CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125289

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D.
OND IO
BLA: 125289
Submission date: June 24, 2008
Drug: golimumab (SIMPONI)
Sponsor: Centocor, Inc.
Indication: Rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis
Reviewing Division: Division of Anesthesia, Analgesia and Rheumatology Products

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

Developmental and Reproductive Toxicity:
The sponsor proposed a pregnancy labeling category of B and the Division agreed with this category although some minor changes in specific wording describing the relevant studies were suggested by the pharm/tox reviewer and supervisor. The human antibody is not active in rodent species. Embryofetal and peri-postnatal studies were conducted in the Cynomolgus monkey with the human antibody and fertility, embryofetal and peri-postnatal studies were conducted in mice with a rodent surrogate antibody.

Essentially no adverse effects to the fetus or offspring were observed in these monkey and mouse studies. Pregnancy category B is generally appropriate when no adverse findings have been identified in developmental studies. In this case, both the human product and a rodent surrogate were tested in animal studies and found to be nonteratogenic. Assignment of pregnancy category B to golimumab is consistent with other therapeutic agents of the same class (antiTNFα). Therefore, I concur with the pregnancy category of B.

The reviewer originally suggested wording for labeling that included description of the [redacted]. However, the supervisor noted in his [redacted] b(4)

This is acceptable since there were no adverse findings in the mouse studies.

Carcinogenicity:
No carcinogenicity studies have been conducted with golimumab. As noted by the reviewer, traditional carcinogenicity studies in rodents with golimumab would not be particularly informative because golimumab does not neutralize rodent TNFα. Potential tumorigenic concerns have already been identified for antiTNFα agents based on the mechanism of action and postmarketing observations. A similar concern would presumably exist for golimumab and so it would be appropriate to include a description of such a concern in labeling.

Conclusions:
I concur that the nonclinical information supports approval of golimumab for the intended indications. The labeling as proposed in the primary review and modified as discussed in the supervisory review is acceptable. No additional nonclinical studies are recommended.

Paul C Brown 3-26-09

Paul C. Brown, Ph.D.
ODE Associate Director for Pharmacology and Toxicology

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER:	**125,289**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**06/24/2008**
PRODUCT:	**Simponi® (Golimumab; Human Anti-Tumor Necrosis Factor alpha; Monoclonal Antibody rTNV148B)**
INTENDED CLINICAL POPULATION:	**Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis**
SPONSOR:	**Centocor, Inc.**
DOCUMENTS REVIEWED:	**eCTD**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia and Rheumatology Products**
PHARM/TOX REVIEWER:	**Gary P. Bond, Ph.D., DABT**
PHARM/TOX SUPERVISOR:	**Adam M. Wasserman, Ph.D.**
DIVISION DIRECTOR:	**Bob Rappaport, M.D.**
PROJECT MANAGER:	**Sharon Turner-Rinehardt**
Date of review submission/sign off:	**February 26, 2009**

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EXECUTIVE SUMMARY

I. Background & Regulatory Issues

Simponi® (golimumab) is a human monoclonal antibody (mAb) with high affinity and specificity for neutralizing both the soluble and transmembrane forms of human tumor necrosis factor alpha (TNFα). The intended clinical population for this DAARP BLA is adult patients with Rheumatoid Arthritis (RA), Psoriatic Arthritis (PsA), and Ankylosing Spondylitis (AS). The applicant, Centocor Inc., also has an approved BLA 103,772 (Remicade®, infliximab) for the same indications. Remicade® is a chimeric immunoglobulin G1 (IgG1) mAb against human TNFα. The fully human mAb Simponi® is at least 2-fold more potent and does not present the potential for human immunotoxicity as it is not a xenogenic drug for humans such as Remicade®. Proposed dosing of 50 mg subcutaneously (SC) every month is supported by nonclinical studies and clinical trials.

II. Recommendations

A. Recommendation on approvability

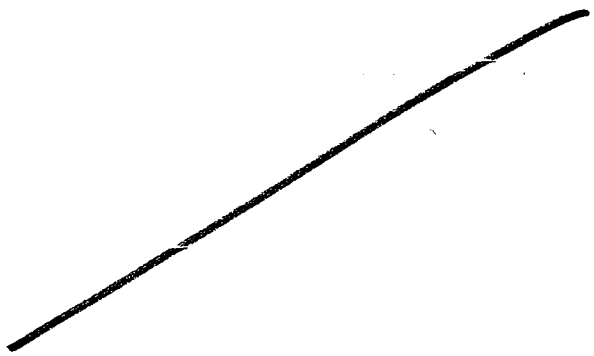
BLA approval is recommended.

B. Recommendation for nonclinical studies

No nonclinical studies recommended.

C. Recommendations on labeling

Suggested deletions are in strikethrough and suggested additions are in red.



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1 Page(s) Withheld

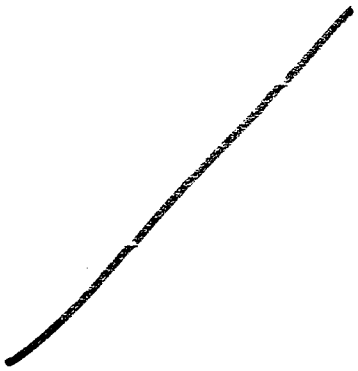
_____ Trade Secret / Confidential (b4)

✓ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Pharm/Tox- 6



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II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The nonclinical safety program for golimumab, a human monoclonal antibody that neutralizes the biological activity of human tumor necrosis factor alpha (TNFα), was designed by the applicant, Centocor Inc., in accordance with the ICH S6 guidelines (Guidance for Industry - Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals; ICH-S6 July 1997). Toxicology studies with golimumab were conducted in cynomolgus monkeys as golimumab was capable of neutralizing TNFα from several nonhuman primates including cynomolgus monkeys, but demonstrated little or no neutralization of dog, rabbit, mouse or rat TNFα. Therefore, the cynomolgus monkey was chosen as a pharmacologically relevant species for nonclinical safety evaluations.

Product labeling for the TNFα inhibitor class of immunosuppressants (e.g., infliximab, etanercept, and adalimumab), most notable adverse reactions in patients receiving anti-TNFα therapy include serious and sometimes fatal blood disorders, infections (opportunistic infection in the form of tuberculosis and histoplasmosis), rare reports of lymphoma and solid tissue cancers, rare reports of serious liver injury, rare reports of drug induced lupus and rare reports of demyelinating central nervous system disorders (progressive multifocal leukoencephalopathy). No such effects were observed in the pivotal nonclinical

studies submitted to support this application. The No Observed Adverse Effect Levels (NOAELs) in the pivotal nonclinical studies were the highest dose tested in those studies. Anticipated pharmacological actions of this immunosuppressive drug and minimal local tolerance/injection site effects were observed at these NOAELs and were considered clinically monitorable and reversible.

The pivotal nonclinical studies in monkeys for golimumab include two 6-month chronic toxicology studies (SC and IV), an embryofetal development study (SC), and a pre- and post-natal development study (SC). Safety pharmacology endpoints were incorporated into intravenous (IV) and subcutaneous (SC) repeated dose toxicology studies conducted with golimumab in cynomolgus monkeys that also included neonatal assessments as part of the SC prenatal and postnatal reproductive toxicology study in monkeys. The safety pharmacology endpoints incorporated into these studies included measures of heart rate, blood pressure and electrocardiograms to assess cardiovascular safety, respiratory rate to assess respiratory safety and body temperature and daily clinical cage side observations to evaluate central nervous system safety. Studies of local tolerance/injection site effects in monkeys were also conducted. Additional supportive studies submitted, the results of which are to be used only in the product label, include chronic repeat dose toxicology studies and reproductive toxicology studies (fertility and early implantation, embryofetal development, and a pre- and post-natal development) in mice using an analogous anti-mouse TNFα mAb (cV1q) as submitted for the Centocor Inc. BLA 103,772 (Remicade®, infliximab) for the same indications.

In the pivotal repeat dose and reproductive toxicology studies ion monkeys, at the highest dose tested (50 mg/kg SC twice weekly), no mortality or golimumab-related clinical signs of toxicity were observed during the dosing and post-dosing periods. No treatment-related effects were observed for the vast majority of biological indices evaluated that included body weight, food consumption, body temperature, physical examination, ECG, blood pressure, heart rate, ophthalmic evaluations, hematology, coagulation and serum chemistry parameters, urinalysis, immunotoxicity evaluations (lymphocyte subsets and Keyhole Limpet Hemocyanin immune response capability analysis), organ weights, macroscopic, histopathologic or immunohistopathologic evaluations. No treatment-related effects were noted for any embryo-fetal or peri- and post-natal reproductive indices.

Notable, treatment related effects observed in the chronic IV monkey study was decreased IgG and IgM antibody responses to a KLH challenge as fewer animals in the golimumab dose groups mounted measurable antibody responses to KLH than in the control group. IgG antibody production was more affected than IgM antibody production with the most noticeable decline present in the 50 mg/kg dose group from day 41-69 with little to no response by day 92. In the chronic SC monkey study, reversible increases in B-lymphocytes were observed. Anti-drug antibody response in golimumab-treated animals was characterized as

inconclusive because the lack of detection of an antibody response in all but a few low dose animals may have been compromised by high serum levels of golimumab. The systemic NOAEL was 50 mg/kg as only anticipated pharmacological effects and reversible and clinically monitorable local injection site effects (minimal irritation) occurred at 50 mg/kg.

Safety Margins (SMs) (SMs - animal NOAEL exposure ÷ proposed human exposure) were considered adequate for human safety as animal:human systemic exposure comparisons were ≥ 75 for AUC-based SMs and ≥ 314 for Cmax-based SMs. SMs of ≥1 are generally considered as adequate for nonclinical-based human safety assessment post first in human dose. Based on this adequate SM criterion, the nonclinical data is considered to support human safety even though *in vitro* pharmacology studies showed that golimumab was up to 72-fold less potent at binding to and neutralizing the effects of cynomolgus TNFα than human TNFα because saturation of target molecules was achieved as nonclinical levels were tested at levels in excess of proposed human levels and the highest nonclinical doses tested were the NOAELs.

The complementary mouse studies with the anti-TNFα mAb, as reported on the label, also indicated acceptable safety margins for reproductive toxicity and for chronic toxicity with suggestion that preneoplastic/neoplastic changes did not occur after chronic dosing. No SMs were calculated from mice studies as a surrogate/different drug was used.

Genotoxicity tests have not been conducted with golimumab. The range and type of genotoxicity studies routinely conducted for pharmaceutical drugs are not applicable to biopharmaceutical antibodies as noted in ICH S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals.

Carcinogenicity tests have not been conducted with golimumab. The carcinogenic potential of golimumab cannot be evaluated in standard 2-year bioassays in rodents because golimumab does not neutralize rodent TNFα. Preneoplastic/ neoplastic lesions were assessed as part of chronic studies in monkeys and mice with no indications of any treatment-related preneoplastic/neoplastic lesions. Based on labeling of similar TNFα blocking drugs, a carcinogenic risk may be expected. Therefore, the carcinogenic potential of golimumab is being evaluated by monitoring of patients for Simponi® and other related TNFα inhibitors.

B. Pharmacologic activity

Golimumab is a fully human mAb that neutralizes the biological activities of human tumor necrosis factor alpha (TNFα). TNFα is produced primarily by activated monocytes, macrophages and T-cells as a membrane-anchored, cell surface protein that is released in soluble form by proteolysis. The bioactive form is a homotrimer that binds to receptors on TNFα-responsive cells and as a result enhances the inflammatory and immune response to environmental stimuli and

trauma. Based on animal experimental models and clinical trials, chronic and uncontrolled expression of TNFα has been linked to diseases such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), inflammatory bowel disease, and psoriasis. Primary pharmacodynamic studies have characterized golimumab binding interactions, mechanism of action, functional effects of TNFα neutralization, Fc-mediated functions, and *in vivo* activity supporting rheumatoid arthritis and other indications. In addition, based on comparative potency assays and species cross-reactivity studies, the cynomolgus monkey was determined to be the most relevant nonclinical test species. *In vitro* pharmacology studies showed that golimumab was up to 72-fold less potent at binding to and neutralizing the effects of cynomolgus TNFα than human TNFα. This potency difference is a nonissue as the doses and the dose regimens selected for the toxicology studies, as supported by toxicokinetic data were sufficient to produce sustained high serum golimumab concentrations that were in excess of the concentrations required to neutralize all TNFα in monkey serum and were in excess of clinical exposure levels as supported by the monkey:human blood level-based SMs of ≥ 75 listed in the previous section .

