CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125496Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Sylvant (siltuximab)

Date: March 4,, 2014
To: File for BLA 125496
From: John K. Leighton, PhD, DABT
Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting and supplemental reviews for Sylvant conducted by Drs. Del Valle, Sheth and Gehrke, and secondary memorandum and labeling provided by Dr. Saber. I concur with Dr. Saber's conclusion that Sylvant may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
03/04/2014

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

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number:	BLA 125496
Supporting document/s:	Original Application
Applicant's letter date:	August 29, 2013
CDER stamp date:	August 29, 2013
Product:	Sylvant (Siltuximab)
Indication:	Treatment of multicentric Castleman's disease (MCD)
Applicant:	Janssen Research & Development, LLC
Review Division:	Hematology and Oncology Toxicology on behalf of the Division of Hematology Products, Office of Oncology Products, Office of New Drugs
Reviewer:	Pedro L. Del Valle, PhD Christopher Sheth, PhD Brenda J. Gehrke, PhD
Supervisor/Team Leader:	Haleh Saber, PhD
Division Director:	John Leighton, PhD, DABT
Project Manager:	Patricia Garvey, R.Ph.

Reference ID: 3464068

Background

This supplemental review is to describe the animal-to-human ratios for sections 8.1 and 13.1 of the label as this information is different from what has been presented in the pharmacology/toxicology review, archived on January 23, 2014.

Amended Information

As described in the BLA review, the AUCs after the first product administration were used to calculate the animal-to-human exposure ratios for reproductive toxicology studies. The human AUC used was 2267 µg•day/mL. Upon further discussion with the Applicant during the labeling negotiation, it was decided that the AUCs after multiple product administration will be more relevant due to product accumulation at the steady state. In addition, there was no anti-drug-antibody related clearance of the product to confound exposure ratios after repeated administration. The following table represents the human AUCs on Day 43 after 3 day administrations every 3 weeks and cynomolgus monkeys AUCs after 25 administrations with the corresponding cynomolgus-to-human AUC ratios.

Table 1 Estimated Safety Margins based on Animal Exposure

Species	Dose (mg/kg)	AUC (168-175day) [a] (µg•day/mL)	Exposure Margin [b]
Cynomolgus monkey	9.2	9674	3.2
	46	22234	7.3

[a] Exposure after the 25th dose in the 6-month study

[b] Based on mean $AUC_{(0-t)}$ of 3044.4 µg•day/mL in MCD patients receiving a 11 mg/kg dose of siltuximab following Day 43 administration (Study C0328T03)

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/s/

PEDRO L DEL VALLE
03/04/2014

HALEH SABER
03/04/2014

MEMORANDUM

Date: January 24, 2014
From: Haleh Saber, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
BLA: 125496
Drug: Sylvant (siltuximab); for Injection, for Intravenous Use
Indications: Treatment of patients with multicentric Castleman's disease (MCD) who are human immunodeficiency virus (HIV-)-negative and human herpesvirus -8(HHV-8) -negative
Applicant: Janssen Research & Development, LLC

Sylvant (siltuximab) is a chimeric IgG1 antibody directed against human interleukin-6 (IL-6). IL-6 is a cytokine involved in both pro- and anti-inflammatory responses. Siltuximab binds to IL6 and prevents the binding of IL6 to the soluble or membrane-bound IL6-receptor, thus inhibiting the IL-6 related signaling pathway. Based on its mechanism of action, the pharmacologic class assigned to siltuximab is "IL-6 antagonist". Overproduction of IL-6 has been linked to systemic manifestation of Castleman's disease (CD). Castleman's disease is a lymphoproliferative disorder that may be localized to a single group of lymph nodes (unicentric) or may occur systemically by affecting multiple groups of lymph nodes and also organs containing lymphoid tissues (multicentric). Multicentric Castleman's disease (MCD) is a rare disease; currently, there are no approved therapies for this disease.

Pharmacology, safety pharmacology (combined with toxicology), pharmacokinetic, and toxicology studies were conducted in *in vitro* systems or in animal species. Genetic toxicology studies were not conducted or needed per ICH S6 guidance. Siltuximab does not bind to IL6 in rodents. General toxicology studies, the embryo-fetal developmental study, and the enhanced pre- and post-natal developmental study (ePPND) were conducted in the cynomolgus monkey, a pharmacologically relevant species. Fertility studies were conducted in mice with an anti-mouse IL6 monoclonal antibody.

Additional endpoints were added to the general toxicology studies to assess the effects of siltuximab on the immune system. These endpoints included immunophenotyping circulating peripheral blood lymphocyte subsets and TDAR assay after KLH administration. Siltuximab-related adverse findings in general toxicity studies were minimal and mainly related to the pharmacology of the antibody; e.g. reduction in the globulin levels (likely due to decreased production of immunoglobulin), lower anti-KLH IgM and IgG levels in the TDAR assay, and reduction in the size of the splenic germinal centers following KLH immunization. These effects suggest the potential for infection secondary to immune modulation. Although not common, first-dose infusion reaction occurred in a monkey (in 1 out of 52) and included moderate facial swelling. The swelling resolved after the infusion ended and no other instances occurred. Skin

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erythema was observed in a few animals in the 6-month toxicology study with corresponding hyperkeratosis or acanthosis of minimal severity.

Infusion reactions, infections, and skin disorders (e.g. pruritus, dry skin, and eczema) have been reported in patients treated with siltuximab. Metabolic disorders reported in patients (e.g. increased cholesterol and triglycerides) were not detected in animal studies reviewed.

There were no drug-related effects in male or female reproductive organs in general toxicology studies. In addition, the fertility studies conducted in mice with a mouse analog of siltuximab did not reveal any potential for impairment of fertility. An embryo-fetal developmental toxicology study was conducted in cynomolgus monkeys with siltuximab and an ePPND study was conducted in the same species with a humanized version of siltuximab. Siltuximab crossed the placenta and resulted in reduced globulin levels in pregnant animals and in the offspring; this may be secondary to a decreased production of immunoglobulin as expected from the pharmacology of this product. The Applicant proposed a category (b) (4) for siltuximab. Based on the pharmacology of the product and the findings in animals, infants born to pregnant women treated with siltuximab may be at an increased risk of infection. Therefore, pregnancy category C is recommended for siltuximab. This is also consistent with the pregnancy category for the approved product Actemra (IL6-receptor antagonist), a drug that inhibits the same pathway.

The nonclinical studies needed to support product labeling were reviewed by Drs. Pedro Del Valle, Christopher Sheth, and Brenda Gehrke. The nonclinical findings are summarized in the "Executive Summary" of the BLA review and reflected in the product label.

Recommendation: I concur with the pharmacology/toxicology reviewers that from a nonclinical perspective, Sylvant may be approved and that no additional nonclinical studies are needed to support approval of Sylvant in patients with MCD.

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/s/

HALEH SABER
01/24/2014

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Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 125496 are owned by Janssen Research and Development, LLC or are data for which Janssen Research and Development, LLC has obtained a written right of reference. Any information or data necessary for approval of BLA 125496 that Janssen Research and Development, LLC 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 reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of BLA 125496.

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1 Executive Summary

1.1 Introduction

Siltuximab is a chimeric (human-murine) immunoglobulin G1κ (IgG1κ) monoclonal antibody being developed for the treatment of multicentric Castleman's disease (MCD). This BLA is seeking approval for use of siltuximab in patients with MCD who are human immunodeficiency virus (HIV)-negative and human herpesvirus-8 (HHV-8)-negative.

Siltuximab binds to soluble human interleukin-6 (hIL-6) on helix D, at the C-terminus of the molecule. IL-6 is a glycoprotein produced by a variety of cell types including lymphocytes, T and B cells, monocytes macrophages, endothelial cells, and malignant cells among others.[1] Its role as both a pro- and anti-inflammatory cytokine during infection, trauma, and immunological challenge is regulated by the formation of a receptor complex with either the IL-6 receptor at the cell surface or with the IL-6 receptor in solution (human soluble receptor, hsIL-6R or gp80). The proposed mechanism of action of siltuximab is that upon binding to hIL-6, siltuximab prevents the binding of hIL-6 to its receptor, (b) (4) Accordingly, the pharmacological class assigned to siltuximab is IL-6 antagonist. Overproduction of hIL-6 is hypothesized to play a central role in MCD, a systemic lymphoproliferative disorder characterized by growth of lymph tissues. Therefore, the use of siltuximab to inhibit the complex formation of IL-6 with the IL-6-receptor in patients with MCD (b) (4)

The Applicant, Janssen Research & Development, LLC, changed the manufacturing process during clinical development from a transfected murine Sp2/0 myeloma cell line (b) (4) to a transfected Chinese hamster ovary cell line (b) (4) A clinical pharmacokinetic (PK) study conducted in healthy volunteers and a single-dose PK comparability study conducted in cynomolgus monkeys provided evidence of the comparability of CHO-derived siltuximab and Sp2/0-derived siltuximab.

1.2 Brief Discussion of Nonclinical Findings

Pharmacology studies showed that siltuximab (CNTO 328) has high selectivity and affinity for human IL-6 ($K_D \approx 34$ pM). Mechanistic studies provided evidence that siltuximab has the ability to block various biologic effects of IL-6 in vitro. Siltuximab had the following effects:

- Neutralized the binding of hIL-6 to hIL-6sR and displaced the hIL-6sR-bound;
- Inhibited the hIL-6 mediated proliferation of murine plasmacytoma 7TD1 cell line;
- Decreased IL-6-mediated Stat-3 phosphorylation levels in human CD3+ T cells
- Inhibited hIL-6-stimulated protein production:
  - acute phase proteins (serum amyloid A (SAA)) in HepG2 human hepatoma cells;

[1] Papanicolaou D, et al., The pathophysiologic roles of interleukin-6 in human disease. *Ann. Intern. Med.* (1998) 128: 127-137

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- secretion of IgM antibodies in Burkitt's lymphoma SKW 6.4 cells

The absorptivity constant for siltuximab IgG was determined experimentally [1.52 $(mg/mL)^{-1}cm^{-1}$)] and it was used in estimating the doses of siltuximab administered in clinical studies that were initiated after 2007. Clinical and nonclinical study reports for studies completed before 2007 reported the proposed siltuximab doses based on a previously calculated absorptivity constant of 1.4 $(mg/mL)^{-1}cm^{-1}$; therefore, nonclinical studies conducted before 2007 included an adjustment to the actual dose given by multiplying them by a factor of 0.92 (1.4:1.52).

Toxicology studies were conducted using cynomolgus monkeys because other species available for toxicity testing (rodents and dogs) are not pharmacologically responsive to siltuximab. A fertility study was conducted with a rat anti-mouse IL6 mAb (CNTO 345) in mice and an enhanced pre- and post-natal development (ePPND) study was conducted with a humanized version of siltuximab (CNTO 136) in cynomolgus monkeys. Studies determining the binding, inhibition, and epitope mapping were performed demonstrating the suitability of CNTO 345 and CNTO 136 for their use as surrogates of siltuximab in toxicology studies. Genotoxicity, carcinogenicity and dedicated safety pharmacology studies were not conducted and were not needed for this application.[2]

Repeat dose toxicology studies of 3-months or 6-months duration with extended recovery periods were conducted using weekly IV infusion administration of 9.6 or 46 mg/kg/dose siltuximab alone or in combination with IL-2 administration. A 1-month study that evaluated CNTO 345 in mice was also completed. The 3-month toxicology study has not been reviewed; major findings are excerpted from the Applicant's submission and reported in this section. No significant toxicities were observed in the 3- or 6-month toxicology studies with siltuximab and the toxicokinetic (TK) results indicated that anti-siltuximab antibodies did not interfere with exposure or study results. Toxicities observed that may be considered consistent with the pharmacology of siltuximab included:

- A trend for immunomodulatory effects with slightly lower anti-KLH IgM and IgG levels present. The effects were more pronounced in the 3-month study compared to the 6-month study although the response to KLH stimulation was strong in both studies.
- Reduction in the size of the splenic germinal centers following KLH immunization in the 3-month study.
- Transient higher levels of albumin and A:G ratios and/or lower globulin levels. The lower globulin levels may reflect lower immunoglobulin production; IL-6 is known to be involved in producing antibodies.
- Reaction to first-dose in 1/52 monkeys with facial swelling that resolved upon completion of the infusion and transient decreases in hematology parameters (neutrophils, monocytes, WBC and/or platelets).

[2] As per recommendations provided in the ICH S6(R1), S7A and S9 Guidance documents regarding biological products intended for the treatment of cancer.

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- Skin erythema in the shoulder region was observed periodically in 20% of monkeys and corresponded microscopically with minimal hyperkeratosis or acanthosis in the 6-month toxicology study.
- Sporadic episodes of low heart rates and blood pressure were experienced by 30% of monkeys in the 3-month toxicology study.

A comparison of systemic exposure estimates between the low (1326.3 µg•day/mL) and high dose group (4508.4 µg•day/mL) in the 6-month repeat-dose study and clinical AUCs (2267.2 µg•day/mL) measured in patients with MCD showed that the exposure to siltuximab in monkeys were approximately 0.6- and 2-times the exposure in patients at the recommended clinical dose of 11 mg/kg based on exposure estimated after the first dose.

No effects were noted for male and female reproductive organs in repeat dose toxicology studies with siltuximab. Two supportive fertility toxicity studies in mice with subcutaneous administration of CNTO 345 were conducted. In one study, drug-treated male mice were mated with untreated females and in the second study drug-treated female mice were mated with untreated males. Administration of CNTO 345 in males showed a dose-related increase in relative weight of prostate with statistical significance in the 100 mg/kg/dose males group. No other parameters were affected including mating or fertility of the male mice, sperm morphology, concentration or motility, or effects on caesarean sectioning and litter parameters of untreated female mice. Administration of CNTO 345 in females showed no effects on mating or fertility, estrus-related cycle duration or effects on caesarean sectioning and litter parameters of treated female mice.

An embryofetal developmental toxicity study in monkeys with siltuximab was conducted using weekly IV infusion of 9.6 or 46 mg/kg/dose. No effects were observed on pregnancy maintenance, fetus viability, body weight, morphometric measurements, external abnormalities, visceral and skeletal findings or effects on lymphoid tissue. Siltuximab crossed the placenta during gestation and exposure of dams and fetuses to siltuximab were similar on the day of caesarean sectioning (GD 140). Exposure in fetuses increased in a higher than dose-proportional manner. Toxicities observed in dams during pregnancy included significant decreases in neutrophil counts along with variable decreases in leukocyte counts and significant increases in alanine aminotransferase levels.

A supportive ePPND toxicity study was conducted with CNTO 136 using weekly IV infusion administration of 10 or 50 mg/kg/dose during gestation days 20-167 (GD 20-167), which included the period of organogenesis (GD 20-50). The study included morphological and functional development evaluation of neonates up to 6 months of age. CNTO 136 did not affect embryofetal development, parturition, postnatal survival, or the growth and development of infants. Significant decreases in globulin concentration with concomitant increases in albumin to globulin ratio occurred in dams and infants; there were no histopathology correlates or changes in any other maternal or neonatal clinical pathology parameter or immunophenotyping or infant immunogenic

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responses. CNTO 136 crossed the blood-placental barrier. Its secretion in the milk of pregnant monkeys was minimal.

Pregnancy category C is recommended for siltuximab. This is based on the following: a) the antibody crossed the placenta and was present at a significant amount in the fetus and b) globulin levels were significantly reduced in the pregnant animals and in the offspring; this may be due to a reduction in immunoglobulin as IL6 is involved in the production of immunoglobulin. Therefore, the infants born to a mother treated with siltuximab may be at increased risk of infections.

Expected specific cross-reactivity staining using biotinylated siltuximab was observed in human tissues from the brain white matter and cerebellum, squamous epithelium of the cornea, axons in the gray and white matter of the spinal cord, and axons of peripheral nerves. There was no unexpected tissue cross-reactivity staining.

1.3 Recommendations

1.3.1 Approvability

RECOMMEND APPROVAL: The submitted pharmacology and toxicology studies using siltuximab (Sylvant) support the safety of its use in patients with multicentric Castleman's disease who are HIV-negative and HHV-8-negative.

1.3.2 Additional Non Clinical Recommendations

No additional nonclinical studies using siltuximab are necessary for the proposed indication.

1.3.3 Labeling

A labeling review is not considered necessary at this time. A separate labeling review may be included following discussions of the proposed package insert with the Applicant.

2 Drug Information

2.1 Drug

CAS Registry Number
541502-14-1

Generic Name (also the INN and USAN Name)
Siltuximab

Code Name
CNTO 328, cCLB8

Chemical Name
Chimeric (human-murine) immunoglobulin G1-kappa, anti-[Homo sapiens interleukin 6 (IL6, IL-6)]

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Structure or Biochemical Description

Siltuximab is a chimeric (human-murine) monoclonal immunoglobulin G1 (IgG1) containing [redacted] (b) (4) Siltuximab is a [redacted] (b) (4)

Molecular Formula/Molecular Weight

The predicted molecular masses for the most common siltuximab glycoforms range from [redacted] (b) (4)

Pharmacologic Class

Interlukin-6 (IL-6) antagonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 011461 was the original submission for CNTO 328 by Centocor (now Janssen Research & Development, Inc.)

2.3 Drug Formulation

Siltuximab drug product is supplied as a sterile lyophilized single-use product for IV infusion. Siltuximab is supplied in an 8 mL (100 mg) or 30 mL (400 mg). Each vial of either presentation of siltuximab is reconstituted with sterile water for injection to provide a 20 mg/mL final concentration for intravenous infusion containing sucrose [redacted] (b) (4) L-histidine/L-histidine monohydrochloride monohydrate [redacted] (b) (4) and polysorbate 80 [redacted] (b) (4) Excipients are maintained at the [redacted] (b) (4)

2.4 Comments on Novel Excipients

The composition of siltuximab final lyophilized product (FLP) is shown in Table 1. The FDA Inactive Ingredient database lists sucrose, L-histidine/L-histidine monohydrochloride monohydrate, and polysorbate 80 in approved drug products for intravenous use at [redacted] (b) (4) respectively. The concentrations of excipients in siltuximab FLP are all below the concentrations in FDA approved drug products.

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Table 1 Composition of Siltuximab FLP with corresponding concentrations after reconstitution

(*Excerpted from Applicant's Submission*)

Component	Amount per vial (mg)[a,b]	Nominal Amount Per Vial (mg)[c]	Concentration post-reconstitution (mg/mL)[d,e]
Siltuximab	(b) (4)	100	20
Sucrose		169	(b) (4)
L-Histidine		3.7	
Polysorbate 80		0.8	

[a] Amounts represent a (b) (4) overfill to offset losses during withdrawal post-reconstitution
[b] Amounts calculated based on measurements of the drug substance as explained in Section 3.2.P.2.1.2 Excipients
[c] Amounts calculated without (b) (4) overfill and based on the measurements of drug substance as explained in footbote b
[d] Concentrations of FLP components following reconstitution with 5.2 mL sterile WFI
[e] Concentrations calculated based on total combined volume in a vial (b) (4) of FLP components (b) (4) and WFI (5.2 mL) after reconstitution and based on the total amounts per vial as explained in footnote a and footnote b

2.5 Comments on Impurities/Degradants of Concern

None.

