CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125276

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

JAN - 7 2010

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology OND IO
BLA: 125276
Submission date: July 8, 2009 (resubmission to Complete Response letter)
Drug: tocilizumab (Actemra), a recombinant humanized IgG1kappa monoclonal antibody to the human interleukin 6 receptor
Sponsor: Hoffman-La Roche
Indication: Adult patients with moderately-to severely- active rheumatoid arthritis who have had an inadequate response to one or more TNF antagonist therapies

Reviewing Division: Division of Anesthesia, Analgesia and Rheumatology Products

Introductory Comments: The pharm/tox reviewer and supervisor found the nonclinical information originally submitted November 19, 2007 to be inadequate to support approval of tocilizumab. The sponsor was issued a complete response letter which requested additional nonclinical information on the effect of tocilizumab on fertility and peri-postnatal development.

Subsequent discussions were held with the applicant on the types of studies that might be adequate to satisfy these informational needs.

Reproductive and developmental toxicity:
Fertility:
The applicant conducted fertility and early embryonic development studies in mice (one male study and one female study) with a murine surrogate antibody to IL-6 receptor. The NOAEL for effects on fertility was the high dose used in this study of 50 mg/kg.

Peri-postnatal:
The applicant conducted a peri-postnatal study in mice with a murine surrogate antibody to IL-6 receptor. The NOAEL for effects on peri-postnatal development was the high dose used in this study of 50 mg/kg.

The doses used in these studies appear adequate based on functional assays of IL-6 activity in mice.

Conclusions and Recommendations:
The applicant conducted adequate studies to address the outstanding nonclinical approvability issues. No further studies are recommended. I agree with the Division pharm/tox conclusion that this BLA can now be approved from a pharm/tox perspective.

I reviewed the labeling negotiated with the sponsor as of Jan. 7, 2009 and find it acceptable from a pharm/tox perspective. Dose ratios comparing human to animal doses for those studies using the murine surrogate antibody are not provided in the labeling since a direct comparison between the dose of human antibody and the dose of the murine antibody is not meaningful.

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

SUPERVISOR'S SECONDARY REVIEW
PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER:	**125276**
PRODUCT:	**ACTEMRA® (tocilizumab)**
INTENDED CLINICAL POPULATION:	**Adults with Rheumatoid Arthritis**
SPONSOR:	**Roche**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia, and Rheumatology Products (HFD-170)**
PHARM/TOX REVIEWER:	**Asoke Mukherjee, Ph.D.**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, Ph.D.** R. Daniel Mellon 12-19-09
DIVISION DIRECTOR:	**Bob A. Rappaport, M.D.**
PROJECT MANAGER:	**Kathleen Davies**

Executive Summary

I. Recommendations

A. Recommendation on approvability

From a nonclinical pharmacology toxicology perspective, BLA 125276 may be approved upon agreement on product labeling.

B. Recommendation for nonclinical studies

None.

C. Recommendations on labeling

The recommended labeling below is based on both the first cycle review as well as the second cycle review. The Sponsor's proposed labeling is from the second cycle. Labeling recommendations were sent to the sponsor at the end of the first cycle and some of the proposed labeling recommendations were accepted at the time.

Sponsor's Proposed Labeling	Recommended Labeling	Rationale/Comment

b(4)

2 Page(s) Withheld

_____ Trade Secret / Confidential (b4)

✓ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Pharm/Tox-1

Sponsor's Proposed Labeling	Recommended Labeling	Rationale/Comment
	———	

b(4)

II. Summary of nonclinical findings

The current submission is the Applicant's response to the complete response letter issued September 17, 2008. The two nonclinical deficiencies are reproduced below:

1. Although requested in the responses for the pre-BLA meeting which was scheduled for October 12, 2007, you have not submitted reports of peri-natal and post-natal developmental toxicology studies nor have you provided adequate justification for why such studies are not possible. To resolve this issue, you must submit reports of such studies using either the monkey or the surrogate model.

2. You have not provided adequate justification as to why the fertility studies in the surrogate model are not possible. Although adequate fertility studies are not feasible in the primate model, you do have a mouse homologous product that can be used to characterize the potential effects on fertility. Therefore, to resolve this issue, you must submit reports of fertility studies in the mouse model.

The complete response letter also requested additional information that was not deemed an approvability issue (verbatim from :

A. You have not submitted the results of carcinogenicity studies with either tocilizumab or the homologous protein. The application states that "the MR 16-1 antibody is also not an appropriate reagent to be used in long-term carcinogenicity studies, as this antibody is a rat monoclonal anti-mouse IL-6R antibody and is considered to be immunogenic in long-term in vivo studies in mice." Submit data to support this statement in order to support your conclusion that this option for carcinogenicity assessment is not viable.

B. As described in ICH S6, to assess in the extrapolation of the toxicology program findings to humans, submit a summary table comparing the binding affinity of tocilizumab to both the human and monkey sIL-6R and mIL-6R and a comparison of the functional potency of tocilzumab at the human arid monkey IL-6R with references to the studies from which the data were obtained.

To address the approval issues, the Applicant conducted a pre- and post natal development study and fertility and early embryonic development studies in both the male and female mouse using the MR16-1 homologous protein (IgG1). MR16-1 has been demonstrated to bind to and block the mouse IL-6R with a K_D value for the sIL-6R of approximately 10 nmol/L. Although the affinity of MR16-1 to the mouse sL-6R is about 14-fold lower than the affinity of tocilizumab for the human sIL-6R (K_D 0.71-0.79 nmol/L), the MR16-1 protein has been shown to suppress the acute phase response to IL-6 at a dose of 0.1 mg/kg in the mouse; therefore, can adequately mimic the pharmacodynamic effects of tocilizumab. Although the MR16-1 protein is a rat anti-mouse protein and anti-MR16-1 antibodies did develop at lower doses, the Applicant was able to dose the animals adequately to provide a characterization of the impact of IL-6R blockade on pre- and postnatal developmental endpoints and fertility in both males and females.

Pre- and Postnatal Development. Mice were treated intravenously with either 15 or 50 mg/kg MR16-1 every three days (72 hours) from gestation day 6 to postnatal day 21. A total of 22% of the dams treated with the low dose died during the study; however, none of the dams in the high dose group died. The cause of death is most lastly an immune reaction to the foreign protein, and is not likely due to the pharmacodynamic effects of the drug. Examination of the pups born from dams in the high dose groups demonstrated no adverse effects of IL-6R blockage in pup viability, development, learning, behavior, immune cell populations (blood, spleen, thymus) or immune function (IgM and IgG responses to KLH antigen). Based on the results of the study, the NOAEL for both maternal and fetal development was the high dose of 50 mg/kg.

Fertility and Early Embryonic Development. In separate studies, male or female mice were treated intravenously with either 15 or 50 mg/kg MR16-1. Female mice were treated intravenously with either 15 or 50 mg/kg MR16-1 every three days from 14 days prior to mating and up to gestation day 6. A total of 12 out of 51 females in the low dose group died during the study; however, only 1 out of 51 females in the high dose group died prior to planned study termination. These deaths were attributed to an immune reaction to the foreign protein. There were adverse effects noted on estrus cycle, fertilization, implantation or early embryonic development that could be attributed to the pharmacodynamic effects of IL-6 receptor blockade. The NOAEL for fertility and early embryonic development was the high dose of 50 mg/kg.

Male mice were treated from 63 days before mating, during the 10 days of mating and 5 days after mating until terminal sacrifice of the males. A total of 10 of 48 (21%) of the males treated with 15 mg/kg MR16-1 died during the study; however, no deaths were noted in the high dose males. The deaths are attributable to an immune reaction to the foreign protein. There were no adverse effects noted in the high dose or surviving low dose animals on testicular or epididymal weights or histopathology. There were no effects on sperm counts or motility or male functional assessments (libido, insemination, epididymal sperm maturation and successful fertilization). There were no adverse effects noted in the untreated females mated to the treated males (endpoints included corpora lutea, live embryos, dead embryos, and post implantation losses). The NOAEL for male fertility was the high dose of 50 mg/kg.

Based on functional studies in the mouse that demonstrated that a dose of 0.1 mg/kg MR16-1 was able to almost completely block a 0.4 mcg/kg mIL-6-induced production of serum amyloid A (SAA) levels in the CD-1 mouse, the dose of 50 mg/kg of MR16-1 likely represents a suprapharmacological blockade of IL-6R in this model. Therefore, the studies adequately characterized the potential impact of IL-6R blockade on both fertility and pre- and postnatal development, including development of the immune response. However, as the tested material does not represent the clinical candidate, exposure margins are not included in the product labeling.

To address the nonapproval issues, the Applicant provided data to demonstrate that tocilizumab binding to the monkey IL-6R was comparable to that of the human IL-6R. In addition, the Applicant also provided data to support the conclusion that carcinogenicity assessment using the MR16-1 analogue protein is unlikely to be successful.

FDA Center for Drug Evaluation and Research
Division of Anesthesia, Analgesia, and Rheumatology Products
10903 New Hampshire Avenue, Silver Spring, MD 20993

PHARMACOLOGY TOXICOLOGY REVIEW OF COMPLETE RESPONSE

BLA number: 125276
Drug: Tocilizumab
Date Submitted: July 8, 2009, Nov 4, 2009 and Date Received: July, 9, 2009 and Nov 4, 2009
Sponsor: Hoffman La Roche Inc.
Reviewer name: Asoke Mukherjee, Ph.D. Amukherjee 12/17/2009
Division name: Division of Anesthesia, Analgesia, and Rheumatology Products
HFD #: 170
Pharmacology/Toxicology Team Leader: Daniel Mellon, Ph.D.
Division Director: Bob Rapapport, M.D.
Project Manager: Sharon Turner-Rinehardt / Kathleen Davies
Review completion date: Oct 30, 2009

Recommendation: The BLA can be approved on the basis of non-clinical data submitted for the Complete Response.

Executive Summary

I. Recommendations

A. Recommendation on approvability: The applicant submitted non-clinical data for a complete response on reproductive safety in mice using the surrogate antibody (MR16-1). The applicant also addressed non-approvability issues on the comparative potency of tocilizumab in monkey and human IL-6R systems and provided data to support why a long-term carcinogenicity study was not possible using the surrogate antibody. The applicant addressed issues related to the complete response. From the non-clinical perspectives, there is no non-clinical outstanding issue for the BLA. Based on the data, the reviewer recommends approval of the BLA for the treatment of rheumatoid arthritis. Recommendations for the label are shown below.

B. Recommendation for nonclinical studies: The reviewer does not recommend any new non-clinical studies for the approval of the BLA.

2 Page(s) Withheld

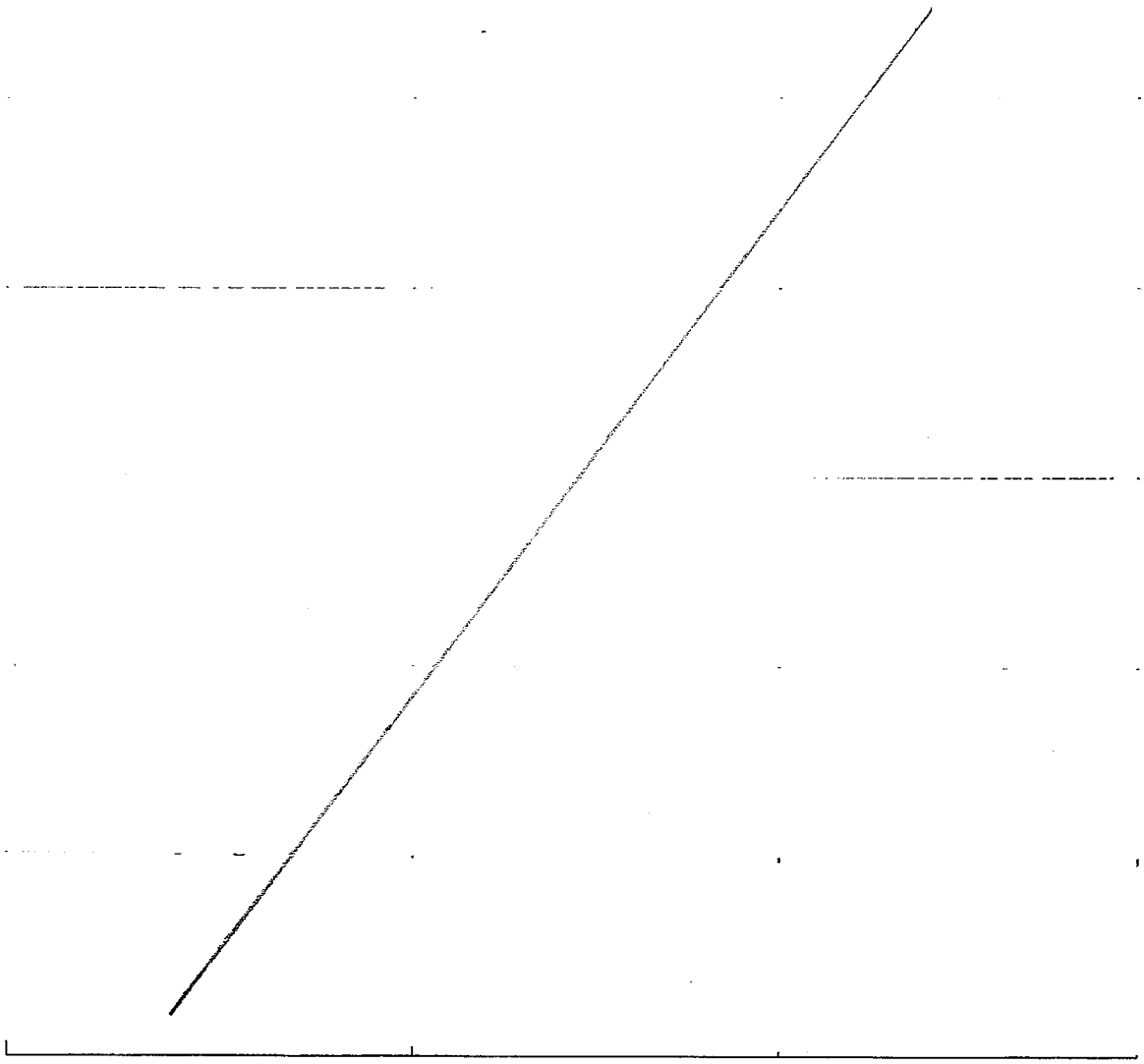
_____ Trade Secret / Confidential (b4)

✓ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Pharm/Tox- 2



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II. Summary of nonclinical findings

Brief overview of nonclinical findings:

The applicant submitted new study reports in response to the Complete Response letter, including studies on male and female fertility, prenatal and postnatal development, immunotoxicity, and behavior and reproductive performance of second-generation of CD-1 mice. The study was conducted using MR16-1, a mouse IL-6 receptor antibody raised in rats. Doses for reproductive studies in mice were 15 and 50 mg/kg/IV/3 days. The applicant obtained input on the dose selection from the review Division.

MR16-1 at 15 mg/kg showed mortality to male and female mice that were related to the treatment. However, mortality was not observed at 50 mg/kg dose. The cause of mortality

could be due to immunologic reactions to foreign proteins. A similar change was not noted at 50 mg/kg and that could be related to the immunosuppressive activity of the surrogate antibody. Mice that survived during the treatment did not show any effect in male and female fertility. F1 mice did not show any treatment-related effect in the behavior, learning parameters and reproductive performance following sexual maturation as well as skeletal variations due to the treatment. However, pups from about 10% of F0 dams at 50 mg/kg showed a slight immunosuppression at 5-6 weeks of age. The functional and clinical significance of the change is not known because there was no effect on the antigen-induced antibody formation in these pups. These studies for the complete response are acceptable for the approval of the BLA.

The applicant also provided data in human and cynomolgus monkey IL-6R systems in vitro that showed high affinity of tocilizumab to its receptor and suggested tocilizumab is effective both in human and monkey IL6 receptor systems with high potency. In addition, MR16-1, a surrogate monoclonal antibody for murine IL-6 receptor showed in vitro affinity and in vivo efficacy in murine system. These non-clinical data clearly justify use of monkey and murine models for the characterization of safety to tocilizumab and MR16-1, respectively.

The applicant provided data for the antibody formation to MR16-1 to justify not conducting a carcinogenicity study using this surrogate protein in CD-1 mice. This issue was raised in the Complete Response as a non-approvability issue. The applicant's position that long-term toxicity and carcinogenicity to MR16-1 in CD-1 mice was not possible due to neutralization of the surrogate antibody is acceptable to the reviewer. Data reviewed in the complete response showed a slight chance of antibody formation at 50 mg/kg within 3 months. The reviewer conceived that mortality at 15 mg/kg, immunosuppression at 50 mg/kg and possibility of antibody formation during the two-year bioassay would compromise the outcome of a carcinogenicity study. In the absence of data, recommendation for the package insert that tocilizumab can induce malignancy due to immunosuppression would provide necessary safety alert to the long-term consequences of the treatment.

No other outstanding issues are there for tocilizumab non-clinical development at this stage. From the non-clinical point of view, reports submitted for the Complete Response are acceptable for the approval of the BLA. The reviewer recommends changes in the proposed package insert.

Studies reviewed within this submission:

1. Recombinant mouse IL-6 induced serum amyloid levels in the mouse model from the liver (hepatocytes), study # PHM09-0012.

2. Cross reactivity to other cytokines that transduce through the same mechanism was investigated by a study titled "Examination of cross-reactivity of MR16-1 with mouse gp 130 family cytokines by SPR, report # 1033689.

3. Measurement of MR16-1 dissociation constant by SPR, report # 1033688.

4. Binding affinity of tocilizumab for human and cynomolgus soluble IL-6Rs, report #PHM08-0205S.

5. Examination of human or cynomolgus IL-6 receptor neutralizing activity of tocilizumab in recombinant Ba/F3 cell proliferation, study # PHM08-0207S.

6. Placental transfer study of MR16-1 in mice, study # PBC036-072.

7. MR16-1: Milk excretion study of MR16-1 in mice, study # PCB036-073.

8. A study for effects of MR16-1 on fertility and early embryonic development to implantation by intravenous administration in male mice, study # SBL036-065.

9. A study for effects of MR16-1 on fertility and early embryonic development to implantation by intravenous administration in female mice, study # SBL036-066.

10. A study for effects of MR16-1 on pre- and post natal development, including maternal function by intravenous administration in mice, study # SBL036-064.

11. A passive cutaneous anaphylaxis study of MR16-1 in mice, study # SBL036-077.

12. Significance of supernumerary ribs in rodent developmental toxicity studies: Postnatal persistence in rats and mice, Chernoff, N., Rogers J, M., Turner C., I. and Francis, B.M.: Fundamental and Applied Toxicology, 17, 448-453, 1991.

PHARMACOLOGY

Brief summary

The applicant conducted in vivo and in vitro studies to justify that MR16-1 and tocilizumab are effective in binding and inhibiting IL-6 signals. MR16-1 at 0.1 mg/kg/IV dose inhibited IL-6 induced acute protein levels in the CD-1 mouse plasma. In vitro techniques based on antigen-antibody recognition system also compared Il-6R and tocilizumab binding constant (K_D) using recombinant human and cynomolgus IL6-R. Data showed that tocilizumab had high affinity in both human and cynomolgus monkeys IL-6 receptors. In addition, tocilizumab showed inhibition of both human and monkey IL-6R mediated cell proliferation at almost equal potency. These data justified conducting non-clinical safety assessment in CD-1 mice and cynomolgus monkeys. The applicant also developed an ELISA assay for the determination of plasma levels in mouse plasma. Data for the plasma levels of MR16-1 (monoclonal antibody to murine IL-6R raised in rats) in several reproductive safety studies showed exposure levels to the treatment. However, the acute pharmacodynamic response for IL-6-induced amyloid protein release in mice at 0.1 mg/kg did not show quantifiable level of MR16-1 in the mouse plasma above the limit of detection, although a physiological effect of IL-6 was inhibited at that dose. Therefore, the reviewer concluded that the applicant made a reasonable attempt to develop a sensitive assay method for the plasma IL-6R antibody detection (MR16-1) based on the available scientific methods at this time.

The complete response letter unrelated to approvability issue asked for a response for the following question:

"As described in ICH S6, to assess in the extrapolation of the toxicology program findings to humans, submit a summary table comparing the binding affinity of tocilizumab to both the human and monkey sIL-6R and mIL-6R and comparison of the functional potency to tocilizumab at the human and monkey IL-6R with references to the studies from which the data were obtained."

Reviewer's Response:

Under the experimental conditions, functional and binding data provided insights to the usefulness of conducting non-clinical safety studies in cynomolgus monkeys and CD-1 mice using tocilizumab and MR16-1, respectively. The experimental data also suggested a similarity of tocilizumab for interacting with the human and monkey IL-6 receptor system. These data also fulfils the responses to the issues with respect to ICH S6 guidelines of the complete response for the BLA. No further action on this issue is required.

Primary pharmacodynamics

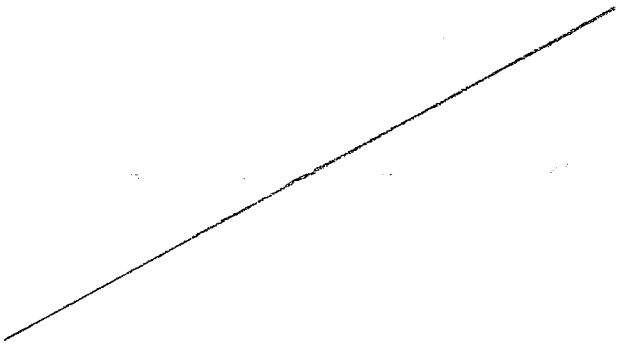
1. The applicant submitted an acute pharmacodynamic study in female CD-1 mice related to IL-6 induced serum amyloid (SSA) production and its inhibition by MR16-1. The title of the study is: Effect of MR16-1 (Lot # MC45021) on mouse induced SAA production" (study # PHM09-0012). Recombinant mouse IL-6 induced serum amyloid levels in the mouse model from the liver (hepatocytes). Amyloid is analogous to acute phase protein that is elevated in RA patients. Mice were treated with MR16-1 at 0.01, 0.03, 0.1, 0.3 and 1 mg/kg/IV into the caudal vein one hour before the IP administration of mouse IL-6 or vehicle. Two hours after the mIL-6 injection, animals were scarified and blood samples were collected for the determination of SSA and MR16-1 levels. The study design from the submission is shown below.

Group	Intravenous administration		Intraperitoneal administration	
	Substance administered	Dose (mg/kg)	Substance administered	Dose (μg/kg)
mIL-6(-) group (n=5)	vehicle-1	–	vehicle-2	–
mIL-6 group (n=6)	vehicle-1	–	mIL-6	4
0.01-mg/kg group (n=6)	MR16-1	0.01	mIL-6	4
0.03-mg/kg group (n=6)	MR16-1	0.03	mIL-6	4
0.1-mg/kg group (n=6)	MR16-1	0.1	mIL-6	4
0.3-mg/kg group (n=6)	MR16-1	0.3	mIL-6	4
1-mg/kg group (n=6)	MR16-1	1	mIL-6	4

Mouse SSA and MR16-1 levels in plasma were assayed by ELISA methods. Methods for Study # 1033691 for plasma MR16-1 determination were also considered acceptable by the product reviewer for the BLA. However, plasma levels at the lowest effective dose were not above the limit of detection. Data suggest that the ELISA method used for the determination

of MR16-1 was not very sensitive for the quantitation of MR16-1 in the mouse plasma. The applicant provided data for plasma levels of SSA in mice as shown below.

Table 1 Plasma SAA production of individual data



b(4)

administration of mIL-6 (dose: 4 μg/kg). ND: Not detected (<6 μg/mL). In the calculation of the mean, SE and median, we used 6 μg/mL as the substitute value for ND.

Above data show that 0.1 mg/kg/IV dose was effective for inhibition of SSA induced by 4 ug/kg/IP dose of mIL-6. The plasma level of MR16-1 at minimal effective dose was below the limit of detection at 0.975 ug/mL. However, the plasma levels at 0.3 and 1 mg/kg of MR16-1 at two hour post mIL-6 challenge were 1.1 and 7.6 ug/mL, respectively. The effective dose for MR16-1 in the acute pharmacodynamic study was 0.1 mg/kg/IV.

It is concluded that MR16-1 was effective in vivo for the biological effect of mIL-6 in the mouse model. Therefore, reproductive safety studies for MR16-1 in mice would provide data on the role of IL-6 in fertility and reproduction using experimental studies. A similar efficacy study for MR16-1 was also conducted in mouse adjuvant-induced amyloidosis that was reviewed for the original BLA application dated Aug 15, 2008.

2. Cross reactivity to other cytokines that transduce through the same mechanism was investigated by a study titled "Examination of cross-reactivity of MR16-1 with mouse gp 130 family cytokines by SPR," report # 1033689.

The applicant investigated the binding of MR16-1 to recombinant mouse IL-11 receptor and recombinant mouse oncostatin M receptor (OSMR) in addition to recombinant mouse IL-6 receptor.

The study was conducted using a techniques in which MR16-1 was immobilized on a chip and amine coupling reagent so that interactions with above receptor would provide a signal that could be quantitated. The difference in the effect before and after injection of IL-6R, IL-11R, and OSMR signified the specificity of binding. Graphical presentation of data suggested that MR16-1 was selective to mouse IL-6R and did not bind to IL-11R or OSMR. However, the applicant did not provide any data table.

3. MR16-1 dissociation (Kd) for binding to mouse recombinant IL-6 receptor was determined in a study titled "Measurement of MR16-1 dissociation constant by SPR," report # 1033688. The applicant used the same procedure described above for the determination of specificity to binding with mouse IL-6 receptor. Goat anti-rat IgG antibody Fc receptor was immobilized on a chip with a sensor that would yield a measurable biochemical response. Binding affinity of MR16-1 between anti-rat IgG and sIL-6R was quantitated. The principle of the method was similar to that used in the radioligand binding studies. Data are shown from the submission below.

10. TABLES

Table 1 **k_a value, k_d value and K_D value for MR16-1 with respect to sIL-6R**

Lot No.	k_a value (10^4 L/mols)	k_d value (10^{-4} /s)	K_D value (10^{-9} mol/L)
Z801J23	32.7 ± 1.5	32.5 ± 1.0	10.0 ± 0.6
MC45021	32.1 ± 1.1	31.5 ± 1.0	9.8 ± 0.6

The data indicate the mean ± SD of 9 repetitions.

K_D is the concentration needed for occupancy of the binding site of the receptor by 50% at equilibrium. K_a and K_b are association and dissociation constants that are determined by $K_d = K_a/K_b$. The above data signified high affinity of MR16-1 to its receptor and the nature of binding was competitive.

4. The applicant provided data for binding of tocilizumab to soluble IL-6 receptors from humans and monkeys in a study titled "Binding affinity of tocilizumab for human and cynomolgus soluble IL-6Rs" in the report #PHM08-0205S. This study report was submitted in response to a non-approvability item in the Complete Response. The methods used in the experiment were similar to that used for above study in which Kd to MR16-1 to mouse IL-6R was determined. Anti-human IgG $F(ab)_2$ dimers were immobilized on a sensor chip. Competition for binding between anti-human IgG and hIL-6 or monkey IL-6 receptor was determined for calculating the K_D for tocilizumab binding to human and monkey IL-6R. Data are provided from the applicant's table below.

Table 1 Binding affinities of tocilizumab for IL-6 receptors

antigen	antibody	ka (1/Ms)	kd (1/s)	KD (M)
SR344	MRA	3.9E+05	9.1E-04	2.3E-09
R&D hIL-6R	MRA	6.2E+05	1.2E-03	2.0E-09
cIL-6R	MRA	1.8E+05	8.5E-04	4.8E-09

Each value represents mean of three measurements.
SR344: soluble human IL-6 receptor produced by in-house CHO (complex type carbohydrate).

5. Examination of human or cynomolgus IL-6 receptor neutralizing activity of tocilizumab in recombinant Ba/F3 cell proliferation, study # PHM08-0207S.

The applicant provided data for IL-6 induced cell proliferation and effect of tocilizumab in vitro. [⊥ was developed by knock in genes for human IL-6 receptor and gp 130 or gp 130. [⊥ for cell proliferation. [⊥ to induce cell proliferation. Cells were also treated with tocilizumab at several concentrations to induce an inhibitory effect on the cell proliferation. The cell growth was determined from the absorbance spectrum of the suspension at 450 nm. The inhibition of growth of cells and IC_{50} were also calculated. Tocilizumab showed IC_{50} for inhibition of the cell proliferation in the human receptor in ——— cells at 6.2 ug/mL and ——— transducing system at 1.2 ug/mL. b(4)

In addition to above cell line, two more cell lines [⊥ were developed that would transduce cynomolgus monkey IL-6R membrane or soluble receptor in the presence of cynomolgus IL-6. Data showed inhibition of cell proliferation at 9.5 and 1.5 ug/mL for membrane and soluble cynomolgus monkey receptor systems, respectively. These data suggest that tocilizumab cross-reacted with both human and monkey IL-6R to inhibit cell proliferation. However, tocilizumab had greater inhibitory potency for human and monkey soluble receptor for IL-6 than membrane bound IL-6 receptors from human or cynomolgus monkeys. Overall, in vitro studies showed almost equal potency for tocilizumab in human and monkey IL-6 receptor systems. b(4)

Data from the applicant's table are shown below.

	IC_{50} values of tocilizumab
	6.2 μg/mL (N=3)
	9.5 μg/mL (N=2)
	1.2 μg/mL (N=3)
	1.5 μg/mL (N=2)

b(4)

The above data suggest that tocilizumab was effective for binding with IL-6R from both human and cynomolgus monkey. Cell proliferation assay showed that tocilizumab had similar potency in neutralizing the effect of IL-6R from human and cynomolgus monkey. However, tocilizumab was more effective in inhibiting the effect of soluble IL-6R than the membrane bound IL-6R for both human and monkey systems.

PHARMACOKINETICS/TOXICOKINETICS

Brief summary: MR16-1 exposure levels and anti-product antibody production were determined. Exposure to MR16-1 in mice was increased with the dose. However, there was a non-linear increase in the exposure with the dose suggesting for an accumulation of MR16-1 at the higher dose. Data from the placental transfer experiments suggest that F0 animals and

F1 fetuses were exposed to MR16-1 during the gestation and rearing period. A similar finding (exposure data) was noted with respect to the exposure to MR16-1 in male and female mice separately in the fertility and early embryonic developmental toxicology studies. It should be noted that MR16-1 also possibly induced immunosuppression so that the anti-product antibody formation was suppressed depending on the dose and length of the treatment with MR16-1 in mice. Longer duration of treatment rendered greater immunosuppression and lowered anti-product antibody formation at 15 mg/kg when data from the fertility and early embryonic development and pre- and postnatal developmental toxicology studies were compared.

Presence of the anti-MR16-1 antibody in F0 mice was observed (surviving animals from pre and post-natal study) only at 15 mg/kg at the end of treatment in a total of 5 mice out of 60 mice deployed in the main and satellite animals. When individual data for animal # 10133, 10138, 10031, 10044 and 10056 were compared for MR16-1 levels and its antibody titers, all these animals showed presence of the anti-MR16-1 antibodies and no detectable MR16-1. These data indicated that MR16-1 could have been cleared via anti-product antibodies in these animals although the anti-product antibody formation did not cause deaths to the animals at 15 mg/kg. In contrast, presence of MR16-1, almost absence of anti-MR16-1 and absence of mortality were observed at 50 mg/kg among main and satellite animals suggesting exposure to the MR16-1 surrogate protein in this dose group. Antigen antibody reactions and neutralization of MR16-1 was observed in about 8% mice treated at 15 mg/kg without mortality. A dose of 50 mg/kg was well tolerated without mortality and with minimal production of anti-product antibody. MR16-1 was excreted in the milk of nursing mice.

Absorption

1. Determination of MRA16-1 concentration in mouse for "A study for effects of MR16-1 on pre- and postnatal development, including maternal function, by intravenous administration in mice, study — 036-064 and analysis # TK-173-G. b(4)

Detail of the study protocol was reviewed under the pre- and postnatal developmental toxicity study. Plasma levels of MR16-1 were determined on gestation days 6, 15 and lactation day 20 to 21 using ELISA methods.

The PK data are summarized below from the applicant's table. The limit of detection was 975 ng/mL.

Dose (mg/kg)	Parameters		MR16-1 concentration (µg/mL)		
			Day 6 of gestation	Day 15 of gestation	Final day of dosing
15	$C_{0.5h}$	Mean	142	100	42.0
		SD	88	34	NC
	$AUC_{(0-72h)}$ (µg·h/mL)		5431.3	1364.1	866.4
50	$C_{0.5h}$	Mean	574	452	612
		SD	79	32	74
	$AUC_{(0-72h)}$ (µg·h/mL)		24531.5	6103.8	25214.6

Individual data from the satellite and main groups are shown below.

Table 2 MR16-1 Concentrations (Satellite group)

Dose (mg/kg)	Time (h)	MR16-1 concentration (μg/mL)					
		Day 6 of gestation		Day 15 of gestation		Final day of dosing	
		Animal No.	Conc.	Animal No.	Conc.	Animal No.	Conc.
15	0.5	10103		10115		10127	-[1]
		10104		10116		10128	[1]
		10105		10117		10129	
		10106		10118		10130	
	Mean		142		100		42.0
	SD		88		34		NC
	24	10107		10119		10131	-[1]
		10108		10120		10132	——[5]
		10109		10121		10133	ND[2]
		10110		10122		10134	-[1]
	Mean		84.2		13.5		ND
	SD		10.0		1.4		NC
	72	10111		10123	ND[2]	10135	-[1]
		10112		10124	ND[2]	10136	ND[2]
		10113		10125	ND[2]	10137	——
		10114		10126	——[5]	10138	ND[4]
	Mean		38.3		ND		15.1
	SD		6.4		NC		26.2
50	0.5	10139		10151		10163	
		10140		10152		10164	
		10141		10153		10165	
		10142		10154		10166	
	Mean		574		452		612
	SD		79		32		74
	24	10143		10155		10167	
		10144		10156		10168	
		10145		10157		10169	
		10146		10158		10170	
	Mean		343		88.1		320
	SD		43		12.8		163
	72	10147		10159	ND[2]	10171	
		10148		10160	ND[2]	10173	[5]
		10149		10161	ND[2]	10173	
		10150		10162	——	10174	
	Mean		240		0.348		384
	SD		NC		0.695		51

b(4)

b(4)

b(4)

b(4)

b(4)

b(4)

-: No sample, ND: <1.95 ng/mL × 500 = 975 ng/mL, NC: Not calculated

[1] Unscheduled death, [2] The dilution of the sample is ×500.

[3] The dilution of the sample is ×5000., [4] The dilution of the sample is ×50000.

[5] Not pregnant

Table 4 MR16-1 Concentrations (Main groups)

MR16-1 concentration (μg/mL)					
0 mg/kg		15 mg/kg		50 mg/kg	
Animal No.	Conc.	Animal No.	Conc.	Animal No.	Conc.
10001	ND [2] [6]	10031	ND [2]	10061	
10002	ND [2]	10032	- [1]	10062	
10003	ND [2]	10033		10063	
10004	ND [2]	10034		10064	[6]
10005	ND [2]	10035		10065	
10006	ND [2] [6]	10036	[6]	10066	
10007	ND [2]	10037	ND [2] [7]	10067	
10008	ND [2] [6]	10038	— [6]	10068	
10009	ND [2]	10039	ND [2]	10069	
10010	ND [2]	10040	- [1]	10070	
10011	ND [2]	10041	- [1]	10071	
10012	ND [2]	10042	- [1]	10072	[6]
10013	ND [2]	10043	— [6]	10073	
10014	ND [2]	10044	ND [2]	10074	
10015	ND [2]	10045	[7]	10075	
10016	ND [2]	10046	[6]	10076	
10017	ND [2]	10047	[7]	10077	
10018	ND [2]	10048	- [1]	10078	
10019	ND [2]	10049	—	10079	
10020	ND [2]	10050	- [1]	10080	
10021	ND [2]	10051	— [6]	10081	
10022	ND [2]	10052	ND [2] [5]	10082	
10023	ND [2]	10053	[3] [7]	10083	[3]
10024	ND [2] [7]	10054	[5]	10084	
10025	— [8]	10055	[3]	10085	[6]
10026	ND [2] [7]	10056	ND [2] [5]	10086	[3]
10027	ND [2] [6]	10057	ND [2] [5]	10087	[3]
10028	— [8]	10058	[6]	10088	ND [2] [3]
10029	ND [2] [6]	10059	[6]	10089	[
10030	— [1]	10060	[6]	10090	[

b(4)

-: No sample, ND: <1.95 ng/mL × 500 = 975 ng/mL,

[1] Unscheduled death, [2] The dilution of the sample is ×500.

[3] The dilution of the sample is ×5000., [4] The dilution of the sample is ×50000.

[5] Animals that aborted their conceptuses (Between Days 17 and 19 of gestation)

[6] Animals that did not deliver (corresponding to Day 23 of gestation)

[7] Dams in which total litter losses occurred (between Days 0 and 3 after delivery)

[8] Animals used for delayed-type hypersensitivity (DTH) reaction (8 days after the final dosing)

The above data indicated an increase in the exposure with time possibly due to the accumulation of the drug. There was variability of exposure on day 15 at 50 mg/kg compared to other observation points. The exposure at 15 mg/kg continued to decline over the time. This could be due to the clearance of MR16-1 by anti-product antibody. However, exposure to animals during the treatment period at 50 mg/kg was higher than that observed at 15 mg/kg.

It was concluded that F0 dams were exposed to MR16-1 both at 15 and 50 mg/kg.

2. Determination of MR16-1 concentration in mouse plasma for "A study for effects of MR16-1 on fertility and early embryonic development to implantation by intravenous administration in male mice".

MR16-1 concentrations in the mouse plasma was determined in satellite animals. Samples were collected from 3 animals in the control group at 0.5 hr post dose on day 1, 63 and 75 from 3 animals at each time point. Blood samples were collected at each of 0.5, 24 and 72 hour post dose from 3 animals/time point at 15 and 50 mg/kg on day 1, 63 and 75 of dosing. Blood samples were also collected from the surviving animals at necropsy for the determination of plasma MR16-1. The assay was conducted as indicated for the pre- and postnatal developmental study reviewed above. The area under the curve of plasma concentration was determined.

Results of the study showed no detectable levels of MR16-1 in the plasma from control animals. MR16-1 concentrations in the satellite animals at 15 and 50 mg/kg are shown below from the applicant's table.

Table 2 MR16-1 Concentrations (Satellite group)

Dose (mg/kg)	Time (h)	MR16-1 concentration (μg/mL)					
		First day of dosing		Day 63 of dosing		Day 75 of dosing	
		Animal No.	Conc.	Animal No.	Conc.	Animal No.	Conc.
15	0.5	10190		10199		10208	-[1]
		10191		10200		10209	-[1]
		10192		10201		10210	
	Mean		265		494		
	SD		55		235		NC
	24	10193		10202		10211	
		10194		10203		10212	
		10195		10204		10213	
	Mean		85.9		378		195
	SD		7.7		158		105
	72	10196		10205		10214	
		10197		10206		10215	
		10198		10207	-[1]	10216	
	Mean		47.9		484		234
	SD		3.8		NC		243
50	0.5	10217		10226		10235	
		10218		10227		10236	
		10219		10228		10237	
	Mean		857		2220		1770
	SD		109		120		170
	24	10220		10229		10238	
		10221		10230		10239	
		10222		10231		10240	
	Mean		296		1650		1060
	SD		52		220		350
	72	10223		10232		10241	
		10224		10233		10242	
		10225		10234		10243	
	Mean		230		1350		896
	SD		5		280		357

b(4)

-: No sample, ND: <1.95 ng/mL × 500 = 975 ng/mL

[1] Unscheduled death

[2] The dilution of the sample is ×500.

[3] The dilution of the sample is ×5000.

[4] The dilution of the sample is ×50000

The calculated exposure data for MR16-1 from the satellite group are shown below from the applicant's table.

Table 3 MR16-1 Toxicokinetic Parameters (Satellite group)

Dose (mg/kg)	Parameters		MR16-1 concentration (µg/mL)		
			First day of dosing	Day 63 of dosing	Day 75 of dosing
15	$C_{0.5h}$	Mean	265	494	581
		SD	55	235	NC
	$AUC_{(0\text{-}72h)(\mu g\cdot h/mL)}$		6925.2	30892.1	18721.5
50	$C_{0.5h}$	Mean	857	2220	1770
		SD	109	120	170
	$AUC_{(0\text{-}72h)(\mu g\cdot h/mL)}$		25173.0	117456.1	79819.0

NC: Not calculated

Animals in the main group also showed measurable levels of MR16-1 as shown from the applicant's table below.

Table 4 MR16-1 Concentrations (Main groups)

MR16-1 concentration (μg/mL)					
0 mg/kg		15 mg/kg		50 mg/kg	
Animal No.	Conc.	Animal No.	Conc.	Animal No.	Conc.
10001	ND [2]	10061	—	10121	
10002	ND [2]	10062	ND [2]	10122	
10003	ND [2]	10063	—	10123	
10004	ND [2]	10064	- [1]	10124	
10005	ND [2]	10065		10125	
10006	ND [2]	10066		10126	
10007	ND [2]	10067		10127	
10008	ND [2]	10068	- [1]	10128	
10009	ND [2]	10069		10129	
10010	ND [2]	10070		10130	
10011	ND [2]	10071	- [1]	10131	
10012	ND [2]	10072		10132	
10013	ND [2]	10073		10133	
10014	ND [2]	10074		10134	
10015	ND [2]	10075		10135	
10016	ND [2]	10076		10136	
10017	ND [2]	10077		10137	
10018	ND [2]	10078		10138	
10019	ND [2]	10079		10139	
10020	ND [2]	10080		10140	
10021	ND [2]	10081		10141	
10022	ND [2]	10082	ND [2]	10142	
10023	ND [2]	10083		10143	
10024	ND [2]	10084		10144	
10025	ND [2]	10085	- [1]	10145	
10026	ND [2]	10086	- [1]	10146	
10027	ND [2]	10087	- [1]	10147	
10028	ND [2]	10088	—	10148	
10029	ND [2]	10089	- [1]	10149	
10030	ND [2]	10090	—	10150	

b(4)

-: No sample, ND: <1.95 ng/mL × 500 = 975 ng/mL

[1] Unscheduled death

[2] The dilution of the sample is ×500.

[3] The dilution of the sample is ×5000.

[4] The dilution of the sample is ×50000.

Above data show that detectable levels of MR16-1 were present at the end of the treatment period in 15 and 50 mg/kg groups. The level was increased with the dose and accumulation of MR16-1 was not evident within 72 hours. However, a peak level was noted at 24 hours postdose. The exposure data showed variability with respect to the sampling day. Overall, the treatment provided exposure to animals for the determination of the impact of the treatment in the sperm function and pregnancy.

3. A study of MR16-1 concentrations in mouse plasma for "A study for effects of MR16-1 on fertility and early embryonic development to implantation by intravenous administration in female mice.

Methods of the study were discussed in the individual study section. Briefly, female mice were treated at 15 and 50 mg/kg/IV during premating, mating and gestation day 6. Blood samples (0.6 mL) were collected at 0.5, 24 and 72 hours post dose on days 1, 12 and last day of dosing from 3 animals/time point from satellite groups. Blood samples were collected at terminal sacrifice also. Blood samples were collected from vena cava under isoflurane anesthesia. Animals were exsanguinated after the collection of samples. Plasma MR16-1 was determined using ELISA methods. Plasma AUC was calculated from the plasma concentration vs. time data. The average exposure to MR16-1 is shown below from the applicant's table.

Dose (mg/kg)	Parameters		MR16-1 concentration (µg/mL)		
			First day of dosing	Day 12 of dosing	Final day of dosing
15	$C_{0.5h}$	Mean	199	415	256
		SD	51	81	111
	$AUC_{(0-72h)}$ (µg·h/mL)		6710.2	10816.5	10537.6
50	$C_{0.5h}$	Mean	586	1190	1240
		SD	143	204	431
	$AUC_{(0-72h)}$ (µg·h/mL)		28832.6	76733.7	59863.4

Data indicate that exposure to MR16-1 was increased with the dose. However, exposure at 50 mg/kg was not dose proportionate (non-linear). The mean trough level at 15 and 50 mg/kg was 21.6 and 331 ug/mL, respectively. The applicant indicated that plasma MR16-1 levels at terminal sacrifice was below the level of quantitation (below 975 ng/mL) in 75% and 11% animals at 15 and 50 mg/kg, respectively. These data indicate that sufficient animals at 50 mg/kg were exposed to MR16-1 during the embryonic phase. However, there was variability in the exposure at the end of dosing period.

Other Pharmacokinetic Studies

1. Determination of anti-MR16-1 antibodies in mouse plasma for "A study for effects of MR16-1 on pre- and postnatal development, including maternal function by intravenous administration."

The antibody to MR16-1 was measured in mice for the segment 3 reproductive safety study. Detail methods were discussed in the review for individual study. Briefly, female mice were treated with MR16-1 intravenously from gestation day 6 to post labor day 21. Animals in the satellite groups were used for the plasma anti-MR16-1 antibody titer levels. Blood samples (0.4 to 0.6 mL) were taken from 4 animals/time point/ dose on day 6 of gestation from satellite animals. Due to deaths in animals at 15 mg/kg, blood samples were collected from 3-4 animals at each time point on gestation day 15. Different animals were used for the blood collection at a different time points. Blood samples were collected from 1-4 animals on the final day (lactation day 21) from satellite animals. Blood samples were also collected from all animals at terminal sacrifice for the determination of anti-MR16-1 antibody titers. Samples were collected from the abdominal vena cava under isoflurane anesthesia.

The anti-MR16-1 antibodies were assayed by a validated ELISA using rabbit anti-MR16-1 antibody as a positive control and biotin labeled MR16-1 as detection antibody. One hundred fold pooled mouse plasma was used for the negative control.

Results:

Satellite animals:

Control animals from satellite groups did not show any detectable levels of anti-MR16-1 antibody on day 6, 15 and final day of dosing. Detectable level of anti-MR16-1 antibody was present in the satellite group at 15 mg/kg at terminal sacrifice on day 21 of lactation for animal #10133 and 10138 as shown from the applicant's table below.

Table 2 Antibody titer of anti-MR16-1 antibody in mouse plasma (Satellite group)

Dose (mg/kg)	Time (h)	Anti-MR16-1 antibody					
		Day 6 of gestation		Day 15 of gestation		Final day of dosing	
		Animal No.	Titer	Animal No.	Titer	Animal No.	Titer
15	0.5	10103	ND	10115	ND	10127	- [1]
		10104	ND	10116	ND	10128	- [1]
		10105	ND	10117	ND	10129	ND [*2]
		10106	ND	10118	- [1]	10130	ND
	24	10107	ND	10119	ND	10131	- [1]
		10108	ND	10120	ND	10132	ND [*2]
		10109	ND	10121	ND	10133	—
		10110	ND	10122	ND	10134	- [1]
	72	10111	ND	10123	ND	10135	- [1]
		10112	ND	10124	ND	10136	ND
		10113	ND	10125	ND	10137	ND
		10114	ND	10126	ND [2]	10138	—
50	0.5	10139	ND	10151	ND	10163	ND
		10140	ND	10152	ND	10164	ND
		10141	ND [2]	10153	ND	10165	ND
		10142	ND	10154	ND	10166	ND
	24	10143	ND	10155	ND	10167	ND
		10144	ND	10156	ND	10168	ND
		10145	ND	10157	ND	10169	ND
		10146	ND	10158	ND	10170	ND
	72	10147	ND	10159	ND	10171	ND
		10148	ND	10160	ND	10172	ND [*2]
		10149	ND [2]	10161	ND	10173	ND
		10150	ND [2]	10162	ND	10174	ND
Cut off (Abs.)		0.152		0.163		0.128 0.191 *	

b(4)

b(4)

ND: Not detected (<Cut off)

-: No sample

* Cut off of Nos.10129, 10132 and 10172

[1] Unscheduled death

[2] Not pregnant

The above data from 36 satellite animals at 15 mg/kg showed 5 deaths (one non-pregnant). Among 30 surviving pregnant mice, only two mice showed positive anti-product antibody

titers at the end of dosing period. However, no anti product antibody was detected at 50 mg/kg dose in pregnant satellite mice.

Main groups:

Data from the main group at the end of dosing period reflected a similar trend. Each of 15 and 50 mg/kg dose groups had 30 animals. Six pregnant animals at 15 mg/kg died and only 3 of the surviving animals showed a positive anti-product antibody response. However, all animals at 50 mg/kg survived and no anti-product antibodies were detected at the end of dosing period. Data from the applicant's table are shown below.

Table 3 **Antibody titer of anti-MR16-1 antibody in mouse plasma (Main groups)**

Anti-MR16-1 antibody						
0 mg/kg		15 mg/kg		50 mg/kg		
Animal No.	Titer	Animal No.	Titer	Animal No.	Titer	b(4)
10001	ND [3]	10031	—	10061	ND	
10002	ND	10032	- [1]	10062	ND	
10003	ND	10033	ND	10063	ND	
10004	ND	10034	ND	10064	ND [3]	
10005	ND	10035	ND	10065	ND	
10006	ND [3]	10036	ND [3]	10066	ND	
10007	ND	10037	ND [4]	10067	ND	
10008	ND [3]	10038	ND [3]	10068	ND	
10009	ND	10039	ND	10069	ND	
10010	ND	10040	- [1]	10070	ND	
10011	ND	10041	- [1]	10071	ND	
10012	ND	10042	- [1]	10072	ND [3]	
10013	ND	10043	ND [3]	10073	ND	b(4)
10014	ND	10044	—	10074	ND	
10015	ND	10045	ND	10075	ND	
10016	ND	10046	ND [3]	10076	ND	
10017	ND	10047	ND [4]	10077	ND	
10018	ND	10048	- [1]	10078	ND	
10019	ND	10049	ND	10079	ND	
10020	ND	10050	- [1]	10080	ND	
10021	ND	10051	ND [3]	10081	ND	
10022	ND	10052	ND [2]	10082	ND	
10023	ND	10053	ND [4]	10083	ND [5]	
10024	ND [4]	10054	ND [2]	10084	ND	b(4)
10025	ND [5]	10055	ND [5]	10085	ND [3]	
10026	ND [4]	10056	—	10086	ND [5]	
10027	ND [3]	10057	ND [2]	10087	ND [5]	
10028	ND [5]	10058	ND [3]	10088	ND [5]	
10029	ND [4]	10059	ND [3]	10089	ND [5]	
10030	ND [5]	10060	ND [3]	10090	ND [5]	

ND: Not detected (<Cut off), -: No sample, [1] Unscheduled death

[2] Animals that aborted their conceptuses (Between Days 17 and 19 of gestation)

[3] Animals that did not deliver (corresponding to Day 23 of gestation)

[4] Dams in which total litter losses occurred (between Days 0 and 3 after delivery)

[5] Animals used for delayed-type hypersensitivity (DTH) reaction (8 days after the final dosing)

2. Anti-MR16-1 antibody analytical report for "A study for effects of MR16-1 on fertility and early embryonic development to implantation by intravenous administration in male mice."

Anti-MR16-1 was assayed from the samples collected for MR16-1 assay from satellite animals at 0.5, 24 and 72 hours post dose on days 1, 63 and 75. AntiMR16-1 was also assayed at terminal sacrifice from the main group animals. Details of the method used for anti-MR16-1 assay was similar to that used for the segment 3 reproductive safety study discussed above.

Anti-MR16-1 titers in the satellite groups are shown from the applicant's table below.

Table 2 Antibody titer of anti-MR16-1 antibody in mouse plasma (Satellite group)

Dose (mg/kg)	Time (h)	Anti-MR16-1 antibody					
		First day of dosing		Day 63 of dosing		Day 75 of dosing	
		Animal No.	Titer	Animal No.	Titer	Animal No.	Titer
15	0.5	10190	ND	10199	ND	10208	-[1]
		10191	ND	10200	ND	10209	-[1]
		10192	ND	10201	ND	10210	ND
	24	10193	ND	10202	ND	10211	ND
		10194	ND	10203	ND	10212	ND
		10195	ND	10204	ND	10213	ND
	72	10196	ND	10205	ND	10214	ND
		10197	ND	10206	ND	10215	ND
		10198	ND	10207	-[1]	10216	ND
50	0.5	10217	ND	10226	ND	10235	ND
		10218	ND	10227	ND	10236	ND
		10219	ND	10228	ND	10237	ND
	24	10220	ND	10229	ND	10238	ND
		10221	ND	10230	ND	10239	ND
		10222	ND	10231	ND	10240	ND
	72	10223	ND	10232	ND	10241	ND
		10224	ND	10233	ND	10242	ND
		10225	ND	10234	ND	10243	ND
Cut off (Abs.)		0.205		0.123		0.117	

ND: Not detected (<Cut off)

-: No sample

[1] Unscheduled death

Anti-MRA titers were not detected at 15 and 50 mg/kg in the satellite animals.

Anti-MR16-1 titers in the main group animal are shown from the applicant's table below.

Table 3 **Antibody titer of anti-MR16-1 antibody in mouse plasma (Main groups)**

Anti-MR16-1 antibody					
0 mg/mL		15 mg/mL		50 mg/mL	
Animal No.	Titer	Animal No.	Titer	Animal No.	Titer
10001	ND	10061	ND	10121	ND
10002	ND	10062	—	10122	ND
10003	ND	10063	ND	10123	ND
10004	ND	10064	- 1)	10124	ND
10005	ND	10065	ND	10125	ND
10006	ND	10066	ND	10126	ND
10007	ND	10067	ND	10127	ND
10008	ND	10068	- 1)	10128	ND
10009	ND	10069	ND	10129	ND
10010	ND	10070	ND	10130	ND
10011	ND	10071	- 1)	10131	ND
10012	ND	10072	ND	10132	ND
10013	ND	10073	ND	10133	ND
10014	ND	10074	ND	10134	ND
10015	ND	10075	ND	10135	ND
10016	ND	10076	ND	10136	ND
10017	ND	10077	ND	10137	ND
10018	ND	10078	ND	10138	ND
10019	ND	10079	ND	10139	ND
10020	ND	10080	ND	10140	ND
10021	ND	10081	ND	10141	ND
10022	ND	10082	—	10142	ND
10023	ND	10083	ND	10143	ND
10024	ND	10084	ND	10144	ND
10025	ND	10085	- 1)	10145	ND
10026	ND	10086	- 1)	10146	ND
10027	ND	10087	- 1)	10147	ND
10028	ND	10088	ND	10148	ND
10029	ND	10089	- 1)	10149	ND
10030	ND	10090	ND	10150	ND

b(4)

b(4)

ND: Not detected (≤Cut off)

-: No sample

Anti-MR16-1 antibodies were not detected at terminal sacrifice in the main group animals at 15 and 50 mg/kg except, male animals #10062 and #10082 at 15 mg/kg showed positive titers for anti-MRA16-1. These two animals also did not show MR16-1 possibly due to neutralization/clearance.

Mating performance data showed #10062 did not mate. However, mating and pregnancy of copulated female was confirmed for #10082.

Above data on anti-MR16-1 suggest that neutralization of MR16-1 did not occur in most of the male mice and male mice were exposed to MR16-1 sufficiently to determine the effect of MR16-1 for male fertility.

3. Determination of anti-MR16-1 antibodies in mouse plasma for "A study for effects of MR16-1 on fertility and early embryonic development to implantation by intravenous administration in female mice."

The study protocol is discussed for the individual study report and the review for plasma MR16-1 level determination. Female mice were treated at 15 and 50 mg/kg/IV Q3 days for 14 days before mating, during mating, and 6 days post mating. Animals were sacrificed on gestation day 13. Plasma samples were collected from satellite animals on day 1, 12 and the last day of dosing from satellite groups at several time points. A final post dose sample was collected at terminal sacrifice from the main group animals for anti-MR16-1 assay. Anti-MR16-1 was assayed by a validated ELISA method. Negative control was diluted pooled mouse plasma and positive control was rabbit anti-MR16-1 antibody.

Control animals did not show any antibody to MR16-1. Data for animals treated at 15 and 50 mg/kg are shown from the applicant's table below.

Dose (mg/kg)	Time (h)	Anti-MR16-1 antibody					
		First day of dosing		Day 12 of dosing		Final day of dosing	
		Animal No.	Titer	Animal No.	Titer	Animal No.	Titer
15	0.5	10208	ND	10216	ND	10225	ND
		10209	ND	10217	ND	10226	ND
		10210	ND	10218	ND	10227	ND
						10228	ND
	24	10207	ND	10219	ND	10229	ND
		10211	ND	10220	ND	10230	ND
		10212	ND	10221	ND	10231	- [1]
						10232	ND
	72	10213	ND	10222	ND	10233	ND
		10214	ND	10223	ND	10234	ND [2]
		10215	ND	10224	- [1]	10235	ND
						10236	- [1]
50	0.5	10249	ND	10258	ND	10267	ND
		10250	ND	10259	ND	10268	ND
		10251	ND	10260	ND	10269	ND
						10270	ND
	24	10252	ND	10261	ND	10271	ND
		10253	ND	10262	ND	10272	- [1]
		10254	ND	10263	ND	10273	ND
						10274	ND [2]
	72	10255	ND	10264	ND	10275	ND [2]
		10256	ND	10265	ND	10276	ND
		10257	ND	10266	ND	10277	ND
						10278	ND
Cut off (Abs.)		0.160		0.196		0.126	

ND: Not detected (<Cut off)

-: No sample

[1] Unscheduled death

[2] Not pregnant

Above data essentially show findings similar to other reproductive safety studies in mice. No anti-MR16-1 was detected during and at the end of approximately 30 days (10 injections) in pregnant mice.

4. MR16-1: Placental transfer study of MR16-1 in mice, study # PBC036-072, study initiation date Feb 20, 2009.

Plasma levels of MR16-1 and anti-MR16-1 were determined in F0 animals in the pre- and postnatal reproductive safety study in mice. In the present study, transport of the monoclonal

antibody (MR16-1) across the placenta was determined. These data would provide insights into the role of MR16-1 in F1 generation of mice during the development.

Pregnant mice were injected with MR16-1 at 50 mg/kg to separate groups on gestation days 11, 15 and 17. MR16-1 levels in the plasma of F0 animals and fetuses were determined on gestation day 18. Therefore, the duration between the injection of drug and collection of blood samples varied. The dose groups are shown from the applicant's table below.

11.3.6 Group Constitution

Group	Day of gestation at dosing	Sampling point	Number of animals (Animal number)
1	Day 11 of gestation	7 days post dose	3 (101, 104 and 105)
2	Day 15 of gestation	3 days post dose	3 (202 to 204)
3	Day 17 of gestation	1 day post dose	3 (301 to 303)

The treatment was given by intravenous injections into the tail vein. Animals were anesthetized by isoflurane at terminal sacrifice and blood samples were collected from the abdominal vein. Animals were sacrificed by exsanguination and fetuses were removed. Blood samples from 3 fetuses per F0 pregnant mice were collected and pooled. Fetal blood samples were collected from an incision in the neck. Fetuses were sacrificed by exsanguination. Plasma levels of MR16-1 were determined by ELISA using techniques similar to other reproductive studies.