C. Nonclinical safety issues relevant to clinical use

In the course of drug development a production cell line switch occurred after [redacted]. In addition, lyophilized formulations used for the early Phase 1 and Phase 2 studies were changed to a liquid formulation used in Phase 3 studies, which will be the final formulation of golimumab. Product quality review determined that, based on a biochemical equivalency study, product made by the two cell lines was equivalent and drug used in nonclinical and clinical development were comparable. The liquid and lyophilized formulations were also found to be comparable in pharmacokinetic comparability studies based on review of nonclinical and clinical pharmacology studies.

b(4)

There are no nonclinical safety issues relevant to clinical use. Although *in vitro* pharmacology studies showed that golimumab was up to 72-fold less potent at binding to and neutralizing the effects of cynomolgus monkey TNFα than human TNFα, the doses and the dose regimens selected for the toxicology studies were considered adequate to indicate human safety. The doses were sufficient to produce sustained high serum golimumab concentrations in excess of the concentrations required to neutralize TNFα in monkey serum, were the highest nonclinical doses tested and were NOAELs, and were in adequate excess of clinical exposure levels.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

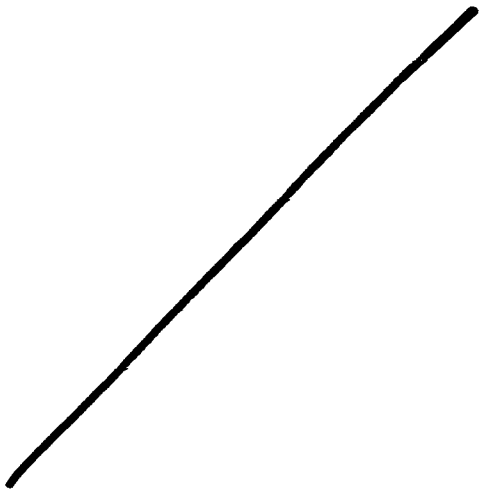
BLA number: 125,289
Review number: 1
Supporting document number/date/type of submission: 0/June 24, 2008/original
Sponsor and/or agent: Centocor, Inc., 200 Great Valley Parkway, Malvern, PA 19355
Manufacturer for drug substance: Centocor B.V., Einsteinweg 101, 2333 CB Leiden, The Netherlands

Reviewer name: Gary P. Bond, Ph.D., DABT
Division name: Division of Anesthesia, Analgesia and Rheumatology Products
Review completion date: February 26, 2009

Drug:

Trade name: Simponi®
Generic name: Golimumab
Code name: CNTO 148, CNTO 148 IgG; Monoclonal Antibody rTNV148B
Chemical name (CAS): Immunoglobulin G1, anti-(human tumor necrosis factor α) (human monoclonal CNTO 148 γ1-chain), disulfide with human monoclonal CNTO 148 κ-chain, dimer
- Human Monoclonal Antibody to Tumor Necrosis Factor Alpha
CAS registry number: 476181-74-5
Molecular formula/molecular weight: [redacted] amino acid residues/ [redacted] Da to [redacted] Da
Structure: Golimumab is a human IgG1 monoclonal antibody composed of two identical heavy chains [redacted] and two identical [redacted] light chains [redacted] in a disulfide-linked heterodimeric structure. The chains are linked together via non-covalent heavy-heavy and heavy-light interactions, and also covalent heavy-heavy and heavy-light disulfide bonds. (see diagram)

b(4)



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Relevant INDs/BLAs/DMFs:

Golimumab (Centocor, Inc.)
IND 9,925 (DAARP – Rheumatoid arthritis), 12,723 (DAARP – Psoriatic arthritis), 12,729 (DAARP – Anklyosing spondylitis), [redacted] b(4)

Infliximab (Centocor, Inc.)
BLA 103,772
- Chimeric (human, [redacted] Monoclonal Antibody (cA2) to Tumor Necrosis Factor alpha b(4)

Other approved TNFα blockers :

BLA 125,057 (adalimumab, HUMIRA)
BLA, 125,160 (certolizumab pegol, CIMZIA)
BLA 103,795 (entanercept, ENBREL)

Drug class: human immunoglobulin (IgG) monoclonal antibody

Intended clinical population: Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis

Clinical formulation: drug product is supplied as a sterile solution in a single-use, 1 mL pre-filled syringe (PFS) with a fixed needle, stoppered with a plunger stopper. The needle is covered with a needle shield. While golimumab PFS is manufactured in two

dosages, a 50 mg/syringe (0.5 mL) and a 100 mg/syringe (1.0 mL), only the 50 mg dose is being proposed for approval. Subcutaneous administration will be by a Centocor Autoinjector [redacted] by a health care professional or the patient. Differing producing cell line and formulation comparison studies successfully linked the early and late phases of the drug development program for golimumab in both the preclinical and clinical phases. See the Product Quality and Clinical Pharmacology reviews for details. b(4)

Drug Substance – The manufacturing process is based on Centocor's mAb platform. It has undergone three to four major variations in the process with respect to site, scale, cell line and unit operation, but generally improved the product throughout development. Biochemical bridging studies were performed between the different process changes. Product made between the various processes was functionally equivalent. In addition, requalification of cell lines was performed after the cell line switch between phase 1 & 2. After phase 1 clinical trials, a production cell line switch occurred to increase bioreactor [redacted] Biochemical equivalency study was performed to demonstrate that product made by the two cell lines was equivalent and drug used in nonclinical and clinical development were considered to be comparable. Refer to Product Quality review for specifics. b(4)

Drug Product - All inactive ingredients in the drug product are present at approved levels or considered to be at safe levels.

Composition of CNTO 148 100 mg and 50 mg PFS

Component	100 mg Dose Amount per dose (mg)	50 mg Dose Amount per dose (mg)	Concentration
CNTO 148	100	50	100 mg/mL
Sorbitol	41.0	20.5	4.1% (w/v)
L-Histidine	0.87	0.44	5.6 mM
PS 80[a]	0.15	0.075	0.015% (w/v)
Water for injection (mL)	1	0.5	NA[b]

[a] PS 80 = Polysorbate 80
[b] NA = Not applicable

In vitro assessment during IND found an increased number of subvisible particles in the prefilled syringes (PFS). Therefore, a recommendation was made to require clinical data to demonstrate that differences between the presentations (initially liquid in vial and later PFS) will not result in clinically meaningful differences in immunogenicity and safety. The report on the safety and immunogenicity of PFS, the proposed dosing method, versus liquid in vial (LIV) was analyzed in the Phase 3 studies. The product made between the

various processes was functionally equivalent. The safety and immunogenicity of the PFS and LIV presentations of golimumab were determined to be similar in the Phase 3 trials per the Medical Officer's review. Refer to Medical review for details.

Also in the course of drug development, lyophilized formulations were used for the early Phase 1 and Phase 2 studies, and a liquid formulation supplied in a glass vial (LIV) was used in all Phase 3 studies or in a PFS used in several healthy volunteer Phase 1 studies and in all Phase 3 (post Week 24) studies. The final product will be liquid formulation of golimumab in PFS fitted with the [redacted] or the same PFS in an autoinjector. The liquid and lyophilized formulations were found to be comparable in a nonclinical pharmacokinetic comparability study. See nonclinical review pharmacokinetic/toxicokinetic section for additional detail. b(4)

Route of administration: subcutaneous

Dose: 50 mg (~1 mg/kg) every month or 4 weeks

Disclaimer: All tabular and graphical information are constructed by the applicant unless cited otherwise. Text may be taken directly and/or modified from the applicant's submission if considered accurate by the reviewer of this BLA.

Data reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of BLA 125,289 are owned by Centocor, Inc. or are data for which Centocor, Inc. has obtained a written right of reference. Any information or data necessary for approval of BLA 125,289 that Centocor, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Centocor, Inc. does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of BLA 125,289.

Studies reviewed within this submission:

Detailed review emphasis was on pivotal studies that dealt with studies in monkeys (e.g., reproductive toxicology, chronic toxicity, and local tolerance) for estimation of human safety and label preparation. The value of immune responses for this humanized protein in animals is of questionable predictive value relative to human safety and is not discussed in any length relative to nonclinical-based human safety as such immune responses were not anticipated or observed in clinical trials.

PHARMCOKINETICS/TOXICOKINETICS (summary reviews only)

Single Subcutaneous Dose Pharmacokinetic Study in cynomolgus Monkeys with CNTO 148 (Phase III liquid formulation) and CNTO 148 (Phase II and Phase III lyophilized

formulations) Anti-TNFα Monoclonal Antibodies (Study Number P-2005-003) – non-GLP

Single Subcutaneous Pharmacokinetic Study in Cynomolgus Monkeys to Compare CNTO 149 (Anti-TNFα mAb) Produced by Cell Lines [redacted] (Study Number P-2002-008) b(4)

REPEAT-DOSE TOXICITY

One-Month Intravenous Dose Toxicity Study in Cynomolgus Monkeys with rTNV148B ([redacted] Study Number: UHAW- 137, Sponsor Study Number: T-2000-007)
- summary review only b(4)

25-Week Intravenous Dose Toxicity Study with CNTO 148 in Cynomolgus Monkeys with a 12-Week Recovery Period (Study Number: UHA00035, Sponsor Study Number: T-2004-006)

6-Month Subcutaneous Dose Toxicity Study with CNTO 148 in Cynomolgus Monkeys with a 12-Week Recovery Period (Study Number: UHAW-159, Sponsor Study Number: T-2002-001)

REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

A Study for the Effect of CNTO 148 on Embryo-Fetal Development in Cynomolgus Monkeys by Twice Weekly Subcutaneous Administration ([redacted] Study Number: [redacted] 050.04, Centocor Study Number: T-2003-005) b(4)

A Study for the Effect of CNTO 148 on Pre- and Postnatal Development, including Maternal Function in Cynomolgus Monkeys by Twice Weekly Subcutaneous Administration (Study Number: [redacted] 27-14, Centocor Study Number: T-2004-007) b(4)

LOCAL TOLERANCE (summary reviews only)

A Single Subcutaneous Dose Pharmacokinetics and Injection Site Irritation Study in Monkeys with rTNV148B (Study Number: UHAW-140, Centocor Study Number: T-2000-008)

A One-Month Subcutaneous Dose Pharmacokinetics and Injection Site Irritation Study in Monkeys with rTNV148B (Study Number: UHAW-141, Centocor Study Number: T-2000-009)

A Multiple Subcutaneous Dose Pharmacokinetic and Immune Response Study of rTNV148B in cynomolgus Monkeys (Study Number: UHAW-151, Centocor Study Number: T-2001-007)

SPECIAL TOXICOLOGY STUDIES (summary review only)

Cross-Reactivity of Biotinylated rTNVl48B (Anti-human TNFa Monoclonal Antibody) with Normal Human Tissues ([redacted] Study No. IM668, Centocor Study No. T-2000-004) b(4)

Studies previously reviewed in another submission: modified sponsor summaries included for these studies originally submitted and reviewed as part of BLA 103,772 (Remicade®/Infliximab - Centocor, Inc.). The approved label text from appropriate studies for BLA 103,772 is incorporated into the label for Simponi®.

REPEAT-DOSE TOXICITY

6-Month Chronic Toxicity Study in Mice with cVlq muG2a(cVlq) Anti-Mouse TNFα Monoclonal Antibody [redacted] Study Number: UHAW- 106, Sponsor Study Number: T-098-004) b(4)

REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

Intravenous Dosage Fertility and General Reproduction Toxicity Study of cVlq muG2a [redacted] Anti-Mouse TNF Antibody in Mice (Sponsor Study Number: T-098-003)

Intravenous Developmental Toxicity Study of cVlq muG2a [redacted] Anti-Mouse TNF Antibody in Mice (Sponsor's Study Number: T-096-011) b(4)

Intravenous Developmental and Perinatal/Postnatal Reproduction Toxicity Study of cVlq muG2a ([redacted] Anti-Mouse TNF Antibody in Mice, including Postnatal Behavioral/Functional and Immunological Evaluations (Sponsor Study Number: T-2001-002)

Studies <u>not</u> reviewed within this submission: all other studies (see original eCTD submission for BLA 125,289) and related information for these studies contained in this submission (e.g., sponsor summaries) that are not related to the nonclinical-based human safety assessment and label information, if cited, are done so to provide general summary information and have not been critically evaluated.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Golimumab is a human mAb that neutralizes the biological activities of human tumor necrosis factor alpha (TNFα). TNFα is produced primarily by activated monocytes, macrophages and T-cells as a membrane-anchored, cell surface protein that is released in soluble form by proteolysis. The bioactive form is a homotrimer that binds to receptors on TNFα-responsive cells and as a result enhances the inflammatory and immune response to environmental stimuli and trauma. Based on animal experimental models and

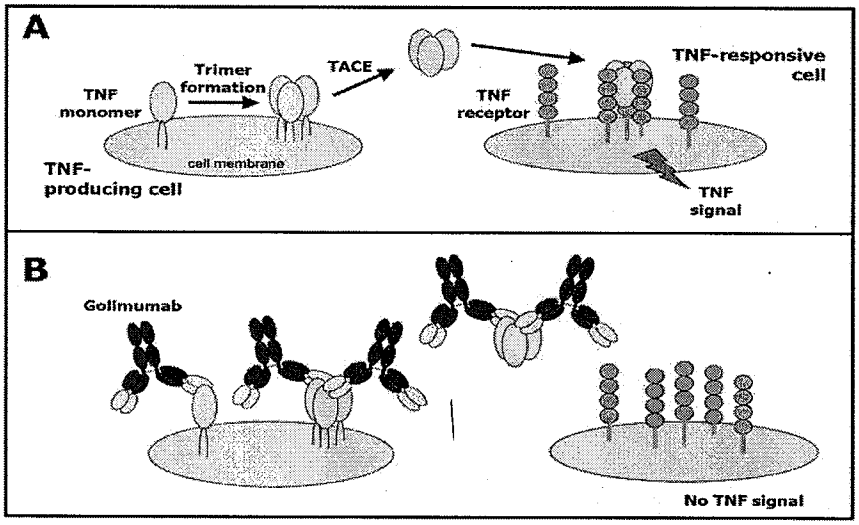
clinical trials, chronic and uncontrolled expression of TNFα has been linked to diseases such as rheumatoid arthritis (RA), inflammatory bowel disease, and psoriasis. Primary pharmacodynamic studies have characterized golimumab binding interactions, mechanism of action, functional effects of TNFα neutralization, Fc-mediated functions, species crossreactivity, and *in vivo* activity supporting rheumatoid arthritis and other indications. Only species crossreactivity with emphasis for selection of most relevant nonclinical animal species, the monkey, will be discussed in any detail.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Golimumab binds with high affinity and specificity to both the soluble and transmembrane bioactive forms of TNFα. The affinity of golimumab for soluble TNFα was 2.4-fold greater than infliximab, while the affinity of both mAbs for transmembrane TNFα was similar. As expected, large complexes were observed between bivalent golimumab and TNFα trimers, and in antibody excess the molecular weight of these complexes was consistent with 3 golimumab molecules bound to 1 or 2 TNFα trimers.

