treatment of rats by consecutive, daily i/v bolus injections with IL-1ra was not associated with any signs of local or systemic toxicity. The NOAEL for IL-1ra under the conditions of this study was 1.17 mg/kg/d, i/v, for 14 days.

Comment: The protocol states that samples of serum were obtained for immunologic evaluation, and shipped to the sponsor for analysis. The results of the sponsor's analysis were not included in the final report, or in the final BLA submission from Amgen.

Study #SY05-T89-02. ***A subchronic, intravenous toxicity study of interleukin 1 receptor antagonist (IL1ra) in Sprague-Dawley rats.***

A second, repeat-dose 14 d i/v toxicity study was conducted at higher doses of IL-1ra in Sprague-Dawley rats. Five rats per sex per dose group were injected daily with i/v bolus injections of vehicle (sterile PBS, pH 7.0), 2, 5, or 20 mg/kg/d IL-1ra. Treatment conditions, animal handling and observations, and evaluations at necropsy were identical to those described for Study #SY05-T89-01, above.

No deaths occurred on study, and there were no overt signs of toxicity. Clinical observations were limited to the presence of red, blood-like material crusted around the nose in the majority of the animals on study. There was no apparent relationship in terms of either incidence or severity to treatment with the test article, as these findings were also present in 3/5 male and 2/5 female rats in the control group at various time points on study.

Group mean body weight values, but not body weight gains were significantly higher in male rats treated with 5 mg/kg/d IL-1ra at days 3, 7, and 10 on study, but not at terminal sacrifice on d 15.

There were no significant differences in either total body weights or body weight gains between the vehicle control and the 2 mg/kg/d or 20 mg/kg/d IL-1ra treated groups of rats. There were no remarkable differences in food consumption between the control and the IL-1ra treated groups.

Treatment with IL-1ra had no remarkable effects on serum biochemistry, urinalysis, or hematologic profiles as compared to rats treated with the vehicle control. At necropsy, several macroscopic findings were noted in animals in all groups, including the control. These were limited to enlargement of either the cervical (animals #77M and 85F, control, and #105F, 5 mg/kg/d) or mediastinal lymph nodes (animals #93F, 2 mg/kg/d and #114F, 20 mg/kg/d). Histologic evidence of mild to moderate, diffuse lymphoid hyperplasia was present in all samples on microscopic examination of prepared tissue section. Other findings on histologic evaluation included trace to mild evidence of hydronephrosis in the kidney, mandibular lymph node hyperplasia, and trace hemorrhage in the thyroid, which were present in animals in all dose groups at approximately equal incidence and severity. Of note was that chronic, interstitial pneumonia of mild severity was present in all 20 female rats, and in 2/20 male rats on study. Similar findings of hydronephrosis and chronic inflammatory changes in the lung were also reported in Study #SY05-T89-01, above.

In summary, treatment of rats for 14 consecutive days with 2, 5, or 20 mg/kg/d IL-1ra by i/v bolus injection was well tolerated, with little evidence of systemic toxicity or pathology. The NOAEL for IL-1ra, under the conditions of this study, was 20 mg/kg/d, by i/v bolus for 14 days.

Comment: The protocol states that samples of serum were obtained for immunologic evaluation, and shipped to the sponsor for analysis. The results of the sponsor's analysis were not included in the final report, or in the final BLA submission from Amgen.

Study #SY05-T89-05. ***Subchronic intravenous toxicity study of interleukin-1 receptor antagonist (IL1ra) in Sprague-Dawley rats.***

The toxicity of higher doses of IL-1ra was evaluated in Sprague-Dawley rats after i/v injection. Ten rats per sex per group were treated by daily, i/v injection for 14 days with vehicle control (sterile PBS plus 0.1 mM EDTA, pH 7.0), 3, 10, or 30 mg/kg IL-1ra. An additional 10 rats/sex were treated for 14 d with either vehicle or 30 mg/kg/d IL-1ra, i/v, then held for a 28 d, treatment-free recovery period. Animals were observed twice daily for mortality, and for clinical and behavioral signs of toxicity. Body weights were recorded at and on Study Day 1 just prior to dosing, then at study days 4, 7, 10, 14, and at terminal sacrifice on d 15. Body weights for the rats in the two recovery groups were recorded at these time points, and at study days 20, 23, 28, 30, 34, 37, 40, and at time of sacrifice on d 43. Food consumption was measured at 2 to 5 day intervals on study. Slit lamp biomicroscopy and fundoscopic, ophthalmic examinations were performed on all animals at baseline (during study week –1) and at study d 10 or 11, and on study d 39 for animals in the recovery groups.

Samples of peripheral blood for immunologic evaluations were collected from all rats on study prior to dose administration on study d 7, and on study d 15 or 16 by retro-orbital puncture of the right eye. Serum was shipped to the sponsor for evaluation of immunologic parameters. Blood samples for determination of hematology and clinical chemistry profiles, and overnight collection of urine samples for urinalysis were collected only at the end of the two week dosing period, and at terminal sacrifice on d 43 for the animals in the recovery groups. At necropsy,

complete gross pathologic evaluations were conduced on each animal to determine any target organs of toxicity. Samples of representative target tissues were preserved in neutral buffered formalin, processed and stained with hematoxylin and eosin, and evaluated microscopically for evidence of organ pathology.

Two rats (animals #81M and #102F, both in the 30 mg/kg/d recovery group) died on study d 7 and 16, respectively. The cause of death for each animal was reported as an overdose of ether anesthesia during the blood collection procedure, and was incidental to IL-1ra treatment.

All remaining animals survived to scheduled sacrifice at the end of the 14 d treatment or 28 d recovery periods. There were no overt, clinical signs of toxicity noted in any of the groups of rats on study. Clinical findings were limited to presence of reddish, blood-like material crusted around the nose, and alopecia of the front paws. These findings occurred at approximately equal incidence in both male and female rats in the control and the IL-1ra treated groups, and were considered unrelated to treatment. Occasional opacities in the right eye were noted in male and female rats, in both recovery groups; these lesions were considered related to the trauma associated with multiple blood collections by retro-orbital puncture.

In general, body weights and body weight gains were not affected by treatment with IL-1ra. All animals gained weight between pre-study and study d 14. Rats were fasted overnight prior to terminal sacrifice on study d 15 or 43, so the final body weights were less than the previous recording the day before. There were no remarkable differences in food consumption between the control and the IL-1ra treated rats at any time point on study.

There were no remarkable differences in erythrocyte parameters, platelet counts, or total leukocyte counts between control and IL-1ra treated rats at either the d 15 or d 43 terminal sacrifices. A statistically significant decrease in lymphocyte differential counts, with a concomitant increase in segmented neutrophils was observed for the female rats in the 10 mg/kg/d dose group as compared to the controls at d 15. This finding was not present in female rats treated with either 3 or 30 mg/kg/d IL-1ra for 14 d, or in male rats at any dose group. At the 43 d recovery sacrifice, there were no remarkable differences between the control and the IL-1ra treated rats in any of the hematologic parameters.

Statistically significant decreases in serum potassium, iron, albumin, A:G ratio, and ALT were observed for male rats in the group treated with 3 mg/kg/d IL-1ra on study d 15, as compared to animals injected for 14 d with the vehicle control ($p \leq 0.05$, ANOVA). The significant decreases in iron, albumin, and A:G ratios were also present in female rats in this same group, as compared to control. Other findings in the female rats treated with either 3 or 10 mg/kg/d IL-1ra for 14 d were a significant increase in BUN, as well as statistically significant decreases in serum alkaline phosphatase, and total protein as compared to the control group ($p \leq 0.05$, ANOVA). Although the mean values for these parameters were statistically significantly different from the control group, they were not outside the normal range of values for this strain of rats. There were no significant, nor remarkable differences in serum biochemistry profiles between the control and the high-dose animals at the end of the 28 d, treatment-free recovery period.

Urinalysis profiles showed no remarkable differences in pH or specific gravity between control and IL-1ra treated rats, either at study d 15 or at the end of the recovery period on study d 43.

There were no macroscopic signs of organ toxicity on gross evaluation at necropsy. Relative thyroid weights were significantly increased in male rats treated for 14 d with 30 mg/kg/d IL-1ra,

as compared to either the vehicle control or the lower dose groups ($p \leq 0.05$, ANOVA). Although not statistically significant, a trend towards increased absolute and relative kidney weights was observed in female rats treated with IL-1ra at study d 15, as compared to control animals. At the end of the 28 d recovery period, relative kidney and pituitary weights in the male rats treated with 30 mg/kg/d IL-1ra for 14 d were significantly elevated over the vehicle control group ($p \leq 0.05$, Student's *t* test). There were no remarkable changes in either absolute or relative organ weights in the female animals at study termination on d 43.

Histologic evaluation of the various organs did not reveal any treatment-related evidence of pathology, with the exception of trace to mild hemorrhage and perivascular inflammation at the site of injection. These findings were present in 1/10 male and 3/10 female rats in the vehicle control group, in 2/10 male and 1/10 female rats in the 10 mg/kg/d group, and in 3/10 male rats after 14 d of treatment with 30 mg/kg/d IL-1ra. Trace to mild inflammation and hemorrhage were still present at the injection site in 4/10 each male and female rats in the control group, and 1/10 IL-1ra treated rats at the end of the 28 d recovery period.

Incidental findings included mild to moderate, tubular dilatation, microconcretions, and pyelonephritis, and trace to mild, chronic inflammation with leukocytic infiltrates present in the kidneys of both male and female rats in all dose groups at the d 15 sacrifice. Hydronephrosis of moderate severity was present in one female control rat in the recovery group at study termination on d 43. Other miscellaneous findings included a trace to mild mononuclear cell and lymphocytic infiltrates in the heart, trace to severe hemorrhage, keratitis, and cataract formation in the eye, and mild to moderate hemorrhage, lymphohistiocytosis, mast cell infiltration, and lymphoid hyperplasia in the lymph nodes. These changes were observed at approximately equal incidence and severity in both the vehicle control and IL-1ra treated animals, and were not considered related to the test article.

In summary, treatment of rats by consecutive, daily i/v bolus injections with IL-1ra was not associated with any signs of local or systemic toxicity. Hemorrhage and perivascular inflammation at the injection site were observed histologically in rats in the control, low, and high-dose groups, and partially resolved at the end of the 28 d recovery period. Based on the injection site inflammatory findings, the NOAEL for IL-1ra under the conditions of this study was 10 mg/kg/d, i/v, for 14 days.

Comment: The protocol states that samples of serum were obtained for immunologic evaluation, and shipped to the sponsor for analysis. The results of the sponsor's analysis were not included in the final report, or in the final BLA submission from Amgen.

Study #SY05-T89-09. ***Subchronic intravenous toxicity study of interleukin-1 receptor antagonist (IL1ra) in Sprague-Dawley rats.***

A second study to evaluate the toxicity of higher doses of IL-1ra was conducted in Sprague-Dawley rats. Ten rats per sex per group were treated by daily, i/v injection for 14 days with vehicle control (sterile PBS plus 0.1 mM EDTA, pH 7.0) or 30 mg/kg IL-1ra. Animals were observed twice daily for mortality, and for clinical and behavioral signs of toxicity. Body weights and core body temperatures were recorded at baseline during the acclimation period, on study d 1 just prior to dosing, then at study days 4, 7, 10, 14, and at terminal sacrifice on d 15.

Slit lamp biomicroscopy and fundoscopic, ophthalmic examinations were performed on all animals at baseline (during study week –1) and at study d 14.

Samples of peripheral blood for immunologic evaluations were collected from all rats on study d 15 by retro-orbital puncture of the right eye. Serum was shipped to the sponsor for evaluation of immunologic parameters. Blood samples for determination of hematology and clinical chemistry profiles and coagulation factors, and overnight collection of urine samples for urinalysis were collected at baseline on study days -7 and -1, and at the end of the two week dosing period on study d 15. At necropsy, complete gross pathologic evaluations were conduced on each animal to determine any target organs of toxicity. Samples of representative target tissues were preserved in neutral buffered formalin, processed and stained with hematoxylin and eosin, and evaluated microscopically for evidence of organ pathology.

All animals survived until scheduled sacrifice on study d 15. There were no clinical signs of overt toxicity noted in either the control or the IL-1ra treated rats at any time point on study. Sporadic incidences of alopecia on the front paws, reddish material crusted around the nares, and hemorrhage, swelling and opacity in the right eye were considered incidental to treatment with the test article. The eye lesions were considered related to the blood collection procedure by retro-orbital puncture.

There were no remarkable differences between the control and the IL-1ra treated rats in final body weights, body weight gains, or food consumption over the duration of the study. Although not statistically different at each time point, a trend toward decreased body temperatures was observed in the control male rats, when compared to the IL-1ra treated animals over the duration of the study. The decreases in body temperature were significantly different from the baseline measurements in both the vehicle and IL-1ra treated male animals between study days -7 and 14 ($p \leq 0.05$, ANOVA).

There were no significant differences in either hematologic or serum biochemistry profiles between the control and IL-1ra treated rats at study d 15, and no remarkable changes from the baseline values for each of these parameters at study days -7 and -1. No remarkable differences between the two groups were noted for PT, APTT, or fibrinogen levels, or urinalysis profiles at either baseline or study d 15.

At necropsy, there were no grossly evident signs of organ toxicity on macroscopic evaluation, and no significant differences in either absolute or relative organ weights were noted between the two groups. Evaluation of bone marrow smears showed no remarkable differences between control and IL-1ra treated rats in the mean myeloid:erythroid ratios.

On histologic evaluation, trace amounts of subacute, inflammatory infiltrates were noted at the site of injection in one male and one female rat each in the group treated with 30 mg/kg/d IL-1ra (animals #288M and #307F, respectively). This was not considered to be either significant, or a treatment-related finding by the reviewing pathologist. Chronic, interstitial pneumonia with infiltrates of mononuclear cells and alveolar macrophages was observed in the lungs of both male and female rats in both the vehicle control and the IL-1ra treated groups. These findings were trace to mild in severity, and occurred in 8/10 male and 10/10 females in the control group, and in 9/10 male and 8/10 female rats after 14 d treatment with 30 mg/kg/d IL-1ra.

