Memorandum:

To File:	BLA 98-0012
From:	Lauren E. Black, Ph.D., Reviewing Pharmacologist 7/14/98
Through:	M. David Green, Ph.D., Branch Chief, Clinical Pharmacology and Toxicology Branch
and	Martin D. Green 7/15/98
Through:	Karen Weiss, M.D., Director, Division of Clinical Trials Design and Analysis
Subject:	Pharmacology Review of the infliximab BLA KW 7-31-97
Product:	**Remicade®,**
	Infliximab (cA2), chimeric (human/murine) IgG1 for use in Crohn's Patients
Sponsor:	Centocor, Inc.
Date:	5/21/98

OVERVIEW:

Chimeric A2 showed no unexpected reactivity (or cross-reactivity) in *in vitro* human tissue cross-reactivity assessment, nor mutagenicity, local intolerance, reproductive, or other systemic toxicities that would preclude its use in Crohn's Disease patients.

Since the chimpanzee is the only species other than humans whose TNFα binds to cA2, safety studies in this species are considered the only studies that can provide relevant safety information on cA2 administration to humans; due to animal use restrictions on this endangered species, these animals may not be necropsied to provide histopathology data, and therefore study outcomes are limited to clinically observable signs, as well well as results from noninvasive testing such as clinical chemistry and hematology assessments. Following some problems attibutable to high doses of ketamine anesthetic required for animal handling, the studies with cA2 in chimpanzees showed that cA2 was well tolerated at doses up to 30 mg/kg/day for at least 3 consecutive days and at doses up to 15 mg/kg/day for at least 5 days. No cA2-related signs of toxicity, including abnormal hepatic or hematologic effects, were observed during these chimpanzee studies.

The nonclinical studies that have been performed have provided support for activity and safety for cA2-treated Crohn's disease patients. The following properties can be attributed to cA2:

- cA2 binds to TNFα homotrimer with high affinity (Ka = 10^{10} M^{-1}); it specifically neutralizes TNFα and does not neutralize lymphotoxin.
- cA2 also binds to both the monomeric subunits of TNFα and transmembrane TNFα; after cA2 binds to cells expressing transmembrane TNFα they can be lysed by the addition of complement or effector cells.
- The stable complex formed between TNFα and cA2 is responsible for blocking TNFα activity.
- cA2 has a long serum half-life (6 days in chimpanzees) and predictable pharmacokinetics.
- Animal safety studies revealed no gross or biochemical evidence of toxicity in normal, adult chimpanzees.
- Doses of an analogous anti-mouse TNFα monoclonal antibody which were high enough to be active in a mouse model of disease, when given to pregnant mice during organogenesis, caused no embryofetal toxicities. These studies were necessitated due to the absence of crossreactivity of cA2 in species other than chimpanzees.

REGULATORY CONCLUSIONS:

These studies are adequate to support the current labeling for short term (2-3 months of active levels) in Crohn's Disease patients

PRODUCT LABELING:

Only minor changes to the sponsor's proposed toxicology labeling are recommended based on review of the file.

CONCLUSIONS:

Based on review of the pharmacology and toxicology data, the safety is adequately supported, and no objection is offered to approving this licensing application.

The review is provided as an attachment to this cover sheet.

REVIEWER: Ph.D.

Lauren E. Black, Ph.D., Reviewing Pharmacologist, DCTDA, CBER

CONCURRENCE: Martin D. Green 8/15/98

Martin David Green, Ph.D., Branch Chief, Clinical Pharmacology and Toxicology Branch, DCTDA, CBER

cc:
M.D. Green, Ph.D., HFM-579
L.E. Black, Ph.D., HFM-579
K. Brorson, Ph.D.
C. Joneckis
Attachment

BIOLOGY OF TUMOR NECROSIS FACTOR ALPHA (TNFα): BACKGROUND

TNFα activity was first detected over 100 years ago when physicians noted that some cancer patients experienced shrinkage of their tumors if they also had a serious bacterial infection. In recent years, however, it has been learned that TNFα has a wide range of biological activities in addition to its ability to kill tumor cells. For example, TNFα has been shown to induce mitogenesis of fibroblasts, induce expression of adhesion molecules on endothelial cells, stimulate interleukin-1 and prostaglandin E2 synthesis in macrophages, trigger a respiratory burst and degranulation in neutrophils, and suppress collagen synthesis by fibroblasts. These widely varied activities, some of which play a role in immune reactions, some in inflammatory reactions, and some in pathophysiological reactions, attest to the complexity of this cytokine.

Various stimuli can induce or suppress TNFα production. Endotoxin, both gram-negative and gram-positive bacteria, tumor cells, several viruses such as HIV, ionizing radiation, cytokines such as interleukin (IL)-1 and interferon-γ, PMA, and various stress-related responses can all induce TNFα production in at least some cell types. The TNFα gene is one of the first genes expressed in T or B cells that have been stimulated through their antigen. Interestingly, TNFα itself has been shown to induce its own synthesis. Agents that can suppress TNFα production include steroids, metalloprotease inhibitors, immunosuppressive agents such as cyclosporin A, pentoxifylline, and certain anti-inflammatory cytokines such as IL-10 and interferon[1].

The pleiotropic nature of TNFα is apparently the result of many variables. These include the levels of expression of two receptors, the amount of soluble TNFα receptors, the amount of soluble versus transmembrane TNFα, the relative influences of the numerous intracellular signaling pathways, the induced expression or suppression of many different TNFα-response genes, and other factors that may affect biological activity of the various induced gene products. Some of the functions of TNFα are associated with normal immune system activities; however, problems arise when TNFα is overexpressed and leads to acute and/or chronic inflammation. Therefore, TNFα is an attractive target for therapeutic intervention for chronic inflammatory indications such as Crohn's disease

---

[1]Human lymphotoxin alpha (LTα; formerly called TNFβ) is a cytokine that is both structurally related to TNFα and binds the same two receptors with similar affinity, and LTα shares numerous biological activities with TNFα. Both cytokines can efficiently kill tumor cell lines, induce adhesion molecule expression on endothelial cells, activate neutrophils, and induce proliferation of fibroblasts. However, there are numerous functional differences between these two cytokines. For example, TNFα, but not LTα, induces proliferation of myelogenous leukemia cells, whereas LTα, but not TNFα, induces proliferation of EBV-infected B cell lines. TNFα, but not LTα, induces HLA antigen expression on vascular smooth muscle cells. Mice deficient in LTα, lack lymph nodes and Peyer's patches while mice deficient in TNFα have not been reported to lack these peripheral lymphoid organs. Both cytokines, which often synergize with each other, induce similar lists of transcription factors, proto-oncogenes, and other inflammatory mediators. (Both the murine antibodies A2 and cA2, derived from A2, were able to neutralize the cytotoxicity of recombinant and natural TNFα, but not LTα. The murine and chimeric forms of A2 recognize the same binding site on TNFα, as demonstrated by their ability to cross-compete for binding to TNFα by immunoassay.)

SUMMARY OF PHARMACOLOGY, ANIMAL PHARMACOKINETICS, AND TOXICOLOGY:

See the review from Dr. Brorson for details covering the construction and molecular pharmacology of cA2. In brief, the monoclonal antibody cA2 is a chimeric, mouse/human hybrid protein with light and heavy chain variable domains from a murine monoclonal antibody with specificity for TNFα and light and heavy chain constant domains from a human $IgG_{1,\kappa}$ isotype antibody. cA2 binds to the human TNFα homotrimer with an association equilibrium constant of approximately 10^{10} M^{-1}; it also binds to the monomer subunit and to the transmembrane form of TNFα with similar affinity.

Species crossreactivity is limited to human and chimpanzee TNFα and this specificity limited the diversity of toxicological and animal efficacy models in which cA2 could be evaluated. cA2 is capable of inhibiting the biological activity of TNFα in a wide variety of *in vitro* cell assays utilizing human fibroblasts, endothelial cells, epithelial cells, neutrophils, and peripheral blood mononuclear cells. The binding of TNFα by cA2 blocks the binding of TNFα to its receptor, thereby neutralizing the biological effects of TNFα. When cA2 binds to cells expressing transmembrane TNFα, the Fc domain is capable of activating complement as well as cytotoxic effector cells, resulting in lysis of the TNFα-expressing cells.

Animal models in which cA2 was evaluated included a murine lethality model in which mice are challenged with human TNFα, as well as transgenic models in which human TNFα is expressed constitutively and results in disease manifested as cachexia and polyarthritis. The administration of cA2 significantly reduced the levels of serum cytokines and clearly reduced histological evidence of disease compared to control mice in a dose-dependent manner. Since no colitis models dependent upon human TNFα have been described, a surrogate anti-mouse TNFα antibody ("cV1q") similar to cA2 (in potency and selectivity relative to LTα) was tested in a transgenic murine colitis model. Administration of the anti-mouse TNFα antibody was capable of reducing the level of disease severity. These results confirm the role of TNFα in the maintenance of an inflammatory response in the mucosa and suggest that antibody neutralization of TNFα might be an effective therapeutic intervention.

The pharmacokinetics of cA2 was determined in chimpanzees, cynomolgus monkeys and in mice yielding terminal half-life values of 6.0, 8.3 and 11.4 days, respectively. In normal mice, the vascular clearance of ^{125}I-human TNFα was prolonged by the presence of cA2 (estimated half-life of 30 min in the absence of cA2 versus 3-4 hours in the presence of cA2). The extended half-life of TNFα in the presence of cA2 was due to the formation of immune complexes; however, no bioactive TNFα could be detected in a cell cytotoxicity assay. The biodistribution, metabolism and clearance of ^{35}S-cA2 in transgenic mice expressing human TNFα was similar to a nonspecific, isotype-matched ^{35}S-labeled control antibody. After two weeks, less than 5% of the radiolabel remained in the vascular compartment and the liver was the primary target organ. Cumulatively, about 20% of the radiolabel was excreted over the two week study, approximately half in urine and half in the feces. An immune response to cA2 could be detected in mice and chimpanzees, although not all animals responded and the titer was quite low in those who did respond.

Safety studies with cA2 in chimpanzees (a relevant species, as cA2 can inhibit chimpanzee TNFα) showed that cA2 was well tolerated at doses up to 30 mg/kg/day for at least 3 consecutive days and at doses up to 15 mg/kg/day for at least 5 days. No cA2-related signs of toxicity were observed during these chimpanzee studies. No chronic toxicity studies currently have been conducted with cA2 in chimpanzees,

nor with cV1q in mice. This is not problematic from a regulatory toxicology perspective since the current label for cA2 does not call for repeated doses that outlast the tolerated chimpanzee exposures.