Drug activity related to proposed indication:

TNF is a proinflammatory cytokine that is expressed primarily by activated monocytes, macrophages and T-cells. It is expressed as a 26 kDa type II membrane-bound protein that self-associates into the bioactive homotrimer and is rapidly released by the protease TNFα converting enzyme, or TNF-alpha converting enzyme (TACE). Two receptors for TNFα have been identified, p55 (also known as TNFR1 or CD120a) which is constitutively expressed on virtually all enucleated cells and p75 (also known as TNFR2 or CD120b) whose expression is limited to immune cells and endothelial cells. The binding of trimeric TNFα to either receptor leads to the clustering of the receptor cytoplasmic domains and initiates signaling. Following the activation of innate recognition pathways, TNFα contributes to immune response by promoting the inflammatory process and directing the organization of immune cells within secondary lymphoid tissues. However, uncontrolled production of TNFα can lead to the chronic inflammation that is the hallmark of diseases such as rheumatoid arthritis, inflammatory bowel disease, and psoriasis. The binding of golimumab to human TNFα prevents receptor binding. (see diagram)



Cartoon illustration of the different forms of TNF, TNF complexed with receptors and the mechanism of action of golimumab

(A) TNF monomer is initially expressed on the cell surface of activated monocytes, macrophages and T cells, followed by self-association into homotrimers and cleavage by TACE to release soluble TNF. The bioactive TNF trimer engages TNF receptors (p55 on most cell types, p75 on immune and endothelial cells) and initiates signaling. (B) Golimumab (shown with variable domains in light blue and constant domains in dark blue) binds TNF monomer and to one or more subunits on the transmembrane or soluble TNF trimer which prevents TNF p55 or p75 receptor binding and subsequent intracellular signaling. **Note:** TNF= TNFα

For comparative purposes, TNFα binding of golimumab and infliximab (Centocor BLA 103,772, Remicade®) is listed in the table. While golimumab is a human mAb, infliximab is a chimeric immunoglobulin G1 (IgG1) mAb against human TNFα. Since trimeric forms of both transmembrane and soluble TNFα are bioactive, binding to both forms of TNFα was evaluated. The dissociation constant equilibrium (KD) measured by surface plasmon resonance (SPR) for the binding of soluble TNFα trimer to immobilized golimumab was 18 picomolar (pM) compared to 44 pM for infliximab. This 2.4-fold difference was primarily due to a slower dissociation rate constant observed for golimumab, but was not statistically significant. SPR was also used to demonstrate that golimumab bound with high affinity to the monomeric form of TNFα In summary, based on the previous and other pharmacology data reported by the sponsor, golimumab binds with higher affinity to soluble forms of human TNFα compared to infliximab, while both antibodies demonstrate similar affinities for cell surface forms of TNFα (transmembrane and soluble TNFα). Both antibodies also form similar high molecular weight complexes with TNFα in solution, with a stoichiometry in antibody molar excess of 3 antibody molecules bound to 1-2 TNFα trimer molecules.

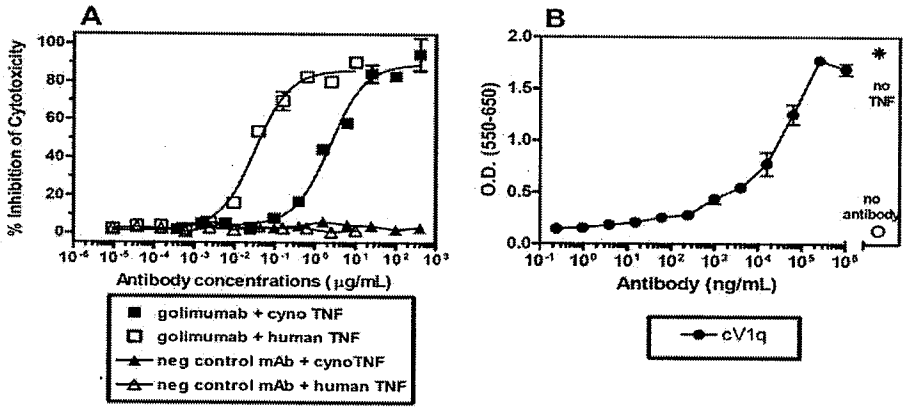
Surface plasmon resonance parameters for the binding of human TNFα trimer to immobilized golimumab and infliximab

Form of Soluble TNFα	Antibody	k_a (low-high) ($M^{-1}s^{-1}$)	k_d (low-high) (s^{-1})	K_D (average) (pM)	K_D (low-high) (pM)
TNFα trimer	golimumab	(3.4 - 4.6) x 10^6	(4.3 - 9.3) x 10^{-5}	18	9-27
	infliximab	(3.2- 4.4) x 10^6	(1.1- 2.0) x 10^{-4}	44	25-63

Note: Binding constants were derived after fitting the data to a simple 1:1 binding model.

Golimumab binds specifically to human TNFα, but shows no binding to other representative members of the TNF ligand superfamily such as lymphotoxin alpha [LTα], lymphotoxin alpha/beta heterotrimer [LTα1/β2], and others that show sequence homology with TNFα. In addition, golimumab did not neutralize LTα-mediated cell cytotoxicity.

Relevant animal species for toxicity and efficacy studies were determined by analysis of golimumab neutralization of either recombinant TNFα or TNFα derived from Lipopolysaccharide (LPS) stimulated monocytes for each animal species tested. Golimumab was most potent against human and chimpanzee TNFα (IC50 ~3 ng/mL), with IC50s 10-72 fold higher against baboon, rhesus, cynomolgus, pigtail macaque and dog TNFα. IC50s were 400-4,300 fold higher against marmoset, cottontop tamarin, rabbit and guinea pig TNFα. No neutralization of rat or mouse TNFα by golimumab was observed. Further experiments with recombinant cynomolgus TNFα demonstrated high affinity binding by golimumab and efficient neutralization (see diagram) though this represents a 34-fold lower affinity and 72-fold lower potency compared with human TNFα .



Golimumab neutralization of recombinant cynomolgus TNF and cV1q neutralization of recombinant mouse TNF in cell cytotoxicity assays.

Panel A: A fixed concentration of cynomolgus or human TNF (100 pg/mL) was pre-mixed with the indicated concentrations of golimumab and incubated with murine WEHI cells. Each data point represents the mean ± SEM of duplicate wells. Data are normalized to media control (no TNF) as 100% inhibition and TNF control (no antibody) as 0% inhibition. Panel B: Varying amounts of cV1q was pre-mixed with 100 pg/mL of mouse TNF and then incubated on murine WEHI cells for 16 hr at 37°C. The cV1q antibody and muTNF alone were included as controls. Each data point represents the mean ± SEM of duplicate wells. **Note:** TNF= TNFα

In addition, since golimumab did not neutralize mouse TNFα, an analogous anti-mouse TNFα mAb (——IgG2a isotype, the functional equivalent of the human IgG1 isotype) designated cV1q was selected and characterized (see above panel B). The cV1q mAb was shown to bind mouse TNFα with high affinity and effect mouse TNFα function similarly to golimumab in monkeys. These observations demonstrate that cV1q shares key characteristics with golimumab and is a suitable analogous anti-mouse TNFα mAb for toxicology studies in mice of which chronic and developmental toxicology studies were submitted to support the infliximab BLA and this application. b(4)

While the above *in vitro* pharmacology studies showed that golimumab was up to 72-fold less potent at binding to and neutralizing the effects of cynomolgus TNFα than human TNFα, the doses and the dose regimens selected for the toxicology studies were considered sufficient to produce sustained high serum golimumab concentrations that were greatly in excess of the concentrations required to neutralize all TNFα in monkey serum and were greatly in excess of clinical exposure levels. The presence of 5 µg/mL of golimumab was shown to be sufficient to completely neutralize up to 25 pg/mL of recombinant cynomolgus monkey TNFα, a 10-fold excess over the concentration of cynomolgus TNFα in normal healthy monkey serum. In the repeated dose toxicity studies, trough serum concentrations of golimumab ranged from approximately 400 to 500 µg/mL after the first dose and from approximately 1200 to 2000 µg/mL at steady state. Therefore, the serum concentrations obtained in the monkeys in the repeated dose studies are well in excess of the concentrations required to neutralize cynomolgus monkey TNFα *in vivo* and, therefore, such nonclinical data can be utilized to demonstrate nonclinical-based human safety of golimumab.

2.6.2.3 Secondary pharmacodynamics

While the sponsor reported that several laboratories have reported significant mitigation of pathological symptoms in [redacted] models of human disease following treatment with cV1q, no such studies were submitted by the applicant. b(4)

2.6.2.4 Safety pharmacology

Safety pharmacology endpoints to assess golimumab were incorporated into the monkey intravenous (IV) and subcutaneous (SC) repeated dose toxicology studies and the SC pre- and post-natal reproductive toxicology study that also included neonatal assessments. The safety pharmacology endpoints incorporated into these studies included measures of heart rate, blood pressure and electrocardiograms to assess cardiovascular safety, respiratory rate to assess respiratory safety and body temperature and daily clinical cage side observations to evaluate CNS safety. No adverse effects of golimumab were observed in these parameters following single and repeated dosing of cynomolgus monkeys via intravenous or subcutaneous routes of administration at doses up 50 mg/kg twice weekly. The NOAEL value for these studies was the highest dose tested of 40 mg/kg. AUC-based safety margins range from 20-94 (no neonate AUCs) and Cmax-based SMs range from 314-1536 with neonates included (SM of 314), indicating more than adequate human safety based as a SM of ≥ 1 is usually acceptable. Study details are included in individual review of the studies in sections 2.6.6.3 & 2.6.6.6 with summary tables in section 2.6.3.

2.6.2.5 Pharmacodynamic drug interactions

No *in vivo* drug-drug interaction studies with golimumab were conducted in nonclinical species. Potential drug-drug interactions (i.e., impact of methotrexate on golimumab PK parameters) were investigated in population PK analyses from 4 Phase 3 studies.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Safety Pharmacology

Test Article: Golimumab

Organ Systems Evaluated	Species/Strain Sex/No. Per Group	Route (Dosing regimen)	Doses (mg/kg)	Noteworthy Findings	GLP Compliance	Study No. Location in CTD
Cardiovascular, respiratory, CNS[a]	Cynomolgus macaque (young adult) 5/sex/group	IV (Once weekly for 1-month)	0, 10, 50	No noteworthy findings for indirect blood pressure, respiratory rate, body temperature or clinical cageside observations. On Day 10, females treated with low or high dose golimumab had slightly lower heart rates (↓8%) and longer RR intervals (↑8%) than controls. The QTc interval for high-dose females on Day 10 was longer than controls (↑10%), and the QRS duration for low dose males on Days 10 and 29 was longer than controls (↑18%). These changes were small in magnitude and were considered not clinically or toxicologically significant. No changes in heart rate or ECG on Day 29 and 59.	GLP	T-2000-007 4.2.3.2
Cardiovascular, respiratory, CNS[a]	Cynomolgus macaque (young adult) 8/sex/group	IV (Once weekly for 6-months)	0, 25, 50	No noteworthy findings for ECG, heart rate, respiratory rate, body temperature or clinical cageside observations. On week 13 low dose males had statistically lower mean blood pressure (↓16%). On week 25 females had statistically lower systolic blood pressure (↓11% at 25 mg/kg, ↓12% at 50 mg/kg). These changes were small in magnitude and were considered not clinically or toxicologically significant. No changes in blood pressure on week 6, 25 or 36.	GLP	T-2004-006 4.2.3.2
Cardiovascular, respiratory, CNS[a]	Cynomolgus macaque (young adult) 8/sex/group	SC (Twice weekly for 6-months)	0, 25, 50	No noteworthy findings for ECG, heart rate, respiratory rate, body temperature or clinical cageside observations. During week 5, mean systolic blood pressure was significantly lower than controls (↓41%) and mean arterial pressure was significantly lower than the controls (↓32%) in the low dose males. These differences were considered unlikely related to golimumab. No changes in blood pressure on weeks 13, 26, 30 and 38.	GLP	T-2002-001 4.2.3.2

Organ Systems Evaluated	Species/Strain Sex/No. Per Group	Route (Dosing regimen)	Doses (mg/kg)	Noteworthy Findings	GLP Compliance	Study No. Location in CTD
Cardiovascular[a]	Cynomolgus macaque (8 – 11 infants/group)	*in utero* and/or via mother's milk (Twice weekly from the end of organogenesis to Day 33 of lactation ~5-months)	0, 25, 50	No noteworthy findings for ECG, heart rate, respiratory rate, body temperature or clinical cageside observations.	GLP	T-2004-007 4.2.3.5.3

[a]Conducted as part of repeated dose toxicity studies

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Because golimumab is a mAb, and thus is expected to be catabolized into individual amino acids then be excreted or reused by the body rather than be metabolized by hepatic cytochrome P_{450} mechanisms, traditional ADME (absorption, distribution, metabolism and elimination) studies were not performed. Nevertheless, PK/TK data from these studies adequately addressed the relationship of systemic exposure to golimumab and its toxicity following subcutaneous (SC) or intravenous (IV) administration in cynomolgus monkeys in accordance with regulatory guidances (refer to ICH S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals).