Incidental findings included hydronephrosis of mild severity on one male and one female control rat each in the vehicle control group (animals #277M and #299F, respectively), and a cyst on the

kidney in one male rat in the IL-1ra treated group (animal #292M). Other miscellaneous findings included a severe, unilateral degeneration, hemorrhage, and a moderate cataract formation in rat #280M in the control group, which was considered likely related to an accidental puncture of the eye during blood collection, and occasional congestion of trace severity in the lymph nodes. These changes were observed at approximately equal incidence and severity in both the vehicle control and IL-1ra treated animals, and were not considered related to the test article.

In summary, treatment of rats by consecutive, daily i/v bolus injections with 30 mg/kg/d IL-1ra was not associated with any signs of local or systemic toxicity. The NOAEL for IL-1ra under the conditions of this study was 30 mg/kg/d, i/v, for 14 days.

Comment: The protocol states that samples of serum were obtained for immunologic evaluation, and shipped to the sponsor for analysis. The results of the sponsor's analysis were not included in the final report, or in the final BLA submission from Amgen.

Study #SY05-T92-01. ***A two-week nose-only inhalation toxicity study of recombinant human interleukin-1 receptor antagonist (IL-1ra) in the rat.***

The purpose of this study was to evaluate the toxicity of repeated administrations of IL-1ra via the inhalation route of exposure. Ten rats/sex/group were exposed for 2 h each day by nose-only inhalation at a flow rate of 20 LPM to either vehicle control (citrate buffered saline plus EDTA and Tween 80), or target concentrations of 7.5 or 75 mg/m^2 IL-1ra as a liquid aerosol, for 14 d. An additional 10 rats/sex were exposed to house-line air only, as a negative control group for 14 d. Exposure levels were monitored by collecting samples gravimetrically twice daily for each chamber and determining the concentration of IL-1ra protein by ELISA. Particle size distribution measurements were analyzed daily with a — cascade impactor, and the two measurements together used to calculate the mean lung deposition of IL-1ra for each test group. The cumulative, mean gravimetric exposure concentrations of IL-1ra for animals exposed to the air only control, the vehicle control, or 7.5 or 75 mg/m^2 IL-1ra were 0, 0, 10, and 99 mg/m^2, respectively. Particle size distributions for the vehicle control, low and high-dose IL-1ra test solutions were —, respectively, resulting in respective calculated mean lung depositions of 0, 0.33, and 3.2 mg/kg/d IL-1ra.

Animals were observed twice daily for mortality and for overt signs of toxicity, and during the exposure period for abnormal signs related to administration of the test article. Detailed physical examinations were performed prior to study initiation, then at days 6 and 13 during treatment. Body weights were recorded prior to treatment at study days -7, -1, and 0, then twice weekly during the exposure period, and food consumption was recorded weekly.

Peripheral blood samples for evaluation of hematology and clinical chemistry profiles, and for analysis of serum IL-1ra and antibody levels were obtained at baseline and on study d 8 via venipuncture of the retrobulbar venous plexus of the eye, and from fasted rats at study termination (24 h after the final IL-1ra exposure).

Complete necropsies were performed on all animals on study d 15, approximately 24 h following completion of the final exposure to the test or control articles. Both gross pathological evaluation of external surfaces and internal organs, as well as weights of selected organ and organ pairs were recorded. Selected tissues were sampled and processed for histopathologic

evaluation of toxicity after hematoxylin and eosin staining of the sections. Tissues selected for microscopic evaluation from each animal included the larynx, lungs with mainstem bronchi, pulmonary lymph nodes, nasal turbinates, pharynx, spleen, trachea, and any gross lesions present. Other tissues, including samples of liver, heart, adrenal glands, kidneys, and brain were preserved in neutral buffered formalin, but not evaluated for evidence of tissue pathology.

Eight female rats (animal numbers and test groups not identified) group died on study d 15 following blood collection, but prior to the scheduled terminal sacrifice. These animals were necropsied as planned, and included in the final study results. An additional male rat (animal #3009M) in the group treated with 7.5 mg/m^2/d IL-1ra died on study d 8 from an apparent overdose of CO_2/O_2 anesthesia during blood collection, and was not included in the final analysis.

Other than the death in male rat #3009M after anesthesia overdose on study d 8, all animals survived the treatment period. There were no overt, clinical signs of toxicity noted in any of the groups of rats at any time point on study. Clinical observations were limited to soft stools of slight to marked severity, reddish discharge and crusted material around the nose, and wet fur. These findings occurred at approximately equal incidence in both male and female rats in all groups, including the air-only and vehicle controls.

There were no significant or remarkable effects of IL-1ra treatment on final body weights, body weight gains, or food consumption as compared to animals in either the vehicle or air-only control groups. Data regarding the hematology and serum biochemistry profiles were provided only for the terminal sacrifice time point. At terminal sacrifice, significant decreases from the air-only control group were noted for hemoglobin and hematocrit levels in male rats in the vehicle control and 75 mg/m^2/d IL-1ra groups, and a significant decrease in red cell counts was noted for male rats in the high dose group only. When the values for the IL-1ra treated rats were compared to animals receiving vehicle control, there were no remarkable or significant differences in erythrocyte parameters. No similar findings were noted in the female rats in any of the dose groups, including both the vehicle and the air-only controls. There were no remarkable differences between the control and the test article groups in mean platelet or total leukocyte counts, or prothrombin time as a measure of coagulation factors. No differential leukocyte counts were reported.

There were no remarkable or significant differences between the IL-1ra treated and the vehicle control animals in serum biochemistry profiles at the d 15 terminal sacrifice. However, when compared to the values obtained for the air-only control group, statistically significant decreases in serum albumin were noted in male rats in both IL-1ra dose groups and the vehicle control, and a significant increase in serum globulin was noted for female rats in the vehicle control group. The toxicological relevance of these findings to treatment with IL-1ra is not considered biologically significant.

No significant differences in either absolute or relative organ weights were noted between the two control groups and the animals treated with either dose of IL-1ra by inhalation for 14 d. On gross pathologic evaluation, 9/10 male and 4/10 female rats in the group treated with 75 mg/m^2/d IL-1ra had areas of reddish discoloration in the lungs. These findings were also present in 6/20 rats in the air-only control group, 8/20 rats in the vehicle control group, and 4/10 male rats and 0/10 females in the group treated with 7.5 mg/m^2/d IL-1ra. Microscopically, these findings were determined to be areas of congestion and/or emphysema within the alveoli, with infiltration of erythrocytes and alveolar macrophages, and subacute inflammation present in the peribronchiolar

areas. Perivascular and peribronchiolar infiltration by lymphocytes was also present in all animals on study, regardless of treatment. Other microscopic findings included the presence of eosinophilic material and erythrocytes in the nasal turbinates and lumens, without evidence of inflammation in the majority of the rats in both control and IL-1ra treated groups, and erythrocytes in the lymph node sinuses, accompanied by sinus ectasia and reticuloendothelial cell hyperplasia at approximately equal incidence in both male and female rats of all dose groups. All animals, regardless of treatment group, had evidence of extramedullary hematopoiesis in the spleen at the end of the treatment period; this finding may represent a physiologic response to the repeated blood collections performed for this study.

In summary, treatment of Sprague-Dawley rats with either 7.5 or 75 mg/m^2/d IL-1ra by nose-only inhalation for 2 weeks was not associated with any evidence of local or systemic toxicities that were related to the test article. The NOAEL for IL-1ra by the inhalation route in this species is therefore 75 mg/m^2/d, for a 14 d treatment.

Study #SY05-T89-11. ***Subchronic (2 week) systemic toxicity study of IL1ra in male and female Rhesus monkeys following repeated subcutaneous injections.***

The toxicity of Il-1ra was evaluated in Rhesus monkeys following twice daily, s/c injections for two weeks. Three monkeys per sex were treated with citrate buffered saline as a vehicle control, or with 5, 20, or 80 mg/kg/d IL-1ra, as divided doses approximately 12 h apart for 14 d. Throughout the dosing period, animals were observed twice daily for mortality and clinical signs of toxicity or behavioral changes. Body weights and temperatures (both rectal and tympanic) were recorded twice during baseline at study weeks -2 and -1, just prior to dose initiation on study d 1, and on study days 8 and 15, prior to sacrifice. Food consumption was recorded qualitatively twice daily. Fundoscopic and slit lamp, biomicroscopic ophthalmic examinations were performed on all animals prior to test article administration, and at study days 12 (females) and 13 (males). Seven lead ECG profiles were determined for each monkey at study weeks -2 and -1 at baseline, and on study d 15 prior to terminal sacrifice.

Samples of peripheral blood for analysis of anti-IL-1ra antibody formation were obtained from all animals on study at baseline (study weeks -2 and -1), and on study days 8 and 15. Serum samples were prepared and stored frozen at -20°C until shipped to the sponsor for further analysis. Urine samples for urinalysis profiles were collected overnight from fasted monkeys at baseline (study weeks -2 and -1), and on study days 8 and 15. Peripheral blood samples for determination of hematology and serum biochemistry profiles were obtained at these same time points.

At necropsy, all animals were subjected to a complete gross pathologic examination, and selected organs or organ pairs were removed, weighed, and sampled for further histologic evaluation. Representative sections of formalin-fixed tissues were embedded in paraffin, sectioned at 4-6 microns, and stained with hematoxylin and eosin for identification of any microscopic lesions.

All animals survived the treatment period until terminal sacrifice on study d 15, with no evidence of overt clinical toxicities. Clinical signs were limited to the occasional presence of soft stool and/or diarrhea in animals in all groups, including the vehicle control. One monkey (animal #78Z, female) in the group treated with 80 mg/kg/ developed a rectal prolapse on study d 4, with blood present in the stool on study d 8. However, these findings were not considered related to treatment with IL-1ra.

There were no significant changes in either final body weights or total weight gains between animals in the control and IL-1ra treated groups, and no remarkable changes in either rectal or tympanic body temperatures over the duration of the treatment. Inappetence was noted in only one monkey (animal #5596M, 80 mg/kg/d IL-1ra) on study d 9 and did not recur at any later time point.

There were no significant or remarkable changes in serum biochemistry profiles, hematologic parameters, or urinalysis in any of the control or IL-1ra treated groups of monkeys. Serum protein electrophoresis did not reveal any changes in levels of albumin, α-, β, or γ-globulins, or A:G ratios between baseline and study days 8 or 15 in any of the groups. There were no treatment-related alterations in urine chemistry profiles observed in any animal on study.

At necropsy, there were no grossly observable lesions in male or female monkeys treated with either 5 or 20 mg/kg/d IL-1ra, or in female monkeys in the vehicle control or 80 mg/kg/d IL-1ra group. One male monkey in the vehicle control group (animal #5597M) had a thickened, and diffusely reddened meninges in the cerebrum. The caudal pole of the occipital lobe of the brain was atrophied, with marked discoloration on the surface. Microscopic evaluation of this lesion revealed mild, focal areas of atrophy and presence of "gitter" cells in both the cerebrum and the meninges. The dura was thickened and fibrotic, with mature bone marrow present, and the cranium was eroded and reddened. These lesions were attributed to a previous head trauma in this animal, and were not considered related to treatment.

One male monkey in the group treated with 80 mg/kg/d IL-1ra (animal #5579M) had a subcutaneous, 1 x 2 cm mass over the right inguinal lymph nodes, with a white, creamy fluid present at gross evaluation. Microscopically, this lesion was determined to be an abscess of moderate severity. This animal also had areas of petechial hemorrhage present on the right testis, with no microscopic correlates found.

Treatment-related histopathologic findings were limited to the presence of chronic, inflammatory areas at the injection site, which appeared to be related in incidence to the dose of IL-1ra administered. Chronic inflammation, although of trace severity in all cases, was observed in 1/6 monkeys each in the vehicle control and 5 mg/kg/d IL-1ra dose groups, in 3/6 monkeys treated for 14 d with 20 mg/kg/d IL-1ra, and in 5/6 monkeys treated with 80 mg/kg/d. The NOAEL for local reactivity at the subcutaneous injection site is considered to be 2.5 mg/kg/injection, or 5 mg/kg/d for 14 d.

Other incidental observations on histologic evaluation included chronic inflammatory infiltrates of mononuclear and/or lymphocytic cells in the esophagus, liver, lung, salivary gland, tongue, and vagina, increased alveolar macrophages in the lung, and mineralization in the adrenal glands. These lesions were present in control and IL-1ra treated monkeys at approximately equal incidence and severity, and was not related to treatment with the test article.

In summary, twice daily injection of IL-1ra at doses of 2.5, 10, or 40 mg/kg (total doses of 5, 20, or 80 mg/kg/d) was well tolerated in Rhesus monkeys, with no evidence of systemic toxicity. The NOAEL, based on hematologic, serum chemistry, and clinical findings is 80 mg/kg/d IL-1ra for 14 d. Based on the increased incidence of chronic inflammatory infiltrates at the injection site in the mid- and high-dose monkeys, the NOAEL for local reactivity at the injection site is considered to be 2.5 mg/kg/injection, or 5 mg/kg/d total dose.

Comment: Unlike the studies conducted in Sprague-Dawley rats (above), there were no remarkable findings in the kidneys of any of the monkeys on study, despite the fact that the doses were equivalent to 2.5 to 4 times higher than those tested in the rats. Taken together, these data suggest that the hydronephrosis, interstitial nephritis, and proteinuria noted in the rodents may be due to a species difference in how the different animals handle large doses of a human protein, and may be unrelated to a direct toxicity induced by IL-1ra.