2.6 Proposed Clinical Population and Dosing Regimen

Siltuximab is indicated for the treatment of patients with multicentric Castleman's Disease (MCD) who are HIV negative and HHV-8 negative. Siltuximab drug product is to be diluted and administered at the recommended dosage of 11 mg/kg given over 1 hour by intravenous (IV) infusion every 3 weeks until treatment failure.

2.7 Regulatory Background

Janssen (formerly Centocor) submitted their original application for siltuximab under IND 011461 on July 18, 2008. The BLA 125496 was received on August 29, 2013.

3 Studies Submitted

3.1 Studies Reviewed

	Study Report	Title
Pharmacology		
1	REStr185-ks	Generation and Characterization of cCLB8, Chimeric Anti-IL-6 Mab
2	REStr510el	Comparison of the Affinities of CNTO 328 and CNTO 136 to human IL-6 as Determined by Biacore and Kinexa
3	Siedeman-j-2002	A Novel Monoclonal Antibody Screening Method Using the Luminex-100 Microsphere System
4	Biol-Res-TR336-dg	Cross-species Reactivity of CNTO 328
5	REStr180-rc	CNTO 328 Inhibition of IL-6 Induced IgM and MCP-1 Secretion
6	REStr233-yc	Epitope Mapping of Monoclonal Antibodies on Human IL-6 by Hydrogen/Deuterium Exchange and Mass Spectrometry
7	RES tr560-rc	CNTO 328 inhibition of Stat-3 phosphorylation: Effects of anti-human IL-6 (CNTO 328), on IL-6 induced Stat 3 phosphorylation in various peripheral blood mononuclear cell populations as assessed by polychromatic flow

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		cytometry
8	DD13306	Characterization of CNTO 345, a Rat Anti-murine IL-6 Monoclonal Antibody
Pharmacokinetics		
9	P-2002-002	Single Intravenous Dose Pharmacokinetic and Immune Response Study in Cynomolgus Monkeys with CNTO 328 (Anti-IL-6 mAb)
Toxicology		
10	T-2003-010	Six month intravenous dose toxicity study of CNTO 328 in cynomolgus monkeys with a three month recovery period
11	T-2010-033	CNTO 345 (Anti-Mouse IL-6 Monoclonal Antibody): Fertility and General Reproduction Toxicity Study in Male Mice
12	T-2010-032	CNTO 345 (Anti-Mouse IL-6 Monoclonal Antibody): Fertility and General Reproduction Toxicity Study in Female Mice
13	T-2005-036	Investigation of the Effect of CNTO 328 on Embryo-Fetal Development in Cynomolgus Monkeys by Once-Weekly Intravenous Administration
14	T-2010-018	A Study for the Effect of CNTO 136 on Embryo-Fetal, Pre- and Postnatal Development in Cynomolgus Monkeys
15	T-2002-013	Cross-Reactivity of CNTO 328 with Normal Human Tissues
16	T-2004-019	Cross-Reactivity Study of Biotinylated CNTO 328 with Selected Normal Human Tissues
17	T-2003-004	Immunohistochemical Staining of Eye Tissues from Cynomolgus Monkeys Treated with CNTO 328

3.2 Studies Not Reviewed

Please refer to Appendix A for the list of 38 studies submitted to the BLA that were not reviewed.

3.3 Previous Reviews Referenced

None.

4 Pharmacology

4.1 Primary Pharmacology

The mechanism of action of siltuximab (also known as cCLB8 or CNTO 328) involves the binding to hIL-6, and neutralization of hIL-6 bioactivity. Relevant data provided to support the proposed mechanism of action for siltuximab was reviewed. These data were provided in the listed individual Study Reports and publications in peer-reviewed journals. Information to support the use of the cynomolgus monkey as a relevant toxicology species to test siltuximab was reviewed. Information to support the use of CNTO 345 (a rat anti-mouse IL-6 monoclonal antibody) and CNTO 136 (a humanized version of siltuximab) as surrogates of siltuximab in toxicology studies was also reviewed. The pharmacology data reviewed is categorized into the following sections.

<u>Siltuximab binding to IL-6</u>

Key findings:

- ELISA analysis confirmed siltuximab binding to hIL-6 is dose-related (Report REStr185-ks).

Reference ID: 3440995

- The most reliable estimates of siltuximab binding affinity for hIL-6 were derived from Biacore experiments, reporting a calculated K_D value of 34 pM (Report REStr510el).
    - A similar K_D value of 40 pM was calculated (from Biacore experiments) for the interactions between CNTO 345 and mouse IL-6 (mIL-6) (Report DD13306).
- The core epitope to which siltuximab binds was determined by protease digestion, hydrogen/deuterium exchange, mass spectrometry (MS) and tandem MS. It was determined the core epitope is located on helix D, at the C-terminus of the hIL-6 molecule (Report REStr233-yc).
    - CNTO 345 was found to bind an epitope located on helix D, at the C-terminus of the mIL-6 molecule (Report DD13306).

Siltuximab neutralization of IL-6 bioactivity

Selected pharmacology studies related to siltuximab-mediated neutralization of IL-6 bioactivity (e.g. receptor binding, Stat-3 phosphorylation, induction of plasmacytoma cell proliferation, induction of acute phase proteins, and induction of IgM secretion) that were reviewed included Reports REStr185-ks, BIOL-RES-tr336-dg, REStr560.rc, REStr180-rc, REStr233-yc, and DD13306, in addition to Siedeman and Petit (2002) [3].

Key findings:

- Siltuximab displaced hsIL-6R-bound biotin-hIL-6 with an IC_{50} of 21 ng/mL
- Siltuximab neutralized hIL-6 mediated proliferation of murine plasmacytoma 7TD1 cells with an EC_{50} of 7.2 ng/mL, approximately 50 pM.
    - CNTO 345 neutralized mIL-6 mediated proliferation of 7TD1 cells with an EC_{50} of 49 ng/mL (Report DD13306).
- Siltuximab neutralized IL-1β/IL-6 induced serum amyloid A protein (SAA) production in human hepatoma (HepG2) cells with an IC_{50} of 34.43 ng/mL, approximately 239 pM.
    - CNTO 345 neutralized mIL-6 induced SAA production in Balb/C mice at doses ≥ 1 mg/kg (Report DD13306).
- Siltuximab produced dose-related inhibition of IgM secretion by Burkitt's lymphoma, SKW 6.4, cells.

A. Report REStr185-ks: Generation and Characterization of cCLB8, Chimeric Anti-IL-6 mAb

This study assessed the binding of siltuximab to IL-6, and demonstrated siltuximab-mediated neutralization of IL-6 stimulated proliferation of 7TD1 murine myeloma cells as well as siltuximab-mediated neutralization of IL-1b/IL-6 induced serum amyloid A secretion in HepG2 cells.

[3] Seideman J., Peritt D. A novel monoclonal antibody screening method using Luminex-100™ microsphere system. *J. Immunol. Meth* (2002) 267:165-171

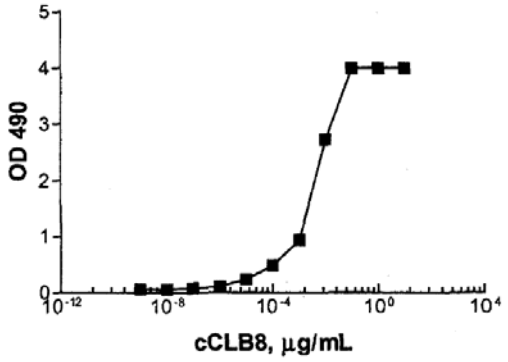
Reference ID: 3440995

Siltuximab binding to IL-6

Methods
Siltuximab was captured onto anti-human IgG, Fc specific coated ELISA plates. The plates were sequentially incubated with biotinylated recombinant human IL-6 (rhIL-6), washed, probed with horseradish peroxidase-labeled streptavidin, developed with substrate and optical densities (ODs) were measured at 490 nM.
Results
Half-maximal binding to 200 ng/mL rhIL-6 occurred at 4 ng/mL siltuximab.

Figure 1 Binding of siltuximab to human IL-6 using solid phase enzyme immunoassay

(*Excerpted from Applicant's submission*)



Siltuximab binding affinity

Methods
Anti-human Fc was coupled to the matrix of a CM-5 sensor chip. Surface plasmon resonance (Biacore) experiments were performed using siltuximab and rhIL-6.
Results
The kinetic constants for siltuximab binding to rhIL-6 were determined by analyzing two Biacore experiments. The calculated K_D value for siltuximab was determined to be 57 or 140 pM.

Table 2 Affinity measurements for siltuximab using Biacore

(*Excerpted from Applicant's submission*)

Sample	k_a ($m^{-1}s^{-1}$) (x 10^6)	k_d (s^{-1}) (x 10^{-5})	K_D(M) (x 10^{-11})	Chi^2
cCLB8	1.1	6.2	5.7	0.111
cCLB8	0.37	5.2	14	0.236

Siltuximab neutralized rhIL-6-mediated cell proliferation

Methods
7TD1 murine plasmacytoma cells were induced to proliferate with rhIL-6, and were co-cultured with siltuximab or the negative control monoclonal antibody (17-1A). Cell

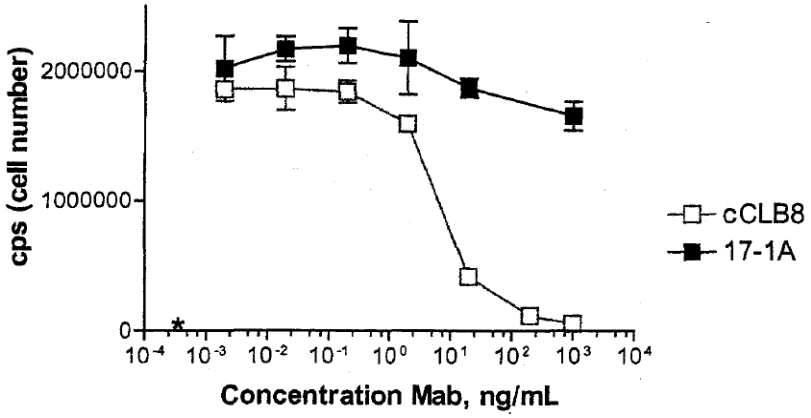
proliferation (measured as counts per second, or cps) was measured by a luminescent ATP assay (ATPLite), which correlated directly with cell number.

Results

Recombinant hIL-6 was shown to stimulate proliferation of 7TD1 cells in the presence of the negative control monoclonal antibody, 17-1A, and siltuximab (cCLB8 in the figure below) was shown to neutralize this effect in a concentration dependent manner. The EC_{50} value for siltuximab was 7.2 ng/mL.

Figure 2 Siltuximab neutralized rhIL-6-induced proliferation of 7TD1 cells

(*Excerpted from Applicant's submission*)



*** represents proliferation of cells in absence of rhIL-6.**

Siltuximab inhibited IL-6-mediated serum amyloid A production

Methods

HepG2 cells were seeded in culture plates. IL-6 and IL-6 soluble receptor (sIL-6R) were preincubated together and then mixed with IL-1β. Siltuximab and a negative isotype control monoclonal antibody (cSF25) were serially diluted and preincubated with the (IL-6, sIL-6R, IL-1β) mixture. The antibody/cytokine mixtures were then cultured with the HepG2 cells and an ELISA was performed to detect SAA in the supernatants.

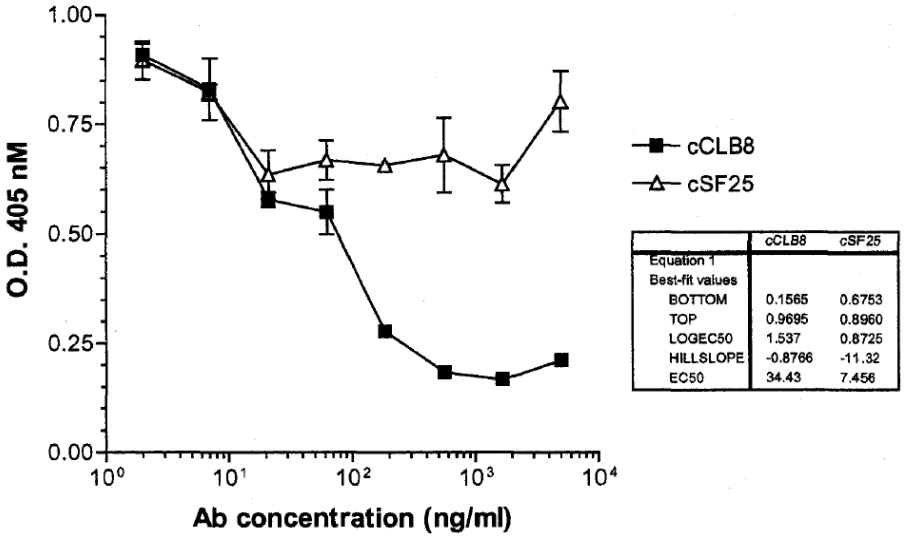
Results

Siltuximab inhibited IL-1β/IL-6-induced production of SAA in a concentration dependent manner. The EC_{50} value for siltuximab was 34.43 ng/mL.

Reference ID: 3440995

Figure 3 Siltuximab inhibited IL-6-induced SAA production in HepG2 cells

(*Excerpted from Applicant's submission*)



B. Report restr510el: Comparison of the Affinities of CNTO 328 and CNTO 136 to human IL-6 as Determined by Biacore and Kinexa

This study comparatively assessed the binding affinities of the chimeric anti-IL-6 monoclonal antibody, siltuximab (CNTO 328), and the humanized monoclonal antibody CNTO 136 to rhIL-6 using two methods, Biacore and Kinexa.

Methods (Biacore)
Anti-human Fc was coupled to the matrix of a CM-5 sensor chip. Surface plasmon resonance (Biacore) experiments were performed using siltuximab, CNTO 136, and rhIL-6.
Results (Biacore)
The K_D values measured using Biacore for rhIL-6 binding to siltuximab (CNTO 328) and CNTO 136 were similar at 34 and 39 picomolar (pM), respectively.

Table 3 Biacore analysis of binding affinity

(*Excerpted from Applicant's submission*)

Antibody	K_{on} ($M^{-1}s^{-1}$)	K_{off} (s^{-1})	K_D (pM)
CNTO 328	$(1.5 \pm 0.12) \times 10^6$	$(4.9 \pm 1.3) \times 10^{-5}$	34 ± 9
CNTO 136	$(1.8 \pm 0.14) \times 10^6$	$(7.1 \pm 1.7) \times 10^{-5}$	39 ± 10

Methods (Kinexa)
Human IL-6-modified beads for capture of CNTO 136 or siltuximab were prepared into columns. Various dilutions of CNTO 136 or siltuximab were mixed with various dilutions of hIL-6 and allowed to flow through the columns. Free CNTO 136 or free siltuximab bound to the beads, and binding was subsequently detected using a Cy5 (Cyanine dye) labeled secondary antibody.

Results (Kinexa)
The K_D values measured using Kinexa for hIL-6 binding to siltuximab (CNTO 328) and CNTO 136 differed by 19-fold.

Table 4 Kinexa analysis of binding affinity

(*Excerpted from Applicant's submission*)

Antibody	K_D (pM)	95% confidence interval (pM)
CNTO 328	3.35	(2.74-4.04)
CNTO 136	0.175	(0.039-0.386)

Differences between the Biacore and Kinexa experimental results (10-fold) may be attributable to differences in the greater time-dependency of the interactions for CNTO 136 than for CNTO 328. Additionally, incubation periods used in Kinexa experiments are measured in days while Biacore uses minutes.

C. Siedeman and Petit, 2002: A Novel Monoclonal Antibody Screening Method Using the Luminex-100 Microsphere System

This study describes the development of a binding competition assay system based on Luminex-100 microspheres (detection limit <500 pg/mL biotin-IL-6, K_D=75 ng/mL) that was used to screen for neutralizing (anti-IL-6) antibody, using hsIL-6R as the target molecule.

Binding assay system

Methods
Carrier-free hsIL-6Rs were coupled to Luminex-100 microspheres and rhIL-6 was labeled with biotin. Increasing amounts of biotin-rhIL-6 were incubated with hsIL-6R coupled microspheres. Binding was calculated using a streptavidin-phycoerythrin (SA-PE) probe and measuring the median fluorescence intensity (MFI).
Results
The binding of biotin-rhIL-6 to hsIL-6R was specific and saturable. The K_D for the reaction was 3.6 × 10 nM (75 ng/mL). B_{max} could not be calculated because the specific activities of phycoerythrin fluorescence and biotin-rhIL-6 were not known.

Figure 4 K_D and B_{max} plot of biotin-rhIL-6 and immobilized hIL-6sR

(*Excerpted from Siedeman and Petit, 2002*)

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Siltuximab mediated inhibition of biotin-rhIL-6 binding to hsIL-6R

Methods
Utilizing the binding assay system described above, hsIL-6R (coupled to microspheres) were incubated with a constant concentration of biotin-rhIL-6 (20 ng/mL) and increasing amounts of siltuximab (square) or an isotype control monoclonal antibody (triangle).
Results
Siltuximab displaced biotin-hIL-6 bound to hsIL-6R with an IC_{50} of 21 ng/mL.

Figure 5 Siltuximab mediated inhibition of biotin-rhIL-6 binding to hIL-6sR

(*Excerpted from Siedeman and Petit, 2002*)

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Comparing the Binding Inhibition Produced by Various Anti-IL-6 Monoclonal Antibodies

Methods
The ability of three anti-IL-6 monoclonal antibodies that bind to different epitopes of IL-6 [siltuximab (square), CLB.IL-6/16 (triangle), and CLB.IL-6/14 (inverted triangle)] to inhibit binding of biotin-rhIL-6 to hsIL-6R was assessed with the Luminex-100 microsphere based assay.
Results
Siltuximab exhibited the greatest inhibitory potency and activity, achieving ≈ 100% inhibition of bound biotin-rhIL-6. CLB.IL-6/16 treatment inhibited ≈ 62% of the bound biotin-rhIL-6. CLB.IL-6/14 exhibited no inhibition of binding.

Figure 6 Binding inhibition of three different anti-IL-6 monoclonal antibodies

(*Excerpted from Siedeman and Petit, 2002*)

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Reference ID: 3440995

D. Report biol-res-tr336-dg: Cross-species Reactivity of CNTO 328

This species cross reactivity study assessed the ability of recombinant IL-6 or conditioned (IL-6 containing) supernatants from peripheral blood mononuclear cell (PBMCs) isolated from a variety of species that were stimulated with interferon gamma (IFNγ) and lipopolysaccharide (LPS) to activate the proliferation of murine 7TD1 myeloma cells. The study also assessed the ability of siltuximab to neutralize the stimulated proliferation.