The majority of Pharmacokinetics/Toxicokinetics (PK/TK) data were generated in support of the Cynomolgus monkey toxicology studies to adequately assess the exposure of golimumab. These toxicology studies and cell line and formulation comparison studies are considered the primary studies for this assessment. TK data from the GLP chronic toxicology studies are most relevant for the intended adult population, but TK data from the developmental toxicology is also relevant for select potential patient populations. PK comparability studies linked the early and late development stage of golimumab used in both nonclinical and clinical phases in regard to differing cell lines producing the drug substance (DS) and differing formulations of lyophilized versus liquid formulations of the drug product (DP). The DSs and DPs utilized throughout the drug development program are considered comparable. In summary, PK and immune response comparability was demonstrated between DS from different golimumab producing cell lines and different DP formulations used throughout the course of development. Taken as a whole, the data from the nonclinical program were considered sufficient to support the clinical development of golimumab. See Product Quality and Clinical Pharmacology reviews for details.

Human PK data used in the nonclinical-based safety assessment included the median steady state Cmax value of 1.71 µg/mL form the pivotal clinical trials for RA, PsA, and AS as provided by the sponsor and the AUC value of 75 µg•day/mL provided by the ClinPharm reviewer.

2.6.4.2 Methods of Analysis

Validated analytical methods were used to quantify golimumab in cynomolgus monkey biological fluids and for anti-golimumab antibody determination. These methods were not reviewed.

2.6.4.3 Absorption

For the purposes of this review, the primary focus of Golimumab PK was in cynomolgus monkeys following repeated SC or IV administrations of golimumab at doses up to 50 mg/kg. Cmax and area under concentration-time curve (AUC) increased in an approximately dose-proportional manner and serum steady-state profiles were indicative of linear PK and lack of accumulation. Similar PK was also observed in humans. Mean terminal half-life ($t_{1/2}$) ranged from 11.16 days to 17.66 days in primary monkey toxicology studies after IV and SC administrations, respectively. No apparent gender differences in the golimumab PK/TK analyses were observed. Refer to individual and summary descriptions of toxicology studies for more detail.

Related to the proposed DS, PK for DS produced by differing cell lines [redacted] used during drug development were compared in a single dose SC study in monkeys (see table). Golimumab manufactured from [redacted] cell line showed similar PK characteristics compared with golimumab manufactured from [redacted] cell line allowing uniform handling of all data. See Product Quality review for more detail. b(4)

Pharmacokinetics: Absorption After a Single Dose

Test Article: rTNV148B/Golimumab

Location in CTD	4.2	
Study No. (Report No.)	P-2002-008 (CP2002T-047)	
Species	Cynomolgus Monkeys	
Lot Number	[redacted] Lot 4841-85	[redacted] Lot 5139-83/2
Route	Subcutaneous	Subcutaneous
Dose (mg/kg)	10	10
Number of Animals	10	10
Sample (whole blood, plasma, serum, etc.)	Serum	Serum
Analyte	CNTO148	CNTO148
Assay	ELISA	ELISA
Pharmacokinetic Parameters, Mean ± SD		
Cmax (µg/mL)	54.26±16.13	55.63±17.58
tmax (day)	2.20±0.79	2.80±1.69
AUC (µg.day/mL)	571.69±229.50	447.61±129.42
$t_{1/2}$ (day)	0.46±0.14	0.47±0.12
CL/F (mL/day/kg)	20.74±9.79	24.17±7.16
Vss/F (mL/kg)	NA	NA
Additional Information	There is no significant difference being observed between two cell lines.	

ELISA: Enzyme linked immunosorbent assay
NA = Not applicable

b(4)

Related to the proposed DP, PK for DP of differing formulations (lyophilized versus liquid) used during drug development were compared in a single dose SC study in monkeys (see table). Both formulations showed similar PK characteristics allowing uniform handling of all data. See Clinical Pharmacology review for details.

Pharmacokinetics: Absorption After a Single Dose

Test Article: rTNV148B/Golimumab

Location in CTD	4.2		
Study No. (Report No.)	P-2005-003 (CP2005T-024, CP2005T-024-A1)		
Species	Cynomolgus Monkeys		
Lot Number	LotD03PG7263 (Phase 2Lyopilized)	Lot 8172:070 (Phase 3Liquid)	Lot 00002 (Phase 3Lyophilized)
Route	Subcutaneous	Subcutaneous	Subcutaneous
Dose (mg/kg)	3	3	3
Number of Animals	15	15	15
Sample	Serum	Serum	Serum
Analyte	CNTO 148	CNTO 148	CNTO 148
Assay	ECLIA	ECLIA	ECLIA
Pharmacokinetic Parameters, Mean ± SD			
Cmax (µg/mL)	22.98±8.95	22.71±5.88	24.24±7.41
tmax (day)[a]	3.00 (2.00 to 7.00)	3.00 (1.00 to 5.00)	5.00 (2.00 to 7.00)
AUC (µg.day/mL)	181.79±70.79	162.74±53.33	194.70±75.37
$t_{1/2}$ (day)[a]	1.08 (0.45 to 1.65)	0.49 (0.47 to 3.15)	1.01 (0.45 to 1.96)
CL/F (mL/day/kg)	19.84±10.16	20.01±5.38	17.54±6.39
Vz/F (mL/kg)	27.46±21.55	21.64±14.97	24.78±20.55
Additional Information	The Phase 3 liq and Phase 2 Lyo were biocomparable; the Phase 3 Lyo and Phase 2 Lyo were similar.		

[a]Refers to median (range) value
ECLIA: ElectroChemiLuminescent ImmunoAssay

Human AUC values – In calculation of nonclinical-based human Safety Margins (SM)s, the AUC value to be used is 75 µg*day/mL. This value is based on the following considerations from the ClinPharm reviewer. From healthy subjects, the mean AUC_{inf} after a single 50 mg dose was ~48 µg*day/mL (assuming linear PK between 50 and 100 mg). For RA patients, assuming linear PK, the mean AUC_{inf} after a single 50 mg dose would be 75 µg*day/mL (average from 0.3, 0.6 and 3 mg/kg single dose data). Although the AUC_{inf} in RA may be less accurate due to a >30% of AUC value was from extrapolation, using the larger AUC value from RA patients is more conservative in

calculating SMs as the human value as the denominator,. Refer to ClinPharm review (Lei Zhang) for documentation of this human value.

2.6.4.4 Distribution

The sponsor reported that the volume of distribution at steady state of golimumab in monkeys was approximately equivalent to or slightly larger than their serum volumes, suggesting that only a small percentage of golimumab was distributed outside the intravascular space. This is consistent with distribution of mAbs. Therefore, biodistribution studies were not conducted for golimumab. Golimumab was shown to cross the placenta into developing fetuses (see embryo-fetal study in monkeys).

The distribution of golimumab to fetuses was examined after SC dosing in an embryofetal development study and a pre- and post-natal study in cynomolgus monkeys (study details in section 2.6.6.6). Fetal exposure to golimumab was proportional to the increase in SC dose administered to the dams. In the embryofetal study, golimumab was shown to cross the placenta and substantial exposure to golimumab was observed in the fetal circulation. Mean fetal/maternal ratio was 0.38 in the 25 mg/kg dosing group (ranging from 0.25 to 0.56) and 1.32 in the 50 mg/kg dosing group (ranging from 0.51 to 3.61) ~50 days after the last maternal dose. The fetal/maternal distribution ratio for one high dose animal was extremely high (3.61) compared to the rest of fetuses in the 50 mg/kg group (0.51-0.59). Excluding this high value would result in a mean distribution ratio of 0.56 for the high dose group. In the peri- and post-natal study, neonates also had significant exposure to golimumab as the mean fetal/maternal ratio on ~30 days after delivery (only comparable sampling days available) was 0.16 and 0.18 for the 25 and 50 mg/kg dose groups, respectively. Fifteen days after birth, mean serum golimumab concentrations in these neonates that were exposed to golimumab *in utero* were 218.57 and 536.53 µg/mL in the 25 and 50 mg/kg dose groups, respectively.

2.6.4.5 Metabolism

As a human IgG1 mAb, golimumab is presumably degraded into small peptides and individual amino acids via catabolic pathways that are typically associated with endogenous IgG. Therefore, classical biotransformation studies as performed for small molecules were not performed. This is consistent with other similar mAbs that have been reviewed.

2.6.4.6 Excretion

Similar to other IgG1 mAbs, golimumab is presumably eliminated via catabolic pathways that is typically associated with endogenous IgG. Thus, routine studies that attempt to assess mass balance were not conducted as the were not expected to be informative (refer to ICH S6 guidance).

2.6.4.7 Pharmacokinetic drug interactions

No *in vivo* drug-drug interaction studies with golimumab were conducted in nonclinical species. Potential drug-drug interactions such as the impact of methotrexate on golimumab PK parameters were investigated in population PK analyses from 4 Phase 3 studies. See Medical review for details.

2.6.4.8 Other Pharmacokinetic Studies

Immune response – Although assessment of antibodies to golimumab in non-human primates may not be considered predictive of immunogenicity expected in humans for the fully human mAb, anti-golimumab antibody development was examined to determine the influence of immune responses on golimumab PK/TK parameters and exposure in toxicity studies as well as to identify potential changes in golimumab immunogenicity following changes in cell line or drug formulation. As noted previously, cell lines were considered comparable as to drug substance produced. As to immunogenicity, treated animals were characterized as having an inconclusive anti-product antibody response because the lack of detection of an antibody response may have been compromised by high serum levels of golimumab. However, antibodies were detected in some low dose animals and animals which had faster clearance. A competitive binding test confirmed that this immune response was specific for golimumab. See individual study reviews in sections 2.6.6.3 and 2.6.6.6 for more detail.

2.6.4.9 Discussion and Conclusions

Golimumab PK/TK parameters were well characterized in cynomolgus monkeys. Approximately dose-proportional pharmacokinetics was observed following administration of golimumab in monkeys. The incidence of detectable anti-golimumab antibody development was higher at lower doses in cynomolgus monkeys, but detection in animals may have been masked by high levels of drug. The PK/TK data generated in support of primary toxicology studies adequately assessed exposure of golimumab. Significantly higher exposures to golimumab were achieved at doses used in toxicology studies than at the proposed dose used in clinical studies (50 mg every 4 weeks). PK and immune response comparability was demonstrated between golimumab cell lines and formulations used throughout the course of development. Taken as a whole, the data from the nonclinical program were sufficient to support the clinical development of golimumab.

2.6.4.10 Tables and figures to include comparative TK summary - see above

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

See individual toxicology studies for tables.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Acute-dose toxicity:

Acute toxicity studies designed to evaluate the potential adverse effect of doses causing major (life-threatening) toxicity were not conducted by the sponsor as the sponsor considered them not appropriate for mAbs that have inherently low toxicity and that can only be tested in non-human primates.

Repeat-dose toxicity:

One month IV monkey study: Male and female cynomolgus monkeys were dosed intravenously with 0, 10, or 50 mg/kg golimumab weekly for 1 month then euthanized 1 week after the 4th dose (3/sex/dose) or after a 5-week recovery period (2/sex/dose). Safety pharmacology evaluations were incorporated into the design of this study and the other toxicology studies with golimumab reported in this section. No mortality or golimumab-related clinical signs of toxicity were observed during the dosing and post-dose recovery periods. No treatment-related effects were observed for body weight, food consumption, body temperature, physical examination, ECG, blood pressure, heart rate, ophthalmic evaluations, hematology, coagulation and serum chemistry parameters, immunotoxicity evaluations for lymphocyte subsets and Keyhole Limpet Hemocyanin (KLH – analysis of ability to have an immune response), organ weights, macroscopic, histopathologic or immunohistopathologic evaluations. Four of the 10 monkeys in the 10 mg/kg treatment group cleared golimumab more rapidly from the sera than the other 6 monkeys. Sera golimumab concentrations for these 4 monkeys, just prior to the fourth dose, were below quantification limits of the assay. These 4 monkeys were shown to have developed immune antibody responses to golimumab. No immune antibody responses to golimumab were observed in the other 6 monkeys at 10 mg/kg or for any of the 10 monkeys administered 50 mg/kg. However, as with the other monkey studies, the immune response results for these animals can be considered inconclusive due to possible assay interference from the presence of high circulating serum concentrations of golimumab at the time points studied in the slower golimumab clearers and at the higher dose. In conclusion, treatment with golimumab by weekly IV injection at doses of 10 and 50 mg/kg for 1 month was well tolerated. The NOAEL was 50 mg/kg with associated, gender combined Cmax values of 2205 and 2448 µg/mL on days 1 and 22, respectively (AUCs not reported). The median terminal half-life was 4 days except in monkeys with antibody response (rapid clearance) where the value was 1.8 days. Steady state conditions were not achieved as will be noted for the other studies where the half-life was approximately 2 weeks.