Study #960040. ***A 4-week toxicity study of IL-1ra administered by subcutaneous injection to Rhesus monkeys, with a 4-week recovery period.***

The toxicity and toxicokinetics of IL-1ra after daily, s/c injections were evaluated in Rhesus monkeys after a one month treatment period. Male and female Rhesus monkeys (3/sex/group) were treated daily by s/c injection with vehicle control (IL-1ra placebo), or IL-1ra at doses of 10, 100, or 200 mg/kg/d x 28 d. Two additional animals/sex in the control and high-dose IL-1ra groups were retained for a 4 week treatment-free recovery period. Clinical observations for signs of morbidity or overt toxicities as well as measurement of food consumption were performed daily, and body weights were determined weekly. Fasting peripheral blood samples for hematologic and serum biochemistry profiles were obtained from all animals prior to study initiation for determination of baseline values, then prior to dosing on study days 7, 14, and 28, and at week 4 of recovery in the appropriate dose groups. Samples for urinalysis and urine chemistries were obtained pre-study, and on days 2, 15, 27, and at terminal sacrifice on either days 29 or 57. Aliquots of urine were collected on study days 2, 15, and 27, and shipped to the sponsor for analysis of urine IL-1ra levels by ELISA. Physiologic parameters (ECG, respiratory and heart rates, blood pressure, body temperatures) were recorded at these same time points. General veterinary examinations, as well as ophthalmologic examinations were performed once during the baseline period, then on d 27 in all animals, and on d 56 in the recovery animals. A full necropsy and gross pathologic evaluation was performed on each surviving animal at terminal sacrifice (weeks 5 or 9 on study for the end-of-treatment and recovery groups, respectively), with organ weights recorded, and tissue samples taken and processed for histopathologic evaluation.

Peripheral blood samples were also obtained from vehicle control and IL-1ra treated monkeys for companion toxicokinetic and antibody development assays. Plasma samples for toxicokinetic analysis were collected prior to dosing, and 0.5, 1, 2, 4, 6, 8, 12, and 24 h after injection on days 1 and 28 of study. Blood samples were also collected 2 h after dosing on days 7, 14, and 21 for analysis of plasma levels of IL-1ra only. Additional serum samples were collected prior to dosing on study days 1, 7, 14, 21, 28, and on d 56 from the recovery animals for analysis of anti-IL-1ra antibody activity.

One male monkey (#R3549M, 100 mg/kg/d IL-1ra dose group) was found dead on study d 6, after receiving 5 doses of the test article. This animal had not shown any clinical signs of toxicity prior to its death. Necropsy of the carcass revealed that this monkey had developed a gastric dilatation and subsequent rupture of the stomach, which was identified as the cause of death, and was not considered related to treatment with IL-1ra.

During the treatment period, 5/10 animals treated with the highest dose of IL-1ra developed skin lesions, beginning during the second to third week of treatment, and continuing through d 45 of

the recovery period in monkey #R3590M. Lesions in three of the five animals began as raised, reddened swelling, with central areas of drainage containing serosanguineous fluid. One animal (#R1PK, ♂) had an area of drainage in the inguinal area that was larger and more severe than that observed in the other monkeys; this animal went on to develop acute swelling in the left carpus and fourth digit of the right hand, two days later. Biopsy of the carpal lesion revealed suppurative, inflammatory changes including hemorrhage, lymphohistiocytic infiltrates, and microabscess formation. The initial lesion noted in male monkey #R3590M presented as redness near the left inguinal area, with a thickening of the surrounding skin. This animal did not develop draining lesions, and microscopic evaluation of the tissues taken at necropsy revealed both lymphohistiocytic and suppurative inflammatory infiltrates, accompanied by hemorrhage into the subcutaneous tissue.

Female monkey #R3969F had abscess formation at three injection sites, which was initially detected at d 25 on study. Biopsy and culture of the abscesses revealed the presence of edema and acute hemorrhage in the immediate sites, accompanied by chronic, inflammatory infiltrates composed of mononuclear cells and eosinophils. Culture specimens were positive for coagulase-positive, hemolytic *Staphylococcus sp.*. This organism is commonly found on the skin of monkeys, and may have been introduced into the subcutaneous tissues during the injection of the test article.

No evidence of skin lesion was present in any of the other monkeys treated with 10 or 100 mg/kg/d IL-1ra, nor in the animals injected with the vehicle control. Other clinical signs that were unrelated to the test article included bruising in several animals at the femoral sites of venipuncture. Sporadic incidences of soft stool were noted in several animals in all dose groups, including the control at various time points on study. The bruising was considered to be related to tissue trauma associated with repeated blood collections, and the findings of soft stool are not uncommon in this strain of monkeys under laboratory conditions.

No significant differences were noted between the vehicle control and the IL-1ra treated animals in food consumption, body weights, body weight gains, or overall general health. There were no treatment related changes in body temperature, blood pressure, heart rate, respiratory rates, or ECG profiles in monkeys treated for four weeks with IL-1ra, as compared to the placebo control group or to baseline values. Although statistically significant differences from the control group were noted in blood pressure and heart rate on d 27 (10 mg/kg/d IL-1ra and 200 mg/kg/d IL-1ra dose groups, respectively), and in body temperature on d 2 in the high-dose group, these findings were still within normal limits for Rhesus monkeys of this age group, and were not considered by the contracting laboratory to be biologically meaningful. Ophthalmologic exams were normal in all monkeys at all time points on study.

Hematologic profiles showed evidence of anemia in all animals between the pre-study and d 7 evaluations, which could be attributed to the multiple blood samples collected for toxicokinetic determinations on days 1 and 2. Decreased erythrocyte counts, hemoglobin and hematocrit were observed at the end of the treatment period in two monkeys (animals #R1PK [♂] and #R3969F) in the group treated with 200 mg/kg/d IL-1ra. These two monkeys also had increases in both the numbers of circulating, nucleated red cells and reticulocytes, suggesting a regenerative anemia. However, both of these animals were found to have skin lesions, including draining, suppurative lesions and hemorrhage during weeks 3 and 4 on study, which may have contributed to the decreases in erythrocyte parameters. There was a trend in all groups of animals to have decreased erythrocyte parameters (red cell counts, hemoglobin, hematocrit) at all time points during the treatment period, as well as significant increases in MCV and MCH in the monkeys

treated with 100 or 200 mg/kg/d IL-1ra. However, these differences were not statistically significant, and had returned to baseline values by d 28 in the control monkeys and by d 56 in the recovery group of animals treated with 200 mg/kg/d IL-1ra.

Other alterations in hematologic profiles included elevations in total leukocyte counts in three monkeys in the high-dose group (animals #R1PK [♂], #R3584F and #R2707F) at d 28, relative to baseline values. Group mean values for this treatment group, however, were not significantly different from either the vehicle control or the lower dose groups ($p > 0.05$, *t*-test). There were no remarkable differences in platelet counts between the control groups and all groups of monkeys treated with IL-1ra. No significant differences in leukocyte differential counts were observed at any time point on study between the control and IL-1ra treated monkeys, although trends towards increasing lymphocyte absolute counts and percentages and decreasing percentages and absolute counts of polysegmented neutrophils were observed in all dose groups over the duration of the study.

No significant differences in prothrombin time or APTT were observed between either baseline and study termination, or between the vehicle control and the IL-1ra treated groups at any time point on study. No remarkable effects of IL-1ra were observed on the macroscopic urinalysis profiles over the duration of the treatment and recovery periods, with the exception of urinary pH, which was significantly decreased from the control group at study days 2 in monkeys treated with 10 or 200 mg/kg/d IL-1ra ($p \leq 0.05$, *t*-test). Urinary pH was also decreased as compared to control on d 27 in the monkeys treated with 10 mg/kg/d IL-1ra, and increased on d 29 in the animals in the high-dose group ($p \leq 0.05$, *t*-test). Urine chemistry profiles showed increased potassium, chloride, calcium, and phosphorous excretion by animals in all dose groups on study d 2, as compared to baseline. Potassium and phosphorus excretion were significantly different from control animals in monkeys treated with 10 mg/kg/d at this time point, and urinary calcium was significantly increased in the 100 mg/kg/d IL-1ra group at study d 2 ($p \leq 0.05$, *t* test, 2-tailed, heteroscedastic). Urinary phosphorous excretion remained significantly elevated over control animals in monkeys in the 10 mg/kg/d IL-1ra dose group over the duration of the study.

There were no definitive, treatment-related changes in clinical chemistry profiles for animals treated with 10, 100, or 200 mg/kg/d IL-1ra over the duration of the study, as compared to either the placebo control group, or to baseline values. Although slight, statistically significant elevations in sodium and chloride were observed in the high-dose monkeys at days 7 and 14 on study as compared to the placebo control group, these findings were still within normal limits for Rhesus monkeys of this age group, and had resolved to baseline by the next measurement.

No remarkable differences in serum levels of hepatic transaminases, g-glutamyl transpeptidase, LDH, BUN, creatinine, potassium, phosphorus, or uric acid were noted between the vehicle control group and monkeys at any dose level of IL-1ra throughout the duration of the study. Serum albumin was significantly increased over control in monkeys treated with 10 mg/kg/d IL-1ra at study d 7; however, this finding was not outside the normal range of values for Rhesus monkeys. There were no other remarkable differences in serum cholesterol or triglyceride levels, or in total protein, globulin, and serum albumin levels, or A:G ratios between the control monkeys and all groups of IL-1ra treated monkeys at any time point during the treatment or recovery periods.

At the d 29 necropsy, the only treatment-related macroscopic pathology findings were minimal to moderate hemorrhage and thickening at the injection site(s). These effects were observed in

monkeys in all groups, including the vehicle control and IL-1ra treated animals, without a dose-relationship in either incidence or severity. On histologic evaluation, these findings correlated with acute hemorrhage with lymphocytic and eosinophil infiltrates, chronic inflammation, and evidence of fibroplasia, fibrin exudates, and suppurative inflammation. The microscopic findings did demonstrate an apparent relationship in severity to the dose of IL-1ra injected, and were most severe (mild to moderate) in the monkeys treated with 200 mg/kg/d at the d 29 sacrifice. The injection site hemorrhage, inflammation, and hemorrhage were only present in 1/ 4 monkeys (animal #R2707F) treated with 200 mg/kg IL-1ra at the end of the treatment-free recovery period; by this point in time, the severity had decreased to minimal.

Incidental gross pathologic findings included paraovarian cysts in the right ovary from one female monkey each in the vehicle control and 200 mg/kg/d IL-1ra dose groups, and focal to more diffuse, reddened areas in the stomach or colon of one monkey each in the 10 mg/kg/d IL-1ra dose group, and in one female monkey treated with 100 mg/kg/d IL-1ra for 28 d. Monkey #R1PK (♂) in the 200 mg/kg/d dose group had numerous wounds, enlarged, inguinal lymph nodes, and excoriating skin lesions, which were associated with an inflammatory reaction at the injection sites. Other miscellaneous findings in monkeys in the recovery groups included multiple tan foci on the liver, and multiple brown to grey nodules in the colon, mid-colon, and cecum of control male monkey #R3664M, and on the mucosal surface of the colon of male monkey #R3590M, in the high-dose group. Histologically, these lesions were described as nodular, granulomatous lesions in the submucosa and subserosa of the gastrointestinal tract. Some of these granulomas contained Trichostrongylidae nematodes (*Oesophagostomum sp.*), which are common parasites of macaques. The liver nodules were found to contain parasitic infestation by cestodes, resulting in small granuloma formations. These findings were considered unrelated to treatment with IL-1ra.

Histologic findings related to treatment were limited to the injection site, and included subcutaneous hemorrhage, inflammation, lymphocytic and eosinophil infiltrates, and fibrosis. The inflammatory lesions appeared to be perivascular in origin, and extended into the lower dermis, perimysium, and underlying fascia. These findings occurred with approximately equal incidence in all of the treatment groups, however, a dose-relationship in the severity of the findings was noted (please see above description). The findings were reversible in all 4 control animals and in 3/ 4 of the monkeys treated with 200 mg/kg IL-1ra at the end of the recovery period.

Other microscopic findings unrelated to IL-1ra treatment included minimal to moderate, subacute or chronic areas of lymphocytic infiltration in the kidneys, gastrointestinal tract, or salivary glands, lymphoid hyperplasia and hyalinized germ centers in the lymph nodes and spleen, and focal areas of hepatocellular vacuolization, necrosis, and mononuclear cell infiltrates in the liver. These findings occurred at approximately equal incidence and severity across all treatment groups, and were considered by the reviewing pathologist to be incidental to treatment with the test articles.

Toxicokinetic evaluation of plasma and urinary IL-1ra levels, and evaluation of serum anti-IL-1ra antibody levels were conducted at various time points on study. Full toxicokinetic profiles were obtained on study days 1 and 28 for animals in all IL-1ra treated groups, from samples collected 0.5, 1, 2, 4, 6, 8, 12, and 24 h post-dosing. Peak plasma IL-1ra levels were also determined on days 7, 14, and 21 from samples collected 2 h after injection of the test article. Urine samples collected 2 h after dosing on days 2, 17, and 27 were also evaluated for IL-1ra levels. All IL-1ra determinations were conducted using a validated ELISA assay system designed to detect human

IL-1ra. Noncompartmental analysis of toxicokinetic profiles was performed using the WinNonlin (Version 1.1) software package.

Toxicokinetic data are available from 23/24 monkeys treated with the control or test articles for the 28 d study duration. Monkey #R3549M (100 mg/kg/d IL-1ra dose group) was found dead on study d 6, and was therefore not available for the final analysis points.