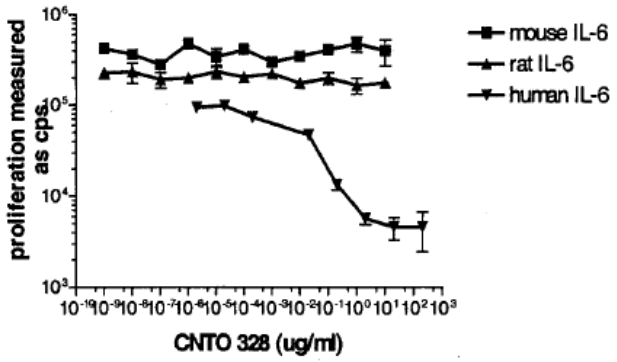
Prior to the neutralization studies the authors demonstrated that human, murine, and rat IL-6 could induce concentration dependent increases in proliferation of 7TD1 cells using the ATPLite bioassay. Conditioned supernatant from human, non-human primates (i.e., chimpanzee, cotton top, marmoset, rhesus, cynomolgus, pigtail, and baboon), guinea pig, beagle dog, and mini pig also induced 7TD1 cell proliferation.

Neutralization of proliferation in 7TD1 cultures stimulated with recombinant human, mouse, or rat IL-6

Methods
Murine 7TD1 plasmacytoma cells were stimulated to proliferate with recombinant human, mouse, or rat IL-6 in the presence of increasing amounts of siltuximab.
Results
Siltuximab neutralized rhIL-6 stimulated proliferation of 7TD1 cells at an IC_{50} of 4.6 ng/mL (30.6 pM). Siltuximab had no effect on 7TD1 cells stimulated with mouse or rat IL-6.

Figure 7 Siltuximab neutralizes IL-6 stimulated proliferation in 7TD1 cells

(*Excerpted from Applicant's submission*)



Neutralization of proliferation in 7TD1 cultures stimulated with conditioned media from stimulated PBMC isolated from different species

Methods
Murine 7TD1 plasmacytoma cells were stimulated to proliferate in various conditioned (IFNγ+LPS stimulated peripheral blood mononuclear cell -PBMCs) media from different species in the presence of increasing amounts of siltuximab.

Reference ID: 3440995

Results
Siltuximab neutralized the 7TD1 cell proliferation stimulated by conditioned media from human, chimpanzee, cotton top, marmoset, rhesus, cynomolgus, pigtail, and baboon, but not beagle dog, guinea pig, mini pig, rat, or mouse.

Table 5 Neutralization potency of siltuximab using PBMCs conditioned supernatants from different species

(*Excerpted from Applicant's submission*)

Species	IC_{50} (ng/ml)	IC_{50} (pM)
Human	4.6	30.6
Chimpanzee (Pan troglodytes)	0.69	4.6
Baboon (Papio cynocephalus)	0.89	5.93
Cynomolgus Monkey (Macaca fascicularis)	1.95	13
Rhesus Monkey (Macaca mulatta)	2.6	17.3
Marmoset (Callithrix jacchus)	2.5	16.7
Pigtailed macaque (Macaca nemestrina)	1.1	7.33
Cotton Top Tamarin (Sanguinus oedipus)	2.0	13.3
Beagle (Canis familiaris)	No effect	No effect
Mini pig (Sus scrofa)	No effect	No effect
Guinea Pig (Cavia porcellus)	No effect	No effect
Mouse	No effect	No effect
Rat	No effect	No effect

E. Report restr180-rc: CNTO 328 Inhibition of IL-6 Induced IgM and MCP-1 Secretion

This study evaluated the ability of siltuximab to inhibit IL-6 induced secretion of human IgM and the chemotactic cytokine monocyte chemotactic protein-1 (MCP-1) from various human cell lines.

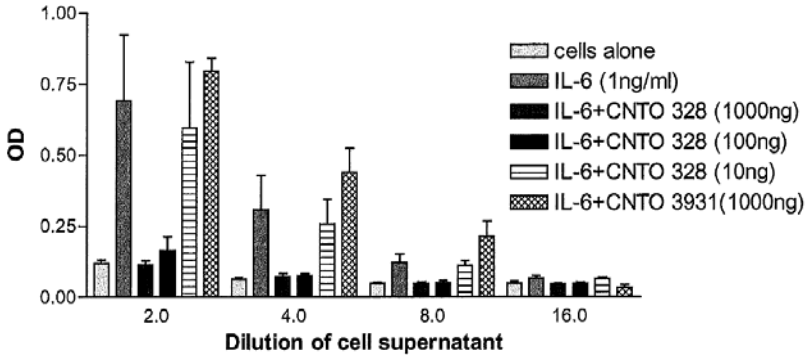
Siltuximab effects on IgM secretion

Methods
SKW 6.4 (Burkitt's lymphoma) cells were cultured alone or with rhIL-6, rhIL-6+siltuximab, or with rhIL-6+CNTO 3931 (an isotype matched control monoclonal antibody). Supernatants (72 hour) were collected and assayed with an ELISA for total IgM.
Results
Results from four different experiments are presented in Figure 8. Dose-related inhibition of IL-6 induced IgM secretion in SKW 6.4 was observed in supernatants from cells treated with siltuximab.

Reference ID: 3440995

Figure 8 Siltuximab inhibited IL-6 induced IgM secretion in SKW 6.4 cells

(*Excerpted from Applicant's submission*)



Siltuximab effects on IL-6 induced chemotactic cytokines secretion

Methods

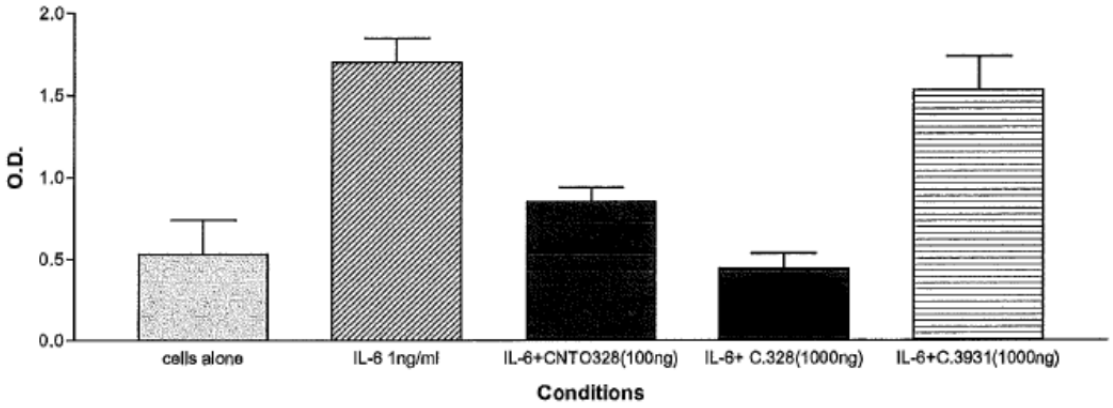
Chemokines are secreted in response to signals such as proinflammatory cytokines where they play an important role in selectively recruiting monocytes, neutrophils, and lymphocytes. U937 myeloid cells were cultured alone or with rhIL-6, rhIL-6+siltuximab, or with rhIL-6+CNTO 3931 (an isotype matched control monoclonal antibody). Supernatants (72 hour) were collected and assayed with an ELISA for total MCP-1.

Results

Results from four different experiments are presented in Figure 9. Dose-related inhibition of IL-6 induced MCP-1secretion in SKW 6.4 was observed in supernatants from cells treated with siltuximab.

Figure 9 Siltuximab inhibited IL-6 induced MCP-1 secretion in U937 cells

(*Excerpted from Applicant's submission*)



F. Report restr233-yc: Epitope Mapping of Monoclonal Antibodies on Human IL-6 by Hydrogen/Deuterium Exchange and Mass Spectrometry

This study was conducted to characterize the epitope recognition of siltuximab and CNTO 136 to hIL-6.

Epitope analysis of siltuximab

Methods
The core epitope of hIL-6 that siltuximab (and CNTO 136) binds to was determined by immunoprecipitation, protease digestion, hydrogen/deuterium exchange, MS/tandem MS.
Results
The binding epitope for siltuximab (and CNTO 136) is located on helix D, at the C-terminus of the hIL-6 molecule.

G. Report restr560-rc: CNTO 328 inhibition of Stat-3 phosphorylation: Effects of anti-human IL-6 (CNTO 328), on IL-6 induced Stat 3 phosphorylation in various peripheral blood mononuclear cell populations as assessed by polychromatic flow cytometry

This study evaluated the ability of CNTO 328 to reduce steady state levels of interleukin-6 (IL-6) induced phosphorylation of Stat-3 (P-stat3) in PBMC (CD3+ T cells or CD19+ B cells) cultured as whole blood and measured using polychromatic flow cytometry.

Methods
Whole blood from 8 healthy volunteers was cultured for 24 hours accordingly: alone; with rhIL-6 (100 pg/mL); with rhIL-6 co-cultured with CNTO 328; or with CNTO 328 added 12 h after rhIL-6. All antibodies for PE CD126, Pacific Blue CD19, FITC CD3, and AlexaFlour 647 Pstat-3 were added simultaneously for nested analysis from flow cytometric samples. Live CD3+ or CD19+ cells were then assessed for intracellular Pstat-3 and IL-6 receptor α.
Results
Recombinant hIL-6 induced upregulation of intracellular Stat-3 phosphorylation in CD3+ cells from healthy volunteers, Figure 10. In both assay conditions containing CNTO 328 and rhIL-6, Stat-3 phosphorylation was reduced in CD3+ cells, Figure 10. These effects were not observed or occurred minimally in CD19+ cells. Data from the presence of IL-6 receptor α was inconclusive.

Reference ID: 3440995

Figure 10 P-Stat 3 expression in whole blood CD3+ cells incubated with rhIL-6 alone or with CNTO 328

(Excerpted from Applicant's submission)

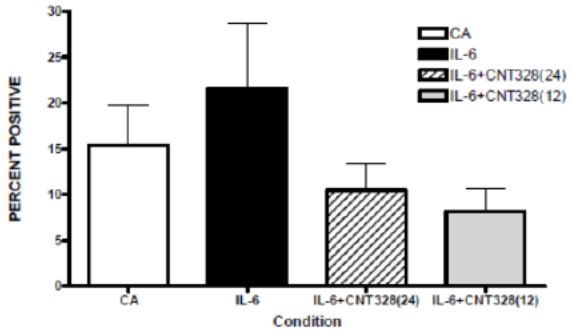
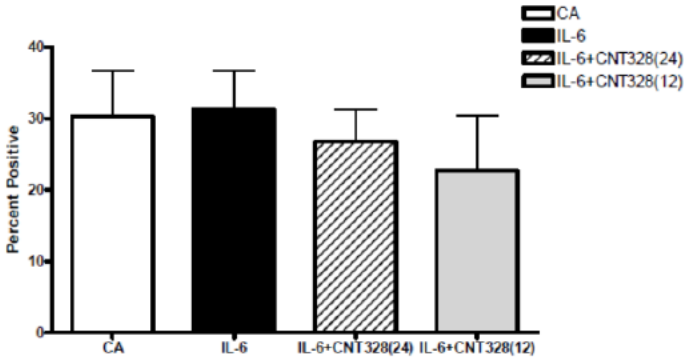


Figure 11 P-Stat 3 expression in whole blood CD19+ cells incubated with rhIL-6 alone or with CNTO 328

(Excerpted from Applicant's submission)



H. Report dd13306: Characterization of CNTO 345, a Rat Anti-murine IL-6 Monoclonal Antibody

This study was conducted to characterize the binding (epitope recognition and affinity determination) of the rat anti-mouse IL-6 (mIL-6) monoclonal antibody (CNTO 345) to recombinant mouse IL-6, in addition to assessing the in vitro (neutralization of IL-6-induced 7TD1 cell proliferation) and in vivo (inhibition of mIL-6 stimulated SAA production in Balb/c mice) activity of the antibody. (b) (4)

Epitope analysis of CNTO 345

Methods

The core epitope of mIL-6 that CNTO 345 binds to was determined by immuno-precipitation, protease digestion and MS and tandem MS.

Results

The epitope's sequence (residues 160-196) encodes a peptide present in helix D of mIL-6. The epitope on mIL-6 recognized by CNTO 345 is similar to the epitope on hIL-6 recognized by siltuximab (CNTO 328) and CNTO 136.

Neutralization of mIL-6-stimulated 7TD1 cell proliferation

Methods

Mouse IL-6-stimulated murine 7TD1 myeloma cell proliferation was measured using the ATPLite bioassay. 7TD1 cells were cultured with 20 pg/mL mIL-6 to achieve approximately 80% maximal proliferation, and CNTO 345 was tested for its ability to neutralize the proliferation.

Results

Mouse IL-6 stimulated 7TD1 cells to proliferate in a concentration dependent manner with an average (n=6 experiments) EC_{50} of 48 pg/mL. CNTO 345 neutralized mIL-6 stimulated 7TD1 cell proliferation with an average (n=4 experiments) EC_{50} of 49 ng/mL.

Figure 12 Mouse IL-6 stimulated proliferation of 7TD1 cells

(*Excerpted from Applicant's submission*)

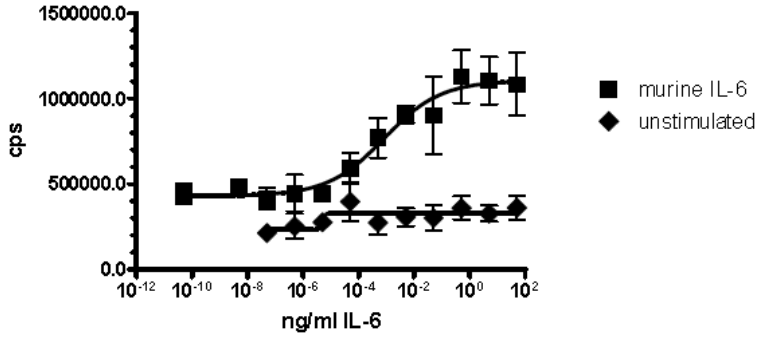
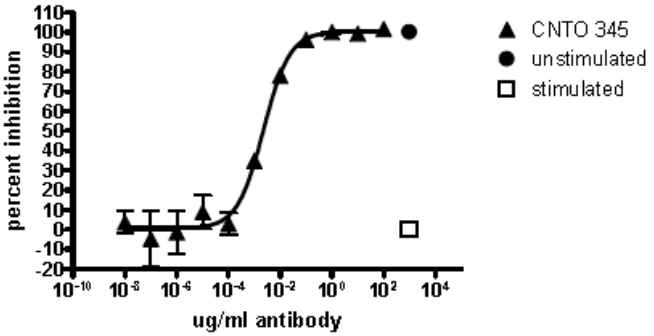


Figure 13 CNTO 345 neutralization of mIL-6 stimulated proliferation of 7TD1 cells

(*Excerpted from Applicant's submission*)



Analysis of binding affinity of CNTO 345 for mIL-6 using Biacore analysis

Biacore analysis was utilized to assess the affinity constant (K_D), on-rate (k_{on}) and off-rate (k_{off}) for the interactions between CNTO 345 and carrier-containing mIL-6. The K_D value was 40 ± 3 pM, and the on-rate (k_{on}) and off-rate (k_{off}) were (1.62 ± 0.04) x 10^6 M^{-1} s^{-1} and (6.46 ± 0.5) x 10^{-5} s^{-1}, respectively.

Reference ID: 3440995

In vivo neutralization of mIL-6 stimulated SAA secretion

Methods

Balb/C mice were given mIL-6 injections (0.25, 1, 2.5, or 5 µg/kg) to stimulate secretion of SAA. CNTO 345 (0.01, 0.1, 1.0 or 10 mg/kg) was tested for its ability to neutralize the SAA production.

Results

Administration of mIL-6 resulted in dose-related increases in serum SAA in Balb/C mice. CNTO 345 administered at ≥ 1 mg/kg resulted in neutralization of SAA secretion in Balb/C mice; SAA levels in these animals were no higher than those observed in unstimulated mice.

Figure 14 Mouse IL-6 stimulated SAA secretion in Balb/C mice

(*Excerpted from Applicant's submission*)

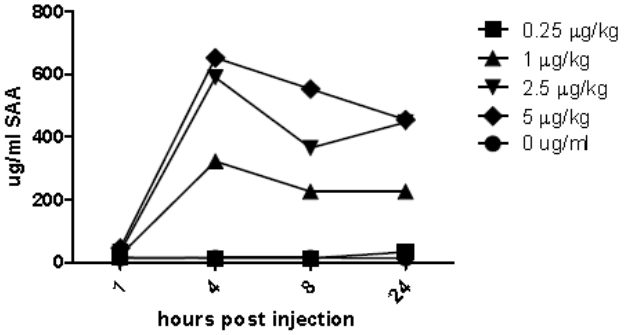
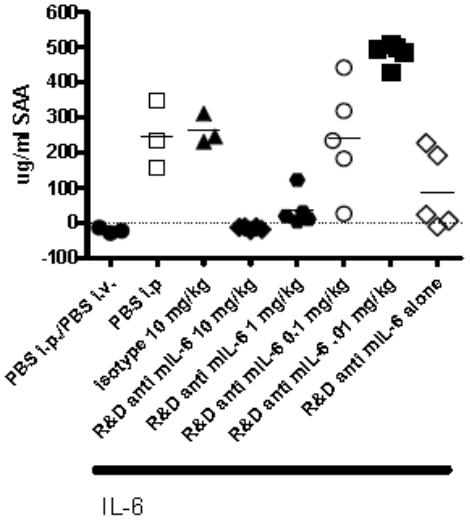


Figure 15 Neutralization of mIL-6 stimulated SAA production in Balb/C mice

(*Excerpted from Applicant's submission*)



4.2 Secondary Pharmacology

Janssen submitted a number of in vitro and in vivo studies conducted with siltuximab, alone or in combination with other drugs, in IL-6 sensitive human tumor models. These studies are not directly related to Castleman's disease but further characterize the

ability of siltuximab to inhibit IL-6 mediated effects (see Appendix A, studies not reviewed).

4.3 Safety Pharmacology

Safety pharmacology evaluations were incorporated into the GLP repeat-dose toxicology studies with siltuximab.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Report p-2002-002: Single IV Dose PK and Immune Response Study in Monkeys
Pharmacokinetics parameters and immune antibody response were evaluated in cynomolgus monkeys following a single IV dose of siltuximab at 1.84, 9.2, or 46 mg/kg. Accelerated clearance occurred in one monkey in each of the 9.2 and 46 mg/kg groups after 35-42 days post-dosing from an apparent anti-drug (ADA) response. No immune response was observed in the 1.84 mg/kg group. Non-compartmental analysis demonstrated the relationship between increasing dose and exposure was greater than dose proportional, and that clearance rates were slow with apparent $t_{1/2}$ values of 10-21 days.

Table 6 Mean PK parameter estimates following a single IV dose in monkeys

Dose Group (mg/kg)	C_{max} (µg/mL)	$AUC_{(0\text{-}tz)}$ (µg.h/mL)	$t_{1/2}$ (day)	V_z (mL/kg)	CL (mL/day/kg)
1.84 (n=3)	68.5	6,447	17.7	143.6	5.8
9.2 (n=2)	440	37,797	10.1	63.0	5.0
46 (n=2)	3,524	263,691	21.1	79.4	2.9

No specific distribution, metabolism, or excretion studies were conducted.

5.2 Toxicokinetics

Refer to individual toxicology studies.