Chronic monkey IV study: Male and female cynomolgus monkeys were administered 0, 25, or 50 mg/kg golimumab intravenously once weekly for 25 weeks with an interim

sacrifice at 13 weeks and a 12-week recovery period. There were no golimumab treatment-related effects on mortality, clinical observations and physical examination, ophthalmic examination, body weights and food consumption, ECG, blood pressure or heart rate, lymphocyte subsets, hematology, blood coagulation, serum chemistry, urinalysis, organ weights, and macroscopic and microscopic pathology. Golimumab administration was associated with dose-responsive decreased IgG and IgM antibody responses to a KLH analysis with IgG antibody production being more affected than IgM antibody production. For example, 8 samples were taken during the dosing and recovery period from day 20 to 253 with the incidence of IgG response 12 of 16, 5 of 16, and 1 of 16 for the control, 25 mg/kg, and 50 mg/kg groups, respectively on day 69. For IgM, the incidence was 6 of 16, 5 of 16, and 1 of 16, respectively, on day 92. Antibodies to golimumab were detected in 1 out of 32 animals dosed with golimumab. The positive response occurred in a female from the 25 mg/kg treatment group that exhibited accelerated clearance of golimumab at the terminal phase relative to the other animals. Anti-golimumab antibodies were not detected in any of the other 31 animals. However, the immune response results for these animals were considered inconclusive due to possible assay interference from the presence of circulating serum concentrations of golimumab at the time points studied. Considering the observed, anticipated pharmacological effects on the immune system, the NOAEL was 50 mg/kg. Mean, gender-combined toxicokinetic values at steady state were 1,620 & 2,627μg/mL (Cmax) and 5,809 & 11,635 μg•day/mL (AUC) for the 25 & 50 mg/kg groups, respectively, with terminal half-lives of 11 and 18 days.

Chronic monkey SC study: Male and female cynomolgus monkeys were dosed subcutaneously with 0, 25, or 50 mg/kg golimumab biweekly for 6 months with interim assessments, including necropsy, at 3 months and terminal assessment at 6 months and 9 months (recovery animals). Treatment-related clinical symptoms and histological observations were reversible dosing site inflammation and edema. Humoral/immunomodulatory responses were not affected by treatment as indicated by KLH analysis (unlike the 6-month IV study in monkeys), lymphocyte subset phenotypes analysis, and immunohistopathology of lymphoid organs. Reversible increases in B-lymphocytes were observed after dosing and golimumab-treated animals were characterized as having an inconclusive anti-product antibody response because the lack of detection of an antibody response in all but 1 low dose female may have been compromised by high serum levels of golimumab. No other notable findings occurred for a complete protocol that included a safety pharmacology component. The systemic NOAEL was 50 mg/kg as only anticipated pharmacological effects and reversible and clinically monitorable local injection site effects (minimal irritation) occurred at 50 mg/kg. Mean, gender combined terminal AUC values were 2622 and 5657 μg•day/mL and Cmax values were 1119 and 2459 μg/mL for the 25 and 50 mg/kg groups, respectively, at the end of the study period with half-lives of 16 and 14 days.

Chronic mouse IV study: Male and female CD-1 mice were treated with 0, 10, or 40 mg/kg of anti-mouse TNFa monoclonal antibody cVlq weekly for 6 months by intravenous injection. Necropsy groups included interim (Week 13), main (Week 26), and recovery (Week 39) groups. There were no cVlq treatment-related effects on clinical

observations, body weight, food consumption, hematology, serum chemistry, ophthalmology, and organ weights. There were no gross or microscopic pathological findings considered cVlq treatment related. Only a few animals had detectable anti-cVlq antibody responses, but the responses were not strong or specific neutralizing responses and had no apparent effect on the clearance of cVlq from the serum. High serum levels of cVlq were detected, indicating exposure. Based on the results of this study, NOAEL was 40 mg/kg.`

Genetic toxicology: Genotoxicity tests have not been conducted with golimumab. The range and type of genotoxicity studies routinely conducted for pharmaceutical drugs are not applicable to biopharmaceutical antibodies as is consistent with ICH S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals. (modified sponsor summary)

Carcinogenicity: Carcinogenicity tests have not been conducted with golimumab. The carcinogenic potential of golimumab cannot be evaluated in standard 2-year bioassays in rodents because golimumab does not neutralize rodent TNFα. On this basis, the carcinogenic potential of golimumab was evaluated by attempting to understand the mechanism of action of the therapeutic mAbs for the TNFα class of drugs and by the monitoring of patients as part of a risk management plan. Preneoplastic/neoplastic lesions were assessed as part of chronic studies in mice and monkeys with no indications of any treatment-related preneoplastic/neoplastic lesions.

Reproductive toxicology: Reproductive toxicology studies have not been conducted with golimumab in the traditional rodent and rabbit nonclinical models as golimumab does not neutralize rodent or rabbit TNFα. Golimumab has been tested for embryofetal and prenatal and postnatal effects in an appropriate nonclinical test species, cynomolgus monkeys. As part of BLA 103,772 (Infliximab), the effects of anti-TNFα treatment on reproductive toxicology were assessed in the mouse using cV1q, an analogous anti-mouse TNFα mAb that allowed for the evaluation of fertility and early embryonic development, embryofetal development, and prenatal and postnatal development.

Embryofetal monkey study: Pregnant female cynomolgus monkeys were dosed by the subcutaneous route twice weekly from days 20 to 51 of gestation at doses of 0, 25, or 50 mg/kg golimumab. High dose group dams gained 8% less weight than controls while low dose dams gained comparable weight as controls. The immunological evaluations did not reveal any treatment-related effects on antibody production. However, drug antibody production was characterized as being an inconclusive anti-product antibody response because detection of an antibody response in all but 1 low dose female may have been compromised by high serum levels of golimumab. The immunohistochemical evaluation for immunoreactivity of spleen, thymus and mesenteric lymph node tissue sections did not reveal any golimumab effects on the B and T lymphocytes and hematopoietic stem cells. Fetal blood levels detected at cesarean section (days 100-103, ~4 half-lives after last maternal dose when maternal levels were 3-5% of levels of last dosing day 51) were variable and the fetal:maternal ratios ranged from 0.25-0.56 (mean of 0.38) at 20 mg/kg and from 0.51-3.61 (mean of 1.32) at 50 mg/kg. The 50 mg/kg group ratio is based on 4

fetal animals with ratios of 0.51, 0.57, 0.59, & 3.61, suggesting that fetal/maternal ratio 50 days after the last dose was more likely to be ~0.5. No effects were observed on fetal viability, fetal weight, placental weight, external measurements, organ weights, or fetal external, placental, viscera or skeletal findings in either the male or female fetuses. The No Observed Adverse Effect Level was 50 mg/kg/day for both pregnant cynomolgus monkeys and for embryo-fetal development with an associated TK values of 27,051 µg•day/mL (AUC) and 1,613 µg/mL (Cmax) in the maternal animals with a half-life of 11 days.

Prenatal and postnatal monkey study: Pregnant female cynomolgus monkeys were dosed by the subcutaneous route twice weekly from day 50 of gestation to day 33 after delivery at doses of 0, 25, or 50 mg/kg golimumab. Nothing remarkable was noted for maternal animals or neonates. The immunological and immunohistochemical evaluations did not reveal any treatment-related effects on antibody production, the B and T lymphocytes and hematopoietic stem cells, and associated organs. However, drug antibody production was characterized as being an inconclusive anti-product antibody response because the lack of detection of an antibody response may have been compromised by high serum levels of golimumab.
The NOAEL was 50 mg/kg/day for both pregnant cynomolgus monkeys and neonatal development associated with maternal Cmax values of 1,482 µg/mL and highest neonate blood levels of 537 µg/mL. Maternal milk levels were 3.6 µg/mL at 50 mg/kg. Up to ~50% of maternal blood levels were measured in the neonate blood.

Developmental toxicology studies in mice: Fertility and early embryonic development, embryofetal, and prenatal and postnatal studies were conducted in CD-1 mice at doses of 10 or 40 mg/kg using an analogous anti-mouse TNFα mAb cV1q with dosing by the intravenous route. These studies showed parental and offspring exposure to cV1q but no anti-TNFα mAb treatment-related effects. A few of the cV1q treated mice died during treatment, but these deaths appeared to be a result of an immune reaction such as hypersensitivity due to the repetitive injection of the cV1q protein. Mice did develop anti-cV1q antibodies and when deaths occurred they tended to occur on the day of dosing following repeated dose administrations. Even though some mice did develop anti-cV1q antibodies during the study, mice continued to be exposed to high serum concentrations of cV1q throughout the treatment periods. In the prenatal and postnatal study, a reduction in the pregnancy rate of F1 mice was considered of none to minimal toxicological relevance as the pregnancy rate (76%) was only slightly below the historical rate (83 to 100%) and another study with cV1q in mice revealed no maternal or developmental toxicity up to 40 mg/kg dosage. In addition, no treatment-related effects were observed in the F2 generation. Based on the immunological parameters evaluated, administration of cVlq to male and female CD-1 mice did not adversely affect the immune function in F1 mice, with the possible exception of a decrease in humoral immune response at the 40 mg/kg dosage level. With the possible exception of this anticipated pharmacological action of cV1q, intravenous administration of cVlq at dosages of up to 40 mg/kg/day in CD-1 mice did not result in developmental toxicity.

Local tolerance: Single and repeated SC injections in monkeys with golimumab at doses up to 50 mg/kg were well tolerated at the injection site with minimal local irritation being observed. Human SC dosing as proposed in this application is 50 mg (~1 mg/kg) at a concentration of 100 mg/mL. The largest SC monkey dose was 50 mg/kg of a similar concentration dose (100 mg/mL). Therefore, the concentration of the dosing solutions in the monkey and human studies are equivalent relative evaluation of potential local tolerance/injections site effects.

A single SC injection of golimumab at a dose of 10 mg/kg (100 mg/mL, 0.1 mL/kg) was well tolerated by cynomolgus monkeys and did not produce a clinically significant degree of irritation at the injection site. Multiple SC injections (twice weekly for 3 weeks) of golimumab at a dose of 50 mg/kg (100 mg/mL, 0.5 mL/kg) were well tolerated by cynomolgus monkeys when administered as repeated SC injections. Minimal signs of local irritation (erythema, edema, pain, heat, and thickening) were observed at the injection sites (no histopathological examination). Multiple SC injections (twice weekly for 1 month) of golimumab at a dose of 10 mg/kg (100 mg/mL, 0.1 mL/kg) were well tolerated by cynomolgus monkeys. Macroscopic observations of the injection sites showed minimal signs of local irritation. Histopathological evaluation of the SC injection sites revealed chronic, mild SC inflammation and vasculitis. These histopathological findings were not observed in the vehicle control monkeys. Therefore, these pathological findings were reported to be related to a local immune response that resulted from multiple SC injections of a foreign protein (human IgG) in cynomolgus monkeys and not related to the anti-TNFα inhibition of golimumab. This conclusion was reported to be supported by the detection of antibodies to golimumab in all the golimumab-treated monkeys, but without comparative testing of a human IgG isotype control, the determination of not being golimumab-related may be not completely defensible. Based on the biweekly SC dosing in monkeys at 50 mg/kg compared to the proposed human dosing of 0.5 mL (1 mg/kg) at the same drug concentration (100 mg/mL) with minimal local effects, the proposed SC dosing is considered as safe and clinically monitorable.

Special toxicology: A GLP *in vitro* cross-reactivity study was performed on cryosections of normal, adult human tissue specimens to determine the potential for biotinylated golimumab to stain human tissues. The study showed that golimumab did not stain the majority of the normal human tissue sections evaluated. There was slight-moderate staining of the adnexal epithelium of the skin from 2 of 3 donors and slight staining of the keratinocytes in the epidermis (stratum malpighi) of nipple skin overlying the breast (mammary gland) from 1 of 3 donors. The staining was only observed at the 10 µg/mL concentration and not at 1 µg/mL. On this basis, golimumab is not considered to possess risk for significant binding to skin after dosing.