STUDY REVIEWS:

MOLECULAR PHARMACOLOGY:

Species Cross-reactivity:

The ability of cA2 to neutralize TNFα from different animal species was evaluated in a series of studies.

. However, cA2 could effectively neutralize only human and chimpanzee TNFα. A small degree of neutralization (approximately 10,000-fold lower potency than for human or chimpanzee TNFα) was observed with dog TNFα. No neutralization was observed using TNFα derived from baboon, rhesus or cynomolgus monkey, pig-tail macaque, marmoset, cotton-top tamarin, pig, rabbit, rat, or mouse at the highest cA2 concentration tested

Several follow up studies were done because the published sequence for baboon TNFα differed from human TNFα by only one amino acid (Chimpanzee TNFα cDNA was cloned for sequencing and the protein sequence was found to be identical to that of human TNFα. Hence it is no surprise that cA2 can neutralize both human and chimpanzee TNFα with equal potency. To confirm earlier experiments with lipopolysaccharide-stimulated baboon whole blood, the cDNA for human TNFα was mutagenized to baboon TNFα and expressed in a transfected cell line. Binding experiments showed that p55-sf2 could bind to the baboon TNFα, but cA2 did not. Similarly, assay, it was shown that p55-sf2 efficiently neutralized the baboon TNFα while cA2 did not neutralize even at concentrations as high as 500 µg/mL.

Effect on Cells Expressing Transmembrane TNFα:

cA2 has been shown to bind transmembrane TNFα with high affinity. To investigate the effects this binding may have on the cells expressing transmembrane TNFα, *in vitro* studies have been performed to evaluate complement and effector cell-mediated lysis of a cell line engineered to express a high number of a cell-surface, modified form of noncleavable transmembrane TNFα.

To evaluate effector cell-mediated lysis

Control cells showed only a background level of lysis in the presence of cA2.

While the role of transmembrane TNFα in chronic inflammation is not fully understood, this result demonstrates that cA2 does not simply neutralize soluble TNFα, but can also promote lysis of the key activated cells that are producing TNFα and other pro-inflammatory cytokines.

Human Tissue Cross-reactivity:

An *in vitro* cross-reactivity study was performed on normal, adult human tissue specimens to determine the potential for cA2 to cross-react with non-target tissues.

Reactivity was observed in Kupffer's cells of the liver, macrophages in the lymph nodes and mononuclear cells in the skin and kidney. Reactivity in these organs was anticipated and considered physiologic because of the presence of TNFα in these cells. Equivocal reactivity was seen in pancreatic acinar cells of 1 of 3 pancreatic specimens only at the highest concentration of the test article. The reason for pancreatic staining is unknown. However, since the observation was equivocal and only seen at the high

concentration, it is considered an insignificant observation.

A second *in vitro* cross-reactivity study on normal human tissues was performed using an improved, more sensitive, assay system to detect cA2.

The tissues evaluated in this study included all the recommended tissues on the list of normal human tissues to be used for immunohistochemical investigations of cross-reactivity in the FDA/CBER "Points to Consider in the Manufacture and Testing of Monoclonal Products for Human Use", (1997) and EC CPMP Guideline "Production and Quality Control of Monoclonal Antibodies". Reactivity was observed with mononuclear and stromal cells in many tissues. This reactivity was consistent with the known cell and tissue expression patterns for TNFα. Reactivity was also observed with smooth musculature associated with vascular walls or selected muscle bundles. Although smooth musculature has been shown to be a source of TNFα production, in this study increasing cA2 concentrations (2 to 500 µg/mL) resulted in increasing binding to the smooth musculature in a concentration-related manner rather than a plateau-like response as seen with antigen-antibody reactions. Therefore, in this study the cA2 reactivity to smooth musculature was considered non-specific. Thus, using a sensitive immunohistochemical assay revealed cA2 reactivity in known cells and tissues that expressed TNFα. No unanticipated cross-reactivity with other cells and tissues was observed.

ANIMAL ACTIVITY MODELS:

Overview: Neutralization of TNFα has been evaluated in a number of different animal models of disease based upon the supposition that TNFα was involved in inflammatory and/or immune responses in a variety of disease settings including sepsis, cachexia associated with malignancy and AIDS, inflammatory bowel disease and rheumatoid arthritis. Notably, the species specificity of cA2 limits the types of animal models that can be utilized to demonstrate neutralization of TNFα *in vivo*. Therefore, cA2 was evaluated in mice challenged with human TNFα and in several transgenic models in which constitutive expression of human TNFα causes cachexia (Tg211) or polyarthritis (Tg197 and Tg5453). In brief, reduced cytokine levels (IL-6 and TNFα) were demonstrated by ELISA in cA2 treated groups; levels of bioactive TNFα were undetectable after cA2 treatment; and histological evidence of joint disease was eliminated in the Tg197 mice. These studies show that cA2 can block, in a dose-dependent manner, the effects of both soluble and transmembrane TNFα *in vivo*. Efficacy studies were extended to a transgenic model of colitis using cV1q, a surrogate antibody that neutralizes mouse TNFα. Disease progression was significantly reduced by treatment with cV1q as measured by weight gain, cell proliferation and histopathology. These results provide "proof of principle" evidence that neutralization of TNFα can effectively reduce the level of inflammation associated with colitis-like disease in this model. Detailed descriptions of the most clinically relevant of these studies follow.

Tg211 Transgenic Mouse Model:

A murine model in which the ability of cA2 to neutralize TNFα could be tested came from the development

of transgenic mice that constitutively express human TNFα. The Tg211 mice carry 5 copies of this modified human TNFα gene per cell and human TNFα protein can be readily detected in the thymus and in the serum (0.01-7 units/mL). Phenotypically, Tg211 mice develop a lethal wasting syndrome resulting in 80-100% mortality at 10-18 weeks after birth. Beginning 3 weeks after birth, mice (n=15) were injected intraperitoneally twice weekly for a total of 8 weeks with 0.5, 2 or 8 mg/kg of cA2, or 8 mg/kg of an isotype-matched IgG1 control antibody. The number of surviving animals and their weight were recorded weekly. The effect of cA2 on the survival of the Tg211 transgenic mice was evaluated. Both the untreated and the control antibody-treated groups had significant mortality beginning at weeks 4-5 and which increased through the end of the study. Of the three cA2 dose groups, only one death was recorded, in the lowest dose group (0.5 mg/kg) at week 6. The results were highly significant for all three cA2 dose groups versus either control group. The weight of each animal was also monitored on a weekly basis to determine the effect of cA2 on the cachectic symptoms that appear in these mice. There was a clear trend in all three cA2 dose groups toward greater weight gain when compared with the two control groups. As with the survival data, there was trend toward a cA2 dose-dependent response in that weight gain in the 0.5 mg/kg dose group was not as high as weight gain in the 2 and 8 mg/kg dose groups. These results from cA2 treatment of Tg211 transgenic mice lead to the following conclusions.

- reduction in mortality was significant in all cA2 dose groups
- increased weight gain was evident in all cA2 groups
- the 0.5 mg/kg cA2 dose was less efficacious than the 2 and 8 mg/kg, suggesting that the effect is dose-dependent

Other Transgenic Mouse Models: The Tg197 and Tg5453 transgenic mice both constitutively express human TNFα and as a result develop a polyarthritis with 100% phenotypic penetrance. The transgenic mouse line designated Tg197, contains about 5 copies per cell of a modified human TNFα gene in which the 3' end is replaced with β-globin sequences as described above, but the wild type promoter is left intact . Expression of the human TNFα gene is deregulated in that mRNA is evident in the brain, thymus, spleen, kidney, lung and joints of the Tg197 mice and human TNFα protein is detectable in the serum. These mice develop a chronic inflammatory polyarthritis beginning at 3-4 weeks of age with subluxation of the joints visible by 10-12 weeks. The transgenic mouse line, Tg5453, share the pattern of tissue expression of human TNFα mRNA observed with the Tg197 mice. A mutated form of soluble human TNFα can be detected in the serum by ELISA but it shows no cytotoxic activity by bioassay. The Tg5453 also share the same phenotypic features seen in the Tg197 mice, although somewhat delayed (polyarthritis beginning at 5-6 weeks after birth). The Tg197 mice express wild type TNFα, while the Tg5453 mice produce a deletion mutant of human TNFα lacking amino acids +1 through +12 which prevents the proteolytic processing and release of the transmembrane form of TNFα. The fact that Tg5453 mice develop polyarthritis is important because it suggests that local effects of TNFα are important in the disease process. cA2 can efficiently bind both the soluble and transmembrane forms of human TNFα and these transgenic mice can be used to evaluate whether cA2 can neutralize both soluble and transmembrane TNFα *in vivo*. Results from the Tg197 studies are described below.

cA2 Study Methods: Beginning two weeks after birth, groups of Tg197 mice received either cA2 or an isotype-matched chimeric IgG1 control antibody as twice weekly intraperitoneal injections of 5 mg/kg. An additional control group consisted of disease-free nontransgenic litter mates of the transgenic mice, who also received the same dose regimen of cA2. Treatment continued for 8 weeks, during which time weight and arthritic score were monitored weekly and any deaths were recorded. Caliper measurements of the

right hind paw were also obtained weekly. At the end of the study, all mice were sacrificed and samples obtained for analysis. Serum samples were stored frozen and later assayed for human TNFα and murine IL-6 by ELISA, as well as for bioactive TNFα using a cell cytotoxicity assay. The right hind limb was taken from representative animals in each group, fixed in formalin, and later sectioned and stained with hematoxylin/eosin. The cA2 antibody is 70% human sequence, and would be expected to be immunogenic, recognized as a foreign protein in mice. Accordingly, immune responses were also evaluated for the purpose of evaluating whether anti-cA2 antibodies were induced, and to determine their effect on efficacy in a surrogate animal model. Immune response analyses could only be evaluated several weeks after the final administration of cA2, since cA2 has a fairly long half-life, and low levels of free cA2 can interfere with the detection of immune responses

Results: The mean weight over time of the Tg197 mice treated with cA2 was equivalent to that of the nontransgenic control group. However, the Tg197 mice that received control antibody showed markedly less weight gain beginning about week 5 and continuing through the end of the study. These differences became significant at week 6 and were highly significant by week 10. Similar results were observed with paw size where differences between the cA2 and control antibody-treated Tg197 became significant at week 4 and remained significant through week 10. The cA2-treated Tg197 mice maintained an arthritic score of 0 (no arthritis observed) throughout the study, while by week 10 the control Tg197 mice were scored as either moderate or severe arthritis; the difference between these groups was significant. These results all demonstrated complete protection of the Tg197 mice treated with cA2 IgG.