6 General Toxicology

6.1 Single-Dose Toxicity

No single-dose toxicology studies were conducted.

Reference ID: 3440995

6.2 Repeat-Dose Toxicity

Study title: Six month intravenous dose toxicity study of CNTO 328 in cynomolgus monkeys with a three month recovery period

Study no.:	t-2003-010
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	June 27, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CNTO 328, lot # 5873-135, Purity: 99.8% lot # 5873-112, Purity: 99.5%

Key Study Findings

- One male treated with 46 mg/kg CNTO 328 was euthanized early on Day 117 due to edema, swelling, lacerations and ulcerations of the right leg and foot. An arterio-venous shunt in the upper right leg of this animal was likely the cause of these adverse clinical signs. It is unclear if these toxicities are related to CNTO 328 administration.
- Toxicities observed with 46 mg/kg included skin erythema in the shoulder region of 2 females and swollen face in one female during the infusion period on the first day of CNTO 328 dosing accompanied with decreases in neutrophils, monocytes, WBC and/or platelets.
- Low heart rate was observed in one male and one female treated with 46 mg/kg CNTO 328 during Week 16; low blood pressure was observed in the same male treated with 46 mg/kg CNTO 328 during Weeks 24 and 29.
- A trend for a lower immune response was observed with treatment of CNTO-328 with slightly lower anti-KLH IgM and IgG levels present in the CNTO 328-treated groups.

Methods

Doses:	0, 9.2, or 46 mg/kg. Dose selection based on 3-month toxicology study results
Frequency of dosing:	Once weekly for 25 weeks
Route of administration:	Intravenous infusion, 2 hours
Dose volume:	2.5 mL/kg
Formulation/Vehicle:	0.9% sodium chloride for injection (saline)
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	Main study: 3/sex/group Recovery: 2/sex/group
Age:	Young adult, specific age not specified
Weight:	Males: 2.5-3.3 kg on Day -3

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Females: 2.1-2.8 kg on Day -5
Satellite groups: None
Unique study design: Animals were dosed according to a staggered schedule, with dosing of the males commencing two days prior to dosing of the females; first day of dosing designated Day 1 for each sex

Observations and times:

Mortality:	Twice daily
Clinical signs:	At least once daily
Body weights:	Three times prior to initial treatment, weekly thereafter, and on the day prior to necropsy; final fasted body weight on the day of necropsy
Food consumption:	Daily
ECG and blood pressure:	Twice prior to initial treatment and Weeks 16, 24, 29, and 36
Physical examination:	Prior to initial treatment and Weeks 16, 24, 29, and 36
Respiratory rate:	Twice prior to initial treatment and Weeks 16, 24, 29, and 36
Ophthalmoscopy:	Prior to initial treatment and Weeks 16, 24, 29, and 36
Hematology:	Weeks -2 and -1, Days 51, 107, 176, 197, and 254
Clinical chemistry:	Weeks -2 and -1, Days 51, 107, 176, 197, and 254
Coagulation:	Weeks -2 and -1, Days 51, 107, 176, 197, and 254
Urinalysis:	At necropsy*
Gross pathology:	At necropsy*
Organ weights:	At necropsy*
Histopathology:	At necropsy*
Anti-therapeutic antibody assessment:	Pretreatment on Day 1 (prior to the first dose) and prior to dosing on Days 57, 113, 169, 176, 197, 225, and 254
Toxicokinetics:	• Days 1 and 169 at predose and 2, 8, 24, 48, and 96 hours after dosing • Samples were also collected prior to treatment on Days 8, 29, 57, 85, 113, and 141 and at 2 hours after infusion on Days 29, 57, 85, 113, and 141 • Samples were taken from recovery animals on Days 176, 183, 190, 197, 211, 225, 239, and 254
KLH analysis:	KLH administered on Days 16 and 30; responses assessed on Days 25, 51, 92, 176, 197, and 254

*Necropsy for main study animals conducted on Day 176 and necropsy for recovery animals conducted on Day 254

Results

Mortality

One male treated with 46 mg/kg CNTO 328 (Animal #3005) was euthanized for humane reasons on Day 117. This animal experienced edema and associated swelling of the right leg on Days 57-115 and 117 that progressively became more severe. Lacerations of the right foot were present on Days 107-115 and 117 and ulcerations were present on the right foot and leg on Day 117. A test facility veterinarian diagnosed an arterio-venous shunt in the upper right leg of this animal that was likely the cause of the adverse clinical signs observed. Microscopic findings for this animal included mild hemorrhage and edema of the skeletal muscle of the right hind limb, and moderate hemorrhage, edema, necrosis of dermis, and acute inflammation of the skin of the right hind limb. Lymphoid hyperplasia and sinus histiocytosis of the iliac and inguinal lymph

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nodes was also observed and corresponded to the enlargement of the right lymph nodes.

Clinical Signs

- Skin erythema (mild or moderate) was observed periodically in the shoulder region of 2 females treated with 46 mg/kg CNTO 328 (Animal #3101 and 3104)
  - Animal # 3101: Mild or moderate skin erythema was reported for the left or both shoulders on Days 99-113, 115-157, and 160-176. Dry flaky skin on the shoulders was reported on Day 176.
  - Animal # 3104: Mild skin erythema was reported for the left shoulder on Days 17-20, 80-113, 115-157, and 160-162.
- Swollen face (moderate) was observed in one female treated with 46 mg/kg CNTO 328 (Animal #3103) during the infusion period on the first day of CNTO 328 dosing. The swelling resolved after the infusion ended and no other instances occurred.

Body Weights and Feed Consumption

Unremarkable

Ophthalmoscopy and ECG

Unremarkable

Blood pressure, heart rate, and respiratory rate

During Week 16, one male (Animal #3004) and one female (Animal #3104) treated with 46 mg/kg CNTO 328 had abnormally low heart rates compared to values recorded at other time points. Blood pressure was low for Animal #3004 treated with 46 mg/kg CNTO 328 during Weeks 24 and 29 compared to values at other time points. The individual values for these animals along with the means for the control and 46 mg/kg groups are presented in the table below.

Table 7 Heart rate and blood pressure for 6-month toxicology study in monkeys

Group/animal	Week					
	-2	-1	16	24	29	36
Heart rate (bpm)						
Males						
Control group mean	232.6	221.8	169.2	138.6	238.5	218.5
46 mg/kg group mean	252.0	227.6	177.0	211.3	NA	NA
Animal #3004	255	235	43	222	250	173
Females						
Control group mean	231.0	241.0	161.6	231.2	242.5	139.5
46 mg/kg group mean	243.4	239.8	131.4	238.4	231.5	225.0
Animal #3104	235	242	36	235	228	228
Mean blood pressure (mmHg)						
Males						
Control group mean	119.2	105.0	102.4	109.4	101.5	95.0
46 mg/kg group mean	88.8	99.6	106.2	88.5	NA	NA
Animal #3004	72	87	127	44	33	68

NA= Not applicable, Animal #3004 was the only recovery animal for this group

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Hematology

Slight decreases in platelets and white blood cell parameters (WBC count, neutrophils, and monocytes) were observed in females treated with CNTO 328, however, there was variability in the control group for these parameters and the toxicological significance of these changes is unknown. There were no consistent hematology findings for males treated with CNTO 328.

Table 8 Hematology in females for 6-month toxicology study in monkeys

Index	Mean				Mean (Percentage deviation from Control)							
	Control (0 mg/kg)				9.2 mg/kg				46 mg/kg			
	Week -2	Day 51	Day 107	Day 176	Week -2	Day 51	Day 107	Day 176	Week -2	Day 51	Day 107	Day 176
Platelets (10^3/µL)	391.8	370.0	409.4	430.8	415.4	362.8	411.2	414.6	358.4	316.2 (↓15)	352.8 (↓14)	346.8 (↓19)
WBC (10^3/µL)	9.48	14.50	11.72	13.58	11.72	9.80 (↓32)	10.54 (↓10)	10.10 (↓26)	10.68	9.22 (↓36)	9.04 (↓23)	9.56 (↓30)
Neutrophils (10^3/µL)	4.118	7.304	5.776	8.068	5.896	3.454* (↓53)	4.054	4.778	5.604	3.442* (↓53)	4.174	4.076
Monocytes	0.328	0.488	0.372	0.360	0.358	0.356 (↓27)	0.416	0.380	0.352	0.308* (↓37)	0.266* (↓28)	0.324

↓=decrease
Significantly different from Control, * p≤0.05

Clinical Chemistry

Unremarkable

Urinalysis

Unremarkable

Gross Pathology

Table 9 Gross pathology for 6-month toxicology study in monkeys

Treatment-Related Macroscopic Findings		No. of animals affected					
		Males			**Females**		
Dose (mg/kg)		0	9.2	46	0	9.2	46
Number of animals examined		0/3/**2**	0/3/**2**	1*/3/**1**	0/3/**2**	0/3/**2**	0/3/**2**
Skin, shoulder	Crust	0/0/**1**	-	-	-	-	0/1/**0**
	Accumulation	0/0/**1**	-	-	-	-	-
	Foci	0/0/**1**	-	-	-	-	-

Number of animals examined and affected: Early deaths*/ Terminal necropsy / **Recovery necropsy**
- = no test-article related changes

Organ Weights

Unremarkable

Histopathology

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Adequate Battery: Yes
Peer Review: No
Histological Findings:
Microscopic findings for the one male treated with 46 mg/kg CNTO 328 (Animal #3005) euthanized early are listed in the mortality section above. Below are the histopathology findings for all other animals.

Table 10 Histopathology for 6-month toxicology study in monkeys

Treatment-Related Microscopic Findings			No. of animals affected					
			Males			**Females**		
Dose (mg/kg)			0	9.2	46	0	9.2	46
Number of animals examined			0/3/**2**	0/3/**2**	1*/3/**1**	0/3/**2**	0/3/**2**	0/3/**2**
Organ	Finding							
Skin/ mammary region	Chronic inflammation of dermis	Minimal	-	-	0/1/**0**	-	0/0/**1**	-
Skin, shoulder	Hyperkeratosis	Minimal	-	-	-	-	-	0/1/**1**
	Acanthosis	Minimal	-	-	-	-	-	0/1/**1**

Number of animals examined and affected: Early deaths*/ Terminal necropsy / **Recovery necropsy**
- = no test-article related changes

Special Evaluation

Antibody Response to T-Cell-Dependent Antigen (KLH)

To access the effect of CNTO 328 on T-Cell-dependent antigen response (TDAR), all monkeys were immunized with a 1 mL emulsion of 100 µg KLH and Incomplete Freund's Adjuvant by intramuscular injection on Days 16 and 30. Blood samples were collected Week -1 and on Days 25, 51, 92, 176 (Week 17), 197, and 254.

Table 11 Mean center point titer values for anti-KLH IgM

Dose	Sex	Day 25	Day 51	Day 92	Day 176	Day 197	Day 254
0 mg/kg	Males	349	343	303	283	281	271
	Females	328	371	344	283	314	263
	Combined	**339**	**357**	**323**	**283**	**303**	**265**
9.2 mg/kg	Males	295	375	312	259	256	267
	Females	260	323	288	256	260	247
	Combined	**277**	**349**	**301**	**257**	**258**	**257**
46 mg/kg	Males	275	297	258	285	NA	NA
	Females	339	399	305	258	241	244
	Combined	**307**	**348**	**287**	**267**	**241**	**244**

NA=Not available

Anti-drug antibody analysis/Immunogenicity

Anti-drug antibody assessment was conducted using a bridging Enzyme ImmunoAssay with blood samples collected pretreatment on Day 1 (prior to the first dose) and prior to dosing on Days 57, 113, 169, 176, 197, 225, and 254. Antibodies to CNTO 328 were not detected at any time during the study in any of the samples for monkeys administered saline or 9.2 or 46 mg/kg CNTO 328; however, high concentrations of circulating CNTO 328 may have interfered with the immunoassay and the detection of anti-drug antibodies.

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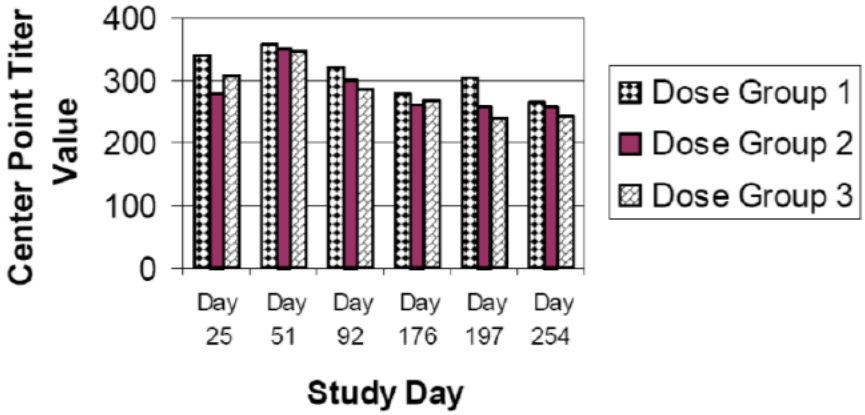
Figure 16 Mean anti-KLH IgM titer levels

(*Excerpted from Applicant's submission*)



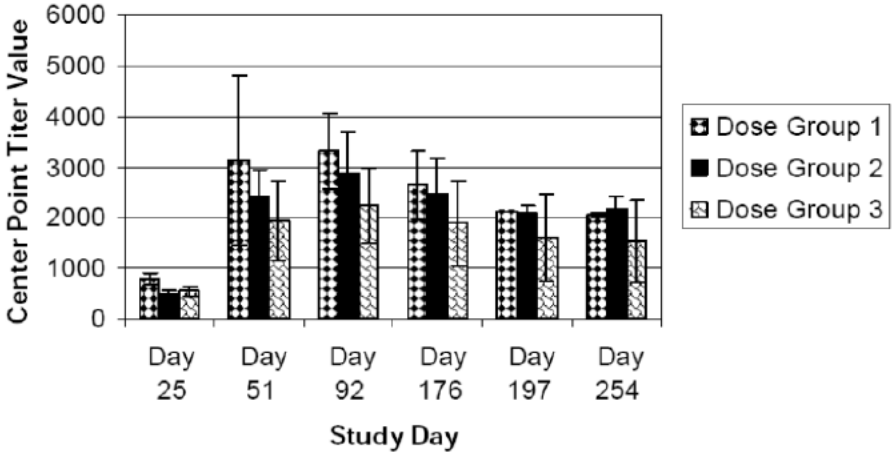
Group 1= 0 mg/kg (control) Group 2= 9.2 mg/kg Group 3= 46 mg/kg

Table 12 Mean center point titer values for anti-KLH IgG

Dose	Sex	Day 25	Day 51	Day 92	Day 176	Day 197	Day 254
0 mg/kg	Males	754	2957	2894	2257	2126	2007
	Females	834	3325	3740	3030	2131	2093
	Combined	**794**	**3141**	**3317**	**2644**	**2129**	**2050**
9.2 mg/kg	Males	533	2656	3058	2606	2199	2170
	Females	473	2220	2656	2276	1996	2136
	Combined	**502**	**2438**	**2857**	**2441**	**2097**	**2153**
46 mg/kg	Males	461	1737	2034	1756	652	618
	Females	595	2139	2424	2011	2083	2001
	Combined	**545**	**1938**	**2229**	**1884**	**1606**	**1540**

Figure 17 Mean center point titer values anti-KLH IgG

(*Excerpted from Applicant's submission*)



Group 1= 0 mg/kg (control) Group 2= 9.2 mg/kg Group 3= 46 mg/kg

Toxicokinetics

The toxicokinetics of CNTO 328 (9.2 and 46 mg/kg) were evaluated on Days 1 and 169 with samples collected at predose and 2, 8, 24, 48, and 96 hours after dosing. Samples were also collected prior to treatment on Days 8, 29, 57, 85, 113, and 141 and at 2 hours after infusion on Days 29, 57, 85, 113, and 141. For recovery animals, samples were taken on Days 176, 183, 190, 197, 211, 225, 239, and 254 during the recovery period.

- C_{max} and AUC values increased with increased dose; the increase in C_{max} for the first dose was approximately dose proportional from 9.2 to 46 mg/kg while the increases in AUC for the first dose and the C_{max} and AUC for the 25th dose were slightly less than dose proportional.
- C_{max} and AUC were higher on Days 169 than Day 1 with accumulation ratios (R) for AUC of 7.84 for the 9.2 mg/kg group and 5.99 for the 46 mg/kg group, indicating that there was accumulation of CNTO 328 with repeated administration.
- Overall, exposure to CNTO 328 was similar between males and females.
- Based on trough concentration observations, steady state was achieved after Day 85 (Dose 13).