Table 1 MR16-1 concentrations in plasma of pregnant mice and fetuses and F/P ratios on day 18 of gestation after single intravenous administration of MR16-1 at 50 mg/kg on day 11, 15 or 17 of gestation

Time after dosage (day)	MR16-1 concentration (ng/mL)		F/P ratio
	Pregnant	Fetus	
1	94,533 ± 21,321	36,300 ± 23,106	0.36 ± 0.16
3	ND	53,067 ± 3,635	NC
7	ND	1277 ± 1158	NC

NC: Not calculated

ND indicate that plasma concentrations of 2 or 3 of 3 animals were not detected (ND: <975 ng/mL).

Data are the mean ± SD of the results from three animals.

Above data show that MR16-1 transported across the placenta within 24 hours after the injection in pregnant mice, continued to accumulate in the fetus up to 3 days after the injection and started to recede. Above data on day one is lower than 343 ug/mL observed in the segment 3 study at 24 hour post dose. Since the large molecule does not distribute to tissues unlike the small molecule, this transplacental transport seems to be a clearance process for

MR16-1 from the maternal blood in the pregnant animals. The data also signify that transplacental transport of IgG to the fetuses took place in the mid gestation and embryonic stage of fetal development in the mice model. However, a similar data was not available for the primate model for tocilizumab that was reviewed for the original BLA application.

There was no anti-MR16-1 antibody detected in the plasma in the pregnant mice and their fetuses at 7-day post dose. Results of the study are shown from the applicant's submission below.

Table 2 Anti-MR16-1 antibody titers in plasma of pregnant mice and fetuses on day 18 of gestation after single intravenous administration of MR16-1 at 50 mg/kg on day 11 of gestation

Pregnant

Time after dosage (day)	MR16-1 antibody titer		
7	ND *(101)*	ND *(104)*	ND *(105)*

Fetus

Time after dosage (day)	MR16-1 antibody titer		
7	ND *(101)*	ND *(104)*	ND *(105)*

ND: Not detected (< cut off absorbance)

Figures in italic show animal number.

5. MR16-1: Milk excretion study of MR16-1 in mice, study # PCB036-073,

Pregnant mice at 10-11 weeks of age allotted to the study. Pregnant mice were allowed to deliver and excretion of MR16-1 in the milk was monitored during post-partum days 5 to 6. The study design is shown below.

11.3.6 Group Constitution

Group	Sample	Sampling point	Number of animals (Animal number)
1	Plasma and milk	Before dosing, 1, 3 and 7 days post dose	5 (101 to 105)

Mice were injected at 50 mg/kg by a single intravenous injection into the tail vein. Blood samples were collected after isoflurane anesthesia from the saphenous vein. Following the blood collection, animals were treated with oxytocin intravenously at 0.05 mL/body and milk was collected from nipples. Plasma and milk levels of MR16-1 were determined. The applicant stated that blank blood and milk samples were collected from untreated animals for comparison. Samples were diluted appropriately for the determination of MR16-1 by ELISA.

Anti-MR16-1 antibodies were also determined in the plasma and milk before dosing and at 7 day post dose. Data taken from the applicant's table are presented below.

Table 1 **MR16-1 concentrations in plasma and milk of lactating mice and M/P ratios after single intravenous administration of MR16-1 at 50 mg/kg on days 5 or 6 after parturition**

Time after dosage (day)	MR16-1 concentration (ng/mL)		M/P ratio
	Plasma	Milk	
Before dosing	ND	ND	NC
1	237,200 ± 53,728	65,700 ± 16,748	0.28 ± 0.03
3	44,520 ± 10,244	11,008 ± 3910	0.24 ± 0.05
7	ND	ND	NC

NC: Not calculated

ND: Not detected (plasma: <975 ng/mL, milk: <390 ng/mL)

Data are the mean ± SD of the results from five animals.

Above data suggest that MR16-1 was excreted in the milk of lactating mice. However, there was no anti-MR16-1 was detected in the plasma or milk in mice following the single injection.

Discussion and Conclusions of PK data and anti-MR16-1 data:

Analytical data showed antibody production at the end of the treatment only at 15 mg/kg in about 8% F0 surviving pregnant mice in the pre- and postnatal developmental toxicology study. The duration of treatment was about 36 days and anti-product antibody could have neutralized MR16-1. There were no data for the antibody production in the dead animals. F0 mice treated at 50 mg/kg showed adequate exposure to MR16-1 and well tolerated. MR16-1 cleared slowly at 50 mg/kg compared to that at 15 mg/kg resulting higher exposure to 50 mg/kg dose when compared to 15 mg/kg.

The anti-product antibody titers were also determined in male mice treated for about 75 days in the fertility and early embryonic development study. Although the duration of treatment in this study was longer than that for the segment 3 study, anti-product antibody was observed only in 2 animals at 15 mg/kg (surviving animals). No anti-product antibody was noted at 50 mg/kg and adequate exposure to MR16-1 was noted at both 15 and 50 mg/kg doses. It seems immunosuppressive nature of MR16-1 regulated the anti-product antibody formation so that a longer duration of treatment prevented anti-MR16-1 antibody formation both at 15 and 50 mg/kg. In a separate study in which female mice were treated at 15 and 50 mg/kg for approximately 30 days (female fertility and early embryonic development study) also did not show anti-MR16-1 antibodies.

Although the anti-MR16-1 antibody neutralized MR16-1 in mice based on the plasma assay data, sufficient exposure to MR16-1 was noted in male and female mice so as to characterize the impact of the treatment on reproduction in mice.

MR16-1 was transported to the fetuses in pregnant mice based on the placental transfer data. Therefore, fetuses were exposed to MR16-1 during the early stage of development. However, no anti-MR16-1 was detected in the F0 dams or fetuses in the placental transfer study in mice. In a separate study, MR16-1 was shown to be excreted in the milk in lactating mice. No anti-MR16-1 was noted in the plasma or milk. These data support the view that MR16-1 was transported in the fetuses and excreted in the milk in pregnant rodents. **Data clearly signify and validate the role of IgG based monoclonal antibodies in the reproductive toxicity in rodents based on the investigation on the mouse IL-6 receptor antibody MR16-1.**

Carcinogenicity

The Complete Response submission addressed the non-approvable items related to justification that carcinogenicity studies were not feasible, as shown below from the action letter.

Complete Response Letter:

"You have not submitted the results of carcinogenicity studies with either tocilizumab or the homologus protein. The application states that the MR16-1 antibody is also not an appropriate reagent to be used in long-term carcinogenicity studies, as this antibody is a rat monoclonal anti-mouse IL6-R antibody and is considered to be immunogenic in long-term in vivo studies in mice. Submit data to support this statement in order to support your conclusion that this option for carcinogenicity assessment is not viable."

The applicant's response:

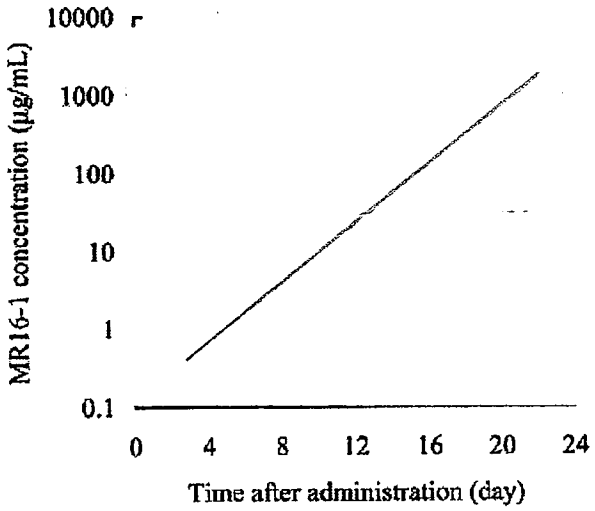
The applicant provided a chronology on the communication with the Agency that mentioned carcinogenicity waiver requests in the IND and BLA. The applicant provided published literature review in the BLA (reviewed for the original BLA application) to justify that carcinogenicity study for tocilizumab was not necessary because the applicant believed that IL-6 possessed anticancer activity. However, the applicant did not consider conducting carcinogenicity study using MR16-1 because the monoclonal antibody is not a drug substance, had selective affinity to interact with murine IL-6 receptor only and it would develop anti-MR16 antibodies.

The applicant stated in the Complete Response submission that the carcinogenicity study using MR16-1 is not an alternative option to the assessment of carcinogenicity to tocilizumab because MR16-1 is rat IgG based antibody and repeated injections to mice for the assessment of carcinogenicity would generate anti-MR16-1 antibodies in mice that would neutralize MR16-1 or develop further complication related to immunogenicity.

The applicant provided following information to support why MR16-1 is not a suitable surrogate for the carcinogenicity assessment.

1. MR16-1 would develop anti-MR16-1 formation the murine model that would interfere with the bioassay. Specifically the applicant stated, "Significant antibody response to MR16-1, leading to exposure depletion and loss of activity over time." For example, after a single IV dose in mice, clearance at 50 mg/kg showed variability and presence of an antibody to MR16-1 would deplete the surrogate. The applicant provided following figure to show clearance pattern in mice after a single IV injection.

Figure 1 Plasma clearance of MR16-1 after a single dose injection



b(4)

Figure 1 Plasma concentration of MR16-1 after single intravenous administration of MR16-1 to female mice at a dose of 5 mg/kg (■ No.1~5) or 50 mg/kg (□ No.6~10)

A similar trend was noted when MR16-1 was injected for about 5 months by intraperitoneal or subcutaneous (SC) route to NZB/W mice that is an experimental model of lupus erythematosus. Data from the applicant's table shown below would provide further information on the antibody titers at 0.5 mg/SC dose/week in mice for 25 weeks.

Table 1 anti MR16-1 antibody titers

Treatment Group	Animal No.	Weeks after first injection (units/ml) 1	6	10	15	21	25
No Treatment	1						
	2						
	3						
	4						
	5						
	Mean	0.07	0.09	0.18	0.34	0.46	1.54
	S.E.	0.02	0.04	0.05	0.09	0.20	1.05
MR16-1 2 mg i.v. (initial dose only) MR16-1 0.5 mg i.p. weekly	1						
	2						
	3						
	4						
	5						
	Mean	0.67	1.26	0.77	0.43	2.36	0.32
	S.E.	0.16	0.50	0.24	0.20	1.96	0.12
Saline i.v. (initial dose only) MR16-1 0.5 mg i.p. weekly	1						
	2						
	3						
	4						
	5						
	Mean	2.27	380.68	379.71	346.65	50.80	2.75
	S.E.	0.98	63.98	11.81	62.36	20.07	1.02
MR16-1 2 mg i.v. (initial dose only) MR16-1 0.5 mg s.c. weekly	1						
	2						
	3						
	4						
	5						
	Mean	0.93	0.78	0.97	1.04	6.58	3.73
	S.E.	0.35	0.34	0.32	0.36	5.55	3.02
		1	6	10	15	21	25
Saline i.v. (initial dose only) MR16-1 0.5 mg s.c. weekly	1						
	2						
	3						
	4						
	5						
	Mean	1.25	462.38	866.29	829.39	200.80	49.46
	S.E.	0.54	123.86	142.41	496.34	121.94	31.99

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Female NZB/NZW F1 mice (10 week-old) were divided to 5 groups as follows.
Group 1: non-treatment
Group 2: MR16-1 (2 mg) was injected i.v. and there after MR16-1 (0.5 mg) was injected i.p. weekly
Group 3: Saline was injected i.v. and there after MR16-1 (0.5 mg) was injected i.p. weekly
Group 4: MR16-1 (2 mg) was injected i.v. and there after MR16-1 (0.5 mg) was injected s.c. weekly
Group 5: Saline was injected i.v. and there after MR16-1 (0.5 mg) was injected s.c. weekly
In groups 3 and 5, MR16-1 injection was terminated at 11 weeks.
In groups 2 and 4, MR16-1 injection was continued by 25 weeks.

Above data showed a potential for developing anti-MR16-1 if an initial IV dose was not given to mice. It appears that the initial IV dose had an immunosuppressive effect that prevented antibody formation in mice.

Reviewer's Response:

The review of the BLA raised an issue whether inhibition of IL-6 pathway could lead to carcinogenic end points. Towards this goal, the Agency asked for a response from the applicant why they think that a long-term carcinogenicity study in mice would not be possible using the murine surrogate MR16-1.

The applicant provided data that demonstrated the anti-product antibody response to MR16-1 in mice was relatively at low doses (0.5 mg/sc/week). At high doses (15 and 50 mg/kg), the antibody response was minimal based on the available data discussed below.

The applicant submitted three reproductive safety studies (reviewed in this review) in mice at 15 or 50 mg/kg/3days/IV for a varied duration. Anti-MR16-1 antibody titers were determined in these studies as summarized.

Animal	Study	Sex	Dose, mg/kg/3 day/IV	Duration of treatment	Anti-MR16-1 antibody
CD-1 mice	Prenatal and post-natal development	Female	15 and 50	About 36 days	2/30 at 15 mg/kg; 0/30 at 50 mg/kg
CD-1 mice	Fertility	Male	15 and 50	About 75 days	2/30 at 15 mg/kg; 0/30 at 50 mg/kg
CD-1 mice	Fertility	Female	15 and 50 mg/kg	About 30 days	0/8 at 15 mg/kg; 0/12 at 50 mg/kg

Above data indicate that both at 15 and 50 mg/kg, male and female mice showed minimal antibody response unlike data presented by the applicant to justify waiver of carcinogenicity study of surrogate MR16-1. However, there were deaths at 15 mg/kg that could be due to anti-MR16-1 antibody formation. A definitive conclusion could not be reached for the cause of deaths due to a lack of data for antibody titers from animals succumbed to death. Comparison of data at 15 and 50 mg/kg suggest that immunosuppression could have prevented antibody formation to MR16-1 at 50 mg/kg. That raises an issue of survival of animals beyond 3 months of dosing. Therefore, either antibody response or the immunosuppressive action of MR16-1 has potential to lead to premature termination of carcinogenicity study. In this regard, long-term safety and further assessment of the potential for this product to alter tumor formation can only be assessed via clinical experience. Safety data provided by the medical reviewer showed that about 164 patients were exposed to tocilizumab for about 4 years so far. Additional post approval data would signify the long-term effect and carcinogenic potential of tocilizumab in the clinical setting. Therefore, further study in mice using MR16-1 for the determination of carcinogenic potential of tocilizumab is not recommended considering feasibility factors.

The reviewer concluded that the applicant met the requirement of addressing the non-approvable issue of the Complete Response. Therefore, further action is not necessary.

Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: MR16-1: A study for effects of MR16-1 on fertility and early embryonic development to implantation by intravenous administration in male mice

Key study findings: MR16-1 had no effect on the male fertility up to 50 mg/kg/IV.

Study no.: —.036-065
Volume #M4, and page #: 1
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: Dec 15, 2008
GLP compliance: Yes
QA reports: yes (X) no ( )
DrugMR16-1, lot # Z801J23 and MC45021, and % purity: 10.9 to 11.3 mg/mL by protein assay; HPLC purity 87.4% to 94.3%. The applicant provided stability data for 8 days in refrigerated storage conditions and 6 hours at room temperature using 1.5 and 11.3 mg/mL solutions prepared with phosphate buffered saline. The stability for lot # Z801J23 was 100.9 to 101.2%. A similar stability data were presented for 14-day storage conditions in the refrigerator. The applicant did not provide any reasons why the stability data were higher than 100%.

Methods

Doses: doses used were 15 and 50 mg/kg.

Species/strain: Crlj:CD1 mice, male weighed 30-42 g and female weighed 19-28 g. Male and female mice were 11 week and 5 week old, respectively, at procurement.

Number/sex/group: 30/sex/group

Route, formulation, volume, and infusion rate: The test and control article was injected intravenously into the tail vein at 10 mL/kg once every 3 days for 63 days before mating, male mice during 10 days of the mating and after 5 days till gross pathological examinations. Therefore, total dosing period was about 77 days.

Satellite groups used for toxicokinetics: A total of 63 mice were allotted to a satellite group. The control group had 9 males and other two groups had 27 male mice as shown from the applicant's table below.

Satellite Groups: 1 control group and 2 test article groups

Group	Test and Control Articles	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Males (Animal No.)
4	PBS[a)]	-	10	-	9 (10181 - 10189)
5	MR16-1[b)]	15	10	1.5	27 (10190 - 10216)
6	MR16-1[b)]	50	10	5	27 (10217 - 10243)

a) PBS was administered in the same manner as test article.

b) Test article from Lot No. Z801J23 was used for the first 4 doses and test article from Lot No. MC45021 was used from the fifth dose.

Study design: Treated male mice were mated with untreated female mice. Animals were housed one male per cage during the treatment period before the mating period and provided food and water ad lib. The study design is shown from the applicant's table below.

Main Groups: 1 control group and 2 test article groups

Group	Test and Control Articles	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals (Animal No.)	
					Males	Females [c]
1	PBS [a]	-	10	-	30 (10001 - 10030)	30 (10031 - 10060)
2	MR16-1 [b]	15	10	1.5	30 (10061 - 10090)	30 (10091 - 10120)
3	MR16-1 [b]	50	10	5	30 (10121 - 10150)	30 (10151 - 10180)

a) PBS was administered in the same manner as test article.

b) Test article from Lot No. Z801J23 was used for the first 4 doses and test article from Lot No. MC45021 was used from the fifth dose.

c) Non-treated

The applicant stated that in a previous study 5 mg/kg dose of MR16-1 suppressed anti-dinitro phenol antibody formation in mice. The high dose was selected at 10-fold higher so as to induce an optimal effect of the drug.

The clinical sign of the animal was recorded twice daily during the dosing days and once daily during the non-dosing period. The food consumption was recorded before dosing and on days 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51, 54, 57 and 60. The food intake was measured from the difference between the amount given and the amount left before the measurement. The daily food consumption was calculated per animal basis. Gross pathology of the treated male mice was conducted for external appearance and internal organs on the treatment day 76 or 77 following isoflurane anesthesia and euthanasia. Blood samples were collected for MR16-1 and its antibody measurements. The organ weight for testes and epididymis was recorded. Testes were fixed in Bouin's fixation medium and epididymis was fixed in buffered formalin. These tissues from all main group animals were stained with H&E stain for histological examinations. The applicant stated that there was no test article related abnormality in any other visceral organs and these organs were not fixed and processed for histological examinations.

Parameters and endpoints evaluated: At necropsy, sperm samples were collected from the right caudal epididymis of ten lowest numbered surviving males from each group and placed into M199 culture medium . Evidence of motility was detected under the microscope to determine of live sperm. Sperms without any motility were counted as dead sperms. The sperm activity was classified by 0 to 4 scores on the basis of the movement as shown from the applicant's table below.

Score	Criteria for the classification of sperm activity
4	Most sperms in 1 visual field very actively moving forward
3	Most sperms in 1 visual field actively moving forward
2	More than half of the sperms moving and gradually moving forward
1	Many non-motile sperms and active sperms moving in a circular or pendulum-like motion
0	Very few active sperms (several active sperms in 1 visual field) or no active sperms
-	No sperm

The sperm count was conducted using a hemocytometer.

Treated male mice were paired with untreated female mice for mating from day 63 of dosing on one to one basis. Mating was confirmed from the presence of sperm in the vaginal smears and the first incidence was considered as gestation day 0. Animals were paired for a maximum of 10 days.

Following index was calculated:

Sperm Viability Index = # live sperm / # live and dead sperms x 100

Total sperm counts in caudal epididymis = mean count/compartment x 16 x 10^4 x suspension volume = n x 10^6/epididymis

Sperm count/mg of caudal epididymis = Total sperm count in caudal epididymis / Removed caudal epididymis weight (mg) x 10^2 = n x 10^4/ mg of epididymis

Abnormal Sperm Index = total count of abnormal sperm / total count of sperm x 100

Copulation Index = # of copulated males / # of paired males x 100

Fertility Index = # of pregnant female / # of copulated males x 100

Preimplantation Loss Index = # of corpora lutea - # of implantation / # corpora lutea x 100

Postimplantation Loss Index = # dead embryos / # implantations x 100

Viability Index for Fetus = # of live embryos / # implantations x 100

Female mice were sacrificed by carbon dioxide inhalation on gestation day 13. External appearance and internal organs were examined macroscopically. Uterus was removed to examine number of corpora lutea, live embryos, dead embryos and post-implantation loss. Live embryos, ovaries, uteri and left kidney (for mouse # 10179) were fixed in 10% buffered formalin and stored.

Male animals that died before the terminal sacrifice were examined for macroscopic changes of the internal organs and external appearance. Testes were fixed in Bouin's solution.

Epididymides, internal organs and brain were fixed in formalin and stored. The applicant's table showed following animals was found dead before the terminal sacrifice:

Group	Animals that died (Animal No.)
2	10064, 10068, 10071 10085, 10086, 10087 and 10089
5	10207, 10208 and 10209

Untreated Female mice:

Clinical signs were observed once a day, the body weight and food consumption was recorded once a week before cohabitation, gestation days 0, 3, 7, 10 and 13. Uncopulated females were sacrificed 13 days after mating period. The applicant stated that the uteri of mice that did not confirm implantation visually were treated with sodium hydroxide to confirm the implantation.

Toxicokinetics:

Blood samples (0.6 mL) were collected for plasma MR16-1 and its antibody determination on days 1, 63 and 75 at 0.5, 24 and 72 hour from 3 animals per time point from the satellite animals. Exposure to MR16-1 was determined from the area under the plasma drug concentration–time curve. Blood samples were also taken at necropsy from the main groups for the assay. Satellite animals were euthanized by exsanguination.

Results

Mortality: Seven male mice at 15 mg/kg died during the treatment period as shown in the table below.

Animal #	Day of death	Time after dose
10064	42	30 min
10068	24	40 min
10071	33	40 min
10085	60	20 min
10086	42	20 min
10087	15	40 min
10089	18	1-2 hours

Clinical signs: One male mouse at 15 mg/kg showed a decrease in the spontaneous activity within 2 hour post dose on day 15. No clinical sign was noted at 50 mg/kg.

Body weight: Male mice in the control and treated groups showed about 38 to 40 g average body weight during the treatment period and there was no treatment related change in the body weight gain.

Food consumption: The food consumption in the control and treated mice was about 6 g/day at the beginning of dosing and about 5.8 g/day at the end of the premating period. Therefore, the treatment had no effect on the food consumption in mice.

Toxicokinetics: Data are reviewed under the PK section.

Necropsy: Gross pathological examinations did not show any treatment related change in the male mice. The organ weight data for testes and epididymis also showed no treatment related change. Histopathological data in testes and epididymis at 15 and 50 mg/kg did not show any treatment related change. The sperm count and viability data are shown from the applicant's table below. The applicant did not provide histopathology data for dead animals.

Table 6 Sperm examination (mean ±S.D.) Study No. SBL036-065

Dose (mg/kg)	Control	15	50
No. of males	10	10	10
Sperm activity[a)]	3.00 ± 0.24	2.65 ± 0.75	2.70 ± 0.54
Sperm viability (%)	75.51 ± 4.86	67.56 ± 9.53	64.47 ± 13.30*
Weight of right caudal epididymis (mg) (A)	21.50 ± 3.22	20.84 ± 1.78	21.04 ± 1.72
Total sperm count in caudal epididymis ($\times 10^6$) (B)	4.76 ± 1.19	4.80 ± 2.49	6.48 ± 1.00*
Sperm count in caudal epididymis ($\times 10^6$/mg) (B/A)	22.07 ± 4.29	22.70 ± 11.73	30.83 ± 4.20**
Abnormal sperm (%)	5.95 ± 1.67	9.29 ± 5.76	4.42 ± 1.54

a): Mean of gradable data (grade score: 0 to 4)
* P<0.05, **<0.01: Significantly different from control

Individual sperm examination data at 15 and 50 mg/kg doses are shown from the applicant's table below.

Appendix 6-2 Sperm examination Study No. SBL036-065

Dose: 15 mg/kg

Animal No.	Sperm activity[a)]	Sperm viability (%) 1st	2nd	Mean	Weight of right caudal epididymis (mg) (A)	Total sperm count in caudal epididymis ($\times 10^6$) (B) 1st	2nd	Mean	Sperm count in caudal epididymis ($\times 10^4$/mg) (B/A)	Abnormal sperm (%) 1st	2nd	Mean
10061				79.4				6.1				4.4
10062				57.3				2.4				21.4
10063				76.0				6.4				8.4
10065				75.4				3.7				7.1
10066				55.4				2.2				8.9
10067				75.7				5.4				6.3
10069				55.0				0.5				18.0
10070				71.1				6.7				6.4
10072				69.8				6.1				4.1
10073				60.5				8.5				7.9
Mean	2.65	69.39	65.67	67.56	20.84	4.61	4.94	4.80	22.70	7.20	11.35	9.29
S.D.	0.75	11.26	8.82	9.53	1.78	2.31	2.76	2.49	11.73	4.66	7.09	5.76
n	10	10	10	10	10	10	10	10	10	10	10	10

b(4)

a): Mean of gradable data (grade score: 0 to 4)
4:Most sperms in 1 visual field very actively moving forward
3:Most sperms in 1 visual field actively moving forward
2:More than half of the sperms moving and gradually moving forward
1:Many non-motile sperms and active sperms moving in a circular or pendulum-like motion
0:Very few active sperms (several active sperms in 1 visual field) or no active sperms

No.10064,10086: Death on Day 42 of dosing
No.10068: Death on Day 24 of dosing
No.10071: Death on Day 33 of dosing
No.10085: Death on Day 60 of dosing
No.10087: Death on Day 15 of dosing
No.10089: Death on Day 18 of dosing

Appendix 6-3 Sperm examination Study No. SBL026-065

Dose: 50 mg/kg

Animal No.	Sperm activity[a]	Sperm viability (%) 1st	2nd	Mean	Weight of right caudal epididymis (mg) (A)	Total sperm count in caudal epididymis ($\times 10^6$) (B) 1st	2nd	Mean	Sperm count in caudal epididymis ($\times 10^6$/mg) (B/A)	Abnormal sperm (%) 1st	2nd	Mean
10121				69.8				7.7				3.0
10122				77.2				8.5				5.7
10123				71.9				5.8				2.0
10124				31.9				5.9				5.1
10125				68.0				5.8				3.6
10126				54.2				6.6				3.2
10127				74.0				6.1				5.0
10128				68.7				5.3				4.2
10129				59.1				6.0				7.3
10130				69.9				7.1				5.1
Mean	2.70	66.91	62.01	64.47	21.04	6.44	6.48	6.48	30.83	4.54	4.23	4.42
S.D.	0.54	11.99	16.05	13.30	1.72	0.78	1.61	1.00	4.20	1.93	1.40	1.54
n	10	10	10	10	10	10	10	10	10	10	10	10

a): Mean of gradable data (grade score: 0 to 4)
4:Most sperms in 1 visual field very actively moving forward
3:Most sperms in 1 visual field actively moving forward
2:More than half of the sperms moving and gradually moving forward
1:Many non-motile sperms and active sperms moving in a circular or pendulum-like motion
0:Very few active sperms (several active sperms in 1 visual field) or no active sperms

b(4)

Control male showed a lowest sperm viability of 65% and the lowest sperm activity of 2.5.

The sperm viability and sperm activity data at 15 mg/kg showed animal #10062, 10066 and 10069 had a slightly lower sperm function than the control.

The sperm viability at 50 mg/kg in animal #10124 was 31.9%, #10126 was 54% and #10129 was 59%. The sperm activity at 50 mg/kg for animal #10124 was 1.5%, #10126 was 2.0 and #10129 was 2.5%.

Considering the data, 3 out of 10 male mice had a lower sperm function at 15 and 50 mg/kg.

Above data showed that MR16-1 had an effect on sperm function. However, further examination of the effect of the treatment on sperm needs to be made on the basis of the fertility data.

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

The fertility data are shown from the applicant's table below.

Table 7 Mating performance

Dose : (mg/kg)		Control	15	50
No. of pairs used for mating	(a)	30	23	30
No. of pairs with successful copulation	(b)	28	22	29
Copulatory index (%)	(b/a)	93.3	95.7	96.7
Mean copulatory interval (days , mean±S.D.)		2.1±1.5	2.0±1.0	2.0±1.3
No. of fertile pairs	(c)	27	22	27
Fertility index (%)	(c/b)	96.4	100.0	93.1

Not significantly different from control

Some of the individual sperm activity and mating performance data are shown in the following table.

Animal #	Dose	Sperm activity	Sperm viability	Copulation	Pregnancy Status
10017	Control	Not known	Not known	Yes, day 3	Not Pregnant
10121	50 mg/kg			Yes, day 3	Pregnant
10123	50 mg/kg			Yes, day 3	Pregnant
10124	50 mg/kg			Yes, day 1	Not Pregnant
10126	50 mg/kg			Yes, day 1	Pregnant
10129	50 mg/kg			Yes	Pregnant
10137	50 mg/kg	Not known	Not known	Yes	Not Pregnant

b(4)

Most of the mated males at 15 and 50 mg/kg induced pregnancy.

Above data suggest that treatment did not impair the mating performance and fertility index in the treated animals compared to the control mice.

The data on implantation and fetal survival on gestation day 13 are shown from the applicant's table below.

No abnormality was observed in the untreated female mice when the body weight, food consumption and gross pathological changes were examined. One female mouse at 50 mg/kg showed dilatation of renal pelvis.

Table 8 Litter data on Day 13 of gestation (mean ± S.D.)

Dose (mg/kg)	Control	15	50
No. of dams	27	22	27
No. of corpora lutea (A)	15.4±2.7	15.8±3.7	15.7±2.5
No. of implantations (B)	13.4±3.2	14.3±4.2	14.5±2.3
Implantation rate (%) (B/A)	90.3±14.8	88.6±20.5	95.7±8.0
Preimplantation loss (%)	9.7±14.8	11.4±20.5	4.3±8.0
Embryo death			
No. of postimplantation loss (D)	1.6±1.7	0.8±0.8	1.2±1.6
Postimplantation loss (%) (D/B)	14.1±21.0	5.9±6.0	8.1±10.9
Implantation site (%)	3.7±19.2	0.0±0.0	0.0±0.0
Placental remnant (%)	10.1±12.3	5.9±6.0	7.8±11.0
Dead embryo (%)	0.3±1.5	0.0±0.0	0.3±1.5
Live embryo			
No. of live embryos (C)	11.9±4.1	13.5±4.1	13.7±2.8
Embryo viability (%) (C/B)	85.9±21.0	94.2±6.0	91.9±10.9

Not significantly different from control

Above data suggested that treatment at 15 and 50 mg/kg did not have any effect on the embryos of surviving animals.

Summary of the study:

Male rats were treated with MR16-1 at 15 and 50 mg/kg/IV for a period of about 77 days. Animals were mated with untreated female mice after 63 days of dosing. The treatment slightly reduced the sperm motility and viability at 15 and 50 mg/kg in 3 out of 10 male mice examined. However, fertility, pregnancy and early embryonic development were comparable with the untreated control.

Based on the data it was concluded that MR16-1 had no effect in the mating performance and fertility of male mice up to 50 mg/kg. The treatment rendered 6 deaths at 15 mg/kg. MR16-1 levels in the plasma confirmed exposure to the drug during the sperm maturation. Anti

MR16-1 antibody was noted in 2 out of 30 mice at 15 mg/kg. However, no anti-MR16-1 was noted at 50 mg/kg. It was concluded that MR16-1 had no effect on the male fertility up to 50 mg/kg.

Study title: MR16-1 : A study for effects of MR16-1 on fertility and early embryonic development to implantation by intravenous administration in female mice.

Key study findings: MR16-1 did not impair fertility in female CD-1 mice up to 50 mg/kg. Mortality was observed at 15 mg/kg.

Study no.: —036-066
Volume # EDR, and page #: 1
Conducting laboratory and location: [b(4)
]

Date of study initiation: Dec 15, 2008
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug: MR16-1, monoclonal antibody to mouse IL-6R raised in rats in OgG1 frame, lot #Z801J23 and % purity: 11.3 mg/mL using a protein assay kit and 87.8% by HPLC assay, the stability of dosing solutions were confirmed at 1.5 and 11.3 mg/mL nominal concentration under the experimental conditions.

Methods

Doses: 15 and 50 mg/kg

Species/strain: Crlj:CD1 (ICR) mice, male mice weighed 30 g to 42 g and female mice weighed 22.8 g to 34 g when the shipment was received.

Number/sex/group: The study design for the main group is shown below from the applicant's table.

Main Groups: 1 control group and 2 test article groups

Group	Test and Control Articles	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals (Animal No.)	
					Males [b]	Females
1	PBS [a]	-	10	-	30 (10001 - 10030)	30 (10031 - 10060)
2	MR16-1	15	10	1.5	30 (10061 - 10090)	30 (10091 - 10120)
3	MR16-1	50	10	5	30 (10121 - 10150)	30 (10151 - 10180)

a) PBS was administered in the same manner as test article.

b) Non-treated

The justification of dose selection was similar to that for other studies because doses for the study were similar to that for other reproductive safety studies. Female mice were treated every 3 days before mating for 14 days and during the mating period and up to day 6 of the gestation. The maximum mating period was 20 days. Therefore, total number of doses used in the study varied. The dosing frequency was also determined on the basis of previous studies and PK data.

Route, formulation, volume, and infusion rate: The test article or the vehicle (phosphate buffered saline) was injected intravenously into the tail vein once every 3 days. The dosing volume was 10 mL/kg and the injection was given at 2 mL/min.

Satellite groups used for toxicokinetics:

Satellite Groups: 1 control group and 2 test article groups

Group	Test and Control Articles	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals (Animal No.)	
					Males [b]	Females
4	PBS [a]	-	10	-	4 (10181 - 10184)	10 (10185 - 10194)
5	MR16-1	15	10	1.5	12 (10195 - 10206)	30 (10207 - 10236)
6	MR16-1	50	10	5	12 (10237 - 10248)	30 (10249 - 10278)

a) PBS was administered in the same manner as test article.

b) Non-treated. No blood was sampled from the males (used for mating only).

Blood samples (0.6 mL) were collected from abdominal vena cava under isoflurane anesthesia on the first day of dosing, day 12 of dosing and final day of dosing from satellite animals. Blood samples were collected at 0.5, 24 and 72 hour post dose from 3 animals/time point/ dose. Blood samples were collected only at 0.5 hour post dose from control animals. Plasma MR16-1 and anti-MR16-1 were determined by ELISA assay. Plasma AUC for MR16-1 was calculated from the plasma concentration data.

Blood samples were also taken at terminal sacrifice on gestation day 13 from the main animal for the determination of plasma MR16-1 and its antibody titers.

Study design: Untreated male and treated female mice were allowed to mate at 1:1 basis. Initial copulation period was 10 days. In the absence of copulation, the female mice were mated with a proven male from the same group for another 10 days. Animals were observed twice a day on the day of dosing and once a day during the study. The body weight and food consumption of female mice was recorded before the dosing and every 3 to 4 days. Daily food consumption was recorded from the difference between the food supplied and remained in the food bowl and calculated as the food consumption per day. The estrus cycle of the rat was monitored 2 weeks before mating, during the mating and up to the day of copulation. The presence of sperms was indicator of copulation and considered as the gestation day 0. Female mice were sacrificed under isoflurane anesthesia on day 13 of the gestation. The external appearance and internal organs were examined macroscopically for the presence of any change.

Parameters and endpoints evaluated: The time taken for copulation from the beginning of mating was recorded. At the terminal sacrifice on gestation day 13, the uterus was removed, number of corpora lutea and implantations were counted. The number of live and dead embryos were assessed.

Following parameters were calculated from the uterine data. Uteri were treated with 0.2% w/v sodium hydroxide to confirm implantations when implantation sites were not visible macroscopically.

Pre-implantation Loss Index = # of corpora lutea- # of implantations / # corpora lutea x 100

Postimplantation Loss Rate = # dead embryos / # of implantations x 100

Embryonic Viability Rate = # Live embryos / # implantations x 100

Implantation Index = #implantations / # of corpora lutea x 100

Copulation Index = # copulated female / # paired females x 100

Fertility Index = # pregnant females / # copulated females x 100

Ovaries and uteri were fixed in 10% formalin. The applicant stated that no other organs were preserved and histopathology data were not collected for any organ due to a lack of macroscopic changes in tissues.

Macroscopic changes for animals that died during the experiment were recorded. Uteri and ovaries were examined for implantations and corpora lutea. Thoraco-abdominal organs, brain, uterus and ovaries were fixed in formalin.

Male mice were sacrificed after mating by carbon dioxide inhalation and exsanguination. External surface and internal organs were examined macroscopically. The presence or absence of sperm was examined in the epididymis. Abnormal testes were fixed in formalin.

Results

Mortality: Following mortality data were provided in the applicant's table

Group	Animals that died (Animal No.)
2	10092, 10094, 10097, 10102, 10103, 10108, 10109, 10112 and 10119
4	10191
5	10224, 10231 and 10236
6	10272

The following table indicates clinical signs in dead animals among the main group. The tabular data from Appendix 1 showed that only one animal had loss of spontaneous activity among dead animals. Since multiple injections of MR16-1 were given before deaths, it is possible that the deaths could be due to immunogenic process of antigen antibody reactions. Deaths of animals at 15 mg/kg were also noted in other studies and it is not an unexpected findings for this study. The applicant referred these deaths as a result of immunoreactions to foreign protein. A positive immuno-reaction was also noted in passive cutaneous reactions in mice that supported this interpretation. In a separate study the applicant was observed deaths

to mice injected with MR16-1 intraperitoneally once a week for 6 weeks. However, data from other studies did not show increase titers of anti-MR16-1.

Animal	Dose	Died on	Time post dose	Clinical sign observed
10092	15 mg/kg	Gestation day 3	40 min post dose	
10094	15 mg/kg	Gestation day 3	40 min post dose	
10097	15 mg/kg	Gestation day 2	40 min post dose	Decrease spontaneous activity
10102	15 mg/kg	Dosing day 9 before copulation	30 min post dose	
10103	15 mg/kg	Dosing day 9 before copulation	30 min post dose	
10108	15 mg/kg	Gestation day 3	30 min post dose	
10109	15 mg/kg	Gestation day 1	30 min post dose	
10112	15 mg/kg	Dosing day 9 before copulation	30 min post dose	
10119	15 mg/kg	Gestation day 1	30 min post dose	

In addition to above deaths, animal #10272 at 50 mg/kg belonged to the satellite group died during early gestation following 8th dose.

Clinical signs: Decrease in the spontaneous activity was noted in one animal before copulation and in 2 animals during the gestation up to 2 hours after dosing at 15 mg/kg. The applicant indicated that the treatment did not show any abnormal clinical sign in any animal at 50 mg/kg. The average estrus cycle frequency was 2.4, 2.6 and 2.6 days in the control, 15 and 50 mg/kg, respective, and the duration was between 5 to 6 days. The treatment did not show an effect on the estrus cycle.

Body weight: The average body weight (g) of dams before mating and gestation period showed that there was no treatment related change. Data are shown below. Dead and non-pregnant animals were excluded in the table.

Treatment Day	Stage	Control	15 mg/kg	50 mg/kg
Day 0	Before mating	29.84, n=30	29.72, n=30	29.60, n=30
Day 12	Before mating	29.95, n=30	29.77, n=27	29.61, n=30
Day 15	Before mating	29.32, n=20	29.54, n=16	29.62, n=17
Day 0	Gestation	43.02, n=26	43.77, n=17	43.77, n=26
Day 13	Gestation	43.02, n=26	43.77, n=17	43.77, n=26

Food consumption: There was no treatment related change in the average food consumption (g/day) as shown in the table below. Data suggest that animals increased food consumption as expected during the pregnancy.

Treatment Day	Stage	Control	15 mg/kg	50 mg/kg
Day 1	Before mating	4.62, n=30	4.54, n=30	4.65, n=30
Day 13	Before mating	5.09, n=30	4.85, n=27	5.10, n=30
Day 1	Gestation	4.57, n=26	4.58, n=23	4.66, n=26
Day 12	Gestation	8.29, n=29	8.13, n=17	8.26, n=26

Toxicokinetics: Data are reviewed under the PK section.

Necropsy: Gross pathology data for female mice were normal for control, 15 and 50 mg/kg.

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

Following animals were non-pregnant

Female #	Dose	Copulatory interval, day	Male #
10036	Control	3	10001
10039	Control	1	10009
10041	Control	4	10011
10043	Control	1	10013
10095	15 mg/kg	1	10065
10100	15 mg/kg	6	10070
10120	15 mg/kg	2	10090
10160	50 mg/kg	1	10130
10170	50 mg/kg	3	10140
10171	50 mg/kg	1	10141
10175	50 mg/kg	1	10145

These data showed that the pregnancy index was similar within the control and treated groups. Therefore, the treatment did not have an effect on the mating performance. The copulatory index and fertility index are shown from the applicant's table below.

Table 6 Mating performance

Dose : (mg/kg)		Control	15	50
No. of pairs used for mating	(a)	30	27	30
No. of pairs with successful copulation	(b)	30	27	30
Copulatory index (%)	(b/a)	100.0	100.0	100.0
Mean copulatory interval (days , mean±S.D.)		3.0±2.7	2.1±1.2	2.8±2.6
No. of fertile pairs	(c)	26	24	26
Fertility index (%)	(c/b)	86.7	88.9	86.7

Not significantly different from control

TOX08-0190

The data on embryos on gestation day 13 are shown from the applicant's table below.

Table 7 Litter data on Day 13 of gestation (mean ± S.D.)

Dose (mg/kg) No. of dams	Control 26	15 18	50 26
No. of corpora lutea (A)	14.3±1.7	13.7±1.5	14.2±2.3
No. of implantations (B)	13.5±1.6	12.9±2.2	13.7±2.4
Implantation rate (%) (B/A)	95.0±5.6	94.0±11.2	96.4±6.8
Preimplantation loss (%)	5.0±5.6	6.0±11.2	3.6±6.8
Embryo death			
No. of postimplantation loss (D)	0.8±0.9	0.5±0.9	0.5±0.7
Postimplantation loss (%) (D/B)	5.8±7.1	4.4±7.0	3.2±4.8
Implantation site (%)	0.0±0.0	0.0±0.0	0.0±0.0
Placental remnant (%)	5.5±7.1	3.9±7.0	3.2±4.8
Dead embryo (%)	0.2±1.4	0.5±2.1	0.0±0.0
Live embryo			
No. of live embryos (C)	12.8±1.9	12.4±2.5	13.3±2.3
Embryo viability (%) (C/B)	94.2±7.1	95.6±7.0	96.8±4.8

Not significantly different from control

Average data did not show any treatment related change in the ovulation, implantation and number of live embryos.

Summary of the study: It is concluded that MR16-1 at 15 and 50 mg/kg/IV to female mice 2 weeks before mating through gestation day 13 did not affect mating performance and fertility. However, like other studies in mice, MR16-1 showed mortality to mice at 15 mg/kg.

Prenatal and postnatal development

Study title: MR16-1: A study for effects of MR16-1 on pre- and postnatal development, including maternal function by intravenous administration in mice

Key study findings: MR16-1 induced mortality to mice at 15 mg/kg/IVq3 days. No treatment related mortality was noted at 50 mg/kg/IVq3 days. No other reproductive abnormality was noted in F0 or F1 mice except some F1 mice born to dams treated with MR16-1 at 50 mg/kg showed a slight immunosuppression associated with a reduction in the peripheral blood cell counts, CD45 and CD49 markers without compromising their ability to induce antibodies to KLH antigen. The biological significance of immunosuppression is not known.

Study no. — 036-064 b(4)
Volume : Electronic submission and page #: 1

Conducting laboratory and location: [redacted] b(4)

Date of study initiation: Dec 8, 2008
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug: MR16-1, rat anti-mouse IL-6R monoclonal antibody (IgG1), **lot** #Z801J23, and % purity: 11.3 mg/mL as protein, purity was 87.4% by HPLC method. The test solution in phosphate buffer was stable for 14 days in the refrigerator followed by 6 hours at room temperature when protected from the light. The test article was diluted once or twice a week to 1.5 and 5 mg/mL with the phosphate buffered saline. Phosphate buffered saline was used as vehicle control.

Methods

Doses: 15 and 50 mg/kg, the doses were chosen because doses were 3 and 10 times higher than that needed for the suppression of delayed hypersensitivity reactions to dinitrophenol in mice.
Species/strain: Crlj:CD1(ICR) mice, mice were mated in the facility.
Number/sex/group: 30 in each group
Route, formulation, volume, and infusion rate: The injection was given intravenously into the tail vein at 2 mL/min once every three days from day 6 of gestation to day 21 after the delivery. The injection days varied slightly due to delivery of dams.
Satellite groups used for toxicokinetics: The satellite groups are shown from the applicant's table below.

Satellite Groups: 1 control group and 2 test article groups

Group	Test and Control Articles	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals [b] (Animal No.)
4	PBS [a]	-	10	-	12 (10091 - 10102)
5	MR16-1	15	10	1.5	36 (10103 - 10138)
6	MR16-1	50	10	5	36 (10139 - 10174)

a) PBS was administered in the same manner as test article.
b) Non-pregnant animals: Nos. 10126, 10129, 10132, 10141, 10149, 10150 and 10172

Study design: The study design is shown from the applicant's table below.

Main Groups: 1 control group and 2 test article groups

Group	Test and Control Articles	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals [b] (Animal No.)
1	PBS [a]	-	10	-	30 (10001 - 10030)
2	MR16-1	15	10	1.5	30 (10031 - 10060)
3	MR16-1	50	10	5	30 (10061 - 10090)

a) PBS was administered in the same manner as test article.
b) 26, 21 and 27 animals in the control, 15-and 50-mg/kg groups, respectively, were confirmed to be pregnant.
Non-pregnant animals: Nos. 10001, 10006, 10008, 10027, 10036, 10038, 10040, 10043, 10046, 10051, 10058, 10059, 10060, 10064, 10072 and 10085

Parameters and endpoints evaluated: Animals were observed for clinical signs twice daily during pregnancy and on the day of dosing during the nursing period. Animals were observed once a day during the non-dosing period. The body weight was recorded when possible on gestation days 0, 6, 9, 12, 15 and 18, post partum days 0, 4, 7, 10, 14, 17 and 22. The food consumption was recorded on gestation days 5, 6, 9, 12, 15 and post partum days 1, 4, 7, 10, 14, 17 and 21. Dams were allowed to deliver. Gestation period and delivery index was recorded.

Delivery Index = # pregnant mice delivered live pups / # pregnant mice x 100.

Thoraco-abdominal organs and brain from groups 2 and 5 animals that died during the study were fixed in formalin. F0 dams that aborted conceptuses or had a total litter loss were euthanized and number of implantations was recorded. F0 dams that did not deliver were euthanized on the day corresponding to day 23 of gestation. Implantations in animals that did not deliver, if any, were recorded, uteri and ovaries for mice that did not deliver were fixed in formalin.

Following organs were collected from all F0 animals that died during the study (except one that died during the blood sampling): Liver, kidney, heart, lungs, spleen, cerebrum, cerebellum, ileum, mesenteric lymph node and internal iliac lymph node.. Tissues were fixed and stained with hematoxylin and eosin for microscopical examinations. The histology slides were peer-reviewed also.

Gross pathology was conducted in the dams (F0) except those used for delayed hypersensitivity reactions on day 22 after the delivery for external appearance and macroscopic changes in the internal organs. Tissues were not subject to histological evaluation due to the absence of any lesions. The number of implantation sites were recorded. Animals # 10025, 10028, 10030 (control animals), 10055, 10056 (15 mg/kg dosed animals), 10083, 10086, 10087, 10088, 10089 and 10090 (50 mg/kg dosed animals) were given one intracutaneous injection of MR16-1, 7 days after final dosing with MR16-1 for immune reactions (delayed type hypersensitivity reaction) and were sacrificed at 24 hours after the intracutaneous injection. Skin reactions at the challenge site were evaluated by Draize scale (from 0-4) for erythema and edema. The cutaneous site was fixed in formalin at necropsy and histopathological examination was conducted following hematoxylin and eosin staining.

Blood samples (0.6 mL) were collected at necropsy for the measurement of plasma MR16-1 and anti MR16-1 from main and satellite animals. Blood samples for TK and anti MR-16-1 were also collected from satellite animals on days 6 and 15 of gestation and final day of dosing from abdominal vena cava under isoflurane anesthesia at 0.5, 24 and 72 hour post dose from different animals at different time points. Stability of MR16-1 in the plasma was examined. Plasma MR16-1 levels at 0.5 hr post dose and AUC in the plasma for 0-72 hours was calculated.

F1 pups were observed at birth for live birth, stillbirth, and external appearance. All pups were observed for clinical signs and mortality. The sex for F1 pups were determined. Following parameters were calculated:

Live Birth Index = # of live F1 pups at birth / # of implantation sites x 100

Viability Index on day 0 = # of live F1 pups after birth / # of F1 pups x 100

Viability Index on day 4 = # of live F1 pups before culling / # of live F1 pups on day 0 after birth x 100

Weaning Index = # of live F1 pups before weaning on day 22 after birth / # of live F1 pups after culling on day 4 after birth x 100

Sex Ratio = # live F1 male / # live F1 pups

Sex Ratio on day 4 after birth = # Live F1 male before culling on day 4 after birth / # live F1 pups before culling on day 4 after birth

Sex Ratio on day 22 = # Live F1 male pups before weaning on day 22 after birth / # of live F1 pups before weaning on day 22 after birth

Approximately four male and four female F1 pups were selected randomly on day 4 after birth from each litter for further study and rest of the F1 pups were sacrificed by carbon dioxide gas inhalation and fixed in formalin. The body weight of surviving F1 pups was recorded on days 0, 4, 7, 14, 22 after birth and continued to days 25, 30, 42, 56, 70 after weaning and at necropsy for selected pups.

The physical and functional development of F1 pups were examined during the lactation period as shown from the applicant's table below.

9.11.2.6 Developmental Examinations During the Lactation Period

Examination of Physical Development (satellite groups were not examined)

Examination	Number of Pups	Examination Period	Judgment Criterion
Pinna detachment	All F1 pups after culling on Day 4 after birth	From Day 4 after birth until completion	Judged to be completed when pinna separates from auditory meatus and becomes elongated
Incisor eruption		Day 10 or 12 after birth	Judged to be completed when incisors have erupted from both the upper and lower jaws
Eyelid opening		Day 14 or 16 after birth	Judged to be completed when both eyelids open completely

Examination of Functional Development (satellite group were not examined)

Examination	Number of Pups	Examination Period	Judgment Criterion
Righting reflex	All F1 pups	From Day 1 after birth until completion	Judged to be accomplished when animal returns to the normal position after being placed on its back
Negative geotaxis	1 male and 1 female from each litter (in principle, pups with the lowest number)	Days 5, 10 and 15 after birth	Animal placed as shown in Figure for 1 minute, and evaluated according to the negative geotaxis criteria (see Table below)
Pupillary reflex		From Day 20 after birth	Judged to be normal when constriction of the pupil is observed after exposure to light following dark acclimation (approximately 30 seconds)

Examination	Number of Pups	Examination Period	Judgment Criterion
Preyer reflex	1 male and 1 female from each litter (in principle, pups with the lowest number)	From Day 20 after birth	Judged to be normal when animal shows pinna reflex in response to exposure to sound at 5000 Hz and 15000 Hz
Pain response		From Day 20 after birth	Judged from the reaction to softly pinching the tail

Table - Negative Geotaxis Scoring

Score	Negative Geotaxis Criteria
1	Falls down immediately
1	Stays at I (less than 1 minute), but falls later
2	Stays at I (less than 1 minute), but falls after turning sideways
2	Stays at I (less than 1 minute), but falls after moving sideways
3	Stays at I (for 1 minute), turns upward
3	Stays at I (less than 1 minute), but falls after turning upward
1	Stays at II (less than 1 minute), but falls later
2	Stays at II (less than 1 minute), but falls after turning sideways
3	Stays at II (less than 1 minute), but falls after moving sideways
3	Stays at II (less than 1 minute), but falls after turning upward
4	Moves to III
1	Stays at I for 1 minute
2	Stays at II for 1 minute

F1 pups died between the births and weaning were fixed in formalin. Similarly, F1 pups from dams that died were fixed in formalin.

Following studies were conducted at post weaning in F1 mice:

Reproductive performance was conducted from one male and one female mouse per litter. F1 mice were allowed to mate at 10 to 11 weeks after the birth. Copulation index (# copulated animals / # paired animals x100), copulatory interval and fertility index (# pregnant mice / # of copulated animals x 100) was calculated. The body weight was recorded on days 0, 7 and 13 of pregnancy. All F1 mice were sacrificed on day 13 of gestation by carbon dioxide inhalation and gross changes were noted. Male mice that did not induce pregnancy when paired with a female were examined for the presence of sperms in the epididymides stamp smears. F1 females were examined for gross changes on external surface and thoracoabdominal organs. Number of corpora lutea and implantations were determined. Pre-implantation loss was calculated. Ovaries and uteri were fixed in formalin. The applicant stated that there was no abnormality in any other organs in treated animals and in the control animals. Therefore, no histopathology was conducted for male and female F1 mice following the reproductive performance study. The noncopulated F1 mouse (10023-F1) was euthanized after the mating period and uteri were examined for implantation sites.

One male and one female F1 mouse per litter was chosen for learning function and behavior assessment using open field test at 5-6 weeks after birth and water maize test at 6-7 weeks after birth.

One male and one female F1 mouse per litter was selected for immunological and hematological examinations. Humoral immune competence to Keyhole Limpet Hemocyanin (KLH) antibody production was examined at 5-6 weeks after the birth from each of 10 liters in each group. KLH antigen was injected intravenously at 0.1 mg/animal and antibody titers were determined on days 7 and 14 after immunization. Hematology and immuno-phenotyping in peripheral blood, thymus and spleen was conducted at 6 to 7 weeks of age by a flow cytometer. F1 mice not used for immuno-competence examinations were used for the hematology and immuno-phenotyping (10015M3 and 10015F3). Parameters for immuno-phenotyping are shown from the applicant's report below.

Parameters	
Peripheral Blood and Spleen:	$CD3e^+$, $CD45R/B220^+$, $CD3e^+CD4^+CD8a^-$, $CD3e^+CD4^-CD8a^+$ and $CD49b/Pan^-NK\ cells^+CD3e^-$
Thymus:	$CD4^-CD8a^-$, $CD4^+CD8a^+$, $CD4^+CD8a^-$ and $CD4^-CD8a^+$

CD3 receptors are complexes with T-lymphocyte receptors and responsible for transduction of events following antigen presentation, CD45 R receptors are common antigen receptors present in all cells of hematopoietic systems except RBC including resting lymphocytes and NK cells with tyrosine phosphatase activity, CD4+ are markers in T-lymphocytes for MHC Class II binding protein for antigen presentation, and CD8+ markers for T-lymphocytes for MHC class I binding protein for antigen presentation.

The selection of animals for reproduction, behavioral study, hematology and immuno-phenotyping was made from assigned numbers.

All weaned F1 pups selected above were examined for the cleavage of the balance-preputial gland and vaginal opening for male and female mice, respectively, on day 30 after the birth. Remaining F1 pups were sacrificed by carbon dioxide in halation at weaning, the carcass was fixed in ethyl alcohol following examinations of gross changes in the external appearance and internal organs. However, no histopathological examinations of thoraco-abdominal organs were performed due to a lack of abnormal gross changes. Skeletal examinations for abnormalities and variations were conducted according to Dawson's method after staining with Alizarin Red S stain.

Results

F_0 in-life:

Several group 2 animals from the main and satellite groups were dead soon after the injection as shown from the applicant's table below:

Animals were found dead at the time points shown in the following table below:

Main Groups

Group	Animal No.	Day of dosing	Total times dosed	Observation time
2	10032	Day 18 (LD6)	7	Approximately 90 minutes after dosing
	10040	Day 15 (GD21)	6	Approximately 40 minutes after dosing
	10041	Day 12 (GD18)	5	Approximately 120 minutes after dosing
	10042	Day 15 (LD3)	6	Approximately 40 minutes after dosing
	10048	Day 15 (LD3)	6	Approximately 50 minutes after dosing
	10050	Day 10 (GD16)	4	On the morning following the day of dosing

GD: Day of gestation LD: Day of lactation

Satellite Groups

Group	Animal No.	Day of dosing	Total times dosed	Observation time
5	10118*	Day 9 (GD15)	4	Approximately 30 minutes after dosing
	10127	Day 16 (LD3)	6	On the morning following the day of dosing
	10128	Day 16 (LD3)	6	On the morning following the day of dosing
	10131	Day 33 (LD21)	12	Approximately 80 minutes after dosing
	10134	Day 15 (GD21)	6	Approximately 70 minutes after dosing
	10135	Day 15 (GD21)	6	Approximately 80 minutes after dosing

GD: Day of gestation LD: Day of lactation
*: Under isoflurane anesthesia for blood sampling

As shown above, 6 animals at 15 mg/kg died soon after the injections during 4 to 7 injections. Animals in the satellite group also showed a similar mortality at 15 mg/kg. The applicant did not state the clinical sign observed at death. No abnormal clinical sign or mortality was noted at 50 mg/kg. No mortality to dams was reported in the control animals.

The average body (g) weight during the gestation and lactation period is shown in the table below.

Gestation day	**Control**	**15 mg/kg**	**50 mg/kg**
0	27.7 (26)	27.4 (21)	27.6 (27)
6	30.3	29.9	30.6
18	52.8 (24)	50.4 (16)	53.2 (21)
Gain 6-18	22.5	20.5	22.6
Lactation day			
0	43.8 (25)	34.3 (16)	35.1 (27)
22	36.8 (23)	35.6 (10)	37.0 (27)

The treatment did not affect the body weight gain during the gestation period.

The food consumption (g/day) during the gestation and lactation period was not affected by the treatment.

There were no gross pathological changes in the F0 dams. Intracutaneous injections of MR16-1 to F0 animals to assess delayed hypersensitivity reactions did not show any erythema or edema due to the injection.

F0 dams delivery:

The delivery data are shown in the table below.