In a second study where dosing in Tg197 mice was given as 0, 1 or 10 mg/kg for 5 weekly doses, samples were collected 1 week after the last treatment (6 weeks after initiation of the study), except for 7 animals from a group of animals treated with 10 mg/kg of cA2 IgG1 which showed marked activity determined by arthritic index. The mice given 10 mg/kg cA2 IgG1 group were maintained for an additional 4 weeks to evaluate the duration of efficacy, and were bled for immune response analysis 10 weeks after the initial cA2 treatment. A statistically significant reduction in the severity of arthritis was observed for the animals receiving cA2 IgG1 compared to control animals at 2- 5 weeks following initiation of the study. Approximately 95% of the animals demonstrated an induced immune response in all cA2 treatment groups. These responses appeared to be primarily directed toward the human constant region, as many of the immune responses could be neutralized using a pool of non-specific human IgG. This study indicates that therapeutic efficacy with 10 mg/kg of cA2 can be observed in the presence of an induced immune response in Tg197 mice. The levels of serum cytokines were also significantly reduced in the cA2-treated Tg197 mice compared to control antibody-treated Tg197 mice. These results included the measurement of human TNFα and murine IL-6 by ELISA and the level of TNFα cytotoxicity by bioassay.

The joint tissue sections following hematoxylin/eosin staining were evaluated based on representative hind limbs from each cA2 treatment group and control mice. Non-transgenic, disease-free mice show a normal joint space between the cartilage layers on each bone surface, with no discernable cell infiltration, hyperplasia or erosion. The cA2-treated Tg197 section looked quite similar to the disease-free animal, with no visible erosion or cell infiltration in the joint space. By contrast, the control antibody-treated Tg197 joints were quite distorted with massive cell infiltration in the joint space as well as hyperplasia along the synovial membrane and erosions in the bone.

Single Dose Escalation Study in Tg197 Mice: To define further the degree and duration of efficacy provided by cA2 in the Tg197 model, a single dose-response study was performed. Three week old Tg197 mice were randomized to one of five treatment groups (based on body weight) that received a single intraperitoneal injection of saline or 1, 3, 10 or 30 mg/kg cA2. The weight and arthritic score for each animal were recorded weekly. The saline, 1 and 3 mg/kg dose groups were sacrificed 5 weeks after dosing due to deteriorating health, while the 10 and 30 mg/kg dose groups were sacrificed 7 weeks post-administration of cA2. A dose-dependent response was observed with arthritic score. In the Tg197 model, it appears that even the lowest dose of cA2 could delay the appearance of joint disease, although the duration of protection was brief. Higher doses of cA2 served to prolong the benefit observed. The relationship between blood levels of cA2 and efficacy in Tg197 mice was examined as follows. The arthritic score began to increase steadily between weeks 3 and 4. In this same timeframe, the serum level of cA2 decreased from 3.2 μg/mL to 0.2 μg/mL. Therefore, cA2 can delay the appearance of joint disease; the duration of protection was dose dependent (the highest dose providing the longest duration of activity); and in the 10 mg/kg dose group, disease progression began as serum levels of cA2 dropped below 3 μg/mL.

Additionally, blood was obtained for immune response analysis at 5 weeks for the animals given saline, 1 or 3 mg/kg of cA2, and at 7 weeks for animals treated with 10 or 30 mg/kg of cA2. No free cA2 was detected in any of these serum samples, however a spike recovery analysis using a baboon positive control antibody was performed on each sample with a negative immune response (for which there was sufficient volume) to determine whether a low level of cA2 (undetectable in the PK assay) might be inhibiting the immune response signal for those animals. For those animals for whom immune response results could be interpreted without free cA2 interference, there was evidence of a decreased immune response at the highest dose level. Approximately 57-62% positive MACA response was observed at the 1, 3 and 10 mg/kg dose groups, and only 25% immune response incidence was detected in the animals receiving 30 mg/kg of cA2, although only 4 animals indicated little or no free cA2 interference. These MACA results appears to indicate, especially in the 10 mg/kg dose group, that clinical efficacy can be observed even when immune responses are present.

Surrogate Anti-TNFα Studies: Tgε 26 Colitis Model

cV1q: Since no colitis animal model has been described in which disease pathogenesis is dependent on the expression or administration of human TNFα, and since cA2 is not crossreactive in any animal with the exception of chimpanzees, the ability of human drug, cA2, to neutralize TNFα and its effect on disease progression in a model of inflammatory bowel disease could not be tested. Instead, the genes for a monoclonal rat anti-mouse TNFα antibody called V1q were cloned and used to construct a rat/murine chimeric monoclonal antibody of IgG2a, κ isotype (cV1q). This antibody can bind and neutralize mouse TNFα, and shares the functional characteristics of the cA2 mouse/human IgG1, κ Fc domain. cV1q was then used in a transgenic mouse model of colitis (described below) as a surrogate for cA2, and demonstrated that neutralization of TNFα altered the severity of disease in this model.

Model choice: Several strains of transgenic mice have been described which develop colitis and the Tgε26 model was selected for evaluation of TNFα neutralization with cV1q. Tgε26 mice, which express the human CD3 ε protein, display an arrest in early intrathymic development that affects the architecture of thymic stromal cells thereby blocking T lymphocyte and NK cell development. Transplantation of wild type bone marrow cells restores the thymic environment of fetal Tgε26 mice, while reconstitution of adult

Tgε26 mice results in compromised T cell development that leads to symptoms and histology similar to inflammatory bowel disease.

Methods: In this model, 8-12 week old Tgε26 mice were treated with 5-fluorouracil (150 mg/kg) 48 hr prior to receiving 5-10x10^6 T cell-depleted bone marrow cells. The bone marrow cells from normal, adult mice were treated with 2 rounds of anti-Thy-1.2 followed by rabbit complement lysis in order to delete mature T cells. The reconstituted Tgε26 mice received PBS (untreated), a nonspecific mouse IgG (0.1 mg) or the anti-TNFα rat/mouse chimeric IgG cV1q (40 mg/kg) via weekly intraperitoneal injections for 4-5 weeks. Disease activity was monitored by weight gain over the 4-5 week treatment period, disease activity index (a combined score encompassing weight loss, stool consistency, blood in the stool and enlargement of the colon), histological examination of colon tissue sections and by incorporation of bromodeoxyuridine to evaluate colonic epithelial cell proliferation.

Results: cV1q treatment positively affected whole body weight from time of bone marrow cell engraftment until sacrifice 4-5 weeks later. Untreated and control Ig-treated mice showed weight loss over this time period, while the majority of mice that received anti-TNFα gained weight. There was also a trend to reduced bromo-deoxyuridine uptake in colon tissue sections from anti-TNFα-treated mice compared to the control Ig and untreated animals. The disease activity index also showed that the anti-TNFα-treated mice had significantly reduced severity of colitis compared to the untreated and the control Ig-treated mice. These results were further supported by visual inspection of the stained colon tissue sections from these mice. The reconstituted Tgε26 mice that received PBS showed increased colon wall thickness, distortion of crypt architecture and cell infiltration/hyperplasia in comparison with a non-reconstituted Tgε26 mouse. These results show that treatment with an antibody capable of neutralizing mouse TNFα can prevent the development of colitis in Tgε26 mice reconstituted with bone marrow cells.

In summary, data from several murine models of inflammation based disease provide a strong rationale for the use of anti-TNFα antibodies to treat inflammatory bowel disease. In a single dose evaluation, disease progression was halted following a dose of 10 mg/kg, and reprogression started around 4-5 weeks following dosing when blood levels dropped below 3 ug/ml, a possible suggestion towards a need for clinical retreatment in Crohn's Disease patients, however, animal models are likely to not emulate all important aspects of human disease etiology.

ANIMAL PHARMACOKINETICS, ADSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION:

Non-compartmental pharmacokinetics were used to characterize the serum concentration versus time curves for free cA2. For the pharmacokinetics studies conducted in chimpanzees, monkeys and mice, the linear trapezoidal rule was employed for the area under the curve (AUC) and the area under the moment curve (AUMC) determinations. The AUC and AUMC were extrapolated to infinity using the quotient of the terminal elimination rate constant and the last measured serum concentration of cA2. The clearance (CL) was calculated by dose/AUC. C_{max} represents the highest median concentration observed following dosing. The terminal elimination rate constant (k_e) was determined using log-linear regression of at least three terminal serum concentrations. The terminal half-life ($T_{1/2}$ Term.) was obtained from 0.693/k_e. The mean residence time (MRT), defined as the AUMC/AUC, was used to calculate the volume of distribution (Vd_{ss} = MRT x CL). Plasma concentration versus time profiles are presented as the mean ± SEM.

cA2 PK in Monkeys for Providing Data on Formulation Equivalence:
Three preclinical studies were conducted in monkeys administered cA2 for which pharmacokinetic samples were collected. T-095-007 was a study which compared a liquid and a lyophilized formulation of cA2. In this study, cynomolgus monkeys (n=4) received 10 mg/kg of either the liquid or lyophilized formulation of cA2. No significant difference in pharmacokinetic parameters was observed between the two formulations using the Mann-Whitney test, providing evidence that they are biologically equivalent. The second cA2 formulation study conducted in monkeys, P-097-012, yielded similar pharmacokinetic results to the two previously described studies. In this study, 3 formulations, one liquid (Lot 94L02AA) and two lyophilized (Lots 95K06ZC and 97A07ZA) lots of cA2 were administered intravenously at 10 mg/kg to three groups (n=10 each) of cynomolgus monkeys. There were no significant differences observed in the pharmacokinetic parameters of C_{max}, AUC, CL, Vd_{ss} and $T_{½}$ between the three formulations of cA2 (P-values from 0.157 to 0.715), and the three formulations had very similar concentration versus time profiles. From these results it was concluded that there were no significant differences between the liquid and the two lyophilized formulations of cA2.

Immune response analyses were performed for one monkey study conducted for the primary purpose of determining the pharmacokinetic profile of cA2 following administration of a single intravenous dose (10 mg/kg) of cA2 in monkeys. In addition, repeated administration of cA2 was performed in order to evaluate monkey anti-cA2 immune response induction following multiple cA2 treatments. No immune responses were detected in any animals in this latter study, although very low levels of free cA2 (<0.5 µg/mL) could still be detected in two of the animals 14 weeks after the final cA2 treatment. This study indicated that cA2 did not appear to be immunogenic in a repeated treatment regimen in monkeys.