There were no apparent, gender-related differences in the mean concentration vs. time profiles of IL-1ra at any dose level tested (data not shown). Both maximal plasma concentrations (C_{max}) and total exposure ($AUC_{0-\infty}$) were approximately linearly related to the dose of IL-1ra administered (Table VI, below). Interestingly, on study d 1 after the first dose of IL-1ra, as the dose of the test article increased, there appeared to be an increase in the T_{max} and $T½_{elim}$. However, this increase was not as apparent after the final dose on d 28. Following multiple dosing with IL-1ra, there was no evidence of bioaccumulation of the agent in animals in the 100 and 200 mg/kg/d dose groups (Table VII). Although an apparent accumulation of IL-1ra was observed in monkeys treated with 10 mg/kg/d IL-1ra for 28 days, this group also developed significant anti-IL-1ra antibody activity, which may have acted as a carrier for the biologic, resulting in a decreased clearance and an apparent increase in both AUC and Cmax. The data for plasma IL-1ra levels after single and multiple dosing are presented in Tables VI and VII, below:

Table VI – Toxicokinetic Evaluation of IL-1ra Plasma Levels in Rhesus Monkeys

A. Day 1

P/K Parameter	Dose of IL-1ra Injected s/c		
	10 mg/kg	**100 mg/kg**	**200 mg/kg**
n	6	6	6
C_{max} (ng/ml)	5841 ± 430[a]	40660 ± 20110	59580 ± 9660
T_{max} (h)	1.7 ± 0.5	2.5 ± 1.2	3.8 ± 1.8
$T_{1/2elim}$ (h)	1.9 ± 0.13	2.1 ± 0.84	2.8 ± 0.73
$AUC_{(0-\infty)}$ (ng+h/ml)	32510 ± 1870	347100 ± 85700	677000 ± 114600
Cl/F (ml/min/kg)	5.1 ± 0.3	5.05 ± 1.2	5.05 ± 0.83

[a] mean, ± S.D. for all parameters

B. Day 28

P/K Parameter	Dose of IL-1ra Injected s/c		
	10 mg/kg	100 mg/kg	200 mg/kg
n	6	5	6
C_{max} (ng/ml)	7849 ± 4250[a]	38390 ± 8850	56040 ± 14070
T_{max} (h)	2.0 ± 1.1	2.0 ± 1.2	2.7 ± 1.2
$T1/2_{elim}$ (h)	2.7 ± 0.69	3.4 ± 0.88	4.7 ± 1.3
$AUC_{(0-\infty)}$ (ng+h/ml)	57150 ± 36760	375600 ± 156500	606400 ± 138700
Cl/F (ml/min/kg)	3.9 ± 2.0	4.9 ± 1.5	5.8 ± 1.4

[a] mean, ± S.D. for all parameters

Table VII – Plasma IL-1ra Concentrations 2 h After Injection in Monkeys

Dose (mg/kg/d)	Plasma Concentration of IL-1ra (ng/ml), ± S.D.				
	Day 1	Day 7	Day 14	Day 21	Day 28
Control	0.88 ± 1.1[a]	5.1 ± 11.5	2.6 ± 5.2	6.3 ± 14.3	6.1 ± 16.4
10	5683 ± 667	3940 ± 1343	4867 ± 1851	7598 ± 5202	7625 ± 4424
100	37260 ± 20180	27580 ± 8740	26260 ± 6120	32040 ± 7640	35830 ± 7000
200	53770 ± 10650	46110 ± 14680	37400 ± 8590	47200 ± 12600	54140 ± 15950

[a] mean, ± S.D. for all parameters

A dose-related increase in urinary excretion of IL-1ra activity, as detected by ELISA was present at all time points in animals treated with 10, 100, or 200 mg/kg/d IL-1ra. On day 2, the mean value for monkeys in the control group was 79,450 ± 238300 ng IL-1ra/ml urine; this finding was due to a single, female monkey (animal #R3647F) with a value of 715500 ng/ml IL-1ra at this time point. At the later time points, this monkey was negative for IL-1ra in the urine. On days 15 and 27, mean urinary IL-1ra levels in the control group were 45.2 ± 114.7 and 151.7 ± 401.7 ng/ml, respectively, and were negligible when compared to monkeys treated with IL-1ra.

Detectable levels of IL-1ra were present in the urine of all monkeys treated with the biologic, and were increased in a manner that was greater than dose-proportional at all time points on study. Urine IL-1ra levels at 2 h after dosing were increased by 0.5 to 3.4-fold on study d 2, as compared to study d 27 in all IL-1ra dose groups. The reason for the apparent decrease in IL-1ra excretion over time was not clear from the data presented. Overall, the percent of the initial dose of IL-1ra recovered in the urine was ≤ 3% at all time points tested.

There was no detectable anti-IL-1ra antibody activity in any of the monkeys treated with the vehicle control, at any time point over the treatment or recovery periods. Anti-IL-1ra antibody titers were detected in both male and female monkeys treated with 10 mg/kg/d, beginning on study d 14 and continuing until terminal sacrifice on day 29. Titers in this group ranged from between 1:50 to 1:400, and were present in 3/3 male and 1/3 female animals. By contrast, a single monkey (animal #R2606F) in the 100 mg/kg/d dose group was positive at a titer of 1:100 on day 14, and negative for anti-IL-1ra antibody at every other time point. The remainder of animals in this group were also seronegative at all time points tested. In the monkeys treated

with 200 mg/kg/d IL-1ra, there was no detectable antibody present in any animal during the treatment period. However, both female monkeys in the recovery group had anti-IL-1ra antibody titers of 1:50 at the end of the treatment-free recovery period. The sponsor did not provide an explanation for these findings.

Comment: The apparent inverse dose-relationship in the development of anti-IL-1ra antibodies was also observed in rats treated for 6 months with 0, 1, 10, or 100 mg/kg IL-1ra by twice daily s/c injection (Study #SY05-T94-04, Table V, above). Several possibilities to explain this effect exist, the first being that inhibition of antibody formation is an extension of the pharmacologic effects of IL-1ra, which acts to selectively suppress T lymphocyte function, including Th_1 sub-populations involved in regulating the immune response. Although unlikely, down-regulation of Th_1 function may inhibit production of antibody in response to the foreign protein. Alternatively, the assay used to detect anti-IL-1ra antibody activity is a sandwich ELISA technique, in which IL-1ra coated plates are exposed to serum samples from treated rats. Anti-IL-1ra antibody present in the samples from the treated animals will bind to the IL-1ra on the plate, and is then detected with a secondary anti-rat antibody conjugated to a chromogenic agent. However, the binding of anti-IL-1ra antibody is competitive, and any free IL-1ra in the sample can inhibit the interaction of the antibodies with the IL-1ra coated to the plates, thereby giving a falsely negative readout. The same methodology — . ELISA) was used to detect antibody formation in both studies.

In summary, treatment of both male and female Rhesus monkeys with IL-1ra for one month was well tolerated, and not associated with any evidence of systemic toxicity at doses of up to 200 mg/kg/d. Transient decreases in erythrocyte counts, hemoglobin, and hematocrit, and increases in MCV and MCH were observed in animals in all dose groups, including the vehicle controls, and were related to multiple blood collections on study days 1 and 2 for toxicokinetic analyses. Slight increases in both total leukocyte and absolute and differential lymphocyte numbers were observed at d 29 in 3/6 monkeys treated with 200 mg/kg/d IL-1ra, as compared to baseline values for this group. However, these findings were not statistically different from either the vehicle control group, or monkeys treated with 10 or 100 mg/kg/d IL-1ra. Pathologic findings related to IL-1ra treatment included macroscopic and microscopic evidence of hemorrhage, inflammation, and fibrosis at the injection site. These findings were transient in nature, and had resolved or were in the process of resolving by the end of the 4 week recovery period. Toxicokinetic evaluation confirmed that animals in all dose groups were being continuously exposed to IL-1ra, and that the plasma levels obtained on study d 28 were not significantly different from those obtained after the first dose of the test article. Based on the local reactivity at the injection site, no NOAEL for this study can be determined. However, the lowest dose tested (10 mg/kg/d) had minimal inflammation at the injection site, and can be considered the maximally tolerated dose in this species for this duration of treatment.

Study #SY05-T90-02. ***A one week, intravenous infusion study of interleukin-1 receptor antagonist (IL1ra) in male Rhesus monkeys.***

The toxicity of IL-1ra, administered by continuous i/v infusion for 7 days was evaluated in male Rhesus monkeys. Two male monkeys were administered placebo control article, diluted in sterile 0.9% saline through a surgically implanted catheter via an infusion pump system, at a flow rate of 5 ml/hr. Three monkeys received the IL-1ra test article at a dose of 150 mg/kg/d, diluted in 0.9% saline at the same flow rate of 5 ml/hr. Clinical observations and individual body

weights and body weight gains were monitored during the in-life phase of the study. Samples of peripheral blood for evaluations of clinical chemistry and hematologic profiles were obtained at baseline (study weeks –2 and -1), study d 1 prior to dosing, and from fasted animals at terminal sacrifice on study d 8. Peripheral blood samples for evaluation of plasma levels of IL-1ra were obtained at baseline during study week –1, on study d 2, 24 hr after initiating test article infusion, on d 4, and just prior to discontinuation of infusion on study d 8. On d 8, all animals were euthanized, and examined at necropsy for evidence of gross pathologic changes. Target organs were removed, weighed, and samples preserved in fixative and evaluated histologically for evidence of microscopic pathology.

No deaths were observed, and no treatment-related signs of clinical toxicity were noted on study. Monkey #5555M received only 6 days of infusion with the control article, due to a loss of catheter patency, but was sacrificed as scheduled on d 8. This animal was treated with Keflin® on study days 4 though 7, due to an apparent systemic infection (gram-positive cocci). No significant deviations from control were noted in treated animals in regard to body weights or food consumption. There were no significant, treatment related changes in hematology or serum biochemistry profiles in the monkeys treated with IL-1ra, as compared to the two animals in the control group. One monkey (animal #5527M, IL-1ra group) had a verified serum iron value of zero at study termination; this finding has previously been reported in monkeys and was not considered related to the test article.

At necropsy, there were no remarkable differences in organ weights between monkeys in the control and the IL-1ra treated groups. Macroscopic evaluation revealed no visible lesions in 1/ 2 control monkeys (animal #5548M) and animals #5371M and #5570M in the IL-1ra treated group. There were no microscopic findings in these three animals on histopathologic examination.

Monkey #5555M in the control group had erythema visible at the catheter site, which had previously been identified as a possible site of infection, and the mesenteric lymph nodes were moderately enlarged. Histologically, chronic granulomatous inflammation and hemorrhage, both mild in severity, as well as moderate thrombosis were present at the catheter site, with endothelial cell loss and partial occlusion of the vessel. There were no microscopic correlates in the enlarged lymph nodes. This animal also had an adhesion of the right, apical lobe of the lung to the chest wall that was not confirmed histologically, as well as both macroscopic and microscopic evidence of a mild lung mite infestation.

Monkey #5527M in the group treated with 150 mg/kg/d IL-1ra had a brown colored, oozing exudate present at the catheter site, and moderately enlarged mediastinal and inguinal lymph nodes. On histopathologic evaluation, the lesion at the catheter site was found to be a multi-focal abscess of mild severity, with moderate inflammation and fibrosis present. The enlarged mediastinal lymph nodes were within normal limits microscopically, while the inguinal lymph nodes were not examined. On gross evaluation, multiple areas of pectoi hemorrhage were present in the lungs, and ranged in size from 0.1 to 1 cm in diameter, which histologically were correlated with multi-focal areas of infiltration by alveolar macrophages. Several small lesions were present at the outer margins of the middle lobe, measuring approximately 0.5 cm each, and a single, 1 x 2 cm lesion was observed on the left apical lobe of the lung. On microscopic examination, these lesions were identified as multi-focal abscesses, which were mild in severity, and likely associated with a septic condition from the catheter area. These findings were considered unrelated to treatment with IL-1ra.

In summary, treatment of adult, male Rhesus monkeys by continuous, i/v infusion with 150 mg/kg/d IL-1ra for one week was well tolerated, with no evidence of systemic toxicity. Local infection, abscess formation, and loss of catheter patency were observed in one control monkey during the treatment period, and in one animal treated with IL-1ra only at the terminal sacrifice. The NOAEL for IL-1ra in Rhesus monkeys after 7 d of continuous, i/v infusion is therefore 150 mg/kg/d, administered at a flow rate of 5 ml/hr.

Comment: The protocol states that samples of serum were prepared from peripheral blood for evaluation of IL-1ra levels and anti-IL-1ra neutralizing activity. However, no data are included in the final report that would confirm that these analyses were ever performed.

Study #SY05-T89-03. ***A range-finding toxicity study of interleukin-1 receptor antagonist (IL1ra) in male cynomolgus monkeys following repeated intravenous injections.***

The objective of this study was to assess the potential toxicity of IL-1ra administered daily by i/v bolus injection for 14 days in cynomolgus monkeys. Three young adult, male monkeys received daily, i/v injection for two weeks with vehicle control (sterile PBS plus 0.1 mM EDTA, pH 7.0), or 0.1, 1, or 10 mg/kg/d IL-1ra. Animals were monitored twice daily for mortality, general well being, behavioral changes, and any overt signs of toxicity. Body weights were obtained just prior to dose administration, at study d 8, and at time of sacrifice, and food consumption was recorded daily. Samples of peripheral blood for clinical pathology, and for immunological evaluation of IL-1ra levels were obtained twice prior to dosing, and at days 8 and 15, prior to terminal sacrifice. At study termination, all animals were sacrificed, and complete necropsies, with both gross and microscopic evaluation of pathology were performed.

All animals survived until terminal sacrifice. Clinical signs were limited to sporadic incidences of inappetence, and changes in stool consistency and/or frequency (diarrhea, soft stool or no stool), which occurred in the both the control and IL-1ra treated monkeys at approximately the same incidence. One monkey (animal #F-1117) in the group treated with 1 mg/kg/d IL-1ra had a cut on the right leg reported on study d 3, and one monkey in the control group (animal #F-1090) had a finding of "left eyelid abnormal" on study d 8, with no further description provided. However, these findings were not reported at any later time point, and were not considered treatment-related. No remarkable differences in body weights, body weight gains, or food consumption over the duration of the study were noted between the control and IL-1ra treated monkeys.