Parameter	Control	15 mg/kg	50 mg/kg
# Animal	30	30	30
# Dead dams	0	6	0
# Nonpregnant	4	7	3
# Pregnant	26	19	27
# Delivered	25	14	27
Delivery Index (%)	92.3	67.5	100
Gestation period (days)	18.6	18.6	18.7
# Implantations	11.2	10.8	11.5
Live birth index (%)	86	69	91
# Pups after culling/litter	7.8	6.4	7.4
Viability Index at birth (%)	89	78	99.4
Viability Index at day 4 (%)	95	80	98.3
Weaning Index (%)	100	100	100
Sex ratio at birth	0.55	0.39	0.53
Sex ratio on day 4	0.53	0.45	0.54
Sex ratio at weaning	0.51	0.49	0.53

Deaths of F1 pups within weaning day 4 is shown from the applicant's table below. Slightly increased deaths of F1 pups were noted in treated animals. However, due to a small difference in number of litters that showed deaths from the control animals, its relationship to the treatment is not known.

Table 6 Clinical signs and external findings in pups (F1) - Before weaning

Dose (mg/kg)	Control	15	50
No. of pups (No. of litters)	271 (24)	125 (14)	287 (27)
From Day 0 to 4 after birth			
No. of pups (No. of litters)	268 (21)	117 (9)	281 (22)
Normal	3 (3)	8 (5)	6 (5)
Death			
From culling to weaning			
No. of pups (No. of litters)	180 (23)	70 (11)	200 (27)
Normal	180 (23)	70 (11)	200 (27)
External findings at birth (F1)			
Malformations (%, mean ± S.D.)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Not significantly different from control

F_0 necropsy:

The applicant presented histopathology data for mice at 15 mg/kg that died before the termination of the experiment. Histopathology data of F0 dams at 15 mg/kg showed a slight thrombus and hemorrhage in lungs, swelling of alveolar epithelium, hyperplasia of internal iliac lymph nodes and extramedullary hematopoiesis in the spleen. These data suggest that there was bleeding in the lungs and the effect could be related to the immune reactions from anti-product antibodies because most of the mice died at 15 mg/kg had received multiple doses before the death. However, there were no data available for MR16-1 levels or antibody titers in the plasma to conclude the cause of deaths other than the histopathological findings. Summary of the histopathology data from the applicant's table are presented below.

b(4)

Table 23-3 Histopathological findings in dams (Satellite group) [H.E. staining] Study No ——.026-064

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Findings	Dose (mg/kg) / Animal No.	15 10127	15 10128	15 10131	15 10134	15 10135
Cerebellum		-	-	-	-	-
Cerebrum		-	-	-	-	-
Heart						
Extramedullary hematopoiesis, epicardium		-	-	-	-	-
Ileum (including Peyer's patch)						
Autolysis		P	P	-	-	-
Kidney (left)		-	-	-	-	-
Kidney (right)		-	-	-	-	-
Liver (left lateral lobe, median lobe)		-	-	-	-	-
Lung (left)						
Fibrin thrombus		-	-	-	-	-
Hemorrhage		-	-	-	-	-
Swelling, alveolar epithelium		-	-	-	-	-
Lung (right)						
Fibrin thrombus		-	-	-	±	-
Hemorrhage		-	-	-	-	-

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

Study No — 033-064

b(4)

F_1 physical development:

No external malformation was noted in the surviving pups.

The body weight (g) of F1 pups up to post-natal day 22 is shown below. Number of pups in each group is shown in the parentheses.

Day	Control	15 mg/kg	50 mg/kg
Day 0 (male)	1.65 (141)	1.55 (54)	1.67 (153)
Day 0 (female)	1.54 (130)	1.49 (71)	1.59 (134)
Day 4 (male)	3.0 (139)	2.91 (41)	3.28 (149)
Day 4 (female)	2.87 (129)	3.03 (53)	3.21 (132)
Day 22 (male)	17.07 (90)	17.06 (30)	17.93 (107)
Day 22 (female)	15.96 (90)	16.35 (34)	16.82 (93)

Data suggest that there was no difference in the weight gain among surviving F1 pups.

Physical development of F1 pups at pre-weaning was examined by the assessment of pinna detachment, incisor eruption and eyelid opening. Male and female F1 pups did not show any treatment related change in these parameters when compared to the control F1 pups. Data for the skeletal changes of F1 pups at weaning are shown from the applicant's table below.

b(4)

Table 9 External, visceral and skeletal findings in pups (F1) at weaning Study No ...036-064

Dose (mg/kg)	Control	15	50
No. of litters	23	8	24
No. of pups	46	15	51
External findings (%, mean ± S.D.)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Visceral findings (%, mean ± S.D.)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Skeletal findings			
Abnormalities (%, mean ± S.D.)	2.17 ± 10.43	0.00 ± 0.00	2.08 ± 10.21
Type and frequency (%)			
Fused sternebrae	2.17	0.00	2.08
Variations (%, mean ± S.D.)	18.84 ± 32.30	31.25 ± 45.81	26.35 ± 31.05
Type and frequency (%)			
Asymmetry of sternebrae	3.62	0.00	0.00
Full supernumerary rib	6.52	25.00	17.36
Knobby rib	2.17	0.00	1.39
Short supernumerary rib	8.70	18.75	13.89
No. of ossified (mean ± S.D.)			
Sternebrae	6.02 ± 0.10	6.00 ± 0.00	6.00 ± 0.00
Sacral and caudal vertebrae	34.52 ± 0.59	34.75 ± 0.60	34.56 ± 0.62

Not significantly different from control

Full and short supernumerary ribs were observed in F1 pups from following F0 dams at weaning on day 22.

Control: 1005, 10010, 10020, 10021, out of 23 litters that represents 17.39% of litters showed either full or short supernumerary ribs. When data were compared for number of full or short supernumerary ribs, a total of 7 out of 46 pups showed the change that was about 15.21% of F1 pups.

15 mg/kg: 10034, 10055, 10056, out of 8 litters that represent 37.5% of litters showed either full or short supernumerary ribs. When data were compared for number of full or short supernumerary ribs, 6 out of 15 pups showed the change that was about 40% of F1 pups.

50 mg/kg: 10063, 10065, 10067, 10074, 10075, 10076, 10077, 10081, 10083, 10084, 10087, out of 24 litters that represent 45.83% of litters showed either full or short supernumerary ribs. When data were compared for number of full or short supernumerary ribs, 17 out of 51 pups showed the change, which was about 33.3% of F1 pups.

When individual F0 dams were counted for the presence of supernumerary ribs in the litters, 4, 3 and 11 F0 dams showed full and short supernumerary ribs in the control, 15 and 50 mg/kg, respectively. In addition, there was an increase of short and full supernumerary ribs at both 15 and 50 mg/kg. These data suggest that the variation of supernumerary ribs at 50 mg/kg was related to the treatment. Since all F1 pups were not counted for variations, the reviewer relied more on the supernumerary ribs as the % litters data provided in the table below.

Supernumerary ribs:

Dose, mg/kg	Short or Full Supernumerary ribs, % Litter	Short, Full % Litter	Supernumerary Ribs, % F1 pups	Short, Full % F1 pups
Control	17.39	17.4, 13.0	15.2	8.7, 6.5
15	37.5	37.5, 37.5	40	20, 20
50	45.83	33.3, 37.5	33.3	15.6, 17.6

In order to conclude that the effect was due to the treatment rather than population variation, the review team asked the applicant for providing historical control data for comparison.

Applicant's Response:

In response to a request from the review team on Oct 29, 2009, the applicant provided historical control data for supernumerary ribs in mice on Nov 4. The applicant provided historical control data from a pre- and postnatal development study in Crlj: CD1 mice from the laboratory between 2000-2008 in which the skeletal abnormality or variations were recorded on weaning day 22 by X-ray images. The applicant referred to the document as #4146 in the submission but the data were provided under Ref # 4145. The tabular form of the data showed 13-40% of litters and 3-12% of F1 pups had the developmental variation with short or full supernumerary ribs. For an embryo-fetal development study in mice, skeletal variations were assessed on day 18 of gestation by Alizarin staining of the skeleton. About 43-57% of the litters had full or short supernumerary ribs and 17-18% of the F1 fetuses had full or short supernumerary ribs at the end of gestation. The applicant's data obtained from the conducting laboratory for full and short extra ribs using staining and radiographic methods are shown in the table below.

Study	Method	Day	Short, Full Supernumerary ribs, % Litter
Segment 2	Alizarin stain	Gestation day 18	57, 43
Segment 3	X-Ray	Postnatal Day 21	40, 13

Above data indicate that between the development at weaning stage the incidences of full supernumerary ribs were reduced. The applicant also submitted a publication by Chernoff et al. (1991) that suggested short supernumerary ribs in mice may fuse together with the full ribs during the development. As a result, the number of extra full ribs remains the same during the development but number of short ribs is reduced. Historical control data set provided by the applicant showed a reduction in the full extra ribs during maturation. Therefore, the significance of the literature finding in relation to the interpretation of results of the historical control is not known.

The applicant also suggested that stress factors may contribute to the high incidences of supernumerary ribs. However, given the fact that control animals were also treated with the vehicle (phosphate buffered saline), the relationship of stress and presence of supernumerary ribs is not known due to a lack of apparent toxicity at 50 mg/kg,

Overall, the historical control data showed high incidences of supernumerary ribs within litters and although there was an increased of supernumerary ribs in the treated animals compared to the control under the experimental situation, the result could be due to population variation rather than a drug effect. **Based on that, the reviewer concluded that the treatment did not show any skeletal variations following the treatment with MR16-1 in mice.**

F_1 behavioral evaluation:

Functional and behavioral development of F1 pups at pre-weaning was assessed by righting reflex, negative geotaxis, pupillary reflex, Preyer's reflex and pain response. These data are shown below from the applicant's table.

Table 8-1 Functional development of male pups (F1) (mean ± S.D.) Study No. ...036-064 b(4)

Dose (mg/kg)	Control		15		50	
Righting reflex (%)						
Day 1	100.0 ± 0.0	(24)	95.8 ± 14.4	(12)	100.0 ± 0.0	(26)
Day 2			100.0 ± 0.0	(12)		
Negative geotaxis a)						
Day 5	3.0 ± 0.3	(23)	2.7 ± 0.7	(10)	2.4 ± 0.9*	(26)
Day 10	3.5 ± 0.5	(23)	3.8 ± 0.4	(9)	3.2 ± 0.6*	(26)
Day 15	4.0 ± 0.0	(23)	4.0 ± 0.0	(9)	4.0 ± 0.0	(26)
Pupillary reflex (%)						
Day 20	100.0 ± 0.0	(23)	100.0 ± 0.0	(9)	100.0 ± 0.0	(26)
Preyer reflex (%)						
Day 20	100.0 ± 0.0	(23)	100.0 ± 0.0	(9)	100.0 ± 0.0	(26)
Pain response (%)						
Day 20	100.0 ± 0.0	(23)	100.0 ± 0.0	(9)	100.0 ± 0.0	(26)

a): Gradable data (grade score: 1,2,3,4)
Day: Day after birth
The number in parentheses indicates the number of litters examined.
*P<0.05: Significantly different from control

A slight reduction in number of negative geotaxis score was noted at 50 mg/kg up to day 10 in male F1 pups. Negative geotaxis was measured from the ability of the animal to retain its body position that prevented from a fall.

A reduction in the negative geotaxis was not observed in the female F1 pups. Perhaps, the control male F1 pups showed a lower score that contributed to the statistical differences between the control and treated male F1 pups. However, biological significance of the change is unknown.

Data at post weaning functional response in open field test are shown from the applicant's table below.

Table 13-1 Open field test (F1) (mean ± S.D.) Study No.

Male

Dose (mg/kg)	Control	15	50
No. of animals	23	9	26
Ambulation	149.3 ± 54.5	152.7 ± 67.0	126.9 ± 45.8
Rearing	23.2 ± 14.6	20.3 ± 13.2	21.1 ± 13.0
Grooming and preening	0.5 ± 0.5	0.9 ± 0.9	0.7 ± 0.6
Defecation	1.9 ± 1.9	2.7 ± 2.5	2.1 ± 1.6

Female

Dose (mg/kg)	Control	15	50
No. of animals	22	9	27
Ambulation	151.9 ± 60.5	146.6 ± 39.8	123.7 ± 40.9
Rearing	21.8 ± 9.4	20.0 ± 9.7	17.4 ± 7.9
Grooming and preening	1.0 ± 0.9	0.3 ± 0.7*	0.7 ± 0.6
Defecation	2.2 ± 2.2	2.2 ± 2.7	1.8 ± 2.0

*P<0.05: Significantly different from control

A significant reduction in the grooming behavior was noted at 15 mg/kg in female F1 pups. The difference could be related to the small number of the sample size in this group and its biological significance in the absence of such changes at 50 mg/kg is not known.

Data for water maize test are shown below.

Table 13-2 Water T-maze test of male animals (F1) (mean ± S.D.)

Dose (mg/kg) / No. of animals		Control 23	15 8	50 25
Straight course	Trial-1			
	Time (sec.)	18.1±10.6 (23)	23.6±22.2 (8)	21.2±16.2 (25)
	Error (b)	0.3±0.6	0.1±0.4	0.5±0.8
	Trial-2			
	Time (sec.)	8.9±4.5 (23)	10.1±4.4 (7)	15.3±13.8 (25)
	Error (b)	0.0±0.0	0.1±0.4	0.4±0.8*
	Trial-3			
	Time (sec.)	9.4±5.4 (23)	10.3±8.9 (7)	14.3±12.1 (25)
	Error (b)	0.1±0.3	0.0±0.0	0.2±0.4
Water T-maze				
1st day	Trial-1			
	Time (sec.)	86.1±44.7 (20)	97.5±50.5 (6)	88.0±36.9 (15)
	Error (s)	3.2±1.5	3.3±2.0	3.3±1.3
	Error (b)	0.7±0.7	0.3±0.5	0.3±0.6
	Trial-2			
	Time (sec.)	62.8±29.7 (20)	95.8±55.9 (5)	77.1±41.4 (18)
	Error (s)	3.0±1.5	4.6±2.3	3.5±2.1
	Error (b)	0.4±0.7	1.0±1.4	1.1±1.8
	Trial-3			
	Time (sec.)	67.8±41.7 (21)	69.4±48.2 (5)	93.5±49.8 (20)
	Error (s)	3.0±1.7	2.4±2.2	4.0±2.0
	Error (b)	0.8±1.1	0.4±0.5	1.0±1.1
2nd day	Trial-1			
	Time (sec.)	58.7±33.1 (21)	76.6±37.7 (5)	84.8±52.4 (19)
	Error (s)	3.0±1.6	3.4±1.7	4.0±3.2
	Error (b)	0.7±1.0	1.0±1.0	1.3±1.9
	Trial-2			
	Time (sec.)	57.0±33.9 (23)	41.8±12.4 (5)	63.5±36.2 (20)
	Error (s)	2.7±1.7	2.8±1.5	3.1±2.2
	Error (b)	0.9±1.2	0.6±0.5	1.1±1.4
	Trial-3			
	Time (sec.)	58.0±34.2 (22)	64.2±42.1 (6)	59.2±30.7 (20)
	Error (s)	2.4±1.4	3.0±1.9	2.5±1.5
	Error (b)	0.8±1.1	0.8±0.8	0.7±0.8
3rd day	Trial-1			
	Time (sec.)	54.6±33.6 (22)	54.7±44.4 (6)	52.2±24.4 (18)
	Error (s)	2.8±2.3	3.0±2.8	2.3±1.3
	Error (b)	0.8±1.3	1.3±1.5	0.6±1.0
	Trial-2			
	Time (sec.)	45.1±29.3 (22)	31.7±8.2 (6)	52.2±29.2 (19)
	Error (s)	1.9±1.4	2.0±0.9	2.4±2.0
	Error (b)	0.4±0.6	0.7±0.5	0.5±1.2
	Trial-3			
	Time (sec.)	46.0±40.4 (21)	53.7±37.0 (6)	45.8±23.2 (18)
	Error (s)	1.8±1.9	2.7±1.4	1.6±1.3
	Error (b)	0.8±1.4	0.7±0.5	0.3±0.7

( ) : No. of animals reached the goal
Error (s) : Select error , Error (b) : Backing error
* P<0.05 : Significantly different from control

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Male F1 mice showed comparable swimming and learning behavior to the control animals. However, all mice showed a quicker response to the learning behavior in successive trials.

Table 13-3 Water T-maze test of female animals (F1) (mean ± S.D.)

Dose (mg/kg) No. of animals		Control 23	15 9	50 25
Straight course	Trial-1			
	Time (sec.)	15.9±13.5 (22)	17.9±18.2 (8)	12.5±9.4 (26)
	Error (b)	0.1±0.4	0.5±1.4	0.3±0.6
	Trial-2			
	Time (sec.)	8.0±3.8 (22)	10.0±6.8 (9)	10.6±8.0 (26)
	Error (b)	0.0±0.2	0.1±0.4	0.3±0.5
	Trial-3			
	Time (sec.)	7.3±7.5 (22)	10.0±6.7 (8)	8.1±5.7 (26)
	Error (b)	0.0±0.0	0.1±0.4	0.1±0.3
Water T-maze				
1st day	Trial-1			
	Time (sec.)	93.1±35.9 (20)	85.8±60.6 (6)	81.3±48.7 (22)
	Error (s)	4.1±1.5	3.7±2.6	3.1±1.7
	Error (b)	0.6±0.8	0.8±1.0	0.6±0.9
	Trial-2			
	Time (sec.)	61.1±22.9 (19)	80.0±38.0 (5)	67.1±37.4 (23)
	Error (s)	3.8±1.2	4.0±2.3	3.5±2.1
	Error (b)	0.9±1.1	1.0±1.0	0.9±1.1
	Trial-3			
	Time (sec.)	59.7±30.7 (18)	71.0±33.4 (7)	62.5±40.7 (24)
	Error (s)	3.4±1.8	4.4±3.2	3.3±2.0
	Error (b)	0.9±1.4	1.0±1.4	0.5±0.8
2nd day	Trial-1			
	Time (sec.)	55.9±40.6 (21)	74.9±53.2 (7)	56.1±34.4 (24)
	Error (s)	3.1±2.5	4.3±1.7	3.0±1.8
	Error (b)	0.9±1.6	0.9±0.9	0.7±0.8
	Trial-2			
	Time (sec.)	68.0±43.2 (21)	66.8±35.6 (6)	63.6±38.3 (25)
	Error (s)	3.7±2.8	3.0±1.3	3.5±2.0
	Error (b)	1.4±2.4	1.0±1.3	1.0±1.4
	Trial-3			
	Time (sec.)	65.7±44.3 (22)	52.0±26.2 (6)	60.1±39.5 (25)
	Error (s)	3.6±3.1	2.7±1.5	3.4±2.4
	Error (b)	1.2±2.3	1.2±1.5	0.7±1.1
3rd day	Trial-1			
	Time (sec.)	42.1±29.1 (18)	62.0±41.9 (6)	47.7±29.9 (23)
	Error (s)	2.3±1.6	3.5±1.9	2.3±1.4
	Error (b)	0.7±1.2	1.0±1.3	0.7±1.0
	Trial-2			
	Time (sec.)	48.7±39.7 (21)	74.7±52.6 (6)	49.1±34.9 (24)
	Error (s)	2.2±1.0	2.5±1.9	2.9±2.2
	Error (b)	0.6±0.9	1.0±1.5	1.0±1.6
	Trial-3			
	Time (sec.)	43.7±35.3 (19)	55.8±18.6 (6)	53.3±37.3 (23)
	Error (s)	2.4±2.3	2.7±1.4	2.8±1.5
	Error (b)	0.6±1.0	0.8±1.0	1.1±1.6

( ) : No. of animals reached the goal
Error (s) : Select error , Error (b) : Backing error
Not significantly different from control

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Data for F1 female mice for the swimming and learning behavior was comparable to the male mice and there was no change in the response time to the swimming error. These data signified that Actemra did not affect the learning and memory functions of F1 mice (second generation).

Data for immuno-competence of F1 pups after weaning are shown from the applicant's table below.

Table 14 Immunocompetence (serum IgG and IgM) (F1) (mean ± S.D.)

Male

Dose (mg/kg)	Day[a]	Anti-KLH antibody titer (×100)[b] IgG		IgM	
Control	7	6.8 ± 0.4	(5)	5.6 ± 0.5	(5)
	14	6.8 ± 1.3	(5)	3.4 ± 0.5	(5)
15	7	7.5 ± -	(2)	6.0 ± -	(2)
	14	6.0 ± -	(2)	4.0 ± -	(2)
50	7	6.0 ± 0.7	(5)	5.4 ± 0.9	(5)
	14	7.0 ± 0.7	(5)	4.0 ± 0.7	(5)

Female

Dose (mg/kg)	Day[a]	Anti-KLH antibody titer (×100)[b] IgG		IgM	
Control	7	6.4 ± 0.5	(5)	5.0 ± 1.2	(5)
	14	7.8 ± 0.8	(5)	3.4 ± 0.9	(5)
15	7	7.5 ± -	(2)	5.0 ± -	(2)
	14	6.5 ± -	(2)	3.5 ± -	(2)
50	7	6.2 ± 1.3	(5)	5.4 ± 0.5	(5)
	14	7.8 ± 0.4	(5)	3.0 ± 1.0	(5)

a): Day after sensitization of KLH
b): The values were calculated from titers log-transformed to base-2.
The number in parentheses indicates the number of animals.
Not significantly different from control

Male and female F1 mice showed production of antibodies to KLH antigen when monitored on days 7 and 14 after KLH injections. IgM titers were slightly lower on day 14 compared to day 7 post sensitization in male and female mice. However, the difference was negligible. MR16-1 treatment to F0 dams did not affect the immunoglobulin production in the F1 mice during the postnatal development.

The average data for hematology for F1 pups after weaning are shown below.

Table 15-1 Hematology in male animals (F1) (mean ± S.D.) Study No. ___036-064

b(4)

Dose (mg/kg)		Control	15	50
	No. of animals	12	3	14
Item	Unit			
Erythrocyte count	$10^6/mm^3$	8.045 ± 0.345	8.053 ± 0.355	7.944 ± 0.246
Leukocyte count	$10^3/mm^3$	4.496 ± 1.850	5.310 ± 2.657	3.366 ± 0.881
Hematocrit value	%	42.14 ± 1.21	43.33 ± 1.12	41.48 ± 1.21
Hemoglobin concentration	g/dL	13.63 ± 0.46	13.97 ± 0.32	13.31 ± 0.30
Platelet count	$10^3/mm^3$	1398.7 ± 152.4	1461.7 ± 39.6	1395.6 ± 121.9
Mean corpuscular volume	fL	52.43 ± 1.55	53.87 ± 1.60	52.24 ± 1.59
Mean corpuscular hemoglobin	pg	16.94 ± 0.50	17.20 ± 0.46	16.78 ± 0.37
Mean corpuscular hemoglobin concentration	g/dL	32.34 ± 0.46	31.93 ± 0.45	32.11 ± 0.56
Reticulocyte ratio	%	3.12 ± 0.39	3.53 ± 0.68	3.16 ± 0.65
Eosinophil count	$10^3/mm^3$	0.105 ± 0.050	0.103 ± 0.072	0.074 ± 0.024
Eosinophil ratio	%	2.43 ± 0.91	1.77 ± 1.11	2.37 ± 1.31
Basophil count	$10^3/mm^3$	0.008 ± 0.010	0.017 ± 0.012	0.004 ± 0.005
Basophil ratio	%	0.18 ± 0.10	0.20 ± 0.10	0.14 ± 0.11
Neutrophil count	$10^3/mm^3$	0.667 ± 0.255	0.523 ± 0.263	0.417 ± 0.091*
Neutrophil ratio	%	16.15 ± 6.48	10.03 ± 1.30*	12.64 ± 2.37

b(4)

*P<0.05: Significantly different from control

b(4)

Table 15-2 Hematology in female animals (F1) (mean ± S.D.) Study No. —036-064

Dose (mg/kg)		Control	15	50
	No. of animals	12	4	11
Item	Unit			
Erythrocyte count	$10^6/mm^3$	8.019 ± 0.329	7.848 ± 0.326	8.012 ± 0.348
Leukocyte count	$10^3/mm^3$	4.357 ± 1.371	3.143 ± 0.525	3.291 ± 0.975
Hematocrit value	%	42.14 ± 2.28	41.98 ± 0.87	42.21 ± 1.57
Hemoglobin concentration	g/dL	13.54 ± 0.73	13.50 ± 0.37	13.48 ± 0.44
Platelet count	$10^3/mm^3$	1228.9 ± 132.8	1238.0 ± 182.4	1201.8 ± 145.0
Mean corpuscular volume	fL	52.57 ± 2.21	53.53 ± 1.73	52.84 ± 1.31
Mean corpuscular hemoglobin	pg	16.88 ± 0.63	17.20 ± 0.41	16.84 ± 0.47
Mean corpuscular hemoglobin concentration	g/dL	32.14 ± 0.68	32.15 ± 0.34	31.89 ± 0.42
Reticulocyte ratio	%	3.16 ± 0.99	3.35 ± 1.06	2.97 ± 0.70
Eosinophil count	$10^3/mm^3$	0.132 ± 0.047	0.105 ± 0.021	0.094 ± 0.027*
Eosinophil ratio	%	3.10 ± 0.85	3.43 ± 1.31	2.97 ± 0.90
Basophil count	$10^3/mm^3$	0.005 ± 0.005	0.003 ± 0.005	0.005 ± 0.005
Basophil ratio	%	0.13 ± 0.07	0.08 ± 0.05	0.12 ± 0.06
Neutrophil count	$10^3/mm^3$	0.689 ± 0.265	0.575 ± 0.106	0.565 ± 0.261
Neutrophil ratio	%	15.11 ± 6.92	18.43 ± 3.16	16.81 ± 4.11

*P<0.05: Significantly different from control

b(4)

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Male F1 mice:

Some of the changes in individual animals are discussed below.

Individual WBC counts in control male F1 mice were between 2.27 to 7.57 x $10^3/mm^3$. The lowest individual WBC counts in F1 male mice at 50 mg/kg was 2.05 x $10^3/mm^3$ (#10080-M3). WBC counts for other male F1 mice were within the range observed in control F1 male mice.

The individual lymphocyte counts in control male F1 mice ranged from 1.79 to 5.78 x $10^3/mm^3$. Male F1 animal # 10080-M3 has lymphocyte counts of 1.60 x $10^3/mm^3$ that was slightly lower than the lower limit in the control mice.

The individual neutrophil counts in control male F1 mice ranged from 0.35 to 1.18 $10^3/mm^3$. Male F1 # 10080-M3 had the neutrophil counts of 0.27 $10^3/mm^3$. Therefore a slight immunosuppression associated with a reduction in the WBC, lymphocyte and neutrophil counts was noted in F1 male # 10080M3.

Female F1 mice:

Some of the changes in individual animals are discussed below.

Individual WBC counts in control F1 female mice were between 2.48 to 6.46 $x10^3/mm^3$. Female F1 mouse # 10080-F3 and #10081-F3 had leukocyte counts of 1.82 and 2.05 x $10^3/mm^3$, respectively.

The individual lymphocyte counts in control F1 female mice ranged from 1.63 to 5.30 $10^3/mm^3$. F1 female mice #10080-F3 and #10089-F3 at 50 mg/kg had neutrophil counts of 1.39 and 1.62 x $10^3/mm^3$, respectively. Between two mice, mouse # 10080-F3 showed greater reduction in the lymphocyte counts that could be treatment related.

The individual neutrophil counts in control female F1 mice were between 0.38 to 1.13 x $10^3/mm^3$. F1 female mice #10081-F3 and #10080-F3 at 50 mg/kg had neutrophil counts of 0.22 x $10^3/mm^3$ and 0.33 x $10^3/mm^3$, respectively. Therefore, F1 female # 10080F3 at 50 mg/kg had a slight immunosuppression associated with a reduction in the WBC, neutrophil and lymphocyte counts.

Above data indicated a slight immunosuppression in the F1 pup # 10080-M3 (male) and #10080-F3 (female) with respect to peripheral blood immune cell counts.

Data for immunophenotyping in peripheral blood, thymus and spleen in F1 male and female mice at 6-7 week of age are shown below.

Male F1 mice:

Some of the changes in individual animals are discussed below.

F1 male showed a lower counts for CD45R/B220 and CD49b/Pan-NK cells that are responsible for activation of resting immune cells and adhesion of immune cells to vascular endothelial cells, respectively. Average data are shown in the table below.

Other markers did not show any treatment related change.

Marker	Control, n=12	15 mg/kg, n=3	50 mg/kg, n=14
CD45/B220, $10^3/mm^3$	1.92	2.0	1.19
CD49b/Pan NK cells, $10^3/mm^3$	0.89	0.92	0.53

CD45/B220 counts:

The cell counts for CD45/B220 markers in F1 control male mice varied from 0.59 to 3.88 x $10^3/mm^3$. F1 male #10018-M3 and #10028-M3 in the control group had 0.97 and 0.59 x $10^3/mm^3$ cell counts for CD45/B220 markers, respectively. F1 male mice #10078-M3, #10080-M3, #10084-M3 and #10089-M3 had CD45/B220 counts of 0.93, 0.65, 0.87 and 0.59 x $10^3/mm^3$ at 50 mg/kg, respectively. A total of two and four F1 male mice in the control and 50 mg/kg, respectively, showed a lower counts of CD45/B220 marker. However, data in the treated animals were not lower than that observed for the control animals. It was concluded

that the treatment of F0 female with MR16-1 at 50 mg/kg did not lower CD45/B220 counts in F1 male mice.

CD49b/Pan-NK cells:

Cell counts for CD49b/Pan NK cells in F1 control mice varied from 0.28 to 1.90 x $10^3/mm^3$. Control F1 male # 10015-M3, #10016-M3, #10018-M3, #10019-M3 and #10030-M3 had CD49/Pan NK cell counts of 0.39, 0.54, 0.45, 0.42 and 0.56, respectively.

The cell counts for CD49b/Pan NK cells at 50 mg/kg were 0.32, 0.35, 0.38 and 0.18 for F1 male # 10078-M3, #10079-M3, #10080-M3 and #10089-M3, respectively. Data suggest that 5 F1 male mice in the control and four F1 male mice in the 50 mg/kg group had a slow expression of the cell adhesion protein. Among F1 mice treated with 50 mg/kg of MR16-1, #10089-M3 showed CD49b/Pan NK cell counts of 0.18 x $10x^3/mm^3$ that could be due to the treatment of F0 female.

Based on the data it was concluded that F1 male #10089-M3 had immunosuppression associated with the reduction in the marker of integrin expression when F0 female mice were treated with MR16-1 at 50 mg/kg.

Female F1 mice:

Average data for cell counts for CD45R/B220 and CD49b/NK cell markers are shown in the table below. Other markers did not show any treatment related change.

Marker	Control, n=12	15 mg/kg, n=4	50 mg/kg, n=11
CD45/B220, $10^3/mm^3$	1.63	1.04	1.06
CD49b/Pan NK cells, $10^3/mm^3$	0.65	0.41	0.47

Individual counts for the control F1 female mice for CD45R ranged from 0.5 to 2.69 x $10^3/mm^3$. Animal #10089-F3 at 50 mg/kg had a CD45R count of 0.47 x $10^3/mm^3$.

Individual counts for CD49b/NK cells ranged from 0.25 to 1.08 x $10^3/mm^3$. However, animal #10089-F3 had a CD49b/NK cell counts of 0.17 x $10^3/mm^3$.

Above data suggest that F1 female #10089-F3 had a slight immunosuppression associated with a reduction in the expression of CD45R and integrin receptors. Other F1 female did not show immunosuppression due to the treatment when compared to the control.

Discussion on immunophenotyping:

Combined peripheral blood cell counts and immunophenotyping data indicated presence of a slight immunosuppression in litters from F0 female #10080 and #10089 at 50 mg/kg.

These changes could be attributed to the treatment of F0 female mice with MR16-1. However, the biological significance of the slight immunosuppression to the immune function is not known because anti-KLH titers in F1 mice (from another batch of F1 mice) were not affected by the treatment. Also, there was no relationship between immunosuppression and MR16-1 plasma levels in F0 dams. Since the number of affected animals was limited, one can conclude that in the absence of any other developmental change in F1 animals, the treatment had a chance for the delay in the immune function of F1 mice rather than an immunotolerance caused by MR16-1.

The following table summarized the immune effect in F1 pups.

Conc MR16-1, ng/mL	Sex	F1 animal #	Dose	Parameter decreased
351	Male	10080-M3	50 mg/kg	WBC, Lymphocyte, neutrophil
29	Male	10089-M3	50 mg/kg	CD49
351	Female	10080-F3	50 mg/kg	WBC, lymphocyte, neutrophil
258	Female	10081-F3	50 mg/kg	WBC, neutrophil
29	Female	10089-F3	50 mg/kg	Lymphocyte, CD45, CD49

F_1 reproduction:

Number of F1 pairs used for mating was 22, 9 and 27 at control, 15 and 50 mg/kg, respectively. Copulatory Index of male and female mating was 95, 100 and 100% for control, 15 and 50 mg/kg, respectively. However, fertility index was 100, 100 and 81.5% for the control, 15 and 50 mg/kg, respectively. Number of copulated, non-copulated, and pregnant animals in each group is shown from the applicant's table below.

b(4)

Table 18 Clinical signs in dams (F1) Study No. — 036-064

Dose (mg/kg)	Control			15		50	
	P	NP	NC	P	NP	P	NP
No. of dams	22	0	1	9	0	22	5
Normal	22	0	1	9	0	22	5

P : Pregnant animal
NP : Non-pregnant animal
NC : Non-copulated animal

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Above data suggest that about 18% of mated F1 animals at 50 mg/kg (F0 dams were treated) were non-pregnant in contrast to about 14% of F0 control animals. Therefore, it is concluded that the mating performance and pregnancy of F1 mice was not affected by the treatment of F0 dams with MR16-1.

F1 male and female mice did not show any abnormality when sacrificed after the weaning and maturation period as shown from the applicant's table below.

b(4)

Table 20 Gross pathological findings in animals (F1) Study No. 036-064

Dose (mg/kg)		Control	15	50
Male	(Used for reproduction test) No. of animals	23	9	27
	Normal	23	9	27
	(Used for learning test) No. of animals	23	8	24
	Normal	23	8	24
	(Used for immunological examination) No. of animals	22	7	24
	Normal	22	7	24
Female	(Used for reproduction test) No. of animals	23	9	27
	Normal	23	9	27
	(Used for learning test) No. of animals	21	8	26
	Normal	21	8	26
	(Used for immunological examination) No. of animals	22	8	21
	Normal	22	8	21

There was no treatment-related change in the clinical signs and body weight of F1 dams during the gestation.

F_2 findings:

F1 pregnant dams were sacrificed on day 13 of the gestation to examine the uterine content. The pregnancy data from the applicant's table are presented below.

b(4)

Table 21 Litter data on Day 13 of gestation (mean ± S.D.) Study No. 036-064

Dose (mg/kg) No. of dams	Control 22	15 9	50 22
No. of corpora lutea (A)	15.5±1.9	16.6±3.1	16.0±2.0
No. of implantations (B)	15.2±2.5	15.7±2.2	15.5±1.8
Implantation rate (%) (B/A)	97.3±9.6	95.6±6.7	96.8±5.6
Preimplantation loss (%)	2.7±9.6	4.4±6.7	3.2±5.6
Embryo death			
No. of Postimplantation loss (D)	0.7±0.9	1.3±1.6	0.5±0.9
Postimplantation loss (%) (D/B)	6.1±11.1	9.2±8.2	3.5±5.4
Implantation site (%)	0.0±0.0	0.0±0.0	0.0±0.0
Placental remnant (%)	6.1±11.1	6.8±5.2	3.2±5.4
Dead embryo (%)	0.0±0.0	3.2±6.6*	0.3±1.5
Live embryo			
No. of live embryos (C)	14.5±3.1	14.3±2.7	14.9±1.8
Embryo viability (%) (C/B)	93.9±11.1	91.0±8.1	96.5±5.4

* P<0.05 : Significantly different from control

There was no treatment related change in the embryos for F1 mice.

Summary of segment 3 reproductive safety study:

It is concluded that the treatment at 15 mg/kg dose of MR16-1 caused mortality to F0 dams that could be due to immune reactions in the lungs. No mortality was observed at 50 mg/kg due to the treatment. Gestation, delivery and rearing of pups were not affected by the treatment. Physical, functional, skeletal development of F1 pups and reproductive performance of the second generation of mice (F1) were not affected by the treatment with MR16-1. Litters from two F0 dams treated at 50 mg/kg showed a slight immunosuppression without affecting the antibody response to KLH antigen.

Special toxicology studies

Study title: MR16-1: A passive cutaneous anaphylaxis study of MR16-1 in mice

Key study findings: Data showed only 2 out of 10 animals had positive antibody response to MR16-1 under the experimental conditions. ELISA techniques (reported separately) for the measurement of anti-MR16-1 also showed similar results for the anti-MR16-1 antibody formation in mice.

b(4)

Study no.: study # .036-077
Volume # EDR Module 4, and page #: 1
Conducting laboratory and location: [redacted]

b(4)

Date of study initiation: March 30, 2009
GLP compliance: No
QA reports: yes (x) no ( )
Drug: MR16-1, lot # Z801J23, and % purity: 11.3 mg/mL
Formulation/vehicle: The applicant stated that the purity and formulation was similar to that used for segment 1 study in female rats.

Methods

Doses: 50 mg/kg IV into the tail vein

Study design: A challenge dose of MR16-1 was injected intravenously at 50 mg/kg 4 hours after passively sensitizing male mouse (Crl: CD1 (ICR) skin with intra-cutaneous injection of MR16-1 antibodies. The body weight of mice ranged from 30 to 42 g and mice were 9 to 10 weeks old. Thirty male mice were used for the PCA reactions.

The study design is shown below.

8.11.2 Four-hour Passive Cutaneous Anaphylactic (4-hr PCA) Study

Group	Antigen*	Dose Level (mg/kg)*	Number of Plasma (Animal No. of Plasma)*	Dilution of Plasma	Challenge Antigen	Dose Level (mg/kg)	Dose Volume (mL/kg)	Number of Animals (Animal No.)
1B	PBS	–	10 (10031 – 10040)	×2	MR16-1	50	10	10 (201 – 210)
2B	MR16-1	15	10 (10091, 10093, 10095, 10098 – 10101, 10104 – 10106)	×2	MR16-1	50	10	10 (211 – 220)
3B	MR16-1	50	10 (10151–10160)	×2	MR16-1	50	10	10 (221 – 230)

*: Plasma from animals administered antigen in the previous study (Study No. SBL036-066) [1]

Briefly, plasma samples from mouse injected with MR16-1 was obtained as the source of anti-MR16-1 antibodies to MR16-1. The applicant indicated that these samples were taken from the surviving female mice for the segment 1 reproductive safety study (— .036-066) that is reviewed under reproductive toxicity section. These plasma samples were diluted two-fold with saline and injected to mice at 0.05 mL/site intracutaneously on the back of shaved skin. Four hours after the sensitization of the skin with serum containing antibodies to MR16-1, mice were challenged intravenously with MR16-1 at 50 mg/kg followed by Evans Blue dye injections at 0.1 mL/mouse. Permeability change to the dorsal skin surface due to antigen induced inflammatory mediator release by antigen-antibody reactions was measured by the extravasation of Evan's Blue dye. Diameter of the blue colored patch on the dorsal side of the skin greater than 5 mm was considered as a positive response. Photographs were taken from the blue area of skin from selected animals. Each diluted donor sample was injected to 2 recipient mice. Plasma samples were collected from the fertility and early embryonic development study in female mice. b(4)

Results:

No abnormal clinical observation was reported in the study. Animals from the control group did not show any dye extravasations due to immunological reactions.

Data are shown below from the applicant's table.

b(4)

Table 2 4-hour passive cutaneous anaphylaxis response in mice

Study No. — 036-077

Group*	Passive sensitization: Antigen**	Dose Level (mg/kg)	Route	Group	Challenge: Antigen	Dose Level (mg/kg)	Route	Incidence of positive reaction
1	PBS	-	i.v.	1B	MR16-1	50	i.v.	0/10
2	MR16-1	15	i.v.	2B	MR16-1	50	i.v.	2/10
3	MR16-1	50	i.v.	3B	MR16-1	50	i.v.	1/10

Notes)

PBS : DULBECCO'S phosphate buffered saline

* : Group numbers indicated are from previous study (Study No. — 036-066)

** : Plasma from animals administered antigen in the previous study (Study No. — 036-066)

i.v. : intravenous

b(4)

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The applicant's conclusion was as follow:

From the results stated above, under the conditions of this study, it is inferred that MR16-1 possesses an antigenic potential, i.e. repeated treatment with MR16-1 induces antibodies, which can trigger anaphylactic reactions in the mouse.

Reviewer's comments:

The reviewer related the result for antibody formation to MR16-1 in the mice model using analytical and bioassay (PCA) techniques. It is clear that MR16-1 antibody formation under the conditions of the fertility and early embryonic development study in female mice was minimal. Moreover, both the analytical and bioassay techniques showed a similar result. Results are compared for each animal below.

Mouse #, Plasma Donor	Dose, MR-16-1 mg/kg/IV	Mouse #, Plasma recipient	Challenge dose, MR16-1 mg/kg/IV	PCA	Plasma Donor's antibody titer
10031	control	201	15	-	
10040	control	201	15	-	
10032	control	202	15	-	
10031	control	202	15	-	
10033	control	203	15	-	
10032	control	203	15	-	
10034	control	204	15	-	
10033	control	204	15	-	
10035	control	205	15	-	
10034	control	205	15	-	
10036	control	206	15	-	

Mouse #, Plasma Donor	Dose, MR-16-1 mg/kg/IV	Mouse #, Plasma recipient	Challenge dose, MR16-1 mg/kg/IV	PCA	Plasma Donor's antibody titer
10035	control	206	15	-	
10037	control	207	15	-	
10036	control	207	15	-	
10038	control	208	15	-	
10037	control	208	15	-	
10039	control	209	15	-	
10038	control	209	15	-	
10040	control	210	15	-	
10039	control	210	15	-	
10091	15	211	50	-	-
10106	15	211	50	+	+
10093	15	212	50	-	-
10091	15	212	50	-	-
10095	15	213	50	-	-
10093	15	213	50	-	-
10098	15	214	50	-	-
10095	15	214	50	-	-
10099	15	215	50	-	-
10098	15	215	50	-	-
10100	15	216	50	+	+
10099	15	216	50	-	-
10101	15	217	50	-	-
10101	15	217	50	-	-
10100	15	217	50	+	+
10104	15	218	50	-	-
10101	15	218	50	-	-
10105	15	219	50	-	-
10104	15	219	50	-	-
10106	15	220	50	+	+
10105	15	220	50	-	-
10151	50	221	50	-	-
10160	50	221	50	-	-
10152	50	222	50	-	-
10151	50	222	50	-	-
10153	50	223	50	-	-
10152	50	223	50	-	-
10154	50	224	50	+	+
10153	50	224	50	-	-
10155	50	225	50	-	-
10154	50	225	50	+	+
10156	50	226	50	-	-
10155	50	226	50	-	-
10157	50	227	50	-	-
10156	50	227	50	-	-
10158	50	228	50	-	-
10157	50	228	50	-	-
10159	50	229	50	-	-
10158	50	229	50		-
10160	50	230	50	-	-
10159	50	230	50	-	-

Conclusion of the study:

The above PCA data provide a bioassay for immunogenicity to MR16-1 at 15 and 50 mg/kg in addition to bioanalytical data on the anti-MR16-1 antibody assay reviewed separately. Using both methods, it is concluded that in a series of reproductive safety studies in mice, MR16-1 did not show an overwhelming antigenicity in female mice used in the segment 1 reproductive safety study (only 2 to 3 animals at each dose showed immunogenicity).

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The original BLA for Actemra was reviewed on Aug 2008 and deficiencies for fertility and early embryonic development and pre- and postnatal development assessments were noted. Additionally, the applicant was asked to compare tocilizumab binding and functional effects for human and cynomolgus monkey's IL-6 receptor systems because the long-term toxicity to tocilizumab was conducted in monkeys. The review Division also asked the applicant for a response why carcinogenicity study could not be conducted when a surrogate antibody for the targeted IL-6R was available.

In the Complete Response, the applicant submitted data for both fertility and early embryonic development and pre- and postnatal development studies in mice, including immuno phenotyping, immune responses, developmental behavior and reproductive functions of newborns from treated F0 mice. These studies were conducted using MR16-1, a surrogate antibody to murine IL-6R raised in rats.

Data from reproductive studies showed that the treatment with MR16-1 had no adverse effects on fertility and postnatal developmental in mice. A slight reduction in the immune cell numbers was noted in newborn mice from the treated mice without affecting the ability to form antibodies to KLH antigen. The above effects were noted at 50 mg/kg/IV dose. A lower dose (15 mg/kg) showed mortality to the treatment due to possibly immunogenicity to MR16-1 in mice. Pregnancy category C is recommended for tocilizumab.

In vitro data for tocilizumab showed that it was effective in the reduction of IL-6R mediated cell proliferation for human and monkey IL-6R systems. The long-term toxicity and reproductive safety of tocilizumab in cynomolgus monkeys would address mechanistic effects of the drug. These data also substantiated the applicants selection of cynomolgus monkeys in toxicity studies. An in vivo study in the murine model for IL-6 induced acute protein expression and its inhibition by MR16-1 antibody also justified the use of mice for the assessment of reproductive toxicity to MR16-1 in the mouse model for the Complete Response.

The applicant provided data to justify that long-term treatment of mice with MR16-1 for the assessment of carcinogenic potential would not be possible due to the development of neutralizing antibodies to MR16-1. The reviewer argued that there was a slight chance to develop antibody to MR16-1 at 50 mg/kg up to 3 months of treatment with MR16-1. However, considering the length of treatment for carcinogenicity assessment, immunogenicity, and mortality to low doses, carcinogenicity assessment for the surrogate antibody would be complicated. The reviewer concluded that further carcinogenicity testing

of MR16-1 in mice would not be required. This issue could be partially resolved by stating that immunosuppression achieved through the treatment with tocilizumab could increase the chances of malignancy in the treated population.

The response for the antibody to MR16-1 was determined from the surviving animals at 15 mg/kg. Based on the data, about 10% animals at 15 mg/kg and most of the animals at 0.5 mg/sc developed antigenicity to MR16-1. Data from the PCA reactions also supported the analytical and immunologic assays. Immunogenicity issue could not completely explain why animals at 15 mg/kg died during the study because there was no data for anti-MR16-1 assay in dead animals. It appears histopathology data would provide more reliable information on the cause of deaths to mice. In the absence of severe pathological changes in the lung from the histopathology data, one can speculate that IL-6 receptor antagonism can trigger an acute syndrome that leads to death in the animal at low dose only. This manifestation was not demonstrated in tocilizumab per se in other animal testing. However, the cause of death remains unknown. During the review process, the Pharmacology Toxicology reviewer consulted Dr. Gerry Feldman, Ph.D., the Product Reviewer for this application regarding the acceptability of anti-MRA assay conducted by the applicant using ELISA and ECL methods. Based on the communication with Dr. Feldman, both assay methods were acceptable for the interpretation of studies reported in the complete response.

Overall assessment of the Complete Response to resolve issues are acceptable. Based on the review of the non-clinical data, the reviewer recommends approval of Actemra with recommendations for the package insert.

Internal Recommendations: Nil

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology
OND IO Paul C. Brown 9-16-08
BLA: 125276
Submission date: November 19, 2007
Drug: tocilizumab (Actemra), a recombinant humanized IgG1kappa monoclonal antibody to the human interleukin 6 receptor
Sponsor: Hoffman-La Roche
Indication: reducing signs and symptoms in adult rheumatoid arthritis patients with moderately to severely active rheumatoid arthritis who had an inadequate response to one or more DMARDs or TNF antagonists or in whom DMARDs are not considered appropriate.

Reviewing Division: Division of Anesthesia, Analgesia and Rheumatology Products

Introductory Comments: The pharm/tox reviewer and supervisor found the nonclinical information submitted to be inadequate to support approval of tocilizumab for the indication described above.

The reviewer had the following recommendation:

> The BLA is not approvable due to lack of any non-clinical data on the carcinogenic risks to IL-6R antibody. It is recommended that the Applicant develop species specific monoclonal antibody to IL-6R and conduct fertility, segment 3 reproductive toxicity and carcinogenicity studies.

The supervisor concluded that the lack of fertility and peri/postnatal data precluded approval at this time. The lack of carcinogenicity data was not considered an approval issue.

In a preBLA meeting, the sponsor was asked to provide a peri-postnatal study or explain why such a study was not possible. Fertility was not discussed.

Reproductive and developmental toxicity:
Fertility:
The sponsor has not conducted fertility studies in a relevant species. Unfortunately, a study was conducted in rats with tocilizumab even though tocilizumab does not bind to rat IL-6R. This information is, therefore, not useful. The sponsor states that no potential for effects on fertility were noted in the toxicity studies conducted with tocilizumab. However, the reviewer and supervisor both note that some literature reports have raised the possibility that IL-6 signaling may play a role in early phases of reproduction.

Embryofetal development:
The sponsor conducted an embryofetal development study in cynomolgus monkey which appears to be a relevant species. No teratogenic effects were noted at doses approximately 6.25 times higher than the human dose based on mg/kg. A slight increase

in embryofetal death appears to have occurred at doses that were greater than or equal to 1.25 times higher than the human dose.

Peri/postnatal:
No studies were conducted to assess effects of tocilizumab in the peri/postnatal timeframe. The embryofetal study may have resulted in fetal exposure to tocilizumab past gestation day 50, which was the last day of dosing. However, this study did not include assessment of the fetuses and parent animals out to full term. The sponsor states that IL-6 knockout mice develop normally and cites a literature report in support (Ref # 4026, Kopf et al, Nature, 368, 339, 1994). However, this report only states that interbreeding of IL-6 deficient mice resulted in the normal number of pups, thus ruling out a crucial effect of IL-6 for embryogenesis. This is inadequate information upon which to make an assessment of the effect of IL-6 on peri/postnatal development. More detailed information from the knockout mouse would be required for it to be of use. Other information in the literature suggests that IL-6 singalling may play a role in late stages of fetal development although details are lacking.

Carcinogenicity:
Long term carcinogenicity studies with tocilizumab or any surrogate antibody have not been conducted. Such studies are not possible with tocilizumab due to its lack of relevance in rodent models and since carcinogenicity studies in nonrodent species are not practical. The sponsor conducted some studies with tocilizumab in mouse xenograft tumor models and provided other literature information on the role of IL-6 in tumor surveillance. This information suggests that in some situations IL-6 acts to increase tumor growth and that inhibition of IL-6 signaling may decrease tumor growth in these cases. These studies do not address all tumor types or stages of tumor development and some role can not be ruled out for IL-6 in increasing tumor development in other cases. However, the data provided to date do not appear to show a cause for concern.

Conclusions and Recommendations:
Reproductive and developmental toxicity:
Fertility:
I agree that the sponsor should provide information on the impact of tocilizumab or a suitable surrogate antibody on fertility. I agree that it is appropriate to have this information prior to approval.

Embryofetal development:
No further studies are necessary.

Peri/postnatal development:
I agree that the sponsor should provide information on the impact of tocilizumab or a suitable surrogate antibody on peri/postnatal development. I agree that it is appropriate to have this information prior to approval.

Carcinogenicity:
I agree with the supervisor that the potential risk of increased tumors can be addressed at this time by wording in the labeling noting that the impact on carcinogenic risk from the modulation of IL-6 activity is not known and may increase the risk.

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

SUPERVISOR'S SECONDARY REVIEW
PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER:	**125-276**
PRODUCT:	**ACTEMRA® (tocilizumab)**
INTENDED CLINICAL POPULATION:	**Adults with Rheumatoid Arthritis**
SPONSOR:	**Roche**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia, and Rheumatology Products (HFD-170)**
PHARM/TOX REVIEWER:	**Asoke Mukherjee, Ph.D.**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, Ph.D.** R. Daniel Mellon 8-20-2008
DIVISION DIRECTOR:	**Bob A. Rappaport, M.D.**
PROJECT MANAGER:	**Sharon Turner-Rinehardt**

I. Recommendations

A. Recommendation on approvability

Dr. Mukherjee has recommended that the BLA not be approved at this time due to the lack of any non-clinical data on the carcinogenic risks following treatment with an IL-6R antibody. Dr. Mukherjee specifically recommended that the Applicant use their species-specific monoclonal antibody to IL-6R (homologous product) to conduct fertility, segment 3 reproductive toxicity and carcinogenicity studies. He does not consider the lack of reproductive and developmental toxicity data necessary for approval, as there are potentially some patients who may benefit from the product who are not of child-bearing potential.

From a nonclinical pharmacology toxicology perspective, I concur that there are inadequate data to support approval of this product at this time. However, it is my opinion that the lack of adequate reproductive and developmental toxicity data should be considered an approval issue, since limiting distribution to only individuals who are not of child-bearing potential is not feasible.

Although traditional carcinogenicity studies would be ideal for any drug product, the ability to conduct such studies for this product is likely very limited, and the potential for this product to increase the risk of malignancy can not be clearly eliminated. As such, this concern can be addressed in the product labeling.

B. Recommendation for nonclinical studies

1. Although requested at the time of the preBLA meeting, the Applicant has not conducted peri-natal and post-natal developmental toxicology studies nor have they provided adequate justification for why such studies are not possible. Consistent with the recommendations made at that time, the Applicant should conduct a peri-natal and post-natal developmental toxicology study using either the monkey or the surrogate model prior to approval of this product.

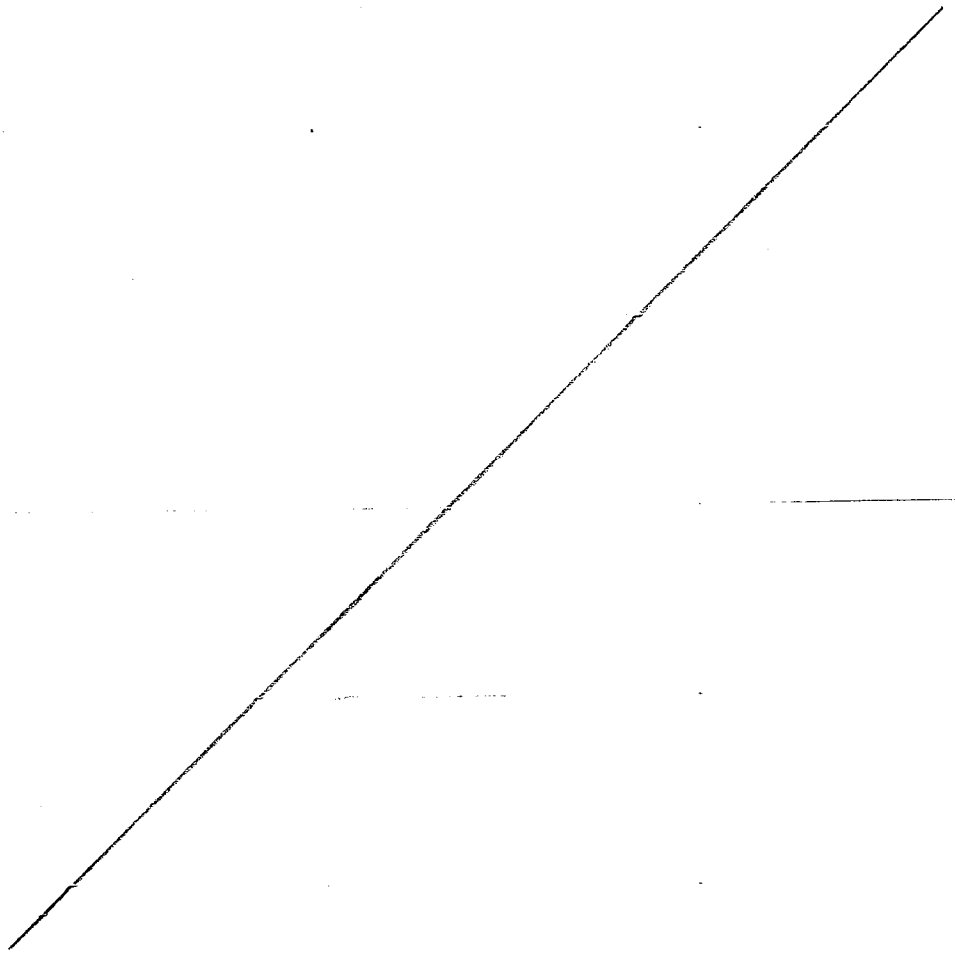
2. Although adequate fertility studies are not feasible in the primate model, the Applicant appears to have a mouse homologous product that can be used to characterize the potential effects on fertility. As the Applicant has not provided adequate justification for why fertility studies in the surrogate model are not possible, such studies should also be completed prior to approval of this product.

3. Although not an approval issue, the Applicant has stated that the homologous protein is not a viable option for carcinogenicity assessment. Specifically, they state that "The MR16-1 antibody is also not an appropriate reagent to be used in long term carcinogenicity studies, as this antibody is a rat monoclonal anti-mouse IL-6R antibody and is considered to be immunogenic in long term in vivo studies in mice." As Dr. Mukherjee's review notes, there are no data to

support this conclusion in the BLA. The Sponsor should be asked to provide data to support this statement in order to support their conclusion that this option for carcinogenicity assessment is not possible. Upon review of these data, this issue may need to be reassessed.

4. Although not an approval issue, the Applicant should submit a summary table comparing the binding affinity of tocilizumab to both the human and monkey sIL-6R and mIL-6R and a comparison of the functional potency of tocilizumab at the human and monkey IL-6R with references to the studies from which the data were obtained. Although not critical for approval due to the existing human experience, these data are necessary to assist in the extrapolation of the findings in the toxicology program to humans, as described in ICHS6.