Animal Pharmacokinetic Comparisons: The pharmacokinetic clearance of cA2 in the three animal species (chimpanzees, monkeys and mice) was predictable and consistent. In chimpanzees, a two-fold higher free cA2 level was observed immediately following the initial treatment with 30 mg/kg than at the same time point following 15 mg/kg of cA2 (mean of 941 vs 443 µg/mL at 5 minutes post-treatment, respectively). After three consecutive daily injections of 30 mg/kg, the difference in drug level immediately before and after the third injection was similar to the C_{max} observed after a single injection. The clearance of cA2 was similar when monitored after 1, 3 or 5 bolus infusions, which suggests that the mechanisms for cA2 clearance were not saturated even at these high dose levels. The clearance rate was slightly faster in the chimpanzees than in monkeys, which may be related to the fact that cA2 can bind chimpanzee TNFα but not cynomolgus monkey TNFα. The pharmacokinetic clearance of cA2 in all of these animal species is slower than the 1-2 day elimination half-life observed for intact murine immunoglobulins in humans, but faster than the 23 day half-life reported for native human IgG1. The half-life of cA2 observed in monkeys (8-10 days) falls within the 3 to 21 day range reported for other human or humanized monoclonal antibodies in monkeys.

Absorption: Absorption is defined as the rate at which a drug leaves its site of administration and the extent to which this occurs. Since cA2 is administered intravenously, it is immediately available for neutralization of circulating TNFα. This was demonstrated in a study conducted in mice administered cA2 or control IgG followed by ^{125}I-labeled recombinant human TNFα. In this study, C3H/HeN female mice were injected intravenously with 1.2 mg/kg of either cA2 or control IgG 30-60 minutes prior to injection with ^{125}I-TNFα (10 µCi, 1.6 µg). A serum sample was obtained 10 minutes following injection of TNFα and was analyzed by gel filtration HPLC. The results indicated that the TNFα was completely bound by cA2 to form

a high molecular weight complex (retention time similar to the thyroglobulin marker with MW= 670,000), whereas the control antibody did not change the elution pattern of the TNFα.

Distribution: The study with ^{125}I-labeled TNFα in C3H/HeN mice also provided information about the pharmacokinetics of cA2-TNFα complexes. As observed in the control samples obtained over time, the free TNFα eluted in the same position as the ovalbumin standard (molecular weight of 44,000). The free TNFα peak rapidly diminished in the 40 and 300 minute samples, yielding an estimated half-life of about 30 minutes. In contrast, the cA2-TNFα complex exhibited a longer half-life of approximately 3-4 hours. Although this half-life is slow relative to free TNFα, it is faster than the half-life of cA2 observed in primates and normal nontransgenic mice (T½ = 8-12 days). The cA2-TNFα also appeared to be stable, since dissociation of the complex to yield free TNFα was not observed at the later time points in these animals.

Information concerning the biodistribution of cA2-TNFα complex was obtained in a similar study. C3H/HeN female mice were injected intravenously with 1.2 mg/kg of either cA2 or control antibody, followed 30-60 minutes later by intravenous injection of ^{125}I-recombinant human TNFα. At the indicated times post-TNFα injection, 5 mice from each group were sacrificed and the major organs were rinsed, weighed and counted. As previously observed, the blood clearance of TNFα was slower in animals treated with cA2 than in animals given a control antibody because the TNFα was complexed with cA2. This conclusion is supported by the reduced TNFα counts in the kidney in the presence of cA2 because a higher molecular weight complex would be less likely to be removed by glomerular filtration in the kidney relative to free TNFα. The primary target organs for clearance of the cA2-TNFα complex appeared to be the liver and the spleen. The distribution of ^{125}I-TNFα in the control animals was primarily in the kidney, liver, lungs and spleen. [This is in agreement with a study conducted in normal mice implanted with Meth A sarcoma cells and control animals in which it is reported that the majority of human TNFα localized in the kidney, lungs and liver.] The radioactivity evident at 40-180 minutes in the gastrointestinal tract may have been due to the metabolism and clearance of ^{125}I, with higher levels observed in the small and large intestines for the animals treated with control IgG than for those receiving cA2.

A pattern was observed in the comparison of the biodistribution of free cA2 to the biodistribution of cA2-TNFα complex. The key differences observed between free cA2 and cA2-TNFα complexes were: a) reduced serum levels of complex; and b) increased levels of complex in liver and spleen. These observations are consistent with faster clearance of immune complex to organs involved in normal protein metabolism. The difference between the amount of radioactivity observed in the liver and spleen for the ^{125}I-TNFα study relative to the ^{125}I-cA2 study is likely due to the relative amounts of those proteins administered (1.6 µg and 1.2 mg/kg, respectively) and the amount of radioactivity incorporated into the cA2-TNF immune complexes. For the mice administered ^{125}I-TNFα, all of the detectable TNFα was incorporated into an immune complex.

Metabolism and Elimination: The biodistribution and elimination of cA2 was evaluated in a study conducted in transgenic Tg197 mice and nontransgenic litter mates that were injected with intrinsically labeled ^{35}S-cA2. In addition, a second group of transgenic Tg197 mice received an isotype matched ^{35}S-labeled control antibody. These antibodies were purified by Protein A in the same manner as clinical grade lots of cA2, and the radiochemical purity was estimated to be approximately 95% by SDS PAGE. Based on the concentration of the formulated antibody and average body weight, each mouse received

approximately 10 mg/kg of antibody and 0.8 μCi of ^{35}S. The groups of Tg197 mice that were sacrificed two weeks after cA2 treatment were housed in a metabolic cage to allow daily collection of urine and feces. Blood samples were counted and also evaluated by EIA for free cA2 concentration. At various time points, mice from each group were sacrificed and the major organs weighed. Approximately 0.3 gram of each tissue was solubilized and counted for ^{35}S.

The time course of cA2 concentrations in the vasculature was evaluated. Tg197 mice treated with cA2 demonstrated a more rapid clearance of cA2 from the blood than the nontransgenic animals treated with cA2, particularly at the 7 and 14 day time points. This result is supported by other pharmacokinetic data. The clearance of radiolabeled control antibody in Tg197 mice matched the clearance of cA2 in nontransgenic mice, thus suggesting that the faster clearance of cA2 in Tg197 mice was due to the presence of human TNFα in these animals and confirming that this effect is likely due to cA2-TNFα complex formation.The radioactivity detected in the urine and feces specimens obtained from Tg197 mice that received ^{35}S-cA2 indicated a fairly consistent daily excretion of cA2 in both the urine and feces. The excretion observed is likely the result of antibody metabolism and incorporation of liberated ^{35}S into sulfate and into bile salts (feces component). These conclusions were verified by gel filtration HPLC analysis of representative serum samples. Insufficient radioactivity was present in the urine and feces samples to determine the molecular size of the excreted radiolabeled molecules, and no free cA2 could be detected in urine in the PK assay.

The results of the organ biodistribution analysis showed no marked differences between ^{35}S-cA2 or control antibody in Tg197 mice or ^{35}S-cA2 in nontransgenic animals. There were some higher levels of cA2 in the liver, spleen, stomach, large and small intestine noted at 1 week following treatment in Tg197 mice, however this difference was not observed at any of the other time points. In summary, it appears that there was little or no prolonged retention of cA2 into any organs in Tg197 transgenic or nontransgenic mice. The cA2 antibody does not appear to target any specific organs when administered to normal or human TNFα-producing mice. The cA2-TNFα complex has a slightly different biodistribution profile than that of TNFα alone. The level of cA2-TNF complex was higher in the liver and lower in the kidney than the levels observed for free TNFα. Since cA2 is composed solely of amino acids and carbohydrates like those found in native immunoglobulins, its metabolism and elimination would be expected to follow the traditional immunoglobulin clearance pathways.

Immune Responses in Animals: The immunogenicity of cA2 compared to related chimeric antibodies and molecular constructs was evaluated in normal inbred Balb/c mice through repeated intravenous (I.V.) and intraperitoneal (I.P.) injections. Four groups of 12-13 week female mice (12 mice per group) were injected with cA2, or cMT412, a chimeric IgG1κ monoclonal antibody specific for the CD4 receptor on human T-cells, or F105, a totally human IgG1κ monoclonal antibody to the gp120 coat protein of HIV-1. In addition, the immunogenicity of molecular construct p55-sf2, a human IgG1 Fc in which each of the heavy chain variable region domains were replaced with the extracellular domain of the p55 receptor for TNFα, was compared to cA2. The p55-sf2 construct can bind to both mouse and human TNFα. The mice received these reagents at 10 mg/kg (250 μg) I.V. in physiological saline at days 0, 7, 21 and 50. The mice were rested for 30 days, then reimmunized I.P. with 100 μg of appropriate antibody or construct emulsified with an equal volume of incomplete Freund's adjuvant.

I.V. administration of cA2 in normal Balb/c mice consistently resulted in a low level IgG antibody response

that did not increase with subsequent I.V. injections. The IgG antibody response also did not increase consequent to an I.P. injection of cA2 in incomplete Freund's adjuvant. The totally human F105 also elicited a low level immune response and demonstrated no anamnestic response when challenged with F105 in adjuvant. However, other mice were able to elicit a strong humoral response to cMT412 and to the p55-sf2 construct. This non-responsiveness to cA2 was demonstrated not to be a consequence of immunological paralysis, since a strong IgG response (1:1600 to 6400) after 14 days was obtained when 5 of the cA2 non-responsive mice were injected with tetanus toxoid in alum. This non-responsiveness of Balb/c mice to cA2 appeared indicative of immune tolerance.

The premise of tolerance was tested by assessing the adoptive transfer of the immune tolerance through splenocyte engraftment. The transfer of spleen cells from cA2 tolerized mice into naive, lethally irradiated mice, followed by I.P. challenge with cA2 in complete Freund's adjuvant did not result in a humoral response, thus further demonstrating the immunological tolerance of the original mice. Naive splenocytes transferred to lethally irradiated mice and challenged I.P. with cA2 in complete Freund's adjuvant were able to induce a humoral response against cA2 after 14 days. A comparison of cA2 immunogenicity in other strains of mice was also conducted to determine whether this non-responsiveness was strain-specific. Balb/c (inbred), CD1 (outbred) and DBA/2 (inbred) mice (n=12/strain) were injected I.V. with 10 mg/kg of cA2 on days 0 and 7. Low IgG geometric mean titers to cA2 were demonstrated by EIA in all three mouse strains. A variety of factors can contribute to immunological tolerance, including route of administration, amount of antigen administered, the epitope specificity of the antigen, and similarity to native antigens. The immunologic processing of the cA2 antibody following I.V. administration in mice is surprising since cA2 is 70% human, thereby mostly foreign to mice, and would be expected to be moderately immunogenic even if administered I.V., as demonstrated by the cMT412 chimeric antibody. In these studies, the evidence indicates that the degree of foreignness may not be as important as certain intrinsic properties of cA2 that regulate its immunogenicity.

