

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
125261

PHARMACOLOGY REVIEW(S)

**PHARMACOLOGY/TOXICOLOGY REVIEW
MEMO**

BLA number: 125261

Sequence number/date/type of submission: 053 / 01-09-2009 / Response to complete response letter deficiencies

Sponsor and/or agent: Centocor, Inc.

Drug substance: Ustekinumab

Tradename: STELARA™

Reviewer name: Jiaqin Yao

Division name: Dermatology and Dental Products

Date: 8-5-2009

No nonclinical deficiencies were identified within the original BLA submission dated on November 28, 2007 and the BLA was approvable from a pharmacology/toxicology perspective. No new nonclinical studies were submitted within the current submission.

The following wording is recommended for the labeling based on the nonclinical information included in the original BLA submission and was agreed upon by the sponsor. Please refer to the original BLA review for additional details, if needed.

8.1 Pregnancy

Pregnancy Category B

There are no studies of STELARA™ in pregnant women. STELARA™ should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. No teratogenic effects were observed in the developmental and reproductive toxicology studies performed in cynomolgus monkeys at doses up to 45 mg/kg ustekinumab, which is 45 times (based on mg/kg) the highest intended clinical dose in psoriasis patients (approximately 1 mg/kg based on administration of a 90 mg dose to a 90 kg psoriasis patient).

Ustekinumab was tested in two embryo-fetal development toxicity studies. Pregnant cynomolgus monkeys were administered ustekinumab at doses up to 45 mg/kg during the period of organogenesis either twice weekly via subcutaneous injections or weekly by intravenous injections. No significant adverse developmental effects were noted in either study.

In an embryo-fetal development and pre- and post-natal development toxicity study, three groups of 20 pregnant cynomolgus monkeys were administered subcutaneous doses of 0, 22.5, or 45 mg/kg ustekinumab twice weekly from the beginning of organogenesis in cynomolgus monkeys to Day 33 after delivery. There were no treatment-related effects on mortality, clinical signs, body weight, food consumption, hematology, or serum biochemistry in dams. Fetal losses occurred in six control monkeys, six 22.5 mg/kg-treated monkeys, and five 45 mg/kg-treated monkeys. Neonatal deaths occurred in one

22.5 mg/kg-treated monkey and in one 45 mg/kg-treated monkey. No ustekinumab-related abnormalities were observed in the neonates from birth through six months of age in clinical signs, body weight, hematology, or serum biochemistry. There were no treatment-related effects on functional development until weaning, functional development after weaning, morphological development, immunological development, and gross and histopathological examinations of offsprings by the age of 6 months.

8.3 Nursing Mothers

Caution should be exercised when STELARA™ is administered to a nursing woman. The unknown risks to the infant from gastrointestinal or systemic exposure to ustekinumab should be weighed against the known benefits of breast feeding. Ustekinumab is excreted in the milk of lactating monkeys administered ustekinumab. IgG is excreted in human milk, so it is expected that STELARA™ will be present in human milk. It is not known if ustekinumab is absorbed systemically after ingestion; however, published data suggest that antibodies in breast milk do not enter the neonatal and infant circulation in substantial amounts.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Animal studies have not been conducted to evaluate the carcinogenic or mutagenic potential of STELARA™. Published literature showed that administration of murine IL-12 caused an anti-tumor effect in mice that contained transplanted tumors and IL-12/IL-23p40 knockout mice or mice treated with anti-IL-12/IL-23p40 antibody had decreased host defense to tumors. Mice genetically manipulated to be deficient in both IL-12 and IL-23 or IL-12 alone developed UV-induced skin cancers earlier and more frequently compared to wild-type mice. The relevance of these experimental findings in mouse models for malignancy risk in humans is unknown.

A male fertility study was conducted with only 6 male monkeys per group administered subcutaneous doses of 0, 22.5, or 45 mg/kg ustekinumab twice weekly prior to mating and during the mating period for 13 weeks, followed by a 13-week treatment-free period. Although fertility and pregnancy outcomes were not evaluated in mated females, there were no treatment-related effects on parental toxicity or male fertility parameters.

A female fertility study was conducted in mice using an analogous IL-12/IL-23p40 antibody by subcutaneous administration at doses up to 50 mg/kg, twice weekly, beginning 15 days before cohabitation and continuing through GD 7. There were no treatment-related effects on maternal toxicity or female fertility parameters.

13.2 Animal Toxicology and/or Pharmacology

In a 26-week toxicology study, one out of 10 monkeys subcutaneously administered 45 mg/kg ustekinumab twice weekly for 26 weeks had a bacterial infection.

Signatures (optional):

Reviewer Signature Jiaqin Yao 8-11-2009

Supervisor Signature Barbara Hill 8-11-09 Concurrence Yes No

**PHARMACOLOGY/TOXICOLOGY REVIEW
MEMO**

BLA number: 125261

Sequence number/date/type of submission: 000 / 11-28-2007 / Original submission

Sponsor and/or agent: Centocor, Inc.