C. Recommendations on labeling (NOTE: prior to discussion with review team and Applicant, final wording may vary)



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3 Page(s) Withheld

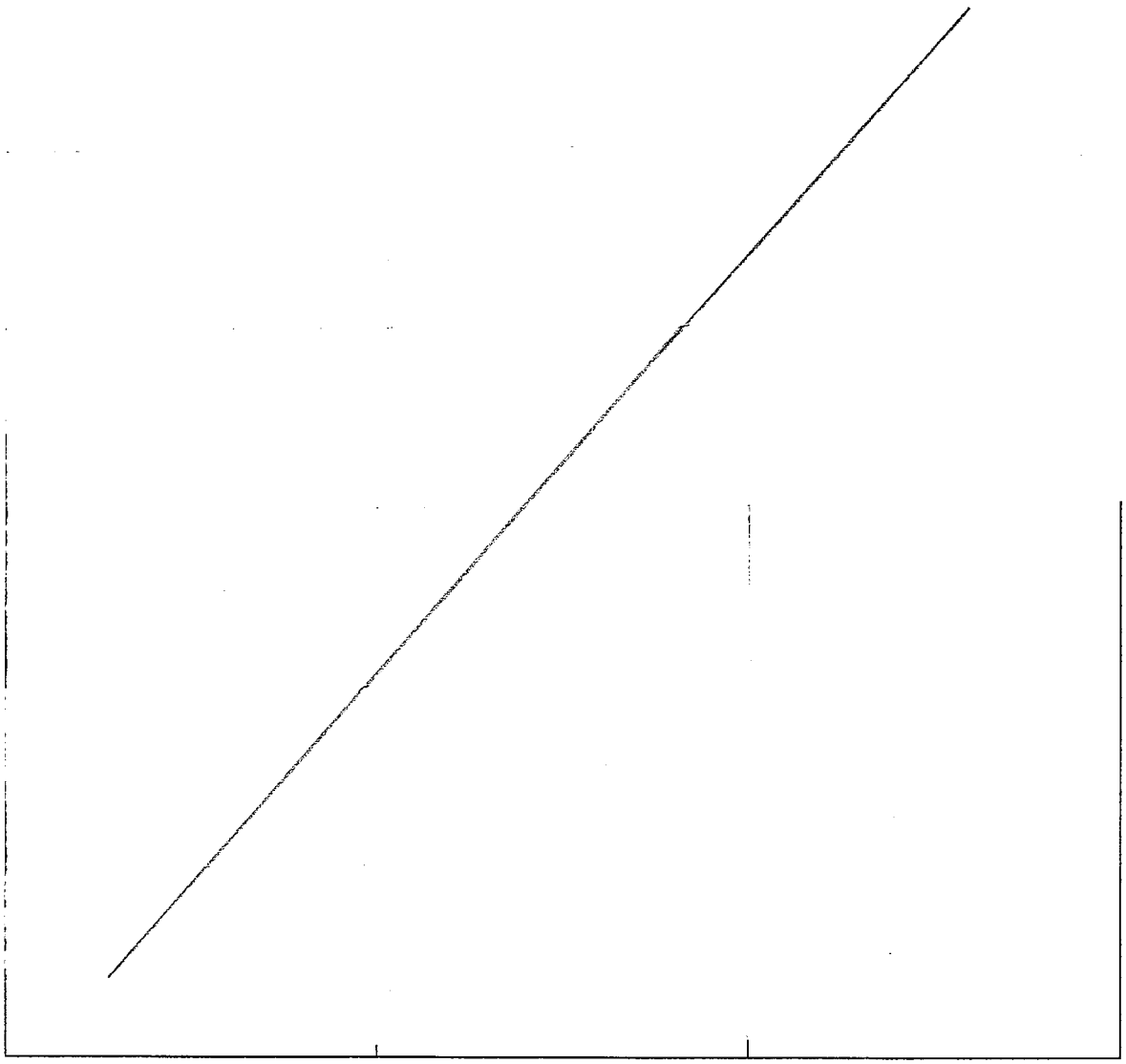
_____ Trade Secret / Confidential (b4)

✓ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Pharm/Tox- 3



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II. Summary of nonclinical findings

A. Pharmacologic activity

Tocilizumab is a recombinant humanized monoclonal antibody that selectively binds to the human interleukin-6 (IL-6) receptor. It is an IgG1 class of antibody that is produced in Chinese Hamster Ovary (CHO) cells and has a H2L2 structure (2 heavy chains and 2 light chains). Tocilizumab binds with comparable affinity to both the soluble and the membrane-bound human IL-6R (2.54 and 2.82 nM, respectively) and therefore inhibits binding of IL-6 to its receptor and subsequent signal transduction events. Tocilizumab does not inhibit TNF-α, IL-1β, IL-15, or IL-2 receptors.

The antibody has no or only a very low level of antibody dependent cellular cytotoxicity (ADCC) and no detectable complement dependent cytotoxicity (CDC). Tocilizumab does not bind to gp130, the accessory protein required for IL-6 to activate cells expressing the IL-6R. Tocilizumab bound to the sIL-6R does not activate gp130 and therefore there does not appear to be any evidence that the product would possess any intrinsic activity to active IL-6 signal transduction events.

Tocilizumab does not bind to rodent or rabbit IL-6R and therefore has no activity in the rat, mouse, or rabbit model. Amino acid sequence homology of the IL-6R in the cynomolgus monkey and human is 97.3%; whereas, homology between the rat and mouse with human IL-6R is only 53 and 54%, respectively. Although data were not provided for binding of tocilizumab to the monkey IL-6R, qualitative functional studies indicate that tocilizumab has activity at both the human and monkey IL-6R but does not have activity at the mouse, rat or guinea pig. Collectively, these data indicate that the most appropriate species for toxicological characterization is the cynomolgus monkey. However, the application does not contain comparative potency data for the effects of tocilizumab at the human and cynomolgus monkey IL-6R (soluble and membrane); therefore, one can not assume that a direct exposure comparison provides a meaningful characterization of the toxicological profile of the product.

Although not an approval issue, the Applicant should submit a summary table comparing the binding affinity of tocilizumab to both the human and monkey sIL-6R and mIL-6R and a comparison of the functional potency of tocilizumab at the human and monkey IL-6R with references to the studies from which the data were obtained. Although not critical for approval due to the existing human experience, these data are necessary to assist in the extrapolation of the findings in the toxicology program to humans, as described in ICHS6.

B. Brief overview of nonclinical finding and safety issues relevant to clinical use

The nonclinical development program submitted by the Applicant was completed primarily in the primate model. Although most of the nonclinical studies were completed with tocilizumab lots from the first generation process, the comparability between generations was provided and deemed acceptable by the CMC review team.

Although the Applicant completed several toxicology studies in non-responder species, these studies are not considered relevant as support for the application by either the Applicant or the FDA. It should be noted that studies in non-relevant species were not requested by the FDA, but were completed by the Applicant at a time when internationally accepted guidelines were either not available or globally recognized.

Repeat-dose toxicology. The pivotal 6-month repeat-dose toxicology study for tocilizumab was completed in the primate model. Cynomolgus monkeys were treated with 0, 1, 10, or 100 mg/kg tocilizumab via intravenous administration once a week for six months. Dr. Mukherjee identified a NOAEL of 1 mg/kg. Plasma concentrations of tocilizumab at this dose were 0.1-0.5-fold the mean human C_{min} at the proposed doses. Dr. Mukherjee noted that at doses of 10 or 100 mg/kg tocilizumab, liver granulomas, and skeletal muscle degeneration were noted.

The doses of 10 and 100 mg/kg in the monkey produced plasma concentrations that were ~25-fold and ~200-fold higher than the mean human C_{min} at the proposed dose of 8 mg/kg. On a mg/kg basis, these doses are only 1.25 and 12.5-fold higher than the proposed clinical dose. Based on these findings, Dr. Mukherjee recommended that liver enzymes, skeletal muscle weakness and creatinine phosphokinase activity be monitored in the clinical population. Although the repeat-dose toxicology study did suggest the potential for alterations in these parameters when dosed at once per week rather than once per month, following discussion with the reviewing rheumatologist, standard of care monitoring should be adequate to identify any concerns in the patient population, should they arise.

Genetic toxicology. The Applicant submitted results from two in vitro genetic toxicology studies which were negative for genotoxic potential (Ames in vitro bacterial reverse mutation assay and an in vitro chromosomal aberration assay in human peripheral blood lymphocytes). It should be noted that these studies were not requested by the FDA, but were completed by the Applicant at a time when internationally accepted guidelines were either not available or globally recognized.

Carcinogenicity studies and labeling recommendations. The Applicant did not conduct carcinogenicity studies with the clinical product formulation; as such studies are not feasible in primates. Both the Applicant and Dr. Mukherjee note that as per ICH S6, "Standard carcinogenicity bioassays are generally inappropriate for biotechnology-derived pharmaceuticals." As Dr. Mukherjee also points out, the guidance document further states that "However, product-specific assessment of carcinogenic potential may still be needed depending upon duration of clinical dosing, patient population, and/or biological activity of the product (e.g., growth factors, immunosuppressive agents, etc.)." Since IL-6 is an immunomodulatory cytokine with diverse activity, it is not known what the effects of chronic blockade of IL-6 would have on tumor surveillance and development. At the preBLA meeting, the Division provided the following comment regarding the need for carcinogenicity assessment:

> Although carcinogenicity assessment may not be feasible for this product, your BLA should include a detailed discussion of why such studies are not possible. In addition, your BLA should discuss the available information you have collected via your own studies as well as those published in the literature regarding the potential impact of IL-6 neutralization on tumor surveillance and tumor development. You should also specifically state how you intend to address the carcinogenicity section of your product labeling.

The Agency agrees that traditional carcinogenicity studies are not feasible for this monoclonal antibody as it does not significantly react with any rodent species. The Applicant also noted that although a surrogate protein has been developed (MR16-1, a rat anti-mouse IL-6R antibody), a bioassay in the mouse would not be feasible because the protein is considered to be immunogenic in long term in vivo studies in the mouse. However, it is not clear from the BLA submission what data this conclusion is based on; therefore, the potential utility of this existing homologous protein for further nonclinical characterization has not been delineated.

The Sponsor should be asked to provide data to support this statement in order to support their conclusion that this option for carcinogenicity assessment is not possible.

In addition, the Applicant states that tocilizumab is not genotoxic nor expected to directly alter DNA, there was no evidence of preneoplastic lesions in the 6-month repeat-dose toxicology study in the primate model, pharmacology studies demonstrate that blockade of IL-6 receptors inhibits proliferation of several tumor cell lines, and IL-6R knock-out models do not report an increased incidence of overt tumors in aged animals (de Hooge et al., 2005; Gomez et al., 2006). Collectively, the Applicant concludes that "These data do not suggest a relevant risk for cancer initiation and progression under therapy with tocilizumab." Although I generally agree with the conclusion of this statement based on the weight-of-evidence, I concur with Dr. Mukherjee that the data is not definitive, as the conclusion is primarily based on data from studies published in the literature that were not conducted under good laboratory practices and that were not specifically designed to characterize the carcinogenic potential of chronic IL-6 blockade. As such, although reassuring, inclusion of the negative study results in the labeling may provide a false sense of security for patients and practitioners. As this is the first product to target IL-6, and the effects of chronic IL-6 blockade have not been definitively characterized, patients should be adequately warned that this immunotherapy may have similar risk for malignancy as other immunosuppressive/immunomodulatory therapeutics. I do not concur with Dr. Mukherjee's conclusion that the Applicant should develop a new homologous protein to further assess the carcinogenic potential of this drug product. The existing homologous protein has been tested in mice for up to 20-weeks, and may be able to be used to assess the impact of IL-6 neutralization. However, although the literature does suggest that neutralization of IL-6 may be of actual benefit in the reduction of some tumors, the potential effects of blockade of IL-6 receptors on the development of malignancies is not fully characterized. Therefore, the labeling should indicate the potential for this product to increase malignancies.

Fertility. Animal fertility studies in a relevant species were not conducted. A fertility and early embryonic development study was completed in the rat model using the clinical product. Interestingly, this study suggested evidence of degeneration of germ cells and decreased spermatozoa in the mid and high dose animals. However, as tocilizumab does not bind to rat IL-6R, the significance of the findings are not clear and the study probably does not provide useful information regarding the potential effect of IL-6R blockade on fertility. I concur with both the Applicant and Dr. Mukherjee that the results of the rat study should not be included in the product labeling.

In the BLA submission, the Applicant proposes that dedicated fertility studies in a relevant species are not needed, since there were no effects on endocrine or reproductive tissues noted in the repeat-dose toxicology studies, that there is no preclinical evidence that IL-6 signaling is involved in the process of reproduction, and the reproductive performance of IL-6 knockout mice is not altered. Dr. Mukherjee does not agree and recommended that the Applicant develop a surrogate species specific anti-IL-6R protein to characterize the effects of IL-6R blockade on fertility and early embryonic development. The Applicant describes the development of a rat anti-mouse IL-6R homologous product, MR16-1, which could be used to obtain fertility data in a relevant species model. Although the Applicant's observations are

reassuring to some extent, they are not direct assessments of the potential impact of tocilizumab on fertility and the Applicant has not provided adequate justification for why such studies can not be completed using the rodent homolog. In the absence of evidence that such studies are not feasible, I concur with Dr. Mukherjee that the Applicant should evaluate fertility via the surrogate model. I also agree that the histopathological observations and literature references proposed by the Sponsor should not be included in the product labeling, as they are only indirect reassurance of a lack of an effect on fertility. I do not agree with the Applicant's conclusion that there are no data in the literature that suggests a role for IL-6 in reproduction; for example, there are preclinical data suggesting a role of IL-6 in testicular development (Potashnik et al., 2005).

Embyro-fetal development. The Applicant submitted results from an embryo-fetal development study conducted in the cynomolgus monkey model. This study did not demonstrate evidence of teratogenicity; however, there was an increased incidence of fetal abortions compared to concurrent controls (incidence in table below).

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

	Group 1 0 mg/kg/day	Group 2 2 mg/kg/day (24 mg/m^2)	Group 3 10 mg/kg/day (120 mg/m^2)	Group 4 50 mg/kg/day (600 mg/m^2))
Toxicokinetic Data[1] **Mean Serum Conc.** **Range Serum Conc.**	N.D.	319.81 [redacted]	1646.45 [redacted]	5813.67 [redacted]
Exposure margin based on mg/kg[2]	--	0.25	1.25	6.25
Exposure Margin based on body surface area comparisons	--	0.08	0.4	2.0
Exposure margin based on mean clinical Cmax[3]		1.7	9	32
Fetal Abortions/Deaths (total)	1/10	1/10	2/10	3/10

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[1] Serum measured 24 hours after final dose (GD50)

[2] Comparison made based on 8 mg/kg dose as proposed by the Applicant (296 mg/m^2).

[3] Clinical C trough values reported by the Applicant in the proposed label are 9.74 ± 10.5 µg/mL 4-weeks after the monthly dose of 8 mg/kg. Clinical Cmax value for the clinical study was reported as 183 ± 85.6 µg/mL. For this table, a conservative approach comparing the clinical Cmax to the animal concentration 24 hours post dose was employed rather than comparison to the human C trough which would produce a potentially false sense of safety.

N.D. = Not detected (< 0.781 mcg/mL)

The Applicant noted that the primate model is known to have a high rate of spontaneous abortions during the early portion of the pregnancy and statistical evaluation of the incidence suggests that up to 2/10 could be attributed to spontaneous events not necessarily related to treatment. Although it is possible that the incidence in the mid dose group may be background, given the apparent dose-related increase in incidence in the mid and high dose groups compared to the concurrent control group, it is also possible that the effects noted at the 10 mg/kg group are treatment-related. As such, I concur with Dr. Mukherjee, that the finding should be included in the product labeling.

The basis for the exposure margins proposed by the Applicant in the product labeling are based on a comparison of the average concentration in the human clinical studies compared to the C trough value of the animal toxicity study (values from the end of the study), as represented in the Applicant's Table 6 reproduced below from the submission (page 5 of nonclinical overview).

Table 6 Animal to human exposure comparison

Species	Cynomolgus Monkey	Cynomolgus Monkey	Cynomolgus Monkey	Cynomolgus Monkey Embryo-fetal study	RA Patients**
Study duration	56 days*	1 month	6 month	GD 20-50	
Dosing frequency	Single Dose	daily	weekly	daily	τ= 4 weeks
Doses	0, 1, 10, 100 mg/kg	0, 2, 10, 50 Mg/kg	0, 1, 10, 100 mg/kg	0, 2, 10, 50 mg/kg	8 mg/kg
Reference	**[3000]**	**[3106]**	**[3107]**	**[3300]**	***
C max (µg/mL)	1971 (F) and 2382 (M)	ND	ND	ND.	183 ± 85.6
C trough (µg/mL) (Male/Female) Values from end of the study	ND	1611/1722 at 10 mg/kg/day (NOAEL) 6833/6121 at 50 mg/kg/day	1935/1936 at 100 mg/kg/week (NOAEL)	1646 at 10 mg/kg/day (NOAEL) 5814 At 50 mg/kg/day	9.74 ± 10.5
AUC τ (µg·h/mL)	ND	ND	ND	ND	35000 ± 15500
C avg (µg/mL)	ND	ND	ND	ND	**52.1**
Margin of safety ****	**11 to 13**	**31** at NOAEL **117** at 50mg/kg/day	**37**	**32** at NOAEL **112** at 50 g/kg/day	

* Single dose study with a 56 day follow-up observation period.

** Tabulated human exposures based on mean predicted human steady-state exposures from the popPK analysis of Phase III data following 8 mg/kg tocilizumab every 4 weeks (a simulation experiment was performed with 48 weeks of treatment) (mean ± SD)

*** See popPK analysis in the Clinical Pharmacology Summary[**9001** hpsum.pdf - 41]

**** The margin of safety for the single dose study is calculated on the basis of a Cmax comparison. In multi-dose studies, PK descriptors differ between preclinical and clinical studies due to differences in the dosing interval. For this reason, a Cavg (Average concentration) was calculated from human AUC. A margin of safety is calculated by comparing Cavg with the C trough values of the relevant safety studies. This comparison is seen as adequate and is a very conservative basis of comparison as it tends to underestimate the actual animal exposure.

C max: maximum plasma concentration
C trough: trough level of plasma concentrations before next dosing
AUC τ: Area under the plasma concentration-time curve within dosing interval
C avg: average plasma concentration during dosing interval τ, calculated as AUC last/τ
ND: not determined

Given the differences in the dosing interval employed in the animal studies and the proposed clinical dosing interval and the lack of direct potency comparisons, a direct comparison of the

exposures between species is difficult. Therefore, I concur with Dr. Mukherjee that the exposure margin is best compared as a pure mg/kg basis.

Peri-natal and post-natal development studies. The Applicant did not submit pre-natal and post-natal development studies (segment III). At the preBLA meeting, the Division provided the following comment regarding the need for segment III studies:

> Your proposed BLA submission does not contain any data regarding the potential pre- and post-natal developmental effects of the drug product (segment III). You should submit studies in either the monkey or a surrogate model to address such effects or provide clear rationale for why such studies are not possible.

As discussed by Dr. Mukherjee, the Applicant submitted their rationale for why such studies are not necessary for this drug product in their original BLA review. Their rationale was summarized and discussed by Dr. Mukherjee.

In a teleconference with the Applicant on August 14, 2008, the Division specifically requested clarification why peri- and post-natal development studies are not possible. The Applicant's response notes that studies with the rodent tocilizumab analogue protein MR16-1 would require a thorough characterization of its PK/PD relationship, which is not available. I concur that such information is essential in order to adequately design and interpret the results of reproductive toxicity studies using MR16-1. However, such information can be obtained and the current lack of such information is not acceptable justification for not conducting the studies.

The Applicant also stated that they believed the embryo-fetal development study conducted in the monkey provided information on exposure to tocilizumab beyond gestation day (GD) 50 due to the slow clearance of the molecule from the body. Although there are levels of exposure out to GD 100 when the fetuses were examined, the exposure was not optimal as the levels were declining from GD 50 to 100, and in the primate, monoclonal antibodies do not cross the placenta in significant amounts until the third trimester, therefore exposures to the fetus would not be deemed optimal. A peri-natal post-natal development study in the primate would obtain information on the effects of exposure from GD 20 to birth (~GD 150-175). In my opinion, the existing primate embryo-fetal development study is not an adequate substitute for the peri- and post-natal development study. As such, there are limited data regarding the potential effects of tocilizumab on the developing immune system and other tissues where IL-6 is reported to have a developmental role. Such data has been completed for approval of other biologic product manufacturers who have pursued rheumatological indications. Specifically, peri-natal and post-natal developmental reproductive toxicology data using either a primate model (modified Segment II/III) or a homologous protein were submitted in support of the BLAs for infliximab, adalimumab, rilonacept, rituximab, and certolizumab pegol. Studies were also completed in support of BLA applications for etanercept and abatacept; however, these products cross reacted with rodents. As such, there is no justifiable reason why such studies should not also be conducted for this product.

Reference List

de Hooge, A. S., van De Loo, F. A., Bennink, M. B., Arntz, O. J., de, H. P., & van den Berg, W. B. (2005). Male IL-6 gene knock out mice developed more advanced osteoarthritis upon aging. *Osteoarthritis.Cartilage., 13,* 66-73.

Gomez, C. R., Goral, J., Ramirez, L., Kopf, M., & Kovacs, E. J. (2006). Aberrant acute-phase response in aged interleukin-6 knockout mice. *Shock, 25,* 581-585.

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER:	**125276**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**11/19/2007**
PRODUCT:	**Actemra**
INTENDED CLINICAL POPULATION:	**Rheumatoid arthritis**
SPONSOR:	**Hoffman-La Roche Inc.**
DOCUMENTS REVIEWED:	**Module 2 and 4 of the eCTD**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia and Rheumatology Products**
PHARM/TOX REVIEWER:	**Asoke Mukherjee** Asoke Mukherjee 8/15/2008
PHARM/TOX SUPERVISOR:	**Daniel Mellon** R Daniel Mellon 8-15-2008
DIVISION DIRECTOR:	**Bob Rappaport**
PROJECT MANAGER:	**Sharon Turner-Rinehardt**

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability:

The BLA is deemed to be a complete response due to lack of any non-clinical data on the carcinogenic risks following treatment with an IL-6R antibody.

B. Recommendation for nonclinical studies:

It is recommended that the Applicant develop species specific monoclonal antibody to IL-6R and conduct fertility, segment 3 reproductive toxicity and carcinogenicity studies. Since MR16-1 was well tolerated in the mouse model after repeated administration, the use of a similar model and species specific antibody could be considered for pursuing issues related to carcinogenic and reproductive risks of tocilizumab.

C. Recommendations on labeling:

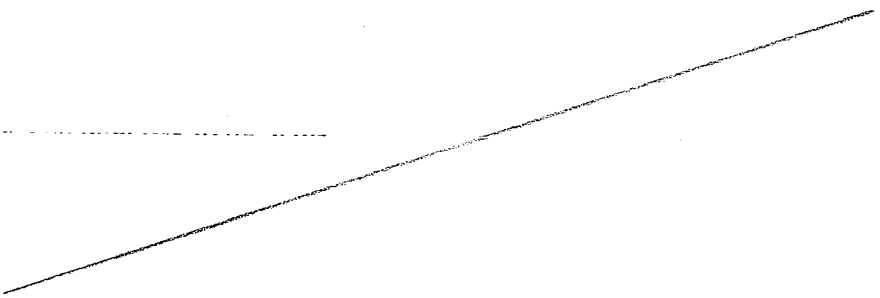
1. Pregnancy Category C for reproductive safety

2. Monitoring liver enzyme, skeletal muscle weakness and creatinine phosphokinase activity in clinical population.

3. If the clinical reviewer considers setting a cancer registry in lieu of non-clinical data for generating clinical data for cancer risk assessment, that needs to be addressed in the package insert.

The proposed Label for pregnancy and nursing should be modified as follows:

PREGNANCY



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1 Page(s) Withheld

_____ Trade Secret / Confidential (b4)

✓ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

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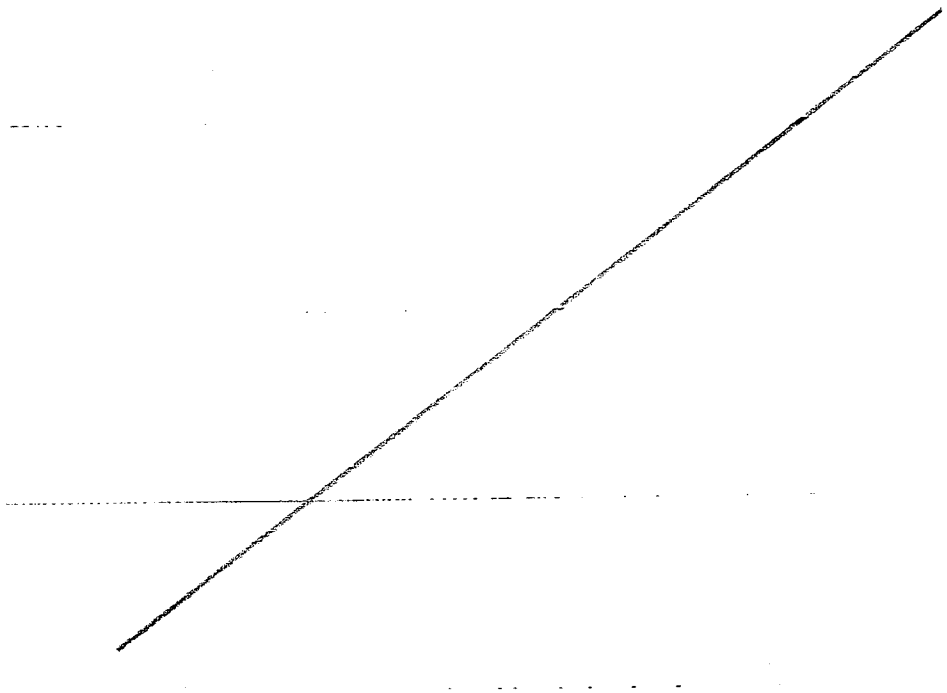

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II. Summary of nonclinical findings

A. Brief overview of nonclinical findings:

Pharmacology and toxicology review of the nonclinical data suggest that the monoclonal antibody to human IL-6R, tocilizumab, is a potent inhibitor of IL-6 by binding to human IL-6 receptor and blocking subsequent signal transduction via that receptor. The BLA is submitted for the marketing of tocilizumab at 8 mg/kg/IV (296 mg/m^2) dose per 4 week. Toxicity study at 1 mg/kg/IV infusion/ week for 6 months did not show organ system toxicity in cynomolgus monkeys. Injection site inflammation was noted in control and treated animals. The clinical observations related to the injection site reactions to the treatment need to be addressed in the package insert.

Above repeat dose toxicity study also showed granuloma in the liver and skeletal muscle degeneration (non-reversible) at 10 mg/kg (120 mg/m^2) and 100 mg/kg (1200 mg/m^2). Therefore, liver and skeletal muscles were target organs of toxicity in the cynomolgus monkeys. Although creatinine phosphokinase activity was not changed, **monitoring of liver enzymes and creatine phosphokinase for the chronic treatment is recommended on the basis of non-clinical data.**

Reproductive toxicity in cynomolgus monkeys at 2 mg/kg/IV during gestation days 20-50 did not show teratogenicity to fetuses. However, at 10 mg/kg (120 mg/m^2, 0.04 x MRHD on mg/m^2 basis) showed reduced viability of fetuses. Therefore, in agreement with the

Applicant's proposed labeling, a Pregnancy category C is recommended for the Pregnancy section of the label.

Adequate information on the fertility, pre-natal and post-natal effect in pregnancy was not provided in the BLA. The review concluded that both fertility, pre and postnatal reproductive toxicity data in relevant species using a species specific IL-6R antibody would be needed to completely characterize reproductive safety.

The potential impact of tocilizumab for the development of tumors is unknown. The Applicant provided information from the literature is not sufficient to rule out that the long term treatment with tocilizumab would not have any effect on the tumor formation of those cells deprived of IL-6 signaling. In addition, cytokines that transduce through gp-130 systems could enhance their activity to induce tumors. Therefore, a direct evidence of the result of a species specific IL-6R antibody in suitable species would be necessary for the assessment of carcinogenic risk to the treatment. The advancement of the methods and techniques to date allows Applicant to develop these non-clinical assays appropriately. In the absence of any data, the reviewer concluded that non-clinical information is insufficient to predict the long term cancer risks to the treatment.

Since monoclonal antibodies mostly circulate in the blood, it is recommended that the dose should be expressed as mg/kg. The animal to human dose ratio and toxicity are shown in the table below.

	Human dose	**Monkey dose**	**Observation in monkey**
mg/kg	8	10	Liver granuloma, skeletal muscle degeneration, fetal deaths
mg/m^2	296	120	
Monkey: Human		0.4 as mg/m^2; 1.25 as mg/kg	

B. Pharmacologic activity:

Tocilizumab is a monoclonal antibody to human IL-6 receptor and structurally based on human IgG_1 immunoglobulin. It was developed for the treatment of rheumatoid arthritis at a maximum dose of 8 mg/kg every 4 weeks. The BLA is submitted for the licensing of the product in the USA. The Sponsor provided information on the role of IL-6 as an inflammatory mediator and progression of certain types of cancer cells. Tocilizumab has been referred to in this BLA as Actemra, RO4877533, and MRA also.

Tocilizumab neutralized human IL-6R present in cell membranes and in the plasma at Kd (binding constant) 2.5 and 0.7 nmol/L, respectively. Data suggest that tocilizumab is more potent to the soluble receptor. Tocilizumab selectively inhibited (bound) IL-6 receptor and it did not show any effect on the transduction of IL-1, IL-15, and TNF.

Tocilizumab inhibited IL-6 and IL-6R complex mediated cell signaling in —— cell line that was refractory to IL-6 or IL-6R alone. The concentration used in the assay was 0.1 ug/mL, the process is known as trans-signaling. Tocilizumab inhibited trans-signaling. b(4)

The Sponsor mentioned the homology of human IL-6R to other species that would provide insight into the selection of appropriate animal models for pharmacodynamic, safety and toxicity.

Human IL6R homology to cynomolgus monkeys and rodents is about 97% and 54%, respectively. Also, tocilizumab did not show an effect on the mouse and rodent cells whereas a mouse IL-6R antibody raised in rats (MR16-1) showed an effect on the inhibition of cell proliferation in mice. These data apparently suggest that cynomolgus monkeys would be appropriate for further non-clinical studies. However, there are limitations for the use of animal models because IL-6 expression under normal conditions may be limited.

To examine and confirm above possibility of selecting monkeys as suitable species, the Sponsor conducted several tissue cross-reactivity studies for Tocilizumab. Data suggest that there were technical inconsistency issues that resulted in variability in the response. However, some of the studies suggest the order of MRA tissue cross-reactivity was Human>Cynomolgus Monkey> Rat. Binding of MRA was mostly related to inflammatory cells, endothelial and epithelial cells.

The Applicant conducted pharmacodynamic studies in collagen-induced arthritis model in cynomolgus monkey (using MRA), wild type mouse model of amyloidosis (using MR16-1) and transgenic mice model expressed human IL-6 (using MRA). Data suggest that MRA did not have a definite anti-inflammatory effect compared to the control in the monkey model at 30 mg/kg/IV. Monkeys also showed neutralization of MRA by anti-MRA antibody. MR16-1 showed an effect in mouse amyloidosis and transgenic model at 0.5 and 2 mg/kg, respectively. These data as well as binding data meet the regulatory requirements of non-clinical pharmacodynamic studies.

Acute safety pharmacology data in anesthetized dogs did not show any cardiovascular or respiratory effect at 66.7 mg/kg, IV. Several cardiovascular parameters in cynomolgus monkeys were not affected at 133.4 mg/kg, IV. Tocilizumab did not show an effect on the ADP induced platelet aggregation in cynomolgus monkeys. No CNS safety data were provided. However, CNS effect is not expected due to low distribution of the drug in adult animals.

C. Nonclinical safety issues relevant to clinical use

1. The Applicant did not conduct carcinogenicity studies. The Applicant was asked to provide rationale why such studies were not possible during the Pre-BLA meeting. The Applicant was also asked to discuss the impact of MRA on tumor surveillance and tumor development. The Applicant argued that IL-6 is involved

in the tumor progression and tocilizumab would prevent cancer. Therefore, carcinogenicity study for tocilizumab was not warranted. Since tocilizumab showed anti product antibodies in rodents, it was not possible to conduct conventional carcinogenicity studies.

Several published papers from the literature were submitted in the BLA. These articles do not reflect that IL-6R inhibition would not have any cancer risks. In fact, an article published by Kong, Isozaki and Sasaki in Gynocologic Oncology 63, 78-84, 1996 stated "It appears that the role of IL-6 in the neoplasm processes is intricate and complicated". The ICH 1997 guidelines on biotechnology product development indicated that "When there is a concern about carcinogenic potential, a variety of approaches may be considered to evaluate risks" and "further studies in relevant animal models may be needed". Therefore, **the reviewer conceived that the carcinogenicity study would be necessary in appropriate species using antibody specific to IL-6R in that species for further assessment of carcinogenic risks to the treatment. Alternatively, a cancer registry needs to be set up to monitor the cancer causing potential of tocilizumab. The package inserts needs to indicate that the carcinogenicity studies in animals were not conducted for tocilizumab. However, immunomodulatory products may cause cancer. Further progression of cancer would be monitored through the cancer registry.**

2. Adequate information on the fertility and pre-natal and post-natal effect in pregnancy was not provided in the BLA.

The Applicant did not conduct pre-natal and post natal segment 3 study in monkeys. The Applicant was asked to provide data or justifications for not conducting the study in the Pre-BLA meeting. The Applicant indicated that normal reproduction in the knock-out mice and absence of reproductive organ toxicity in 6-month and segment 2 studies do not warrant segment 3 study in monkeys. Moreover, long half-life of MRA would expose the fetus to the drug that would provide information on the effect of the drug on labor and delivery.

The reviewer recommends that considering the role of IL-6 as a growth factor, there is a need to generate data for both segment 1 and segment 3 studies in appropriate species. Although the half-life of the drug is about 9 days or longer in monkeys, distribution studies in monkeys showed that most of the drug was removed from tissues soon after 2 days. The Applicant also did not provide data on the distribution of the drug in female reproductive organs. Data in the knock-out models are not confirmatory models for not conducting studies recommended because knock-out models are not totally predictive of biological responses as discussed in the review section for reproductive risks. **The issue of immunomodulatory nature of IL-6 and the role of IL-6 as a growth factor strongly implicate the need to collect data for fertility and late pregnancy for MRA in suitable models. The reviewer recommends that the Applicant develop species specific monoclonal antibody to IL-6R, conduct fertility and segment 3 reproductive safety studies.**

3. The Applicant and the site inspection report indicated that an ——— was used in the process development that has carcinogenic risks. However, it was confirmed during the review that the ——— was not present in the clinical batch that would be marketed if the product is approved. b(4)

4. The Applicant submitted published data on the role of IL-6 for the transcription of COX-2 in mouse osteoblast cells. The role of IL-6R receptor antagonism by tocilizumab on COX-2 inhibition and its relationship to cardiovascular safety is not known. Also, conventional non-clinical models are not predictive for the cardiovascular risks of COX-2 inhibition. **Therefore, close monitoring of clinical data for cardiovascular safety would be necessary for the post marketing safety of the drug if it is approved.**

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

BLA number: 125276
Review number: One
Sequence number/date/type of submission: 000/ Nov 19, 2007, Original BLA
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Hoffman-La Roche Inc.
Manufacturer for drug substance: Chugai Pharma Manufacturing Company Ltd, 16-3 Kiyohara Kogyodanchi, Tochigi, Japan

Reviewer name: Asoke Mukherjee, Ph.D.
Division name: Division of Anesthesia, Analgesia, and Rheumatology Products
HFD #: 170
Review completion date: June 12, 2008

Drug:

Trade name: Actemra
Generic name: tocilizumab
Code name: RO4877533, MRA
Chemical name: Recombinant humanized anti-human interleukin 6 (IL-6 receptor) monoclonal antibody
CAS registry number: 375823-41-9
Molecular formula/molecular weight: 148 kDa
Structure:

Generic Name:	Tocilizumab
Code Number:	Drug Substance: RO4877533 Drug Product: Ro 487-7533/F01 (for 200 mg strength) Ro 487-7533/F05 (for 400 mg strength) Ro 487-7533/F04 (for 80 mg strength) Placebo: Ro 487-7533/F03 (matching 200 mg strength)
Chemical Name:	Recombinant humanized anti-human IL-6R monoclonal antibody
Chemical Structure:	H2L2 polypeptide structure consisting of two light chains and two heavy chains held together by disulfide bonds. Each light chain and heavy chain consists of 214 and 448 amino acids, respectively.
Empirical Formula:	
Molecular Weight:	Approximately 149 kDa
Description:	Colorless to pale yellow liquid

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Relevant INDs/BLAs/DMFs:

IND#	Status	Division	Indication	Status Date	Sponsor
11972	Active	DAARP	Treatment of adult-onset rheumatoid arthritis	11/04/2004	Hoffman-La Roche

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There are no currently approved BLAs for this product.

Drug class: Monoclonal antibody to IL-6 receptor

Intended clinical population: Adult patients with rheumatoid arthritis

Clinical formulation: The formulation from the Applicant's table is shown below.

Table 1 Composition of ACTEMRA Drug Product – 80 mg Dosage Strength

Ingredient	Specification	Quantity/Vial	Concentration (mg/mL)	Function
Tocilizumab	In house specifications	80 mg[c]	20	Active ingredient
Polysorbate 80	Ph.Eur./USP/JP	2 mg	0.5	b(4)
Sucrose	Ph.Eur./USP/JP	200 mg	50	b(4)
Disodium phosphate dodecahydrate	Ph.Eur./USP/JP	5.11 mg[d]	q.s.[d]	pH buffer
Sodium dihydrogen phosphate dihydrate	Ph.Eur./USP/JPE[b]	6.70 mg[d]	q.s.[d]	pH buffer
Total volume adjusted with WFI[a]	Ph.Eur./USP/JP	4 mL	q.s.	b(4)

a. Water for injection.

b. Japanese Pharmaceutical Excipients.

c. May vary dependent on protein content. Calculated on the basis of the actual tocilizumab protein content and actual measurement of tocilizumab drug substance density.

d. pH of solution approx 6.5 and 15 mmol/L for phosphate buffer.

Route of administration: Intravenous infusion

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

Studies reviewed within this submission:

Six-month repeated dose intravenous toxicity study of MRA in cynomolgus monkeys, Tox-02-0169 (J99-0451)

One-month repeated dose intravenous toxicity of MRA in rats, Tox-02-0164 (J97-0141)

MRA monoclonal antibody: Tissue cross-reactivity study in human tissues, study #KEIANKEN97-203, TOX02-0150(JITSU 97-0680)

Cross-reactivity study of FITC-MRA with normal human tissues, TOX04-0068

MRA monoclonal antibody tissue cross-reactivity study in cynomolgus monkey, TOX02-0149

MRA monoclonal antibody tissue cross-reactivity study in rats, TOX02-0148

Cross-reactivity study of MRA (humanized monoclonal IgG1 antibody) with normal human tissues, TOX04-0015

Cross-reactivity study of MRA (humanized monoclonal IgG1 antibody) with normal cynomolgus monkey tissues, TOX04-0016

Study of species cross-reactivity of MRA and MR16-1 in vitro, PHM-02-0275 (JITSU 99-0396)

MRA: in vivo cross-reactivity study in cynomolgus monkeys, KEIANKEN99-206

Cross-reactivity of MRA to cynomolgus monkey IL-6 receptor, PHM02-0192.

A study for effects of MRA administered intravenously on fertility and early embryonic development to implantation in rats, TOX02-0173 (J99-0018)

A study for the effects of MRA on embryo-fetal development in cynomolgus monkeys by intravenous administration, TOX00-0012 (J99-0385).

A study for effects of MRA administered intravenously on embryo-fetal development in rabbits, TOX02-0177A.

A study for effects of MRA administered intravenously on embryo-fetal development in rats, TOX02-0174.

The IL-6 cytokine system in embryonic development, embryo-maternal interactions and carcinogenesis, Seiler P and Plenz G: Eur Cytokine News, 12 (1), 15-21, 2001.

Linkage of IL-6 with neutrophil chemo-attractant expression in virus-induced ocular inflammation, Fenton R.R, Molesworth-Kenyon S, Oakes J.E. and Lausch R.N: Invst. Opthalmol. Vis. Sci. 43 (3), 737-743, 2002.

Impaired neutrophil responses and CD+ helper cell 1 development in IL-6 deficient mice infected with candida albicans, Romani L, Mencacci A, Cenci E, Spaccapelo R, Toniatti C, Puccetti P, Bistoni F and Poli V: J. Exp. Med., 183 (4), 1345-1355, 1996.

IL-6 is required for protective immune response to systemic E. coli infection, Dalrymple S.A, Slattery R, Aud D.M, Krishna M, Lucian L.A and Murray R: Infec and Immunity 64 (8), 3231-3235, 1996.

IL-6 deficient mice are highly susceptible to Listeria monocytogenes infection: correlation with inefficient neutrophilia, Dalrymple S.A, Lucian L.A, Slattery R, McNeil T, Aud D.M, Fuchino S, Lee F and Murray R: Infec and Immun, 63 (6), 2262-2268, 1995.

Impaired antifungal effector activity but not inflammatory cell recruitment in interleukin-6 deficient mice with invasive pulmonary Aspergillus, Cenci E, Menacci A, casagrande A, Mosci P, Bistoni F and Romani L: J. Infec. Dis, 184 (5), 610-617, 2001.

The role of interlukin-6 in mucosal IgA antibody responses in vivo, , Ramsay A.J, Husband A.J., Ramshaw I.A., Bao S, Matthaei K, I, Koehler G and Kopf M: Science , 264, 561-563, 1994.

Single dose intravenous toxicity study of MRA in cynomolgus monkeys, TOX 02-0161.

Investigation of intestinal IgA productivity in six-month repeated-dose toxicity study of MRA in cynomolgus monkeys, TOX02-0207 (JITSU99-0182).

Immuno-toxicological investigation in the six-month repeated dose intravenous toxicity study of MRA in cynomolgus monkeys, TOX02-0205 (JITSU99-0180).

One-month and six-month repeated dose intravenous toxicity studies of MRA in cynomolgus monkeys-analysis of serum creatinine phosphokinase isozymes, TOX03-0002 (J99---18).

Proinflammatory mediators and genetic background in oncogene mediated tumor progression by Russell J.P, Engiles J.B and Rothstein J.L: J. Immunology, 172, 4059-4067, 2004.

Co-operative functions between nuclear factors NFkB and CCAT/enhancer-binding protein B (C/EBBP) regulate the IL-6 promoter in autocrine human prostate cancer cells, Xiao W, Hodge D.R, Wang L, Yang X, Zhang X and Farrar W.L: Prostate 61, 354-370, 2004.

An interleukin-6 gene promoter polymorphism influences the biological phenotype of ovarian cancer, Hefler L.A, Grimm C, Ackermann S, Malur S, Radjabi-Rahat A.R, Leodolter S, Beckmann M.W, Zeillinger R, Koelbl H, Tempfer C.B: Cancer research, 63, 3066-3068, 2003.

Interleukin-6 is essential for the in vivo development of B lineage neoplasm, Hilbert D.M, Kopf M, Mock, B.A, Kohler G and Rudikoff S: J. Expt. Med, 182, 243-248, 1995.

Cytotoxicity of IL-6-PE40 and derivatives on tumor cells expressing a range of interleukin 6 receptor levels, Siegall C.B, Fitzgerald D.J and Pastan I: J. Biol. Chem., 265(27), 16318-16323, 1990.

TGF-B suppresses tumor progression in colon cancer by inhibition of IL-6 trans-signaling, Becker C, Fantini M.C, Schramm C, Lehr H.A. et al.: Immunity, 21, 491-501, 2004.

IL-6 signaling promotes tumor growth in colorectal cancer, Becker C, Fantini M.C., Wirtz S, Nikolaev A et al.: Cell Cycle, 4: 217-220, 2005.

Association of common polymorphisms in inflammatory genes interleukin (IL)-6, IL-8, TNF-alpha, NFkB1 and peroxisome proliferator-activated receptor-Gamma with colorectal cancer, Landi S, Moreno V, Gioia-Patricola L, Guino E et al.: Cancer Research, 63:3560-3566, 2003.

Cross-link between cancer and immune cells: role of STAT3 in the tumor microenvironment, Yu H, Kortylewski and Pardoll D: Nature Review Immunology, 7: 41-51, 2007.

IL-6 regulated in vivo dendritic cell differentiation through STAT-3 activation, Park S.J, Nakagawa T, Kitamura H and Atsumi T et al.: J. Immunology 173: 3844-3854, 2004.

Fibrosarcoma cells transduced with the IL-6 gene exhibit reduced tumorigenicity, increased immunogenicity, and decreased metastatic potential, Mullen C.A, Coale M.M, Levy A.T., Stetler-Stevenson W.G. et al.: Cancer research 52: 6020-6024, 1992.

In vivo and in vitro characteristics of interleukin 6-transfected B16 melanoma cells, Sun W.H, Kreisle R.A, Phillips A.W. and Ershler W.B: Cancer research 52: 5412-5415, 1992.

Paradoxical effects of cytokines in tumor immune surveillance and tumor immune escape, Onfray-Salazar F, Lopez M.N and Naranjo A.M: Cytokine Growth factor reviews, 18: 171-182, 2007.

Reverse mutation study of MRA in bacteria, TOX02-0172 (JITSU97-0035)

Chromosomal aberration assay of MRA in human peripheral blood lymphocytes, TOX02-0171 (JITSU97-0086)

Effects of MRA on gastrointestinal propulsion of BaSO4 in mice, TOX02-0155 (J96-0511)

Effects of MRA on urine volume and electrolyte excretion in rats, TOX02-0157 (J96-0460)

Effects of MRA on respiratory and cardiovascular systems in anesthetized dogs, TOX02-0123 (J96-0520)

Effects of MRA on respiratory and cardiovascular systems and gastrointestinal motility in anesthetized cynomolgus monkeys, TOX02-0127 (J97-0017)

Effects of MRA on electrocardiogram in anesthetized cynomolgus monkeys, TOX02-0158 (J99-0722)

Disposition of MRA in monkeys: Plasma concentration and urinary excretion after single intravenous administration of MRA, ADM02-0133 (J97-0369)

Plasma concentration after single intravenous administration of MRA in female monkeys, ADM02-0157 (J99-0081)

Plasma concentration after repeated intravenous administration of MRA in monkeys, ADM02-0163 (J99-0080)

Plasma concentration after single subcutaneous administration of MRA in monkeys, ADM04-0014

Disposition of ^{125}I-labelled MRA in monkeys (2)- quantative distribution after single intravenous administration of ^{125}I-labelled MRA, ADM02-0203 (J99-0212)

Characterization of metabolites of 125I-MRA (3) characterization of metabolites in plasma after single intravenous administration of ^{125}I-labelled MRA in monkeys,ADM02-0148 (J97-0370)

Disposition of ^{125}I-MRA in cynomolgus monkeys-plasma, concentration, excretion in urine and feces after single intravenous administration of ^{125}I-MRA, ADM02-0202 (J97-0579)

Effect of MRA and IL-6 on the expression of drug metabolizing enzyme in human liver, ADM03-0155

Effects of MRA on isolated smooth muscle, TOX02-0159 (J96-0512)

Preventive effect of MRA on joint destruction in established monkey collagen-induced arthritis, PHM04-0217 (— 09-91) b(4)

Disease amelioration in IL-6 transgenic mice using anti-IL-6 receptor antibody, PHM04-0094 (JITSU01-0889)

Preventive effect of MR16-1 on adjuvant induced mouse amyloidosis model, PHM01-0158S

MRA dissolution constant analysis, PHM00-0041

Measurement of MRA dissociation constant by Biacore, PHM05-0235

Binding of MRA to membrane-bound human IL-6 receptor, PHM02-0151

Dissociation of IL-6 from IL-6/soluble IL-6 receptor complex by MRA, PHM03-0243

Examination of neutralizing activity of MRA against human gp1130 family cytokine receptors, PHM04-0114

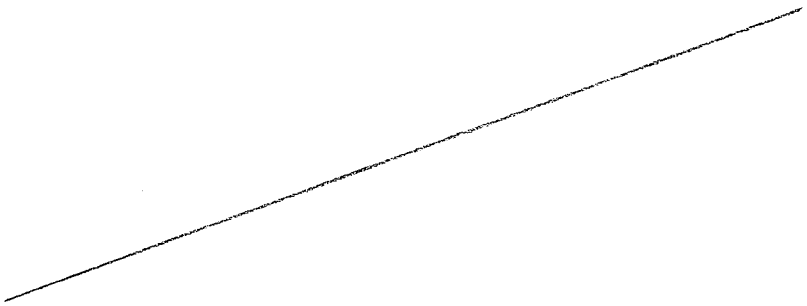
Effect of MRA on signal transduction by TNF alpha, IL-1 beta, IL-15 and IL-2, PHM04-0203

Inhibitory activity of MRA on IL-6 and soluble IL-6 receptor complex signaling, PHM02-0148

Transcriptional induction of cyclooxygenase-2 in osteoblast is involved in interleulin-6-induced osteoclast formation, Tai H, Miyaura C, Pilbeam C.C, Tamura T et al.: Endocrinology, 138 (6): 2372-2379, 1997

Studies not reviewed within this submission:

A written review of following studies were not provided because after a preliminary review these reports or publications did not appear to contribute additional information in relation to relevance of the animal model, repetition of the information already captured from other citations and studies.



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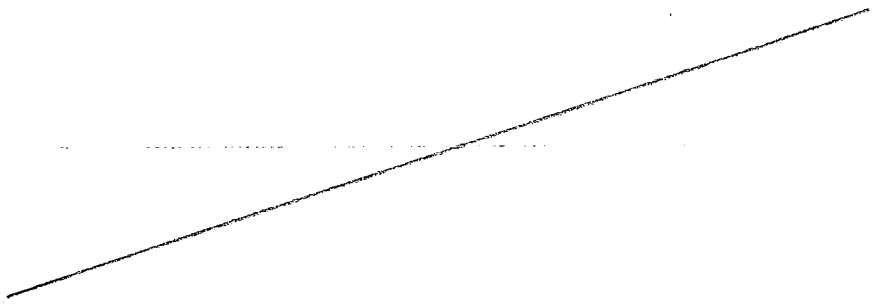

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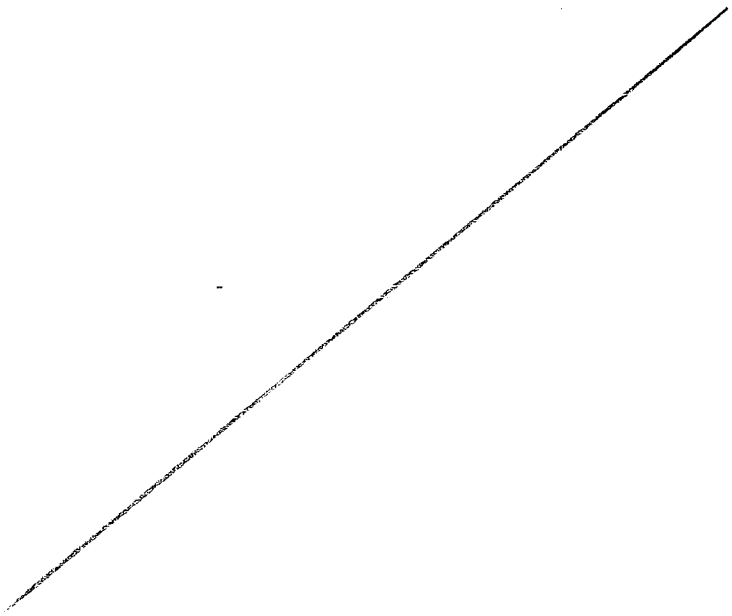

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2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Tocilizumab is a monoclonal antibody to human IL-6 receptor and structurally based on human IgG1 immunoglobulin frame. It was developed for the treatment of rheumatoid arthritis. The Applicant provided information on role of IL-6 as an inflammatory mediator and progression of certain type of cancer cells. Tocilizumab referred in this BLA as Actemra, RO4877533, and MRA also.

Tocilizumab neutralized human IL-6R present in cell membranes and in the plasma at Kd (binding constant) 2.5 and 0.7 nmol/L, respectively. Data suggest that tocilizumab is more potent to the soluble receptor. Tocilizumab selectively inhibits signal transduction via the IL-6 receptor and it did not show any effect on the transduction of IL-1, IL-15, and TNF. Tocilizumab inhibited IL-6 and IL-6R complex mediated cell signaling in ——— cell line that was refractory to IL-6 or Il-6R alone. The concentration used in the assay was 0.1 ug/mL. b(4)

The Applicant examined the homology of human IL-6R to other species to provide insight into the selection of appropriate animal models for pharmacodynamic, safety and toxicity.

Human IL6R homology to cynomolgus monkeys and rodents is about 97% to 54%, respectively. Also, tocilizumab did not show an effect on the mouse and rodent cells whereas a mouse IL-6R antibody raised in rats (MR16-1) showed an effect in mouse. These data apparently suggest that cynomolgus monkeys would be appropriate for further non-clinical studies.

To examine and confirm above possibility of selecting the monkey as an appropriate species, the Applicant conducted several tissue cross-reactivity studies for tocilizumab. Data suggest that there were procedural issues with these studies. However, some of the studies suggest the order of MRA tissue cross-reactivity of human>cynomolgus monkey> rat. Binding of MRA was related to inflammatory cells, endothelial and epithelial cells.

The Applicant conducted pharmacodynamic studies in collagen-induced arthritis model in cynomolgus monkey (using MRA), wild type mouse model of amyloidosis (using MR16-1) and transgenic mice model expressed human IL-6 (using MR16-1) to improve survival. Data suggest that MRA did not have definite anti-inflammatory effect compared to the control in the monkey model at 30 mg/kg/IV. Monkeys also showed neutralization of MRA by anti-MRA antibody. MR16-1 showed an effect in mouse amyloidosis and transgenic models at 0.5 mg/kg and 100 ug/mouse, respectively. A minimal anti MR16-1 antibody was detected in mice after repeated administration.

Above data clearly suggest that there was a need to develop surrogate IL-6R antibody selective to a species and to develop the pharmacodynamic, toxicity in that species to better understand the role of IL-6. Considering the significance of IL-6 in arthritis as indicated in the submission, data submitted for pharmacodynamic characterization of

MRA in monkeys are insufficient. However, binding studies and other pharmacodynamic studies met the regulatory requirements for pharmacology.

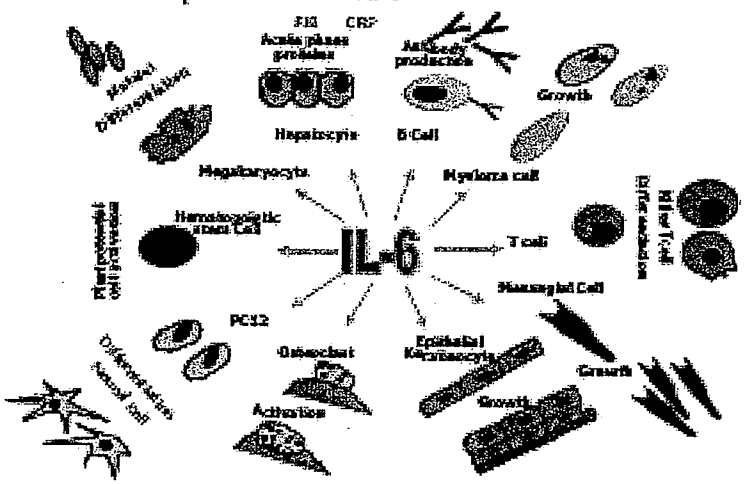
2.6.2.2 Primary pharmacodynamics

Mechanism of action: The role of IL-6 and its inhibition of IL-6 receptor monoclonal antibody (MRA, tocilizumab or Actemra, RO4877533) are summarized below.

IL-6 is a cytokine protein secreted by inflammatory cells, myeloma cells, vascular endothelial cells, T and B-lymphocytes. Published data related IL-6 to progression of myeloma as well as inflammatory conditions. IL-6 is involved in several secondary events in arthritis including expression of fibrinogen, C-reactive protein and serum amyloid proteins.

The Applicant developed humanized antibody to IL-6R based on IgG1k. The BLA was submitted for the treatment of arthritis. The Applicant provided a schematic to illustrate the role if IL-6 in cellular systems.

Figure 1 Pleiotropic effects of IL-6



IL-6 binds to either the IL-6 membrane receptor or the soluble receptor to form an IL-6 receptor complex. The complex further binds to two molecules of another glycoprotein known as gp-130 (known as dimmer) before it sets on further events of gene expression. IL-6R activation also involves STAT-3 transcription factor phosphorylation that led to immunosuppression and lack of tumor surveillance. A citation by Yu et al. Nature Reviews Immunology, 7, 41-51, 2007 provided more insight to the process. When IL-6R (soluble or membrane bound) and tocilizumab binds, the receptor antibody complex fails to activate gp-130 protein and prevents IL-6 receptor activation process.

The binding of tocilizumab to whole cell IL-6R was investigated in COS-7 cells transfected with human IL-6R. Flow cytometric data confirmed the binding of tocilizumab to its receptor.

Since gp-130 protein is involved in the activation of several receptor systems, any cell that lacks IL-6 receptor can get activated by IL-6 if soluble form of IL-6 receptor is present in the plasma and binds to IL-6. This process is known as trans-signaling. Tocilizumab inhibited trans-signaling up to 0.1 ug/mL in ——— cell line in vitro. IL-6 or sIL-6 R alone did not induce proliferation of the cell. However, IL-6 (100 ng/mL) and IL-6R (500 ng/mL) complex induced proliferation of the cell. MRA inhibited the proliferation of cell induced by IL-6 and IL-6R complex at 0.1 ug/mL and higher concentrations. b(4)

MRA binding of membrane bound IL-6R in myeloma cell line ——— with a Kd of 2.54 to 2.82 nmol/L. The Kd for soluble receptor and antibody binding is 0.713 nmol/L. Data indicated that tocilizumab binds strongly to the soluble receptor. Tocilizumab did not bind to IL-11R, oncostatin M (OSM) receptor, leukemia inhibitory factor receptor (LIF) receptor and ciliary neurotropic factor (CNTF) receptor. Tocilizumab also did not inhibit direct effect of IL-1, IL-15 and TNF response. Above data suggest that tocilizumab is selective to IL-6 receptor. b(4)

The homology of amino acid sequences in humans and animals were summarized on page 35 of non-clinical written summary of the submission. It is indicated that human IL-6R concurs at 97.3% in cynomolgus monkeys. In contrast, rodents showed only 54% homology. These data suggest that rodent models are not appropriate for the non-clinical development of tocilizumab. Indeed, the tissue cross-reactivity studies reviewed below (JITSU99-0396) support the conclusion that tocilizumab does not bind to IL-6R in mice as shown from the Applicant's table below.

VI. TABLES

Table 1. Species cross-reactivity of MRA and MR16-1.

Antibody	Mouse	Rat	Human
MRA	–	–	+
MR16-1	+	–	–

Drug activity related to proposed indication:

1. Preventive effect of MRA on joint destruction in established monkey collagen-induced arthritis, study # PHM04-0217.

Female monkeys were sensitized with bovine type II collagen in complete Freund's adjuvant at 8 mg in 2 mL by intracutaneous injection in the dorsal area on days 1 and 21. Mean oval area of proximal interphalangeal joints was measured as a measure of

inflammation. Inflammation in the phalangeal joint was one of the criteria of randomization subsequent to local injection. Furthermore, X-ray and body weights were taken to determine baseline conditions. The dose groups are shown below.

11.11 Study Design

There were 1 control group and 1 test substance group.