Immune response analyses conducted in animal models were primarily performed to evaluate the pharmacokinetic or efficacy results observed in these studies. Due to the high doses administered to the chimpanzees, the long half-life of cA2, and the limited number of immune response samples which were obtained following the treatments, it was not possible to assess the immunogenicity of cA2 in these primates. The cA2 antibody did not appear to be immunogenic in cynomolgus monkeys, even following repeated administration. Repeated I.V. treatment with cA2 in normal mice indicated that cA2 when administered I.V. can be tolerogenic, as demonstrated by a lack of immune response induction following splenocyte engraftment and I.P. challenge with cA2 adjuvant. In Tg197 mice, cA2 administered I.P. is fairly immunogenic with ~60% of the animals demonstrating an immune response after a single bolus dose of 1-10 mg/kg, and >90% after multiple treatments. Additionally, cA2 administered I.P. in Tg197 mice was less immunogenic at the highest dose of 30 mg/kg; activity at 10 and 30 mg/kg was seen in the animal disease model despite the presence of MACA (murine anti chimeric antibodies) response in some animals.

TOXICOLOGY AND SAFETY

Toxicology Study List - Centocor Study Reports

T-090-027 A Five Day Intravenous Dose Safety Study of Chimeric Anti-TNF (cA2) in Chimpanzees

1993T-090-029 Evaluation of the Safety Profile of a Single Intravenous Dose of Chimeric anti-TNF (cA2) in Combination with a Single Intravenous Dose of Centoxin® inChimpanzees, 1993

T-091-033 cA2 (Chimeric Anti-TNF): Three-Day Intravenous Dose Study in Chimpanzees (Pan troglodytes), 1993

T-094-002 Acute Dose Tolerance Study of Chimeric A2 in Beagle Dogs, 1994

P-094-003 Acute Pilot Pharmacokinetic Study of Chimeric A2 in Dogs, 1994

T-091-008 Single Dose Intravenous Toxicity Study of Chimeric Anti-TNF (cA2) in Rats, 1993

T-091-031 Single Dose Intravenous Toxicity Study of Chimeric A2 (C168J) in Rats, 1993

T-091-036 Single Dose Intravenous Toxicity Study of Chimeric A2 (C168A) in Rats, 1993

T-090-028 Seven Day Intravenous Toxicity Study of Chimeric Anti-TNF (cA2) in Rats, 1993

T-091-011 Seven Day Intravenous Toxicity Study of Chimeric anti-TNF (cA2) in Rats, 1993

T-091-032 One Week Intravenous Toxicity Study of Chimeric A2 (C168J) in Rats, 1992

T-096-001 Acute Intravenous Irritation Study of Chimeric A2 (cA2) in Rabbits, 1996

T-096-004 Acute Intramuscular Irritation Study of cA2 in Rabbits, 1996

T-096-010 Intravenous Dosage-Range Developmental Toxicity Study of cV1q muG2a (C258A) Anti-Mouse TNF Antibody in Mice, 1997

T-096-011 Intravenous Developmental Toxicity Study of cV1q muG2a (C258A) Anti-Mouse TNF Antibody in Mice, 1997

T-096-007 Mutagenicity Test on Chimeric A2 (cA2) in a Chromosome Aberrations Study in Human Whole Blood Lymphocytes with a Confirmatory Assay with Multiple Harvests, 1996

T-096-008 Mutagenicity Test with Chimeric A2 (cA2) in the *Salmonella-Escherichia Coli*/Mammalian-Microsome Reverse Mutation Assay Preincubation Method with a Confirmatory Assay, 1996

T-096-002 Mutagenicity Test on Chimeric A2 (cA2) in an *In Vivo* Mouse Micronucleus Assay, 1996

Safety Introduction:

Ideally, *in vivo* nonclinical toxicology assessment of a potential therapeutic monoclonal antibody should be performed in species that have the relevant antigen and/or show a similar pharmacological response to the monoclonal antibody as humans. However, *in vitro* neutralization assays, used to evaluate species cross-reactivity of cA2, demonstrated that neutralization of TNFα cytotoxic activity was similar only in humans and chimpanzees. Weak neutralization of dog TNFα was observed with cA2 at a potency about 10,000-fold lower than observed for human and chimpanzee TNFα. No inhibition of TNFα was observed, even at high cA2 concentrations, with rat, mouse, rabbit, cynomolgus, rhesus, pig-tail macaque, baboon, marmoset, cotton-top tamarin or pig. Although the dog showed weak reactivity at high cA2 concentrations, studies were initiated to investigate if this species could be used, in addition to the chimpanzee, to evaluate potential toxicity of cA2. Unfortunately in dogs, cA2 during the first infusion induced an immediate dermal hypersensitivity response (urticaria and angioedema) in association with high plasma histamine levels. Therefore, toxicity studies in dogs were not considered feasible[2].

Therefore, the safety studies in chimpanzees performed by Centocor are considered to be the relevant studies to assess the safety of cA2 administration to humans. Single and multiple dose nonclinical safety assessment studies have been completed in chimpanzees and are summarized below.

---

[2] Although chimeric A2 does not neutralize rat TNFα cytotoxic activity *in vitro,* single and multiple dose toxicity studies were performed in rats only to evaluate potential non-specific effects; no response that were relevant to clinical development were noted and they are not included here for that reason.

Single Intravenous Dose of cA2 in Combination with a Single Intravenous Dose of Centoxin® in Chimpanzees (Centocor Study T-090-029)

Methods: This interaction study was performed primarily to support the potential use of cA2 administered in combination with Centoxin® (a specific human monoclonal antibody to the Lipid A moiety of endotoxin from gram-negative bacteria) for the clinical indication of sepsis. The chimpanzees (*Pan troglodytes)* were housed socially. Two chimpanzees (1 male, 1 female) were administered 5 mg/kg of Centoxin® (Lot No. 89L04ZA) and 30 mg/kg of cA2, intravenously, at concentrations of 5 mg/mL. Centoxin® was administered at a dose volume of 1 mL/kg. Immediately after the completion of the Centoxin® dose, cA2 was administered at a dose volume of 6 mL/kg. Centoxin and cA2 were administered at infusion rates of approximately 4 mL/minute. Both antibodies were filtered through a 0.2 µm low protein binding filter just prior to administration. The chimpanzees were fasted overnight and sedated with ketamine prior to administration of the antibodies. Anesthesia was maintained with halothane, nitrous-oxide and oxygen mixture during the 50 minute infusion of the antibodies and for approximately 30 minutes after completion of the cA2 infusion.

Both chimpanzees were monitored for approximately two weeks following completion of their infusions. Each animal was examined for pharmacotoxic signs continually during the infusion, frequently after dosing and once daily during a two week non-treatment observation period. Blood pressure, heart rate and respiration rate were measured just prior to, during and for approximately 30 minutes after completion of the cA2 infusion and on Day 2 (except respiration rate). Body temperature was measured just prior to, and at approximately 30 minutes after completion of the cA2 infusion and on Day 2. Body weights were measured pretest, just prior to dosing, Day 2 and once weekly during the two week non-treatment observation period. Electrocardiographic tracings were obtained just prior to, during and for approximately 30 minutes after completion of the cA2 infusion, and on Days 2 and 15. Serum samples were collected for analysis of cA2 serum concentrations and chimpanzee anti-cA2 antibody response during the study. Ophthalmoscopic examinations were performed pretest, on Day 2 and at the end of the two-week non-treatment observation period. Hematology and clinical chemistry parameters were monitored pretest, just prior to dosing, on Day 2 and once weekly during the two week non-treatment observation period. Urinalysis was performed pretest and on Days 2 and 15.

Results: cA2 at 30 mg/kg when administered concomitantly with Centoxin® at 5 mg/kg was well tolerated by the two treated chimpanzees. No test article related clinical signs of toxicity or body weight changes were observed during the course of the study. No test article treatment related changes were observed for blood pressure, heart rate, respiratory rate, body temperature, hematology, serum chemistry or urinalysis values. Ophthalmologic and electrocardiographic examinations revealed no changes which were attributable to the administration of cA2 when administered concomitantly with Centoxin®. Analysis of serum samples for immune response detected no chimpanzee anti-cA2 antibodies, however the presence of cA2 could interfere with detection of low levels of anti-cA2 antibodies. Thus, cA2 at a single dose of 30 mg/kg when administered concomitantly with a single dose of Centoxin® at 5 mg/kg was well tolerated. No signs of toxicity were observed during dosing or during the two-week non-treatment observation period for the treated chimpanzees.

Single/Multiple Intravenous Dose Safety Study of cA2 in Chimpanzees (Centocor Study T-090-027)

Methods: In this study chimpanzees (*Pan troglodytes*) were administered single and multiple doses of cA2. The chimpanzees were housed socially. One chimpanzee (male) was administered 30 mg/kg cA2,

prepared from the C168A cell clone, intravenously once in a dosage volume of 6 mL/kg and an additional chimpanzee (male) received 6 mL/kg of 0.9% Sodium Chloride for Injection (saline) once to serve as a control animal. Two chimpanzees (1 male, 1 female) were similarly administered 30 mg/kg of cA2 intravenously, once daily for 3 consecutive days. One (1) additional chimpanzee (female) received 6 mL/kg of saline once daily for 3 consecutive days, to serve as a control animal. Chimeric A2 (5 mg/mL) or saline was administered at an infusion rate of approximately 4 mL/minute. Three (3) chimpanzees (2 males, 1 female) were intravenously administered 15 mg/kg of cA2 in a dosage volume of 3 mL/kg. Two (2) of these animals were dosed for 5 consecutive days and 1 animal was dosed for 4 consecutive days. One (1) additional chimpanzee (female) received 3 mL/kg of saline intravenously for 5 consecutive days, to serve as a control animal. In these animals, chimeric A2 (5 mg/mL) or saline was administered at an infusion rate of approximately 2 mL/minute. The antibody was filtered through a 0.2 μm low protein binding filter just prior to administration. The chimpanzees were fasted overnight and anesthetized with ketamine prior to administration of the cA2 or saline. Initially, anesthesia was maintained with repetitive doses of ketamine during the 35 to 50 minute infusion of cA2 or saline, and for approximately 30 minutes after completion of the infusion. For reasons presented below, the method of anesthesia was modified to employ a low sedative dose of ketamine followed by a gaseous mixture of halothane, nitrous-oxide and oxygen. All chimpanzees were monitored for approximately 2 weeks following completion of their last dose.