There were no treatment-related differences in hematologic parameters between the control and the IL-1ra treated animals at any time point on study. A trend toward decreasing erythrocyte parameters (red cell counts, hemoglobin and hematocrit levels) was observed in all groups of monkeys between the baseline values (study week –1) and the values at study days 8 and 15. Reticulocyte counts were performed only at study d 15, and showed no differences between the control and the IL-1ra treated monkeys, however, the counts did appear to be increased slightly outside of the normal range for cynomolgus monkeys of this age group. These differences were felt to be due to repeated blood sampling, and were approximately equal in severity across all dose groups, including the vehicle control. There were no remarkable differences either from baseline, or between the study groups in platelet counts, total or differential leukocyte counts, or coagulation factor profiles (prothrombin time, APTT, fibrin and fibrin degradation products, and ACT). No significant differences in clinical biochemistry or urinalysis profiles were observed in

the IL-1ra treated monkeys, when compared to either baseline values or the vehicle control group at any time point on study.

At necropsy, there were no lesions noted that were related to treatment with IL-ra. Trace to mild evidence of hemorrhage and subacute inflammation was present at the injection site in all animals, regardless of treatment with IL-1ra or vehicle control article. Animals in all groups had evidence of brown pigment deposition in either the mediastinal or mesenteric lymph nodes on microscopic inspection, without an apparent relationship in either incidence or severity to the dose of the test article administered. One monkey (animal #F-1090) in the control group had macroscopic evidence of multiple small (1-5 mm diameter), white nodule on the surface of the heart, which was determined microscopically to be an epidermal inclusion cyst of unknown origin.

In the IL-1ra treated monkeys, macroscopic findings at necropsy included a 2mm nodule on the surface of the spleen of monkey #F-1178 in the low dose group, which was determined histologically to be an area of lymphoid hyperplasia. The left caudal and middle lobes of the lung of monkey #F-1180 in this same group had adhesions to the pleural surface and the crura of the diaphragm, which correlated microscopically with areas of acariasis.

Small (5 mm diameter) lesions on the surface of the heart, with an apparent thickening of the epicardium on cut surface evaluation were present in monkey #F-1102, and marked congestion along the coronary artery was present in animal F-1117, both in the 1 mg/kg/d dose group. Additionally, monkey #F-1087, in the group treated with 10 mg/kg/d IL-1ra had fine petechial hemorrhages present on the epicardial surface. However, the tissue was within normal limits for all three animals on histopathologic evaluation. Monkey #F-1117 also had two areas in the colon which were hemorrhagic both on gross and microscopic evaluation.

Other spontaneous, histologic lesions included multifocal areas of mineralization in the seminal vesicles in one monkey each in the control, 1, and 10 mg/kg/d IL-1ra dose groups, trace to mild areas of inflammation in the lung, thyroid, esophagus, and kidney, and areas of hemorrhage, sarcoscystis, or atrophy in skeletal muscle. These findings have been reported occasionally in laboratory monkeys of this strain and age group, and were considered by the reviewing pathologist to be unrelated to treatment with IL-1ra.

In summary, treatment of male cynomolgus monkeys with daily, i/v bolus injections of IL-1ra ws not associated with any local or systemic toxicity, after 14 d of treatment. The NOAEL for IL-1ra under the conditions of this study is 10 mg/kg/d, i/v, for 14 days.

Comment: The protocol states that samples of serum were prepared from peripheral blood for evaluation of IL-1ra levels and anti-IL-1ra neutralizing activity. However, no data are included in the final report that would confirm that these analyses were ever performed.

Study #SY05-T89-08. ***Subchronic systemic toxicity study with recovery period of interleukin-1 receptor antagonist (IL1ra) in Rhesus monkeys.***

The toxicity of IL-1ra administered by twice daily, i/v injections was evaluated in Rhesus monkeys after a two week treatment period. Male and female Rhesus monkeys (3/sex/group) were treated daily by s/c injection with vehicle control (IL-1ra placebo), or IL-1ra at doses of 3,

10, or 30 mg/kg/d (1.5, 5, or 15 mg/kg/injection) for 14 days. Three additional animals/sex in the control and high-dose IL-1ra groups were retained for a 4 week, treatment-free recovery period. Clinical observations for signs of morbidity or overt toxicities, as well as measurement of food consumption were performed twice daily, and body weights were determined weekly. Fasting peripheral blood samples for hematologic and serum biochemistry profiles were obtained from all animals prior to study initiation for determination of baseline values, then prior to dosing on study days 8, 15, 29, and d 43 of recovery in the appropriate dose groups. Samples for urinalysis and urine chemistries were obtained at these same time points. Aliquots of peripheral blood were collected twice at baseline during study weeks –2 and –1, then prior to dosing at study weeks 1, 2, 4, and 6, and shipped to the sponsor for analysis of anti-IL-1ra antibody levels by ELISA. Physiologic parameters (ECG, respiratory and heart rates) were recorded at study weeks –1, 2, and 6. General veterinary examinations, as well as ophthalmologic examinations were performed once during the baseline period, then on d 13 or 14 in all animals, and on d 42 in the recovery animals. A full necropsy and gross pathologic evaluation was performed on each surviving animal at terminal sacrifice (weeks 3 or 7 on study for the end-of-treatment and recovery groups, respectively), with organ weights recorded, and tissue samples taken and processed for histopathologic evaluation from animals in the control and high-dose groups only.

One male and one female monkey in the control/recovery group (animals #5226M and #31T, respectively) were sacrificed in moribund condition on study d 15. Additionally, one male monkey (animal #5577M, 30 mg/kg/d IL-1ra/recovery dose group) was sacrificed in moribund condition on study d 9, after receiving 8 doses of the test article. Clinical signs of soft or bloody stool, diarrhea, dehydration, mucus in stool, inappetence and overt bleeding from the rectum, were observed in these animals prior to sacrifice. Shigellosis was confirmed at necropsy in all three monkeys by gross and microscopic examination of the colon, and was not considered related to treatment with IL-1ra.

Other clinical signs included sporadic incidences of soft stool and/or diarrhea and inappetence in animals in all groups, including those treated with the vehicle control. On study d 13, monkey #5242M in the control/recovery group was observed to be either convulsing or shaking; however, this was the only incidence of this finding, and there were no obvious findings on evaluation of the clinical or gross pathology indicative of a seizure. Monkey #153Q (female) in the 30 mg/kg/d IL-1ra/recovery group developed a transient rash on the trunk area, which was observed only on study d 14. At terminal sacrifice on study d 43, there was no evidence of the rash on either macroscopic or microscopic evaluation.

Comment: The rash in animal #153Q was only observed on the final day of dosing with IL-1ra. It may have been an early sign of hypersensitivity; however, this animal was allowed to recover for the 28 d, treatment-free period at which point the rash had resolved. It is not known whether the rash would have persisted or worsened if treatment was continued, nor what the histologic evaluation of the affected area would have shown.

No significant differences were noted between the vehicle control and the IL-1ra treated animals in mean values for body weights and body weight gains, or in overall general health. Ophthalmologic exams were normal in all monkeys at all time points during the treatment and recovery periods. However, incidental findings included monkey #5414F (vehicle control group), who had a focal area of aggregated pigment on the corneal endothelium during the pre-treatment screening examination, which was not present on subsequent examinations. Monkey #5409F in the 10 mg/kg/d IL-1ra treatment group had multiple, white and gold particulate flecks

In summary, treatment of both male and female rats with IL-1ra prior to mating, and throughout the duration of the pregnancy was not associated with any overt toxicities or pathology in the F0 generation, and no evidence of developmental or other abnormalities in the F1 generation. Based on the decreased body weights and food consumption in female rats during GD14-20, the NOAEL for IL-1ra in this study is 50 mg/kg/d, administered as twice daily s/c injections for 14 d prior to mating and for the duration of the pregnancy.

Study #960044. ***A study of fertility and early embryonic development to implantation of recombinant-methionyl human interleukin-receptor antagonist (r-met-HuIL-1ra) administered subcutaneously in rats.***

A confirmatory study to evaluate the effects of IL-1ra on fertility and general, reproductive capacity was conducted in male and female Sprague-Dawley rats. Twenty-five rats per sex per group were injected s/c once daily with vehicle (IL-1ra excipient, composition not specified), or 10, 100, or 200 mg/kg IL-1ra. Male rats were injected daily for 29 days prior to mating, and throughout mating until one day prior to euthanization. Female rats were treated for 14 days prior to mating, then throughout mating until GD7. Females with no evidence of copulation were dosed until one day prior to euthanization.

Animals were observed twice daily for morbidity and mortality, and once daily for evidence of overt toxicities. Detailed clinical observations were recorded daily for both male and female rats throughout the study period. Individual body weights were measured twice weekly for male rats throughout the duration of the study, and for female rats prior to mating. Once evidence of copulation had been documented, body weights for female rats were determined on gestation days (GD) 0, 3, 7, 10, 13 and 15. Food consumption was measured at the same time points as body weights, except during the period of cohabitation.

Females in whelp were sacrificed on GD15, and the uterus from each female was opened and examined macroscopically for the number of viable and non-viable fetuses, implantation sites, pre-and post-implantation losses, and early and late resorptions. The numbers of corpora lutea on each ovary were recorded for each animal. Uteri with no macroscopic evidence of implants were preserved in 10% aqueous ammonium sulfide for detection of early embyolethality as described by Salewski[13].

The F0 male rats were euthanized on study d 63, and evaluated for gross and microscopic evidence of pathology. The right testis and epididymis of each rat was excised, weighed, and sperm isolated for evaluation of morphology and motility, following opening of the right epididymis and washing with pre-warmed PBS. Analysis of at least 200 motile and non-motile spermatozoa was performed microscopically for each animal, and the percent motility reported. The left testis and accessory sex organs from each animal were removed and weighed, homogenized, and evaluated for determination of spermatid counts and sperm production rates using the methods of Blazak *et al.*[14].

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[13] Salewski, V.E. 1964. Farbemethode zum makroskopischen nachweis von implantations stellen am uterus der ratte. *Naunyn-Schm. Archiv. FurExper. Pathologic und Pharm.*, **247**:367.

[14] Blazak, W.F., T.L. Ernst, and B.E. Stewart. 1985. Potential indicators of reproductive toxicity: Testicular sperm production and epididymal sperm number, transit time, and motility in Fischer F344 rats. *Fund. Appl. Toxicol.*, 5:1097-1103.

in the paramacular regions of both eyes throughout the pre-study and treatment periods. The findings in both of these animals were not considered related to treatment with the control or test articles.

There were no treatment related changes in heart rate, respiratory rates, or ECG profiles in monkeys treated for two weeks with IL-1ra, as compared to the placebo control group or to baseline values. Although several monkeys in all dose groups showed decreases in heart rate and longer QT intervals on different occasions, these findings were still within normal limits for Rhesus monkeys of this age group, and were considered by the contracting laboratory to be secondary to the effects of the ketamine anesthetic used for sedation.

Hematologic profiles showed evidence of progressive anemia and increasing reticulocyte counts in all animals between the pre-study and d 15 evaluations, which could be attributed to the multiple blood samples collected for clinical pathology and immunologic evaluations over the study duration. Although there was an apparent, two-fold increase in reticulocyte counts between the vehicle control group and the monkeys treated with 30 mg/kg/d IL-1ra at study days 8 and 15, this difference was neither statistically significant, nor accompanied by a change in myeloid:erythroid rations in the bone marrow. There were no significant or remarkable differences between the mean values for the control or the other IL-1ra treated groups at any time point on study. Both mean and individual values for red cell parameters had returned to approximately baseline at the end of the 4 week recovery period for animals in the control and the 30 mg/kg/d IL-1ra treated groups. There were no remarkable differences between the control and IL-1ra treated monkeys in total or differential leukocyte counts and platelet counts over the duration of the study, or in myeloid:erythroid ratios from bone marrow samples obtained at terminal sacrifice.

No remarkable effects of IL-1ra were observed on the macroscopic urinalysis profiles, or urine chemistries over the duration of the treatment and recovery periods, as compared to monkeys treated with the placebo control. There were no significant differences observed between the control and IL-1ra treated groups in serum biochemistry profiles, electrolytes, or serum protein electrophoresis over the duration of the treatment and recovery periods. On study days 8 and 15, decreases in A:G ratios from baseline were present in both male and female monkeys in all treatment groups including the control, but were not statistically significantly different from the study week –2 or week –1 values.

At the d 15 necropsy, the only treatment-related macroscopic pathology findings were minimal to mild hemorrhage and thickening at the injection site(s). These effects were observed in monkeys in all groups, including the vehicle control and IL-1ra treated animals, without a dose-relationship in either incidence or severity. On histologic evaluation, these findings correlated with acute hemorrhage with mild to moderate edema present in the subcutaneous tissue, and evidence of acute to chronic inflammatory infiltrates of trace to mild severity.

Comment: Monkey #5595M, in the group treated with 30 mg/kg/d IL-1ra was inadvertently sacrificed on study d 15, rather than continuing on through the 28 d recovery period. Animal #5577M in this same group was sacrificed moribund on study d 9, leaving only one male monkey in the high-dose/recovery group for evaluation at study termination.

No significant differences in either absolute or relative organ weights between the IL-1ra treated monkeys and those receiving the placebo control group were noted at either the d 15 or d 43

sacrifices. Other than the injection site reactions, there were no remarkable, treatment-related findings on gross pathologic evaluation. Incidental gross pathologic findings included congestion, discoloration, changes in texture, and/or small areas of atelectasis in the lungs of monkey #5242M, in the 3 mg/kg/d IL-1ra dose group, and animal #5220M in the high-dose group. Similar findings in the lung were also present in monkey #5577M, in the high-dose recovery group (sacrificed moribund on study d 15). Histologically, these animals had evidence of mild, multi-focal bronchial pneumonia, which was considered unrelated to treatment. Twp female monkeys (animal #5302F, 10 mg/kg/d IL-1ra and animal #5686F, 30 mg/kg/d IL-1ra) had pleural adhesions present in the different loves of the lung, which on rnicroscopic evaluation were associated with multifocal, chronic inflammation of trace severity.

Histologic findings related to treatment were limited to the injection site, and included subcutaneous hemorrhage, inflammation, lymphocytic and neutrophilic infiltrates, and fibrosis. The inflammatory lesions appeared to be perivascular in origin, and occurred with approximately equal incidence and severity in all of the treatment groups (please see above description). The findings were reversible in all 3/ 4 surviving control animals and in all 4 surviving monkeys treated with 30 mg/kg IL-1ra, at the end of the recovery period.