2.6.6.2 Single-dose toxicity - no studies conducted (see 2.6.6.1 – Overall Toxicology Summary)

2.6.6.3 Repeat-dose toxicity – Full set of data tables for all studies in section 2.6.7 – Toxicology Tabulated Summary

One month IV monkey study: see 2.6.6.1 - Overall Toxicology Summary for study summary

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Study title: 25-Week Intravenous Dose Toxicity Study with CNTO 148 in Cynomolgus Monkeys with a 12-Week Recovery Period

Key study findings:

- Male and female Cynomolgus monkeys were administered 0, 25, or 50 mg/kg CNTO 148 intravenously once weekly for 25 weeks with an interim sacrifice at 13 weeks and a 12-week recovery period.
- There were no CNTO 148 treatment-related effects on mortality, clinical observations and physical examination, ophthalmic examination, body weights and food consumption, ECG, blood pressure or heart rate, lymphocyte subsets, hematology, blood coagulation, serum chemistry, urinalysis, organ weights, and macroscopic and microscopic pathology.
- CNTO 148 administration was associated with dose responsive decreased IgG and IgM antibody responses to a KLH challenge with IgG antibody production being more affected than IgM antibody production.
- Antibodies to CNTO 148 were detected in 1 out of 32 animals dosed with CNTO 148. The positive response occurred in a female from the 25 mg/kg treatment group. The immune response positive female exhibited accelerated clearance of CNTO 148 at the terminal phase relative to the other animals. Anti- CNTO 148 antibodies were not detected in any of the other 31 animals. However, the immune response results for these animals were considered inconclusive due to possible assay interference from the presence of circulating serum concentrations of golimumab at the time points studied.
- Considering anticipated pharmacological effects, the NOAEL was considered to be 50 mg/kg. Mean, gender-combined toxicokinetic values at steady state were 1,620 & 2,627µg/mL for Cmax and 5,809 & 11,635 µg•day/mL for AUC for the 25 & 50 mg/kg groups, respectively, with terminal half-lives of 11 and 18 days.

Study no.: Study Number: UHA00035, Sponsor Study Number: T-2004-006
Volume # and page #: eCTD
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: June 29, 2004 (report date May 18, 2006)
GLP compliance: yes
QA report: yes (x) no ( )

Drug, lot #, and % purity: CNTO 148, D03PG7263, 98.9% (within standard)
- Imject Mariculture Keyhole Limpet Hemocyanin (KLH), lot # FA67499,

Methods:

Doses: 0, 25, & 50 mg/kg

Species/strain: male and female Cynomolgus monkeys

Number/sex/group or time point (main study): 3/sex/group

Route, formulation, volume, and dose duration: bolus intravenous injection, liquid in 0.9% sodium chloride, 1-2 mL/kg, weekly for up to 25 weeks

Satellite groups used for toxicokinetics or recovery: all animals for TK, 3/sex/group for interim sacrifice, 2/sex/group for 12-week recovery

Group Number	Number of Animals		Test Article	Dosage Level (mg/kg)	CNTO 148 Concentration (mg/mL)	Dosage Volume (mL/kg)	Dosing Regimen	Necropsy Day
	Males	Females						
1	8	8	0.9% Sodium Chloride Injection	0	0	2	IV, once weekly for up to 25 doses	3 per sex per group on Day 92
2	8	8	CNTO 148	25	25	1		3 per sex per group on Day 176
3	8	8		50	25	2		2 per sex per group on Day 253

Age: not reported

Weight: groups means 2.28-2.44 kg (males) and 2.26-2.36 (females) day before dosing initiation

Sampling times:

Unique study design or methodology (if any):
- On Day 13, all animals were administered a 1-mL dose of 10 mg/mL Keyhole Limpet Hemocyanin (KLH)/mL intramuscularly

Observation and Times:

Dose formulation: Determination of concentration and stability of the formulated test article was conducted by the Sponsor.

Clinical signs: morbidity/mortality (2x/day), clinical observations (1x daily)

Body weights: Body weights were recorded at least three times prior to initial treatment (including one measurement on Day -1), approximately weekly thereafter, and on the day prior to necropsy. A final, fasted body weight was taken on the day of necropsy for the calculation of organ to body weight percentages.

Food consumption: daily

Ophthalmoscopy: performed by a board-certified veterinary ophthalmologist prior to the initial treatment and during Weeks 6, 13, 25, and 36.

EKG: Electrocardiogram (ECG) tracings were obtained using 12 leads prior to initial treatment and during Weeks 6, 13, 25, and 36. Ten-second tracings were obtained at each time point using a chart speed of 50 mm/second. The ECG tracings were evaluated by a board-certified veterinary cardiologist.

- Indirect blood pressure and heart rate were measured for all animals prior to initial treatment and during Weeks 6, 13, 25, and 36.

Blood collection schedule and analysis:

Time Point	Clinical Pathology						*In Vitro* ADMET
	Hematology	Serum Chemistry	Coagulation	KLH Analysis	CBC with Differential[d]	PK[b] and Immune Response[a]	Lymphocyte Subset Assay
Week -3					X		X
Week -2	X	X	X				X
Week -1	X	X	X	X			X
Day 1 prior to tx						X[a]	
Day 1 at 1 and 6 hours post-tx						X[b]	
Day 2					X	X[b]	X
Day 3						X[b]	
Day 4						X[b]	
Day 8 prior to tx						X[b]	
Day 15 prior to tx						X[b]	
Day 20				X			
Day 22 prior to tx						X[b]	
Day 27				X			
Day 29 prior to tx					X	X[b]	X
Day 34	X	X	X	X			
Day 41				X			
Day 57 prior to tx						X[b]	
Day 69				X			
Day 85 prior to tx						X[a]	
Day 85 at 1 and 6 hours post-tx						X[b]	
Day 86						X[b]	
Day 87						X[b]	
Day 88						X[b]	
Day 89						X[b]	

KLH = Keyhole Limpet Hemocyanin; CBC = complete blood count; PK = pharmacokinetics. tx = treatment.

[a] One tube of 3.0 mL of whole blood was collected for PK and for Immune Response analysis.
[b] One tube of 2.0 mL of whole blood was collected for PK analysis.
[c] Blood samples were obtained from all animals (necropsy and non-necropsy).
[d] CBC with differential was not analyzed for reticulocytes.

Time Point	Clinical Pathology						*In Vitro* ADMET
	Hematology	Serum Chemistry	Coagulation	KLH Analysis	CBC with Differential[d]	PK[b] and Immune Response[a]	Lymphocyte Subset Assay
Day 92 prior to nx[c]	X	X	X	X		X[a]	X
Day 113 prior to						X[b]	
Day 141 prior to						X[b]	
Day 169 prior to tx						X[a]	
Day 169 1 and 6 hr post-tx						X[b]	
Day 170						X[b]	
Day 171						X[b]	
Day 172						X[b]	
Day 176 prior to nx[c]	X	X	X	X		X[a]	X
Day 183						X[b]	
Day 190						X[b]	
Day 197						X[b]	
Day 204						X[a]	
Day 211						X[b]	
Day 218						X[b]	
Day 225						X[b]	
Day 232						X[a]	
Day 239						X[b]	
Day 246						X[b]	
Day 253	X	X	X	X		X[a]	X
Volume of Whole Blood/	1.3 mL	1.8 mL	1.3 mL	2.0 mL	1.3 mL	3.0 mL[a] 2.0 mL[b]	2.0 mL
Anticoagulant	EDTA	None	Sodium Citrate	None	EDTA	None	Sodium Heparin

KLH = Keyhole Limpet Hemocyanin; CBC = complete blood count; PK = pharmacokinetics, tx = treatment; nx = necropsy.

[a] One tube of 3.0 mL of whole blood was collected for PK and for Immune Response analysis.

[b] One tube of 2.0 mL of whole blood was collected for PK analysis.

[c] Blood samples were obtained from all animals (necropsy and non-necropsy).

[d] CBC with differential was not analyzed for reticulocytes.

Hematology: indices evaluated listed in table

Total leukocyte count (WBC)
Erythrocyte count (RBC)
Hemoglobin concentration (HGB)
Hematocrit value (HCT) [a]
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH) [a]
Mean corpuscular hemoglobin concentration (MCHC) [a]
Platelet count (PLT)
Relative and absolute reticulocyte count (RTC, ARTC)
White blood cell differential
Hyperchromasia (HYPER)
Macrocytosis (MACRO)
Relative and absolute polymorphonuclear neutrophil count (PLY, APLY)
Relative and absolute lymphocyte count (LYM, ALYM)
Relative and absolute monocyte count (MNO, AMNO)
Relative and absolute eosinophil count (EOS, AEOS)
Relative and absolute basophil count (BSO, ABSO)
Relative and absolute large unstained cells count (LUC, ALUC)

[a] Calculated values.

Prothrombin time (PT)
Activated partial thromboplastin time (APTT)

Clinical chemistry: indices evaluated listed in table

Glucose (GLU)	Calcium (CAL)	Total bilirubin (TBIL)
Urea nitrogen (BUN)	Phosphorus (PHOS)	Triglycerides (TRG)
Creatinine (CRE)	Sodium (NA)	Alanine aminotransferase (ALT)
Total protein (TPR)	Potassium (K)	Aspartate aminotransferase (AST)
Albumin (ALB)	Chloride (CL)	Alkaline phosphatase (ALK)
Globulin (GLOB) [a]	Total cholesterol (CHOL)	Gamma glutamyltransferase (GGT)
Albumin/Globulin ratio (A/G) [a]		

[a] Calculated values.

Toxicokinetics and immune antibody response: sampled as listed on schedule table

Toxicokinetics - Non-compartmental analysis (NCA) was employed to calculate the pharmacokinetic parameters of CNTO 148. The maximum serum concentration, Cmax, was obtained from inspection of the individual serum concentration versus time profile. The partial AUC during the defined time period (0-7 days for the first dose, 84-91 days for the 13th dose and 168-175 days for the 25th dose) was obtained by the trapezoidal rule. The terminal rate constant (hz) was determined by least-squared regression analysis of the log-linear portion of the terminal phase. The terminal half-life, t1/2, was calculated from the ratio of 0.693 and hz. The clearance at steady state (CLss) was calculated as dose/AUCtau, in which tau is the time period of one dose interval (7 days), following the final dose. The volume of distribution at steady state (Vss) was calculated by MRT*CLss.

Immune antibody response - The immune response assay consisted of a bridging enzyme immune assay (EIA). Detection of antibodies to CNTO 148 relied upon their ability to cross-link solid phase CNTO 148 with a labeled solution phase molecule of CNTO 148. Therefore, the assay was designed to detect antibodies to CNTO 148 independent of isotype or subclass.

KLH analysis: Serum samples were analyzed for anti-KLH antibodies (ability to illicit an IgG and IgM immune response) using validated ELISA procedures as listed on the previously listed schedule table.

Lymphocyte Immunophenotyping: Blood was processed using a whole blood lysis technique. Samples were analyzed by FACS (fluorescence–activated cell sorter). The relative abundance of circulating mononuclear leukocytes was determined using flow cytometric cell surface marker analysis with the antibody combinations as described in the table.

Antibody	Subset Evaluated
CD2/CD20	Lymphocyte Purity Estimate; Total B-lymphocytes
CD3/CD4	T-helper lymphocytes
CD3/CD8	T-cytotoxic/suppressor lymphocytes
CD3/CD14	Monocytes
CD3/CD16	Natural killer (NK) cells
CD3/CD44	Memory T-lymphocytes
CD3/CD45RA	Naïve T-lymphocytes

Urinalysis: Urine samples were collected prior to necropsy. Indices evaluated listed in table.

Macroscopic evaluations	
Microscopic evaluations	
Test Strip Analysis, including:	
Glucose	Ketones
Bilirubin	Urobilinogen
Blood	Specific Gravity
pH	Nitrites
Protein	Leukocytes

Gross pathology: Three animals per sex per group were euthanized on Days 92 and 176, and the remaining animals (two per sex per group) were euthanized on Day 253. Tissues collected and examined listed in table.
- sections of select tissues were collected for immunohistochemistry (listed on table)
- sections of the spleen, thymus, Peyer's patch, tonsil, and lymph nodes (axillary, mesenteric, mandibular, and inguinal) were preserved for immunohistopathologic evaluation (T and B cell distribution).

Administration Site (last)	Intestine, Small [a]	Sciatic Nerve [a]
Adrenal Glands	Duodenum	Skeletal Muscle (left bicep brachii) [a]
Aorta	Jejunum	Skin
Bone Marrow – Sternum	Ileum	Mammary Region
Brain [a]	Kidneys [a]	Spinal Cord [a]
Cerebrum	Liver [a]	Cervical
Cerebellum	Lungs with bronchi [a]	Thoracic
Brain Stem	Lymph Nodes [a, d]	Lumbar
Esophagus [a]	Axillary	Spleen [a, d]
Eyes with Optic Nerve [b]	Inguinal	Stomach [a]
Females	Mandibular	Cardiac
Cervix	Mesenteric	Fundic
Ovaries [a]	Males	Pyloric
Uterus [a]	Epididymides	Thymus [d]
Vagina	Prostate Gland	Thyroid Glands
Mammary Gland [a]	Seminal Vesicle	Tonsils [d]
Femur with Articular Surface [c]	Testes [a, e]	Tongue
Gallbladder	Pancreas [a]	Trachea
Heart [a]	Parathyroid Glands	Urinary Bladder
Intestine, Large	Peyer's patch [d]	
Cecum	Pituitary Gland	Macroscopic Lesions
Colon [a]	Salivary glands	Animal Identification [f]
Rectum	Mandibular	

[a] Samples collected for Immunohistochemistry.
[b] Fixed in Davidson's Solution.
[c] Collected at necropsy but not sectioned histologically.
[d] Samples collected for Immunohistopathology.
[e] Fixed in Modified Davidson's Solution.
[f] Collected at necropsy to retain identification.