Each animal was examined for pharmacotoxic signs continually during the infusion, frequently after dosing and once daily during the non-treatment observation period. Blood pressure, heart rate and respiration rate were measured just prior to, during and for 30 minutes after completion of each infusion. Body temperature was measured just prior to and at approximately 30 minutes after completion of each infusion. Body weights were measured pretest, just prior to each dose and once weekly during the non-treatment period. Electrocardiographic tracings were obtained after the first and last doses for each treatment regimen. Ophthalmoscopic examination was performed pretest and at the end of the treatment regimen. Routine hematology, clinical chemistry and urinalysis parameters were monitored pretest, frequently during the dosing period and/or weekly during the non-treatment observation period. Serum samples were collected for analysis of cA2 serum concentrations and chimpanzee anti-cA2 antibody response during the study.

Results: Complications from anesthetic methods were noted in several animals, necessitating a change in anesthetic combination medications partway through this study. Three (3) control chimpanzees were administered 0.9% Sodium Chloride for Injection (saline). One (1) chimpanzee was included at each treatment regimen (1 dose, 3 doses and 5 doses). The 2 control chimpanzees administered 1 or 3 doses were asymptomatic during the dosing and non-treatment observation periods. The third control chimpanzee received 5 daily administrations of saline. During each day of dosing for this animal, increasing doses of ketamine were required to maintain anesthesia. On the fifth dose day, anesthesia could not be maintained during the infusion with repetitive doses of ketamine and, therefore, a halothane, nitrous-oxide and oxygen inhalation anesthesia mixture was administered. Shortly thereafter, this animal went into shock (decreased blood pressure). The animal was immediately administered Reomacrodex (plasma volume expander), adrenaline, atropine and Haemacell (plasma substitute). The animal responded to this treatment and fully recovered. This incident was considered related to the relatively high cumulative dose of ketamine (34 mg/kg) that was administered to this animal during the infusion period. This event may have been precipitated by the addition of the gaseous mixture, however, as discussed

below, similar observations were made in other animals that received relatively high doses of ketamine without administration of the gaseous anesthetic mixture.

During the dosing period and throughout the 2-week non-treatment observation period, no signs considered related to cA2 treatment were observed for the 3 chimpanzees dosed at 15 mg/kg/day for 4 (1 animal) or 5 days. One (1) of the 3 chimpanzees exhibited difficulty breathing and a slow heart rate approximately 1 hour after receiving its fourth daily infusion. The animal was immediately administered Reomacrodex, di-Adreson-F (corticosteroid), atropine and prophylactic amoxycillin (for 4 days to prevent possible aspiration-pneumonia). The animal responded to the treatment and fully recovered. This incident was considered related to the relatively high dose of ketamine (68 mg/kg) that was administered to this animal to maintain anesthesia for the intravenous cA2 infusion. Because of this event it was decided not to submit the animal to anesthesia the following day; therefore, the initially scheduled fifth (final dose) was not administered to this chimpanzee.

One (1) chimpanzee administered a single 30 mg/kg dose of cA2 exhibited cyanosis caused by depressed respiration, approximately 1.5 hours after completion of the infusion. The animal was immediately administered di-Adreson-F and atropine. The animal responded to the treatment and fully recovered. As with the other saline- and cA2-treated animals that displayed a similar event, this observation was considered related to the ketamine anesthesia. In order to maintain anesthesia during the 80 minute infusion/observation period, repetitive doses of ketamine were administered so that a relatively high total dose (43 mg/kg) had been given to this chimpanzee.

Following Methods/anesthetic Modification: Results: Because of the untoward events observed in a portion of the cA2- and saline-treated chimpanzees (all of which were considered related to the ketamine anesthesia), the anesthesia procedure was modified to a low sedative dose of ketamine followed by inhalation of mixture of halothane, nitrous-oxide and oxygen to maintain anesthesia during these relatively long infusion/observation periods (65 to 80 minutes). Two (2) additional chimpanzees were administered cA2 at 30 mg/kg/day for 3 consecutive days using the modified anesthesia procedure. No adverse clinical signs were observed during the dosing period or during the 2-week non-treatment observation period. No changes considered cA2 treatment-related were observed for body weight, blood pressure, heart rate, body temperature, hematology, serum chemistry or urinalysis values. Ophthalmologic and electrocardiographic examinations revealed no changes which were attributable to the administration of cA2. Analysis of serum samples for immune response detected no chimpanzee anti-cA2 antibodies, however the presence of cA2 could interfere with detection of low levels of anti-cA2 antibodies. However, an immune response was detected in one saline control chimpanzee which suggests that a small amount of cA2 was inadvertently administered to this animal.

The occurrence of respiratory depression in 1 chimpanzee each in the saline control, 15 mg/kg and 30 mg/kg groups was considered a result of the quantity of ketamine administered and not the cA2 treatment. Thus, following the improvement of anesthetic administration, study results indicated that cA2 was well tolerated in chimpanzees at dosage levels of 15 mg/kg for 4 to 5 consecutive days and 30 mg/kg once, or for 3 consecutive days. This is supported by the lack of any similar event in other animals following a modification of the anesthesia procedure, including animals dosed at the highest dosage level of 30 mg/kg/day for 3 consecutive days.

Three-Day Intravenous Dose Study of cA2 in Chimpanzees (Centocor Study T-091-033)

Methods: In this study, two chimpanzees (*Pan troglodytes*), 1 male, 1 female, were administered 30 mg/kg of cA2, intravenously once daily for 3 consecutive days. Two (2) additional chimpanzees (1 male, 1 female) received saline once daily for 3 consecutive days to serve as controls. Chimeric A2 (5 mg/mL) or saline was administered at an infusion rate of approximately 4 mL/minute and at a dose volume of 6 mL/kg. cA2 was filtered through a 0.2 μm low protein binding filter prior to administration. The chimpanzees were fasted overnight and initially sedated with ketamine and then maintained under halothane, nitrous-oxide and oxygen anesthesia for the dose administration. All chimpanzees were monitored for approximately 6 weeks (non-treatment observation period) following completion of the third (last) dose. Each animal was examined for pharmacotoxic signs continually during the infusion, frequently after dosing and twice daily during the non-treatment observation period. Blood pressure (systolic), respiratory rate, heart rate, electrocardiograms were recorded just prior to, during and for 10 to 30 minutes after the first and third doses, on day 4 and just prior to study termination. Body weights were measured during the pre-dose period, just prior to each dose and once weekly during the non-treatment period. Ophthalmoscopic examination was performed pre-dose, on day 6 and at the end of the non-treatment period. Routine hematology and clinical chemistry parameters were monitored pre-dose, prior to dosing on days 1, 2, on day 4 and once weekly during the non-treatment period. Urinalysis was performed pre-dose, on day 4 and once at the end of the non-treatment period. Serum samples were collected for analysis of chimeric A2 serum concentrations and induced immune response.

Results: During the dosing period and throughout the 6-week non-treatment observation period no overt signs of toxicity were observed. No changes considered cA2-treatment-related were observed for body weight, respiratory rate, heart rate, hematology, serum chemistry or urinalysis evaluations. A slight elevation in systolic blood pressure was observed after the first dose. The significance of this elevation is unclear because blood pressure can vary significantly when measured in chimpanzees during anesthesia. Electrocardiographic and ophthalmologic examinations revealed no changes considered cA2 treatment-related. Analysis of serum samples for immune response detected no chimpanzee anti-cA2 antibodies, however the presence of cA2 could interfere with detection of low levels of anti-cA2 antibodies.

Thus, cA2 was well tolerated in chimpanzees administered intravenous infusions at 30 mg/kg/day for 3 consecutive days. No signs of toxicity were observed during the study. A slight elevation in systolic blood pressure was observed only after the first dose. No other cA2-related changes in physiological parameters, electrocardiographic or clinical pathology examinations were observed during the study.

Immune Response in Chimpanzees

Samples were collected for immune response in three safety studies conducted in chimpanzees. As safety studies, the dosage regimens were fairly high, ranging from a single bolus (1X) of 30 mg/kg to five consecutive, daily treatments (5X) with 15 mg/kg of cA2. The immune response samples were collected at different intervals, but no longer than 75 days post-treatment. In addition to routine EIA analysis of these sera, all samples were spiked with a fixed amount (250 ng/mL) of an affinity purified baboon anti-cA2 positive control antibody to assess whether free cA2 was present in the test sera. Free cA2 levels were seen to persist throughout the post-treatment sampling time frame, as demonstrated by little or no signal generated by the baboon positive control antibody spike. There was one chimpanzee (Centa) in Study T-090-027 which did demonstrate a moderate immune response 19 days after the initial treatment with a titer of 81. This animal was designated to receive saline control, administered on 5 sequential days,

however pharmacokinetic analyses confirmed that 993 ng/mL of free cA2 was observed at day 11 in this animal, indicating that an inadvertent low level treatment with cA2 probably occurred.

cA2 Pharmacokinetics in Chimpanzees

Three safety studies were conducted in chimpanzees (T-090-027, T-090-029, and T-091-033) in which serum samples were collected for pharmacokinetic analyses. In each of these studies, super-pharmacologic dose levels of cA2 were administered to test the safety of cA2 treatment in primates. In the first study (T-090-027), a bolus of 15-30 mg/kg of cA2 was administered intravenously to chimpanzees for up to five consecutive days (Heidt, 1993b). In the second study (T-090-029), cA2 (30 mg/kg) was administered in combination with a monoclonal antibody (Centoxin®, 5 mg/kg) directed against the lipid A moiety of endotoxin from Gram-negative bacteria. In the third study (T-091-033), chimpanzees received bolus treatment with 30 mg/kg of cA2 for three consecutive days. The pharmacokinetic results from these studies will be discussed separately since the dose regimens and PK sample time periods were somewhat different for each protocol.