Other microscopic findings unrelated to IL-1ra treatment included minimal to moderate, subacute or chronic areas of lymphocytic infiltration in the kidneys, liver, tongue, gastrointestinal tract, or salivary glands, and focal areas of brown pigmentation, consistent with mite infestation in the lungs. These findings occurred at approximately equal incidence and severity across all treatment groups, and were considered by the reviewing pathologist to be incidental to treatment with the test article.

In summary, treatment of both male and female Rhesus monkeys with IL-1ra for 14 d was well tolerated, and not associated with any evidence of systemic toxicity at doses of up to 30 mg/kg/d. Transient decreases in erythrocyte counts, hemoglobin, and hematocrit were observed in animals in all dose groups, including the vehicle controls, and were related to multiple blood collections. Pathologic findings related to IL-1ra treatment included macroscopic and microscopic evidence of hemorrhage, inflammation, and fibrosis at the injection site. These findings were transient in nature, and had resolved or were in the process of resolving by the end of the 4 week recovery period. The NOAEL for this study for IL-1ra administered by i/v injection is therefore 30 mg/kg/d, given as divided doses of 15 mg/kg, twice daily for 14 days.

Comment: Serum samples for determination of anti-IL-1ra antibody were collected as part of the protocol design, and shipped to the sponsor for further analysis. There are no data in the BLA submission that would indicate whether these assays were ever conducted.

Study #SY05-T89-07. ***An antigenicity study of interleukin-1 receptor antagonist (IL1ra) in male Rhesus monkeys following repeated intravenous administrations.***

The objective of this study was to determine the antigenic potential and toxicologic properties of IL-1ra following daily, i/v injections for two weeks. Four mature, male Rhesus monkeys received daily, i/v injection of IL-1ra at a dose of 10 mg/kg/d for 19 days. Animals were observed twice daily for overt signs of toxicity and mortality, behavioral changes, and general appearance. Body weights were obtained prior to initial dose administration, and then weekly

during the study duration. Food consumption was determined qualitatively each day throughout the treatment period. At the end of the study, monkeys were returned to the research colony.

Blood samples for determination of hematology and serum biochemistry profiles were obtained at baseline at week –1, and prior to dosing on study d 1, then at study d 14 during the treatment period. Samples of peripheral blood for immunological evaluation were collected prior to dosing on study d 1, then on study days 10, 12, 14, and 19. Serum samples for immunologic evaluation were prepared from the blood, and shipped to the study sponsor for further analysis.

There were no remarkable effects of IL-1 ra treatment on clinical signs, body weights, or food consumption over the duration of the treatment period. Sporadic incidences of soft stool or diarrhea were noted in 3/ 4 monkeys over the study duration, but were not considered related to the test article. Three of the 4 monkeys gained weight over the study duration; the fourth monkey had a loss of 0.09 kg, which was not considered biologically significant.

A progressive decrease in erythrocyte parameters (total red cell counts, hemoglobin, hematocrit), without a corresponding increase in MCV, MCH, or MCHC was observed in all monkeys from baseline to study d 14. No reticulocyte counts were measured. Erythrocyte morphology showed evidence of slight to moderate anisocytosis, and polychromasia at the end of the treatment period. The decreases in erythrocyte parameters and changes in morphology are consistent with a regenerative response by the bone marrow, in response to the multiple blood samplings.

No remarkable changes from baseline were noted in any of the serum biochemistry parameters for any monkey on study. There were no significant changes from baseline in either absolute or differential white cell counts, over the duration of the study. Male monkey #37T had an elevated total leukocyte count (16.6 x 10^3/mm^3) at study week –1; however, no differences in differential count profiles between this animal and the other monkeys in the group were noted at this time point. At the day 1 and d 14 time points, the total white cell counts for this animal had decreased to 6.6 and 6.9 x 10^3/mm^3, respectively, and were well within the range observed for the group.

In summary, treatment of male Rhesus monkeys with 10 mg/kg IL-1ra by a single, daily i/v injection for 14 d was not associated with any signs of either overt, clinical, or systemic toxicity. The NOAEL for IL-1ra under the conditions of this study is therefore 10 mg/kg/d, i/v.

Comment: The purpose of this study was defined by the contracting laboratory as to determine the antigenicity of IL-1ra after repeated, daily, i/v injections. Serum samples for immunologic evaluations were collected as part of the protocol design, and shipped to the sponsor for further analysis. There are no data in the BLA submission that would indicate whether these assays were ever conducted.

Study #SY05-T93-01. ***Fertility and general reproduction (Segment I) study of interleukin-1 receptor antagonist (IL-1ra) administered by subcutaneous injection to rats.***

The effects of IL-1ra on fertility and general, reproductive capacity were evaluated in male and female Sprague-Dawley rats in a segment I, reproductive toxicity design. Forty rats per sex per group were injected s/c twice daily with vehicle (citrate-buffered saline plus 0.1% polysorbate 80, pH 6.5), or 12.5, 50, or 200 mg/kg IL-1ra, administered as divided doses (6.25, 25, or 100 mg/kg/injection). Male rats were injected daily for 64 days prior to mating, up until study d 85.

Female rats were treated for 15 days prior to mating, then throughout pregnancy. Animals were observed twice daily for morbidity and mortality, and once daily for evidence of overt toxicities. Detailed clinical observations were recorded once weekly for males and females prior to and during mating, and daily for females during gestation and lactation. Individual body weights were measured weekly for male rats throughout the duration of the study, and for female rats prior to mating. Once evidence of copulation had been documented, body weights for female rats were determined on gestation days (GD) 0, 7. 14, and 20 and lactation days 1, 7, 14, and 21. Food consumption was measured at the same time points as body weights, except during the period of cohabitation.

Twenty females per group were sacrificed on GD20, and the fetuses harvested by Cesarean section for evaluation of litter size and gender distribution, and external abnormalities. The F0 female rats were evaluated for any gross evidence of pathology, and any internal gross lesions were preserved for future microscopic evaluation. The uterus from each female was opened and examined macroscopically for the number of viable and non-viable fetuses, implantation sites, early and late resorptions. The numbers of corpora lutea on each ovary were recorded for each animal. Uteri with no macroscopic evidence of implants were preserved in 10% aqueous ammonium sulfide for detection of early embyolethality as described by Salewski[12].

The remaining 20 female rats per group were allowed to litter normally, and the pups were evaluated for external abnormalities. Litters were culled to a maximum of 8 pups (4/sex where possible) on lactation d 4. The culled pups were weighed, euthanized by CO_2 inhalation, and discarded. The remaining pups were examined daily for viability, and individual pup weights were measured on lactation days 1, 4, 7, 14, and 21. The gender of the pups was determined on lactation d 0, and verified on lactation days 4 and 21.

The F0 male rats were euthanized on study d 88 or 89, and evaluated for gross and microscopic evidence of pathology. Testes and accessory sex organs were retained and preserved for microscopic evaluation. Surviving F0 female rats, and the F1 pups were euthanized on lactation d 21, and evaluated for gross evidence of pathology.

Two male rats (animals #1275M and #1174M, in the 50 and 200 mg/kg/d dose groups, respectively) were euthanized *in extremis* prior to study termination. Male rat #1275M was found to have an apparent, mechanical or traumatic opening in the palate, and was sacrificed moribund on study d 16. Rat #1174M, in the 200 mg/kg/d IL-1ra dose group was euthanized on study d 84, after exhibiting reddish-colored urine, dehydration, scant feces, dark material around the nose and mouth, and decreased activity and ruffled appearance. At necropsy, multiple calculi were present in the bladder of this rat, accompanied by reddened and thickened mucosal surface of the bladder. These findings were considered spontaneous development of bladder stones, and were not considered related to IL-1ra treatment.

There were no other treatment related signs of overt or clinical toxicity in the remainder of the male rats, or in the pregnant dams over the duration of the study. Incidental findings included alopecia, scabbing at the injection site(s), and dark material around the eyes and/or nose, and were present in approximately equal incidence and severity in all dose groups, including the controls. There were no remarkable differences in mean body weights, body weight gain, or food consumption between male rats in the vehicle control and the IL-1ra test groups at any time point

[12] Salewski, V.E. 1964. Farbemethode zum makroskopischen nachweis von implantations stellen am uterus der ratte. *Naunyn-Schm. Archiv. FurExper. Pathologic und Pharm.*, **247**:367.

during the treatment period. Statistically significant decreases in food consumption, and an increase in mean body weight gain as compared to control were observed during weeks 8-9 for male rats in the 200 mg/kg/d and 12.5 mg/kg/d dose groups, respectively. However, since there were no corresponding changes in either mean body weights or food intake for the respective groups, these changes were considered incidental to IL-1ra treatment.

A significant decrease (5%; $p \leq 0.05$, ANOVA) in mean body weight gain as compared to control was recorded on GD20 for female rats in the 200 mg/kg/d IL-1ra dose group. This was the only reported incidence of this finding, and was considered by the contracting laboratory to be unrelated to treatment with the test article. However, this group also displayed a statistically significant decrease in food consumption during gestation days 14-20, so a relationship to the test article cannot be definitively ruled out. Decreases in food consumption that were significantly different from the control group were also observed in the pregnant female rats treated with 12.5 mg/kg/d IL-1ra during GD7-14 and GD14-20.

There were no statistically significant effects of IL-1ra treatment on the parameters evaluated in the rats delivered by Cesarean section on GD20, including the mean numbers of corpora lutea, implantation sites, pre-implantation losses, early and late resorptions, post-implantation losses, nor on the numbers of viable fetuses or the fetal sex ratios. A trend towards increased pre-implantation losses and decreased implantation sites and viable fetuses per litter was observed for female rats treated with 200 mg/kg/d IL-1ra; however, additional analyses showed that these findings were within the normal range of historical control data for this strain of rats at this laboratory.

Fetal external malformations were limited to open eyelids, maxillary micrognathia, and microphthalmia in one fetus in the control group. No developmental variations were observed in any of the fetuses.

In the female rats that continued to parturition, there were no differences observed between the control and the IL-1ra treated animals with respect to copulation and fertility indices, estrous cycles, precoital intervals, gestation durations, or pregnancy rates. No signs of prolonged delivery or unusual nesting behavior were noted in any of the study groups. One female rat (animal #4446, 200 mg/kg/d IL-1ra) was observed to have a red, vaginal discharge on GD22, however, no viable pups were delivered. At necropsy on day 25 post-breeding, two implantation scars were noted in the uterus of this female.

At necropsy, there were remarkable macroscopic findings in the surviving male rats treated with either vehicle control, or 12.5, 50, or 200 mg/kg/d IL-1ra for 84 days. In the F0 female rats sacrificed at lactation d 21, there were no gross pathologic abnormalities noted in either the control or IL-1ra treated groups, and the mean number of implantation scars in the uterus was similar among the groups.

There were no remarkable differences between the control and IL-1ra treated groups the mean numbers of live and dead pups delivered, the number of litters with live offspring, mean live litter size or sex ratios at the time of whelping. No external abnormalities were noted in the developing F1 pups either at the time of birth, or during lactation. Mean pup weights, weight gains, and viability were comparable between the groups throughout the lactation period. At necropsy on lactation d 21, there were no treatment-related, gross abnormalities observed in any of the F1 offspring.

Complete necropsies were performed on all F0 female rats at GD15 following harvest of the fetuses, and on all male rats and females with no evidence of mating at the completion of the study. Tissues were collected from each animal and preserved in neutral, 10% buffered formalin for future evaluation of microscopic pathology, if warranted.

All male rats survived to scheduled sacrifice. There were no remarkable signs of clinical toxicity, and no evidence of irritation at the s/c injection sites throughout the duration of the study. Isolated and transient decreases in mean body weights, as compared to the control group were noted for males treated with 100 mg/kg/d IL-1ra during study days 0-4, and in the 200 mg/kg/d IL-1ra dose group during study days 53-56. However, there were no corresponding changes in food consumption in these groups at these time points; therefore, the findings are felt to be incidental to treatment with IL-1ra. At necropsy, there were no differences in either absolute or relative organ weights between the control and the IL-1ra treated groups. No test-article related macroscopic findings observed, with the exception of reddened areas at the injection sites; however, no histologic evaluation of the tissues was conducted. Other macroscopic observations in the F0 male rats included renal calculi and/or dilated renal pelves, small coagulating glands or seminal vesicles, dark red lungs, and splenic adhesions. These findings were limited to singular or infrequent occurrences, and were approximately equal in incidence between the control and the IL-1ra treated groups.

There were no remarkable effects of IL-1ra treatment on male reproductive performance. Mating indices for male rats treated with vehicle control, 10, 100, or 200 mg/kg/d IL-1ra were 96%, 96%, 92%, and 96%, respectively. Male fertility indices were 100%, 96%, 95.7%, and 96%, respectively, for these same dose groups. There was no evidence of adverse effects of IL-1ra treatment on mean testicular or epididymal sperm numbers, sperm production rate, differential morphology, or sperm motility. A statistically significant increase ($p \leq 0.01$, ANOVA with Dunnett's test) in the mean number of sperm present and the mean sperm production rate was observed in rats treated with 200 mg/kg/d IL-1ra, as compared to the control or the lower dose groups. However, mean testicular weights and sperm motility in this group were comparable to control, therefore the findings were considered incidental to treatment with IL-1ra.

One female rat (animal #51317F, 200 mg/kg/d IL-1ra) was found dead on study d 48. On the day prior to death, this animal was observed to be hypoactive, with drooping eyelids and soft stool. Macroscopic evaluation at necropsy revealed that this female was non-gravide, and had enlarged and reddened adrenal glands and iliac lymph nodes, dilated renal pelves, focal white areas in both kidneys, a calculus in the urethra, and a distended urinary bladder and ureter with reddened contents. The death of this female was attributed to renal calculi, and was not considered related to IL-1ra treatment.