Group	Treatment	Dosage (mg/kg/day)	Dose Volume (mL/kg/day)	Concentration (mg/mL)	Number of Animals (Animal No.) Females
1	Control	–	0.556	–	6 (1 to 6)
2	MRA	30	0.556	54.0	8* (7 to 14)

A dose of 30 mg/kg/IV was chosen to avoid neutralization of MRA by anti-MRA antibody. A total of 4 injections were given at once a week starting day 37 of sensitization so that the treatment was initiated in the established phase of the experimental arthritis. X-ray changes were observed on days 34, 37 and 66 of sensitization. Animals were sacrificed on day 66 after sensitization. The body weight was recorded once a week during dosing and on the day of necropsy. Animals were observed twice for clinical signs on dosing days. Longitudinal and transverse axes of phalangeal joint of fore and hind limbs were measured several times before and during the treatment to determine the anti-inflammatory effect. Bone specific alkaline phosphatase activity was determined several times as a bone metabolic marker. IL-6, IL-6 receptor, MRA levels, anti-MRA titers were determined. Histopathology of bone of digits was conducted.

Results:

Anti-MRA was detected in 7 animals and MRA levels were not detected in animals # 10 and 11 from the third week of dosing. The Applicant stated that anti-inflammatory activity of the joint was less than other animals in monkey #10 and #11 due to neutralization of MRA. The Applicant stated that the X-ray score analysis did not show measurable changes because of variability of data even before the treatment. IL-6 expression was increased in animals after MRA treatment.

X-ray data are shown from the Applicant's table below.

b(4)

Study No. 105-51

Table 4-1 X-ray findings in female cynomolgus monkeys

Group	Animal No.	Findings	Day 1 of dosing: Forelimb Left	Forelimb Right	Hindlimb Left	Hindlimb Right	Total	Day 29 of dosing: Forelimb Left	Forelimb Right	Hindlimb Left	Hindlimb Right	Total
Control	1	Arthritis	2	0	3	1	6	3	0	4	4	11
	2	Arthritis	0	2	1	4	7	0	2	1	4	7
	3	Arthritis	3	4	4	4	15	4	4	4	4	16
	4	Arthritis	1	1	1	0	3	3	3	3	4	13
	5	Arthritis	0	0	2	1	3	0	0	2	1	3
	6	Arthritis	3	2	3	4	12	3	2	3	4	12
MRA 30 (mg/kg)	7	Arthritis	0	0	0	0	0	0	0	0	0	0
	8	Arthritis	4	3	4	4	15	4	4	4	4	16
	9	Arthritis	0	1	1	1	3	1	0	0	0	1
	10	Arthritis	4	3	4	4	15	4	3	4	4	15
	11	Arthritis	2	2	2	4	10	4	4	4	4	16
	12	Arthritis	0	0	2	2	4	0	0	1	2	3
	13	Arthritis	2	1	3	2	8	2	3	3	3	11
	14	Arthritis	0	1	4	3	8	0	1	4	3	8

Arthritis: Bone destruction on joint areas, accompanied by the disappearance of articular cavity and/or the degeneration of articular tissue.
The score means the number of fingers at which the finding was observed.

Data for the area of the joint are shown from the Applicant's table below.

b(4)

Study No. 105-51

Table 5-1 Mean oval area of the proximal interphalangeal joints in female cynomolgus monkeys

(unit: mm²)

Group	Animal No./Day	Days of sensitization*: Day -1	Day 22	Day 84	Days of dosing**: Day 1	Day 8	Day 15	Day 22	Day 29
Control	1	26.76	25.74	26.87	41.12	42.80	42.05	43.08	42.52
	2	24.71	27.26	30.06	30.80	30.05	30.02	30.02	29.92
	3	28.57	26.65	40.34	42.93	41.11	41.19	40.40	39.52
	4	22.65	22.75	27.71	31.05	35.79	42.88	42.58	41.87
	5	28.49	25.11	26.65	27.22	27.86	28.13	28.04	25.05
	6	31.62	28.25	32.78	33.29	32.06	31.25	31.70	31.27
MRA 30 (mg/kg)	7	29.15	25.05	27.65	27.43	27.34	25.85	23.55	23.50
	8	24.77	30.46	42.00	41.02	38.15	34.09	31.12	30.42
	9	21.42	22.62	28.41	26.95	25.19	24.98	22.25	22.81
	10	22.36	23.24	40.81	43.79	41.07	36.34	34.87	33.51
	11	24.47	24.10	33.10	41.92	46.45	47.03	47.90	47.35
	12	24.82	25.65	28.16	27.92	26.63	26.27	25.06	25.02
	13	28.72	23.25	35.11	40.50	44.54	42.33	39.52	37.84
	14	25.42	27.09	30.76	31.02	29.52	28.42	27.15	27.20

Notes) *: The day of the first sensitization was designated as Day 1 of sensitization.
**: The first day of dosing was designated as Day 1 of dosing.
Days 1, 8, 15, 22, and 29 of dosing corresponded to Weeks 1, 2, 3, 4, and 5 of dosing, respectively.

Data for mean oval area of the inflamed joint in above table showed no increase in the area in animals # 7, 8, 9, 10 and 12 between days 1 and 29 of treatment. However, a similar trend was also observed in control animals. Therefore the lack of inflammatory response in treated animals was not conclusive and did not result from efficacy of MRA.

Data for MRA levels from Applicant's table are shown below.

Table 9-1 Plasma concentrations of MRA

(Unit: μg/mL)

Group	Animal No./Day	Days of dosing*					
		Day 1	Day 4	Day 8	Day 15	Day 22	Day 29**
MRA	7						
30 (mg/kg)	8						
	9						
	10						
	11						
	12						
	13						
	14						

b(4)

Notes) N.D. < 0.781 μg/mL

*: Days 1 and 4 of dosing were included in Week 1 of dosing, and Days 8, 15, 22, and 29 of dosing corresponded to Weeks 2, 3, 4, and 5 of dosing, respectively.

**: Day 29 of dosing was day of necropsy.

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Animal #10 showed efficacy despite neutralization of MRA. Therefore, it is difficult to conclude if the effect in the treated animals was due to MRA or simply due to variability of the response.

Applicant's data for histopathology were also inconclusive due to variability of responses between the control and treated arm.

Overall, quantitation of the efficacy for the determination of anti-inflammatory activity is questionable due to variability of responses. Therefore, it was concluded that MRA had no anti-inflammatory effect in this model.

2. Disease amelioration in IL-6 transgenic mice using anti-IL-6 receptor antibody, study # PHM04-0094 (JITSU01-0889).

The Applicant developed a mouse transgenic model that expressed human IL-6 excessively (IL-6Tgm). In this mouse model several characteristics of Castleman's disease e.g. IgG1 plasmacytosis, mesangial proliferation, nephritis, anemia, thrombocytopenia and autoantibody production were generally observed. This model was considered to be an experimental model for Castleman's disease as well as IL-6 dependent autoimmune model. The role of mouse IL-6R antibody raised in rats (MR-16-1) was investigated. Il-6Tgm mice received rat anti-DNP antibody (KH5) and served as the control for Castleman's disease.

The applicant felt necessary to investigate the effect of IL-6R in another species. The Applicant developed a mouse specific antibody in rats (MR-16-1) that binds to mouse membrane bound and soluble IL-6 R. Tocilizumab does not bind to IL-6R in mice.

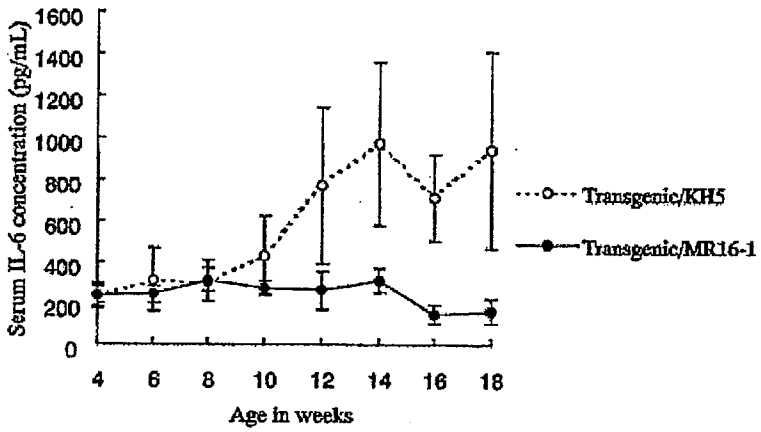
The in vitro activity of MR16-1 and tocilizumab in mouse and human IL-6R systems was compared for further determination of the specificity of monoclonal antibodies.

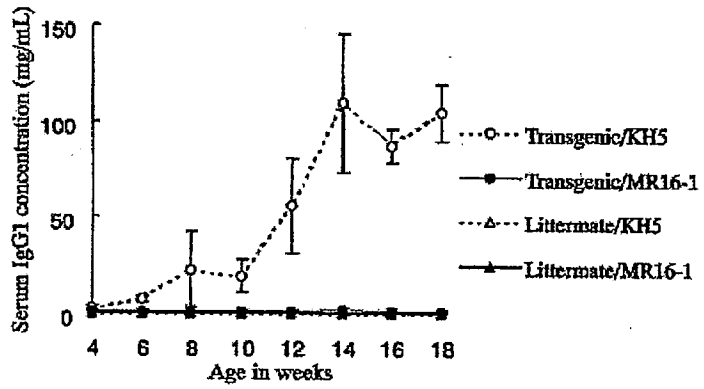
Animals were treated at 2 mg/mouse IV at 4 weeks of age. Thereafter, MR16-1 was administered at 100 ug/mouse subcutaneously twice a week up to 18 weeks of age. Total number of treatments was 28 at 100 ug/mouse. A slight decrease in the body weight was observed at about 8 weeks of age in the MR16-1 treated mice compared to the appropriate control. About 80% survival was noted in IL-6 expressed transgenic mice. However, MR16-1 treated animals survival was 100% up to week 18. Urinary protein was not observed in MR-16-1 treated animals. Improvement in the RBC counts, platelet counts was noted. The WBC counts varied in the control and transgenic mice treated with MR16-1. Serum IL-6 concentrations and IgG1 titers were substantially reduced in MR16-1 treated mice. Data are shown from the applicant's figures and table below.

Table 2. Changes in serum anti-rat IgG antibody titer.

	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks	14 weeks	16 weeks	18 weeks
Transgenic /KH5 (n=7)	0.15	0.78	1.69	7.41	>100	>100	>100	>100
Transgenic /MR16-1 (n=6)	0.22	0.34	0.45	0.38	0.43	0.50	0.39	0.30
Littermate /KH5 (n=5)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	3.55
Littermate /MR16-1 (n=6)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.20

The mean antibody titers (Units/mL) are shown. In groups in which there were individuals that exceeded 100, >100 is shown.
N.D. : not determined


Serum IL-6 concentration (pg/mL)
1600
1400
1200
1000
800
600
400
200
0
4
6
8
10
12
14
16
18
Age in weeks
Transgenic/KH5
Transgenic/MR16-1


Serum IgG1 concentration (mg/mL)
150
100
50
0
4
6
8
10
12
14
16
18
Age in weeks
Transgenic/KH5
Transgenic/MR16-1
Littermate/KH5
Littermate/MR16-1

Based on the survival and laboratory parameters, it was concluded that MR16-1 treated hIL-6 transgenic mice showed increase in the survival and improvement of disease conditions.

However, no increase in anti-rat antibody titer was observed in Il-6 transgenic mice treated with MR16-1. The sponsor concluded that MR16-1 was immunologically tolerated in the transgenic mice.

3. Preventive effect of MR16-1 on adjuvant induced mouse amyloidosis, study # PHM01-0158S.

Amyloid proteins derived from acute phase proteins are deposited in several organs in mice injected with Amyloid enhancing factor (AEF) with adjuvant. This is considered to be a model that expressed some of the characteristics of rheumatoid arthritis due to autoimmunity.

Mice were injected with MR16-1 (mouse IL-6R antibody raised in rats) at 100 mg/kg IP. The treatment completely prevented amyloid deposition on weeks 2 and 5 in the histological examinations in liver, spleen and kidney. A similar response was noted at 0.5 and 2 mg MR16-1 in 2 weeks.

Summary of Primary Pharmacodynamics.

The Applicant examined the effect of MRA in an auto-immunity model in cynomolgus monkeys. However, the anti-inflammatory effect was not substantiated when compared to the control and anti-MRA antibody production was also noted in monkeys.

Transgenic mice expressing human IL-6 showed improvement of survival at 100 ug/SC/week x 18 weeks of MR16-1 (murine antibody to IL-6R in rats). The model was considered for Castleman's disease. In another experiment in mice, amyloid protein deposition was inhibited at 0.5 mg/IP injection (single injection) in liver, spleen and kidney. Deposition of amyloid protein is considered as secondary to acute phase protein secretion in arthritis.

Tissue cross-reactivity:

1. **MRA monoclonal antibody: Tissue cross-reactivity study in human tissues, study #KEIANKEN97-203, TOX02-0150(JITSU 97-0680)**

The study was conducted at [ ] in May 1997. The study was conducted in compliance with OECD guidelines and an audited report was submitted. Tissue cross reactivity to 3 human donor tissues was conducted for MRA IL-6 receptor monoclonal antibodies. Tissues were collected from the external sources and frozen for the preparation of frozen sections using a cryostat microtome. Tissues were collected from patients diagnosed for cancers or cardiovascular diseases, where tissue was surgically removed as well as from normal subjects. A list of donor summary was

b(4)

provided on page 37 of the report. Tissues were fixed in acetone at room temperature. The test antibody was conjugated with FITC (Fluorescein isothiocyanate) that can be excited at 488 nM to develop fluorescence for detection. Mouse anti-FITC monoclonal antibody was used for determination of specificity of binding to tissues. Antibody to mouse IgG raised in rabbits and goat were used for non-specificity of binding of mouse antibodies. Tissues were stained with hematoxylin and eosin to confirm the suitability of the use of tissues.

Five sections were obtained from each tissue and three sections were treated with IL-6 antibody at 1:10, 1:50 and 1:500 dilutions. The negative control had no test antibody.

Results of the study are shown below in a tabular form (Table 1 below).

2. **Cross-reactivity study of FITC-MRA with normal human tissues, study # TOX 04-0068.**

MRA was conjugated with FITC to make FITC-MRA. Tissue cross-reactivity to cryosections of normal human tissues was determined. Two concentrations of FITC-MRA were used e.g., 5 and 20 ng/mL. The study was conducted at ⌜ ⌟ according to GLP. The positive controls in the assay were IL-6 receptor protein (IL-6R), TIB-196, human myeloma that expressed IL-6 receptor and cryosections of colon from Crohn's disease that expressed IL-6R. Jurkat cells (T-lymphocyte lymphoma cell line that does not express IL-R and PTHrP (hypercalcemia of malignancy peptide) were used as a non-IL-R expressing negative control. The assay without the test antibody was a negative control for the assay. FITC bound antibody of IgG1 with a different specificity to antigen was used as negative control of the MRA antibody. b(4)

Results are shown as the positive response to binding of FITC-MRA to several positive controls and a lack of binding to the negative control. The panel of tissues was selected on the basis of recommendations as per the "Points to Consider Guidelines in the Manufacturing and Testing Monoclonal Antibodies" document published by the FDA. Specific staining was observed in the cytoplasm and membrane in mononuclear cells. Tissue cross reactivity data is shown below in Table 1 below.

3. **Cross-reactivity study of MRA (humanized monoclonal IgG1 antibody) with normal human tissues, TOX04-0015.**

The study was conducted at ⌜ ⌟ during Nov 19, 2003 to Feb 2004 according to GLP. Human cryosections were used for the cross-reactivity study at 10 and 200 ug/mL. Tissues were obtained from human biopsy or autopsy samples. Procedure for the cross-reactivity study was not exactly similar to other studies reviewed here particularly immunoperoxidase-related staining was used. The positive control was human IL-6 receptor adhered to UV activated resin (recombinant human IL-6 receptor b(4)

was used). PThrP was used as a negative control protein. —— cell line was used as a positive control due to IL-R expression. b(4)

Based on results of the binding study of MRA, no specific binding to MRA was observed in human tissues. Endothelial, epithelial, hematological, dendritic and neural cells showed binding to murine IL-6R antibody as positive controls. Jurkat cells were used as a negative control due to lack of IL-6R. Omission of the test antibody served as an assay control. Antibody with different antigen specificity was served as a negative control for the antibody.

The study did not show any binding of MRA to human tissues. However, control preparations showed positive or negative response as anticipated. Data are shown in Table 2 below.

4. **MRA monoclonal antibody tissue cross-reactivity study in cynomolgus monkey, TOX02-0149**

The study was conducted at ⌜ ⌟ according to OECD guidelines. Tissues were frozen and cryosections were prepared. MRA was conjugated with FITC. Frozen sections were obtained from male and female monkey tissues and incubated with the antibody at 1:50, 1:10 and 1:500 titer values. Tissues without antibodies served as the negative control. Esophagus tissues treated with or without anticytokeratin antibody was used as a positive control for the immunohistochemistry analysis. Tissues were stained with hematoxylin and eosin to examine the suitability of its use in the cross-reactivity studies. b(4)

Results of the study showed tissues stained with H&E were suitable for cross-reactivity study. Data suggest that positive antibody binding was noted in endothelial cells, epithelial cells, macrophage (cytoplasm), cytoplasm of neutrophil and membrane of lymphocytes. Tissues where above cells were involved as a positive response to specific tissue cross-reactivity are shown in Table 1 below.

5. **Cross-reactivity study of MRA (humanized monoclonal IgG1 antibody) with normal cynomolgus monkey tissues, TOX04-0016.**

The study was conducted at ⌜ ⌟ between Dec 18, Feb 5, 2004. The study was conducted according to GLP guidelines. b(4)

The binding to IL-6R by MRA, a monoclonal directed against human IL-6 receptor (HuIL-6R), was determined at 10 and 200 ug/mL. The Applicant stated that tissues used for the cross-reactivity study in the monkey were similar to that used in the human tissue cross-reactivity study. The positive controls were HuIL-6 adhered to UV-activated resin slides and —— cell line. The negative controls were PTHrProtein that does not bind to IL-6R antibody and Jurkat cell line that lacks expression of IL6R. Omission of the b(4)

antibody (test substance) and inclusion of IgG with different antigenicity also served as negative controls.

Positive control showed positive staining and negative control showed a lack of binding with the test substance.

Result of the experiment showed no binding and cross-reactivity in monkey tissues (see Table 2 below).

6. **MRA: in vivo cross-reactivity study in cynomolgus monkeys, report # KEIANKEN99-206, TOX02-0151.**

The study was conducted at [redacted] between Sept 6, 1999 and May 26, 2000. The study was conducted according to OECD guidelines. b(4)

In vivo cross-reactivity of MRA was investigated in cynomolgus monkeys. The test substance was infused intravenously at 10 mg/kg/day as a single dose to one male and two female monkeys. The age of the animal was approximately 5 years and weighed between 3 to 4.9 kg. Animals were inspected for clinical signs. ECG was recorded two weeks prior to the dosing, 1 and 2 hour post dose. Animal # A588F was sacrificed one hour post dose, animal # Y44F was sacrificed 48 hour post dose and animal # A607M was scarified 14 days post dose. Macroscopic examinations of several organs were conducted. Tissues were treated with FITC- conjugated anti-human IgG for interactions with MRA. These tissues were frozen and cryosections were prepared. Human lymph node sections were used as a positive control. All tissues were treated with anti-human IgG-FITC at various concentrations. Negative controls received the buffer only. The binding of MRA and anti-IgG-FITC was visualized by alkaline phosphatase-related fluorescence.

Data presented in table 4 of the report suggest that cardiac, liver, lung, lymph node, spleen and kidney tissues from monkeys were moderate to strongly positive for the binding to MRA within 2 hours of the treatment. No binding to conducting tissues in the heart was noted. Also, ECG report did not show any abnormality. Detailed examination of the histology slides revealed that the binding of MRA in monkey tissues were reflection of endothelial, epithelial and mononuclear cells of tissues. Most of the negative tissues showed negative results. Data are shown in Table 2 below.

7. **Cross-reactivity of MRA to cynomolgus monkey IL-6 receptor, PHM02-0192.**

The study was conducted at Chugai Pharmaceutical Co. Ltd., between July 10, 2002 and Sept 27, 2002.

The study was conducted for the determination of MRA on the T-lymphocyte proliferation induced by phytohemagglutinin. The study would signify the role of IL-6R on the cell function and antagonism by MRA in monkey T-lymphocytes. Peripheral blood was collected, T-lymphocytes were purified and suspended to 10^6 cell/mL. Cells

were treated with PHA and IL-6 for proliferation in the presence of ^{3}H-thymidine. The effect of MRA treatment on cell proliferation was determined at 0.2, 1, 5, and 25 ug/mL.

Data suggest that MRA inhibited ^{3}H-thymidine incorporation at 1, 5 and 25 ug/mL.

Based on the data it was concluded that MRA cross reacted to monkey lymphocyte IL-6R. Data are shown in Table 2 below.

8. MRA monoclonal antibody tissue cross-reactivity study in rats, TOX02-0148

The study was conducted at [redacted] in 1997 according to OECD guidelines. b(4)

Tissue cross-reactivity to MRA IL-6R antibody to Sprague Dawley tissues from male and female rats was determined. Cryosections of frozen tissues were used in the study. MRA IL-6R antibody was conjugated with FITC. The procedures of the assay were similar to that reviewed above. The test substance was used at 1:10, 1:50, 1:100 and 1:500, 1:1000, 1:2000 and 1:4000 for the determination of optimum titer to be used in the main assay. Sections were incubated at 1:10, 1:50, and 1:500. Omission of the MRA antibody and binding of CD11B antibody to rat spleen was considered to be negative and positive controls. The cross-reactivity was observed in myeloid cells, endothelial cells, epithelial cells and neutrophils. The Applicant stated that the affinity of MRA antibody in rat tissues was weaker than human and monkey tissues. Tissue cross-reactivity data are shown in Table 1 below.

9. Study of species cross-reactivity of MRA and MR16-1 in vitro, Report # PHM-02-0275 (JITSU 99-0396).

The study was conducted at [redacted] between Dec 7, 1998 to May 28, 1999. The report is an English translated copy. b(4)

MRA (humanized IL-6R monoclonal antibody) produced in CHO cells by recombinant DNA techniques. The Applicant also indicated that MR16-1 is anti-mouse IL6-R antibody in rats was also developed as IgG1 antibody. Tissue cross-reactivity of MRA and MR16-1 was conducted. In addition, human IL-6 antibody raised in mouse (IgG1) was used as a positive control.

Spleen tissues from rats, mice and human blood cells were used in the study. T-cells from mouse, rat spleen, and human blood were separated for the study. Phytohemagglutinin (PHA) and human IL-6 were added together for growth activation and to induce T-cell proliferation assay and ^{3}H-thymidine incorporation. The growth inhibition was determined in the presence of MRA and MR16-1 and human IL-6 antibody raised in mice (B-E8). Data presented graphically showed that a dose dependent increase in the T-lymphocyte proliferation from mouse, rat and human was achieved by PHA in the absence and presence of IL-6. Data showed that MRA and MR16-1 inhibited T-lymphocyte proliferation in human and mouse, respectively. The

pharmacological effect of the MRA antibody was verified in this assay. Data are shown below in bar diagram and in Table 2 below.

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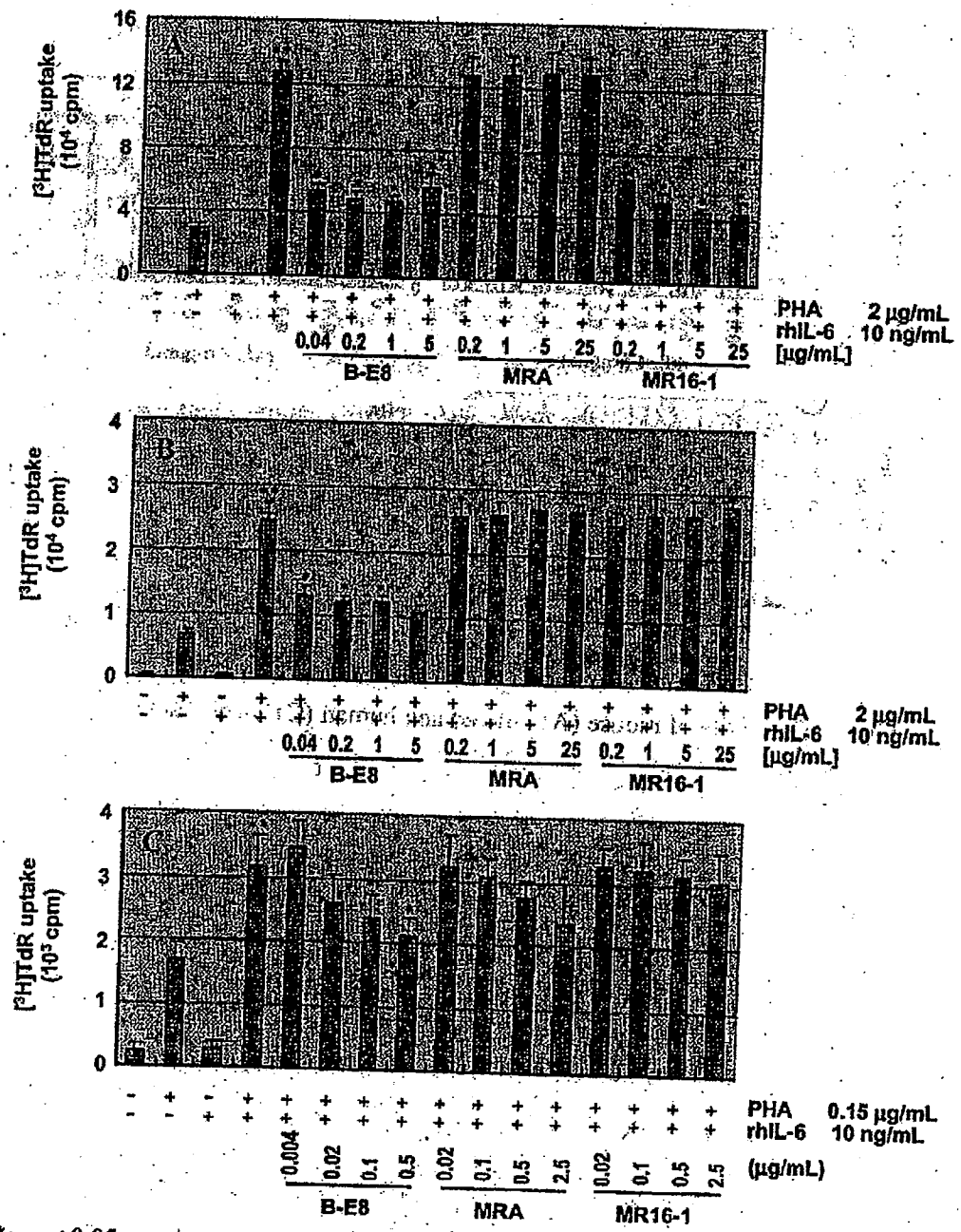

**: $p < 0.05$ vs. group treated with PHA alone (unpaired *t* test)
*: $p < 0.05$ vs. PHA+rhIL-6 control group (Dunnett)
mean ± SD, n = 6

Fig. 2. Inhibitory effects of MRA and MR16-1 on DNA synthesis in T cells from different species, accelerated by IL-6 in the presence of PHA.
Mouse: A, rat: B, human: C

Table 1: Binding of MRA-FITC conjugates to tissues from human, cynomolgus monkeys and rats

Tissue	Human, TOX02-0150	Human, TOX04-0068	Cynomolgus monkey, TOX02-0149	Sprague Dawley rats, TOX02-0148
Laboratory	[redacted]	[redacted]	[redacted]	[redacted]
Adrenal	-	-, + for mononuclear cell infiltrate	Not determined	
Bladder	+ (luminal epithelium)		Not determined	
Blood vessel	+ (endothelium)		Not determined	
Bone marrow	+	+, weak	+	+, myeloid cells
Breast	± (weak)			
Brain			+, female, blood vessel	-
Cerebellum	-	-		
Cerebral cortex	-			
Colon	-	-, + for mononuclear cells in colon		
Blood	+			
Blood, neutrophil		-	+	-
Blood, lymphocyte		+	+	-
Blood, monocytes		-		
Blood Eosinophil		-		
Blood, platelet		-		
Eye		-		
Fallopian tube	+ (epithelial)	-		
Cardiac muscle	-			
GI, Ileum				
GI, Intestine, large		-		
GI, Esophagus		-		
GI, small intestine		-	-	-
GI-mononuclear cells in GALT		+		
Heart		-	-	-
Kidney	-	+, mononuclear cells	+, female, epithelial cells	+, weak in tubular epithelium, F
Liver	+ (endothelial cells)	+, mononuclear cells	+, Blood vessel	-
Lung	+ (epithelial cells and macrophage)	-	+, female, epithelial and macrophage	-
Lymph node	+	+, mononuclear cells	-	-, weak in vascular endothelium
Nerve, peripheral		-		

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Tissue	Human, TOX02-0150	Human, TOX04-0068	Cynomolgus monkey, TOX02-0149	Sprague Dawley rats, TOX02-0148
Ovary	-	-	+	
Pancreas	+ (epithelial)	-		
Parathyroid	-	-		
Placenta	+			
Pituitary	-	-		
Prostrate	+ (acinar epithelial)	-		
Salivary gland		-		
Skin	-	-		
Spinal cord	-	-		
Spleen	+ (red pulp)		+, red pulp, macrophage in white pulp	+, neutrophil and germinal center
Stomach	-	-		
Striated muscle	-	-		
Testis	± (weak, interstitial cell)	-		-
Thymus		-	+	-
Thyroid	+ (acinar epithelial)	-		
Tonsil, mononuclear cell		+		
Ureter	± (mesenchymal cells)	-		
Uterus	+		+	
Uterus, endometrium	+ (endometrial gland)			
Urinary bladder		+ for mononuclear cell		

- = did not bind, ± = weak binding, + = strong binding

Table 2

Tissue cross-reactivity of MRA or MR-16-1 in several species and several methods

Tissue	**Normal Human Tissue, TOX04-0015**	**Human, Rat, Mouse, JITSU-0396**	**Cynomolgus monkey, TOX02-0151**	**Cynomolgus monkey, TOX04-0016**	**Cynomolgus monkey, PHM0192**
Laboratory	[redacted]	[redacted]	[redacted]	[redacted]	**Chugai lab**
Technique	**Immunoperoxidase staining**	**^{3}H-Thymidine Incorporation**	**MRA-FITC conjugation**	**Immunoperoxidase staining**	**^{3}H-Thymidine Incorporation**
Adrenal	-			-	
Blood	-			-	
Bone marrow	-			-	
Brain, cerebrum	-			-	
Brain, cerebellum	-			-	
Breast	-			-	
Eye	-			-	
GI tract	-			-	
Heart	-		+	-	
Kidney	-		+	-	
Liver	-		+	-	
Lung	-		+	-	
Lymph node	-		+	-	
Ovary	-			-	
Fallopian tube	-			-	
Pancreas	-			-	
Parathyroid	-			-	
Peripheral nerve	-			-	
Pituitary	-			-	
Placenta	-			-	
Prostate	-			-	
Salivary Gland	-			-	
Skin	-			-	
Spinal Cord	-			-	
Spleen	-		+	-	
Skeletal muscle	-			-	
Testis	-			-	
Thymus	-			-	
Thyroid	-			-	
Tonsil	-			-	

b(4)

Tissue	Normal Human Tissue, TOX04-0015	Human, Rat, Mouse, JITSU-0396	Cynomolgus monkey, TOX02-0151	Cynomolgus monkey, TOX04-0016	Cynomolgus monkey, PHM0192
Laboratory	———	———	———	———	Chugai lab
Technique	Immunoperoxidase staining	^{3}H-Thymidine Incorporation	MRA-FITC conjugation	Immunoperoxidase staining	^{3}H-Thymidine Incorporation
Ureter	-			-	
Uterus	-			-	
T-lymphocyte		+ (Human), - for rat and mouse		-, spleen	+, proliferation

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+ = positive binding

Conclusion of cross-reactivity studies:

Several studies in human, monkey, and rat tissues suggest that:

1. tissue cross-reactivity was dependent on the methods,
2. species selectivity of binding to MRA was human>monkey>rat, and
3. binding of MRA was related to inflammatory, endothelial, and epithelial cells.

2.6.2.3 Secondary pharmacodynamics

The Applicant conducted in vitro studies in isolated guinea-pig ileum and no inhibition of contractions to spasmogen was observed in the smooth muscle at 200 ug/mL of MRA (study # Tox02-0159-J96-0512). The Applicant stated that no effect on general behavior and body temperature was noted in rodents up to 133.4 mg/kg/IV.

2.6.2.4 Safety pharmacology

Neurological effects: No CNS effect was reported for spontaneous motor activity, CNS stimulation or sedation in rodent models.

Cardiovascular effects: Effects of MRA on respiratory and cardiovascular systems in anesthetized dogs, TOX02-0123.

Male adult dogs were treated with MRA at 66.7 mg/kg or the vehicle intravenously. Several parameters were monitored as shown in the data table below from the Applicant's submission. The dose was selected considering clinical doses between 5 to 40 mg/kg.

Table 2 Effect of MRA(66.7ml/kg,i.v.) on hemodynamics and respiratory rate in anesthetized dogs

Parameters	Time					
	Before	15min	30min	60min	90min	120min
MBP(mmHg)	131±5	132±6	132±5	131±4	131±4	132±6
HR(beats/min)	150±5	153±11	151±7	147±3	147±3	150±5
AoF(l/min)	1.42±0.15	1.76±0.19	1.56±0.14	1.42±0.10	1.38±0.10	1.37±0.15
SVR(dynes·sec/cm^5)	7584±634	6161±464	6863±496	7502±375	7753±417	7903±666
LVSP(mmHg)	148±6	150±8	149±7	149±5	150±5	151±7
LVdP/dt max(mmHg/sec)	2450±194	2550±275	2450±225	2363±80	2425±80	2525±125
RBF(ml/min)	105±16	105±16	111±18	110±11	108±12	106±18
RVR(dynes·sec/cm^5)	106480±15327	108017±14555	103839±18995	103880±19703	107259±24887	111925±25611
CBF(ml/min)	137±19	173±25	148±18	131±9	121±8	118±11
CVR(dynes·sec/cm^5)	81306±11822	64599±8921	74904±10786	79371±9394	86790±10633	91620±10235
RR(strokes/min)	18±5	20±6	19±5	16±3	16±3	16±4

Each value represents the mean ± S.E. of 4 animals.

There was no significant difference between the value of before and after administration(2-wayANOVA/Dunnett test).

MBP, mean arterial blood pressure; HR, heart rate; AoF, aortic blood flow; SVR, systemic vascular resistance; LVP, left ventricular systolic pressure; LVdP/dt max, maximum rate of rise of left ventricular pressure; RBF, renal artery blood flow; RVR, renal vascular resistance;CBF, common carotid artery blood flow; CVR, common carotid vascular resistance; RR, respiratory rate.

Based on the data there was no effect of the drug on cardiovascular and respiratory parameters.

Study # TOX02-0127 was conducted in cynomolgus monkeys. MRA at 133.4 mg/kg IV dose showed no effect up to 90 min post injection on the respiratory rate, mean blood pressure, heart rate, left ventricular pressure, aortic blood flow, systemic vascular resistance, ECG, and GI motility in cynomolgus monkeys. However, a transient increase in the aortic blood flow was noted in the vehicle and MRA treated animals. No treatment related changes were observed. Data are shown from the Applicant's table below.

Table 2 Effect of MRA (133.4mg/kg,i.v.) on hemodynamics and respiratory rate in anesthetized monkeys

Parameters	Time						
	Before	15min	30min	45min	60min	75min	90min
MBP(mmHg)	93±3	92±3	78±10	83±4	89±1	86±2	91±4
HR(beats/min)	188±9	182±13	179±11	187±13	187±15	189±14	196±15
AoF(l/min)	0.46±0.09	0.64±0.11	0.50±0.12	0.49±0.10	0.46±0.11	0.44±0.11	0.42±0.11
SVR(mmHg/l/min)	216±45	153±29	165±24	181±27	211±40	216±40	239±45
LVP(mmHg)	127±19	141±15	113±16	133±14	131±13	130±12	130±17
LVdP/dt max(mmHg/sec)	2315±828	2915±1134	2189±815	2352±896	2204±852	2133±817	2167±833
CBF(ml/min)	24±4	33±14	30±9	30±9	32±9	30±10	32±11
CVR(mmHg/l/min)	4057±763	3904±1326	2930±596	3316±1020	3314±928	3553±1131	3676±1301
RR(strokes/min)	83±17	93±14	87±20	89±19	87±18	93±13	93±14

Each value represents the mean ± S.E. of 3 animals.

MBP, mean arterial blood pressure; HR, heart rate; AoF, aortic blood flow; SVR, systemic vascular resistance; LVP, left ventricular systolic pressure; LVdP/dt max, maximum rate of rise of left ventricular pressure; CBF, common carotid arterial blood flow; CVR, common carotid vascular resistance; RR, respiratory rate.

Study # J99-0722 was conducted at 1, 3, and 50 mg/kg IV to anesthetized male cynomolgus monkeys. ECG, CPK, and platelet aggregation was monitored. The dosing schedule is shown below.

The first experiment

Administration day	Dose levels (mg/kg)			
	Animal No. 1	Animal No. 4	Animal No. 7	Animal No. 8
Week 0	1	10	50	50
Week 2	1	10	50	50
Week 4	1	10	50	50

Results of the test showed no effect on the ECG, CPK and ADP induced platelet aggregation.

Pulmonary effects: See above.

Renal effects: Effects of MRA on urine volume and electrolyte excretion in rats, TOX-02-0157.

Intravenous injection up to 133.4 mg/kg as a single dose did not affect the urine volume, osmolarity, pH, or excretion of sodium, potassium, or chloride ions up to 6 hours post dose.

Gastrointestinal effects: Study # TOX02-0155. The effect of MRA on the GI motility was investigated following a 50% barium sulphate meal in mice. Intravenous dose of vehicle or 1.33, 13.34 and 133.4 mg/kg MRA was injected. Thirty minutes after the barium meal, GI transport of barium was determined. MRA had no significant effect on the GI motility compared to the vehicle.

Abuse liability: No data were submitted nor required. Based on the target of this antibody, there is no reason to believe there is any abuse liability of this product.

2.6.2.5 Pharmacodynamic drug interactions

Tocilizumab is selective to IL-6R and did not interfere with the signal transduction of other cytokines as reviewed under the pharmacology section.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the Applicant]

In vivo primary pharmacodynamics is shown in the table below.

Model	Dose, conc, %	Remarks
Mouse, amyloidosis	0.5, 2 mg, IP, single IP injection MR16-1	No amyloid deposition in liver, spleen, or kidney
Mouse, human IL-6 transgenic	2 mg, IV followed by 100 ug/week, SC for 18 weeks of MR16-1	Improvement of survival and laboratory parameters compared to control

Cynomolgus monkeys, bovine Type II collagen induced arthritis	30 mg/kg, IV, 4 injections of MRA	Neutralization of MRA was noted. The effect was not consistent when compared to the control.
—— , myeloma cell line	Kd 2.5- 2.8	Membrane receptor binding
sIL-6R	Kd 0.7	Soluble receptor binding
Amino acid homology	Monkey 97.3%, Rodents 54%	Human IL-6R comparable to cynomolgus monkey

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2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Single and repeat dose PK studies in cynomolgus monkeys showed non-linear kinetics. No gender difference in the exposure was noted. Anti-MRA antibody was detected after 2-6 weeks of injections. Subcutaneous route showed about 72% bioavailability. The elimination half-life was about 9 days in cynomolgus monkeys. Some studies also showed higher half-life depending on the doses.

After single intravenous dose, distribution of ^{125}I-MRA in male cynomolgus monkeys showed high retention of radiolabel up to 28 days in the thyroid. High level of radioactivity in thyroid could be due to interaction of ^{125}I to thyroid hormones. Among other organs, adrenals, lungs, kidneys, liver, spleen, bone marrow, and synovium also showed considerable radioactivity within 2 days of the injection and gradually declined. It is interesting to note that minimal distribution of radioactivity was noted in the CNS. Male reproductive organs also showed distribution of MRA. The Applicant did not conduct the distribution study in female monkeys.

The Applicant used cross-reactivity of the ^{125}I-MRA to human IL-6R to determine intact nature of MRA in plasma. It was reported that most of the radioactivity eluted from the plasma samples in the metabolism study represented intact MRA. Cumulative elimination data for the radioactivity suggested about 75% elimination through the urine and about 2% elimination in the feces. However, the excretion of radioactivity in urine could be due to excretion of ^{125}I because very low level of radioactivity was noted in the TCA precipitated samples. It is understandable because a large molecular weight protein is not filtered through the kidney. The metabolism study did not identify the degraded fraction of MRA in the plasma. However, the Applicant indicated that clearance of MRA could be through the binding of MRA to the glycoprotein receptor, internalization into the cell and eventual degradation by lysosomal proteases.

IL-6 showed a role on the expression of CYP isozymes in human hepatocytes in vitro. However, the role of MRA on the metabolism of coadministered small molecular drugs is unknown in the animal models. The in vitro studies conducted in human hepatocytes also did not address precise role of MRA in the context of drug metabolism. The Applicant conducted clinical pharmacology studies to address drug-MRA interactions. The

Applicant indicated that MRA could excrete through breast milk, transfer to waning animals through binding to Fc receptor in GI tract.

2.6.4.2 Methods of Analysis

[see under individual study reviews]

2.6.4.3 Absorption

3. Disposition of MRA in monkeys: Plasma concentration and urinary excretion after single intravenous administration of MRA, ADM02-0133

Intravenous injections of MRA were given to male cynomolgus monkeys at 0.5, 5, and 50 mg/kg. Pharmacokinetics and urinary excretion of MRA was determined. Data showed that anti-MRA antibody were present from day 7 onwards dose dependently starting at 0.5 mg/kg. The tissue uptake of MRA was low based on the volume of distribution. $T_{1/2}$ was between 5.6 to 8.2 days. A non-linearity in the exposure was noted based on the normalized exposure and mean residence time. Urinary excretion of MRA was less than 2% up to 28 days after the injection (see more data under excretion). Data suggest that MRA did not excrete in the urine unchanged as anticipated being a large protein. MRA lot # 96F01 was used in the study.

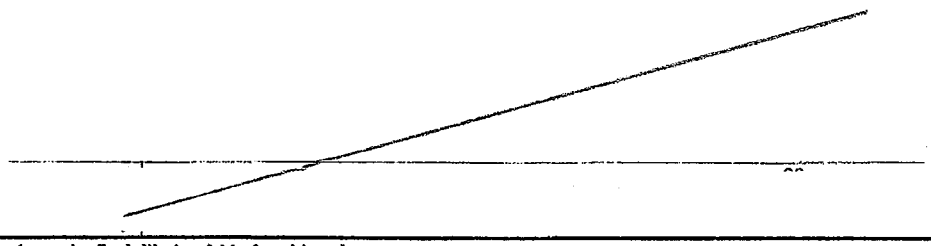
Pharmacokinetic data and anti-MRA levels are shown from the Applicant's table below.

Table 1 Phamacokinetic parameters of MRA following single intravenouse administration of MRA to male cynomolgus monkeys at doses of 0.5, 5.0 or 50 mg/kg (n = 3)

Dose (mg/kg)	AUC (mg · h/mL)	MRT (INF) (h)	Cl total (mL/h/kg)	Vdss (mL/kg)
0.5	0.3915 ± 0.1030	30.58 ± 6.127	1.331 ± 0.3041	38.71 ± 4.364
5	10.33 ± 2.006	127.5 ± 34.51	0.4960 ± 0.09449	61.17 ± 18.13
50	256.4 ± 122.1	311.3 ± 200.9	0.2297 ± 0.1137	57.59 ± 10.28

Dose (mg/kg)	t 1/2 (α) (h)	t1/2 (β) (d)	t1/2 (γ) (d)	Uex(0-28d)(%)
0.5	21.28 ± 7.536			N.D.
5	10.00 ± 7.073	5.621 ± 2.567	2.688	1.709 ± 0.65313
50	10.16 ± 11.82	8.196 ± 2.687	1.720	0.7352 ± 0.4280

Table 2 Anti-MRA antibody production in cynomolgus monkeys after intravenous administration of MRA at doses of 0.5, 5 or 50 mg/kg



b(4)

Each value shows the final dilution fold of positive plasma.
-- : represents negative result at 1 : 20 dilution of plasma.

1. Plasma concentration after single intravenous administration of MRA in female monkeys, study # ADM02-0157 (J99-0081)

Data from the female monkeys are shown below. For comparison, data for male monkeys were collected from the study #97-0369. Male and female monkeys did not show significant difference in the elimination half-life.

Table 1 Pharmacokinetic parameters of MRA following single intravenous administration of MRA to male and female cynomolgus monkeys at a dose of 5 mg/kg (n=3, each)

	AUC (mg•h/mL)	MRT(INF) (h)	Cltotal (mL/h/kg)	Vdss (mL/kg)	t1/2(α) (h)	t1/2(β) (d)
Female	10.3 ± 1.2	160 ± 28	0.488 ± 0.060	73.6 ± 16.2	9.95 ± 9.17	4.65 ± 1.40
Male [2)]	10.3 ± 2.0	127 ± 35	0.496 ± 0.094	61.2 ± 18.1	10.00 ± 7.07	5.62 ± 2.57

There is no difference between female and male.

3. Plasma concentration after repeated intravenous administration of MRA in monkeys, ADM02-0163

Male cynomolgus monkeys were treated at 5 mg/kg, IV once a week for 8 weeks. Steady state kinetics was achieved after 5 injections. Anti-MRA antibody was detected after the 6 weeks of administration in one of the 3 monkeys. Data suggest the exposure remained almost the same between doses and the elimination half life was about 10 days after multiple injections. Pharmacokinetic data are shown from the Applicant's table below.

Table 1 Pharmacokinetic parameters of MRA after single or 8 weeks repeated intravenous administration of MRA once a week for 8 weeks to male cynomolgus monkeys at a dose of 5 mg/kg (n = 3)

	AUC(0-7d) (mg*h/mL)			
1 st dose	9.28 ± 2.17			
	AUC(0-7d)-AUC(7-14d) (mg*h/mL)	t1/2(α) (h)	t1/2(β) (d)	t1/2(γ) (d)
8 th dose	9.66 ± 0.75	4.15 ± 0.31	9.57 ± 2.91	2.33

t1/2(α) and t1/2(β) were calculated using plasma concentration of MRA from 0.5h to 42, 21 or 28 day after 8th administration.
t1/2(γ) was calculated on the data of two animals.

4. Plasma concentration after single subcutaneous administration of MRA in monkeys, ADM04-0014.

The pharmacokinetics of subcutaneous injections of MRA was investigated in cynomolgus monkeys at 1, 5, and 15 mg/kg as single dose. MRA absorbed slowly to the systemic circulation independent of dose. The exposure was non-linear. The bioavailability of 5 mg/kg subcutaneous dose was 72%. Anti-MRA antibody was present from day 16 post dose depending on the dose. Pharmacokinetic data from the Applicant's table are shown below.

Table 2 Pharmacokinetic parameters of MRA in plasma after a single subcutaneous administration of MRA to cynomolgus monkeys

Dose (mg/kg)	Cmax (μg/mL)	Cmax/Dose (kg/mL)	Tmax (d)	AUCinf (mg·hr/mL)	AUCall (mg·hr/mL)	CLf (mL/hr/kg)	MRTinf (d)	T1/2 (d)	Bioavailability*1 (%)
1	4.88 ± 1.29	0.00488 ± 0.00129	2.08 ± 1.26	0.737 ± 0.210	0.620 ± 0.156	1.45 ± 0.44	5.11 ± 1.56	2.84 ± 1.09	N.C.
5	30.1 ± 11.8	0.00603 ± 0.00236	3.00 ± 0.82	7.86 ± 2.26	7.45 ± 2.11	0.687 ± 0.238	8.57 ± 0.88	4.65 ± 0.36	72.1
15	145 ± 8	0.00966 ± 0.00051	2.75 ± 0.50	55.2 ± 10.2	N.C.	0.278 ± 0.045	14.4 ± 2.6	9.54 ± 1.74	N.C.

Data were expressed as the mean ± S.D. of four animals.
N.C. :Not calculated
*1: The bioavailability was estimated by dividing the mean AUCall in the subcutaneous administration (5 mg/kg) from mean AUCall of three animals in the intravenous administration (5 mg/kg).

Significantly different from 1mg/kg group by Tukey's test: *: p<0.05
Significantly different from 5mg/kg group by Tukey's test: #: p<0.05

Table 3 Concentrations of anti-MRA antibody in plasma after a single subcutaneous administration to cynomolgus monkeys

Time	Plasma concentration of anti-MRA antibody (μg/mL)		
	1 mg/kg	5 mg/kg	15 mg/kg
Before	N.D. ± ---	N.D. ± ---	N.D. ± ---
4 d	N.D. ± ---	N.D. ± ---	N.D. ± ---
7 d	N.D. ± ---	N.D. ± ---	N.D. ± ---
10 d	N.D. ± ---	N.D. ± ---	N.D. ± ---
16 d	N.D. ± ---	N.D. ± ---	N.D. ± ---
22 d	0.418 ± 0.547	0.529 ± 0.714	N.D. ± ---
28 d	0.628 ± 0.726	0.581 ± 0.816	N.D. ± ---

Data were expressed as the mean ± S.D. of four animals.
N.D. means the case where three or more than out of four animals were under the quantitation limit (< 0.391 μg/mL).

2.6.4.4 Distribution

Disposition of ^{125}I-labelled MRA in monkeys (2)- quantative distribution after single intravenous administration of ^{125}I-labelled MRA, ADM02-0203

IV injection of ^{125}I-labelled MRA was given to male cynomolgus monkeys at 5 mg/kg as a single injection. The experimental design is shown below from the Applicant's table.

7. Experimental design

Group* no.	Time for sampling tissue	Number of the animal
1	0.5 h	01101 - 01103
2	2 day	02101 - 02103
3	7 day	03101 - 03103
4	14 day	04101 - 04103
5	28 day	05101 - 05103

* Each group consists of 3 male animals.

Blood samples were drawn from saphenous vein at 0.5 hour, 2, 7, 14 and 28 days after the injection. Animals were sacrificed, liver and knee joints were removed for perfusion of the liver through its artery and collection of synovial fluids, respectively. In addition, following tissues were collected as shown below.

liver, kidneys, adrenal gland, spleen, pancreas, mesenteric lymphonodus, fat (around the abdominal wall), testis, epididymis, seminal vesicle, bladder, prostate gland, femoral bone (removed bone marrow), epiphysis (region of femoral bone), bone marrow (region of femoral bone), muscle (rectus), skin, submaxillary gland, trachea, thyroid gland, lungs, heart, aorta, thymus, tongue, eye balls, cerebrum, cerebellum, pituitary gland, membrana synovialis, synovia, stomach, small intestine, large intestine, contents of stomach, contents of small intestine, and contents of large intestine.

After processing tissues as appropriate, total radioactivity of samples were counted by a gamma-counter to determine the radioactivity as % of the dose. Distribution of MRA in tissues was determined from the ratio of radioactivity in TCA precipitated tissues to the total radioactivity. The amount of MRA in tissues determined as ug.eq/g tissue using specific radioactivity of the labeled MRA.

Distribution of radioactivity in tissues is shown from the Applicant's table below.

Table 1 Tissue concentrations of total radioactivity after single intravenous administration of ^{125}I-MRA(5mg/kg) to male cynomolgus monkeys

Tissue	0.5h			2 d			7 d			14 d			28 d		
	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.
Blood	82.595	±	9.147	31.856	±	2.101	12.544	±	1.368	4.268	±	0.861	0.055	±	0.042
Plasma	149.634	±	17.324	59.577	±	1.602	23.640	±	2.218	8.019	±	1.549	0.104	±	0.066
Blood corpuscle	22.958	±	4.297	10.586	±	0.187	4.176	±	0.515	1.348	±	0.182	0.037	±	0.032
Cerebrum	0.917	±	0.259	0.444	±	0.096	0.173	±	0.026	0.062	±	0.018	0.001	±	0.001
Cerebellum	1.004	±	0.311	0.615	±	0.142	0.221	±	0.022	0.074	±	0.010	0.007	±	0.012
Pituitary gland	6.249	±	0.887	8.611	±	1.843	2.710	±	0.437	0.990	±	0.364	0.042	±	0.031
Eye ball	0.510	±	0.065	1.743	±	0.511	0.989	±	0.107	0.356	±	0.058	0.040	±	0.014
Tongue	1.279	±	0.055	6.300	±	0.975	3.089	±	0.747	1.039	±	0.288	0.021	±	0.006
Submaxillary gland	2.038	±	0.582	5.964	±	1.236	2.501	±	0.4887	1.035	±	0.275	0.061	±	0.004
Thyroid	4.448	±	1.958	810.016	±	774.469	1012.13	±	1022.63	3025.89	±	863.82	1746.62	±	1377.86
Thymus	2.244	±	0.971	5.241	±	0.881	1.650	±	0.501	0.704	±	0.149	0.130	±	0.140
Trachea	4.319	±	2.873	8.805	±	2.159	4.642	±	1.768	1.301	±	0.167	0.173	±	0.073
Lung	13.541	±	2.483	15.834	±	7.120	6.089	±	1.015	1.471	±	0.155	0.039	±	0.008
Heart	5.864	±	0.848	8.747	±	0.375	3.156	±	0.227	1.020	±	0.320	0.026	±	0.013
Liver	10.329	±	6.840	2.350	±	0.315	0.987	±	0.195	0.619	±	0.050	0.052	±	0.019
Kidney	13.051	±	2.445	8.962	±	1.221	3.127	±	0.389	0.951	±	0.150	0.062	±	0.011
Adrenal	19.382	±	2.329	8.105	±	1.025	3.390	±	0.506	1.052	±	0.233	0.043	±	0.020
Spleen	14.978	±	1.242	7.300	±	1.394	2.686	±	0.227	0.926	±	0.161	0.141	±	0.107
Pancreas	2.760	±	0.409	3.965	±	0.652	1.204	±	0.259	0.457	±	0.095	0.006	±	0.009
Bladder	2.677	±	0.684	5.516	±	1.596	3.996	±	0.314	1.663	±	0.311	0.037	±	0.018
Muscle	0.510	±	0.065	0.700	±	0.319	0.301	±	0.161	0.240	±	0.047	0.002	±	0.002
Femoral bone	0.471	±	0.221	0.422	±	0.145	0.200	±	0.021	0.076	±	0.021	0.006	±	0.003
Epiphysis	3.192	±	1.289	2.287	±	1.318	0.905	±	0.035	0.361	±	0.180	0.018	±	0.013
Membrane synovialis	2.176	±	0.991	4.751	±	1.867	3.451	±	0.924	1.119	±	0.363	0.075	±	0.037
Synovia	5.006	±	2.288	12.677	*		6.019	±	3.784	2.320	±	1.043	1.333	±	2.015
Bone marrow	21.118	±	9.856	6.968	±	2.490	2.006	±	0.803	1.121	±	0.511	0.034	±	0.031
Mesenteric lymphbnodus	2.271	±	0.388	6.870	±	0.443	2.654	±	0.411	0.960	±	0.123	0.082	±	0.055
Fat	2.280	±	0.509	1.809	±	0.973	0.828	±	0.313	0.255	±	0.021	0.003	±	0.005
Skin	1.134	±	0.305	2.449	±	0.337	2.781	±	0.775	1.077	±	0.123	0.047	±	0.034
Testis	3.401	±	2.036	4.594	±	0.864	1.658	±	0.492	0.487	±	0.205	0.016	±	0.017
Epididymis	2.326	±	0.204	7.968	±	1.747	2.425	±	0.770	0.810	±	0.389	0.031	±	0.018
Prostate gland	1.739	±	0.284	7.929	±	2.063	3.807	±	0.458	1.025	±	0.159	0.032	±	0.024
Seminal vesicle	2.205	±	0.124	7.273	±	1.949	3.216	±	0.846	1.129	±	0.103	0.057	±	0.046
Aorta	2.067	±	1.126	8.846	±	6.469	3.551	±	1.102	0.971	±	0.116	0.068	±	0.026
Stomach	1.649	±	0.478	9.499	±	1.451	2.623	±	0.177	1.253	±	0.240	0.035	±	0.031
Small intestine	1.485	±	0.059	4.975	±	0.507	1.242	±	0.045	0.468	±	0.009	0.023	±	0.005
Large intestine	1.509	±	0.135	3.137	±	2.487	1.907	±	0.210	0.675	±	0.038	0.018	±	0.007
Contents of stomach	0.177	±	0.036	0.895	±	0.542	0.577	±	0.323	0.100	±	0.052	0.004	±	0.004
Contents of small int.	0.122	±	0.005	0.302	±	0.113	0.088	±	0.024	0.045	±	0.011	0.002	±	0.002
Contents of large int.	0.100	±	0.033	0.580	±	0.022	0.159	±	0.020	0.077	±	0.025	0.007	±	0.005

Unit : μ g eq. / g

Each value represents the mean ± S.D. of 3 animals. (*: 2 animals)

Most of the MRA was distributed to blood cells, lungs, liver, kidney, adrenal, spleen, and bone marrow and thyroid. Minimal tissue distribution was note in CNS and thymus within 0.5 hours. With the exception of thyroid and blood, minimal radioactivity was noted in most of the tissues after 2 day onwards. It is interesting to note that a high amount of radioactivity was noted in the bone marrow and synovium up to 2 day also.

Tissue concentration of total radioactivity in ug.eq/g of tissues at 0.5 hr, 2, 7, 14 and 28 days after the injection is shown from the Applicant's table below.