A total of 6 chimpanzees from study T-090-027 received cA2, and an additional 3 chimpanzees received a saline control. As shown in Table 8, the dosing ranged from 30 mg/kg for 1 or 3 sequential days to 15 mg/kg for 4-5 days. Two animals were given only one treatment (either 30 mg/kg cA2 or a saline control) although they were intended to be treated for 5 days. One other animal was treated with 15 mg/kg of cA2 for 4 days, but had been scheduled to be dosed for 5 sequential days. The pharmacokinetic data for this animal were similar to those from the other animals in the 5 day treatment group, so the data for all three animals receiving 15 mg/kg of cA2 were averaged.

Approximately a two-fold higher free cA2 level was observed immediately following the initial treatment with 30 mg/kg than at the same time point following 15 mg/kg of cA2 (mean of 941 vs 443 µg/mL at 5 minutes post-treatment, respectively). The C_{max} observed after three daily injections was also predictable. The chimpanzees treated with 3 daily doses of 30 mg/kg cA2 had mean cA2 levels of 783 and 1563 µg/mL immediately before and after the third dose, and the difference (780 µg/mL) approximated the peak cA2 concentration observed after the a single 30 mg/kg dose (825 µg/mL). The serum concentrations observed following 15 and 30 mg/kg of cA2 were comparable to those levels observed to occur in humans at these doses, thus indicating that chimpanzees are a good model for evaluating the safety of cA2 administration.

The two other chimpanzee studies, T-090-029 and T-091-033, verified the single and multi-dosing results obtained with 30 mg/kg of cA2 in T-090-027, although in both cases the pharmacokinetic sampling covered a longer time frame than in the initial study. In T-090-029, two chimpanzees received 30 mg/kg of cA2 in combination with Centoxin® at 5 mg/kg (Centocor Technical Report 91060084-R01). A longer sample collection regimen allowed more extensive pharmacokinetic analysis for a single cA2 treatment than was performed for T-090-027. The results from both studies were very similar. From the comparison of the PK results obtained from T-090-027 and T-090-029, it appeared that the co-administration of Centoxin® did not alter the pharmacokinetic clearance of cA2 administered at 30 mg/kg. The linearity of the terminal logarithmic concentration versus time plot for free cA2, following a bolus dose of 30 mg/kg, suggested that the pharmacokinetic behavior of cA2 is linear (concentration independent).

In study T-091-033, two chimpanzees were treated with three consecutive daily doses of 30 mg/kg of cA2.

Although there was some variation between animals at the early time points, in general the serum concentration versus time curves reflected the results seen in T-090-027. As seen in the earlier study, there was some accumulation of free cA2 such that higher levels (~2-fold) were observed shortly after the third administration of cA2 relative to the amount observed following the first treatment (1750 vs. 888 µg/mL, respectively).

The median values C_{max}, AUC, CL, Vd_{ss} and $T_{1/2}$ from the three chimpanzees administered a single dose of 30 mg/kg of cA2 were as follows with ranges in parentheses - Cmax was 974 ug/ml (825-1850); AUC_{0-} was 121,004 ug/ml x hr (115,038-127,605); CL was 7.1 ml/hr/kg (6-8.6); Vd_{ss} was 1590 ml (1345-1601; and $T_{1/2}$ Term was 144 hr (140-193).

Local Tolerance

Acute Intravenous Irritation Study of cA2 in Rabbits (Centocor Study T-096-001)

The objective of Centocor Study T-096-001 was to assess the intravenous irritancy potential of cA2 following a single infusion dose to rabbits. In addition, the effect of cA2 on subcutaneous tissue was examined to assess the effect of potential extravasation during administration of cA2.
Sixteen (16) New Zealand White male rabbits were administered a three-hour infusion of either cA2 (5 mg/mL), Human Serum Albumin, USP (0.5 mg/mL) or 0.9% Sodium Chloride for Injection, USP (saline). The reconstituted dosing solutions of cA2 and Human Serum Albumin (HSA) were filtered through a 15 µm filter prior to dosing. The intravenous infusions were injected into the auricular vein of the left or right ear. The results of this study demonstrate that a three-hour intravenous infusion of cA2 (at a concentration of 5 mg/mL) was well tolerated and produced no vascular irritation when administered to rabbits. Subcutaneous administration of cA2 (5 mg/mL) resulted in a slightly greater irritation than similarly injected HSA or saline.

Acute Intramuscular Irritation Study of cA2 in Rabbits (Centocor Study T-096-004)

A study was performed to assess the intramuscular irritancy potential of cA2 following a single dose to rabbits. Seven (7) New Zealand White male rabbits were injected with 1.0 mL of cA2 (5 mg/mL), Human Serum Albumin, USP (0.5 mg/mL) or cefoxitin sodium (Mefoxin®; positive control) into separate sites of the sacrospinalis muscle. The reconstituted dosing solutions of cA2 and Human Serum Albumin (HSA) were filtered through a 15 µm filter prior to dosing. At 24, 48 or 72 hours post-dose, the rabbits were euthanized and the injection sites examined and scored for signs of irritation. Thus, this scheme provided three injection sites per time interval for the test and control articles. Overall grading of irritation was based on group average scores. The results of this study demonstrate that cA2 (at a concentration of 5 mg/mL) administered intramuscularly to rabbits produced a degree of irritation which was less than that from Mefoxin®, an approved intramuscularly administered therapeutic drug.

Reproduction Studies

Reproductive and developmental nonclinical toxicity studies have not been performed with cA2 because cA2 has limited species cross-reactivity (besides human TNFα, cA2 only neutralizes chimpanzee TNFα with similar potency). Based on the lack of cross-reactivity, standard reproductive/developmental toxicity studies of cA2 in rats and rabbits were considered inappropriate to provide relevant information on cA2's potential to produce adverse reproductive or developmental effects in humans. Since the effects of cA2 on developing human fetuses are not known, the benefit of administering the material to pregnant women should be considered relative to the potential risks. Appropriate warnings should be included in the

package insert.

It has been reported that pregnant mice administered a monoclonal antibody specific to murine TNFα showed no outcomes of gross or morphologic abnormalities. In addition in the most extreme model of inhibition of TNFα, transgenic mice deficient in TNFα (TNFα knock-out), the mice develop normally and have no gross structural or morphological abnormalities. Lymphoid and hematopoietic development in these mice are normal, whereas transgenic mice deficient in lymphotoxin α (previously called TNFβ) lack lymph nodes and Peyer's patches. This suggests that TNFα does not have a critical role in organogenesis, but the presence of lymphotoxin α may be critical for lymphoid organ development. In this last regard, cA2's specificity for TNFα over LTα is viewed as a point favoring developmental safety.

In an effort to provide potentially relevant information on the reproductive and developmental effects when TNFα is inhibited, comprehensive developmental toxicity studies with an anti-mouse TNFα monoclonal antibody in mice were performed. The anti-mouse TNFα monoclonal antibody, chimeric V1q muG2a, was cloned

Like cA2, cV1q is TNFα specific and does not neutralize lymphotoxin α. Results of the intravenous developmental toxicity studies of cV1q in mice are summarized in this section.

Intravenous Dosage-Range Developmental Toxicity Study of cV1q Anti-Mouse TNF Antibody in Mice (Centocor Study T-096-010)

The study was designed to provide information for the selection of cV1q dosages to be used in a subsequent developmental toxicity study (Centocor Study T-096-011) in mice. Forty-eight (48) female CD-1 mice were randomly assigned to three dose groups (16 mated female mice per dose group). Chimeric V1q (1.3 mg/mL) was supplied in vials and administered at doses of 10, 20 or 40 mg/kg/day at dose volumes of 7.7, 15.4 or 30.8 mL/kg, respectively. [*Importantly, 40 mg/kg was seen to be active in reducing TNF-associated disease in the study performed in Tgε26 mice (see section on animal activity studies).]* Chimeric V1q was administered intravenously once on Day 6 of presumed gestation. Clinical observations and body weights were recorded daily during the study. Blood samples were collected from four mice per dose group at 24 hours, 4, 7 and 12 days after dosing for determination of cA2 serum levels. At the same time points as blood collection, four mice per dose group were Caesarean-sectioned, submitted for gross necropsy and examined for number and distribution of corpora lutea, implantation sites, viable and nonviable embryos or live and dead fetuses and early and late resorptions. The body weight of each fetus was recorded.

Results: No deaths or adverse clinical observations occurred during the study. All cV1q - treated dose groups exhibited a pregnancy rate within the normal rate observed historically at the testing laboratory. No significant body weight changes among the dose groups were observed during the study. At necropsy, one 40 mg/kg mouse had a large spleen and extreme dilation of the left renal pelvis filled with a white substance. The significance of these findings is unclear. Based on the testing laboratory's historical control database, the average number of nonviable embryos/resorptions and percentage of nonviable or resorbed conceptuses per litter were slightly increased and average fetal body weights appeared slightly

decreased in the 40 mg/kg dose group. No changes considered cV1q -related were observed in the 10 and 20 mg/kg dose groups. Analyses of the serum showed that a single intravenous dose (10, 20 or 40 mg/kg) of cV1q was cleared from the circulation by Day 18 of presumed gestation (12 days post-dose). Thus, based on the results of this study no clear maternal toxicity was produced. An increase in the percentage of nonviable and resorbed conceptuses in the 40 mg/kg/day dose group was observed. It was left to the final study performed under GLP and reported below to clarify the finding seen in this pilot trial.

Notably, analysis of the maternal serum showed that significant cV1q serum levels are sustained following a single dose (10 to 40 mg/kg) administered on day 6 of gestation up to day 13 of gestation. Therefore, the cV1q dosage regimen employed in this study maintained serum levels of cV1q during the entire period of major organogenesis in the mouse (gestation days 6 to 18). Analyses of the fetal samples on day 14 of gestation (48 hours after the second cV1q dose) revealed cV1q levels (>32 ug/mL), thus indicating placental transfer of the antibody and that the fetuses were exposed to cV1q during the organogenesis period. c V1q was not detected in the serum at the end of the study. Therefore, it was recommended to administer cV1q twice (on Day 6 and Day 12 of presumed gestation) at doses up to 40 mg/kg in the subsequent developmental toxicity study to sustain cV1q serum levels during the entire period of organogenesis in the mouse.