Organ weights: listed organs weighed prior to fixation

Adrenal Glands	Pituitary Gland
Brain	Prostate Gland
Heart	Seminal Vesicles
Liver with gallbladder (drained)	Spleen
Lung	Testes
Kidneys	Thymus
Ovaries	Thyroid Glands (including Parathyroid Glands)

Histopathology: all animals were examined. All tissues, except those specified in the immunohistopathological assessment of lymphoid tissues were trimmed, embedded, and sectioned. Slides were stained with hematoxylin and eosin.
- in addition, slides of lung, liver, and ileum sections from 25 mg/kg Male No. 2008 were stained with Gamori's methenamine sliver (GMS), acid-fast, and Gram stains.

Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no (x)

Bone Marrow Smear Analysis - bone marrow smears from the sternum were prepared from each animal and allowed to dry. Both slides from each animal were stained with Wright-Giemsa and coverslipped. These were not evaluated.

Results:

Dose formulation: Analyses indicated acceptable concentration and stability.

Treatment: All animals were dosed without incident as previously described. The study days on which doses were administered were 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, 92, 99, 106, 113, 120, 127, 134, 141, 148, 155, 162, and 169.
- from days 98–106, 50 mg/kg Female No. 3106 was treated topically with Prepodyne® for a lacerated fourth digit on the right hand. On Days 150–159, 50 mg/kg Male No. 3008 was given intramuscular injections of 5 mg/kg Baytril® (enrofloxacin) once daily for an infected laceration of the second digit on the right hand.

Mortality: none

Clinical signs: no treatment-related effects
- mild to moderate skin erythema and soft feces observed in all groups, including controls
- no treatment-related effects observed during individual physical examinations

Body weights: no treatment-related effects

Food consumption: no treatment-related effects

Ophthalmoscopy: no treatment-related effects

EKG: No qualitative ECG abnormalities or treatment-related effects on indirect blood pressure or heart rate was observed. Values for systolic, diastolic, and mean arterial pressure and heart rates were similar between CNTO 148-treated monkeys and their respective controls throughout the study.

Hematology: Slightly increased lymphocyte counts in several, but not all, individual males and females in the 25 and 50 mg/kg groups were observed during one or more days of the study. The response was quite variable and of a low magnitude so as not to result in overall statistical differences in group means. This effect was not observed to the same extent in the control animals. This result agrees with those observed during lymphocyte subset analysis (see Lymphocyte Immunophenotyping).

There was no effect of CNTO 148 administration on blood coagulation.

Clinical chemistry: no treatment-related effects

Immune antibody response: Of all the animals, one female monkey dosed with 25 mg/kg CNTO 148 developed antibodies to CNTO 148 during the study. The immune response peaked at Day 169 in this animal at a titer of 5120 (optical density). An antigen competition study confirmed the positive immune response to CNTO 148 in this animal. This animal also exhibited accelerated CNTO 148 clearance at the terminal phase, relative to the other animals.

The immune response analysis was considered to be inconclusive for the CNTO 148 treated animals with no detectable antibodies to CNTO 148 as high serum concentrations of CNTO 148 can interfere with antibody detection. Therefore, spike recovery experiments were conducted to investigate the potential interference from serum CNTO 148 in the serum samples that had a negative immune response result. For animals having no positive immune response sera, days 1 (prior to administration), 92, 176, and 253 sera were prepared at a 1/10 dilution with 50 ng/mL of the positive control antibody and tested again to demonstrate the ability to recover a known positive spike. The spike recovery experiments indicated that assay interference was present in all of the serum samples except that of one 25 mg/kg male on Day 253. Furthermore, the pharmacokinetic analysis indicated that circulating CNTO 148 remained detectable in the terminal sera of all CNTO 148-treated animals except one 25 mg/kg female and male.

KLH analysis: CNTO 148 administration was associated with a decreased incidence of IgG and IgM antibody responses to a KLH challenge (see tables). Fewer animals in the 25 and 50 mg/kg dose groups mounted measurable antibody responses to KLH than in the control group. IgG antibody production was more affected than IgM antibody production with the most noticeable decline present in the 50 mg/kg dose group from day 41 to 69 with little to no response by day 92.

Mean Center Point Values[a] of anti-KLH IgG

Group No.	CNTO 148 Dosage (mg/kg)		Day 20	Day 27	Day 34	Day 41	Day 69	Day 92	Day 176	Day 253
1	0	Mean	ND	674	667	618	603	562	579	ND
		S.D.	NA	75	98	84	47	55	51	NA
		No.[b]	0/16	11/16	14/16	14/16	12/16	7/16	5/10	0/2
2	25	Mean	653	611	616	643	594	604	650	ND
		S.D.	NA	162	129	120	73	121	98	NA
		No.[b]	2/16	10/16	12/16	10/16	5/16	3/16	3/10	0/2
3	50	Mean	ND	585	636	610	578	ND	ND	ND
		S.D.	NA	80	95	90	NA	NA	NA	NA
		No.[b]	0/16	8/16	10/16	9/16	1/16	0/16	0/10	0/2

No. = number; S.D. = standard deviation; ND = not detected; NA = not applicable.

[a] A minimum of six dilutions of each serum sample were prepared ranging from 250 to 1500 fold. The center point titer for each sample was determined using one half the maximum absorbance at 450 nm for the dilution set.

[b] No. of responders/No. of animals.

Mean Center Point Values[a] of anti-KLH IgM

Group No.	CNTO 148 Dosage (mg/kg)		Day 20	Day 27	Day 34	Day 41	Day 69	Day 92	Day 176	Day 253
1	0	Mean	261	272	269	220	221	224	205	ND
		S.D.	57	59	51	22	39	55	NA	NA
		No.[b]	16/16	16/16	15/16	15/16	9/16	6/16	2/10	0/2
2	25	Mean	257	266	269	222	218	242	259	387
		S.D.	57	59	64	58	42	84	NA	NA
		No.[b]	16/16	14/16	11/16	12/16	8/16	5/16	2/10	1/2
3	50	Mean	215	228	233	204	247	230	ND	ND
		S.D.	37	39	44	13	54	NA	NA	NA
		No.[b]	13/16	14/16	14/16	14/16	5/16	1/16	0/10	0/2

No. = number; S.D. = standard deviation; ND = not detected; NA = not applicable.
[a] A minimum of six dilutions of each serum sample were prepared ranging from 250 to 1500 fold. The center point titer for each sample was determined using one-half the maximum absorbance at 450 nm for the dilution set.
[b] No. of responders/No. of animals.

Lymphocyte Immunophenotyping: Detailed lymphocyte subset analysis revealed that through Day 253, CNTO 148 administration resulted in slight dose-dependent increases in most animals in the number of naïve T-lymphocytes, T-helper lymphocytes, T-cytotoxic/suppressor lymphocytes, total T-lymphocytes, and B-lymphocytes. Males were generally affected more often, earlier, and longer than females. These increases were not toxicologically significant. There were no apparent CNTO 148-associated alterations in Natural Killer (NK) cells or monocytes through Day 253. See table in Section 2.6.7 for details.

Urinalysis: no treatment-related effects

Gross pathology: no treatment-related effects

Organ weights: no treatment-related effects

Histopathology: no treatment-related effects

Immunohistopathology - There were no CNTO 148-related findings in any of the lymphoid organs.

One 25 mg/kg male: One 25 mg/kg male had several anatomical lesions on Day 253 that may have been secondary to CNTO 148 treatment. These lesions included granulomatous hepatitis, histiocytic ileitis, pulmonary hemorrhage, and glomerulopathy. Lung, liver, and ileum sections were stained with special stains in an attempt to detect and/or identify microorganisms. Silver stained sections of liver, ileum, and lung revealed the presence of fungal organisms consistent with *Histoplasma capsulatum*. Disseminated histoplasmosis in this animal was likely associated with CNTO148 administration. In addition, this individual animal had a greatly enlarged spleen at the time of necropsy. There were no clinical observations, physical examination findings, food consumption, or body weight changes that suggested an active illness in this animal prior to day

253. On Day 253, there were several abnormal clinical chemistry results (elevations in alanine aminotransferase, aspartate aminotransferase, globulin, and potassium and decreased albumin levels) that correlated with the hepatic and renal lesions noted in this animal. On Day 253, this male had a total white blood cell count that was 0.80 to 0.87× baseline. This was largely due to a decrease in neutrophils (i.e., neutrophil counts on Day 253 were 0.27 to 0.45× baseline). Absolute lymphocyte counts on Day 253 in this animal were similar to baseline and this was also true for lymphocyte subsets. However, on Day 253, immunophenotyping revealed that monocyte counts in this animal were 39% of baseline (compared to 74%–156% of baseline in control males on Day 253) with a lower Natural Killer (NK) cell percentage (18% of baseline) than the other 25 mg/kg male on Day 253 (224% of baseline) or the two control males on Day 253 (79%–134% of baseline). There was no evidence of suppressed humoral response to T-dependent antigen challenge in this animal; the highest center point values for anti-KLH IgG and IgM observed during the study were detected in this animal. This male was also the only "recovery" animal from any dose group to register a detectable level of anti-KLH IgM antibody on Day 253. While treatment-related effects cannot be absolutely ruled out, the toxicological relevance of these findings is unknown based on its solitary occurrence (one animal).

Toxicokinetics: CNTO 148 exposure appeared to increase with dose in an approximately dose proportional manner over the dose range of 25 to 50 mg/kg. No gender significant differences were observed in the PK parameters. Steady state was achieved by day 113 (dose 17). Some drug accumulation was observed following weekly 25 or 50 mg/kg doses. At steady state, mean gender-combined PK values were 1,620 & 2,627µg/mL for Cmax and 5,809 & 11,635 µg•day/mL for AUC for the 25 & 50 mg/kg groups, respectively. The mean terminal half-life values were 11 & 18 days, respectively. Individual animal data and summary data tables follow.

Individual and Mean (SD) CNTO 148 Pharmacokinetic Parameter Estimates Following Weekly IV Doses of 25 mg/kg in Cynomolgus Monkeys

		1st Dose on Day 1		13th Dose on Day 85		25th Dose on Day 169				
Gender	ID	Cmax µg/mL	AUC(0-7d) µg.day/mL	Cmax µg/mL	AUC(84-91d) µg.day/mL	Cmax µg/mL	AUC(168-175d) µg.day/mL	CLss mL/day/kg	Vss mL/kg	t1/2* day
Male	2001	746.89	1859.88	1242.51	4764.11	NS	NS	NS	NS	NS
	2002	841.15	1923.97	973.44	3660.74	NS	NS	NS	NS	NS
	2003	657.62	1441.17	1126.74	3903.14	NS	NS	NS	NS	NS
	2005	760.01	1715.32	981.85	3820.41	2364.37	5260.11	4.75	33.65	NS
	2006	614.02	1716.68	1156.76	5288.96	1957.05	7973.52	3.14	41.03	NS
	2007	825.34	1964.16	1188.04	3285.10	1093.17	3817.96	6.55	83.68	3.82
	2008	800.58	1467.23	1096.94	4534.32	1124.03	4829.94	5.18	81.21	7.97
	2009	919.49	2098.64	1425.23	4727.11	1737.45	4351.74	5.74	35.89	NS
	N	8	8	8	8	5	5	5	5	2
	Mean	770.64	1773.38	1148.94	4247.98	1655.21	5246.66	5.07	55.09	5.90
	SD	99.28	233.92	145.70	679.84	547.45	1616.45	1.27	25.13	NA
Female	2101	409.60	1692.56	1172.43	4259.46	NS	NS	NS	NS	NS
	2102	754.44	1863.49	1077.63	3755.87	NS	NS	NS	NS	NS
	2103	804.33	1870.59	1216.87	4121.41	NS	NS	NS	NS	NS
	2104	1361.12	1266.49	306.83	88.54	NA	NA	NA	NA	NA
	2105	955.18	2730.14	1547.11	6921.45	2545.74	12737.55	1.96	NA	NS
	2106	1020.23	2451.87	1505.05	5554.26	1026.41	983.89	25.41	5.72	NS
	2107	850.52	2006.69	1406.75	6490.31	1576.35	8177.53	3.06	47.33	21.68
	2108	474.83	1489.99	1113.78	4682.43	1159.37	4152.38	6.02	82.98	NA
	N	8	8	8	8	4	4	4	3	1
	Mean	828.78	1921.48	1168.31	4484.22	1576.97	6512.84	9.11	45.34	21.68
	SD	303.22	480.48	390.87	2110.63	687.03	5087.87	11.00	38.66	NA
N		16	16	16	16	9	9	9	8	3
All Mean		799.71	1847.43	1158.62	4366.10	1620.44	5809.40	6.87	51.44	11.16
All SD		220.02	372.99	285.14	1519.69	573.20	3385.15	7.12	28.52	9.35

NS = No Sample available
NA= Not Applicable
* Only 2 recovery animals per sex from each group were used to calculate t1/2.