There were no other treatment related signs of overt or clinical toxicity in the remainder of the pregnant dams over the duration of the study. Incidental findings included reddened and swollen ears in female rats in all dose groups, although the incidence was slightly increased in the group treated with 200 mg/kg/d IL-1ra. There were no remarkable differences in mean body weights, body weight gain, or food consumption between female rats in the vehicle control and the IL-1ra test groups at any time point during the treatment period. Statistically significant increases in food consumption as compared to control were observed during study days 21-25 for female rats in the 200 mg/kg/d dose group. However, since there were no corresponding changes in either mean body weight or body weight gain for this group, this finding was considered incidental to IL-1ra treatment.

One female rat in the control group (animal #51277F), and two female rats in the group treated with 100 mg/kg/d IL-1ra (animals #51252F and #51261F) had no evidence of mating, and were euthanized at study termination on GD15. However, all three females were gravid, and animal #51252 actually delivered two live pups with no apparent malformations, prior to euthanasia.

No remarkable effects of IL-1ra treatment were observed on female reproductive performance. Estrous cycling and the days between pairing were not significantly different from the control group in any of the IL-1ra dose groups. The female mating indices for animals treated with vehicle control, 10, 100, or 200 mg/kg/d IL-1ra were 96%, 100%, 92%, and 96%, respectively. The female fertility indices were 100%, 96%, 96%, and 96%, respectively, for these same dose groups. There was no evidence of adverse effects of IL-1ra treatment on the intrauterine survival of the F1 fetuses. Pre-and post-implantation losses, numbers of viable fetuses, corpora lutea, and implantation sites were comparable between the dose groups, with the exception of a decreased mean pre-implantation loss in the group treated with 10 mg/kg/d IL-1ra, as compared to the control group. This difference was statistically significant ($p \leq 0.01$, ANOVA with Dunnett's test) from control; however, since no similar changes were observed at the higher dose levels, the biologic significance of this finding is unclear.

At necropsy, there were no treatment-related differences on macroscopic evaluation between the control rats and the females treated with 10, 100, or 200 mg/kg/d IL-1ra. No differences in absolute organ weights were noted between the dose groups. The only statistically significant effect of IL-1ra treatment was an increase in mean relative, pituitary weights for the group treated with 100 mg/kg/d IL-1ra, as compared to the control female rats ($p \leq 0.05$, ANOVA). However, since no similar findings were present in the highest dose group, this effect was considered incidental to treatment with IL-1ra.

In summary, treatment of both male and female rats with IL-1ra prior to mating, and throughout GD7 of pregnancy was not associated with any overt toxicities or pathology in the F0 generation, and no adverse effects on either male or female reproductive performance were observed. Male and female fertility indices ranged from 92 to 100%, and were not significantly different between dose groups. Spermatogenic endpoints (testicular and epididymal sperm numbers, sperm production rate, sperm motility and differential sperm morphology) were not affected by IL-1ra treatment. There were no treatment-related effects on intrauterine survival of the F1 generation, noron the numbers of implantation sites or corpora lutea in the ovaries. Based on these findings, the NOAEL for IL-1ra in this study is 200 mg/kg/d, administered as daily s/c injections to male rats for 29 d prior to mating, and to female rats for 14 d prior to mating, and until GD7 of the pregnancy.

Study #SY05-T92-03. ***Developmental toxicity study in rats with interleukin-1 receptor antagonist (IL-1ra).***

The teratogenic and developmental effects of IL-1ra were evaluated in female Sprague-Dawley rats in a combined segment II/III, reproductive toxicity design. Forty mated, female rats per group were injected s/c twice daily with vehicle (citrate-buffered saline plus 0.1% polysorbate 80, pH 6.5), or 12.5, 50, or 200 mg/kg IL-1ra, administered as divided doses (6.25, 25, or 100 mg/kg/injection) from GD6 to GD17. Animals were observed twice daily for morbidity and mortality, and once daily for evidence of overt toxicities. Individual body weights were measured on GD0, GD6, GD9, GD12, GD15, GD18, and GD20, and on lactation days 1, 7, 14, and 21. Food consumption was measured at the interval time points between body weight measurements.

Twenty-one females per group were sacrificed on GD20, and the fetuses harvested by Cesarean section for evaluation of litter size and gender distribution, and external abnormalities. The F0 female rats were evaluated for any gross evidence of pathology, and any internal gross lesions were preserved for future microscopic evaluation.

The uterus from each female was opened and examined macroscopically for the number of viable and non-viable fetuses, implantation sites, early and late resorptions. The numbers of corpora lutea on each ovary were recorded for each animal. Uteri with no macroscopic evidence of implants were preserved in 10% aqueous ammonium sulfide for detection of early embyolethality as described by Salewski[15].

Fetuses harvested from the pregnant dams were examined for external malformations, the gender and weight recorded, and the crown-rump length determined. Approximately one-half of the fetuses from each litter were preserved in Bouin's solution and examined for visceral malformations by the Wilson technique[16]. The remainder of the fetuses were fixed in isopropyl alcohol, cleared, and stained with Alizarin red S for evaluation of skeletal abnormalities[17].

The remaining 19 female rats per group were allowed to litter normally, and the pups were evaluated for external abnormalities. Litters were culled to a maximum of 8 pups (4/sex where possible) on lactation d 4. The culled pups were weighed, euthanized by CO_2 inhalation, and discarded. The remaining pups were examined daily for viability, and individual pup weights were measured on lactation days 1, 4, 7, 14, and 21. The gender of the pups was determined on lactation d 0, and verified on lactation days 4 and 21.

On lactation d 6, each dam was evaluated behaviorally for litter retrieval. The dams were removed from the nesting box, and the pups were aligned at the farthest end of the nest. Dams were returned to the nest, and the number of pups retrieved to the nest within a five minute period recorded. Behavioral, developmental, and functional evaluations were performed on the F1 offspring during the lactation. Developmental landmarks included pinnae detachment, surface righting response, cliff aversion, eye opening, and startle and auditory responses. Following weaning, 20 pups per sex per dose group were randomly selected as parental animals for the F2 generation, and examined for testes descent or vaginal opening. Behavioral testing, including open-field testing for emotionality and water maze testing for learning and memory recall were also conducted on these animals.

At approximately 12 weeks of age, each F1 female was placed with a single, F1 male from the same treatment group. Sibling matings were avoided, and copulation was confirmed by the presence of a copulatory plug within the vagina, or a sperm-positive vaginal smear. If no evidence of mating was detected after 10 consecutive days of cohabitation, the breeding pair was separated, and the female was housed with a second, proven male from the same treatment group. If no evidence of copulation was obtained after the second mating, the breeding pair was

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[15] Salewski, V.E. 1964. Farbemethode zum makroskopischen nachweis von implantations stellen am uterus der ratte. *Naunyn-Schm. Archiv. FurExper. Pathologic und Pharm.*, **247**:367.

[16] Wilson, J.G. 1965. Methods for administering agents and detecting malformations in experimental animals. *In: Teratology Principles and Techniques*, J.G. Wilson and J. Workany, eds., University of Chicago Press, Chicago, IL; pp. 262-277.

[17] Dawson, A.B. 1926. A note on the staining of cleared specimens with Alizarin red S. *Stain Technol.*, **1**:123-124.

separated and the female retained until study termination. Females with no evidence of mating and all F1 male rats were weighed and observed weekly. Mated F1 females were observed daily throughout gestation and lactation, and body weights were recorded on GD0, GD7, GD14, and GD20. Following parturition, the F1 females were weighed on lactation days 1, 7, 14, and 21.

On the day of delivery, the F2 generation pups were weighed, genders were determined, and the numbers of viable and non-viable pups recorded. On lactation d 4, litters were randomly culled, as described above. Pup viability was determined daily throughout lactation, and individual pup weights were measured on lactation days 1, 4, 7, 14, and 21. At study termination, the F2 generation pups were euthanized on lactation d 21 by CO_2 inhalation, and discarded.

All females in the F0 generation survived to scheduled euthanasia. There were no overt, clinical signs of toxicity related to IL-1ra observed over the duration of the study. Incidental findings included alopecia, scabbing at the injection site(s), and were observed sporadically in all dose groups. There were no remarkable effects of IL-1ra treatment on mean body weights, body weight gains, or food consumption in the F0 females over the duration of the study. Statistically significant decreases in mean maternal body weight gains were observed during GD0-GD6 for F0 females in the 12.5 mg/kg/d dose group; however, this finding was present prior to treatment with IL-1ra, and was therefore considered incidental. In addition, a statistical decrease in mean body weight gain was observed for rats in the 200 mg/kg/d dose group during lactation days 7-14.

The pregnancy rate for the F0 females was 97.5%, 100%, 97.5%, and 95% for female rats in the vehicle control, 12.5, 50, and 200 mg/kg/d IL-1ra dose groups, respectively. All female reproductive parameters evaluated at Cesarean section, including mean number of corpora lutea, implantation sites, viable fetuses, early and late resorptions, post-implantation losses, fetal sex ratios, and mean fetal body weights were comparable between the vehicle control and IL-1ra treated groups.

Morphologic evaluation of the fetuses revealed a low incidence of malformations in all dose groups, including the vehicle control. These included micrognathia, cleft palate, microphthalmia and/or anophthalmia, and incomplete development of the renal papillae. Slight, although not statistically significant increases in the number of fetuses per litter with distended ureter(s) were noted in the 50 and 200 mg/kg/d dose groups. However, the percentage of fetuses affected was slightly above the historical control ranges for animals of this strain at this laboratory. The incidence of other developmental variations, including skeletal abnormalities was comparable between the dose groups.

In the group of rats allowed to proceed to parturition, an apparent dystocia was noted in female #3883F in the 50 mg/kg/d IL-1ra dose group. Red vaginal discharge was present in this animal for 2 days prior to delivery, and ten viable and three non-viable pups were delivered on GD23. No other signs of difficult deliveries were noted in the remainder of the F0 female rats, and no unusual nesting behaviors were noted in any of the study groups. Litter retrieval by the dams on lactation d 6 was comparable between the dose groups, with similar percentages of pups retrieved to the nest within the 5 minute test period. At necropsy, there were no treatment-related gross abnormalities noted in the F0 females. The mean number of implantation scars in the uterus was similar between the dose groups.

The number of live and dead pups, as well as the number of litters with live offspring and the mean litter size were comparable between the vehicle control and IL-1ra treated groups of rats.

F1 pup viability remained similar between the dose groups throughout lactation and weaning. A statistically significant difference in the ratio of male:female pups was observed at parturition in the dose group treated with 50 mg/kg/d IL-1ra, as compared to the control group; however, this finding was not present in the F1 offspring in this dose group delivered by Cesarean section, nor in the highest dose group, and was therefore considered incidental to treatment.

There were no developmental abnormalities or malformations noted in the F1 generation pups throughout the lactation and weaning period. Mean F1 pup weights were similar between the control and IL-1ra dose groups throughout lactation and weaning, and no significant differences in developmental landmarks were noted in offspring from control or IL-1ra treated dams. Behavioral testing did not reveal any apparent, treatment-related changes in open-field ranging, or water maze swimming trials between F1 pups from dams in the control or IL-1ra treated groups.

During the post-weaning maturation, breeding, and gestation and lactation periods, there were no apparent differences in F1 mean body weights, body weight gains, food consumption, or overt toxicity between F1 offspring from vehicle control or IL-1ra treated dams. Following mating, there were no statistically significant differences in the copulation or fertility indices of the F1 males or females. Precoital intervals and duration of gestation were comparable between the dose groups, and there were no signs of prolonged parturition or unusual nesting behavior in the F1 females in any of the dose groups.

Pregnancy rates were 80%, 80%, 95%, and 100% for F1 offspring from dams in the vehicle control, 12.5, 50, and 200 mg/kg/d dose groups, respectively. At necropsy on lactation d 21, there were no treatment-related abnormalities on gross pathologic evaluation of the F1 females, and the mean numbers of implantation scars were comparable between the dose groups.

One female (animal #3910-14F) in the group of F1 offspring from F0 dams treated with 50 mg/kg/d IL-1ra had a total litter loss of the F2 generation between parturition and lactation d 2. This female lost five pups between days 0 and 1, and ten pups between lactation days 1 and 2. The losses in this animal resulted in a statistically significant decrease in pup survival between lactation days 0 and 4 for the F1 group from the 50 mg/kg/d Il-1ra dosed F0 females. However, there were no similar losses of F2 generation pups in any of the other females in this group, nor in any of the animals in the F1 generation from the highest dose group, therefore this finding was considered incidental to treatment of the F0 dams. F2 generation pup weights were similar among the groups throughout lactation, and there were no external developmental abnormalities noted in the F2 pups in any dose group.

In summary, treatment of the F0 generation dams with IL-1ra from GD6 through GD17 had no significant maternal toxicity. There were no remarkable effects on F1 generation pup viability, body weights, body weight gains, external, visceral, or skeletal malformations, or developmental landmarks, maturation, or behavioral indices. There were adverse effects of IL-1ra treatment on the F0 generation dams on the fertility and reproductive capacity of the F1 generation, and on the F2 pup viability, body weights, external observations, and survival to weaning. Under the conditions of this study, the NOAEL for IL-1ra effects on developmental toxicity in the Sprague-Dawley rat is $\geq$ 200 mg/kg/d.

Study #SY05-T92-04. ***Developmental toxicity study in rabbits with interleukin-1 receptor antagonist (IL-1ra).***

The objective of this study was to evaluate the potential embryotoxic or teratogenic effects of IL-1ra when administered by twice daily, s/c injection to pregnant, New Zealand white rabbits in a modified, segment II reproductive toxicity design. Twenty female rabbits per group were artificially inseminated with 0.5 ml of diluted semen, instilled into each female's vagina. Semen from the same donor male was used to inseminate an equal number of females in each study group. Immediately following insemination, the female rabbits were injected i/v with human chorionic gonadotropin to stimulate ovulation. The day of insemination was considered day 0 of gestation.