Intravenous Developmental Toxicity Study of cV1q Anti-Mouse TNF Antibody in Mice (Centocor Study T-096-011)

Methods: The purpose of this study was to determine the potential effects of cV1q on pregnancy and embryo-fetal development in the mouse. Ninety-nine (99) presumed pregnant female CD-1 mice were randomly assigned to three dosage groups (33 mated female mice per dosage group) and were scheduled to be administered intravenously with control vehicle (1X Dulbecco's Phosphate Buffered Saline) or cV1q (4.93 mg/mL) once on day 6 of gestation and once on day 12 of gestation. Chimeric V1q was administered at dosages of 10 and 40 mg/kg/dose. Both dosages of cV1q and the control vehicle were administered at a dose volume of 10 mL/kg. Due to a shortage of available test material on gestation day 12, cV1q was administered to only 22 mice in the 10 mg/kg/dose group and 23 mice in the 40 mg/kg/dose group. All 32 surviving mice in the control group were dosed on gestation day 12. Eight (8) mice from each dose group were Caesarean-sectioned on day 14 of gestation and maternal blood and fetal samples were collected and analyzed for cV1q concentration. The remaining mice in each dose group were Caesarean-sectioned on day 18 of gestation and maternal blood samples were collected from three mice per dose group and submitted to the Sponsor for analysis of cV1q serum concentration. At least 17 pregnant mice per group were Caesarean-sectioned on day 18 of gestation and the observations and measurements from these mice were used to evaluate any potential effects of cV1q treatment on development of the conceptus.

All study mice were observed daily during the study for signs of toxicity. On days 6 and/or 12 of presumed gestation the mice were also observed for abortions and premature deliveries before injection and approximately one hour after injection. Maternal body weights were measured during the study. Following Caesarean-sectioning on day 18 of gestation, the thoracic, abdominal and pelvic viscera of each mouse were grossly examined. The number of corpora lutea in each ovary was recorded. The uterus was excised and examined for pregnancy, number and distribution of implantations, early and late resorptions and live and dead fetuses. The fetuses were weighed, examined for gender and gross external alterations. Approximately one-half of the fetuses were placed in Bouins' fixative and examined internally to confirm

gender and screen for any visceral alterations using a dissecting microscope. The remaining fetuses were stained with alizarin red S and evaluated for skeletal alterations using a dissecting microscope.

Serum levels: Analyses of maternal serum samples collected on days 14 and 18 of gestation showed that cV1q (10 or 40 mg/kg) administered on days 6 and 12 of gestation produced significant cV1q serum levels during this time period. Mean cV1q mu2a serum levels for the 10 and 40 mg/kg/dose groups on day 18 of gestation were 0.8 ± 0.4 and 7.2 ± 0.9 ug/mL, respectively but had reached as high as 38 and 160 ug/ml on day 14 of gestation.

Results: No deaths, adverse clinical observations, body weight changes considered cV1q treatment-related occurred during the study. No changes considered cV1q -related were observed at necropsy. There were no cV1q -related effects on the number of corpora lutea, implantations, litter sizes, fetal weights, fetal sex ratios, resorptions or dead or resorbed conceptuses/litter. Detailed external, visceral and skeletal examinations revealed no cV1q -related effects. Thus based on the results of this study, cV1q anti-mouse TNFα monoclonal antibody administered at 10 or 40 mg/kg/dose to pregnant mice on days 6 and 12 of gestation produced no maternal or developmental toxicity. *Since the specificity, pharmacologic activity, and kinetics of cV1q were well defined as analogous to cA2, these findings were considered by FDA reviewers to be suitable for reporting in the package insert for the drug.*

Mutagenicity Studies

The mutagenic potential of cA2 was evaluated in three separate assays. Human lymphocytes were used to evaluate *in vitro* the potential of cA2 to induce chromosomal aberrations. In the *Salmonella - Escherichia coli*/microsome plate assay (Ames Test), cA2 was evaluated for its potential ability to induce reverse mutations. Potential clastogenic effects were determined *in vivo* using the mouse micronucleus test. *No genotoxic effects of cA2 were noted in the following study reports.*

Chromosome Aberration Study in Human Whole Blood Lymphocytes (Centocor Study T-096-007)

Human whole blood lymphocyte cultures in appropriate growth medium were incubated with cA2 at concentrations of ( ). Chimeric A2 was supplied in vials as a lyophilized powder and reconstituted to a concentration of ( ). Dilutions to appropriate test concentrations were made with sterile water for injection, USP. Treatment of duplicate human lymphocyte cultures with each concentration of cA2 was carried out in both the absence and presence of S-9 reaction mixture (used for the purposes of metabolic activation; the S-9 liver fraction was obtained from male Sprague-Dawley rats treated with Aroclor 1254). Positive control agents ( ) were included in the assays. Following incubation (hours) the cells were harvested and slides prepared for analysis of chromosome aberrations. The results of the initial assay were confirmed in independently conducted repeat a ays.

Chimeric A2 did not induce a significant increase in cells with chromosomal aberrations or in polyploidy at any concentration tested either in the presence or absence of an exogenous metabolic activation system. Treatment of the cultures with positive control agents induced statistically significant increases in the proportion of cells with chromosome aberrations. Therefore, under the conditions of the assay, cA2 did not exhibit mutagenic activity in the human whole blood chromosomal aberration analysis.

Salmonella - Escherichia coli/Mammalian-Microsome Reverse Mutation Assay Preincubation Method (Centocor Study T-096-008)

Chimeric A2 at concentrations of [illegible] µg/mL of preincubation reaction mixture was tested in this bacterial mutagenicity assay ([illegible]Test). The assay was performed using [illegible] both in the presence and absence of an exogenous metabolic activation system. Chimeric A2 was supplied in vials as a lyophilized powder and reconstituted to a concentration of [illegible] The tester strains were exposed to cA2 using the treat and plate modification of the [illegible]Test. The treat and plate modification of the preincubation method was used to allow the test article to be separated from the tester strains following a defined exposure period (approximately[illegible]minutes). This rinsing step allowed for the removal of test article components (i.e., histidine), prior to plating, that could interfere with selective conditions of the assay. The assay was conducted in both the presence and absence of [illegible]eaction mixture (used for the purposes of metabolic activation; the [illegible] fraction was obtained from male Sprague-Dawley rats treated with Aroclor 1254).[illegible] concentrations of cA2 along with concurrent vehicle (sterile water for injection, USP) and positive controls ([illegible]using three plates per concentration were tested in the assay. Following an approximate [illegible]nour incubation (37°C) period, the numbers of revertant colonies per plate were counted. The results of the initial assay were confirmed in an independently conducted repeat assay.

Chimeric A2 did not cause a positive increase in the number of revertants per plate of any of the tester strains either in the presence or absence of an exogenous metabolic activation system. The positive controls did produce significant increases in the number of revertants. Therefore, under the conditions of the assay, cA2 did not exhibit mutagenic activity in the

In Vivo Mouse Micronucleus Assay (Centocor Study T-096-002)

In the micronucleus assay, cA2 was administered by intravenous injection to mice at doses of 25, 50 and 100 mg/kg. Chimeric A2 was supplied in vials as a lyophilized powder and reconstituted to a concentration of 5 mg/mL. Separate groups of mice were intravenously injected with a vehicle control, Human Serum Albumin, USP (0.5 mg/mL) and a positive control (cyclophosphamide; final reconstituted concentration of 8 mg/mL). The cA2 doses of 25, 50 and 100 mg/kg were administered at dose volumes of 5, 10 and 20 mL/kg, respectively. The dose volumes for the positive and vehicle controls were 10 and 20 mL/kg, respectively. Ten (10) CD-1 mice (5 males and 5 females) were included in each treatment group for each time-point evaluated. At 24, 48 or 72 hours post-dose, the mice were euthanized and bone marrows harvested and slides prepared for analysis of micronuclei and polychromatic erythrocyte (PCE) to normochromatic (NCE) cell ratio. Chimeric A2 did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes of mice at any dose level and time-point evaluated in this assay. The positive control induced significant increases in micronuclei in bone marrow polychromatic erythrocytes. Therefore, under the conditions of this assay, cA2 did not exhibit mutagenic activity in the mouse micronucleus assay.

Oncogenic/Carcinogenic Potential

No carcinogenicity studies have been performed with cA2 because of its restrictive species cross-reactivity. Carcinogenicity studies in chimpanzees are unlikely to provide meaningful carcinogenicity information because only small numbers of chimpanzees can be evaluated and only limited antemortem

and no postmortem assessments can be performed on them. No findings suggestive of carcinogenic or mutagenic activity were observed in laboratory studies (*in vivo* and *in vitro)* performed with cA2, nor have any carcinogenic or mutagenic effects been associated with any of the constituents used to formulate cA2. *Therefore, carcinogenicity studies are considered to be unwarranted, a decision consistent with the ICH document detailing approached to safety characterization of biotechnologically-derived products.*

Toxicology Summary

Chimeric A2 showed no unexpected reactivity (or cross-reactivity) in *in vitro* human tissue cross-reactivity assessment, nor mutagenicity, local intolerance, or other systemic toxicities that would preclude its use in Crohn's Disease patients.

Since the chimpanzee is the only species other than humans whose TNFα bind to cA2, safety studies in this species are considered the only studies that can provide relevant safety information on cA2 administration to humans; due to animal use restrictions on this endangered species, these animals may not be necropsied to provide histopathology data, and therefore study outcomes are limited to clinically observable signs, as well well as results from noninvasive testing such as clinical chemistry and hematology assessments. Following some problems attibutable to high doses of ketamine anesthetic required for animal handling, the studies with cA2 in chimpanzees showed that cA2 was well tolerated at doses up to 30 mg/kg/day for at least 3 consecutive days and at doses up to 15 mg/kg/day for at least 5 days. No cA2-related signs of toxicity, including abnormal hepatic or hematologic effects, were observed during these chimpanzee studies.

The nonclinical studies that have been described in this section provided support for activity and safety for cA2-treated Crohn's disease patients. The following characteristics have been established regarding cA2 and its neutralization of human TNFα.

- cA2 has a long serum half-life (6 days in chimpanzees) and predictable pharmacokinetics.
- Animal safety studies revealed no gross or biochemical evidence of toxicity in normal, adult chimpanzees in studies where chimpanzees were administered 30 mg/kg, and monitored for 6 weeks, the appropriate time (compared with a t1/2 in chimpanzees of 6 days) for the drug to have cleared to a level below clinically active levels. Given the initial high exposure levels in this toxicity evaluation, this six week study is sufficient for judging risk of patients with active cA2 levels for 2-3 months, but not on a chronic basis.
- Doses of an analogous anti-mouse TNFα monoclonal antibody which were high enough to active in a mouse model of disease, when given to pregnant mice during organogenesis, caused no embryofetal toxicities. These studies were necessitated due to the absence of crossreactivity of cA2 in species other than chimpanzees.

These studies are adequate to support the current labeling for short term use in Crohn's Disease patients.