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Individual and Mean (SD) CNTO 148 Pharmacokinetic Parameter Estimates Following Weekly IV Doses of 50 mg/kg in Cynomolgus Monkeys

		1st Dose on Day 1		13th Dose on Day 85		25th Dose on Day 169				
Gender	ID	Cmax µg/mL	AUC(0-7d) µg.day/mL	Cmax µg/mL	AUC(84-91d) µg.day/mL	Cmax µg/mL	AUC(168-175d) µg.day/mL	CLss mL/day/kg	Vss mL/kg	t1/2* day
Male	3001	2053.81	5588.40	2035.45	8096.69	NS	NS	NS	NS	NS
	3002	1667.54	5108.11	3338.66	14232.67	NS	NS	NS	NS	NS
	3003	1759.34	4384.78	3527.75	13376.53	NS	NS	NS	NS	NS
	3004	1607.84	4739.72	1997.22	8291.32	2887.34	14950.24	3.34	55.57	NS
	3005	2256.92	4800.75	4459.04	12808.19	3457.08	13274.64	3.77	56.85	NS
	3006	1660.31	3995.21	3039.51	9262.78	2025.05	8772.06	5.70	60.06	NS
	3007	2300.03	6223.07	4485.31	13296.87	3770.00	11517.80	4.34	65.04	17.38
	3008	1657.71	3951.84	2195.35	8217.08	1954.47	7057.83	7.08	89.94	18.44
	N	8	8	8	8	5	5	5	5	2
	Mean	1870.44	4848.98	3134.79	10947.76	2818.79	11114.51	4.85	65.49	17.91
	SD	287.70	780.62	1011.91	2703.18	820.67	3220.45	1.53	14.15	NA
Female	3101	1787.83	4364.62	2745.26	13417.11	NS	NS	NS	NS	NS
	3102	1689.10	4168.40	2398.57	10233.76	NS	NS	NS	NS	NS
	3103	1760.16	4168.43	2565.37	10744.77	NS	NS	NS	NS	NS
	3104	1648.82	4118.98	3000.61	12834.02	2883.21	11907.24	4.20	46.51	NS
	3105	1978.33	5075.99	3125.66	9943.87	2515.77	12540.60	3.99	45.11	NS
	3106	1783.79	4523.43	2921.05	10935.51	2136.23	11460.57	4.36	67.03	NS
	3107	1619.83	4478.27	1971.45	9630.72	2129.16	9038.76	5.53	117.90	15.60
	3108	2170.69	5097.00	2616.46	13779.90	2518.22	15828.50	3.16	80.84	19.21
	N	8	8	8	8	5	5	5	5	2
	Mean	1804.82	4499.39	2668.05	11439.96	2436.52	12155.14	4.25	71.48	17.40
	SD	184.64	391.44	370.53	1648.90	315.09	2444.27	0.85	29.92	NA
All N		16	16	16	16	10	10	10	10	4
All Mean		1837.63	4674.19	2901.42	11193.86	2627.65	11634.83	4.55	68.49	17.66
All SD		235.98	623.27	774.60	2177.94	619.72	2750.56	1.21	22.29	1.56

NS = No Sample available
NA= Not Applicable
* Only 2 recovery animals per sex from each group were used to calculate t1/2.

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Study title: 6-Month Subcutaneous Dose Toxicity Study with CNTO 148 in Cynomolgus Monkeys with a 12-Week Recovery Period

Key study findings:

- Male and female cynomolgus monkeys were dosed subcutaneously with 0, 25, or 50 mg/kg CNTO 148 biweekly for 6 months with interim assessments, including necropsy, at 3 months and 6 months and 9 months (recovery animals)
- Treatment-related clinical symptoms and histological observations were reversible dosing site inflammation and edema
- Humoral/immunomodulatory responses were not affected by treatment
- Reversible increases in B-lymphocytes were observed after dosing and CNTO 148-treated animals were characterized as having an inconclusive anti-product antibody response because the lack of detection of an antibody response in all but 1 low dose female may have been compromised by high serum levels of CNTO 148
- No other notable findings occurred for a complete protocol that included a cardiovascular safety pharmacology component

- The NOAEL was considered to be 50 mg/kg as only reversible and clinically monitorable injection site effects occurred at 50 mg/kg
- Mean, gender combined terminal AUC values were 2622 and 5657 µg•day/mL and Cmax values were 1119 and 2459 µg/mL for the 25 and 50 mg/kg groups, respectively, at the end of the study period with half-lives of 16 and 14 days.

Study no.: Study Number: UHAW-159, Sponsor Study Number: T-2002-001
Volume #, and page #: eCTD
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: July 9, 2002 (report date January 19, 2004)
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: CNTO 148 (human tumor necrosis factor α [TNFα] monoclonal antibody [mAb]), lot 5813-104, 99.1%

Methods:

Doses: 0, 25, & 50 mg/kg 2x weekly
- stability and concentration of the formulated test article was conducted by the Sponsor and were within acceptable limits

Species/strain: Cynomolgus monkey

Number/sex/group or time point (main study):
3/sex/group with necropsy at 14 & 27 weeks

Route, formulation, volume, and infusion rate: subcutaneous, liquid (0.9% NaCl for control group, dose volumes of 0.5 mL/kg (control and high dose) and 0.25 mL/kg (low dose)
- biweekly doses were administered to alternating sides of the back (5 cm^2 area)

Satellite groups used for toxicokinetics or recovery:
- 2/sex/group for recovery (necropsy week 39); all animals for TK evaluation

Study Design

Group Number	Number of Animals		Test Article	Dosage Level (mg/kg)	Dosage Volume (mL/kg)	Dosing Regimen	Necropsy Day
	Males	Females					
1	8	8	0.9 % Sodium Chloride, Injection	0	0.50	SC, twice weekly for up to 52 doses	3/sex/group during Week 14
2	8	8	CNTO 148	25	0.25	SC, twice weekly for up to 52 doses	3/sex/group during Week 27
3	8	8	CNTO 148	50	0.50	SC, twice weekly for up to 52 doses	2/sex/group during Week 39

Age: young adult

Weight: mean group range of 2.43-2.58 kg (males) and 2.43-2.51 kg (females) on

first day of dosing

Sampling times: blood sampled during the first 3 weeks, weeks 12-14, 25-27, & during the recovery period for TK and Immune (antibody) analysis with some days only for TK analysis (see sampling tables below for more detail)

Unique study design or methodology: see methods

- KLH analysis
  - Keyhole Limpet Hemocyanin (KLH) in Incomplete Freund's Adjuvant with distilled water – 100 mcg (1 mL) administered by intramuscular (IM) injection on day 12 (day after 4th dose) and day 30 (day after 9th dose)
  - analysis was conducted predosing and days 25 & 53 and weeks 14, 25, 29, & 37
- Lymphocyte subset assay
  - Immunophenotyping of lymphocyte subsets was conducted predosing, and on days 2, 23, 86, 177, 205 (recovery) & 261 (recovery)
- Tissue collections for immunohistochemical detection of test article and immunohistopathologic assessment of lymphoid tissues was conducted

Observation and Times:

Clinical signs:

- Moribundity/mortality checks were performed twice daily (A.M. and P.M.).
- Clinical observations were performed once daily with special attention being given to the subcutaneous administration sites for signs of irritation.
- A physical examination, including a record of general condition, rectal body temperature, respiratory rate, heart rate, and capillary refill time, was performed for each animal prior to initial treatment (Day -12) and during Weeks 5, 13, 26, 30, and 38.

Body weights: three times prior to initial treatment, on Day 1, weekly thereafter, and on the day prior to necropsy

- A final, fasted body weight was taken for organ weight ratio calculations.

Food consumption: measured and recorded daily beginning one week prior to treatment

Ophthalmoscopy: prior to treatment (Day -19) and during Weeks 4, 13, 26, and 38

- included macroscopic examinations of the anterior portion of the eye, the optic media, and the ocular fundus

EKG: Electrocardiogram (ECG) tracings were obtained using 12 leads prior to initial treatment and during Weeks 5, 13, 26, 30, and 38. One 10-second tracing was obtained at each time point using a chart speed of 50 mm/sec.

- Indirect blood pressure and heart rate were measured for all animals prior to Initial treatment and during Weeks 5, 13, 26, 30, and 38

Blood sample collection: (see tables for more detailed description)

- fasting was conducted prior to sample collection.
- sampling was conducted pre dosing, week 3, 14, & 27 for hematology, serum chemistry, and coagulation analysis

Blood Sample Collection Schedule

Time Point	Clinical Pathology							
	Hematology	Serum Chemistry	Coagulation	KLH Analysis	Lymphocyte Subset Assay	Hematology for WBC[a]	TK and Immune Response[b,c]	Background TK
Week -4					X	X		
Week -3	X	X	X		X			
Week -2	X	X	X	X	X			
Week -1					X	X		
Day 1 prior to tx							X[b]	
Day 1 at 15 minutes and 6 hours post-tx							X[c]	
Day 2 at 24 hours post-tx					X	X	X[c]	
Day 3 at 48 hours post-tx							X[c]	
Day 4 prior to tx and 15 minutes post-tx							X[c]	
Day 8 prior to tx and 15 minutes post-tx							X[c]	
Day 15 prior to tx and 15 minutes post-tx							X[c]	
Day 22 prior to tx	X	X	X				X[b]	
Volume of Whole Blood/ Time Point	1.3 mL	1.8 mL	1.3 mL	2.0 mL	2.0 mL	1.3 mL	3.0 mL[b] 2.0 mL[c]	1.5 mL
Anti-coagulant	EDTA	None	Sodium Citrate	None	Sodium Heparin	EDTA	None	None

[a] Hematology for WBC was not analyzed for reticulocytes.
[b] One tube of 3.0 mL of whole blood was collected for TK and for Immune Response analysis.
[c] One tube of 2.0 mL of whole blood was collected for TK analysis.

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Time Point	Clinical Pathology							
	Hematology	Serum Chemistry	Coagulation	KLH Analysis	Lymphocyte Subset Assay	Hematology for WBC[a]	TK and Immune Response[b,c]	Background TK
Day 22 at 15 minutes post-tx							X[c]	
Day 23					X	X		
Day 25 prior to tx				X				X
Day 53 prior to tx				X				
Day 57 prior to tx								X
Day 81 prior to tx							X[b]	
Day 81 at 15 minutes and 6 hours post-tx							X[c]	
Day 82 at 24 hours post-tx							X[c]	
Day 83 at 48 hours post-tx							X[c]	
Day 85 prior to tx							X[c]	
Day 86					X	X	X[b]	
Day 92 or prior to nx[d] (Week 14)	X	X	X	X			X[b]	
Day 113 prior to tx								X
Volume of Whole Blood/ Time Point	1.3 mL	1.8 mL	1.3 mL	2.0 mL	2.0 mL	1.3 mL	3.0 mL[b] 2.0 mL[c]	1.5 mL
Anti-coagulant	EDTA	None	Sodium Citrate	None	Sodium Heparin	EDTA	None	None

[a] Hematology for WBC was not analyzed for reticulocytes.
[b] One tube of 3.0 mL of whole blood was collected for TK and for Immune Response analysis.
[c] One tube of 2.0 mL of whole blood was collected for TK analysis.
[d] Blood samples were obtained from all animals (necropsy and non-necropsy).

Time Point	Clinical Pathology							
	Hematology	Serum Chemistry	Coagulation	KLH Analysis	Lymphocyte Subset Assay	Hematology for WBC[a]	TK and Immune Response[b,c]	Background TK
Day 141 prior to tx								X
Day 165 prior to tx							X[b]	
Day 169 prior to tx							X[b]	
Day 176 prior to tx				X			X[b]	
Day 177					X	X		
Day 179 prior to tx, and at 15 minutes and 6 hours post-tx							X[c]	
Day 180 at 24 hours post-tx							X[c]	
Day 181 at 48 hours post-tx							X[c]	
Day 182 (72 hr post Day 179 tx)							X[b]	
Day 183 prior to tx or prior to nx[d] (Week 27)	X	X	X					
Volume of Whole Blood/ Time Point	1.3 mL	1.8 mL	1.3 mL	2.0 mL	2.0 mL	1.3 mL	3.0 mL[b] 2.0 mL[c]	1.5 mL
Anti-coagulant	EDTA	None	Sodium Citrate	None	Sodium Heparin	EDTA	None	None

[a] Hematology for WBC was not analyzed for reticulocytes.
[b] One tube of 3.0 mL of whole blood was collected for TK and for Immune Response analysis.
[c] One tube of 2.0 mL of whole blood was collected for TK analysis.
[d] Blood samples were obtained from all animals (necropsy and non-necropsy).

Time Point	Clinical Pathology							
	Hematology	Serum Chemistry	Coagulation	KLH Analysis	Lymphocyte Subset Assay	Hematology for WBC[a]	TK and Immune Response[b,c]	Background TK
Day 190							X[b]	
Day 197							X[b]	
Day 205	X	X	X	X	X		X[b]	
Day 211							X[c]	
Day 218							X[c]	
Day 225							X[b]	
Day 232							X[c]	
Day 239							X[c]	
Day 246							X[c]	
Day 253							X[c]	
Day 261					X	X	X[c]	
Approximately Day 267[e] or prior to nx	X	X	X	X			X[c]	
Volume of Whole Blood/ Time Point	1.3 mL	1.8 mL	1.3 mL	2.0 mL	2.0 mL	1.3 mL	3.0 mL[b] 2.0 mL[c]	1.5 mL
Anti-coagulant	EDTA	None	Sodium Citrate	None	Sodium Heparin	EDTA	None	None

[a] Hematology for WBC was not analyzed for reticulocytes.
[b] One tube of 3.0 mL of whole blood was collected for TK and for Immune Response analysis.
[c] One tube of 2.0 mL of whole blood was collected for TK analysis.
[e] Serum collected for TK analysis on Day 267 was also analyzed for Immune Response.