Appendix 1 Tissue concentrations of total radioactivity 0.5 hr after single intravenous administration of ^{125}I-MRA(5mg/kg) to male cynomolgus monkeys

		Concentration (μ g eq. / g)		
Tissue	Animal No.	01101	01102	01103
Blood		85.213	72.424	90.148
Plasma		150.146	132.059	166.696
Blood corpuscle		22.089	19.162	27.623
Cerebrum		0.810	0.729	1.212
Cerebellum		0.895	0.762	1.355
Pituitary gland		7.099	6.319	5.330
Eye ball		0.463	0.484	0.584
Tongue		1.311	1.215	1.310
Submaxillary gland		1.840	1.580	2.693
Thyroid		6.589	2.748	4.007
Thymus		3.128	1.205	2.398
Trachea		7.635	2.604	2.717
Lung		14.761	10.651	15.120
Heart		6.794	5.133	5.666
Liver		7.036	5.758	18.192
Kidney		10.929	12.499	15.725
Adrenal		19.857	16.853	21.437
Spleen		15.146	13.661	16.127
Pancreas		2.868	2.308	3.104
Bladder		3.249	1.919	2.864
Muscle		0.551	0.434	0.544
Femoral Bone		0.371	0.318	0.724
Epiphysis		2.494	2.403	4.679
Membrana synovialis		1.855	1.386	3.288
Synovia		3.930	7.633	3.454
Bone marrow		31.968	12.719	18.668
Mesenteric lymphonodus		2.718	2.022	2.072
Fat		1.763	2.296	2.780
Skin		0.985	1.485	0.931
Testis		5.741	2.429	2.033
Epididymis		2.561	2.198	2.218
Prostate gland		1.766	2.009	1.443
Seminal vesicle		2.298	2.065	2.253
Aorta		1.399	1.434	3.367
Stomach		1.485	1.275	2.188
Small intestine		1.475	1.431	1.548
Large intestine		1.579	1.376	1.632
Contents of stomach		0.217	0.147	0.167
Contents of small int.		0.127	0.117	0.121
Contents of large int.		0.100	0.064	0.130

Appendix 2 Tissue concentrations of total radioactivity 2 days after single intravenous administration of ^{125}I-MRA(5mg/kg) to male cynomolgus monkeys

		Concentration (μ g eq. / g)		
Tissue	Animal No.	02101	02102	02103
Blood		31.017	30.304	34.246
Plasma		58.118	61.291	59.321
Blood corpuscle		10.531	10.433	10.794
Cerebrum		0.542	0.440	0.350
Cerebellum		0.733	0.654	0.457
Pituitary gland		10.732	7.400	7.701
Eye ball		1.896	1.173	2.161
Tongue		5.368	7.313	6.219
Submaxillary gland		6.159	7.091	4.642
Thyroid		19.651	1567.545	842.852
Thymus		4.371	5.560	6.091
Trachea		11.197	8.206	7.005
Lung		17.127	15.178	15.198
Heart		9.142	8.395	8.705
Liver		2.703	2.247	2.099
Kidney		9.957	9.329	7.599
Adrenal		9.262	7.745	7.309
Spleen		8.904	6.618	6.378
Pancreas		4.073	4.557	3.266
Bladder		3.722	6.778	6.047
Muscle		0.404	0.657	1.038
Femoral Bone		0.257	0.479	0.529
Epiphysis		1.976	2.597	2.168
Membrana synovialis		6.453	5.046	2.754
Synovia		N.S.	16.027	9.326
Bone marrow		9.810	5.922	5.172
Mesenteric lymphonodus		7.181	6.363	7.067
Fat		0.688	2.437	2.301
Skin		2.063	2.681	2.604
Testis		3.618	4.901	5.262
Epididymis		6.830	9.979	7.095
Prostate gland		7.267	10.241	6.278
Seminal vesicle		8.115	8.660	5.045
Aorta		16.310	5.367	4.862
Stomach		10.546	10.108	7.843
Small intestine		5.407	5.101	4.416
Large intestine		3.795	0.388	5.229
Contents of stomach		0.908	0.346	1.430
Contents of small int.		0.419	0.193	0.294
Contents of large int.		0.562	0.605	0.574

N.S. : No sample

Appendix 3 Tissue concentrations of total radioactivity 7 days after single intravenous administration of ^{125}I-MRA(5mg/kg) to male cynomolgus monkeys

		Concentration (μg eq. / g)		
Tissue	Animal No.	03101	03102	03103
Blood		13.885	11.150	12.596
Plasma		25.550	21.207	24.163
Blood corpuscle		4.263	3.623	4.641
Cerebrum		0.149	0.200	0.171
Cerebellum		0.242	0.222	0.198
Pituitary gland		2.564	3.201	2.365
Eye ball		1.101	0.888	0.978
Tongue		3.599	2.231	3.436
Submaxillary gland		3.135	2.176	2.492
Thyroid		2177.748	592.972	265.671
Thymus		2.163	1.161	1.626
Trachea		6.628	3.239	4.058
Lung		7.260	5.541	5.467
Heart		3.083	2.974	3.410
Liver		1.205	0.829	0.928
Kidney		2.679	3.323	3.380
Adrenal		3.421	2.870	3.880
Spleen		2.873	2.433	2.753
Pancreas		1.187	0.993	1.431
Bladder		4.143	3.635	4.210
Muscle		0.557	0.236	0.410
Femoral Bone		0.177	0.218	0.205
Epiphysis		0.945	0.886	0.884
Membrana synovialis		4.262	2.446	3.646
Synovia		9.618	2.074	6.365
Bone marrow		2.388	1.083	2.547
Mesenteric lymphonodus		3.118	2.336	2.507
Fat		0.975	0.469	1.040
Skin		3.466	1.940	2.937
Testis		1.761	1.123	2.090
Epididymis		2.967	1.542	2.761
Prostate gland		3.456	3.641	4.325
Seminal vesicle		2.351	3.256	4.041
Aorta		4.822	2.864	2.967
Stomach		2.812	2.460	2.598
Small intestine		1.213	1.294	1.219
Large intestine		2.131	1.873	1.716
Contents of stomach		0.685	0.832	0.214
Contents of small int.		0.073	0.075	0.116
Contents of large int.		0.177	0.162	0.137

Appendix 4 Tissue concentrations of total radioactivity 14 days after single intravenous administration of ^{125}I-MRA(5mg/kg) to male cynomolgus monkeys

		Concentration (μ g eq. / g)		
Tissue	Animal No.	04101	04102	04103
Blood		3.515	5.207	4.083
Plasma		6.426	9.519	8.112
Blood corpuscle		1.162	1.357	1.526
Cerebrum		0.041	0.075	0.069
Cerebellum		0.062	0.079	0.080
Pituitary gland		0.592	1.305	1.074
Eye ball		0.320	0.395	0.352
Tongue		0.706	1.220	1.190
Submaxillary gland		0.753	1.298	1.084
Thyroid		2546.581	2507.995	4023.094
Thymus		0.610	0.626	0.875
Trachea		1.109	1.396	1.399
Lung		1.339	1.641	1.432
Heart		0.782	1.384	0.894
Liver		0.675	0.577	0.605
Kidney		0.798	1.097	0.957
Adrenal		0.845	1.305	1.006
Spleen		0.740	1.021	1.017
Pancreas		0.360	0.549	0.462
Bladder		1.323	1.934	1.733
Muscle		0.290	0.232	0.198
Femoral Bone		0.055	0.077	0.096
Epiphysis		0.250	0.568	0.264
Membrana synovialis		0.700	1.313	1.343
Synovia		1.188	3.241	2.532
Bone marrow		0.541	1.504	1.317
Mesenteric lymphonodus		0.820	1.049	1.010
Fat		0.276	0.235	0.254
Skin		1.014	1.219	0.998
Testis		0.293	0.702	0.467
Epididymis		0.603	0.972	0.856
Prostate gland		0.842	1.120	1.113
Seminal vesicle		1.019	1.224	1.144
Aorta		0.860	1.092	0.962
Stomach		0.984	1.328	1.447
Small intestine		0.458	0.473	0.473
Large intestine		0.649	0.719	0.658
Contents of stomach		0.071	0.070	0.160
Contents of small int.		0.040	0.037	0.058
Contents of large int.		0.056	0.071	0.104

Appendix 5 Tissue concentrations of total radioactivity 28 days after single intravenous administration of ^{125}I-MRA(5mg/kg) to male cynomolgus monkeys

		Concentration (μg eq. / g)		
Tissue	Animal No.	05101	05102	05103
Blood		0.056	0.012	0.096
Plasma		0.148	0.028	0.137
Blood corpuscle		0.060	0.000	0.050
Cerebrum		0.002	0.000	0.000
Cerebellum		0.000	0.000	0.021
Pituitary gland		0.072	0.010	0.044
Eye ball		0.032	0.031	0.056
Tongue		0.027	0.019	0.016
Submaxillary gland		0.057	0.061	0.065
Thyroid		331.909	1823.559	3084.398
Thymus		0.023	0.078	0.288
Trachea		0.096	0.240	0.184
Lung		0.048	0.034	0.035
Heart		0.038	0.013	0.028
Liver		0.031	0.056	0.068
Kidney		0.071	0.050	0.066
Adrenal		0.061	0.021	0.048
Spleen		0.051	0.260	0.112
Pancreas		0.000	0.016	0.003
Bladder		0.052	0.017	0.042
Muscle		0.000	0.003	0.003
Femoral Bone		0.006	0.003	0.009
Epiphysis		0.030	0.004	0.021
Membrana synovialis		0.115	0.042	0.067
Synovia		0.349	0.000	3.651
Bone marrow		0.041	0.000	0.060
Mesenteric lymphonodus		0.052	0.146	0.049
Fat		0.008	0.000	0.000
Skin		0.085	0.019	0.037
Testis		0.033	0.014	0.000
Epididymis		0.039	0.010	0.044
Prostate gland		0.029	0.009	0.057
Seminal vesicle		0.078	0.005	0.089
Aorta		0.084	0.082	0.038
Stomach		0.048	0.000	0.057
Small intestine		0.018	0.025	0.027
Large intestine		0.012	0.025	0.017
Contents of stomach		0.005	0.000	0.007
Contents of small int.		0.002	0.000	0.004
Contents of large int.		0.002	0.011	0.008

Based on the data, considerable radioactivity was detected in adrenal, lungs, kidneys, liver, spleen, thyroid, bone marrow, and synovium on day 2 after the injection. Among these organs, thyroid and blood continued to show higher radioactivity beyond day 7. However, minimal CNS distribution was noted. Intermediate level of radioactivity was also observed in the male reproductive organs. Since the study was conducted in male animals only, the distribution of MRA in female reproductive organ is unknown.

It was concluded that thyroid could be the major organ for retention of the drug or its metabolite. The effect could also be due to the binding of iodine to thyroid hormone.

2.6.4.5 Metabolism

3. **Characterization of metabolites of 125I-MRA (3) characterization of metabolites in plasma after single intravenous administration of ^{125}I-labelled MRA in monkeys, ADM02-0148.**

Radioactivity of ^{125}I-MRA was determined in the plasma samples at 0.5 hr, 2, 7, 14 and 28 days after a single IV injection at 5 mg/kg in male cynomolgus monkeys. Based on the gel filtration chromatography of the samples, it was determined that most of the radioactivity in the plasma sample was due to intact MRA up to day 14. A slight amount of UG-1 (peak represents molecular weight higher than MRA and may represent soluble IL-6R and MRA complex) and UG-2 (peak represents lower than molecular weight lower than MRA) were noted in the chromatography that was almost negligible as shown in the figure below.

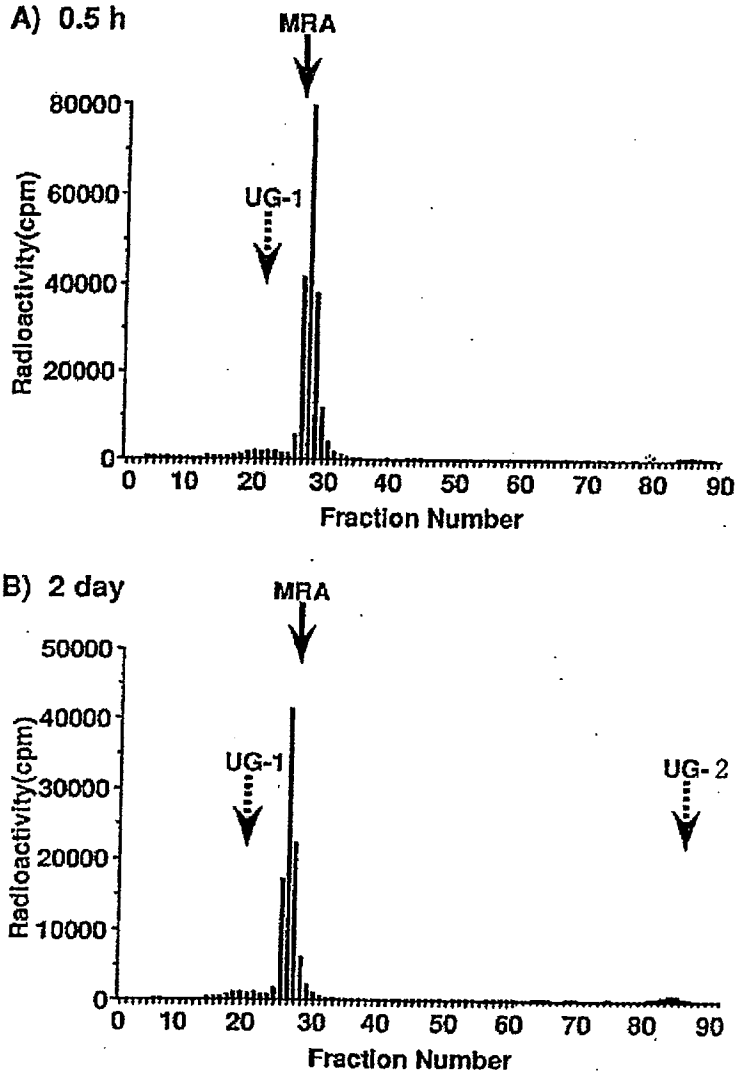


Fig. 1 Gelfiltration chromatograms on Superdex 200 column of radioactivity in plasma samples after intravenous administration of ^{125}I-MRA at the dose of 5 mg/kg in monkeys

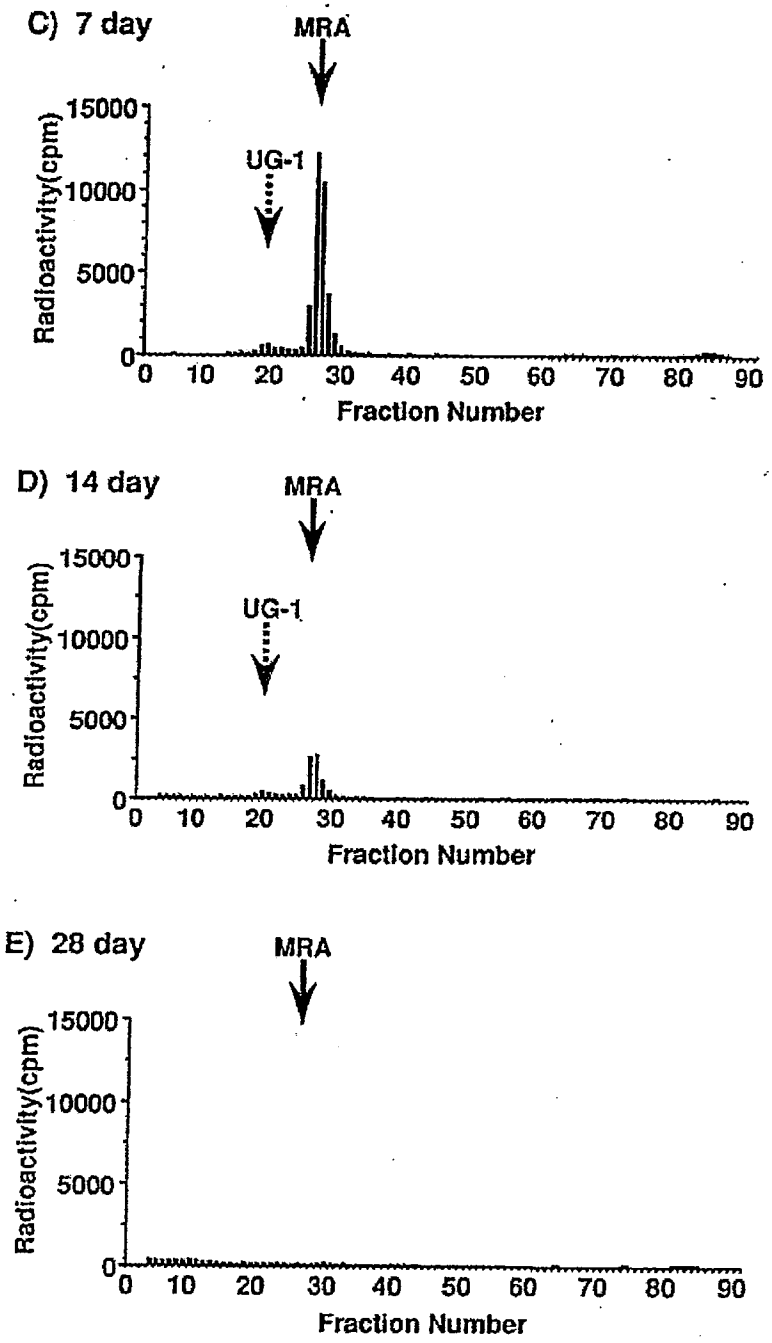


Fig. 2 Gelfiltration chromatograms on Superdex 200 column of radioactivity in plasma samples after intravenous administration of ^{125}I-MRA at the dose of 5 mg/kg in monkeys

Reviewer: Asoke Mukherjee, BLA No. 125276

Radioactivity data from the Applicant's table are shown below.

Table 1 Ratio of radioactivity in each peak to sum of radioactivity in peak after gelfiltration chromatography

Peak	Ratio (%)				
	0.5 h	2 day	7 day	14 day	28 day
UG-1	4.6	6.9	5.8	5.0	-
MRA	95.4	90.9	94.2	95.0	-
UG-2	-	2.1	-	-	-

- : No peak

Plasma samples from 3 animals given 125I-MRA intravenously at the dose of 5 mg/kg were analyzed by Superdex 200 column.
See Fig.1 and Fig.2 for the position of each peak.

Table 2 Ratio of SR-344 reactive radioactivity in each peak to SR-344 reactive radioactivity of intact 125I-MRA

Peak	Ratio (%)				
	0.5 h	2 day	7 day	14 day	28 day
UG-1	0.0	0.0	0.0	0.0	-
MRA	92.2	92.3	96.1	94.1	-

- : No peak

Plasma samples from 3 animals given 125I-MRA intravenously at the dose of 5 mg/kg were analyzed by Superdex 200 column.
See Fig.1 and Fig.2 for the position of each peak.

Table 3 Ratio of SR-344 reactive radioactivity in each peak to SR-344 reactive radioactivity of intact 125I-MRA

Peak	Ratio (%)				
	0.5 h	2 day	7 day	14 day	28 day
MRA	93.5	94.6	97.1	101.0	97.0
TYX	24.7	22.8	23.6	22.7	25.0
125I	0.0	0.0	0.0	0.0	0.0

Plasma samples from 3 animals given 125I-MRA intravenously at the dose of 5 mg/kg were analyzed by Asahipak GS-320H column.
See Fig.3 and Fig.4 for the position of each peak.

The Applicant stated that ^{125}I-thyroxine (TYX) was also present in the plasma samples due to the injection of iodine labeled protein as a procedural artifact.

^{125}I-MRA cross-reactivity to SR-344 (soluble hIL-6 receptor reactive radioactivity) confirmed the intact nature of the radiolabeled MRA in the eluted plasma samples.

Based on the data, it is concluded that MRA was not metabolized within day 14 after the IV administration when measurable radioactivity was detected in the plasma.

2.6.4.6 Excretion

Disposition of ^{125}I-MRA in cynomolgus monkeys-plasma, concentration, excretion in urine and feces after single intravenous administration of ^{125}I-MRA, ADM02-0202 (J97-0579).

Male cynomolgus monkeys were injected with ^{125}I-MRA at 5 mg/kg, IV. The experimental design was similar to that described in the absorption and distribution study. Animals were monitored in the metabolism cage for the fecal and urinary excretion of radioactivity up to 28 days. Cumulative radioactivity excretion data are shown in the Applicant's table below.

Table 3 Cumulative excretion of total radioactivity in urine and feces, and TCA-precipitable radioactivity in urine after single intravenous administration of ^{125}I-MRA at a dose of 5 mg/kg to male cynomolgus monkeys

Time after administration (day)	Cumulative excreted radioactivity (% of dose)			
	Urine (Total)[1]	Urine (TCA)[2]	Feces (Total)[1]	Urine (Total)[1] + Feces (Total)[1]
0 ~ 2	15.2 ± 4.26	1.80 ± 0.90	0.17 ± 0.127	15.4 ± 4.37
0 ~ 7	44.7 ± 4.52	3.78 ± 1.83	0.97 ± 0.159	45.7 ± 4.35
0 ~ 14	61.9 ± 2.88	4.52 ± 2.03	1.62 ± 0.366	63.5 ± 2.50
0 ~ 21	70.7 ± 1.53	5.00 ± 2.13	2.20 ± 0.755	72.9 ± 1.01
0 ~ 28	74.3 ± 2.11	5.24 ± 2.21	2.46 ± 0.846	76.8 ± 1.28

Each value represents the mean ± S.D. of 3 animals.
1) Total radioactivity
2) TCA-precipitable radioactivity

Data showed that about 74% and 2.5% of the total radioactivity was excreted up to day 28 in the urine and feces. However, the excreted radioactivity was due to ^{125}I excretion.

2.6.4.7 Pharmacokinetic drug interactions

Effect of MRA and IL-6 on the expression of drug metabolizing enzyme in human liver, ADM03-0155.

The expression of CYP3A4, CYP2C9 and CYP2C19 did not show changes when untreated human hepatocytes were incubated for 72 hours. In order to determine the effect of MRA and circulating IL-6 on the CYP expression, 250 ug/mL MRA and 12.5 mg/mL of IL-6 were incubated with hepatocyte generating systems. mRNA levels of CYP2D6, CYP2B6, CYP3A4, CYP2C19 and CYP2C9 were not changed with either treatment. The effect on expression of CYP1A2 mRNA at 250 ug/mL MRA and 12.5 ng/mL of IL-6 was reduced. The effect of IL-6 alone on the mRNA expression of CYP1A2, CYP2B6, CYP2C9, CYP2E1, and CYP3A4 changed depending on the concentrations of IL-6. Therefore, IL-6 partly regulated CYP expression probably by the activation of STAT transcription factor and neutralization of the blockade of IL-6 receptor could influence metabolism of other drugs used concomitantly. However, a definite conclusion of the drug interactions between MRA and other drugs could not be made unless in vivo studies are conducted in relevant species. It should be noted that anti-MRA could also pose a problem in the determination of drug-drug interactions in animals in vivo. Clinical pharmacology section of the BLA showed some study reports with respect to drug interactions and these studies would be reviewed under a separate review.

2.6.4.8 Other Pharmacokinetic Studies

The Applicant submitted several studies on the analytical method development for the assay of MRA, preparation of radio-labeled MRA, binding of MRA to Fc receptor to glycoproteins.

Fc region of MRA binds to Fc receptor expressed on the human cell membrane. The binding potential of Fc region of MRA on human mononuclear cells was compared for

MRA manufactured by Ukima and Utsunomiya methods. The method consisted of competition of labeled and unlabelled MRA binding on human mononuclear cell suspension. Total radioactivity and cell bond radioactivity (precipitated form) was determined using a gamma-scintillation counter. Non-specific binding of radioactive MRA was determined by displacing radioactive MRA binding from the non-specific site using high concentrations of MRA. K_d of binding for MRA obtained from two methods of used for the manufactured batches were compared for consistency of potency of the antibody are shown below.

Kd values of MRA to Fc receptor on human mononuclear cell

	Kd (nmol/L)	
	Ukima	Utsunomiya
1	1.23	1.43
2	1.70	2.10
3	1.37	1.25
Mean	1.43	1.59
S.D.	0.24	0.45

2.6.4.9 Discussion and Conclusions

Intravenous injections of MRA showed non-linear kinetics similar to that indicated in the clinical pharmacology studies. No gender difference in the kinetics was noted. Anti-MRA antibody was detected in monkeys. The biological half-life of MRA in monkeys was about 9 -10 days. However, some studies in monkeys also showed half-life of about 13 days. Human PK data showed about 8-14 days T ½ at 8 mg q 4 weeks at steady state.

Reviews on PK/TK data on male cynomolgus monkeys suggest that MRA distributed mostly to lungs, liver, kidney, adrenal, spleen, thyroid, bone marrow, synovium, and male reproductive organs. Minimal distribution was noted in CNS. Most of the radioactivity was receded after 2 days of injection. Radioactivity in the plasma represented intact MRA. Urine was the major route of elimination of radioactivity probably as iodide. High level of radioactivity was noted in thyroid. The Applicant indicated that ^{125}I exchange with thyroid hormones could be possible. The Applicant did not study the distribution of ^{125}I-MRA in female monkeys. Small molecular drug-MRA interactions in non-clinical models were not investigated. However, some of the drug interactions with omeprazole, warfarin, and cyclosporine were conducted in clinical pharmacology studies.

Selection of animal models based on the tissue cross-reactivity and anti-MRA antibody formation was an important issue for the species selection. Data from monkeys based on the understanding of the role of MRA in monkeys with respect to cross-reactivity studies,

the magnitude of anti-MRA response and toxicity studies questioned the application of non-clinical model for the development of humanized monoclonal antibody.

The Applicant indicated that IgG based products could be excreted through breast milk and transferred to weaning animals through internalization process following binding of the IgG protein to Fc receptor in the small intestine. This process could expose the weaning animals to MRA. However, its biological significance to the post-natal development is unknown because the Applicant did not conduct any studies.

In the primate model, it is concluded that IV administration of MRA resulted in anti-product antibody production to MRA, retention of MRA were evident in specific tissues that slowly decreased after 2 days, and non-linearity of kinetics was observed.

2.6.4.10 Tables and figures to include comparative TK summary

See tabulated summary below.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the Applicant]

The table below shows summary of PK information in cynomolgus monkeys from several studies.

Dose, mg/kg	Route	Frequency	$T_{1/2}$, day	Conc, week 26 though, ug/mL	AUC, mg.hr/mL	Anti-MRA Antibody
0.5	IV	Single			0.39	-
1	SC	Single	2.8		0.73	+
5	SC/IV	Single	4.6/5.6		7.8/10.4	+
5	IV	8 doses	9.5		9.6	+
15	SC	Single	9.5		55	-
50	IV	Single	8.2		256	
10	IV	Q weekly x 6 mo	6.8	187-244		+
100	IV	Q weekly x 6 mo	10.4-13.4	1935		-

- = Negative for anti-MRA antibody
+ = Positive for anti-MRA antibody

Human PK data from single and multiple dose studies as shown in the clinical pharmacology section are shown from the Applicant's table for comparison.

6. APPENDIX

Appendix 1 Overview of Clinical Pharmacology Studies: Single Dose Studies and Additional Studies

Protocol #	Product ID/Batch	Study Objective	Study Design	Subjects No. (M/F) Type Age: Mean (Range)	Treatment (mg/kg) (Infusion Duration)	Mean Pharmacokinetic Parameters (±SD) Substrate Drug					
						C_{max} (µg/mL)	AUC_{last} (µg*h/mL)	CL (mL/h/kg)	$t_{1/2}$ (h)	Vss (mL/kg)	t_{max}* (h)
BP19461 (part 1 only)	RO4877533/ MR5C06	ST, PK, ECG	Double blind, randomized, placebo-controlled, two centers, SAD	36 (17/19) HV (18-61)	2 10 20 28 (1 h)	41.9± 3.33 242± 31.3 410± 81.3 558± 79.2	3210± 410 37800± 6000 77800± 17100 115000± 10900	0.609± 0.0635 0.243± 0.0421 0.217± 0.0517 0.192± 0.0215	54.0± 10.9 201± 29.8 277± 35.5 293± 48.0	50.0± 6.51 67.5± 9.41 85.7± 20.3 81.4± 11.3	3.84± 2.65 4.05± 2.12 3.82± 2.39 3.03± 1.39
LRO300	RO4877533/ R7F03	ST, anti-genicity, PK, efficacy	Double blind, randomized, placebo-controlled, six centers, SAD	45 (15/30) P (35-74)	0.1 1 5 10 (1 h)	1.96± 1.26 17.9± 4.7 123± 21 273± 121	18± 13 1180± 839 18100± 3530 43600± 17000	- 0.742 0.262± 0.039 0.256± 0.125	- 52.9 135.9 158.0	- 49.7 49.7± 7.5 58.7± 22.4	1.192 4.0 1.183 4.25
MRA001JP	RO4877533/ R7F03 MRSF02	ST, PK, MTD determinati on	Single blind, randomized, placebo controlled, single center, SAD	28 (28/0) HV (20-29)	0.15 0.5 1 2 (1 h)	2.4± 0.61 8.49± 1.17 19.5± 2.73 37.6± 8.78	10.7± 5.7 285± 73.3 1010± 222 2530± 569	# 1.26± 0.210 0.826± 0.073 0.633± 0.145	17.4± 15.6 33.1± 3.71 49.4± 5.14 74.3± 9.00	# 58.4± 7.1 57.3± 10.9 65.9± 8.3	1.5± 0.6 1.6± 0.5 1.8± 1.3 2.8± 2.2
MRA220JP	RO4877533/ MR4C05	ST, PK efficacy (DDI)	Open label, non-randomized, single center	31 (6/25) P 50 y (23-69)	8 (1 h)	137±22.7	20600± 5750	0.404± 0.125	136± 25.7	79.9± 16.8	2.7± 1.6
MRA221JP	RO4877533/ MR4C05	PK, ST (renal impair-ment)	Open label, non-randomized, multiple center	14 (3/11) P 64 y (56-74)	8 Normal (n=2) Mild (n=4) Moderate(n=5) Severe (n=3) (1 h)	176 174± 29.1 177± 18.9 172± 35.0	23400 20800± 9330 24800± 7710 28700±10100	0.34 0.49± 0.35 0.34± 0.11 0.29± 0.10	119 101± 38.4 143± 51.5 148± 14.5	64.1 65.5± 9.55 62.5± 5.74 62.1± 16.8	NC
MRA004JP	RO4877533/ MR9D02	ST, PK, ECG	Open label, non-randomized, single center	6 (6/0) HV 22 y (20-23)	2 (2 h)	26.4± 5.78	2940± 593 (infinity)	0.709± 0.165	82.1± 4.68	85.0± 19.4	6± 0

Mean PK parameters rounded to 3 sig. figs where appropriate, t_{max} rounded to 2 signif. figs. HV: healthy volunteers; M/F: male/female; MAD: multiple ascending doses; MTD: maximum tolerated dose; P: patients; PD: pharmacodynamics; PK: pharmacokinetics; SAD: single ascending dose; ST, safety and tolerability
*: time zero equal to start of 1 h infusion; #: parameters not reported as area extrapolated too high for all subjects (≥ 48%); ** area extrapolated very high

Appendix 2 Overview of Clinical Pharmacology Studies: Multiple Dose Studies

Protocol # (Country)	Product ID/batch	Study Objective	Study Design	Subjects No. (M/F) Type Age: Mean (Range)	Treatment (mg/kg) (Infusion Duration; Dosing Frequency)	Mean Pharmacokinetic Parameters (±SD) Substrate Drug					
						C_{max} (µg/mL)	AUC_{last} (µg*h/mL)	CL (mL/h/kg)	$t_{1/2}$ (h)	V_{ss} (mL/kg)	C_{min} (µg/mL)
MRA002JP	RO4877533/ MR9D01	Safety, PK, efficacy	Open label, non-randomized, multiple center, MAD	15 (4/11) P 53 y (32-72)	2						
					1st dose	43.6± 10.1	3440± 822	0.510± 0.083	74.4± 18.3	55.0± 13.0	1.91
					2nd dose	44.0± 9.09	3570± 801		77.0± 13.9		1.96
					3rd dose	27.9± 12.3	3010± 1070		86.6± 18.4		3.00± 1.39
					4						
					1st dose	49.0± 12.6	4660± 2180	0.698± 0.526	96.9± 50.2	102± 24.0	5.68± 2.52
					2nd dose	55.1± 1 2.3	5670± 2750		122± 64.2		9.84± 4.28
					3rd dose	49.5± 10.1	6040± 3200		140± 71.1		15.5± 5.18
					8						
					1st dose	82.5± 32.4	10700± 4070	0.279± 0.095	160± 34.3	137± 31.6	14.8± 7.30
					2nd dose	106± 36.6	17000± 8230	9	192± 45.6		26.2± 16.3
					3rd dose	130± 48.1	19900± 8900		242± 71.4		37.1± 20.4
					(2 h; 2 weeks)			***		***	
MRA009JP	RO4877533/ MR0I02, MR1B03 MR1G02	PK, safety, efficacy	Double blind, randomized, placebo controlled, parallel group, multiple site	162**** (37/125) P 54 y (21-74)	4	72.3± 16.1	9030± 3980 (mainly 2 points only)	NC	NC	NC	NC
					8 based on 3rd administration (1 h; 4 weeks)	160± 36.5	32100± 10800	0.22± 0.07	171± 41.5	52.2± 9.90	9.3± 8.7

Mean PK parameters are rounded to 3 significant figures where appropriate, except for t_{max} which is rounded to 2 significant figures

HV: healthy volunteers; M/F: male/female; MAD: multiple ascending doses; MTD: maximum tolerated dose; NC: not calculated; P: patients; PD: pharmacodynamics; PK: pharmacokinetics; SAD: single ascending dose

*: time zero equal to start of 1 h infusion; #: parameters not reported as area extrapolated too high for all subjects (≥ 48%)

** area extrapolated very high

*** values were recalculated (i.e. normalized by body weight) for this appendix

**** Full Analysis Set (FAS)

Table 10 Summary of Mean (± SD) Predicted AUC_τ, C_{max} and C_{min} for the First Dosing Interval and at Steady-State following 4 and 8 mg/kg Tocilizumab every 4 Weeks

Pharmacokinetic Parameter	4 mg/kg every 4 weeks			8 mg/kg every 4 weeks		
	First Dosing Interval	Steady-State	R_{AC}	First Dosing Interval	Steady-State	R_{AC}
AUC_τ (h·µg/mL)	11800 ± 5000	13000 ± 5800	1.11	28800 ± 11600	35000 ± 15500	1.22
C_{max} (µg/mL)	86.8 ± 40.9	88.3 ± 41.4	1.02	174 ± 81.9	183 ± 85.6	1.06
C_{min} (µg/mL)	0.76 ± 0.91	1.49 ± 2.13	1.96	4.14 ± 4.29	9.74 ± 10.5	2.35

R_{AC}: accumulation ratio; a simulation experiment was performed with 48 weeks of treatment and the two doses tested in Phase III (4 and 8 mg/kg every 4 weeks) with 10 studies (replicates) (n = 1793 patients per replicate); AUC_τ: AUC within dosing interval

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

A single dose toxicity study in cynomolgus monkeys and 28-day toxicity study in Sprague Dawley rats up to 50 and 100 mg/kg/IV respectively did not show any organ system toxicity due to the drug. However, injection site inflammation was noted in the control and drug treated animals.

A 6-month repeat dose toxicity to IL-6 antibody was evaluated in cynomolgus monkeys at 1, 10, and 100 mg/kg, IV infusion per week with a 2 month recovery. The highest dose of 100 mg/kg was tolerated without treatment related mortality. However, one male monkey at 10 mg/kg showed gingival inflammation that subsided following extraction of the tooth. The relationship of gingival inflammation to the treatment is not clearly known due to low prevalence of the observation, but is not likely due to the treatment as it involved a single tooth. **A slight granuloma in the liver at 10-100 mg/kg was observed and it was reversible. Degeneration of the skeletal muscle was noted at 10-100 mg/kg that was not completely reversible.** However, creatinine phosphokinase activity was not affected by the treatment as shown in Report #TOX03-0002. Antibody to IL-6 antibody was detected at 1 and 10 mg/kg only. The half-life of MRA was 10-13 days in this study. Intestinal IgA production was not affected by the treatment in monkeys. MRA did not show biologically relevant modulation in the CD4+, CD8+, and CD 20+ lymphocytes. The NOEL was 1 mg/kg, IV. Inflammation in the injection site was noted in control and treated animals.

It was concluded that granuloma of the liver and skeletal muscle degeneration was noted in monkeys at 10 and 100 mg/kg, IV for 6 months, and the NOEL was 1 mg/kg. Inflammation at the injection site was noted in control and treated animals.

Genetic toxicology:

Although genetic toxicology studies were not required for this BLA, the Applicant conducted and submitted two genetic toxicology studies. MRA was not mutagenic in either the Ames bacterial reverse mutation assay or the chromosomal aberration assay in peripheral lymphocytes from human volunteers.

Carcinogenicity:

The Applicant did not conduct any carcinogenicity study.

The Applicant was asked to provide rationale why such studies were not possible during the Pre-BLA meeting. The Applicant was also asked to discuss the impact of MRA on tumor surveillance and tumor development. The Applicant argued that IL-6 is involved in the tumor progression and tocilizumab would prevent cancer. Therefore, carcinogenicity study for tocilizumab was not warranted. Since tocilizumab showed anti product antibodies in rodents, it was not possible to conduct conventional carcinogenicity studies.

Reproductive toxicology:

A total of four reproductive safety studies were submitted in the BLA. Fertility and early embryonic development study in male and female Sprague Dawley rats showed no effect at 5, 16 and 50 mg/kg/day/IV in the segment 1 study.

Three segment II studies were conducted in rats, rabbits and cynomolgus monkeys for the determination of the effect of treatment on organogenesis and fetal development. Sprague Dawley rats did not show teratogenicity, variations or embryo-toxicity at 5, 16 and 50 mg/kg/day, IV when treated between gestation days 7 to 17. The NOEL was 50 mg/kg/day, IV. The validity for the selection of high dose is uncertain due to absence of maternal toxicity.

The rat model may not be appropriate due to weak tissue cross-reactivity of MRA in rats and the Applicant stated that rat IL-6R did not bind to MRA. Therefore, both segment 1 and segment 2 reproductive safety studies in rats would not have any impact to determine the reproductive safety of MRA.

Japanese White rabbits were treated at 0.5, 5, and 50 mg/kg/day, IV from gestation days 6 to 18. A retardation of sternum development was noted at all doses. Increased fetal deaths were noted at 5 mg/kg. A NOEL was not established. The MRA trough levels on day 19 were 212 and 3388 ug/mL at 5 and 50 mg/kg, respectively. Detectable level of MRA was not noted at 0.5 mg/kg on day 19. However, titer values of anti-MRA were 32500, 115500 and 6663 at 0.5, 5, and 50 mg/kg, respectively on day 19.

It is interesting to note that the treatment showed anti-MRA antibodies on days 19 and 28 that had an impact on organogenicity in rabbits. Most of the effect was noted at 5 mg/kg e.g. body weight, food consumption, embryo-lethality and deaths. Implication of the finding to the clinical situation is unknown because in the clinical data minimal anti-MRA was noted in patients and monkeys did not show anti-MRA antibodies yet fetal deaths were noted. The Applicant also did not provide any tissue cross-reactivity data of MRA in rabbits. Based on the incompatibility to IV MRA, the rabbit study considered to be unnecessary and would not provide meaningful data to determine reproductive safety of MRA.

The third teratogenicity and embryotoxicity study was conducted in cynomolgus monkeys at 2, 10 and 50 mg/kg/day/IV between gestation days 20 to 50. Data suggested that MRA-induced abortion and or fetal deaths were noted at 10 and 50 mg/kg. The NOEL was 2 mg/kg. No anti-MRA was detected in most of the treated animals. Plasma trough MRA levels were 319, 1646 and 5813 ug/mL at 2, 10, 50 mg/kg/day, IV, respectively, at the end of gestation day 50. Data suggest that the treatment with MRA to pregnant monkeys had an adverse effect on the viability of fetuses. Based on the data in cynomolgus monkeys, Pregnancy Category C should be designated to tocilizumab. The reviewer recommends that only segment 2 reproductive safety data in monkeys should be included in the label, in concurrence with the Applicant's proposed labeling.

Comments for effect of MRA on early, late pregnancy and post natal development of fetuses:

The Applicant was asked to conduct segment 3 reproductive safety study or to justify for a waiver at Pre-BLA meeting on Oct 9, 2007. The Applicant did not conduct segment 3 reproductive safety study in appropriate model and provided their position for not conducting the study as discussed in the Pre-BLA meeting. The Applicant stated that reasons for not conducting segment-3 study were as follows:

1. knock-out mouse showed normal reproduction
2. 6-month toxicity study in monkeys did not show any organ system toxicity
3. Segment-2 reproductive safety data in monkeys did not show teratogenicity
4. Due to a long half-life of MRA, the effect of MRA on fetuses would be predicted from the segment-2 reproductive safety study in monkeys.

Therefore, further assessment of pre and post natal safety in animal study would not be warranted.

However, review of data in the BLA and referenced literature suggest that

1. Delivery and post-natal survival of F_1 generation could be compromised due to immunosuppressive effect of MRA.
2. MRA to have anti-growth effect that could manifest late abortion and affect post-natal development.

3. In addition, IL-6 is an important growth factor for early embryogenesis and implantation to late gestation.

Based on the above the reviewer recommends conducting both segment 1 and segment 3 reproductive safety studies in appropriate model using surrogate antibody if necessary so as to determine the reproductive safety of MRA and to write the package insert appropriately for the target patient population.

Special toxicology: No special toxicity study was submitted for a review.

2.6.6.2 Single-dose toxicity

Study Title: Single dose intravenous toxicity study of MRA in cynomolgus monkeys, TOX02-0161.

The study was conducted between May 20, 1996 to Dec 9, 1996 at Chugai Pharmaceutical Company, Nagano, Japan according to GLP.

MRA at 6.67 mg/mL from Lot # MRA 96D02 was used in the study. The drug substance was diluted with 20 mM phosphate buffered saline at pH 7. Male and female cynomolgus monkeys were used in the study. Animals were 2.5-4.0 years old at procurement. The study design is shown below.

Dose, mg/kg	Concentration, mg/mL	Volume, mL/kg	Male	Female
0	0	15	1	1
1	6.67	0.15	1	1
10	6.67	1.5	1	1
100	6.67	15	1	1

The Applicant stated that the high dose was 100 fold higher than the expected clinical dose. The control animals were treated with the vehicle. Animals were treated by single IV injection in the saphenous vein at 10 mL/min. Clinical observations and mortality were recorded up to 3 hours post dose on the day of dosing. Animals were observed twice daily up to 28 days and once daily up to 56 days. The body weight was recorded at predose, days 1, 3, 7, 14, 21, 28, 35, 42, 49, and 56 after dosing. Standard hematology parameters were determined at predose and on day 14. Standard serum chemistry data were collected at predose, and on days 1, 7, 14, 28, and 42 after dosing. Blood samples were drawn from saphenous vein. Autopsy was not performed after day 56 because no clinical signs were observed. Blood samples were collected for plasma MRA measurement at predose, at 1 and 8 hour post dose, days 1, 3, 7, 14, 21, 28, 35, 42, 49, and 56 post dose. Anti-MRA antibody was detected on days 7, 14, 21, 28, 42, and 56 post dose.

Results of the study showed no clinical signs or mortality. No treatment related change in the body weight hematology and serum chemistry was detected after the single dose.

The MRA PK parameters are shown below from the Applicant's table. Measurable amount of MRA was not detected at 1 mg/kg after day 3. The average half-life was about 7 days.

Table 9
Pharmacokinetic parameters in single-dose intravenous toxicity study of MRA in cynomolgus monkeys

Dose (mg/kg)	Sex	Animal No.	Parameter		
			AUC (mg · hr/ml)	Vdss (ml/kg)	t1/2 (β) (day)
10	Male	00301	0.83	87.74	5.34
	Female	50301	1.36	74.58	7.34
100	Male	00401	14.23	80.17	8.37
	Female	50401	11.99	79.81	6.79

Anti-MRA antibody was detected at 1 and 10 mg/kg up to day 56 beginning day 14 in male moneys and no anti-MRA antibody was detected in female monkeys.

It is concluded that single dose up to 100 mg/kg/IV was tolerated in cynomolgus monkeys without toxicity.

2.6.6.3 Repeat-dose toxicity

Study title: Six-month repeated dose intravenous toxicity study of MRA in cynomolgus monkeys

Key study findings: Granuloma of liver and skeletal muscle degeneration was noted at 10 and 100 mg/kg. The NOEL was 1 mg/kg for systemic toxicity. Inflammation at the injection site was noted in the control and treated animals.

Study no.: Tox-02-0169(J99-0451)
Volume # DVD, and page #: 1
Conducting laboratory and location: Fugi Gotemba Research Laboratory, Chugai Pharmaceutical Co Ltd., 135 Komakado, Shizuoka, Japan
Date of study initiation: Feb 24, 1997 (approx)
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #MRA96G01, and % purity: Certificate of analysis of the drug product was not attached in the report.

Methods

Doses:

Dose (mg/kg)	MRA concentration (mg/mL)	Dosing volume (mL/kg)	Number of animals (cage number: animal number) Male	Female
0	0	20	4(001-004:00101-00104)	4(018-021:50101-50104)
1	0.055	20	4(005-008:00201-00204)	4(022-025:50201-50204)
10	0.50	20	4(009-012:00301-00304)	4(026-029:50301-50304)
100	5.00	20	5(013-017:00401-00405)	5(030-034:50401-50405)

Species/strain: Chinese cynomolgus monkeys

Number/sex/group or time point (main study): see study design above

Route, formulation, volume, and infusion rate: Continuous infusion at 2 mL/min into cephalic vein once a week for 6 months

Satellite groups used for toxicokinetics or recovery: Recovery groups are shown below. Recovery period was for 2 months.

Group	Male	Female
Control	1	1
1 mg/kg	0	1
10 mg/kg	1	1
100 mg/kg	2	2

Age: Approximately 4 years of age

Weight: 2.6 to 4.0 kg for male and 2.3 to 3.1 kg for female monkeys

Sampling times: Blood samples were collected from the saphenous vein at pre study, and on weeks 4, 9, 13, 17, 21, and 25 from main study animals. Blood samples were also collected on weeks 2, 3, 6, and 7 from recovery animals. Hematological and blood chemistry parameters were evaluated. Some of the samples were used for the assay of coagulation parameters, IL-6 levels, and platelet aggregation.

Plasma MRA levels were measured at pre test, weeks 1, 2, 4, 6, 8, 12, 16, 20, and 26 after the treatment and on weeks 1, 2, 3, 4, 5, 6, 7, and 8 from recovery animals. Anti-MRA antibodies were also measured at predose, weeks 2, 4, 8, 12, 16, 20, and 26 during the treatment period and, on week 2, 3, 6, and 8 weeks of the recovery period.

Unique study design or methodology (if any): Rectal temperature was recorded before dosing and on weeks 6, 12, and 25 after the initiation of treatment.

Observations and times:

Mortality: See clinical sign parameter

Clinical signs: General conditions and behavior was observed once a day and three times on the day of injection.

Body weights: The body weight was recorded once a week before dosing, during dosing and during the recovery period.

Food consumption: Daily food intake was recorded from the residual food.

Ophthalmoscopy: Ophthalmological examinations were conducted in ketamine anesthetized animals following dilatation of the pupil by Mydrin P. Both anterior, posterior chambers and retina were examined at pre dose, week 24 of the treatment and on week 7 in the recovery animals. Slit lamp and retinal camera were used for the determination of ophthalmic changes in the eye.
EKG: ECG was recorded at pre dose, weeks 11 and 24 of the treatment period.
Hematology: The Applicant provided a list of hematological parameters evaluated. The parameters were standard for a toxicity study. The morphology of blood cells was examined under a light microscope to understand the maturation process of cells under the influence of IL-6 antibodies. It should be noted that the in vivo cytogenetic study for biologics are generally not conducted due to lack of transport of the large molecules access the cell membrane. However, the maturation process of blood cells in the peripheral stream would be understood by the morphological examinations. At autopsy, sternal bone marrow cells were collected and 500 nucleated cells were examined for myeloid and erythroid cell ratios. Coagulation parameters e.g., prothrombin time, thrombin time and APTT were examined along with the effect of the drug for the ability of platelets to aggregate in vitro.
Clinical chemistry: Standard clinical chemistry parameters from 16 hour fasted monkeys were determined. In addition, serum IL-6 levels were measured at several time points during the pre- treatment, treatment, and recovery period.
Urinalysis: Urine samples were collected for 18 hours. Food and water were withheld during the collection period. The urine samples were collected at predose,
Gross pathology: Animals were sacrificed by exsanguination under anesthesia with Ketalar and Nembutal injections at seven days after the last dose and at the recovery period. Macroscopic changes in the systemic organs were recorded. Animals that died before the schedule termination were autopsied as soon as possible. The organ weight of selected organs was recorded as shown in the table below. Organ weights were normalized to the body weight.
Organ weights (specify organs weighed if not in histopath table):
Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no (x)

Protocol specified tissues were fixed for the histopathological examinations. Testes and epididymides were fixed in Bouin's solution. Eyes were fixed in glutaraldehyde. Other tissues were fixed in formalin. Tissues were stained with hematoxylin-eosin stain for light microscopic examinations.

One male and one female from each group were selected for electron microscopic changes of liver and kidney. Liver and kidney tissues from one male and one female animal from the recovery group except animals from 1 mg/kg dose group were also examined by electron microscopy. Tissues for the EM study were fixed in 2.5% glutaraldehyde solution and osmium tetroxide solution.

Results

Mortality: One male (#202) treated with 1 mg/kg died on day 171. Distension of GI tract was noted. The Applicant stated that hemorrhage in the oral cavity and foamy discharges from the nasal cavity were noted. Subcutaneous hemorrhage was noted in several sites of the body. Possibility of antigen–antibody response as a contributory factor to the death was minimal because other monkeys with a similar titer did not show untoward response. Thymus atrophy was noted at higher dose in the absence of any mortality in other animals. Therefore, immunosuppression would not be the cause of death of the monkey. The cause of death of the animal was considered as incidental.
Clinical signs: One male (301) at 10 mg/kg showed gingival inflammation at upper left canine tooth around 96 day of the treatment. The inflammation subsided following extraction of all canine teeth for the monkey.

Body weights: No treatment related changes in the body weight were reported. The average body weight (kg) in male and female monkeys is shown below.

Dose, mg/kg	Pretreatment		Day 88		Day 179	
	Male	Female	Male	Female	Male	Female
Control	3.1	2.6	3.5	2.7	3.8	2.7
1	3.5	2.5	3.8	2.7	4.3	2.9
10	3.2	2.6	3.5	2.7	3.8	2.7
100	3.2	2.6	3.6	2.8	3.8	2.9

Food consumption: No treatment related changes in the food consumption was recorded.

Ophthalmoscopy: There was no treatment related change in the ophthalmic examinations except one male monkey #403 at 100 mg/kg showed cloudiness (decreased transparency) in the left eye on week 24. The finding could be incidental. Given a small number of animals deployed in the study and the occurrence at the high dose in only in male, it is likely that the change might be incidental.

EKG: No treatment related ECG change was noted among animals in which ECG was recorded.

Hematology: Some of the average values of hematology parameters on week 25 are shown below.

Dose mg/kg	RBC 10^6/uL		Platelet 10^3/uL		Hb g/dL	
	Male	Female	Male	Female	Male	Female
Control	5.45	5.23	332	297	13.6	12.5
1	5.13	5.16	280	346	13.0	12.6
10	5.54	4.96	293	327	13.6	12.3
100	5.53	5.13	300	325	13.4	12.3

Above data showed no treatment related change in the hematology parameters at the end of treatment. Average data for the differential counts (%) are shown below. Although it appeared that there was a decrease in the lymphocyte counts and increase in the neutrophil counts, the change was spontaneous rather than the treatment effect. It was concluded that the treatment did not have any effect on the hematology parameters (M=Male) and F=Female).

Dose	**Neutrophils**			**Lymphocytes**			**Monocytes**		
mg/kg	**M/F**	**M/F**	**M/F**	**M/F**	**M/F**	**M/F**	**M/F**	**M/F**	**M/F**
	-2 Wk	13 Wk	25 Wk	-2 Wk	13 Wk	25 Wk	-2 Wk	13 Wk	25 W
Control	34.8/24.9	20.2/44.0	14.5/29.9	59.0/69.7	72.9/51.7	77.5/66.3	3.3/3.0	2.7/3.0	3.5/2
1	42.3/31.5	18.7/48.8	21.9/37.5	52/62.1	75.7/45.5	73.5/56.7	3.2/3.8	2.6/4.2	2.6/3
10	42.0/27.9	29.5/48.2	26.8/49.2	51/66.0	64.0/46.0	66.9/45.5	3.7/3.2	3.6/2.9	4.0/2
100	35.2/24.5	25.0/41.4	34.2/51.2	60.2/70	70.6/54.9	61.2/44.5	2.8/3.3	2.4/2.4	2.8/2

Average data for plasma coagulation parameters are shown below.

Dose	**PT, sec**			**APTT, sec**		
mg/kg	**M/F**	**M/F**	**M/F**	**M/F**	**M/F**	**M/F**
	-2 wk	13 wk	25 wk	-2 wk	13 wk	25 wk
Control	10.9/10.9	10.5/10.2	11.1/10.6	20.2/19.5	19.7/19.3	19.1/19.1
1	10.7/10.9	10.4/10.4	10.8/10.8	19.7/21.0	19.0/20.2	19.3/19.4
10	10.8/10.7	10.7/10.4	10.4/10.6	18.3/19.0	18.0/18.2	18.7/17.9
100	11.3/11.0	11.1/10.7	11.0/10.7	20.1/19.4	20.0/18.9	19.7/18.3

Based on the average data, there is no treatment related change for PT and APTT time.

The average data for fibrinogen level (g/L) are shown below.

Dose, mg/kg	**fibrinogen, g/L**	
	Males/Females	**Males/Females**
	17 wk	**23 wk**
Control	1.99/1.87	2.09/2.06
1	2.04/1.96	2.09/2.08
10	1.81/1.73	1.85/1.96
100	1.80/1.54	1.91/1.79

Male monkeys showed 1.80 to 2.31 g/L fibrinogen level and the treatment had no effect on the fibrinogen level. Female monkeys showed 1.79 to 2.19 g/L in the control female monkeys. Although, female monkeys showed a slightly lower fibrinogen level at 10 and 100 mg/kg, the effect is considered to be unrelated to the treatment due to variability of the data within treated groups.

Male and female monkeys did not show any treatment related change in the myeloid and erythroid ratio on week 26.

Overall, it is concluded that the treatment had no effect on the hematology in the monkey.

Clinical chemistry:

The average serum chemistry data in male monkeys did not show any treatment related adverse change.

Female monkeys showed a slight increase in the average TG level from 24 mg/dL in the control to 42 mg/dL at 100 mg/kg at the end of week 25. The sodium level at 10 and 100 mg/kg doses was increased to 147.9 and 148.7 meq/L, respectively, on week 25 from the control level of 144.9 meq/L. Although, the data were statistically significant, clinical significance of the change is unknown.

Plasma level of cytokine:

Male and female monkeys showed traces or undetectable level of cytokine IL-6 in the control monkeys and there was no change during the treatment period.

Urinalysis: There was treatment related changes in the urine analysis.

Gross pathology: Some of the gross autopsy findings are shown below.

	Males (mg/kg)				**Females (mg/kg)**			
Lesion	**0**	**1**	**10**	**100**	**0**	**1**	**10**	**100**
Thymus, atrophy	0	2, slight-marked	3,slight	1, marked				
Injection site, dark red area	0	0	1, slight	0	1, slight	0	1, slight	1, mod
Lung, bronchus adhesion	2, slight-mod	0		1, slight				
Uterus, dark red content					0	0	0	1, slight

One control male showed parasite in the large intestine. Male monkeys showed some sign of immunosuppression in thymus gland.

Organ weights (specify organs weighed if not in histopath table): The organ weight data did not show any treatment related change in male and female animals at the end of week 26.

Reviewer: Asoke Mukherjee, BLA No. 125276

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no (x)

Some of the histological changes are shown below.

	Males (mg/kg)				Females (mg/kg)			
Lesion	**0**	**1**	**10**	**100**	**0**	**1**	**10**	**100**
Injection site, inflammation and hemorrhage	3, v. slight-slight	2, v. slight-slight	3, v. slight	1, v. slight	2, v. slight	1, v. slight	3, v. slight	3, v.v. slight
Recovery group	1		1	2	1, slight	0	0	0
Liver granuloma	0	0	0	1, v. slight	0	0	1, v. slight	0
Recovery group	0		0	0	0	0	0	0
Ileum, duodenum, yellow pigment in mucosa	0	0	1, v. slight	1, v. slight				
Recovery	0		0	0				
Duodenum, smooth muscle degeneration, inflammation	0	0	0	0	0	0	0	1, v. slight
Recovery group	0		0	0	0	0	0	0
Spleen, hyaline deposition	3, v. slight-slight	3, v. slight-slight	3,, v. slight-slight	3, slight	1, v. slight	2, v. slight	1, v. slight	1, v. slight
Recovery group	1, v. slight		1, v. slight	1, v. slight	1, slight	0	1, slight	2, slight
Thymus, decreased lymphocyte	0	0	2, slight	0	0	1, v. slight	0	0
Recovery group	0		0	0	0	1, v. slight	0	0
Skeletal muscle, femoral, degeneration	0	0	1, slight	1, slight	0	0	1, slight	0

	Males (mg/kg)				Females (mg/kg)			
Lesion	**0**	**1**	**10**	**100**	**0**	**1**	**10**	**100**
of muscle fiber								
Recovery group	0		0	0	0	0	1, slight	0
Pituitary gland, cyst in pars intermedia					1, slight	1, slight	3, slight	2, slight
Pituitary gland, cyst in anterior lobe	1, slight	0	0	1, slight				
Recovery	0		0	0	0	0	0	1, slight

Male:

Inflammatory changes in the injection site were observed in the control and treated animals. These changes were not completely reversible during 8 weeks of recovery. A very slight granuloma was observed at 100 mg/kg in the liver that was reversible. **Degeneration of skeletal muscle fiber in femoral region was noted at 10 and 100 mg/kg and it was reversible. It is unlikely related to the infusion because the infusion was given through the cephalic vein.** Some animals in the control and treated groups showed pituitary cyst. It was not associated with the treatment based on the distribution of the lesion across the group and gender.

Female:

Female monkeys also showed injection site inflammation and reversible granuloma in the liver. Skeletal and duodenal smooth muscle degeneration was noted at 10 and 100 mg/kg, respective. Skeletal muscle degeneration was not reversible completely.

Conclusion of histopathology findings:

1. Based on the data, injection site inflammation due to procedure and vehicle was expected and it was not completely reversible during next 8 weeks after the treatment.

2. A very slight liver granuloma was present at 10-100 mg/kg and was reversible.

3. A slight degeneration of skeletal muscle fiber between 10-100 mg/kg was noted and it not completely reversible during next 8 weeks after the treatment.

The Applicant indicated in the method section that EM study was conducted for liver and kidney of selected animals. Considering the histopathology data obtained by light

microscopy the EM would not provide additional information on reversible inflammatory changes in the liver. However, EM data were not submitted in the report.

Toxicokinetics:

Plasma levels of the monoclonal antibody in male animals during the treatment period are shown below from the Applicant's table # 314. Based on the data a dose proportionate increase in the plasma level was noted between 10 and 100 mg/kg. The plasma level of the monoclonal antibody was minimal at 1 mg/kg. Also, it appears that a steady state was reached about 3 month after the initial of the treatment. The data also did not show an accumulation of the antibody at the end of treatment. The recovery animals did not show a complete washout of the antibody at the end of 8 weeks as shown below.