Inseminated, female rabbits were injected s/c twice daily with vehicle (citrate-buffered saline plus 0.1% polysorbate 80, pH 6.5), or 12.5, 50, or 200 mg/kg IL-1ra, administered as divided doses (6.25, 25, or 100 mg/kg/injection) from GD6 to GD18. On GD7, female rabbit #3528F in the group treated with 12.5 mg/kg/d IL-1ra jumped during the injection, and an undetermined amount of IL-1ra was not administered. There were several incidences during the dosing period where the animals did not receive the full amount of test article, as evidenced by an undetermined amount of test article observed on the animal's coat at the site of injection. These events occurred at approximately equal incidence in all study groups, including the vehicle control, and were not considered to have compromised the integrity of the study or the interpretation of the study results.

Animals were observed twice daily for morbidity and mortality, and once daily for evidence of overt toxicities, including physical or behavioral abnormalities. Individual body weights were measured on GD0, GD6, GD9, GD12, GD15, GD19, GD24,and GD29. Food consumption was measured daily, and body weight gains were measured at the interval time points between body weight measurements.

All females in each dose group were sacrificed on GD29 by i/v injection of sodium pentobarbital, and the fetuses harvested by Cesarean section for evaluation of litter size and gender distribution, and external abnormalities. The dams were evaluated for any gross evidence of pathology, and a representative tissue section of an injection site, as well as any internal gross lesions were preserved for future microscopic evaluation. Any female rabbits exhibiting gross lesion(s) at the injection site were photographed prior to euthanasia.

The uterus from each female was opened and examined macroscopically for the number of viable and non-viable fetuses, implantation sites, early and late resorptions. The numbers of corpora lutea on each ovary were recorded for each animal. Uteri with no macroscopic evidence of implants were preserved in 10% aqueous ammonium sulfide for detection of early embyolethality as described by Salewski[18].

Fetuses harvested from the pregnant dams were examined for external malformations, the gender and weight recorded, and the crown-rump length determined. Approximately one-half of the fetuses from each litter were preserved in Bouin's solution and examined for visceral

[18] Salewski, V.E. 1964. Farbemethode zum makroskopischen nachweis von implantations stellen am uterus der ratte. *Naunyn-Schm. Archiv. FurExper. Pathologic und Pharm.*, **247**:367.

malformations by the Wilson technique[19]. The remainder of the fetuses were fixed in isopropyl alcohol, cleared, and stained with Alizarin red S for evaluation of skeletal abnormalities[20].

All females survived to scheduled sacrifice on GD29. The pregnancy rates were 75%, 85%, 90%, and 85% for rabbits treated with vehicle control, 12.5, 50, or 200 mg/kg/d IL-1ra, respectively. No overt, clinical signs of toxicity related to IL-1ra were observed over the duration of the study. Incidental findings included alopecia, scant or soft feces, scabbing, and apparent, subcutaneous edema at the injection site(s), and were observed in all dose groups. However, there was an apparent, dose-related increase in the injection site reactivity, since the subcutaneous edema was observed in 20/20 rabbits treated with 200 mg/kg/d IL-1ra, and in 1/20 rabbits each in the vehicle control and 12.5 mg/kg/d IL-1ra dose groups, and in 7/20 females in the group treated with 50 mg/kg/d IL-1ra.

There were no remarkable effects of IL-1ra treatment on mean body weights, body weight gains, or food consumption in the females over the duration of the study.

At necropsy, subcutaneous hemorrhage at the injection site(s) was the most common macroscopic finding, and occurred at low incidence in the females in the control, 12.5, nd 200 mg/kg/d IL-1ra dose groups. Additionally, areas of white to tan, firm tissue present in the subcutis were observed adjacent to the injection site in all 20 rabbits treated with 200 mg/kg/d IL-1ra, and in 1/20 rabbits treated with 12.5 mg/kg/d IL-1ra. Histologically, the injection site reactions consisted of hemorrhage, chronic inflammatory infiltrates with vacuolated macrophages present, necrosis and/or presence of eosinophilic, granular material, and fibrosis. The necrosis was reported as minimal to mild in severity, and was present in 3/20 of the rabbits in the highest dose group. Incidental findings included paraovarian cysts, small kidneys or areas of depression(s) on the surface of the kidney, and scabbing of the skin and were present in either single or low incidences in all dose groups, including the vehicle control.

All female reproductive parameters evaluated at Cesarean section, including mean number of corpora lutea, implantation sites, viable fetuses, early and late resorptions, post-implantation losses, fetal sex ratios, and mean fetal body weights were comparable between the vehicle control and IL-1ra treated groups. No treatment-related malformations or developmental variations were noted in any of the fetuses from either the vehicle control or IL-1ra treated test groups. Morphologic evaluation of the fetuses revealed a low incidence of spontaneous malformations in all dose groups, including the vehicle control. These findings included anophthalmia and/or microphthalmia and domed head, in one fetus each from dams treated with either vehicle control or 12.5 mg/kg/d IL-1ra, respectively, and were not considered related to the test article. Animal #3566, in the group treated with 50 mg/kg/d IL-1ra had one early resorption, eight late resorptions, and one fetus that was morphologically normal at GD29. With the exception of this animal, the incidence of either early or late resorptions was comparable between the dose groups.

Visceral abnormalities included a major blood vessel variation (left carotid artery arising from brachiocephalic trunk; not incompatible with sustaining life), a hemorrhagic ring around the iris

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[19] Wilson, J.G. 1965. Methods for administering agents and detecting malformations in experimental animals. *In. Teratology Principles and Techniques*, J.G. Wilson and J. Workany, eds., University of Chicago Press, Chicago, IL; pp. 262-277.

[20] Dawson, A.B. 1926. A note on the staining of cleared specimens with Alizarin red S. *Stain Technol.*, 1:123-124.

in the eye, and a retrocaval ureter in one fetus each from dams in the vehicle control group. Two additional fetuses from the control group dose group also had the same blood vessel variation, accompanied by accentuated lobular markings in all lobes of the liver. Six fetuses from four dams in the group treated with 12.5 mg/kg/d IL-1ra, and one fetus in the mid-dose group had the blood vessel variation in the left carotid artery. This finding was present in three of 11 pups from dam #3592F in the 12.5 mg/kg/d IL-1ra dose group. Atalectasis was present in all lobes of the lungs of 4/6 pups from dam #3588, and the hemorrhagic ring(s) in the iris were present in one pup from each of two dams in this dose group. Two pups from dam #3533 in the 50 mg/kg/d IL-1 ra dose group had retrocaval ureters on the right kidney, as did one pup each from dams #3562 and #3578 in the 200 mg/kg/d dose group. In all cases, these findings were not outside the range or normal variations for this strain of rabbits, and were not considered related to IL-1ra treatment.

Skeletal variations included present of extra (13^{th}) and/or rudimentary rib(s) or rib pairs, slight to moderate malalignment of the sternebrae, presacral (27) vertebrae, variations in the position of the costal cartilage, and bent hyoid arches. Skeletal malformations included fused ribs, extra site(s) of ossification in the sternebrae, curvature of the lumbar spine, and fused ribs with associated costal cartilage. These findings were present at approximately equal incidence in slight to moderate severity, in fetuses from dams in all dose groups, including the vehicle control.

In summary, treatment of pregnant female rabbits with IL-1ra from GD6 through GD18 had no evidence of significant, systemic maternal toxicity. A dose-related increase in injection site hemorrhage, edema, and chronic inflammation was observed in dams in the 50 and 200 mg/kg/d IL-1ra dose groups. Based on the injection site findings in 20/20 female rabbits in the highest dose group and 7/20 rabbits in the 50 mg/kg/d IL-1ra dose group, the NOAEL for IL-1ra effects on maternal toxicity is 12.5 mg/kg/d. There were no remarkable effects of IL-1ra treatment of the dams on F1 generation pup viability, body weights, body weight gains, external, visceral, or skeletal malformations. Under the conditions of this study, the NOAEL for IL-1ra effects on embryotoxicity and teratogenicity in the New Zealand white rabbit is $\geq$ 200 mg/kg/d, administered as twice daily, divided doses from GD6 through GD18.

SUMMARY AND CONCLUSION:

In summary, the pharmacokinetic, acute and sub-chronic toxicities of IL-1ra were evaluated in rats, cynomolgus and Rhesus monkeys. Subcutaneous administration of either 10 or 100 mg/kg IL-1ra to adult, male cynomolgus monkeys resulted in serum concentration levels and exposures approximately equivalent to those achieved following i/v administration. Elimination half-lives were approximately 3 hours by either route of administration, and the volume of distribution at steady state was equivalent to the extracellular fluid space. Mean plasma clearances were in a range consistent with the rate of glomerular filtration in the monkey, suggesting that renal tubular secretion and degradation is the main route of elimination of IL-1ra in this species. Intravenous or or s/c administration of 1-100 mg/kg IL-1ra to adult, male Sprague-Dawley rats resulted in dose-related, linear increases in maximal serum concentration levels and total exposures, as defined by $AUC_{(0-\infty)}$. Total exposures at each dose level after s/c injection were approximately equivalent to those achieved following i/v administration, with mean bioavailability ranging from 75 to 100%. Elimination half-lives were approximately 1 hour by either route of administration, and the volume of distribution at steady state was equivalent to the extracellular fluid space. Mean plasma clearances were in a range consistent with the rate of glomerular filtration in the

rat, suggesting that renal tubular secretion and degradation is also the main route of elimination of IL-1ra in this species. Subcutaneous administration of either 10 or 100 mg/kg IL-1ra to male New Zealand White rabbits resulted in serum concentration levels and exposures approximately equivalent to those achieved following i/v administration. Elimination half-lives were approximately 1-2 hours by either route of administration, and the volume of distribution at steady state was equivalent to the extracellular fluid space. Development of significant antibody titers against IL-1ra was observed in the two rabbits initially injected with 100 mg/kg by the s/c route, resulting in prolonged MRT and elimination half-life of IL-1ra following a second, i/v dose. Anti-IL1ra antibody titers were undetectable in animals treated with either 100 mg/kg IL-1ra by the i/v route followed by the s/c exposure, or in all rabbits treated with the 10 mg/kg dose regardless of sequence.

In general, treatment with IL-1ra was well-tolerated at doses of up to 100 mg/kg/d by either s/c or i/v injection for 14 days, and at doses of 2, 20 or 200 mg/kg/d, s/c in rats for 6 months. Toxicities were limited to mild to moderate, gross and histologic evidence of dose-related inflammation, hemorrhage, and fibrosis at the injection site and occurred in all species, regardless of route of administration. These findings were reversible on discontinuation of treatment with IL-1ra. The NOAEL for these effects in the rat was 2 mg/kg/d after daily s/c injection for 6 months, and 30 mg/kg/d following i/v injection for 14 or 28 days. The NOAEL in Rhesus monkeys was 150 mg/kg/24 hours by continuous i/v infusion for 7 days, 10 to 30 mg/kg/d by i/v bolus injection for 14 days, and 5 mg/kg/d by s/c injection for 14 days (as twice daily, divided doses of 2.5 mg/kg/injection). Antibodies to IL-1ra developed in both rats and cynomolgus monkeys by one month of treatment with daily IL-1ra injections, but diminished over time on study and did not appear to be neutralizing. No developmental, embryotoxic, or teratogenic effects of IL-1ra were observed in either Sprague-Dawley rats or New Zealand white rabbits at doses of up to 200 mg/kg/d, administered by s/c injection throughout the critical period of organogenesis. There were no effects of IL-1ra on reproductive capacity of either male or female rats in the F0 or F1 generations, and no effects of IL-1ra on male fertility parameters (sperm counts, morphology, motility, and production rates) were observed following 29 d of treatment with 100 mg/kg IL-1ra. In summary, IL-1ra toxicities are limited mainly to local injection site reactivity. These data support the safety of IL-1ra treatment in the clinic, and its licensure for the treatment of rheumatoid arthritis.

COMMUNICATIONS TO THE SPONSOR:

The following items are for comment or clarification by the sponsor:

1. We note from our review of the preclinical toxicology studies that samples of serum were obtained in studies #SY05-T89-10, #SY05-T89-12, #SY05-T89-01, #SY05-T89-02, #SY05-T89-05 and #SY05-T89-09 in rats and studies #SY05-T90-02, #SY05-T89-03, #SY05-T89-08, and #SY05-T89-07 in Rhesus monkeys, and shipped to the study sponsor for the determination of anti-interleukin-1 receptor antagonist seropositivity. However, there are no data in the BLA submission that would indicate that these assays were ever performed. Please comment on the disposition of the samples from the indicated studies. Were these samples ever analyzed, and what were the results of the assays?

2. We also note the apparent inverse dose-relationship in the development of anti-IL-1ra antibodies, in both rats treated for 6 months with 0, 1, 10, or 100 mg/kg interleukin-1 receptor antagonist by twice daily s/c injection (Study #SY05-T94-04), and in Rhesus monkeys treated for 28 days with 10, 100, or 200 mg/kg/d interleukin-1 receptor antagonist by a single, s/c daily injection. Several possibilities to explain this effect exist, the first being that inhibition of antibody formation is an extension of the pharmacologic effects of IL-1ra, which acts to selectively suppress T lymphocyte function, including Th_1 sub-populations involved in regulating the immune response. Although unlikely, down-regulation of Th_1 function may inhibit production of antibody in response to the foreign protein. Alternatively, the assay used to detect anti-IL-1ra antibody activity is a ELISA technique, in which IL-1ra coated plates are exposed to serum samples from treated rats. Anti-IL-1ra antibody present in the samples from the treated animals will bind to the IL-1ra on the plate, and is then detected with a secondary anti-rat antibody conjugated to a chromogenic agent.

   Please comment as to the mechanism of the apparent, inverse dose-relationship in antibody production. Please provide any information you may have that shows if the binding of anti-IL-1ra antibody is competitive, and whether any free IL-1ra in the sample can inhibit the interaction of the antibodies with the IL-1ra coated to the plates, thereby giving a falsely negative readout.

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Key Words: interleukin-1, interleukin-1 receptor antagonist, rheumatoid arthritis, toxicity

concurrences: OTRR/C,P-T/MGreen

cc: OTRR/C,P-T/MGreen