Table 13 Toxicokinetics for 6-month toxicology study in monkeys

(*Excerpted from Applicant's submission*)

9.2 mg/kg Group

		1st dose (protocol time Day 1)		25th dose (protocol time Day 169)					
Gender	Animal ID	Cmax (µg/mL)	AUC(0-7d) (µg.day/mL)	Cmax (µg/mL)	AUC(168-175d) (µg.day/mL)	CLss (mL/day/kg)	Vss (mL/kg)	t1/2 (day)	R (AUC)
Male	2001[b]	303.12	1225.70	1291.37	6820.37	1.47	78.27	NA[d]	5.56
	2002	330.34	974.99	3605.75	15600.85	0.64	4.39	NA[d]	16.00
	2003	250.09	1266.63	2614.33	13288.88	0.75	39.52	NA[d]	10.49
	2004	345.80	1173.12	2916.89	9699.18	1.03	13.02	31.66	8.27
	2005	322.70	1021.42	3168.16	10860.89	0.92	17.29	21.12	10.63
Female	2101[b]	384.04	1693.78	1606.44	7771.36	1.29	34.32	NA[d]	4.59
	2103[a]	432.98	1515.95	2296.59	11572.10	0.86	NA[a]	NA[a]	7.63
	2104	392.89	1490.15	2888.17	8116.34	1.23	23.00	19.19	5.45
	2105	441.17	1952.76	1497.24	7345.57	1.36	35.47	17.20	3.76
	2106[b]	284.43	948.09	1236.50	5665.66	1.77	172.25	NA[d]	5.98
N		10	10	10	10	10	9	4	10
Mean		348.76	1326.26	2312.14	9674.12	1.13	46.39	20.15[c]	7.84
SD		63.08	331.14	853.51	3142.63	0.35	51.77	17.20-31.66[c]	3.70

[a]The log-linear regression of the terminal phase of the concentration-time profile following the 25th dose on Day 169 for animal 2103 could not be calculated due to the abnormal concentration values observed during terminal phase. Therefore, the Vss and t1/2 can not be calculated.
[b]The time point on Day 176 for animals 2001, 2101, 2106 was excluded from the regression analysis of the log-linear portion of the terminal phase of the concentration-time profile following the 25th dose on Day 169 due to an abnormal high concentration value observed.
[c]Median and range, instead of mean and SD, were calculated for t1/2.
[d]Not available

46 mg/kg Group

		1st dose (protocol time Day 1)		25th dose (protocol time Day 169)					
Gender	Animal ID	Cmax (µg/mL)	AUC(0-7d) (µg.day/mL)	Cmax (µg/mL)	AUC(168-175d) (µg.day/mL)	CLss (mL/day/kg)	Vss (mL/kg)	t1/2 (day)	R (AUC)
Male	3002	4588.60	4141.39	4844.00	19357.09	2.58	19.38	NA[c]	4.67
	3003	1412.86	2221.94	4538.07	15014.42	3.33	21.49	NA[c]	6.76
	3004	1751.43	1585.46	6321.94	26604.42	1.88	62.63	20.78	16.78
	3005	1897.24	1870.45	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]
	3006	1877.30	3598.41	4962.15	19038.96	2.63	12.15	NA[c]	5.29
Female	3101	1959.74	7483.11	5668.60	27773.29	1.80	16.08	NA[c]	3.71
	3102	1830.34	5850.57	5173.01	22603.14	2.21	60.78	NA[c]	3.86
	3103	2271.90	8948.25	5129.74	20799.49	2.40	7.99	NA[c]	2.32
	3104	1870.78	5910.28	8639.78	29892.09	1.67	22.16	18.40	5.06
	3105	1202.27	3474.36	6880.92	19020.93	2.63	47.18	16.73	5.47
N		10	10	9	9	9	9	3	9
Mean		2066.25	4508.42	5795.36	22233.76	2.35	29.98	18.40[a]	5.99
SD		933.72	2474.09	1302.55	4893.31	0.52	21.07	16.73-20.78[a]	4.24

[a]Median and range, instead of mean and SD, were calculated for t1/2.
[b]Not available (animal was euthanized on Day 117 due to the arterio-venous shunt).
[c]Not available

Dosing Solution Analysis

The CNTO 328 concentrations in the dosing solutions for the control (0 mg/kg), 9.2 mg/kg, and 46 mg/kg dose groups were determined for Weeks 1, 5, 13, 17, and 25. CNTO 328 was not detected at any time in the dosing solutions for the control animals. All of the 9.2 and 46 mg/kg dosing solutions had CNTO 328 concentrations that were within the Test Facility's acceptable limits of 90-110% of theoretical concentration.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

9.1.1. Fertility in Male mice

Study title: CNTO 345 (Anti-Mouse IL-6 Monoclonal Antibody): Fertility and General Reproduction Toxicity Study in Male Mice

Study no.: T-2010-033
Study report location: eCTD 4.2.3.5.1
Conducting laboratory and location: (b) (4)
Date of study initiation: November 22, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: CNTO 345; Lot #: AHV221006B; Purity: 98.5%

Key Study Findings

- No CNTO 345-related mortality or effects were noted on clinical signs or body weight.
- Dose-related increases in relative weight of prostate were observed, reaching statistical significance in the 100 mg/kg/dose males group.
- Increases in relative organ weight of epididymis, seminal vesicles and testis in the 40 mg/kg/dose were not present at the high dose of 100 mg/kg/dose.
- No CNTO 345-related effects occurred on mating and fertility parameters, caesarean-sectioning or embryos from the F1 generation.

Methods

Doses:	0, 40, 100 mg/kg/dose. Dose selection based on previous studies conducted with CNTO 345
Frequency of dosing:	Once weekly for 4 weeks (before cohabitation), during cohabitation and continuing through the day before necropsy (for a maximum of 7 weeks)
Dose volume:	12.3 mL/kg
Route of administration:	Subcutaneous
Formulation/Vehicle:	Dulbecco's phosphate buffered saline (Mg and Ca free)
Species/Strain:	Mouse/Crl:CD1(ICR)
Number/Sex/Group:	25 mice/sex/group
Satellite groups:	6 males/group for use in TK sample collection before and 24 hours after dose administration on the 1st, 3rd and last day of dosing
Study design:	Females were not given the vehicle or drug formulation and were sacrificed on GD 13. Cohabitation period of maximum 17 days Male euthanasia and gross necropsy after completion of the cohabitation period
Deviation from study protocol:	None of the deviations affected the interpretation of study findings

Additional Methods

Blood collection: blood samples were collected from all main study mice at termination for serum CNTO 345 concentration bioanalysis and for anti-CNTO 345 antibody analyses.

Female examination: estrous cycling evaluated by examination of vaginal cytology for 14 days before initiation of drug treatment initiated in males, for 14 days before cohabitation and then until spermatozoa were observed in a smear of the vaginal contents and/or a copulatory plug was observed in situ during the cohabitation period. Caesarean-sectioning and litter evaluation on GD 13. Euthanasia and gross necropsy conducted on GD 13.

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Observations and Results

Mortality

None in male mice treated with vehicle or CNTO 345.

Clinical Signs

The majority of clinical signs were observed in one male given 40 mg/kg/dose. Clinical signs included urogenital area mass, urine stained abdominal fur, alopecia on the head, a scab on the ear(s) or head, torn ear(s), lacrimation, ptosis, sparse hair coat on the head, red or slight red eye(s), and a moist and red abrasion on the head and frequent scratching on the head. One male given 100 mg/kg/dose presented with a urogenital area mass and urine stained abdominal fur. Clinical signs were not considered CNTO 345-related based on the lack of dose-dependence and low incidence.

Body Weight

Mean body weight and body weight gain were unremarkable.

Feed Consumption

Not evaluated

Mating and Fertility

All mating and fertility parameter values were comparable among the control and CNTO 345-treated groups.

Necropsy of Males

All male mice were euthanized after completion of the cohabitation period and the gross necropsy conducted was unremarkable.

Organ Weight of Males

The increase in relative weight of prostate was dose-related and statistically significant in the 100 mg/kg/dose males compared to control. The increase in relative weight of epidydimides, seminal vesicles without fluid and testis was statistically significant but not dose-related in the 40 mg/kg/dose males except for the testis.

Table 14 Fold Change in Ratios of Organ Weight to Terminal Body Weight in Male mice compared to Control Group

	CNTO 345 (mg/kg/dose)	
	40	100
N	25	25
Terminal Body Weight	1	1
Epididymis left	**1.12**↑	1.06
Testis left	1.11↑	1.07
Seminal Vesicles without Fluid	**1.15**↑	1.06
Epididymis Right	1.06	0.95
Testis Right	1.12↑	1.05
Prostate	1.33↑	**1.59**↑

Values in **bold** denotes statistically different from control

Histopathology of Males

Adequate Battery: Yes
Peer Review: Yes
Histological Findings: No CNTO 345-related findings were noted.

Sperm Evaluation
Sperm concentration and motility were evaluated using a sample collected from the left cauda epididymis. All parameter values were comparable among the control and CNTO 345-treated groups.

F0 Generation Untreated Female Mice

Mortality

One female assigned for mating with male mice dosed with 100 mg/kg/dose was found dead on GD 3. Pregnancy status and cause of death could not be determined. This death was not CNTO 345-related since females were not given the vehicle or drug formulation.

Maternal Body Weight

Mean maternal body weight and body weight gain were unremarkable.

Estrous Cycling, Mating and Fertility

The estrous cycling, number of estrous stages per 14 days, mice with 6 or more days of diestrus or with 6 or more consecutive days of estrus, and all mating and fertility parameter values were comparable among females assigned to mate with males in the control and CNTP 345-treated groups.

Necropsy of Dams

All dams were euthanized on GD 13 and the gross necropsy conducted was unremarkable.

Cesarean Section Data and Litter Observation (Embryos)

CNTO 345 has no effect on any caesarean-sectioning or litter parameters when given to male mice at doses up to 100 mg/kg/dose for up to 7 weeks, Table 15.

Table 15 Summary of Caesarean-Sectioning and Litter Observations of Untreated Female Mice

Parameter	CNTO 345 (mg/kg/dose)		
	0	40	100
Number of female mice	25	25	24
Number of early deceased	0	0	1
Number of pregnant females (%)	23 (92.0)	25 (100.0)	22 (91.7)
Number of pregnant females caesarean-sectioned on GD 13	23	25	22
Corpora lutea (mean)	12.9	13.3	13.9
Implantation sites (mean)	12.7	13.2	13.7
Percent pre-implantation loss (mean)	1.5	1.7	1.6
Viable embryos (mean)	12.1	12.5	12.6

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Non-viable embryos (mean)	0.6	0.7	1.0
Percent post-implantation loss (mean)	5.5	5.4	7.3
Number of females with any non-viable embryos (%)	8 (34.8)	10 (40.0)	12 (54.5)
Number of females with viable fetuses (%)	23 (100.0)	25 (100.0)	22 (100.0)
Placentae appeared normal (%)	23 (100.0)	24 (96.0)	22 (100.0)

Toxicokinetics

Serum samples were collected before dosing on Days 1, 22 and 43 and after dosing on Days 2, 23 and 44. No CNTO 345 was detected in serum samples before dosing started on Day 1. Mean CNTO 345 serum concentrations increased in an approximately dose-proportional manner in the dose range from 40 to 100 mg/kg/dose. Systemic exposure to CNTO 345 accumulated in male mice more than 2.5 X over the weekly treatment during 7 weeks.

Table 16 Summary of Serum Concentrations of CNTO 345 in Male Mice Dosed Weekly for Seven Weeks

(Excerpted from Applicant's Submission)

	Mean CNTO 345 Concentration (µg/mL)	
Protocol Time	**40mg/kg**	**100mg/kg**
Day 1 Predose	< 0.50	< 0.50
Day 2 (24hr postdose)	197.26	558.29
Day 22 Predose	404.61	853.10
Day 23 (24hr postdose)	464.33	1244.02
Day 43 Predose	554.69	1089.00
Day 44 (24hr postdose)	617.55	1491.76

Lowest Quantifiable Concentration in a Sample = 0.50 µg/mL

Dosing Solution Analysis

CNTO 345 was supplied at 8.1 mg/mL concentration. Samples from dosing solutions prepared for Day 1 and Day 43 were analyzed for protein concentration. All results were within the target protein concentration.

Table 17 Summary of Dosing Solution Analysis

	CNTO 345		
Dose (mg/kg/dose)	0	40	100
Protein Concentration (mg/mL)	0	3.25	8.1
Day 1	0	3.21	8.3
Day 43	0	3.24	8.2

9.1.2. Fertility in Female Mice

Study title: CNTO 345 (Anti-Mouse IL-6 Monoclonal Antibody): Fertility and General Reproduction Toxicity Study in Female Mice

Study no.:	T-2010-032
Study report location:	eCTD 4.2.3.5.1
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	November 22, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CNTO 345; Lot #: AHV221006B; Purity: 98.5%

Key Study Findings

- No CNTO 345-related mortality or effects were noted on clinical signs, body weight, mating and fertility parameters, or caesarean-sectioning or embryos.

Reference ID: 3440995

Methods

Doses:	0, 40, 100 mg/kg/dose. Dose selection based on previous studies conducted with CNTO 345
Frequency of dosing:	Once weekly for 15 days (before cohabitation), during cohabitation (maximum of 14 days) and on Day 0 and 6 of presumed gestation (total of 7 doses)
Dose volume:	12.3 mL/kg
Route of administration:	Subcutaneous
Formulation/Vehicle:	Dulbecco's phosphate buffered saline (Mg and Ca free)
Species/Strain:	Mouse/Crl:CD1(ICR)
Number/Sex/Group:	25 mice/sex/group
Satellite groups:	6 females/group for use in TK sample collection before and 24 hours after dose administration on the 1st, 3rd and last day of dosing
Study design:	Males were not given the vehicle or drug formulation Cohabitation for maximum 14 days Male euthanasia without gross necropsy after completion of the cohabitation period Females were sacrificed on GD 13
Deviation from study protocol:	None of the deviations affected the interpretation of study findings

Additional Methods

Blood collection: blood samples were collected from all main study mice at termination for serum CNTO 345 concentration bioanalysis and for anti- CNTO 345 antibody analysis.

Female examination: estrous cycling evaluated by examination of vaginal cytology for 14 days before initiation of drug treatment, for 14 days before cohabitation and then until spermatozoa were observed in a smear of the vaginal contents and/or until a copulatory plug was observed in situ during the cohabitation period. Caesarean-sectioning and litter evaluation on GD 13. Euthanasia and gross necropsy conducted on GD 13.

Observations and Results

Mortality

No CNTO 345-related mortality occurred. One 40 mg/kg/dose female mouse was inadvertently dosed 3X on Day 20 (during the cohabitation period) and found dead. This death was defined as accidental and data were not included for analysis.

Reference ID: 3440995

Clinical Signs

Clinical signs of limited use of a hindlimb/hindpaw occurred in 2 females given 100 mg/kg/dose before cohabitation and/or gestation. These clinical signs were considered CNTO 345-related but not adverse based on the low frequency and incidence.

Body Weight

Mean body weight and body weight gain were unremarkable during the pre-cohabitation and gestation periods.

Feed Consumption

Not evaluated

Estrous Cycling, Mating and Fertility

The estrous cycling, number of estrous stages per 14 days, mice with 6 or more days of diestrus or with 6 or more consecutive days of estrus, and all mating and fertility parameter values were comparable among the control and CNTO 345-treated groups.

Necropsy of Dams

All dams were euthanized on GD 13 and the gross necropsy conducted was unremarkable.

Cesarean Section Data and Litter Observation (Embryos)

CNTO 345 has no effect on any caesarean-sectioning or litter parameters at doses up to 100 mg/kg/dose given for up to 7 weeks, Table 18. The percent pre and post-implantation loss values were highest in the 100 mg/kg/dose group; however, all values were well within the historical control range for the Testing Facility (b) (4)

Table 18 Summary of Caesarean-Sectioning and Litter Observations of Female Mice treated with vehicle control or CNTO 345

Parameter	CNTO 345 (mg/kg/dose)		
	0	40	100
Number of female mice	25	25	24
Number of early deceased	0	1	0
Number of pregnant females (%)	23 (92.0)	21 (84.0)	25 (100.0)
Number of pregnant females caesarean-sectioned on GD 13	23	21	25
Corpora lutea (mean)	13.0	12.8	13.2
Implantation sites (mean)	12.8	12.8	12.9
Percent pre-implantation loss (mean)	1.7	0.7	2.2
Viable embryos (mean)	12.4	12.3	12.1
Non-viable embryos (mean)	0.4	0.5	0.8
Percent post-implantation loss (mean)	3.2	3.6	6.7
Number of females with any non-viable embryos (%)	7 (30.4)	8 (38.1)	14 (56.0)
Number of females with viable fetuses (%)	23 (100.0)	21 (100.0)	25 (100.0)
Placentae appeared normal (%)	23 (100.0)	21 (100.0)	25 (100.0)

Toxicokinetics

Serum samples were collected before dosing on Days 1, 15 and 43 and after dosing on Days 2, 16 and 44. No CNTO 345 was detected in serum samples before dosing started on Day 1. Mean CNTO 345 serum concentrations increased in a less than dose-proportional manner in the dose range from 40 to 100 mg/kg/dose. Systemic exposure to CNTO 345 accumulated in female mice more than 2.1 X over the weekly treatment during 7 weeks.

Table 19 Summary of Serum Concentrations of CNTO in Female Mice Dosed Weekly for up to 7 doses

(Excerpted from Applicant's Submission)

	Mean CNTO 345 Concentration (µg/mL)	
Protocol Time	**Dose=40mg/kg**	**Dose=100mg/kg**
Day 1 Predose	< 0.50	< 0.50
Day 2 (24hr post dose)	288.17	556.50
Day 15 Predose	331.86	699.70
Day 16 (24hr post dose)	585.21	1257.96
Day 43 Predose	544.58	1092.61
Day 44 (24hr post dose)	672.11	1218.49

Lowest Quantifiable Concentration in a Sample = 0.50 µg/mL

Dosing Solution Analysis

CNTO 345 was supplied at 8.1 mg/mL concentration. Samples from dosing solutions prepared for Day 1 and Day 43 were analyzed for protein concentration. All results were within the target protein concentration.

Table 20 Summary of Dosing Solution Analysis

	CNTO 345		
Dose (mg/kg/dose)	0	40	100
Protein Concentration (mg/mL)	0	3.25	8.1
Day 1	0	3.15	8.0
Day 43	0	3.16	8.2

Reference ID: 3440995

9.2 Embryonic Fetal Development

Study title: Investigation of the Effect of CNTO 328 on Embryo-Fetal Development in Cynomolgus Monkeys by Once-Weekly Intravenous Administration

Study no.:	T-2005-036
Study report location:	eCTD 4.2.3.5.2
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	November 22, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CNTO 328; Lot #: D04PL7405; Material Master: 305731; Purity: 99.7%

Key Study Findings

- No CNTO 328-related mortality or effects were noted on clinical signs, body weight, food consumption or effects on pregnancy maintenance including placenta parameters.
- CNTO 328-related and significant decreases in neutrophil counts, along with variable decreases in leukocyte counts and significant increases in alanine aminotransferase levels, occurred in dams at 9.2 and 46 mg/kg/dose on GD 90 and the day of C-section (GD 140-141).
- No CNTO 328-related effects were noted on fetuses including viability, body weight, morphometric measurements, external abnormalities, visceral and skeletal findings or effects on immunohistochemistry results for CD3, CD4, CD8, CD20, or CD34 staining in the lymph node, spleen or thymus of any fetuses.
- CNTO 328 crossed the placenta during gestation and exposure of dams and fetuses to CNTO 328 were similar. Exposure increased in a dose-proportional manner in dams and increased in a higher than dose-proportional manner in fetuses.

Reference ID: 3440995

Methods

Doses:	0, 9.2, 46 mg/kg/dose. Dose selection based on the 6-month toxicology study results
Frequency of dosing:	Once weekly from GD 20 though GD 118 for a total of 15 doses
Dose volume:	0.5 to 2.5 mL/kg
Route of administration:	Intravenous infusion 1-2 mL/kg/min
Formulation/Vehicle:	Sterile saline for injection
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis*)
Number/Sex/Group:	15 pregnant monkeys/group
Satellite groups:	None
Study design:	Males were not given the vehicle or drug formulation Mating conducted for three consecutive days; coitus was confirmed visually or by the presence of sperm in a vaginal smear. The mid-point of the mating period was designated as Day 0 of gestation Pregnancy was diagnosed and monitored by an ultrasound examination including fetal heart beat and fetal size
Caesarean sectioning (CS):	Conducted on GD 140 or 141 to remove fetus and placenta. Dams were returned to the animal colony at the end of study. No gross pathology conducted
Fetal examinations:	Fetus and placenta weight; sex, morphometric measurements, visceral and skeletal examinations
Blood collection:	Blood was collected from all females for hematology, serum chemistry, and serum CNTO 328 concentrations bioanalysis once predose on GD 20 and 90, on the day of scheduled cesarean section, at abortion, embryonic death or at delivery before scheduled CS Additional blood was collected on GD 20 predose to measure serum monkey chorionic gonadotropin (mCG) and confirm pregnancy. Also samples from the fetal cord blood were collected before fetal necropsy
Immunohistochemistry:	Small portions from fetuses' spleen, thymus and mesenteric lymph node were used for immuno-histochemical staining (CD3, CD4, CD8, CD20, and CD34)
Deviation from study protocol:	None of the deviations affected the interpretation of study findings

Reference ID: 3440995

Observations and Results

Maternal Animals

Mortality

No mortality of dams occurred during the study.