Table 314 Plasma concentration on cynomolgus monkeys in 6-month intravenous toxicity study of MRA

Duration:Dosing period
Sex:male (Unit: μg

Dose (mg/kg/w)	Animal No.	Week	Pre	1W	2W	4W	6W	8W	12W	16W	20W	26W
1	00201		0.0									
	00202		0.0									
	00203		0.0									
	00204		0.0									
	mean		0.0	1.4	2.3	2.0	0.8	1.0	1.6	1.4	2.3	4.1
	S.D.		0.0	1.1	1.7	2.3	1.7	2.1	3.1	2.8	3.6	4.9
10	00301		0.0									
	00302		0.0									
	00303		0.0									
	00304		0.0									
	mean		0.0	60.5	92.3	141.8	127.5	129.1	164.2	146.2	155.9	187.2
	S.D.		0.0	2.6	11.0	33.7	54.9	89.4	118.4	116.9	114.9	131.2
100	00401		0.0									
	00402		0.0									
	00403		0.0									
	00404		0.0									
	00405		0.0									
	mean		0.0	699.0	1057.6	1428.8	1442.0	1486.2	1758.7	1764.0	1842.8	1935.4
	S.D.		0.0	75.5	88.3	175.8	164.3	135.8	210.7	298.7	178.0	340.1

N.D. (Not Detected) < 0.78125 μg/ml
Mean and S.D. were calculated by treated N.D. as zero.

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Table 315 Plasma concentration on cynomolgus monkeys in 6-month intravenous toxicity study of MRA

Duration:Recovery period
Sex:male

(Unit: μg/ml)

Dose (mg/kg/w)	Animal No.	R-1W	R-2W	R-3W	R-4W	R-5W	R-6W	R-7W	R-8W
	Week	27W	28W	29W	30W	31W	32W	33W	34W
10	00303	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
100	00404								
	00405								
	mean	1331.7	1004.2	653.8	478.9	331.8	236.2	160.8	107.8
	S.D.	816.8	609.9	387.3	285.5	147.9	100.8	82.9	65.0

N.D. (Not Detected) < 0.78125 μg/ml
Mean and S.D. were calculated by treated N.D. as zero.

b(4)

The elimination half-life of the drug was about 13 days in male monkeys. Due to a long half-life of the antibody, a complete removal of the drug was not achieved during the recovery period. The elimination half-life data are shown below from the Applicant's table.

Table 318 Elimination half-life on cynomolgus monkeys in 6-month intravenous toxicit

Sex:male

Dose (mg/kg/w)	Animal No.	t1/2 (day)
10	00303	-
100	00404	14.7
	00405	12.1
	mean	13.4

-:Impossible to calculate
t1/2 calculated during Week 27 to Week 34

Female:

Table 316 Plasma concentration on cynomolgus monkeys in 6-month intravenous toxicity study of MRA

Duration:Dosing period
Sex:Female (Unit

Dose (mg/kg/w)	Animal No.	Week	Pre	1W	2W	4W	6W	8W	12W	16W	20W	
1	50201		0.0									
	50202		0.0									
	50203		0.0									
	50204		0.0									
		mean	0.0	0.2	0.8	0.9	0.0	0.0	0.3	0.3	0.3	
		S.D.	0.0	0.5	0.6	1.0	0.0	0.0	0.7	0.7	0.6	
10	50301		0.0									
	50302		0.0									
	50303		0.0									
	50304		0.0									
		mean	0.0	67.8	110.1	152.9	167.6	177.9	258.5	245.7	205.8	
		S.D.	0.0	12.8	27.0	60.4	51.9	55.5	77.5	54.9	46.1	
100	50401		0.0									
	50402		0.0									
	50403		0.0									
	50404		0.0									
	50405		0.0									
		mean	0.0	647.8	1096.0	1656.4	1572.6	1594.9	1993.4	1867.6	1764.7	
		S.D.	0.0	46.8	78.3	170.0	150.3	87.7	267.9	156.4	206.9	

b(4)

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b(4)

N.D. (Not Detected) < 0.78125 μg/mL
Mean and S.D. were calculated by treated N.D. as zero.

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Table 317 Plasma concentration on cynomolgus monkeys in 6-month intravenous toxicity study of MRA

Duration:Recovery period
Sex:female

(Unit: μg/mL)

Dose (mg/kg/w)	Animal No.	R-1W	R-2W	R-3W	R-4W	R-5W	R-6W	R-7W	R-8W
	Week	27W	28W	29W	30W	31W	32W	33W	34W
1	50304	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
10	50304								
	50404								
100	50405								
	mean	1324.9	860.3	546.7	419.2	242.8	173.5	114.7	77.0
	S.D.	691.1	462.9	401.7	335.4	238.6	201.9	151.3	108.8

b(4)

N.D. (Not Detected) < 0.78125 μg/mL
Mean and S.D. were calculated by treated N.D. as zero.

The elimination half-life data in female monkeys are shown below.

Table 319 Elimination half-life on cynomolgus monkeys in 6-month intravenous toxicity study of MRA

Sex: female

Dose (mg/kg/w)	Animal No.	t1/2 (day)
1	50204	-
10	50304	6.8
100	50404	6.7
	50405	14.1
	mean	10.4

-: Impossible to calculate
t1/2 calculated during Week 27 to Week 34

The kinetic profile of the drug in female animals was similar to that in the male monkeys.

Based on the data, the elimination half-life of the days was between 10-13 days.

Other:

Positive antibody titers were noted at 1 and 10 mg/kg from week 2 of the treatment up to the end of treatment period in several male and female monkeys. One male #00303, female #50204 and #50304 also showed anti IL-6 antibody response during the recovery period. However, except a slight positive response on week 2, none of male and female monkeys treated at 100 mg/kg showed antibodies to IL-6 antibody during the treatment and recovery period. It was possible that immune cells were suppressed under the influence of IL-6 antibodies and did not produce antibodies at 100 mg/kg dose of IL-6 monoclonal antibody. The Applicant stated that no administration related abnormalities in the fibrinogen level or platelet aggregation or platelet counts was noted.

The treatment had no effect to normal body temperature in male and female monkeys.

The Applicant reported IgA production in the small intestine samples from monkeys treated with the vehicle and 100 mg/kg (TOX02-0207, JITSU 99-0182) by immuno-histochemical methods. Data showed that MRA treatment did not affect intestinal mucosal IgA production in monkeys treated with MRA at 100 mg/kg. Data might reflect that the IV treatment did not transport the antibody to the local site to interfere with the synthesis and release of IgA in the intestinal mucosa as opposed to an inhibition of mucosal IgA production observed in the IL-6R knock-out mouse model.

Immuno-toxicological investigations were conducted in lymphocytes from peripheral blood from monkeys (TOX-02-0205, JITSU99-0180). The dose groups and time point for blood collection for immuno-phenotyping study is shown below.

Table 1 Experimental groups of cynomolgus monkey

Dose (/week)	Number of animals (Animal No.)	Bleeding points after first administration
0 mg/kg	♂ : 4(00101-00104)	
	♀ : 4(50101-50104)	-1 day
1 mg/kg	♂ : 4(00201-00204)*	1 week
	♀ : 4(50201-50204)	1 month
10 mg/kg	♂ : 4(00301-00304)	3 month
	♀ : 4(50301-50304)	6 month
100 mg/kg	♂ : 5(00401-00405)	
	♀ : 5(50401-50405)	

* Animal No. 00202 was died before completion of the administration.

Data for several subsets of lymphocytes were presented. CD4+ and CD8+ lymphocyte counts were increased slightly at 1 mg/kg at 6 month in male monkeys. The treatment had no effect on CD4+ and $CD8^+$ lymphocytes in female monkeys. These data indicated that the basal counts of CD4+ and $CD8^+$ lymphocyte were unaffected by the treatment with IL-6R antibody in monkeys.

MRA treatment showed a statistically significant reduction in CD20+ B-lymphocytes in male monkeys (B lymphocyte maturation and differentiation isotype receptor) at 10 and 100 mg/kg at the end of 3 and 6 months of the treatment. However, female monkeys did not show any treatment related effect on the CD20+ cells. Female monkeys also showed a reduction of T lymphocyte proliferation at 1 and 10 mg/kg at the end of 6 months. Male monkeys did not show any effect on the mitogen induced T lymphocyte proliferation. Neutrophil chemotaxis and phagocytosis were not affected by the treatment.

In the opinion of the reviewer, MRA did not have clinically relevant or biologically significant effect on immune cell counts and functions in normal male and female monkeys. However, the Applicant concluded that the treatment with MRA "clearly affected the immune system within a range reflecting the pharmacological action of MRA in cynomolgus monkeys. It was concluded that there were no problems concerning Immunotoxicity."

Creatinine phosphokinase activity measurement (TOX03-0002, J99-0018) activity did not show changes that could be attributed to the skeletal muscle degeneration.

Conclusion of 26 week toxicity study in monkeys:

A 6-month repeat dose toxicity to IL-6R antibody was evaluated in cynomolgus monkeys at 1, 10 and 100 mg/kg/IV infusion dose per week with a 2 month recovery. The highest dose 100 mg/kg was tolerated without treatment related mortality. However, one male monkey at 10 mg/kg showed gingival inflammation that was subsided following extraction of the tooth. The relationship of gingival inflammation to the treatment is not clearly known due to low prevalence of the observation. A slight granuloma in the liver at 10-100 mg/kg was observed and it was reversible. Degeneration of the skeletal muscle was noted at 10-100 mg/kg that was not completely reversible. However, creatinine phosphokinase activity was not affected by the treatment as shown in Report #TOX03-0002. Antibody to IL-6 antibody was detected at 1 and 10 mg/kg only. The half-life of the antibody was 10-13 days. Intestinal IgA production was not affected by the treatment in monkeys. MRA did not show biologically relevant modulation in the CD4+, CD8+ and CD 20+ lymphocytes. The NOEL was 1 mg/kg/IV. Inflammation in the injection site was noted in control and treated animals.

Study title: One-month repeated dose intravenous toxicity of MRA in rats

Key study findings: No systemic toxicity was detected up to 50 mg/kg/day IV for 28 days in rats. Non-reversible injection site inflammation and hemorrhage was observed in placebo and MRA treated animals. Neutralizing antibody was detected at the end of 28 day recovery period. The NOEL for MRA could not be established due to its effect in the injection site that could be due to MRA and its antibody also. Overall, this is not a suitable model to determine systemic toxicity to MRA.

Study no.: Tox-02-0164 (J97-0141)
Conducting laboratory and location: Chugai Pharmaceutical Co., Ltd, Nagano 399-46, Japan
Date of study initiation: Aug 6, 1996
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot # MRA96D02, and % purity: Not provided

Methods

Doses: The study design is shown below.

Group	Dose, mg/kg/day	Volume, ml/kg	#Animal/sex
1	Control, phosphate buffer vehicle	1.5	15
2	2	1.5	15

3	10	1.5	15
4	50	7.5	15

Satellite group

Group	Dose, mg/kg/day	Volume, mL/kg	#Animal/sex
1	2	1.5	3
2	10	1.5	3
3	50	7.5	3

The treatment was given once daily for 4 weeks at 2 mL/min. The dose was selected on the basis of a two-week study. However, the Applicant stated that no toxicity was noted up to 50 mg/kg in the 2-week study. Satellite groups were allotted for the determination of PK parameters. The treatment was given intravenously because it is the expected clinical route. The Applicant stated about 28-day recovery in PK section. However, it is not clear from the report how many animals were observed for the recovery. It appears from the data table that 5 animals/sex/group was observed for recovery.

Species/strain: Male and female Sprague Dawley rats, Slc: SD
Number/sex/group or time point (main study): See study design table above.
Route, formulation, volume, and infusion rate: See study design, MRA was diluted with 20 mM phosphate buffer. The dosing solution was stable for 15 days.
Satellite groups used for toxicokinetics or recovery: See study design above.
Age: Six weeks at the beginning of the treatment
Weight: Males 175-199 g; Females 133-159 g
Sampling times: Plasma concentrations of MRA were determined from blood samples collected at 5 min, 1, 4, 8 and 24 hours on days 6, 13, and 20 of the of the main groups. Samples were also taken on days 3, 7, 14 and 28 of recovery animals. Blood samples were drawn from the jugular vein. Plasma MRA was assayed by ELISA. Plasma half-life of MRA was determined from the concentration-time curve.
Unique study design or methodology (if any): The antibody to the drug substance (anti-MRA antibody) was also determined at terminal sacrifice and end of recovery.

Observations and times:
Mortality: Mortality and general conditions were observed once daily before the daily treatment and twice after treatment each day.
Clinical signs: See above.
Body weights: The body weight was recorded at before the initiation of the treatment, 3 times per week during the treatment.
Food consumption: The food consumption was recorded before starting the treatment and twice per week during the treatment period.
Ophthalmoscopy: Ophthalmological examinations were conducted before the beginning of treatment and on week 3 by a slit lamp. Eyes were dilated by mydriatic agent e.g., Midrin for examination of the posterior chamber and fundus of the eye.
EKG: Not recorded

Hematology: Blood samples were taken on weeks 2 and 3 during the treatment, week 3 of the recovery for hematological examinations before dosing the animals. Blood samples were collected by puncturing metatarsal vein. At the end of treatment and recovery, coagulation parameters (PT, APTT) were determined. Bone marrow smears were prepared from 5 rats/sex at the end of treatment period and from all rats at the end of recovery period for the determination of myeloid and erythroid ratios.
Clinical chemistry: Blood samples were drawn from the abdominal aorta at the end of treatment and recovery period. Standard clinical chemistry parameters were determined.
Urinalysis: Urine samples were collected on week 3 of the treatment and on week 3 of from the recovery animals from all animals from each group. Animals were placed in a metabolism cage for the collection of 24-hour pooled urine sample. Following parameters were examined:

pH, proteins, ketone bodies, bilirubin, occult blood, cells, specific gravity, presence of any crystals or casts, urine volume and electrolytes.

Gross pathology:

At the end of terminal sacrifice or recovery animals were sacrificed by exsanguination. Gross changes in protocol specified organs were recorded. Organ weights of protocol specified organs were recorded.

Organ weights (specify organs weighed if not in histopath table): See table
Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no (x)

Tissues were fixed in formalin, testes and epididymides were fixed in Bouin's solution, eye and Hardarian gland were fixed in 4% glutaraldehyde. The histopathology was conducted by light microscopy following staining slides with hematoxylin and eosin. Some of the liver tissues were stained with Nile blue. Histopathology of the dead animals was also conducted.

Results

Mortality: Male #00307 at 10 mg/kg died on day 8 and evidence of pulmonary edema, hemorrhage in the brain, **and fracture of the cranial bone noted**. The Applicant stated that head injury due to handling could have resulted in the injury and death of the rat.

Clinical signs: Hemophthalmia was noted in one female #50410 at 50 mg/kg and one male at 2 mg/kg (#00204). No other treatment related change was noted.

Body weights: The average body weight (g) data are shown in the table below. Day 0 referred to the first day of treatment. There was no treatment related change in the body weight gain in most animals. Female rats at 50 mg/kg showed a 11% reduction in the body weight gain. The change was reversible.

Dose, mg/kg	Day 0, Male	Day 0, Female	Day 27, Male	Day 27, Female
Control	188	145	323	214
2	187	145	320	213
10	188	146	321	210
50	189	143	327	205

Food consumption: The average food consumption (g/day/animal) was not affected by the treatment in male rats. A slight decrease in the food consumption from 15.2 to 14.5 was noted in female rats between day 0 and day 27 at 50 mg/kg. The water consumption was also reduced from 23.2 mL/day/animal on day 0 to 19.1 mL/day/animal on day 27 at 50 mg/kg dose in female rats.

Ophthalmoscopy: One male rat at 2 mg/kg showed dark red area in the cornea. However, it is incidental and appeared to be unrelated to the treatment. No treatment related ocular finding was noted in the fundus.

EKG: No EKG was recorded.

Hematology: Hematology data did not show treatment related change on weeks 2 and 3 in male and female rats.

No treatment related change in the coagulation parameters was noted in male and female rats.

Data from the bone marrow smears did not show any treatment related change in male and female rats.

Clinical chemistry: Blood chemistry data at the end of week 4 of treatment did not show treatment related change in male and female rats although some parameters were decreased statistically significantly.

Urinalysis: Male rats did not show significant change in the urine chemistry. Female rats showed a slight decrease in the average calcium excretion at 50 mg/kg (3.9 mg/kg) when compared to the control (5.08 mg/kg); however, it was reversible.

Gross pathology: Male rats at 10 mg/kg showed a slight nodule in the liver and red foci in kidney 1 and 2 out of 10 rats, respectively. Relevance of these changes is not known.

Organ weights (specify organs weighed if not in histopath table): Organ weight data with or without normalization to the body weight did not show any treatment related change in male and female rats.

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no (x)

Male:

Some of the histopathological changes in male rats are injection site hemorrhage and inflammation in the control and MRA-treated animals. Slight vasculitis was still present at the end of 28-day recovery. A slight granuloma in lung was noted at 2 and 10 mg/kg in 1/10 rats at each dose. One male rat out of 10 rats showed a slight epidermal cyst in the bronchus at 50 mg/kg. However, one female rat in the control group also showed epidermal cyst in the bronchus. Therefore epidermal cyst in bronchus considered to be unrelated to the treatment.
Female:

Female rats also showed a slight hemorrhage and inflammation at the site of injection in placebo and MRA treated rats. Inflammation at the injection site was also present in the placebo and MRA treated animals at the end of the 28-day recovery period. A slight calcification of cortico-medullary junction in the kidney was noted at 50 mg/kg. Recovery animal did not show any change in the kidney. Thinning of retina (slight) was also noted in one female rat at 50 mg/kg (#50410). No other female animals in the treated, placebo or recovery groups showed a similar change.

Summary of histopathology data:

There were incidental findings in the histopathology report. However, male and female rats showed inflammation and hemorrhage at the site of injection in control and treated animals that did not recover. NOEL was not established for systemic toxicity due to anti-MRA and its effect at the injection site.

Toxicokinetics: The mean half-life was calculated to be 9.7-10.6 days in male and female rats. Gender difference in the kinetics was not observed. Although the Applicant presented data for plasma level with time, PK parameters were not calculated. Data from Applicant's table are shown below. Measurable MRA was present at the end of recovery period.

Item : Plasma concentration (μ g/ml) of MRA

<male>

Dose (mg/kg)	Animal No.	administration period* days after administration period 0	6	13	20	27	recovery period** days from Day-0 (days after recovery period) 30(2)	34(6)	41(13)	48(20)	55(27)
2	00501										
	00502										
	00503										
	mean	13.8	67.2	112.7	128.5	170.5	131.1	101.2	63.2	40.7	27.2
	S.D.	1.2	10.2	16.8	9.2	7.3	15.7	11.8	10.6	8.6	8.2
10	00601										
	00602										
	00603										
	mean	80.3	353.6	589.8	694.9	863.3	692.0	519.6	329.0	207.1	135.6
	S.D.	10.4	34.6	61.3	18.7	27.7	84.1	50.8	20.6	15.5	7.9
50	00701										
	00702										
	00703										
	mean	407.8	1955.5	2925.8	3420.9	3409.4	2624.7	1815.7	1187.6	719.0	457.3
	S.D.	43.0	301.8	157.2	214.3	116.9	253.0	178.0	96.5	49.9	42.7

b(4)

<female>

Dose (mg/kg)	Animal No.	administration period* days after administration period 0	6	13	20	27	recovery period** days from Day-0 (days after recovery period) 30(2)	34(6)	41(13)	48(20)	55(27)
2	50501										
	50502										
	50503										
	mean	13.2	64.3	113.5	127.1	156.2	121.5	98.9	57.2	36.5	24.3
	S.D.	0.7	14.7	12.8	19.6	24.3	15.9	11.2	11.1	9.0	6.0
10	50601										
	50602										
	50603										
	mean	78.0	348.9	569.1	822.0	806.7	671.1	474.7	294.9	189.1	132.4
	S.D.	5.3	10.0	43.1	100.6	81.2	15.8	64.4	33.8	16.0	20.5
50	50701										
	50702										
	50703										
	mean	409.2	1788.1	3086.8	3411.8	3312.4	2805.1	1609.8	1194.4	690.2	447.7
	S.D.	64.7	108.2	29.9	376.9	139.3	71.5	273.2	39.8	68.2	26.1

b(4)

*Each value was measured after 24 hours of each administration

**Each value was measured after 24 hours of each recovery day

Other: MRA antibody was detected in one 2 mg/kg dose male rat at the end of 4 weeks treatment and in several recovery animals at 2, 10, and 50 mg/kg. Data suggest that neutralizing antibodies could interfere with the toxicity assessment of the drug. The Applicant's data table is shown below.

Table 110
1-month intravenous toxicity study of MRA in rats

Item : Antibody titration — Stage: Recovery(4week) — Control: MRA 0 mg/kg — Carrier : Vehicle

Test Article	MRA	MRA	MRA	MRA
Dose	0 mg/kg	2 mg/kg	10 mg/kg	50 mg/kg
Number of Animals	5	5	5	5
Male	00111 -	00211	00306	00411
	00112 -	00212	00308	00412
	00113 -	00213	00309	00413
	00114 -	00214	00310	00414
	00115 -	00215		00415
Female	50111 -	50211	50311	50411
	50112 -	50212	50312	50412
	50113 -	50213	50313	50413
	50114 -	50214	50314	50414
	50115 -	50215	50315	50415 -

b(4)

b(4)

< satellite animal >

Test Article	MRA	MRA	MRA
Dose	2 mg/kg	10 mg/kg	50 mg/kg
Number of Animals	3	3	3
Male	00501	00601	00701
	00502	00602	00702
	00503	00603	00703
Female	50501	50601	50701
	50502	50602	50702
	50503	50603	50703

b(4)

b(4)

- ; Negative result at 1 : 20 dilution of sera + ; Positive result (titers)

Conclusion of one-month toxicity study in rats:

Daily dose of MRA at 2, 10 and 50 mg/kg/day for 28 days did not show any systemic toxicity related to the treatment. However, placebo and MRA treated animals showed non-reversible inflammation and hemorrhage at the site of injection. Antibody to MRA was present at the end of 28 day recovery in some animals. MRA-antibody reaction could lead to injection site reactions also. The use of this model for MRA toxicity is questionable due to anti-MRA antibody formation and lack of cross-reactivity to MRA in rats.

Histopathology inventory (optional)

Study	6-mo Tox	28-day Tox,
Species	Monkey	Rat
Adrenals	*X	*X
Aorta	X	X
Bone Marrow smear	*X	X
Bone (femur)	X	X
Brain	*X	*X
Cecum	X	X
Cervix		
Colon	X	X
Duodenum	X	X
Epididymis	X	X
Esophagus	X	X
Eye	X	X
Fallopian tube		
Gall bladder	*X	
Gross lesions	X	X
Harderian gland		X
Heart	*X	*X
Ileum	X	X
Injection site	X	X
Jejunum	X	X
Kidneys	*X	*X
Lachrymal gland	X	X
Larynx		
Liver	*X	*X
Lungs	*X	*X
Lymph nodes, cervical		
Lymph nodes, axillary	X	
Lymph nodes mandibular		
Lymph nodes, mesenteric	X	X
Lymph nodes,, sub-mandibular	*X	
Lymph nodes, Iliac		X
Mammary Gland	X	X
Nasal cavity		
Optic nerves		
Ovaries	*X	*X
Pancreas		X
Parathyroid	*X	X
Parotid gland	X	X
Peripheral nerve (sciatic)	X	
Pharynx		
Pituitary	*X	*X

Study	6-mo Tox	28-day Tox,
Prostate	*X	*X
Rectum	X	X
Salivary gland		*X
Sciatic nerve	X	X
Seminal vesicles	*X	*X
Skeletal muscle	X	X
Skin	X	X
Spinal cord	X	X
Spleen	*X	*X
Sternum	X	
Stomach	X	X
Testes	*X	*X
Thymus	*X	*X
Thyroid	*X	X
Tongue	X	X
Tonsil	X	
Trachea	X	X
Urinary bladder	X	X
Uterus	*X	*X
Vagina	X	X
Zymbal gland		

X, histopathology performed
*, organ weight obtained

2.6.6.4 Genetic toxicology

Study title: Reverse mutation study of MRA in bacteria

Key findings: MRA was not mutagenic in Ames assay.

Study no.: KEIANKEN96-212, TOX02-0172 (JITSU 97-0035)

Volume #M4 and page #: 1
Conducting laboratory and location: ⌜ ⌟ Chugai b(4)
Pharmaceutical Company, ⌜ ⌟
Date of study initiation: July 26, 1996
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug: MRA, lot #MRA96D03, and % purity: 7.57 mg/mL

Methods

Strains/species/cell line: *Salmonella typhimurium* TA 100, TA 98, TA 1535, TA 1537 and *E. coli* WP2uvrA

Reviewer: Asoke Mukherjee, BLA No. 125276

Doses used in definitive study: 47.3, 94.6, 189, 379, 757 and 1514 ug/plate in the absence of S-9 liver homogenate mixtures; 47.3, 94.6, 189, 379, 757, 1514 ug/plate in the presence of S-9 mixtures.

Basis of dose selection: Preliminary cytotoxicity tests were conducted in TA 100 and *E. coli* WP2uvr cell lines in the absence and presence of S-9 liver homogenate mixtures using preincubation methods. The maximum feasible dose of 1514 ug/plate was chosen that correspond to 0.2 mL of the stock solution.

Negative controls: 20 mM Phosphate buffered saline was used as a negative control.

Positive controls: N-ethyl-N'-nitro-Nitrosoguanidine (ENNG), sodium azide (SA), 2-Nitrofluorene (2-NF), ICR-191 (2-methoxy acridine) were used in the absence of S-9 liver mixtures. 2-Aminoanthracene was used in the presence of S-9 mixtures.

Incubation and sampling times: Mixture containing 0.1 mL of the tester strain, 0.1 or 0.2 mL of the test substance was incubated with or without S-9 mixtures for 20 min at 37°C. After the incubation 2 mL of molten agar in the presence of histidine, biotin or tryptophan (for *E. coli*). The incubation mixture was added to 30 mL agar plate for further incubation for 48 hours at 37°C. Revertant colonies were counted by automatic colony counter. Replicate plates were used for each concentration.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Results of the test was considered positive when the mean revertant colonies of two plates were twice the negative control and a dose dependent increase in the colony counts.

Study outcome: MRA did not increase the number of revertant colonies at 1514 ug/plate in the absence and presence of S-9 in the preliminary tests in TA 100 and *E. coli* strains. A maximum dose of 757 ug/plate was selected due to cytotoxicity at 757 ug/plate in the absence of S-9. However, data up to 1514 ug/plate was also obtained to confirm the cytotoxicity observed in the preliminary experiment. Data for one of the replicate experiment in the absence of S-9 mixtures are shown below.

Table 2. Bacterial reverse mutation test of MRA in the absence of S9.

Test compound	Concentration (μg/plate)	Number of revertants / plate (Mean) TA100	TA98	TA1535	TA1537	WP2uvrA
MRA	0[1]	145	15	11	16	33
	0[2]	126	24	12	8	26
	47.3	130	15	16	15	33
	94.6	148	18	15	19	29
	189	140	15	14	20	30
	379	132	26	9	17	29
	757	131+[1]	18+[1]	12	17	25
	1514	130+[1]	16	13	20	31
ENNG[3]	2	-[4]	-	-	-	2200
	3	1383	-	-	-	-
2NF[5]	1	-	1097	-	-	-
SA[6]	0.5	-	-	168	-	-
ICR-191	1	-	-	-	3248	-

1)-4) See Table 1.
5) 2-nitrofluorene
6) sodium azide
+1~+4 See Table 1.

Based on the above data, MRA was not mutagenic in the absence of S-9 mixtures. A similar result was obtained in the presence of S-9 mixtures as shown below.

Table 3. Bacterial reverse mutation test of MRA in the presence of S9.

Test compound	Concentration (μg/plate)	Number of revertants / plate (Mean) TA100	TA98	TA1535	TA1537	WP2uvrA
MRA	0[1]	141	19	13	21	27
	0[2]	146	21	9	18	34
	47.3	122	15	14	16	27
	94.6	143	28	15	13	30
	189	148	28	11	18	31
	379	133	24	8	21	27
	757	137	18	9	18	30
	1514	150	21+[1]	11	19	35
2AA[3]	0.5	-[4]	153	-	-	-
	1	592	-	-	-	-
	2	-	-	88	139	-
	10	-	-	-	-	289

1)-4) ,+1~+4 See Table 1.

Based on the data, it was concluded that MRA was negative in the Ames Assay.

Study title: Chromosomal aberration assay of MRA in human peripheral blood lymphocytes

Key findings: MRA is not mutagenic in the in vitro chromosome aberration assay in human lymphocytes.

Study no.: KEIANKEN96-217, TOX-02-0171 (JITSU 97-0086)

Volume #M4, and page #: 1
Conducting laboratory and location: Chugai Pharmaceutical Co. LTD., b(4)
Date of study initiation: Oct 15, 1996
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug: MRA, lot # MRA96D03, and % purity: 7.57 mg/mL

Methods

Strains/species/cell line: Peripheral human blood was collected from three healthy adult male volunteers. Lymphocytes were isolated and treated with phytohemagglutinin.

Doses used in definitive study: 189.3, 388.5 and 757 ug/ml for 24 hour exposure

Basis of dose selection: A preliminary study was conducted to determine the dose that could induce 50% inhibition of cell proliferation, mitotic index, and cell survival using Alamar blue dye fluorescence measurement technique. The concentration of the test substance that reduced fluorescence by 50% compared to the negative control was selected as a cytotoxic dose.

Negative controls: 20 mM phosphate buffered saline

Positive controls: Mitomycin C (MMC)

Incubation and sampling times: Cells were incubated with MRA at 47.3 to 757 ug/mL for 24 hours. Colcemid solution was added two hour before the harvest time to arrest the cell division. Metaphase cells were treated with 0.075 M potassium chloride solution for cell lysis due to the hypo tonicity. Cells were fixed in methanol and acetic acid, air dried on slides and stained with Giemsa stain. About 300 metaphase cells per concentration were scored for structural aberrations and numerical aberrations.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Incidences of aberrant cells per dose were compared with the control for statistically significant changes. A dose dependent increase in the aberrant cell was considered for a positive response.

Study outcome: About 10% inhibition of metaphase cells was observed at 757 ug/mL. There was no statistically significant increase in the aberrant cells as shown from the Applicant's table below.

Table. 2 Chromosome aberration test of MRA in human peripheral blood lymphocytes

Test compound	Exposure time(hr)	Conc. (μ g/ml)	No. of cells observed	Aberrant cell (%)						TAG (%)	TA (%)	AF	Polyploid (%)
				gap	ctb	cte	esb	cse	others				
MRA	24	0*	300	1.3	3.3	0	0	0	0	4.7	3.3	0.04	0
		189.3	300	2.3	3.3	0	0.3	0	0	5.7	3.3	0.04	0
		378.5	300	2.3	4.3	0	0	0	0	5.7	4.3	0.04	0
		757	300	2.3	5.0	0	0.7	0	0	8.0	5.7	0.06	0.3
Mitomycin C	24	0.1	300	7.7	43.3	12.7	17.3	0	0.3	58.7	56.0	0.97	0

*: Solvent control (20mM phosphate buffer, 250mM NaCl, pH7.0)
Abbreviations: ctb; chromatid break, cte; chromatid exchange, csb; chromosome break, cse; chromosome exchange,
TAG; total aberrations including gap, TA; total aberrations excluding gap, AF; aberration frequency per cell.

Based on the data, MRA was considered to be non-mutagenic in the chromosome aberration assay in human lymphocytes.

2.6.6.5 Carcinogenicity

The Applicant did not conduct any carcinogenicity studies. The Division communicated to the Applicant the following recommendations for a Pre BLA meeting on Oct 12, 2007 as follows:

"Although carcinogenicity assessment may not be feasible for this product, your BLA should include a detailed discussion of why such studies are not possible. In addition, your BLA should discuss the available information you have collected via your own studies as well as those published in the literature regarding the potential impact of IL-6 neutralization on tumor surveillance and tumor development. You should also specifically state how you intend to address the carcinogenicity section of your product labeling."

Reviewer: Asoke Mukherjee, BLA No. 125276

The Applicant submitted the following references in support of their response, full reference of these papers are included on the list of studies reviewed.:

1. Proinflammatory mediators and genetic background in oncogene mediated tumor progression by Russell et al, J. Immunology. 172, 4059, 2004 (#1039)

   RP3 is an oncogene protein obtained from tissues of thyroid cancer and Hashimoto's thyroiditis. RP3 induces among several cytokines IL-6 for the development and progression of thyroid tumor.

2. Co-operative functions between nuclear factors NFkB and CCAT/enhancer-binding protein B (C/EBBP) regulate the IL-6 promoter in autocrine human prostate cancer cells, The Prostate 61, 354, 2004 (#1040)

   IL-6 activates the transcription factor NFkB in prostate cancer cells and regulates further production of IL-6 as an autocrine mediator for the growth of cancer cells. Authors also indicated that increased IL-6 was found in serum and prostate cancer tissues.

3. An interleukin-6 gene promoter polymorphism influences the biological phenotype of ovarian cancer, Cancer Research 63, 3066, 2003 (#1041).

   The authors indicated that polymorphism at G/C base in the promoter region of IL-6 gene contributed to ovarian cancer in humans.

4. Interleukin 6 is essential for the in vivo development of B lineage neoplasms, J. Exp. Med, 182, 243, 1995 (#1042).

   IL-6 gene mutation was induced to investigate the role of IL-6 on plasma cell tumors. Mice with IL-6 $^{-/-}$ were completely resistant to plasma cell tumors. Data suggest the role of IL-6 on b-cell malignancies.

5. Cytotoxicity of IL-6-PE40 and derivatives on tumor cells expressing a range of interleukin 6 receptor levels, J. B. Chem. 265, 16318, 1990 (#1043).

   The role of IL-6 in tumor cells was investigated by the cytotoxic effect of IL-6-mutant endotoxins combination in myeloma and hepatoma cell line. The research was conducted to induce cytotoxicity to IL-6R bearing tumor cells as a proof-of-concept study for developing therapeutic agent to treat myeloma and liver cancer. These data indicate that IL-6R is expressed in cancer cells.

6. TGF-B suppresses tumor progression in colon cancer by inhibition of IL-6 trans signaling, Immunity, 21, 491, 2004 (#1044).

   The authors indicated that over expression of TGF-Beta in mice induce T lymphocyte infiltration and colorectal cancer. The effect was synergized by IL-6

mediated transduction mechanism via STAT-3 activation. The study showed that IL-6 was involved in colorectal cancer.

7. IL-6 signaling promotes tumor growth in colorectal cancer, Cell Cycle, 4, 217, 2005 (#1045).

   This article is analogues to #1044.

8. Association of common polymorphisms in inflammatory genes interleukin (IL)-6, IL-8, TNF-alpha, NFkB1 and peroxisome proliferator-activated receptor-Gamma with colorectal cancer, Cancer research, 63, 3560, 2003 (#1046).

   This report showed that patients with colorectal cancer or rectal cancer had prevalence of single nucleotide polymorphism at IL-6-174C promoter region. Data suggest a relationship to the expression of IL-6 and cancer.

10. Crosslink between cancer and immune cells: role of STAT3 in the tumor microenvironment, Nature Reviews Immunology, 7, 41, 2007 (#1047).

    This review discussed the role of oncogene protein STAT-3 regulation among other processes by IL-6 and promotion of tumors. The reviewer suggested that certain inflammatory and immune responses can promote cancer. IL-6 and COX-2 are among key players in tumor initiation and progression induced by chronic inflammation by activating STAT-3, inhibiting dendritic cell maturation, and tumor surveillance by immune cells. IL-6 is an important mediator of tumor promotion involving NFκB and STAT-3 activation processes. It should be emphasized that these transcription factor activation processes also regulate several other inflammatory mediators including IL-10, TGF-B, and COX-2.

11. IL-6 regulates in vivo dendritic cell differentiation through STAT-3 activation, J. Immunology, 173, 3844, 2004 (#1048).

    This publication supplements data referred to the Reference # 1047. The authors stated that dendritic cells remain in a suppressed state in the absence of any infection and undergo maturation process when activated to combat the antigen. The suppression of dendritic cell activation is mediated by IL-6 through STAT-3 transcription factor activation. There are other cytokines may be involved in the activation of dendritic cells, also such as IL-10. The experimental data in the publication led to the understanding that IL-6 induces a state of immunosuppression and decreases T-lymphocyte activation. However, the **authors raised concerns about the experimental findings** because **IL-6 is also known as a mediator of autoimmunity and inflammation.** As a result of the conflicting understanding of the role of IL-6 in inflammation and immunity, the authors indicated that **"cytokines act as a two-edged sword".**

12. Fibrosarcoma cells transduced with the IL-6 gene exhibit reduced tumorigenicity, increased immunogenicity, and decreased metastatic potential, Cancer Research, 52, 6020, 1992 (#1049).

    Authors injected transformed murine fibrosarcoma cells that secrete IL-6 to normal mice. IL-6 secretion reduced tumor cells in the normal mice but not in the nude mice suggesting that IL-6-related reduction in tumorigenicity was T lymphocyte dependent.

13. In vivo and in vitro characteristics of interleukin 6-transfected B16 melanoma cells, Cancer Research, 52, 5412, 1992 (#1050).

    The authors reported anti-tumor activity of human IL-6 in B16 melanoma cells. Cells were transfected with human IL-6 DNA to induce IL-6. C57BL/6 mice were injected with the transformed cells and tumor growth was monitored. Tumor cells showed greater adhesion to the stromal matrix and less vascular response. The authors concluded that IL-6 showed an anti-tumor activity in the mouse model.

14. Receptor synergy of interleukin-6 (IL-6) and insulin-like growth factor-1 in myeloma cells that highly express IL-6 receptor, Blood, 103, 2291, 2004 (#1051).

    The authors showed existence of IL-6 and insulin-like growth factor (IGF1) receptors coexistence on myeloma cell line with IL-6 transduction events independent of STAT-3 activation. STAT-3 activation is a common signaling event to IL-6R activation. However, IL-6 in U266 cell line phosphorylated IGF-1 receptors that were not blocked by a JAK inhibitor. IGF-1 activation by IL-6 eventually suppresses p53 gene activity and tumor survival. These data suggest multiple pathways of the tumor progression in myeloma cell line.

15. Paradoxical effects of cytokines in tumor immune surveillance and tumor immune escape, Cytokine and Growth Factor Review, 18, 171, 2007 (#1052).

    In this review the role of several pro-inflammatory cytokines e.g. IL-6, IL-10, TGF-B, INF-alpha, IL-2 in tumor progression was discussed. The authors indicated that IL-6 is involved in tumorigenic process. However, it has a role on the in vivo anti-tumor immune response also.

Summary of literature findings:

The authors provided role of IL-6 on the pathogenesis of cancer. IL-6 was detected in human thyroid cancer and prostate cancer cells. Polymorphism of IL-6 gene promoter also contributed to ovarian cancer in humans. IL-6 knock-out mice showed resistance to B-lymphocyte malignancies. IL-6R also expressed in human cancer cells. Experimental and human cancer in the colon showed high IL-6 expression. Myeloma cell line showed increased transduction of IL-6R and suppression of p53 gene. These data support the role

of IL-6 in cancer progression. However, IL-6 also showed anti-tumor activity in the melanoma cell model.

The role of IL-6 in cancer is mediated through STAT-3 transcription factor. Dendritic cells remain in an immature state by the influence of IL-6. This process promotes tumor formation due to lack of surveillance on tumor forming cells. Authors also suggested that besides IL-6, many others process i.e., COX-2 activation also contributes to the tumor progression due to lack of tumor surveillance by dendritic cells under the influence of IL-6 and other cytokines.

However, exact physiological role of IL-6 for immuno-modulation and cancer progression is unknown because IL-6 has a role in both immunosuppression as well as inflammation.

The Applicant stated the following rationale for risks of malignancies:

1. Non-clinical data in monkeys do not suggest that chronic IL-6 receptor blockage with tocilizumab predisposes patients for risk to developing malignancies.
2. Published data suggest a significant role of IL-6 in the pathophysiology of cancer induction and progression.
3. As tocilizumab does not bind to the rodent IL-6R, conventional long term cancer studies in rats or mice are inappropriate to assess a function-related carcinogenic potential of tocilizumab and are not requested by international guidelines.

The reviewer's comments on the Applicant's response to carcinogenicity issue:

1. The Applicant provided several published papers that indicate the role of IL-6 in the development of malignancy. Based on these data, it appears that an IL-6R monoclonal antibody should not pose a significant carcinogenic risk. However, the role of IL-6 as a cytokine in the immune system is still controversial and can play a dual role in mitigating cancer and causing cancer. In light of the unclear role of IL-6 in tumor formation/growth, there is a need to collect data in a suitable rodent species using homologous antibodies for the understanding the role of IL-6R antibodies in cancer. The need for more data is immense due to the patient population at risk to the treatment, duration of the treatment, unknown nature of intervention of the cytokine in immune systems, role of other cytokines in the presence of MRA that transduce through the same gp-130 protein systems.
2. The Applicant argued that rodent model is not relevant to human IL-6R antibody. However, they attempted to investigate the role of IL-6R in the rodent model of reproductive safety. It appears there is inconsistency to their approach to the non-clinical understanding of the role of IL-6R. The reviewer also perceived that MR16-1, a monoclonal antibody to murine IL-6R raised in rats could be explored for this purpose.
3. The ICH 1997 guidelines on biotechnology product development indicated that "When there is a concern about carcinogenic potential, a variety of approaches

may be considered to evaluate risks" and "further studies in relevant animal models may be needed".

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: A study for effects of MRA administered intravenously on fertility and early embryonic development to implantation in rats

Key study findings: MRA had no effect on male and female fertility in rats up to 50 mg/kg/Day, IV.

Study no.: KEIANKEN 96-216, Tox02-0173 (J99-0018)
Volume # M4 and page #: 1
Conducting laboratory and location: Chugai Pharmaceutical Co. Ltd., Nagano 399-46, Japan
Date of study initiation: Aug 23, 1996
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug: MRA, lot # MRA96F01, and % purity: 5.57 mg/mL

Methods

Doses: The dose groups are shown below.

Dose, mg/kg/day	# Rat/sex	Volume of injection, mL/kg
0, vehicle	18	8.98
5	18	0.898
16	18	2.87
50	18	8.98
No vehicle or drug (untreated), mated with fertile males from control and 50 mg/kg	17 (female) x2=34	Nil

Species/strain: Sprague Dawley rats, male and female rats were 7 and 6 weeks old at the procurement. Male rats weighed 261 to 289 g and female rats weighed 193 to 217 g at the beginning of the experiment.

Number/sex/group: See table above

Route, formulation, volume, and infusion rate: The drug substance was diluted in 20 mM phosphate buffer and injected intravenously into the tail vein at 2 mL/min. Control animals received the vehicle. Male rats were treated 28 days before mating and 15 days during mating period (total maximum 43 days). Female rats were treated 14 days before mating, during mating and 7 days during pregnancy (total 36 days, average 24 days). The Applicant stated that 34 female rats were not treated with vehicle or the drug

substance and were considered as untreated female rats. Untreated females were mated with males with proven fertility from control and 50 mg/kg groups.

Satellite groups used for toxicokinetics: None

Study design: Male and female rats were housed 1:1 for mating. Mating was confirmed by the presence of sperm cells in the vaginal smears (day 0 of pregnancy). Male rats were sacrificed after mating was confirmed. Blood samples, liver, testes, and epididymides were dissected. Sperm count, sperm morphology, sperm viability, and motility were determined from epididymides at necropsy. Following macroscopic examinations of the organ, liver, testes, and epididymides were appropriately fixed. The protocol indicated that testes and epididymis from rats that did not mate with female rats or mated with sterile female rats were examined histologically following hematoxylin and eosin staining.

Female rats were sacrificed on day 13 of pregnancy. Blood samples and liver were collected. Ovaries and uteri were examined for the determination of pregnancy status, corpora lutea, number of implantations, and number of live or dead embryos. Liver, ovaries, and uteri were fixed appropriately. The Applicant stated that rats did not copulate were sacrificed at the end of schedule mating period.

The Applicant stated that untreated female rats were allowed to mate with male rats with confirmed mating performance from the control and 50 mg/kg groups. These control female rats were sacrificed on day 13 of pregnancy. Ovaries and uteri were dissected to determine pregnancy, corpora lutea, implantations, live or dead embryos.

Parameters and endpoints evaluated: Clinical observations were recorded after the treatment twice a day during the treatment period. The body weight of male rats was recorded twice a week. The body weight of female rats were recorded before treatment, days 3, 7, 10, 14 before mating, once every day during day 0 to day 8 of pregnancy, and on day 13 of pregnancy. Food consumption was noted before mating on day 28 of the treatment in male rats and day 14 of the treatment for female rats, during days 1-7 of pregnancy. Blood samples were collected on day 43 in male rats and on day 8 of pregnancy in female rats for hematological examinations. The estrous cycle was monitored daily up to day 14 of dosing (before mating). Details of the procedures and observations are described under the study design.

Results

Mortality: No unscheduled deaths were reported in the study.

Clinical signs: No treatment-related clinical signs were reported in the study.

Body weight: The average body weight of male rats is shown in the table below.

Day	Control	5 mg/kg	16	50 mg/kg
0	277	277	276	276
28	383	394	389	378

44	416	436	427	415

Above data do not suggest any treatment-related change at the end of Day 44 (premating and mating period). However, a slight reduction in the body weight was noted at 50 mg/kg on day 28 that was statistically not significant.

The average body weight (g) in female rats is shown below.

Day	Control	5	16	50 mg/kg
0	205	203	205	205
14	223	227	223	224
0, Pregnancy (after mating)	230	238	232	232
13, Pregnancy	290	288	289	290

Above data suggest that female rats had no effect on the body weight gain before and after mating.

Food consumption:

Male rats did not show treatment related change in the average food consumption up to mating period except a slight reduction in the food intake at 50 mg/kg throughout the observation period compared to rats in other groups.

The average food consumption in female rats did not show any treatment related change before mating and during the pregnancy.

Hematology: Hematology data at the end of treatment or terminal sacrifice did not show any treatment related change compared to the control in male and female rats.

Toxicokinetics: The Applicant did not determine the PK in the study.

Necropsy: see below.

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

Estrous cycle before mating was monitored. Data were expressed as number of rats that showed regular estrus cycle. Treated female rats at 5, 16, and 50 mg/kg showed less number of animals with regular estrus cycle when compared to the control as shown in the table below.

Percentage of female rats with regular estrus cycles

Control	5 mg/kg	16 mg/kg	50 mg/kg
94.4%	50%*	72.2%	77.8%

* Statistically significant $P<0.001$

Pregnancy data are shown below.

Treatment (mg/kg/day)	**0**	**5**	**16**	**50**
Male (Assigned)	18	18	18	18
Copulated	17	17	17	17
Copulation Index (%)	94.4%	94.4%	94.4%	94.4%
#Pregnant Female/ Total	17/18	16/18	15/18	17/18
Fertility Index	100%	94.1%	88.2%	100%
Sperm Count (10^6 /uL)	2.36	2.05	2.13	2.20
Viability	1.6	1.4	1.4	1.4
Sperm Motility	251	245	229	248
Sperm abnormality (%)	2.6	3.0	7.1	6.0**
Sperm abnormality (staining)	5.75			7.19

** P<0.01

The Applicant examined sperm in control and 50 mg/kg treated rats following staining with eosin, fast green, napthol yellow and acetic acid (John et al., Stain Technology, 45, 231, 1970). Data showed that control rats had 5.7% abnormal sperms versus 7.2% in the 50 mg/kg dose group. Based on the data it appeared that the sperm morphology changed in the treated animals. However, its biological significance is not clear.

The average weight of liver, testes, and epididymides was not affected by the treatment.

The pregnancy data from Applicant's table #17 are shown below.

Table 17
Fertility Study of MRA Administered Intravenously in Rats

Item : Litter Data with Caesarean Section (F0) - Summary

Control : MRA 0 mg/kg

Test Article	MRA	MRA	MRA	MRA
Dose	0 mg/kg	5 mg/kg	16 mg/kg	50 mg/kg
No. of Dams	17	16	15	17
Corpora Lutea				
No per Litters:Mean±S.D.	15.2±1.7	15.4±1.4	15.4±2.1	15.4±1.8
Implantations				
No per Litters:Mean±S.D.	14.8±1.6	15.1±1.4	14.7±2.2	14.6±1.8
% to Corpora Lutea:Mean±S.D.	97.4±4.0	97.6±3.2	95.9±8.8	95.6±6.5
Live Embryos				
Total	237	213	202	241
No per Litters:Mean±S.D.	13.9±1.8	13.3±2.1	13.5±1.8	14.2±1.9
% to Implantations:Mean±S.D.	94.6±7.9	88.4±11.1	92.0±9.0	96.7±4.5
Dead Embryos				
Total	14	28	19	8
No per Litters:Mean±S.D.	0.8±1.1	1.6±1.7	1.3±1.5	0.5±0.6
% to Implantations:Mean±S.D.	5.4±7.9	11.6±11.1	8.0±9.0	3.3±4.5

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Above data did not suggest any treatment related effect on the implantation

Mating with untreated females:

Seventeen untreated females/group were mated with vehicle and 50 mg/kg treated males that already mated with corresponding female rats. Results of the study would provide data on the effect of the treatment on male fertility. Data in male and female animals on mating performance showed that the treatment had no effect on male fertility as shown below from Applicant's table # 18 and 19.

Table 18

Fertility Study of MRA Administered Intravenously in Rats

Item : Reproductive Performance (F0) - Summary

Control : MRA 0 mg/kg

Test Article	MRA	MRA
Dose Male	0	50
Female	Intact	Intact
	mg/kg	mg/kg
Male		
Mating		
No. of Animals Mated (A)	17	17
No. of Copulated Animals (B)	17	17
Copulation Index (%) (B/A)x100	100.0	100.0
No. of Fertile Animals (C)	17	17
Fertility Index (%) (C/B)x100	100.0	100.0
Female		
Mating		
No. of Animals Mated (A)	17	17
No. of Copulated Animals (B)	17	17
Copulation Index (%) (B/A)x100	100.0	100.0
No. of Pregnant Animals (C)	17	17
Fertility Index (%) (C/B)x100	100.0	100.0

Table 19

Fertility Study of MRA Administered Intravenously in Rats

Item : Litter Data with Caesarean Section (F0) - Summary

Control : MRA 0 mg/kg

Test Article	MRA	MRA
Dose - Male	0	50
Female	Intact	Intact
	mg/kg	mg/kg
No. of Dams	17	17
Corpora Lutea		
No per Litters:Mean±S.D.	15.9±2.5	14.9±1.4
Implantations		
No per Litters:Mean±S.D.	13.8±2.6	14.1±1.8
% to Corpora Lutea:Mean±S.D.	88.2±17.3	94.4±10.3
Live Embryos		
Total	218	229
No per Litters:Mean±S.D.	12.8±2.8	13.5±2.2
% to Implantations:Mean±S.D.	92.1±7.5	95.8±9.3*
Dead Embryos		
Total	17	10
No per Litters:Mean±S.D.	1.0±0.8	0.6±1.2
% to Implantations:Mean±S.D.	8.0±7.5	4.4±9.3*

Significantly different from control : * P<0.05 (Wilcoxon test)

Testes and epididymides from male rats that did not copulate or copulated with infertile females were examined for histopathological changes. Data showed that male # 00302 and # 00406 at 16 and 50 mg/kg had degeneration in the germ cell and decreased spermatozoa. However, based on the fertility and post implantation data, it is unlikely that the treatment had an effect on the fertility of male and female rats.

Conclusion of the study:

The effect of MRA on fertility and early implantations was investigated in male and female rats at 5, 16 and 50 mg/kg/day/IV doses. There was no treatment related effect on the male and female fertility in rats. However, sperm analysis data showed a slightly higher morphological change at 16 and 50 mg/kg. Female rats also showed a slight increase in irregular estrus cycle. The significance of the sperm analysis and estrus cycle data is not known given the fact that fertility index was comparable between control and treated animals. It was concluded that MRA had no effect on male and female fertility in rats.

The Applicant stated in the non-clinical overview that "Animal fertility studies in a relevant species were not conducted." According to the Applicant, the need to conduct segment 1 reproductive study (fertility and early embryonic development) in an appropriate model was not warranted because of following justifications:

1. The Applicant stated in the non-clinical toxicology summary that IL-6 deficient mice did not show deficiency in the reproductive performance and 6-month toxicity study in monkeys did not show toxicity in reproductive organs. Therefore, MRA would not have any effect on the fertility and early embryonic development.
2. Two literature references # 4026 and #1031 concluded that there is no non-clinical evidence to justify that IL-6 signaling is involved in process of reproduction. These papers are briefly discussed below.

It should be noted that cross reactivity study in cynomolgus monkey (Tox02-0149) showed binding to ovarian tissues. However, its relation to fertility in the female is not known.

The Applicant referenced two published papers as summarized below.

Ref # 4026, Kopf et al, Nature, 368, 339, 1994.

IL-6 deficient mice (IL-6 $^{-/-}$) showed normal breeding. The knock-out mouse model showed 20-40% decrease in the T-lymphocyte population, 5 to 10 fold reduction in the virus-induced IgG production and some reduction in the mucosal IgA response. However, IgM production remained normal. Data suggested that IL-6 deficiency had some effect on cytotoxic T lymphocyte function and B-lymphocyte functions. Based on the information in the knock-out mouse model, MRA appears to be immunomodulatory

and lacks an effect on fertility. IL-6 $^{-/-}$ mice also showed acute phase proteins in the liver in response to IL-6.

Ref # 1031, Seiler et al, Eur. Cytokine Netw, 12, 15, 2001.

The review indicated that IL-6 is an important cytokine growth factor for the development of embryo at pre and post implantation in rodents and humans. However, IL-6 deficient mice developed normally.

Reviewer's comments:

1. The Applicant conducted cross-reactivity study # 02-0275 in mice using anti-mouse IL-6R antibody raised in rats (MR-16-1). Data suggest that IL-6 induced T-lymphocyte proliferation was inhibited by the surrogate antibody. Therefore, the use of mouse model and MR-16-1 (or murine surrogate antibody) would be an appropriate model system to investigate the effect of MRA on fertility.
2. The use of rat as a species for fertility study was not the best species although slight cross-reactivity was detected in Sprague Dawley rats using MRA.
3. There is strong evidence in the literature that IL-6 is involved in the blastocytosis, early pregnancy, and implantation. Therefore, it is important to conduct an appropriate study and to write the label accordingly.
4. MRA could have an effect on masking gp130 protein that is involved in the signaling of IL-6 and other closely related growth factors.
5. The Applicant stated that rat IL-6R did not bind to MRA and the rat model may not be the most desirable model for segment 1 reproductive safety study.

Embryofetal development

Study title: A study for effects of MRA administered intravenously on embryo-fetal developments in rats

Key study findings: No treatment-related teratogenicity, variation or embryocidal effect was noted up to 50 mg/kg/Day, IV for gestation days 7 to 17 in Sprague Dawley rats, NOEL was 50 mg/kg. This study is inappropriate due to lack of cross-reactivity of MRA to rats.

Study no.: Tox02-0174
Volume # M4 and page #: 1
Conducting laboratory and location: ⌜ ⌟ Chugai Pharmaceuticals LTD, ⌜ ⌟ b(4)
Date of study initiation: March 4, 1997
GLP compliance: Yes
QA reports: yes (x) no ( )

Drug: MRA, lot #MRA96F01, and % purity: 5.57 mg/mL, It was diluted with 20 mM phosphate buffer to appropriate concentrations.

Methods

Doses: 5, 16 and 50 mg/kg/IV, day, the Applicant stated that the high dose was chosen from previous studies where a decrease in erythrocytes and food consumption were noted at 50 mg/kg.

Table of Groups

group	dose (mg/kg)	MRA concentration (mg/mL)	dosing volume (mL/kg)	No. of animals (pregnant)	Animal Nos.
control	0	0	5.00	20(19)	50101-50120
MRA 5 mg/kg	5	1.57	3.200	20(20)	50201-50220
MRA 16 mg/kg	16	5.17	3.07	20(20)	50301-50320
MRA 50 mg/kg	50	15.7	3.21	20(20)	50401-50420

Species/strain: Sprague Dawley female rats, Slc: SD, Mated female rats were deployed in the study.

Number/sex/group: 20/group

Route, formulation, volume, and infusion rate: Animals were treated by intravenous injections through the tail vein during gestation days 7 to17 at 2 mL/min. The control animals were treated with 20 mM saline by IV route.

Satellite groups used for toxicokinetics: No satellite groups were used in the study.

Study design: See above

Parameters and endpoints evaluated:

Animals were observed twice a day before and after the dose or once a day when the test substance was not injected. All rats were monitored for the general health evaluation such as clinical signs, abortions, premature deliveries, and deaths before and after dosing and once a day other than the dosing period. The body weight was recorded on day 0 of gestation, days 6-9, and on gestation day 21. The food consumption was recorded at pre dose, gestation days 6 to 19 and on day 21 from the residual food. The daily food consumption was calculated. Animals were sacrificed by carbon dioxide inhalation, uteri and ovaries were removed. Number of implantations, number of corpora lutea, number of live fetuses, number of dead embryos, number of early fetal deaths and number of late fetal deaths were recorded.

Live fetuses were sacrificed by carbon dioxide inhalation, external examinations were conduced, weight, and sex were recorded. Visceral examinations were conducted for half of fetuses after fixing fetuses in Bouin's fixative according to Wilson's methods. The

rest of the fetuses were digested by methanol, stained in Alizarin Red and examined for skeletal changes by Dawson's methods.

Results

Mortality (dams): No treatment-related mortality was observed.

Clinical signs (dams):

A mass in the left axilla, loss of fur and crusting was noted on GD 10 and GD 19 in one dam from the low dose group. No other abnormalities were noted in any of the other animals.

Body weight (dams):

There was a statistically significant ($P<0.05$) increase (100%, ↑) in the body weight gain between gestation days (GD) 9-11 in the dams from the 5 mg/kg/day dose group compare to those of the control rats. An increase in the body weight gain (70%, in mid dose group and 27% in the high dose group) was also noted during that time in the dams from the mid and high dose group compared to the control rats (not statistically significant). This trend of increase in the body weight gain in the test article treated animals continued until the termination of the study. The reason for the increase in the body weight gain in the dams could not be explained; however, the effect is considered to be test article related due to the absence of this finding in the control. The body weight data are graphically presented in the Applicant's figure below.