Clinical Signs and Pregnancy Monitoring (Abortions, Embryonic Death, Early Delivery)

External genital bleeding occurred in two dams in the 46 mg/kg group; one event was related to embryonic death on GD 51. The other incidence of external genital bleeding did not correspond with an effect on maintenance of pregnancy. Similar number of dams in all groups presented placental signs of genital bleeding; this type of clinical sign is commonly observed in cynomolgus monkeys during early gestation.

Table 21 Summary of Clinical Signs and Pregnancy Monitoring during Gestation Days 20 through 140

	CNTO 328		
Dose (mg/kg/dose)	0	9.2	46
Number of dams	15	15	15
External genital bleeding	1	0	2
Placenta signs genital bleeding	12	13	13
Abortions			
≤GD 30	1	3	0
≥GD 100	1	0	0
Embryonic death			
GD 40	0	0	1
GD 51	0	0	1
Early delivery			
GD 138	0	1	0

One abortion in the control group and three abortions in the 9.2 mg/kg/dose group occurred between the start of dosing on GD 20 and GD 30; one of the dams that aborted in the 9.2 mg/kg/dose had no detectable monkey chorionic gonadotropin on GD 20 prior to start of dosing and it was considered unrelated to CNTO 328. A second dam in the same group presented external genital bleeding on GD 30, the same day of the abortion. An additional abortion occurred in the control group on GD 121. Two embryonic deaths occurred in the 46 mg/kg/dose group; one of those related to external genitalia bleeding. One early delivery in the 9.2 mg/kg/dose group occurred on GD 138; the neonate that was alive (male, 227 g) on the day of birth, presented no external abnormalities. The percentage of abortions (b) (4), embryonic death (b) (4) and early delivery (b) (4) were within the range of control historical data of the testing laboratory.[4]

[4] Control Background Data of Cynomolgus Monkeys (1994 to 2005), in-house data of (b) (4)

Body Weight and Food Consumption

No significant differences in mean body weight, weight gain or food consumption were observed between the control and CNTO 328-treated dam groups.

Hematology and Coagulation

Blood samples for hematology and coagulation evaluation were collected from all dams before treatment start on GD 20, predose on GD 90 and on the day of CS. Neutrophil counts were decreased in a dose-dependent manner on GD 90 and significantly decreased on the day of CS. Percent neutrophils followed similar patterns of decreased levels.

Table 22 Changes (%) of Hematological Parameters Compared to Control

	CNTO 328					
Dose (mg/kg/dose)	9.2			46		
Number of dams	15			15		
Sample Day	GD 20	GD 90	CS	GD 20	GD 90	CS
Neutrophil counts ($10^6/mm^3$)	0	↓25.7	**↓30.7**	↓6.3	**↓37.5**	**↓33.2**
Neutrophil (%)	↓1.2	↓11.4	↓10.3	↓10.7	**↓29.7**	**↓20.0**
Leukocyte counts ($10^3/mm^3$)	0	**↓12.8**	↓22.8	0	↓8.0	↓17.5
Lymphocyte counts ($10^3/mm^3$)	↓5.1	↓4.3	↓14.1	↑16.3	↑16.9	0
Lymphocyte (%)	↓1.3	↑5.8	↑8.9	↑9.6	**↑23.9**	**↑21.5**

CS: Day of Caesarean Sectioning. Values in **bold** denotes statistically different from control

Significantly low leukocyte counts occurred in the 9.2 mg/kg/dose group on GD 90; low leukocyte counts also occurred in the same group on the day of CS and in the 46 mg/kg/dose group but it was not statistically significant due to high inter-individual variability. Lymphocyte counts were slightly decreased in the 9.2 mg/kg/dose and slightly increased in the 46 mg/kg/dose group; however, the percent of lymphocytes was significantly increased in the high-dose group on GD 90 and the day of CS.

Clinical Chemistry

Blood samples for serum chemistry evaluation were collected from all dams before treatment start on GD 20, predose on GD 90 and on the day of CS. Alanine aminotransferase values were increased in a dose-dependent manner on GD 90 and the day of CS. Values for inorganic phosphorous, calcium and potassium were slightly decreased in both CNTO 328 dose groups at most of the sampling times.

Table 23 Changes (%) of Serum Chemistry Parameters Compared to Control

	CNTO 328					
Dose (mg/kg/dose)	9.2			46		
Number of dams	15			15		
Sample Day	GD 20	GD 90	CS	GD 20	GD 90	CS
Alanine Aminotransferase (IU/L)	↓6.1	↑5.0	↑26.7	↑15.6	**↑95.7**	↑141.3
Inorganic Phosphorous (mg/dL)	↓12.9	↓6.9	↓3.9	**↓15.8**	↑2.8	↓2.6
Calcium (mg/dL)	↓1.2	↓1.4	↓4.8	↓1.2	↓1.4	↓5.5
Potassium (mEq/L)	0	↓3.5	↓7.3	↓1.3	0	↓13.3

Values in **bold** denotes statistically different from control

Monkey Chorionic Gonadotropin (mCG) Assay (non-GLP)

Blood samples for monkey chorionic gonadotropin evaluation were collected from all dams before treatment start on GD 20. Group mean serum mCG values were similar among the groups and ranged from 44.9 to 902.3 mIU/mL. One dam in the 10 mg/kg/dose presented values of 0.00 mIU/mL on GD 20 suggesting that this dam aborted before initiation of CNTO 328 dosing.

Table 24 Mean mCG values on GD 20 before Initiation of CNTO 328 dosing

	CNTO 328		
Dose (mg/kg/dose)	0	9.2	46
Number of dams	15	15	15
Mean mIU/mL ± SD	378.1 ± 280.79	317.8 ± 205.32	312.1 ± 185.45

Necropsy

Females that underwent caesarean sectioning were returned to animal colony.

Cesarean Section Data (Fetal Viability and Weight, Placental data)

Caesarean section was conducted on GD 140 or 141. All fetuses removed were alive. Sex distribution of fetuses was similar among groups. No significant differences were noted in mean fetal body weight, placental weight, placental diameter, placenta findings, amniotic fluid volume, or umbilical cord length between CNTO 328-treated groups and control.

Table 25 Summary of Caesarean Sectioning after 15-weekly doses

	CNTO 328		
Dose (mg/kg/dose)	0	9.2	46
Number of dams	13	11	13
Viability (%)	100	100	100
Sex (number; percent) M F	 6; 46.2 7; 53.8	 8; 72.7 3; 27.3	 6; 46.2 7; 53.8
Fetal weight (g) mean ± SD	289.9 ± 23.74	285.2 ± 27.96	296.8 ± 35.40
Placental weight (g) mean ± SD	69.52 ± 280.79	317.8 ± 205.32	312.1 ± 185.45
Placental diameter (cm) mean ± SD Primary Secondary	 9.7 ± 1.53 7.7 ± 1.41	 10.3 ± 0.95 7.6 ± 1.13	 10.2 ± 1.27 6.5 ± 1.70
Placenta findings (number of dams) Single Misshapen	 5 0	 2 0	 2 1
Amniotic fluid volume (mL) mean ± SD	49.6 ± 16.0	50.9 ± 24.3	53.5 ± 20.5
Umbilical cord length (cm) mean ± SD	14.8 ± 3.40	14.1 ± 2.26	14.5 ± 3.84

Offspring (Malformations, Variations, etc.)

No external abnormalities were noticed in any fetuses. Morphometric fetal measurements were similar between groups except a significantly greater head width value in the 46 mg/kg/dose group compared to control. This change may be considered of toxicological insignificance because it is not related to retarded development of fetuses. No visceral findings or differences in fetal absolute or relative organ weight were noticed. A skeletal variation of short supernumerary rib was observed in 1/11 and 1/13 fetuses at 9.2 and 46 mg/kg/dose, respectively. Full supernumerary rib was also present in 1/11 fetuses at 9.2 mg/kg/dose. These skeletal variations are commonly observed in monkeys and may be considered of toxicological insignificance because there was no significant difference in frequency of occurrence when compared to control or published historical data[5].

Table 26 External, Visceral and Skeletal Examinations of Fetuses of Monkey Dams after 15-weekly doses of CNTO 328

	CNTO 328		
Dose (mg/kg/dose)	0	9.2	46
Number of fetuses	13	11	13
	N (%)		
Morphometric measurements Head width value (mean ± SD)	4.5 ± 0.14	4.4 ± 0.13	**4.6 ± 0.21**
External abnormalities	0 (0.0)	0 (0.0)	0 (0.0)
Visceral findings Abnormalities Variations	 0 (0.0) 0 (0.0)	 0 (0.0) 0 (0.0)	 0 (0.0) 0 (0.0)
Skeletal findings Abnormalities Variations Short supernumerary rib Full supernumerary rib	 0 (0.0) 0 (0.0) 0 (0.0)	 0 (0.0) 1 (9.1) 1 (9.1)	 0 (0.0) 1 (9.1) 0 (0.0)

Values in **bold** denotes statistically different from control

Immunohistochemistry Evaluation

Tissue samples from fetuses' spleen, thymus and mesenteric lymph node were used for immunohistochemistry staining of CD3, CD4, CD8, CD20, and CD34. Staining changes were graded very slight, slight, moderate, and marked. The immunohistochemistry staining pattern was similar among groups for all tissues. No CNTO 328-related effects on immunohistochemistry were observed.

Toxicokinetics

Blood samples for analysis of CNTO 328 serum concentrations were collected from all dams before treatment start on GD 20, predose on GD 90, on the day of CS, at abortion, embryonic death or at delivery before scheduled caesarean section. Additionally, samples from the fetal cord blood were collected before fetal necropsy.

[5] Nonhuman Primate Embryo-Fetal Development at GD100 in Cynomolgus monkey (b) (4) referenced in: (b) (4)

Reference ID: 3440995

Table 27 Summary of Mean CNTO 328 Serum Concentrations in Monkeys

	Dose (mg/kg/dose)		Dose Proportionality
	9.2	46	
GD 90 – Mean (µg/mL) ± SD	260.3 ± 108.01	1424.4 ± 378.94	5.5
C-Section – Mean (µg/mL) ± SD	134.1 ± 42.77	727.1 ± 261.54	5.4
Fetal cord blood – Mean (µg/mL) ± SD	106.6 ± 26.00	725.7 ± 254.50	6.8
Fetal/Maternal Ratio	0.82 ± 0.17	1.07 ± 0.36	

Exposure in dams to CNTO 328 on GD 90, based on mean serum concentrations, was dose–proportional from 9.2 to 46 mg/kg/dose. Exposure to CNTO 328 on the day of CS was also dose proportional; however, mean serum concentrations were approximately half of the concentrations determined on GD 90. Exposure in fetuses to CNTO 328, based on mean fetal cord blood concentrations, was lower than in dams at 9.2 mg/kg/dose (ratio of 0.82) and it was similar for fetuses and dams at 46 mg/kg/dose (ratio of 1.1) on the day of CS. The increases in exposure in fetuses were greater than dose-proportional.

Dosing Solution Analysis

CNTO 328 was supplied at 20 mg/mL concentration. Samples from dosing solutions prepared for Day 1 taken at the time of dosing from the first and last animal were analyzed for protein concentration. All results were within the target protein concentration.

Table 28 Summary of Dosing Solution Analysis

	CNTO 328		
Dose (mg/kg/dose)	0	9.2	46
Protein Concentration (mg/mL)	0	20	20
Day 1 – first animal	0	19.5	19.6
Day 1 – last animal	0	19.9	19.6

9.3 Prenatal and Postnatal Development

Study title: A Study for the Effect of CNTO 136 on Embryo-Fetal, Pre- and Postnatal Development in Cynomolgus Monkeys

Study no.: T-2010-018
Study report location: eCTD 4.2.3.5.2
Conducting laboratory and location: (b) (4) [redacted]
Date of study initiation: June 14, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: CNTO 136 –antihuman IL-6 mAb; Lot #: D08PF7671 and D09PB7682; Purity: 98.5% and 98.8%, respectively

Reference ID: 3440995

Key Study Findings

- Significantly decreased values in globulin with concomitant increases in albumin to globulin ratio occurred sequentially; first in dams during the period of GD 34 through LD 30, and then in infants during the period of day after birth, day 30, 90 and 120 (LD 30 through 120); there were no histopathology correlates or changes in any other maternal or neonatal clinical pathology parameter or immunophenotyping or infant immunogenic responses.

Reference ID: 3440995

Methods

Doses:	0, 10, 50 mg/kg/dose
Frequency of dosing:	Once weekly from GD 20 though parturition (GD 167) for a total of 22 doses
Dose volume:	0.2 to 1.0 mL/kg
Route of administration:	Intravenous infusion 4-6 mL/min
Formulation/Vehicle:	Sterile saline for injection
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis*)
Number/Sex/Group:	20 pregnant monkeys/group; additional 3 pregnant monkeys were included in the 10 mg/kg/dose group because of early pregnancy loss of 5/20
Satellite groups:	None
Study design:	Males were not given the vehicle or drug formulation Mating conducted for three consecutive days; coitus was confirmed visually or by the presence of sperm in a vaginal smear. The mid-point of the mating period was designated as Day 0 of gestation Pregnancy was diagnosed and monitored by an ultrasound examination including fetal heart beat and fetal size Dams had infants by natural delivery or emergency C-section and kept infants through the lactation day (LD) 210
Observations/Measurements of Maternal Animals:	All surviving dams were examined up to the day of infant necropsy and then returned to stock.
Observations/Measurements of Infants:	All surviving infants were examined up to Day after Birth (DB) 210 (± 3 days). Each viable infant (including those prematurely born) was examined for viability, sex, external formation, body weight, morphological development (external measurement and skeletal development), functional development, heart rate, ophthalmology, and behavioral assessments
Antigenic Challenge to Infants:	Two injections of 0.5 mL keyhole limpet hemocyanin (KLH) at 3 mg/mL concentration were given intramuscularly into two sites in the thigh once on each DB 120 and 180
Blood collection:	The blood collection schedule for all analysis is presented in Appendix/Attachments B.

Reference ID: 3440995

	Blood was collected from all females, infants and fetuses for hematology, serum chemistry, TK and antidrug antibody (ADA) analysis. Additional blood samples were collected on GD 20 predose and GD 21 to measure serum monkey chorionic gonadotropin (mCG) and confirm pregnancy. Blood was collected from infants for anti-KLH IgG and IgM analysis.
Immunophenotyping:	Blood was collected from all females, infants and fetuses for lymphocyte immunophenotyping using antibodies against CD3, CD4, CD8, CD20, CD16, CD14, CD3-CD14+, and natural killer cells (NK, CD159a). Flow cytometry was used for assessment of the relative percent of positive cells.
Histopathology and Immunohistochemistry:	Portions from infant's lymphoid organs (thymus, spleen, Peyer's patches/GALT and lymph nodes [mandibular, axillary, mesenteric, and inguinal] were evaluated for histopathology and immuno-histochemical staining of CD3 and CD20
Deviation from study protocol:	None of the deviations affected the interpretation of study findings

Observations and Results

F_0 Dams

Survival:	19/20, 22/23, 19/20 in the control, 10 and 50 mg/kg/dose groups, respectively
Clinical signs:	No CNTO 136-related clinical observations
Body weight:	Unremarkable
Feed consumption:	Unremarkable
Uterine content:	Not examined
Necropsy observation:	Not conducted
Pregnancy monitoring:	No CNTO 136-related increases in gestation length, fetal loss, stillbirth, premature birth, or infant death
Clinical pathology:	No CNTO 136-related effects on hematology or coagulation. ↓ globulin concentration (10.3% to 19.4%) with concomitant increases in albumin to globulin ratio in the 10 and 50 mg/kg/dose during the period of GD 34 through LD 30
Toxicokinetics:	Steady state exposure reached by GD 69; dose-proportional exposure
Dosing Solution Analysis:	Within target concentrations
Other:	No CNTO 136-related effects on immunophenotyping or ADA

Reference ID: 3440995

F_1 Generation

Survival:	8/20, 10/23, 6/20 in the control, 10 and 50 mg/kg/dose groups, respectively
Clinical signs:	No CNTO 136-related clinical observations
Body weight:	Unremarkable
Feed consumption:	Not evaluated
Heart rate examinations:	Unremarkable
Ophthalmology evaluations:	Unremarkable
Clinical pathology:	No CNTO 136-related effects on hematology or coagulation. ↓ globulin concentration (10.5% to 21.1%) with concomitant increases in albumin to globulin ratio in the 10 and 50 mg/kg/dose during the period of DB 30 through DB 120
Physical development:	No CNTO 136-related effects
Neurological assessment:	No CNTO 136-related effects
Reproduction:	Not evaluated
Other:	No CNTO 136-related effects on immunogenic reactions, immunophenotyping, or ADA

Observations and Results

Maternal Animals Observations

Mortality

Three dams, one from each group, were euthanized for humane reasons. One female in the control group had a stillborn infant breeched with undetectable heartbeat and the dam was euthanized on GD 163. One female in the 10 mg/kg/dose group delivered a viable infant on GD 161; however, the dam presented extensive blood loss because of retained placenta and was euthanized. Changes in hematology and serum chemistry parameters were considered related to the dam moribundity. One dam in the 50 mg/kg/dose group aborted on GD 131 presenting with extensive blood loss. An emergency C-section was conducted to remove the fetal remains, which were discarded due to autolysis. The dam was found dead the next day. These deaths were considered unrelated to CNTO 136 based on similar incidence in all groups.

Clinical Signs and Pregnancy Monitoring (Abortions, Embryonic Death, Early Delivery)

No CNTO 136-related clinical observations were noted in dams aside from incidental observations commonly observed in monkeys including urogenital discharges. There were no CNTO 136-related increases in gestation length, fetal loss, stillbirth, premature birth, or infant death. The percent of abortions in the 10 mg/kg/dose was slightly higher than the range (0.0 to 29.2%) of the historical database of the testing facility; however, because the transport of maternal IgG is minimal during the first trimester of

Reference ID: 3440995

pregnancy[6], the higher percent of abortions in the 10 mg/kg/dose group was considered unrelated to CNTO 136.

Table 29 Summary of Pregnancy Loss, Stillbirths, Premature births and Infant loss

	CNTO 136		
Dose (mg/kg/dose)	0	10	50
	Ratio - Incidence/number of dams (%)		
Abortions –GD 20 – GD 50	0/19 (0.0)	7/23 (30.4)	1/20 (5.0)
Fetal loss – GD >50	1/19 (5.3)	1/16 (6.3)	1/20 (5.0)
Stillbirth – birth of nonviable fetuses	4/18 (22.2)	2/15 (13.3)	2/18 (11.1)
Post-birth infant death	2/14 (14.3)	0/13 (0.0)	2/16 (0.0)
Premature birth [a]	2/14 (14.3)	0/13 (0.0)	1/16 (6.3)
Total infant loss and pregnancy	8/20 (40.0)	10/23 (43.5)	6/20 (30.0)

[a] One premature birth in Group 1 and one in Group 3 were born alive and later euthanized because humane reasons

Body Weight and Food Consumption

No significant differences in mean body weight, weight gain or food consumption were observed between the control and CNTO 136-treated dam groups.

Hematology, Coagulation and Serum Chemistry

Blood samples for hematology, coagulation and serum chemistry evaluation were collected from all dams before treatment start on GD 20, predose on GD 34, 62, 146, and 160 and on LD 0-3, 13, 30, 41, 75 and 120.
No CNTO 136-related effects were noted in the hematology, coagulation, or serum chemistry parameters from maternal animals at scheduled intervals except for significant decreases in globulin concentration (10.3% to 19.4%) with concomitant increases in albumin to globulin ratio in the 10 and 50 mg/kg/dose group during the period of GD 34 through LD 30. The biological significance is unclear as there were no maternal histopathology correlates or changes in any other clinical pathology parameter or immunophenotyping; however, neonatal decreases in globulin occurred during DB 30 through 120 after similar changes were observed in dams.

Table 30 Summary of Percent Changes in Serum Chemistry Parameters of Dams treated with CNTO 136

	CNTO 136 (mg/kg/dose)											
	10						50					
Day	GD 34	GD 62	GD 146	GD 160	LD 30	LD 120	GD 34	GD 62	GD 146	GD 160	LD 30	LD 120
Albumin (g/dL)	↑7.7	↑9.4	↑11.1	↑3.7	↑2.5	0	↑2.6	↑6.3	↑7.4	↑3.7	↑2.5	↓2.3
Globulin (g/dL)	**↓12.9**	**↓19.4**	**↓15.2**	**↓14.3**	**↓13.8**	↓7.1	**↓12.9**	**↓16.1**	**↓15.2**	**↓17.1**	**↓10.3**	↓3.6
A:G ratio	**↑23.1**	**↑27.3**	**↑22.2**	**↑25.0**	**↑14.3**	↑13.3	**↑15.4**	**↑18.2**	**↑22.2**	**↑25.0**	↑14.3	↑6.7

Values in **bold** denotes statistically different from control

[6] Simister NE., Placental transport of immunoglobulin G. *Vaccine* (2003) 21: 3365-69.

Immunophenotyping using Flow Cytometry

Blood samples for immunophenotyping using antibodies against CD3, CD4, CD8, CD20, CD16, CD14, and NK evaluation were collected from all dams before treatment start on GD 20, predose on GD 34, 62, 146, and 160 and on LD 0-3, 13, 30, 41, 75 and 120. Flow cytometry was used for assessment of the relative percent of positive cells.

No CNTO 136-related effects were noted in the absolute counts of peripheral blood or relative percentages of peripheral blood in total T-lymphocyte (CD3), helper T-lymphocyte (CD4), suppressor/cytotoxic T-lymphocyte (CD8), total B-lymphocyte (CD20), monocytes co-expressing CD16 (CD16), natural killer cells (NK, CD159a), monocytes (CD14), and CD3-CD14+ population relative to the mononuclear cells.

Monkey chorionic gonadotropin (mCG) Assay (non-GLP)

Blood samples for mCG were collected from all dams before treatment start on GD 20 and on GD 21 approximately 24 hours after the first dose of CNTO 136. The cut off for mCG concentrations that were consistent with pregnancy was 0.039 ng/mL. Group mean serum mCG values on GD 20 were similar among the groups and ranged from 1.1 to 301.9 ng/mL. One dam in the 0 mg/kg/dose presented values <0.039 ng/mL on GD 20 suggesting that this dam aborted before initiation of CNTO 136 dosing. Group mean serum mCG values on GD 21, 24 hours after the first dose, were also similar among the groups and ranged from 0.7 to 585.5 ng/mL.

Table 31 Mean mCG values on GD 20 before Initiation of CNTO 136 dosing

	CNTO 136		
Dose (mg/kg/dose)	0	10	50
Number of dams	19	23	20
Mean ng/mL ± SD	78.2 ± 72.97	72.9 ± 68.40	88.0 ± 88.12

<u>Infant Animals Observations</u>

Mortality

One premature birth in the control group and one in the 50 mg/kg/dose group were born alive and later euthanized for humane reasons. A second premature birth in the control group was found dead on DB 10. A naturally delivered infant in the 50 mg/kg/dose group was found dead on DB 21; this infant presented macroscopic findings of dark red multifocal to coalescent discoloration of the lungs/bronchi, moderately decreased thymus, dark red discoloration of the tail, and an abscess in the abdominal cavity. Histopathology findings noted include acute inflammation and intravascular leukocytosis of the axillary lymph node, sinusoidal leukocytosis of the liver and granulocytic hyperplasia of the bone marrow, consistent with septicemia. Macro and microscopic findings that occurred in dead infants were not considered CNTO 136-related based on the incidence in the control and the treated groups.

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Observations at Birth and Clinical Signs

No CNTO 136-related external abnormalities occurred in any of the examined infants. Phimosis, inability to retract the penis foreskin, was observed in 1, 4, and 3 infants from the control, 10 and 50 mg/kg/dose groups, respectively. All cases resolved before infant necropsy on DB 210 except for one infant in the 50 mg/kg/dose group. Phimosis was not considered to be CNTO 136-related because it was not associated with adverse findings in the urinary system or function, the condition was also observed in the control group, and it may be a normal physiologic condition in humans[7]. No CNTO 136-related clinical observations were noted in infants aside from incidental observations commonly observed in monkeys.

Body Weight

Infant body weight was evaluated on the DB and once weekly through DB 210 (± 3 days). No significant differences in mean body weight or weight gain were observed between the control and CNTO 136-treated infant groups.

Morphological Development

External Parameters

External parameters were measured in all infants, including those stillborn, found dead or euthanized for humane reasons, once between DB 25 to 30, 90 to 100, and 170 to 180. No significant differences in mean head width, head circumference, distance between the eyes, crown-rump length, tail length, chest circumference, paw and foot length (right-side), and ano-genital distance were observed between the control and CNTO 136-treated infant groups.

Skeletal Development

Skeletal development was performed using radiographs once between DB 25 to 30 in all infants including those stillborn, found dead or necropsied before scheduled termination. No CNTO 136-related effects were noted on the skeletal development of the infants.

Functional Development and Behavioral Assessment

Physical evaluations for pupillary reflex, Preyer reflex, pain response, and grip strength, and behavioral assessment parameters were performed on all infants once between DB 25 to 30, 90 to 100, and 170 to 180. No CNTO 136-related effects were noted on the functional development or any behavioral assessment of the infants.

Heart Rate and Ophthalmology Examinations

Heart rate measurements were performed on all infants once between DB 25 to 30, 90 to 100, and 170 to 180. Ophthalmology examinations were performed on all infants once between DB 90 to 100, and 140 to 160. No CNTO 136-related effects were noted on heart rate or ophthalmology examinations of the infants. Incidental findings of capsular opacity or cataract were considered spontaneous, background lesions.

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[7] McGregor TB, Pike JG, Leonard MP., Phimosis – a diagnostic dilemma? *The Canadian Journal of Urology* (2005) 12: 2598-2602.

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Hematology and Coagulation

Blood samples for the evaluation of hematology and coagulation parameters were collected from all infants on DN 30, 90, 120, 180 and on the day of necropsy (DB 210 ±3 days).
No CNTO 136-related effects were noted in the hematology and coagulation parameters from infants at scheduled sample collection.

Serum Chemistry

CNTO 136-related and significantly lower globulin values with concomitant increases in A:G ratio were noted on DB 30, 90 and 120 in infants from the 10 and 50 mg/kg/dose groups. Values for globulin and A:G ratio returned to similar values compared to control group by DB 180 and 210. The biological significance is unclear as there were no histopathology correlates or changes in any other clinical pathology parameter, immunophenotyping or immunogenic responses; however, neonatal decreases in globulin occurred after similar changes were observed in dams from GD 34 through LD 30.

Table 32 Summary of Percent Changes in Serum Chemistry Parameters of Infants from Dams treated with CNTO 136

	CNTO 136									
Dose (mg/kg/dose)	10					50				
Day after Birth	30	90	120	180	210	30	90	120	180	210
Albumin (g/dL)	↓5.3	↑2.6	↑5.0	↓2.4	↓2.4	↑2.7	↑2.6	0	↓2.4	0
Globulin (g/dL)	**↓10.5**	**↓15.8**	**↓10.5**	↑5.0	0	**↓10.5**	**↓21.1**	**↓15.8**	↓5.0	↓5.0
A:G ratio	**↑10**	**↑18.2**	**↑19.1**	↓4.8	0	**↑10**	**↑27.3**	**↑23.8**	↑9.5	↑4.8

Values in **bold** denotes statistically different from control

Immunophenotyping using Flow Cytometry

Blood samples for immunophenotyping using antibodies against CD3, CD4, CD8, CD20, CD16, CD14, and NK evaluation were collected from all infants on DN 30, 90, 120, 180 and on the day of necropsy (DB 210 ±3 days). Flow cytometry was used for assessment of the relative percent of positive cells.

No CNTO 136-related effects were noted in the absolute counts of peripheral blood or relative percentages of peripheral blood in total CD3, CD4, CD8, CD20, CD16, NK, CD14, and CD3-CD14+ cells from infants.

Gross Pathology

No CNTO 136-related macroscopic observations were noted at necropsies of early decedent dams, neonates, infants, stillbirths or scheduled necropsies of infants.

Organ Weights

No CNTO 136-related changes occurred in absolute or relative organ weight of infants.

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Histopathology and Immunohistochemistry

Portions from infant's lymphoid organs (thymus, spleen, Peyer's patches/GALT and lymph nodes [mandibular, axillary, mesenteric, and inguinal] were evaluated for histopathology and immunohistochemical staining of CD3 (T-cells) and CD20 (B-cells). Microscopic findings were graded by the pathologist as present and on a scale 1 to 4, minimal<mild<moderate<marked, according to the intensity and extent of change.

Adequate Battery: **YES**
Peer Review: **YES**
Histological Findings:
No CNTO 136-related microscopic observations in lymphoid tissues, liver or bone marrow or changes in immunohistochemistry for T- and B-cells were noted in neonatal cynomolgus monkeys following weekly IV administration of CNTO 136 to the maternal monkeys during organogenesis of the fetuses, Table 33.

Table 33 Summary of Histopathology Findings Incidence and Group Mean Severity

(Excerpted from Applicant's Submission)

ORGAN/Diagnosis	Summary Data		
Group/Dose	Group 1 0 mg/kg	Group 2 10 mg/kg	Group 3 50 mg/kg
THYMUS			
Cyst(s)	1/12	1/13	2/14
LYMPH NODE, AXILLARY			
Follicle- Increased cells		1/13 (0.1)	
Sinus histiocytosis	2/12 (0.2)	1/13 (0.1)	2/14) (0.1)
Sinus erythrocytosis	5/12 (0.4)	1/13 (0.1)	4/14 (0.3)
Pigmentation	1/12 (0.1)	1/13 (0.2)	
Extramedullary hematopoiesis		2/13 (0.2)	
LYMPH NODE, MANDIBULAR			
Follicle- Increased cells	10/12 (1.3)	8/13 (1.2)	10/14 (1.1)
Extramedullary hematopoiesis		1/13 (0.1)	
SPLEEN			
Follicles - Increased cells	7/12 (0.7)	8/13 (0.8)	9/14 (0.8)
LYMPH NODE, MESENTERIC			
Follicle- Increased cells	6/12 (0.6)	10/13 (1.0)	8/14 (0.6)
LYMPH NODE, INGUINAL			
Hemorrhage, focal			1/14 (0.1)
Sinus histiocytosis	3/12 (0.2)	2/13 (0.2)	2/14 (0.1)
Follicle- Increased cells	1/12 (0.1)	5/13 (0.4)	4/14 (0.3)
PEYER'S PATCH/GALT			
Germinal center - Increased cells	5/11 (0.5)	4/13 (0.4)	3/13 (0.3)
LIVER, RIGHT			
Lymphoid infiltrates, focal			2/14 (0.1)
Hepatocellular vacuolation, centrilobular			1/14 (0.1)
Extramedullary hematopoiesis	2/12 (0.2)	1/13 (0.1)	4/14 (0.3)
Inflammation, acute	1/12 (0.1)		
LIVER, LEFT			
Hepatocellular vacuolation, centrilobular			1/14 (0.1)
Extramedullary hematopoiesis	4/12 (0.3)	1/13 (0.1)	6/14 (0.4)
BONE MARROW	N= 12	N= 12	N= 14

*Data obtained from the Histopathology and Immunohistopathology Data (Tables 7-9). The value in ( ) is the group mean severity calculated by total severity/N.

Toxicokinetics in Dam and Infants

Exposure to CNTO 136, as measured by serum concentrations, increased as the dose increased from 10 to 50 mg/kg/dose in an approximately dose-proportional manner during gestation (GD 20 through GD 139). CNTO 136 serum concentrations declined during lactation because dams were not dosed during this time, Table 34. CNTO 136 half-life of approximately 20 days was similar in dams at both doses. CNTO 136 concentrations in milk measured during LD 30 to 75 were very low in both groups. CNTO 136 serum concentrations in infants were highest after birth and declined over time until the day of necropsy on DB 210. CNTO 136 half-life in infants (23 to 26 days) was slightly longer than the half-life in dams, Table 34.

Antidrug Antibody (ADA) Evaluation in Dam and Infants

All dams in the control, 10 and 50 mg/kg/dose groups and their corresponding infants tested negative for antibodies to CNTO 136 suggesting it was not immunogenic in cynomolgus monkeys.

Table 34 Summary of CNTO 136 Serum Concentrations in Dams and Infants and Breast Milk

Dose (mg/kg/dose)	10	50	Dose Proportionality
CNTO 136 concentration	Mean (µg/mL) ± SD		
Maternal Animals			
GD 20	229 ± 20.3	1059 ±	4.6
GD 69	320 ± 65.4	1543 ±	4.8
GD 139	317 ± 79.8	1467 ±	4.6
LD 0-3	125 ± 59.3	363 ± 153.6	2.9
LD 75	4 ± 2.1	13 ± 6.9	3.3
LD 120	0.9 ± 0.58	3 ± 1.9	3.3
LD 210	0.01	0.2 ± 0.23	NC
Half-life – mean day ± SD	19 ± 2.2	20 ± 4.0	NC
Milk LD 30-75	<0.1	<0.1	NC
Infant Animals			
DB 0-3	81 ± 34.4	378 ± 206.6	4.7
DB 75	9 ± 3.87	47 ± 17.6	5.2
DB 120	3 ± 1.59	13 ± 5.7	4.3
DB 210	0.25 ± 0.51	1 ± 1.1	4.0
Half-life – mean day ± SD	26 ± 7.3	23 ± 4.9	NC

GD: Gestation day; DB: Day after birth; NC: Not calculated

Antigenic Challenge Evaluation in Infants

Serum concentrations of monkey anti-KLH IgG and IgM were similar among infants from the control, 10 and 50 mg/kg/dose groups at all sample collection intervals. No CNTO 136-related effects were noted on the ability of the infants to mount a response to foreign antigen stimulus.

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Dosing Solution Analysis

CNTO 136 was supplied at 50 mg/mL concentration. Samples from dosing solutions prepared for Day 1 and Day 160 taken at the time of dosing the first of each group were analyzed for protein concentration. All results were within the target protein concentration.

Table 35 Summary of Dosing Solution Analysis

	CNTO 136		
Dose (mg/kg/dose)	0	10	50
Protein Concentration (mg/mL)	0	50	50
Day 1	0	52.5	51.1
Day 160	0	50.3	50.8

10 Special Toxicology Studies

1 Study title: Cross-Reactivity of CNTO 328 with Normal Human Tissues

Study no.: T-2002-013
Study report location: BLA Section 4.2.3.7.7
Conducting laboratory and location: [redacted] (b) (4)
Report Date: June 16, 2013
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: CNTO 328 biotinylated, PAI No. A4030, Lot No. 5884:47, 2 mg/mL concentration; negative control used was Human IgG1, myeloma plasma, kappa, biotinylated.

Cryosections of normal human tissues (3 or more donors) were stained with two antibody concentrations (2 µg/mL and 10 µg/mL). Expected specific cross-reactivity was observed localized in the membrane of the following tissues and cell types:

- Brain, white matter and cerebellum
- Eye, in the squamous epithelium of the cornea
- Spinal cord, in axons in the gray and white matter
- Peripheral nerves, in axons

The following tissues were evaluated:

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Adrenal	Lung	Spinal Cord
Blood Cells	Lymph Node	Spleen
Blood vessels (endothelium) [1]	Ovary	Striated muscle (skeletal)
Bone Marrow	Fallopian Tube (oviduct)	Testis
Brain – cerebrum (cortex)	Pancreas	Thymus
Brain – cerebellum	Parathyroid	Thyroid
Breast (mammary gland)	Peripheral Nerve	Tonsil
Eye	Pituitary	Ureter
Gastrointestinal Tract [2]	Placenta	Urinary Bladder
Heart	Prostate	Uterus- (endometrium)
Kidney (glomerulus, tubule)	Salivary Gland	Uterus- cervix
Liver	Skin	

[1] Blood cells included granulocytes, lymphocytes, monocytes and platelets.

[2] Gastrointestinal tract included the colon (large intestine), esophagus, small intestine, and stomach.

2 Study title: Cross-Reactivity Study of Biotinylated CNTO 328 with Selected Normal Human Tissues

Study no.: T-2004-019
Study report location: BLA Section 4.2.3.7.7
Conducting laboratory and location: [redacted] (b) (4)
Report Date: March 5, 2005
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: CNTO 328 biotinylated, PAI No. A5943, Lot No. 2004PRO158B, 2.29 mg/mL concentration; negative control used was Human IgG1 biotinylated, [redacted] (b) (4) No. A5712, Lot No. 61052.

Cryosections of normal human tissues (3 or more donors) were stained with two antibody concentrations (2 µg/mL and 10 µg/mL). No specific cross-reactivity was observed on the selected normal human tissue panel examined

Tissues evaluated included brain (cerebrum cortex and cerebellum), eye, peripheral nerve, spinal cord, and thymus.

3 Study title: Immunohistochemical Staining of Eye Tissues from

Reference ID: 3440995

Cynomolgus Monkeys Treated with CNTO 328

Study no.:	T-2003-004
Study report location:	BLA Section 4.2.3.7.7
Conducting laboratory and location:	[redacted] (b) (4)
Report Date:	August 6, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CNTO 328 Lot No. 5884-47, 20 mg/mL concentration; C437 anti-idiotypic antibody, 6.49 mg/mL; negative Control Antibody, [redacted] (b) (4)

Eye tissues from 2 control monkeys and 2 treated monkeys with CNTO 328 at 46 mg/kg/dose under study T-2002-007 "Three Month Intravenous Dose Toxicity Study of CNTO 328 in Cynomolgus Monkeys with One Month Recovery Period" were used for this study.

Eye tissue from monkeys in the HD treatment group had staining in the stroma (sclera, optic nerve and adjacent tissue, and cornea) and blood vessels (wall and intravascular protein) when stained with the anti-idiotypic antibody. The corneal epithelium had no staining but there was a staining on the corneal surface. The staining of stroma and vasculature was interpreted as the pharmacokinetic distribution of CNTO 328 in eye tissues and not membrane epitope binding.