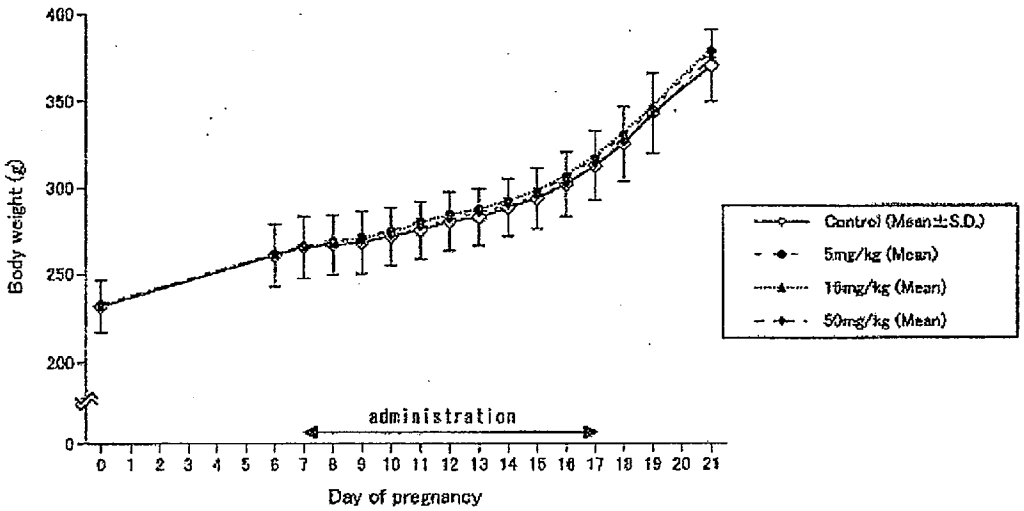

Fig.1 Body weight (g) of pregnant rats in study for effects of MRA administered intravenously on embryo-fetal development in rats

Food consumption (dams): There were no differences in the feed consumption in any of the animals from the different dose groups.

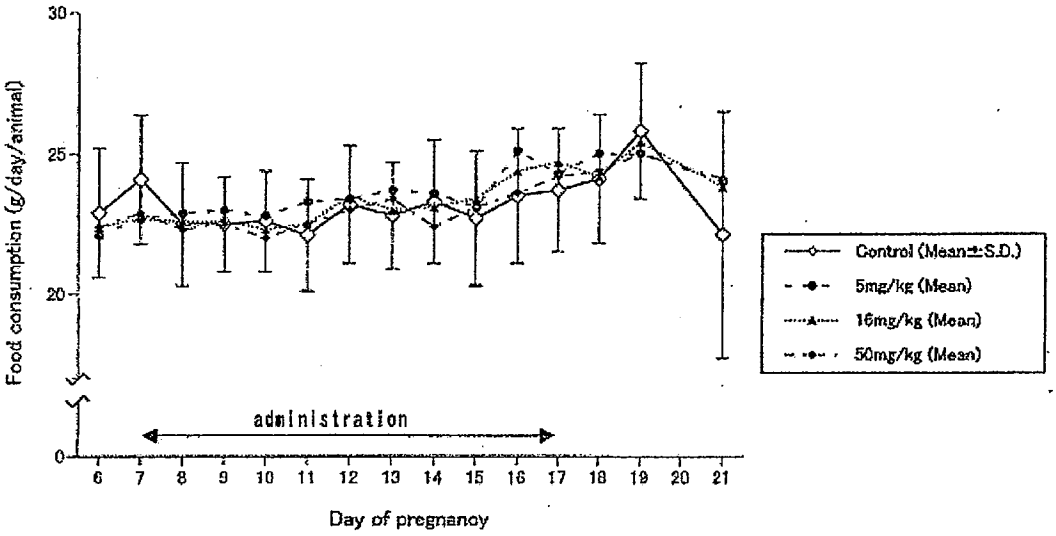

Fig.2 Food consumption of pregnant rats in study for effects of MRA administered intravenously on embryo-fetal development in rats

Toxicokinetics: No TK data were provided.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

Gross Pathology: The gross pathology observation consisted of a subcutaneous mass formation in 1/20 dams from the low dose group (5 mg/kg), this was associated with enlargement of the local lymph nodes, atrophy of thymus, and pallor of the liver and spleen. Another dam from the mid dose group (1/20) had white spots on the liver. There were no such findings in the control animals. These findings appeared to be incidental because a one-month toxicity study up to 50 mg/kg did not show systemic toxicity. Also, in the absence of the histopathological data it is difficult to understand the nature of these lesions and therefore it is not known whether the findings are test article related or not.

The C-sectioning and litter parameters such as corpora lutea, implantation sites, litter sizes, placenta, fetal body weight, and resorptions and teratological findings were evaluated (refer to Applicant's tables 6-9). The major findings from the c-section parameters and fetal malformation analyses are summarized below.

Summary of Findings: Terminal and Necropsy Evaluation, Offspring:

Reviewer: Asoke Mukherjee, BLA No. 125276

Some of the data for fetuses, its variations and malformation are shown below.

Parameter	Dose (mg/kg/day)			
	0	0.5	5.0	50
Total Number of Live Fetuses	273	277	282	273
Number of Dams	19	20	20	20
Live fetuses/Dam	14.4	13.85	14.1	13.65
Embryonic death/Dam	0.63	0.45	0.8	1.2
Death/ Total,	12	9	15	24
% implantation	8%	6%	9 %	15%, 2-fold ↑
Embryonic death /Total	11	8	15	24
% implantation	8%	6%	9 %	15%, 2-fold ↑
Body weight live fetuses (g)	5.4 ± 0.2	5.4 ± 0.2	5.5 ± 0.2	5.4 ± 0.2
male	5.1 ± 0.2	5.1 ± 0.2	5.2 ± 0.2	5.2 ± 0.2
female				
Visceral malformations				
Dilation of renal pelvis	none	none	none	1(1) 0.6%
Persistent umbilical artery	1(1) 0.7%	1(1) 0.6%	none	2(2), 1.1%
Skeletal Alterations				
Thoracic vertebra/ Ossification center splitting	1(1), 0.6%	1(1), 0.7%	2(1), 1.7 %	none
Ribs/Lumber rib	6(3), 4%	5(3), 4%	9(5), 7.2 %	4(4), 3%
Shortening of 13th rib	**none**	**none**	**none**	**1(1) 0.8**
Ossa digitorium manus/Retardation/ Fetus (Litter) % live fetuses	none	none	1(1) 0.7%	none
Sternum/Ossification center/splitting/Retardation/ Fetus (Litter) % live fetuses	1(1) 0.8%	none	2(2) 1.4 %	none
Ossa digitorium pedes	6(4)	9(4)	4(3)	10(5)
Retardation/ Fetus (Litter) % live fetuses	4%	7%, 1.7-fold ↑	3%	7%, 1.7-fold ↑

The body weight of surviving fetuses was comparable between pups delivered by control and MRA-treated animals. However, there was an increase in the fetal death at 50 mg/kg. The Applicant did not provide historical control data for dead fetuses/pregnant rats. However, the published data showed up to 3.4 fetal death/pregnant were observed

in 95 studies during 1992-1994 in Sprague Dawley rats. Although number of dead fetuses was higher at 50 mg/kg, the incidences were not too high to consider as a treatment-related incidence.

The visceral malformations were associated with an increase in the dilatation of renal pelvis and persistent umbilical cord at high dose. These findings are not considered test article related due to its occurrence at smaller prevalence. The published data suggest that dilatation of renal pelvis could occur as high as 19.7% among fetuses in Sprague Dawley rats. Also, umbilical artery finding was incidental because 2/59 fetuses were observed in control, low and mid dose. Also, there was no impact of the finding to the survival of pups.

The skeletal alterations consisted of shortening of the 13th rib in one fetus at 50 mg/kg. The reviewer considered it incidental because Sprague Dawley rats showed 17-22% supernumerary ribs and the finding was considered to be incidental.

The skeletal variations included retardation in ossification in the thoracic vertebra, sternum, and ossa digitorium manus and pedes. Due to the higher incidences of the variations in the fetuses both in control and treated groups, its relationship to the treatment is unknown.

Offspring (malformations, variations, etc.): See above

Based on the data it is concluded that MRA up to 50 mg/kg/IV did not affect organogenesis in Sprague Dawley rats. NOEL was 50 mg/kg. The validity for selection of the high dose was uncertain due to lack of maternal toxicity. This study is inappropriate due to lack of cross-reactivity of MRA to rats.

Study title: A study for effects of MRA administered intravenously on embryo-fetal development in rabbits

Key study findings: A reduction of the sternum development was noted at 0.5, 5, and 50 mg/kg/Day between gestation days 6 to 18. Increased fetal death was noted at 5 mg/kg. NOEL was not established. The treatment showed anti-MRA antibodies that contributed to excess fetal deaths. This model is not appropriate for the segment 2 study of MRA.

Study no.: TOX02-0177A
Volume # M4 and page #: 1
Conducting laboratory and location: Chugai Pharmaceutical Co Ltd., 1-135 Komakado, Gotenba-shi, Shizuoka, Japan
Date of study initiation: May 19, 1997
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug: MRA, lot #MRA96F02, and % purity: 5.33 mg/mL

Methods

Doses: The study design is shown below. The doses were chosen from the preliminary study at which 5 mg/kg showed a decrease in the body weight and increase mortality in fetuses. However, 50 mg/kg did not show adverse effects.

Table of Groups

group	dose (mg/kg)	MRA concentration (mg/mL)	dosing volume (mL/kg)	No. of animals (pregnant)	animal Nos.
control	0	0	9.381	25(17)	50101-50125
0.5 mg/kg	0.5	5.33	0.094	25(17)	50201-50225
5 mg/kg	5	5.33	0.938	22(16)	50301-50322
50 mg/kg	50	5.33	9.381	28(20)	50401-50428

Species/strain: Pregnant Japanese White Rabbits, JW/CSK strain, animals were mated with male from same strain. Rabbits weighed 2.82-4.15 kg and body weight was 4-6 months.

Number/sex/group: See above

Route, formulation, volume, and infusion rate: The test substance was diluted in 20 mM phosphate buffered saline for injections. The vehicle group received the buffer. The dose was injected via auricular vein once a day between gestation days 6-18 at 6 mL/min.

Satellite groups used for toxicokinetics: None, MRA plasma levels were determined from main animals on day 6, day 7 before dose, day 18 at 24 hour post dose, and on day 28. MRA levels were determined ELISA at detection limit of 0.78 ug/mL.

Anti-MRA antibody was determined on days 6, 19 and 28 by ELISA.

Study design: See above

Parameters and endpoints evaluated:

Clinical conditions were observed twice a day before and administration of the drug. The body weight was recorded on days 5-9, 11, 13, 15, 17, 19, 22, 25, and 28. Food consumption was recorded before the treatment and during gestation days 6-28.

Caesarean section was done on gestation day 28 under pentobarbital anesthesia. Abdomen was opened and uterus was examined for implantations, live fetuses, dead embryos, resorptions. Ovaries were fixed in formalin and corpora lutea were counted.

Sex and body weight of fetuses were determined, external examinations were performed after scarifying the live fetus by carbon dioxide inhalation. Abdominal and thoracic

viscera were examined after fixing by Bouin's fixative. Skeleton was stained with Alizarin red and examined for skeletal malformations and deformations after removing the soft tissue.

Results

Mortality (dams): No mortality was reported in the study.

Clinical signs (dams): One dam at 5 mg/kg aborted (#50318) aborted on gestation day 28. No other treatment related clinical sign was noted.

Body weight (dams): The average body weight (g) is shown in the table below.

Gestation day	**Control**	**0.5 mg/kg**	**5 mg/kg**	**50 mg/kg**
# Pregnant	17	17	16	20
5	3.45	3.40	3.43	3.39
6	3.45	3.42	3.46	3.41
19	3.68	3.65	3.58	3.61
Gain day 6-day 19	0.23	0.23	0.12	0.20
28	3.88	3.87	3.71	3.75
Gain day 19-28	0.20	0.22	0.13 (65% of control)	0.14 (70% of control)

Above data show that pregnant rabbits at 5 and 50 mg/kg has less weight gain at the end of the study.

Food consumption (dams): The average daily food consumption (g/day/animal) data are shown below.

Gestation day	**0 mg/kg**	**0.5 mg/kg**	**5 mg/kg**	**50 mg/kg**
6	188	195	188	201
19	189	193	75	190
Change in food consumption day 6-19	1	-2	-113	-11
28	166	172	166	131
Change in food consumption day 19-28	-23	-21	91	-59

The food consumption data suggest that the control and the treated animals did not consume enough food during the treatment period. On the other hand, mid and high dose animals showed greater reduction in the food consumption. One explanation of the effect could be relationship of the treatment and procedure to the food consumption. The

intravenous injection could induce pain and irritancy that contributed to the reduced food consumption during the treatment and post-treatment period. This explanation could be well perceived if an untreated group (with no injection) would have included in the study design. The effect on the food consumption also reflected on the body weight at mid and high doses.

Toxicokinetics: The Applicant provided data on MRA (ug/mL) on table 5 and anti-MRA on table 6. The average data on MRA levels (ug/mL) are shown below.

Dose (mg/kg)	**0.5**	**5**			**50**		
Day	7	7	19	28	7	19	28
n	25	22	9	1	28	28	21
Average concentration (ug/mL)	6.03	68.8	212.9	3.3	675	3388	486

The level was below the limit of quantitation on days 19 and 28 at 0.5 mg/kg. The level was about 6 ug/mL on day 7 at 5 mg/kg. However, several animals showed negligible amount in the plasma on day 19 samples. The level was almost non-quantifiable on day 28. MRA levels at 50 mg/kg increased dose proportionately on days 7 and 19. On day 28, the plasma levels of MRA were reduced at 50 mg/kg. However, it was detectable unlike that in 0.5 and 5 mg/kg dose groups.

The average anti-MRA antibody titer is shown below.

Dose	**0.5 mg/kg**			**5 mg/kg**			**50 mg/kg**		
Day	7	19	28	7	19	28	7	19	28
N	2	25	25	2	22	22	2	27	27
Average	20	32500	28500	260	115500	116136	20	6663	9411

N=number of data points

The data for anti-MRA formation suggest that ant-MRA titer was noted in most of the treated animals on day 19 and day 28 of gestation. However, the titer level was much higher at 5 mg/kg than 0.5 and 50 mg/kg. One explanation of relatively lower titer level of anti-MRA antibody at 50 mg/kg could be due to immunosuppression that led to lower antibody formation.

The Applicant correlated embryo-fetal toxicity with the PK and anti-MRA antibody level. When MRA, anti-MRA, food consumption and fetal death was tabulated, a marginal reduction in the food consumption and fetal deaths were noted at 0.5 mg/kg. Since the caesarean data between control and 0.5 mg/kg was comparable, embryo-fetal deaths at 0.5 mg/kg was not affected due to the MRA and its antibody titer.

The relationship between MRA, anti-MRA, food consumption, and embryo-fetal deaths were correlated at 5 mg/kg, the food consumption and fetal deaths became substantially high as shown in the Applicant's table 8 below.

Table 8

Study for effects of MRA administered intravenously on embryo-fetal development in rabbits

Item : Summary of plasma MRA level, anti MRA antibody titration, food consumption and fetal mortality

〈Dose:5mg/kg〉

Animal No.	Plasma MRA level (μg/mL) Day of pregnancy or post coitum 6 7 19 28	Anti MRA antibody titration Day of pregnancy or post coitum 6 19 28	Decrease of food consumption (< 20 or 100g/day)	No. of implantations	Dead fetuses No.	%
50301						
50302						
50303						
50304						
50305						
50306						
50307						
50308						
50309						
50310						
50311						
50312						
50313						
50314						
50315						
50316						
50317						
50318						
50319						
50320						
50321						
50322						

- : 〈0.78125μg/mL - : 〈20 - : not decreased less than 100g/day

b(4)

There could be several explanations for above findings. The MRA-anti-MRA complex could trigger a biological response that contributed to embryo toxicity directly or indirectly through the release of cytokines so as to affect food intake and embryo-fetal development.

The data for 50 mg/kg group are similarly presented in Applicant's table 9 below.

Table 9

Study for effects of MRA administered intravenously on embryo-fetal development in rabbits

Item : Summary of plasma MRA level, anti MRA antibody titration, food consumption and fetal mortality

〈Dose:50mg/kg〉

Animal No.	Plasma MRA level (μg/mL) Day of pregnancy or post coitum 6	7	19	28	Anti MRA antibody titration Day of pregnancy or post coitum 6	19	28	Decrease of food consumption (< 20 or 100g/day)	No. of implentations	Dead fetuses No.	%
50401											
50402											
50403											
50404											
50405											
50406											
50407											
50408											
50409											
50410											
50411											
50412											
50413											
50414											
50415											
50416											
50417											
50418											
50419											
50420											
50421											
50422											
50423											
50424											
50425											
50426											
50427											
50428											

- : <0.78125 μg/mL - : <20 - : not decreased less than 100g/day

b(4)

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

Litter data following caesarean section are shown from Applicant's table 10 below.

Body weight of live pups was affected at 5 and 50 mg/kg. However, data at 5 mg/kg were affected most. Number of live fetuses was reduced at 5 mg/kg without affecting the live fetuses at 50 mg/kg. However, total fetal deaths (embryonic and fetuses) was greater than the control at 5 mg/kg.

Table 10
Study for effects of MRA administered intravenously on embryo-fetal development in rabbits
Item : Litter Data with Caesarean Section (P0) - Summary

Control : MRA 0 mg/kg

Test Article	MRA	MRA	MRA	MRA
Dose	0 mg/kg	0.5 mg/kg	5 mg/kg	50 mg/kg
No. of Dams	17	17	16	20
Corpora Lutea				
No per Litters:Mean±S.D.	8.3±1.0	8.9±1.1	8.9±1.5	8.6±1.6
Implantations				
No per Litters:Mean±S.D.	6.9±1.6	7.6±1.2	8.0±1.5	7.2±2.2
% to Corpora Lutea:Mean±S.D.	83.7±16.5	85.1±10.9	89.8±9.7	82.8±19.8
Live Fetuses				
Male	57	60	41	69
Female	49	62	40	59
Total	106	122	81	128
No per Litters:Mean±S.D.	6.2±2.0	7.2±1.6	5.1±3.3	6.4±2.1
% to Implantations:Mean±S.D.	89.0±19.9	93.8±12.8	61.8±37.8*	89.5±12.7
Sex Ratio (%) Male/Total	53.8	49.2	50.6	53.9
Death				
Total	12	7	47	16
% to Implantations:Mean±S.D.	11.0±19.9	6.2±12.8	38.2±37.8*	10.5±12.7
Embryonic Death				
Total	11	6	20	8
% to Implantations:Mean±S.D.	9.8±15.8	5.5±12.8	15.5±26.9	5.9±9.3
Early Fetal Death				
Total	0	0	13	4
% to Implantations:Mean±S.D.	0.0±0.0	0.0±0.0	9.8±25.0*	2.4±6.0
Late Fetal Death				
Total	1	1	14	4
% to Implantations:Mean±S.D.	1.2±4.9	0.7±2.7	12.9±29.5	2.3±7.0
Body Weight of Live Fetuses(g)				
Male : Mean±S.D.	40.6±4.2	38.6±5.0	33.7±7.7	37.2±7.6
Female : Mean±S.D.	38.1±3.4	36.8±3.7	33.7±6.1	36.7±7.5

Significantly different from control : * P<0.05, ** P<0.01

Offspring (malformations, variations, etc.):

Visceral examinations of live fetuses did not reveal a treatment effect with the exception of ventricular septal defects in control and some treated animals. One fetus at 0.5 mg/kg also showed supernumerary coronary orifice. However, no treatment-related visceral malformations were noted. These data are presented in the Applicant's table 11.

Table 11
Study for effects of MRA administered intravenously on embryo-fetal development in rabbits
Item : Visceral Examination of Fetuses (F1) - Summary

Control : MRA 0 mg/kg

Test Article	MRA	MRA	MRA	MRA
Dose	0 mg/kg	0.5 mg/kg	5 mg/kg	50 mg/kg
No. of Dams	17	17	13	20
No. of Fetuses	106	122	81	128
No. of Dams with Anomalous Fetuses	1(5.9%)	2(11.8%)	1(7.7%)	1(5.0%)
No. of Fetuses with Anomaly	1	2	1	1
Mean (%)	0.7	1.4	1.1	0.6
S.D.	3.0	3.9	4.0	2.8
Ventricular septal defect	1(1) a)	1(1)	1(1)	0(0)
Mean (%)	0.7 b)	0.7	1.1	0.0
S.D.	3.0	2.7	4.0	0.0
Hypoplasia of lung	0(0)	0(0)	0(0)	1(1)
Mean (%)	0.0	0.0	0.0	0.6
S.D.	0.0	0.0	0.0	2.8
Supernumerary coronary orifice	0(0)	1(1)	0(0)	0(0)
Mean (%)	0.0	0.7	0.0	0.0
S.D.	0.0	3.0	0.0	0.0

Figures in parentheses represent No. of litters
a) No. of animals with anomaly :
b) No. of animals with anomaly X 100 / No. of animals examined, on litter basic

The skeletal variation and malformation data showed retardation of sternum development at 0.5, 5, and 50 mg/kg as shown in Applicant's table 14.

Table 14
Study for effects of MRA administered intravenously on embryo-fetal development in rabbits
Item : Skeletal Variation of Fetuses (F1) - Summary

Control : MRA 0 mg/kg

Test Article	MRA	MRA	MRA	MRA
Dose	0 mg/kg	0.5 mg/kg	5 mg/kg	50 mg/kg
No. of Dams	17	17	13	20
No. of Fetuses	106	122	81	128
No. of Dams with Varied Fetuses	10(58.8%)	12(70.6%)	9(69.2%)	11(55.0%)
No. of Fetuses with Variation	22	30	21	35
Mean (%)	19.6	23.9	22.1	21.0
S.D.	22.9	25.1	19.4	27.8
Sternum				
- Asymmetry	1(1) a)	1(1)	0(0)	1(1)
Mean (%)	0.6 b)	0.8	0.0	2.5
S.D.	2.4	8.5	0.0	11.2
- Retardation	5(1)	9(8)	13(6)	14(5)
Mean (%)	4.2	7.1	14.0	12.0
S.D.	17.3	8.3	20.1	22.4

Figures in parentheses represent No. of litters
a) No. of animals with variation :
b) No. of animals with variation X 100 / No. of animals examined, on litter basic

Summary and conclusion of the teratogenicity study in rabbits:

Effect of MRA on segment II reproductive toxicity was investigated at 0.5, 5, and 50 mg/kg/day, IV infusion to Japanese White rabbits. The animals were treated between gestation days 6-18 and sacrificed on gestation day 28 to determine the effect of the drug on organogenesis. The body weight gain and food consumption was reduced at 5 and 50 mg/kg at the end of gestation day 28. Pharmacokinetic data showed anti-MRA antibody formation on day 19 and day 28 at 0.5, 5, and 50 mg/kg. However, the anti-MRA titer was higher at 5 mg/kg compared to other doses. The Applicant presented a relationship to the loss of weight gain and embryo lethality with that of anti-MRA antibody formation at 5 mg/kg. A treatment related increase in the retardation of sternum development was noted at 0.5, 5, and 50 mg/kg although the data were not statistically significant and historical control data were not presented in the submission.

The effect of anti-MRA and formation of anti-MRA-MRA complex (if any) on the food intake and fetal death could be due to induction of an intrinsic effect of the complex on the fetal development. However, a direct relationship of the pharmacological effect of the complex could not be determined in the absence of further investigation. The role of anti-MRA antibody at 50 mg/kg was relatively less than that at 0.5 mg/kg. It is possible that MRA could induce an immunosuppressive effect that reduced the anti-MRA titer level and its biological response. It should be noted that the relevance of findings in rabbits to the human could be minimal because the cross-reactivity of MRA to rabbit tissues is unknown and anti-MRA antibody formation in humans is not expected due to humanized nature of the monoclonal antibody.

The reviewer also perceived that IV infusion and local irritancy at the site of injection also could contribute to the reduced food intake and fetal deaths.

It is concluded that the treatment showed reduced development of sternum at 0.5, 5, and 50 mg/kg, IV. The no effect dose (NOEL) was not determined. The study was conducted at MTD.

This model is not appropriate for the segment 2 study of MRA.

Study title: A study for the effects of MRA on embryo-fetal development in cynomolgus monkeys by intravenous administration.

Key study findings: Pregnant animals were treated between gestation days 20 to 50. MRA was not teratogenic in cynomolgus monkeys. However, an embryocidal effect was noted at 10 and 50 mg/kg. NOEL was 2 mg/kg/day, IV.

Study no.: TOX00-0012 (J99-0385)
Volume # M4 and page #: 1
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: Not provided in the report
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug: MRA, lot # RMR7D21, and % purity: 5.81 mg/mL,

Methods

Doses: The study design is shown below. Pregnant monkeys were treated with the vehicle or MRA at 2, 10, and 50 mg/kg/day. The pregnancy was determined on the basis of ultrasound techniques under ketamine anesthesia. The high dose was chosen on the basis of a one-month toxicity study in which neutropenia was noted at 50 mg/kg.

The experimental groups were as shown below.

Group	Test Article	Dose Level (mg/kg/day)	MRA Concentration (mg/mL)	Dose Volume (mL/kg/day)	No. of Pregnant Animals (Animal nos.)
1	Control	0	0	8.606	10 (10001-10010)
2	MRA	2	5.81	0.344	10 (10101-10110)
3	MRA	10	5.81	1.721	10 (10201-10210)
4	MRA	50	5.81	8.606	10 (10301-10310)

Species/strain: Pregnant cynomolgus monkeys that were 4 to 9 years old at mating, weighed 2.5 to 4.29 kg. Monkeys were obtained from [redacted] b(4)

Number/sex/group: Ten

Route, formulation, volume, and infusion rate: The drug substance was diluted with 20 mM phosphate buffered saline and injected intravenously at 0.344, 1.721, and 8.606 mL/kg/day volume. The intravenous infusion rate was 2 mL/min. The vehicle group received 20 mM phosphate buffer solution. The treatment was given once a day from gestation day 20 to gestation day 50 for the determination of the effect of treatment on organogenesis. The intravenous infusion was given into femoral or saphenous veins.

Satellite groups used for toxicokinetics: No satellite group was allotted in the study.

Study design: See above

Parameters and endpoints evaluated:

Clinical signs were observed three times a day during the treatment and once before and after the treatment period. The heart beat of the fetuses were monitored on days 25, 30, 40, 50, 60, 70, 80 and 90. The body weight and food consumption was recorded from gestation day 0 to gestation day 99-100 at several time points. Standard hematological parameters were determined at predose, end of dosing on gestation day 51 and post dose on gestation day 100.

The plasma levels of MRA and antibodies to MRA was determined from 5 animals/group from blood samples collected at predose, days 20, 29, 50 (24 hr after first dose), on days 65, 80 and at necropsy. The blood samples were collected from femoral or cephalic veins. However, blood samples were collected from umbilical vein at caesarean section for the determination of MRA and its antibodies from dams as well as fetuses.

Caesarean section was performed on gestation days 100-102 under ketamine and isoflurane anesthesia. Fetus and placenta were removed for further analysis. Caesarean section was performed sooner for dams that had dead fetus. Animals were not sacrificed after the caesarean section.

Fetal viability, sex, body weight, placental weight, external examination, brain, and thoraco-abdominal organs examined following the caesarean section. Fetuses were sacrificed by IP injections of sodium pentobarbital, and organ weights for the following organs were noted: brain, thymus, heart, lungs, spleen, liver, kidney, adrenal gland, testes, ovary, and uterus.

The Applicant stated that eye, stomach, small intestine, large intestine, skin and above organs were fixed in formalin. The remaining fetus was digested with ethyl alcohol and stained with Alizarin Red. Skeletal abnormality, variations, and ossification were noted.

Results:

A comprehensive data of dams are shown in the table below.

Parameters	Dose (mg/kg/day)			
	0	**2**	**10**	**50**
Abortions/ Day of abortions	1/10 GD 27	1/10 GD 50	2/10 GDs 25, 30	3/10 GDs 30, 40, 65
Clinical Observation/ Non placenta-related genital bleeding/ Day of bleeding	-	1/10 GDs 60-65	-	1/10 GDs 60-65
Body Weight Changes (g) mean ± SD; (Days 7-21)	NC	NC	NC	NC
Food Consumption (g/day) mean ± SD; (Days 7-21)	NC	NC	NC	NC
Hematological Parameters from GD 51 (% control of mean values)				
Erythrocytes counts	NC	6% ↑	6% ↑	NC
Hb value	NC	12%↑	14%↑	NC
HCT value	NC	11%↑	NC	NC
Leukocyte counts	NC	30%↓	NC	NC
Plasma Concentration of MRA in Dams (µg/mL)				
GD 20	ND	43 ± 20	192 ± 19	914 ± 170
GD 50	ND	319 ± 148	1646 ± 250	5813 ± 625
GD 100	ND	ND	20 ± 20	102 ± 40
Plasma Concentration of MRA in fetuses (µg/mL)				
GD-100	ND	ND	8 ± 8	61 ± 13
Plasma Concentration of anti MRA antibody in Dams (µg/mL)				
GD 50	ND	20 ± 45	ND	1 ± 2
GD 100	ND	3 ± 4	ND	ND
Plasma Concentration of anti MRA antibody in fetuses (µg/mL)				
GD 100	ND	1 ± 2	ND	ND

Clinical signs: No dams died in any group. However, there were a dose-related increase in the abortions or embryo-fetal death of 1/10, 1/10, 2/10, and 3/10 fetuses died from the 0, 2, 10, and 50 mg/kg/day dose group, respectively, as shown in the table below.

Dose	**Animal#**	**Observation**
Control	10009	Aborted, day 27
2 mg/kg	10101	Embryonic death, day 50
2 mg/kg	10106	Genitalia bleeding, days 62-67
10 mg/kg	10202	Embryonic death, day 30
10 mg/kg	10208	Abortion, day 25
50 mg/kg	10301	Abortion, day 30
50 mg/kg	10306	Fetal death, day 65
50 mg/kg	10307	Fetal death, day 40

Abortion or death could be treatment related specially at 50 mg/kg.

Body weight: There were no differences in the average body weight changes and body weight gains in between the dams from the different treatment groups indicating no test article related changes in the body weights as shown in the table below.

Day	Control	2 mg/kg	10 mg/kg	50 mg/kg
0	3.2	3.0	3.4	3.2
20	3.3	3.1	3.5	3.3
50	3.4	3.1	3.7	3.5
100	4.3	3.9	4.3	4.2

Food consumption: There were no differences in the feed consumption in dams from any of the dose groups suggesting that the feed consumption was not affected by the test article.

Hematological Examinations: There was a significant decrease in the average Hb, HCT, erythrocyte, and reticulocyte counts in one of the high dose dams at GD 100 (compared to the values of the erythrocytes parameters at GD 19 and GD 51) indicating anemia. This dam aborted at GD 65 (#10306, 50 mg/kg). It is, however, not known whether the changes in the erythrocyte related parameters were associated with the abortion. There was a statistically significant increase in the average erythrocyte counts, Hb, and HCT and a decrease in the platelets at gestation day (GD 51) in the low and mid dose groups. No such changes were noted at GD 100 suggesting recovery.

Therefore, individual data showed anemia in one monkey at 50 mg/kg. However, no neutropenia was observed as mentioned in the one month dose finding study.

Toxicokinetics:

There was a dose proportional increase in the exposure of MRA in plasma (refer to Applicant's table 5). The highest exposure of MRA was noted around Day 50 in all test article treated groups. There was relatively a low level of MRA plasma concentration at GD 100 in dams from the mid and high dose groups. MRA was noted among, 3/5 and 5/5 fetuses from mid and high dose groups, respectively. However, fetuses in control and low dose did not detect the presence of MRA. Data are shown below from the Applicant's table #5.

b(4)

Table 5 Plasma MRA concentration (μg/mL) Study No. — 54-12

Dose (mg/kg/day)	Animal number	Plasma MRA concentration (μg/mL) GD18 or 19 (before dosing)	GD20 (a)	GD29 (a)	GD50 (a)	GD65	GD80	GD100-102 (b)	Fetuses GD100-102 (b)
Control	10001	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	10002	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	10003	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	10004	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	10005	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Mean	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	S.D.	-	-	-	-	-	-	-	-
2	10102	N.D.							
	10103	N.D.							
	10104	N.D.							
	10105	N.D.							
	10106	N.D.							
	Mean	N.D.	43.02	216.44	319.81	57.57	6.74	N.D.	N.D.
	S.D.	-	20.31	31.99	148.71	33.27	4.02	-	-
10	10201	N.D.							
	10203	N.D.							
	10204	N.D.							
	10205	N.D.							
	10206	N.D.							
	Mean	N.D.	192.38	1065.01	1646.45	436.46	134.16	20.11	7.78
	S.D.	-	19.76	205.83	250.63	134.84	64.50	28.23	8.43
50	10302	N.D.							
	10303	N.D.							
	10304	N.D.							
	10305	N.D.							
	10308	N.D.							
	Mean	N.D.	914.28	3736.27	5813.67	1397.14	487.24	102.07	61.35
	S.D.	-	169.48	260.14	625.38	121.82	94.84	40.87	13.53

GD : Day of gestation
a) : at 24 hours after dosing
b) : At cesarean section
N.D. : Not detected (<0.781 μg/mL)

b(4)

b(4)

The anti-product antibody production was noted in dam #10103 from GD 50 at 2 mg/kg. Traces of antibody were detected in #10106 at 2 mg/kg and fetuses from above two dams. The rest of the monkeys did not show anti-product antibodies. Therefore, MRA was virtually not neutralized during the treatment period in pregnant monkeys. Data are shown in table 6 below.

Table 6 Plasma anti-MRA antibody concentration (μg/mL) Study No. ___ 54-12

Dose (mg/kg/day)	Animal number	Plasma anti-MRA antibody concentration (μg/mL) GD18 or 19 (before dosing)	GD20 (a)	GD29 (a)	GD50 (a)	GD65	GD80	GD100-102 (b)	Fetuses GD100-102 (b)
Control	10001	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	10002	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	10003	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	10004	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	10005	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Mean	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	S.D.	-	-	-	-	-	-	-	-
2	10102	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	10103	N.D.	N.D.	N.D.	[redacted]				
	10104	N.D.	N.D.	N.D.					
	10105	N.D.	N.D.	N.D.					
	10106	N.D.	N.D.	N.D.					[redacted]
	Mean	N.D.	N.D.	N.D.	20.62	27.26	7.36	2.79	1.22
	S.D.	-	-	-	45.61	60.27	14.83	4.14	2.05
10	10201	N.D.	N.D.	N.D.	N.D.	[redacted]	N.D.	N.D.	N.D.
	10203	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	10204	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	10205	N.D.	N.D.	N.D.	N.D.	N.D.	[redacted]	[redacted]	N.D.
	10206	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	[redacted]	N.D.
	Mean	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.54	N.D.
	S.D.	-	-	-	-	-	-	0.950	-
50	10302	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	10303	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	10304	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	10305	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	10308	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Mean	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	S.D.	-	-	-	-	-	-	-	-

GD : Day of gestation
a) : at 24 hours after dosing
b) : At cesarean section
N.D. : Not detected (<0.391 μg/mL)

b(4)

b(4)

Gross Necropsy of fetuses and Placental findings:

There were no gross necropsy findings in the fetuses.

Number of live births was 9/10, 9/10, 8/10, 7/10 at control, 2, 10 and 50 mg/kg, respectively. Fetal weight, placental weight, various external examination parameters were comparable between control and treated fetuses. The amniotic fluid volume was 72, 64, 56, and **54** mL at control, 2, 5 and 50 mg/kg, respectively. There was no statistically significant change in the organ weight in the control and MRA treated fetuses. Dam #10303 showed lower weight of uterus (13 g) among any other monkeys deployed in the study.

Single placenta were noted in 1/10, 2/10, 2/10, and 3/10 dams from the 0, 2, 10, and 50 mg/kg/day dose group indicating a dose-related and therefore a test article-related change. The Applicant stated that the finding was within the background incidences in primates.

Offspring (malformations, variations, etc.):

Fetuses from control and treated animals did not show any external visceral and skeletal abnormalities or variations as shown in table 10 below.

Table 10-1 Summary of alterations in fetuses — Study No. — 54-12

Dose (mg/kg/day)		Control	2	10	50
No. of fetuses		9	9	8	7
External abnormalities	n	0	0	0	0
	(%)	0.0	0.0	0.0	0.0
Placental abnormalities	n	1	3	2	3
	(%)	11.1	33.3	25.0	42.9
Single placenta	n	1	2	2	3
	(%)	11.1	22.2	25.0	42.9
Discoloration of secondary placenta	n	0	1	0	0
	(%)	0.0	11.1	0.0	0.0
Visceral abnormalities	n	0	0	0	0
	(%)	0.0	0.0	0.0	0.0
Visceral variations	n	0	0	0	0
	(%)	0.0	0.0	0.0	0.0
Skeletal abnormalities	n	0	1	0	0
	(%)	0.0	11.1	0.0	0.0
Absebce of 12th ribs	n	0	1	0	0
	(%)	0.0	11.1	0.0	0.0
Skeletal variations	n	3	1	0	3
	(%)	33.3	11.1	0.0	42.9
Cervical ribs	n	0	1	0	0
	(%)	0.0	11.1	0.0	0.0
Lumbar ribs	n	3	0	0	3
	(%)	33.3	0.0	0.0	42.9

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

Data for fetal skeletal examination did not show abnormality between control and treatment groups. Skeletal variations in the control and treated animals were observed. However, its clinical significance to MRA treatment is unknown.

Monkey #10103 and #10106 showed anti-MRA antibody levels and detectable levels of MRA were not observed in these monkeys on gestation days 65 onwards perhaps due to the presence of anti-MRA antibody. However, #10103 and #10106 did not show any change in the fetal weight compared to other fetuses. Skeletal examinations of #10103

showed bilateral absence of 12th rib. The data suggests that the presence of anti-MRA could contribute to the variation. However, control and MRA at 10 and 50 mg/kg also showed skeletal variations. Therefore, presence of anti-MRA did not show any impact to the organogenesis in monkeys.

Conclusion:

Based on the data, it is concluded that IV infusion of MRA at 2, 10, and 50 mg/kg/day between gestation days 20-50 was well tolerated to pregnant cynomolgus monkeys. No teratogenicity or variations due to the treatment was noted. However, based on the fetal data, high incidences of abortion and fetal deaths were noted at 10 and 50 mg/kg (20-30%) without significant maternal toxicity. The NOEL would be 2 mg/kg in the study. Therefore, Pregnancy category C would be designated due to embryocidal effect in the absence of teratogenicity in pregnant cynomolgus monkeys.

Prenatal and postnatal development

The Applicant did not submit a Segment III reproductive safety study report. As noted earlier, the FDA informed the Sponsor as the time of the pre-BLA meeting in 2007 of the following: "Your proposed BLA submission does not contain any data regarding the potential pre- and post-natal developmental effects of the drug product (segment III). You should submit studies in either the monkey or a surrogate model to address such effects or provide clear rationale for why such studies are not possible."

The Applicant provided following literature references to address the issue for not conducting the segment 3 study.

1. The role of interlukin-6 in mucosal IgA antibody responses in vivo, Science, 264, 561, 1994 (#1054).

   IL-6 knock-out mice showed an inhibition of intestinal mucosal IgA production in response to ovalbumin. IgA production was associated with mucosa-associated lymphoid tissue and B-lymphocytes. Therefore, IL-6 deficiency contributed to an immuno-compromised state.

2. Impaired immune and acute-phase response in interleukin-6-deficient mice, Nature, 368, 339, 1994 (#4026).

The authors developed IL-6 resistant genes by homologus recombination and development of IL-6 deficient mice. Authors reported that IL-6 deficient mice developed normally. However, these mice did not respond to vaccination and immunity to infections with Listeria monocytogenes and stomatitis virus infections. Impaired immune responses in IL-6 deficient mice were also responsible to reduced acute responses to tissue injuries. Six-week old mice showed 20-40% lower number of lymphocytes without altering pattern of T-cell receptors.

Data indicate that IL-6 is an important cytokines that has protective roles.

3. Linkage of IL-6 with neutrophil chemo-attractant expression in virus induced ocular inflammation, Fenton et al., Invest. Ophthalmol. Vis. Sci., 43, 737, 2002 (#4027).

   IL-6 -/- mouse model showed reduced corneal inflammation and opacity to HSP-1 (herpes virus) than wild type mouse. Data suggest that IL-6 is an important pro-inflammatory cytokine and induces corneal cells to produce macrophage inflammatory proteins (MIP). These proteins further recruit neutrophils to induce corneal opacity. Supplementation of IL-6 to virally infected eyes in the IL-6 knock out model restored the inflammation. Data showed the role of IL-6 in inducing inflammatory response. However, the citation also discussed the possible role of IL-6 for anti-inflammatory activity. **IL-6 deficient mice also showed greater inflammatory response to endotoxemia**. The author indicated that IL-6 can also produce anti-inflammatory effect by inducing glucorticoids.

4. IL-6 deficient mice are highly susceptible to *Listeria monocytogenes* infection: correlation with inefficient neutrophilia, Infec and Immun, 63, 2262, 1995 (#4030).

   IL-6 deficiency in the knock-out mouse showed greater opportunistic infections due to suppression of neutrophilia. rIL-6 showed a protective effect even in the absence of lymphocytes suggesting that IL-6 protective effects were not mediated by lymphocytes.

5. Impaired neutrophil response and $CD^{+}T$ helper cell 1 development in IL-6 deficient mice infected with *Candida albicans*, J.Exp.Med, 183, 1345, 1996 (#4031).

   The authors summarized that IL-6 deficient mice were more susceptible to *Candida albicans* infection than the wild type mice. IL-6 Knock out mice showed lack of neutrophil and macrophage responses to infection.

6. Impaired antifungal effector activity but not inflammatory cell recruitment in interleukin-6 deficient mice with invasive pulmonary aspergillosis, J. Infec. Dis, 184, 610, 2001 (#4040).

   IL-6 $^{-/-}$ mice were infected with fungus, *Aspergillus fumigatus*, intranasally. Results showed that IL-6 deficient mice were more susceptible to infections. Exposure to exogenous IL-6 restored antifungal activity.

7. IL-6 is required for protective immune response to systemic *Escherichia coli* infection, Infec and Immunity, 64, 3231, 1996 (#4041).

IL-6 knock-out mouse model showed increased susceptibility to *E. coli* infection and mortality due to infections. However, IL-6 knock-out mice and wild type mice had a similar response to LPS–induced shock. The authors concluded that IL-6 deficiency induced immune suppression due to deficiency in neutrophilia. Also, LPS–induced release of several mediators in addition to IL-6 that would be responsible for shock-like effects in mice. Prophylactic treatment with recombinant IL-6 showed reversal of neutrophilia and accumulation of bacteria in tissues in IL-6 knock-out mice.

Above publications suggest that IL-6 deficient transgenic mice induce immunosuppression by reducing neutrophilia and increased the susceptibility to opportunistic infections. However, wild type mice and IL-6 deficient transgenic mice respond to LPS. IL-6 deficient mice showed greater response to endotoxins. IL-6-deficient mice also showed a reduced IgA response. Authors suggested that IL-6 supplementation reduced above effects of IL-6 deficiencies.

The Applicant's addressed following issues to support why they did not conduct segment 3 reproductive safety study as requested in the Pre-BLA correspondence dated Oct 9, 2007.

1. IL-6 does not play a critical role in organ, tissue development and alteration of immune system based on data from 6-month repeat dose toxicity in monkeys and literature citations on IL-6 $^{-/-}$ mice.
2. Biological responses of IL-6 deficiency in knock-out mouse model could be reversed by rIL-6.
3. Absence of IL-6 during fetal or postnatal life did not lead to sustained or irreversible functional deficiencies as referenced in #4026 in the mouse model.
4. Slow clearance of IL-6 from monkey plasma after 30 days of treatment during organogenicity did not show teratogenicity of fetuses at the end of gestation day 100.

Reviewer's comments:

The Sponsor's justification for why the segment 3 study in appropriate animal species would not be necessary is not acceptable for the non-clinical requirement of BLA approval due to following reasons:

1. IL-6 deficiency in the knock-out mouse model showed opportunistic infections and was reversed by supplementation of rIL-6. Therefore, there are chances of opportunistic infections that would lead to severe consequences during postnatal development.
2. Literature cited by the Applicant suggests that IL-6 could induce glucocorticoids. Therefore, lack of IL-6 due to the treatment with IL-6R antibody during late pregnancy could contribute to reduced postnatal growth and development due to the glucocorticoids deficiency.

3. Since IL-6 $^{-/-}$ mouse showed increased susceptibility to infections, the normal reproductive performance in IL $^{-/-}$ mice cited in Reference #4026 is not a sufficient justification for not conducting the segment 3 reproductive safety study.

2.6.6.7 Local tolerance

Local tolerance to Tocilizumab in monkeys has been discussed in the repeat dose toxicity study.

2.6.6.8 Special toxicology studies

No special toxicological studies were submitted..

2.6.6.9 Discussion and Conclusions:

Tocilizumab is a monoclonal antibody to human IL-6 receptor and structurally based on human IgG1 immunoglobulin. It was developed for the treatment of rheumatoid arthritis at a maximum dose of 8 mg/kg every 4 weeks. The BLA is submitted for the licensing the product in the USA. The Applicant provided information on its role as an inflammatory mediator and progression of certain types of cancer cells. Tocilizumab referred in this BLA as Actemra, RO4877533, and MRA.

Tocilizumab neutralized human IL-6R present in cell membranes and in the plasma at Kd (binding constant) 2.5 and 0.7 nmol/L, respectively. Data suggest that tocilizumab is more potent to the soluble receptor. Tocilizumab selectively inhibited IL-6 receptor and it did not show any effect on the transduction of IL-1, IL-15, and TNF. Tocilizumab inhibited IL-6 and IL-6R complex mediated cell signaling in . ——— cell line that was refractory to IL-6 or IL-6R alone. The concentration used in the assay was 0.1 ug/mL. Tocilizumab inhibited trans-signaling. b(4)

The Applicant mentioned the homology of human IL-6R to other species that would provide insight into the selection of appropriate animal models for pharmacodynamic, safety and toxicity.

Human IL-6R homology to cynomolgus monkeys and rodents is about 97% and 54%, respectively. Also, tocilizumab did not show an effect on mouse and rodent cells whereas a mouse IL-6R antibody raised in rats (MR16-1) showed an effect on the inhibition of cell proliferation in mice. These data apparently suggest that cynomolgus monkeys would be an appropriate species for further non-clinical studies. However, there are limitations for the use of animal models because IL-6 expression under normal conditions may be limited.

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The Applicant conducted several tissue cross-reactivity studies for Tocilizumab. Data suggest that there was technical inconsistency issues that resulted in variability in the response. However, some of the studies suggest the order of MRA tissue cross-reactivity was human>cynomolgus monkey>rat. Binding of MRA was mostly restricted to inflammatory cells, endothelial and epithelial cells.

The Applicant conducted pharmacodynamic studies in collagen-induced arthritis model in cynomolgus monkey (using MRA), wild type mouse model of amyloidosis (using MR16-1), and transgenic mice model expressed human IL-6 (using MRA). Data suggest that MRA did not have a definite anti-inflammatory effect compared to the control in the monkey model at 30 mg/kg, IV. Monkeys also showed neutralization of MRA by anti-MRA antibody. MR16-1 showed an effect in mouse amyloidosis and transgenic model at 0.5 and 2 mg/kg, respectively. These data as well as binding data meet the regulatory requirements of non-clinical pharmacodynamic studies.

Acute safety pharmacology data in anesthetized dogs did not show any cardiovascular or respiratory effect at 66.7 mg/kg, IV. Several cardiovascular parameters in cynomolgus monkeys were not affected at 133.4 mg/kg, IV. Tocilizumab did not show an effect on the ADP induced platelet aggregation in cynomolgus monkeys. No CNS safety data were provided. However, CNS effect is not expected due to low distribution of the drug in adult animals.

Tocilizumab showed non-linear increase in the exposure upon IV injections. Antibody to the product was noted even after a single injection. Subcutaneous bioavailability was about 72%. ^{125}I-MRA distributed to adrenals, lungs, kidneys, liver, spleen, bone marrow, synovium, thyroid, and male reproductive organs and slowly eliminated after 2 days following a single injection at 5 mg/kg. The Applicant did not investigate distribution of drug in female monkeys. However, it did not show gender difference in the kinetics. Intact tocilizumab was observed in the plasma following IV doses. The Applicant stated that tocilizumab possibly internalized in the cell through glycoprotein receptors for further degradation. The elimination half-life was about 9-13 days in monkeys depending on the dose and treatment duration. The Applicant indicated that MRA could excrete through the milk due to IgG nature of the antibody and transfer to weaning animals through breast feeding. The Applicant conducted clinical pharmacology studies for drug interactions and no non-clinical in vivo drug interaction studies were conducted. Human PK data at 8 mg/kg every 4 weeks showed Cmax and AUC of 183 ug/mL and 35000 ug.h/mL, respectively.

A 6-month repeat dose toxicity to IL-6 antibody was evaluated in cynomolgus monkeys at 1, 10 and 100 mg/kg/IV infusion dose per week with a 2 month recovery. The highest dose 100 mg/kg was tolerated without treatment-related mortality. However, one male monkey at 10 mg/kg showed gingival inflammation that subsided following extraction of the tooth. The relationship of gingival inflammation to the treatment is not clearly known due to low prevalence of the observation. A slight granuloma in the liver at 10-100 mg/kg was observed that was reversible. Degeneration of the skeletal muscle was noted at 10-100 mg/kg that was not completely reversible. However, creatinine phosphokinase

activity was not affected by the treatment as shown in Report #TOX03-0002. Antibody to IL-6 antibody was detected at 1 and 10 mg/kg only. The half-life of the antibody was 10-13 days in this study. Intestinal IgA production was not affected by the treatment in monkeys. MRA did not show biologically relevant modulation in the CD4+, CD8+, and CD20+ lymphocytes. The NOEL was 1 mg/kg, IV. Inflammation in the injection site was noted in control and treated animals.

It was concluded that granuloma of the liver and skeletal muscle degeneration was noted in monkeys at 10 and 100 mg/kg, IV for 6 months. The NOAEL was 1 mg/kg. Inflammation at the injection site was noted in control and treated animals.

MRA was not mutagenic in Ames assay and chromosomal aberration assay in peripheral lymphocytes from human volunteers.

Reproductive safety studies were conducted in rats for fertility (segment 1) and teratogenicity (segment 2), in rabbits for teratogenicity (segment 2) and cynomolgus monkeys for segment 2. Among these studies only the segment 2 study in monkeys would be considered appropriate based on the tissue cross-reactivity results and high level of anti-MRA antibody in rats and rabbits. Therefore, study results for the reproductive safety in monkeys need to be addressed in the package insert.

The embryotoxicity study (segment 2) in cynomolgus monkeys was conducted at 2, 10, and 50 mg/kg/day, IV between gestation days 20 to 50. Abortion and or fetal deaths were noted at 10 and 50 mg/kg. However, teratogenicity or variations was not noted. NOAEL was 2 mg/kg. No anti-MRA was detected in most of treated animals in this study. Plasma trough MRA levels were 319, 1646, and 5813 ug/mL at 2, 10, 50 mg/kg/day, IV, respectively, at the end of gestation day 50. Data suggest that treatment with MRA to pregnant monkeys had an adverse effect on the viability of fetuses at relatively non-maternally toxic doses. Based on the data in cynomolgus monkeys, Pregnancy category C should be assigned to tocilizumab. The reviewer recommends that only the segment 2 reproductive safety data in monkeys should be included in the label.

It should be noted that anti-MRA antibody was detected in monkeys in several studies including the 6-month repeat dose toxicity study described above. However, pregnant monkeys did not show anti-MRA antibody except 2 animals at 2 mg/kg. It is possible that MRA had an inhibitory effect on the immune system that minimized the antibody formation under the experimental conditions.

The Applicant did not conduct pre-natal and post natal segment 3 study in monkeys. The Applicant was asked to provide data or justifications for why the studies were not possible at the Pre-BLA meeting. The Applicant indicated that normal reproduction in IL-6 knock-out mice and absence of reproductive organ toxicity in 6-month and segment 2 studies do not warrant segment 3 study in monkeys. Moreover, long half-life of MRA would expose the fetus to the drug that would provide information on the effect of the drug on labor and delivery.

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The reviewer recommends that considering the role of IL-6 as a growth factor, there is a need to generate data for both segment 1 and segment 3 studies in appropriate species. Although the half-life of the drug is about 9 days or longer in monkeys, distribution studies in monkeys showed that most of the drug was removed from tissues soon after 2 days. The Applicant also did not provide data on the distribution of the drug in female reproductive organs. Data in the knock-out models are not definitive justification for not conducting studies recommended because knock-out models are not totally predictive of biological responses as discussed in the review section of the individual study. **The issue of immunomodulatory nature of IL-6 and the role of IL-6 as a growth factor strongly implicate the need to further data for fertility and late pregnancy for MRA in suitable models.**

The Applicant did not conduct carcinogenicity studies. The Applicant was asked to provide rationale why such studies were not possible during the Pre-BLA meeting. The Applicant was also asked to discuss the impact of MRA on tumor surveillance and tumor development. The Applicant argued that IL-6 is involved in the tumor progression and tocilizumab would prevent cancer. Therefore, carcinogenicity study for tocilizumab was not warranted. Since Tocilizumab showed anti product antibodies in rodents, it was not possible to conduct conventional carcinogenicity studies.

The review of published literature and the Applicant's presentation did not clearly explains that tocilizumab would be free from tumor progression or tumor development on long term treatment in suitable species. The understanding of the literature is not well supported to predict that tocilizumab would have no role towards the development of cancer. If IL-6 is implied as a growth factor, mechanistically inhibition of IL-6R would deprive the tissue of its effect that could result in tumor growth. IL-6 receptor transduction involves several other cytokines. If IL-6R is inhibited selectively by antibodies, the role of other cytokines to the progression of tumor can not be predicted unless there is a direct evidence of IL-6R antibody in carcinogenicity studies. Therefore, **the reviewer conceived that the carcinogenicity study would be necessary in appropriate species using antibody specific to IL-6R in that species for further assessment of carcinogenic risks to the treatment. Alternatively, a cancer registry needs to be set up to monitor the cancer causing potential of Tocilizumab. The package inserts needs to indicate that the carcinogenicity studies in animals were not conducted for Tocilizumab. However, immunomodulatory products may cause cancer. Further progression of cancer would be monitored through the cancer registry.**

The Applicant did not provide exposure data in several studies in monkeys for determination of long term toxicity and reproductive safety. Therefore, monkey to human exposure ratios could not be determined. Instead the dose ratios between animal to human should be provided in the label as mg/kg because biological products mostly circulates in the blood with a minimal tissue distribution.

2.6.6.10 Tables and Figures

Tables are presented in the individual section of the review.

2.6.7 TOXICOLOGY TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the Applicant]

Species	Duration	Dose	Observation
Sprague Dawley rats	One-month	2, 10, 50 mg/kg, IV infusion	No treatment related Mortality, injection site inflammation in placebo and drug treated animals. Antibody to MRA was noted.
Cynomolgus monkeys	Single dose	1, 10 and 100 mg/kg	No mortality due to the treatment
Cynomolgus monkeys	Six-month	1, 10 and 100 mg/kg, IV infusion	No mortality due to the treatment, liver granuloma and skeletal muscle degeneration at 10 and 100 mg/kg, NOEL 1 mg/kg, inflammation at injection site of placebo and drug treated animals

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Pharmacology and toxicology review of the data suggest that the monoclonal antibody to human IL-6R, tocilizumab, is a potent inhibitor of IL-6 by binding to human IL-6 receptor. The BLA is submitted for the marketing of tocilizumab at 8 mg/kg, IV (296 mg/m^2) dose per 4 week. Toxicity study at 1 mg/kg/IV infusion/week for 6 months did not show systemic organ system toxicity in cynomolgus monkeys. Injection site inflammation was noted in control and treated animals. The injection site reaction could be due to the procedure and antigenicity of MRA in monkeys. The clinical observations related to the injection site reactions to the treatment need to be addressed in the package insert.

The repeat dose toxicity study also showed granuloma in the liver and skeletal muscle degeneration (non-reversible) at 10 mg/kg (120 mg/m^2) and 100 mg/kg (1200 mg/m^2). Therefore, liver and skeletal muscles were target organs of toxicity in the cynomolgus monkeys. Although creatinine phosphokinase activity was not changed, **monitoring of liver enzymes and creatine phosphokinase for the chronic treatment is recommended on the basis of non-clinical data.**

Reproductive toxicity in cynomolgus monkeys at 2 mg/kg/IV during gestation days 20-50 did not show teratogenicity to fetuses. However, at 10 mg/kg (120 mg/m^2, 0.04 x MRHD)) showed reduced viability of fetuses. Pregnancy category C would be designated for the Pregnancy section of the label.

Adequate information on the fertility and pre-natal and post-natal effect in pregnancy was not provided in the BLA. The review concluded that fertility, pre and postnatal reproductive toxicity data in relevant species using a species specific IL-6R antibody would be needed to completely characterize reproductive safety.

The role of tocilizumab in the development of tumors is unknown. The information provided in the literature is not sufficient to rule out that the long term treatment with tocilizumab would not have any effect on tumor formation of those cells deprived of IL-6 signaling. Direct evidence of the result of a species specific IL-6R antibody in a suitable species would be necessary for the assessment of carcinogenic risk to the treatment. The advancement of the methods and techniques to date allows Applicant to develop these non-clinical assays appropriately. In the absence of any data, the reviewer concluded that non-clinical information is insufficient to predict the long term cancer risks to the treatment. **On the basis of the non-clinical deficiency and from non-clinical perspectives, the BLA is not recommended for approval.**

The animal to human dose ratio and toxicity are shown in the table below.

	Human dose	Monkey dose	Observation in monkey
mg/kg	8	10	Liver granuloma, skeletal muscle degeneration, fetal deaths
mg/m^2	296	120	
Monkey: Human		0.4 as mg/m^2; 1.25 as mg/kg	

Unresolved toxicology issues (if any):

During the BLA review process, the Applicant indicated that an unapproved — that has carcinogenic risk was used in the process development of Tocilizumab. The Applicant has eliminated the use of this — in the manufacturing process for the to-be-marketed product. b(4)

Reviewer: Asoke Mukherjee, BLA No. 125276

Recommendations:

The BLA is not approvable due to lack of any non-clinical data on the carcinogenic risks to IL-6R antibody. It is recommended that the Applicant develop species specific monoclonal antibody to IL-6R and conduct fertility, segment 3 reproductive toxicity and carcinogenicity studies.

Suggested labeling:

1. Pregnancy Category C for reproductive safety

2. Monitoring liver enzyme, skeletal muscle weakness and creatinine phosphokinase activity in clinical population.

3. If the clinical reviewer considers setting a cancer registry in lieu of non-clinical data for generating clinical data for cancer risk assessment, that needs to be addressed in the package insert.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS: NIL