11 Integrated Summary and Safety Evaluation

Pharmacology studies presented evidence that siltuximab binds to human and monkey IL-6 and inhibits the interaction of IL-6 with its receptor, neutralizing some of the IL-6 biological activity.

The pharmacokinetics of siltuximab in monkeys showed that exposure to siltuximab increased with dose with a half-life of 10 to 24 days; however, increases in exposure to siltuximab in toxicology studies were not always in a dose-proportional manner. Exposure values, as estimated by C_{max}, were lower in pregnant monkeys compared to non-pregnant monkeys. Siltuximab serum concentrations in monkey mothers were similar to siltuximab concentrations in blood samples from the cord at the caesarean sectioning. Anti-siltuximab antibodies were developed in 2/9 monkeys in the PK study and in 1/28 monkeys in the 3-month toxicology study (not reviewed). No ADAs were detected in the 6-month toxicology study although the high serum concentrations of siltuximab may have interfered with the detection of ADA. Assessment of ADAs in the monkey EFD was not conducted.

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Table 36 Comparison of Exposure to Siltuximab in Pregnant and Non-Pregnant monkeys

Species	Dose (mg/kg)	6-Months Study (µg/mL) [a]	6-Months Study (µg/mL) [b]	EFD Study (µg/mL) [c]
Cynomolgus monkey	9.2	349	2312	260
	46	2066	5795	1424

[a] Cmax after the first dose in the 6-month study
[b] Cmax after the 25th dose in the 6-month study
[c] Cmax on GD 90 (before the 10th dose) in the EFD study

The estimated exposure margins represented by the doses administered during the 6-month or during the EFD toxicology studies exceed the estimated prediction for a 660 mg clinical dose every 3 weeks in MCD patients responding to siltuximab therapy, Table 37. No adverse or significant toxicology effects were noted at the high dose in the 6-month or the EFD toxicology studies in monkeys.

Table 37 Estimated Safety Margins based on Animal Exposure

Species	Dose (mg/kg)	AUC (0-7day) [a] (µg•day/mL)	Exposure Margin [b]
Cynomolgus monkey	9.2	1326.3	0.6
	46	4508.4	2.0

[a] Exposure after the first dose in the 6-month study
[b] Based on first dose calculated mean $AUC_{(0-t)}$ of 2267.2 µg•day/mL in MCD patients receiving a 11 mg/kg dose of siltuximab (Study CNTO328MCD2001)

Species	Dose (mg/kg)	EFD Cmax [a] (µg/mL)	Exposure Margin [b]
Cynomolgus monkey	9.2	260	1.2
	46	1424	6.5

[a] Cmax on GD 90 (before the 10th dose) in the EFD study
[b] Based on first dose calculated mean Cmax of 218.8 µg/mL in MCD patients receiving a 11 mg/kg dose of siltuximab (Study CNTO328MCD2001)

IL-6 biological effects include down regulation of albumin production and primary induction of C-reactive protein by the liver; mediation of signal transduction and activation of transcription-3 phosphorylation (STAT-3) as well as production of globulin proteins.

The toxicity findings observed in the repeat-dose toxicology studies in monkeys, in general, reflect the intended pharmacology of siltuximab of inhibiting the IL-6 interaction with its receptor and neutralizing some of the IL-6 biological activity. No serious

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adverse or significant toxicological effects were noted in repeated-dose toxicology studies, Table 38.

Table 38 Summary of Major Findings from Repeat-Dose Toxicology Studies

GLP Toxicology Study	Weekly Doses (mg/kg)	Major Findings
3-Month IV (T-2002-007) (Study was not reviewed; major findings excerpted from Applicant's submission)	0, 9.2, 46	• Transient significantly higher albumin and A:G ratios in LD and HD males and HD females, and lower globulin values in LD and HD males and females on Day 30. • Transient immunomodulatory effects with lower and dose dependent anti-KLH IgG titers at all-time points and slightly lower anti-KLH IgM titers on Days 30, 36 and 50. • Increases in B-lymphocytes (CD20+), total lymphocytes, T-lymphocytes (CD3+), T-helper lymphocytes (CD3+/CD4+), T-cytotoxic/suppressor lymphocytes (CD3+/CD8+), and naïve T-lymphocytes (CD3+/CD45RA+) on Day 2. • CNTO 328 was detected in the CSF and increased in a dose-proportional manner. • Reduction in size and number of germinal centers (follicular atrophy) in the spleens of 2/3 HD males and 1/3 HD females from the Day 30 necropsy and in 1/3 HD males and 1/3 control females from the Day 87 necropsy. No effects observed in recovery animals.
6-Month IV (T-2003-010)	0, 9.2, 46	• Facial swelling of one HD female suggestive of reaction to the first dose resolved upon completion of the infusion and transient decreases in neutrophils, monocytes, WBC and/or platelets. • Skin erythema in the shoulder region was observed periodically in 20% of HD monkeys and corresponded microscopically with minimal hyperkeratosis or acanthosis. • Slight decreases in platelets and white blood cell parameters (WBC count, neutrophils, and monocytes) were observed in HD females • Sporadic episodes of low heart rates and/or blood pressure were experienced by 30% of HD monkeys. • A trend for immunomodulatory effects in HD monkeys to have slightly lower anti-KLH IgM and IgG levels was present.
EFD IV (T-2005-036)	0, 9.2, 46	• Significant decreases in neutrophil counts along with variable decreases in leukocyte counts and/or significant increases in alanine aminotransferase levels occurred in LD and HD dams on GD 90 and the day of CS (GD 140-141). • CNTO 328 crossed the placenta during gestation and exposure of dams and fetuses to CNTO 328 were similar

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12 Appendix/Attachments

A. Studies submitted that were not reviewed

	Study Report	Title
Pharmacology		
1	Biol-Res-TR670-jn	Effect of CNTO 328 on the Proliferation of Human Non-Small-Cell Lung Cancer Cell Lines in vitro
2	BM2013CRO-T-021	Investigation of IL-6 Posttranslational Modifications and Selectivity Analysis of IL-6 Binding Antibodies
3	Cavarretta-IT-2006	The Antiapoptotic Effect of IL-6 Autocrine Loop in a Cellular Model of Advanced Prostate Cancer is Mediated by Mcl-1
4	REStr179-sj	Generation of Human IgG Monoclonal Antibodies Reactive to Human IL-6 Using GenPharm Transgenic Mouse Technology
5	REStr560-rc	CNTO 328 Inhibition of Stat-3 Phosphorylation: Effects of Anti-human IL-6, on IL-6 Induced Stat 3 Phosphorylation
Secondary Pharmacodynamics		
6	Biol-Res-TR316-mt	Combined Blockade of Human and Mouse IL-6 Inhibits Cancer Cachexia in Nude Mice
7	Biol-Res-TR318-jn	CNTO 328 Inhibits the Growth of Human Prostate Cancer Xenografts in Nude Mice
8	Biol-Res-TR322-jz	Effect of CNTO 328 on IL-6-Induced Angiogenesis in Mouse Matrigel Models
9	Biol-Res-TR486-jn	Effect of CNTO 328 on Docetaxel-Mediated Inhibition of PC3 Tumor Growth
10	Biol-Res-TR543-jn	Evaluation of CNTO 328 for Combination Activity with Mitoxantrone Against DU-145 Human Prostate Carcinoma
11	Biol-Res-TR645-rv	Evaluation of the Effects of CNTO 328 Combined with Mitoxantrone in DI145 Human Prostate Xenografts in Male Nude Mice
12	Biol-Res-TR646-jn	Evaluation of the Effects of CNTO 328 Combined with Mitoxantrone in a Nude Mouse Model of PC-3 Human Prostate Carcinoma
13	Biol-Res-TR674-cn	Effect of CNTO 328 on the Growth of Subcutaneous H460 Human Non-Small Cell Lung Carcinoma Cells in Female CD1 Nude Mice
14	Biol-Res-TR691-rv	Inhibition of CCL2 and IL-6 in a Preclinical Model of Bone-metastatic Prostate Cancer
15	Biol-Res-TR697-rh	Effect of Bortezomib Alone and in Combination with CNTO 328 on the Growth of IL-6 Sensitive Human Myeloma Tumors in SCID Mice
16	Biol-Res-TR718-cn	Efficacy of CNTO 328 Alone or in Combination with Erlotinib in H460 Human NSCLC Mice Model in Nude Mice
17	Hunsucker-S-2011	Blockade of Interleukin-6 Signaling with Siltuximab Enhances Melphalan Cytotoxicity in Preclinical Models of Multiple Myeloma
18	Mauray-S-2000	Epstein-Barr Virus-dependent Lymphoproliferative Disease: Critical Role of IL-6
19	Smith-P-2001	Anti-Interleukin-6 Monoclonal Antibody Induces Regression of Human Prostate Cancer Xenografts in Nude Mice
20	Steiner-H-2006	Regulation of Growth of Prostate Cancer Cells Selected in the Presence of Interleukin-6 by the Anti-interleukin-6 Antibody CNTO 328
21	Stone-R-2012	Reference and Supplementary Appendix: Paraneoplastic Thrombocytosis in Ovarian Cancer
22	Voorhees-PM-2007	Inhibition of Interleukin-6 Signaling with CNTO 328 Enhances the Activity of Bortezomib in Preclinical Models of Multiple Myeloma
23	Voorhees-PM-2009	Targeted Inhibition of Interleukin-6 with CNTO 328 Sensitizes Pre-clinical Models of Multiple Myeloma to Dexamethasone-mediated Cell Death

Reference ID: 3440995

24	Wallner-L-2006	Inhibition of Interleukin-6 with CNTO 328, an Anti-Interleukin-6 Monoclonal Antibody, Inhibits Conversion of Androgen-Dependent Prostate Cancer to an Androgen-Independent Phenotypein Orchiectomized Mice
25	Weissglas-M-1007	The Role of Interleukin-6 in the Induction of Hypercalemia in Renal Cell Carcinoma Transplanted in Nude Mice
26	Zaki-M-2004	CNTO 328, a Monoclonal Antibody to IL-6, Inhibits Human Tumor-Induced Cachexia in Nude Mice
Pharmacokinetics		
27	CP2002V-035a	Validation of an Assay for the Quantification of CNTO 328 (Anti-IL-6) in Monkey Serum for the Quantification of CNTO 328 in Monkey Cerebrospinal Fluid
28	CP2003V-001a	Validation of an Assay to Detect Antibody Immune Responses to CNTO 328 (Human Anti-IL-6) in Human and Non-human Primate Serum or Plasma
29	CP2010V-005	Validation for the Quantification of CNTO 345 in Mouse CD1 Serum Using an Electrochemiluminescent-based Immunoassay (ECLIA) Method on the Meso Scale Discovery Platform
30	P-2004-061	Single Intravenous Dose Pharmacokinetic Study in Cynomolgus Monkeys with CNTO 328 (Anti-IL-6 mAb) Produced by Cell Line (b) (4)
Toxicology		
31	T-2009-025	CNTO 345 (Anti-Mouse IL-6 Monoclonal Antibody): One Month Multiple Dose Toxicokinetic and Tolerability Study in Mice
32	T-2002-007	Three Month Intravenous Dose Toxicity Study of CNTO 328 in Cynomolgus Monkeys with One Month Recovery Period
33	T-2002-008	An Intravenous Dose-Range Finding Study Using Proleukin® (IL-2) in Cynomolgus Monkeys
34	T-2002-010	Three Month Intravenous Dose Toxicity Study of CNTO 328 in Cynomolgus Monkeys in Combination with IL-2 Therapy
35	BTTR023	Effects of a Rat Anti-Mouse Interleukin-6 Monoclonal Antibody (CNTO 345) on Local Growth, Invasion and Metastases of SCC VII (Squamous Cell Carcinoma) Cells in C3H/HeN Mice
36	BTTR024	Effects of a Rat Anti-Mouse Interleukin-6 Monoclonal Antibody (CNTO 345) on Establishment of Experimental Pulmonary Metastases of SCC VII (Squamous Cell Carcinoma) Cells in C3HIHeN Mice
37	BTTR025	Effects of a Rat Anti-Mouse Interleukin-6 Monoclonal Antibody (CNTO 345) on Progression of Experimental Pulmonary Metastases of SCC VII (Squamous Cell Carcinoma) Cells in C3HIHeN Mice
38	T-099-001	Cross-Reactivity of Biotinylated cCLBS (Anti-human IL-6 Monoclonal Antibody) with Normal Human and Cynomolgus Monkey Tissues

Reference ID: 3440995

B. Additional information from the fertility studies

CNTO 345 (Anti-Mouse IL-6 Monoclonal Antibody): Fertility and General Reproduction Toxicity Study in Male Mice

Summary of Mating and Fertility Parameters for Male mice treated with vehicle control or CNTO 345

Male Mice Doses	CNTO 345 (mg/kg/dose)		
	0	40	100
Number of mice in cohabitation	25	25	25
Days in cohabitation [a] (mean)	2.5 ± 1.3	2.3 ± 1.3	2.8 ± 2.1
Mice that mated - N (%)	25 (100)	25 (100)	25 (100)
Number of mice with confirmed mating dates	24	25	24
Fertility Index [b] - N / N (%)	23 / 25 (92.0)	25 / 25 (100.0)	22 / 24 (91.7) [c]
Mated with Female [d] - N / N (%) Days 1-7 Days 8-14 Days 15-21	 24 (100) 0 (0.0) 0 (0.0)	 25 (100) 0 (0.0) 0 (0.0)	 23 (95.8) 1 (4.2) 0 (0.0)
Mice Pregnant / Mice in Cohabitation - N / N (%)	23 / 25 (92.0)	25 / 25 (100.0)	22 / 24 (91.7)

a: Mice with a confirmed mating date and mice that did not mate
b: Number of pregnancies / number of mice that mated
c: Excludes values for a female found dead on gestation day 3
d: Mice with confirmed mating date

Summary of Sperm Motility Count and Density for Male mice treated with vehicle control or CNTO 345

Male Mice Doses		CNTO 345 (mg/kg/dose)		
		0	40	100
Mice tested	N	25	25	25
LEFT CAUDA EPIDIDYMAL SPERM MOTILITY				
Number motile	Mean ± SD	606.2 ± 366.9	627.8 ± 332.7	467.2 ± 326.3
Motile percent	Mean ± SD	84.9 ± 14.2	89.9 ± 6.7	84.8 ± 10.1
Non-motile (static count)	Mean ± SD	84.1 ± 53.3	61.6 ± 47.4	67.4 ± 36.8
Total count [a]	Mean ± SD	690.3 ± 373.3	689.4 ± 343.6	534.6 ± 343.0
CAUDA EPIDIDYMAL SPERM COUNT				
Sperm count [b]	Mean ± SD	92.6 ± 61.7	109.6 ± 30.0	119.8 ± 37.0
Sperm density	Mean ± SD	1438.22 ± 969.94	1575.83 ± 430.75	1837.95 ± 479.93

a: Sum of number of motile and non-motile count. Groups of five fields were evaluated until a sperm count of at least 200 was achieved or 20 fields were evaluated
b: 20 fields were evaluated

Summary of Estrous Cycling of Untreated Female Mice

Male Mice Doses		CNTO 345 (mg/kg/dose)		
		0	40	100
Females mice tested	N	25	25	25
PRECOHABITATION ESTROUS CYCLING				
Estrous stages / 14 Days	Mean ± SD	2.7 ± 0.6	2.7 ± 0.6	2.7 ± 0.8
Mice with ≥6 consecutive days of diestrus	N (%)	1 (4.0)	3 (12.0)	2 (8.0)
Mice with ≥6 consecutive days of estrus	N (%)	1 (4.0)	1 (4.0)	1 (4.0)

CNTO 345 (Anti-Mouse IL-6 Monoclonal Antibody): Fertility and General Reproduction Toxicity Study in Female Mice

Summary of Estrous Cycling Observations for Female mice treated with vehicle control or CNTO 345

Female Mice Doses		CNTO 345 (mg/kg/dose)		
		0	40	100
Females mice tested	N	25	25	25
PREDOSE ESTROUS CYCLING				
Estrous stages / 14 Days	Mean ± SD	2.1 ± 0.8	2.0 ± 0.9	2.3 ± 0.8
Mice with ≥6 consecutive days of diestrus	N (%)	9 (36.0)	8 (32.0)	6 (24.0)
Mice with ≥6 consecutive days of estrus	N (%)	1 (4.0)	1 (4.0)	1 (4.0)
PRECOHABITATION ESTROUS CYCLING				
Estrous stages / 14 Days	Mean ± SD	2.6 ± 0.7	2.0 ± 1.0	2.1 ± 1.0
Mice with ≥6 consecutive days of diestrus	N (%)	4 (16.0)	10 (40.0)	6 (24.0)
Mice with ≥6 consecutive days of estrus	N (%)	0 (0.0)	1 (4.0)	1 (4.0)

Summary of Mating and Fertility Parameters for Female mice treated with vehicle control or CNTO 345

Male Mice Doses	CNTO 345 (mg/kg/dose)		
	0	40	100
Number of mice in cohabitation	25	24 [a]	25
Days in cohabitation [b] (mean)	2.4 ± 0.8	2.5 ± 1.0	2.4 ± 1.0
Mice that mated - N (%)	25 (100)	24 (100)	25 (100)
Number of mice with confirmed mating dates	23	24	24
Fertility Index [c] - N / N (%)	23 / 25 (92.0)	21 / 24 (87.5)	25 / 25 (100.0)
Mated with Female [d] - N / N (%) Days 1-7	23 (100)	24 (100)	25 (100.0)
Mice Pregnant / Mice in Cohabitation - N / N (%)	23 / 25 (92.0)	21 / 24 (87.5)	25 / 25 (100.0)

a: One female death by overdosing during the cohabitation period
b: Mice with a confirmed mating date and mice that did not mate
c: Number of pregnancies / number of mice that mated
d: Mice with confirmed mating date

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/s/

PEDRO L DEL VALLE
01/23/2014

HALEH SABER
01/23/2014

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR BLA or Supplement

BLA Number: 125496 **Applicant: Janssen R&D, LLC.** **Stamp Date: August 30, 2013**

Drug Name: siltuximab **BLA Type: Original BLA**

On **initial** overview of the BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		Monkey is the only animal species available for toxicity testing that is pharmacologically responsive to siltuximab. Repeat-dose and developmental toxicology studies conducted in sexually immature monkeys were completed before the ICH S6 R1 addendum was published. An additional fertility study was conducted with a rat anti-mouse IL6 mAb in mice and a pre- and post-natal development study was conducted with a humanized version of siltuximab in cynomolgus monkeys.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		Product was administered intravenously or subcutaneously for all toxicology studies.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)		X	Impurity issues will be addressed, as needed, during the review.
11	Has the applicant addressed any abuse potential issues in the submission?		X	Not applicable.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?		X	Not applicable.

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? __YES__

If the BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

No issues identified

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Not applicable

Pedro L. Del Valle, PhD — October 16, 2013
Reviewing Pharmacologist — Date

Haleh Saber, PhD — October 16, 2013
Team Leader/Supervisor — Date

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

PEDRO L DEL VALLE
10/16/2013

HALEH SABER
10/16/2013