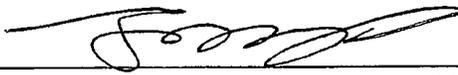
Reviewer name: Jiaqin Yao

Division name: Dermatology and Dental Products

Date: 11-25-2008

The Clinical Reviewers requested to add some nonclinical information in the labeling on the UV-induced tumors in IL-12/IL-23p40 knockout (KO) mice, because the animal studies demonstrated the association between IL-12 deficiency and the susceptibility to UV-induced skin tumors in mice. The following information can be found in my previous review, "Compared to the wild-type mice, IL-12/IL-23p40 KO mice developed UV-induced tumors earlier and more frequently, and tumors generated in IL-12/IL-23p40 knockout mice grew faster in vivo and had greater intrinsic invasion potential (Maeda et al, 2006). Similarly, the development of UV-induced tumors was more rapid and the tumor multiplicity and tumor size were significantly greater in IL-12p35 KO mice than the wild-type mice; the incidence of malignant transformation of UVB-induced papillomas to carcinomas was higher in IL-12p35 KO mice in terms of carcinoma incidence; UVB-induced DNA damage in the form of cyclobutane pyrimidine dimers was removed or repaired more rapidly in the wild-type mice than the IL-12p35 KO mice (Meeran et al, 2006)." Therefore, beside what has been recommended in the Section 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility in my previous review, "Published literature showed that administration of murine IL-12 caused an anti-tumor effect in mice that contained transplanted tumors and IL-12/IL-23p40 knockout mice or mice treated with anti-IL-12/IL-23p40 antibody had decreased host defense to tumors", the following statement is also recommended: "Compared to the wild-type mice, IL-12/IL-23p40 knockout mice developed UV-induced tumors earlier, more frequently, and with bigger size and more aggressive potential."

Signatures (optional):

Reviewer Signature 

Supervisor Signature Barbara Hill ¹¹⁻²⁵⁻⁰⁸ Concurrency Yes No

Pharmacology/Toxicology Supervisory Memorandum

BLA number: 125261
Sequence number/date/type of submission: 000 / November 28, 2007 / original submission
Sponsor and/or agent: Centocor
Supervisor name: Barbara Hill *Barbara Hill 10-2-08*
Division name: Division of Dermatology and Dental Products
Date: October 2, 2008
Drug: Ustekinumab (CNTO 1275)
Drug class: IL-12/IL-23 receptor inhibitor, fully human IgG1 monoclonal antibody that binds to the p40 protein subunit of IL-12 and IL-23
Indication: Moderate to severe plaque psoriasis

Introduction and discussion:

Per the statistical review written by Dr. Kathleen Fritsch, the efficacy of ustekinumab 45 mg and 90 mg in the treatment of moderate to severe psoriasis has been demonstrated in two Phase 3 clinical studies. The sponsor's proposed dosing regimen for ustekinumab is an initial dose and then one dose administered 4 weeks later, followed by doses every 12 weeks. The sponsor's proposed doses are 45 mg/dose for subjects weighing 100 kg or less and 90 mg/dose for subjects weighing more than 90 kg. Per the clinical pharmacology review written by Dr. Abimbola Adebowale, the clinical pharmacology and biopharmaceutics information provided in the submission is acceptable provided the applicant adequately addresses the recommended 3 step dosing regimen based on exposure-response analysis. The recommended 3-step dosing regimen is: a) for patients weighting <70 kg, the recommended dose is 45 mg initially and 4 weeks later, followed by dosing every 12 weeks, b) for patients weighting ≥ 70 kg and <100 kg, the recommended dose is 67.5 mg initially and 4 weeks later, followed by dosing every 12 weeks, and c) for patients weighting ≥ 100 kg, the recommended dose is 90 mg initially and 4 weeks later, followed by dosing every 12 weeks.

The sponsor conducted several nonclinical toxicology studies to support the safety of ustekinumab. These studies have been reviewed by Dr. Jiaqin Yao. The pivotal chronic toxicology and reproductive toxicology studies include the following:

- 1) 26-Week subcutaneous dose toxicity and toxicokinetic study with CNTO 1275 in cynomolgus monkeys with a 12-week recovery period (T-2001-004)
- 2) CNTO 1275: Male fertility study in cynomolgus monkeys following twice-weekly subcutaneous injections (T-2005-015)
- 3) CNTO3913: Mouse subcutaneous female fertility and general reproduction toxicity study of a mouse anti-IL-12/23p40 monoclonal antibody (T-2007-003)
- 4) A study of the effects of subcutaneous administration of CNTO 1275 on embryo-fetal development in cynomolgus monkeys (T-2002-005)
- 5) A study of the effects of 12B75 on embryo-fetal development in cynomolgus monkeys (T-2001-001)

- 6) A study for the effect of CNTO 1275 on embryo-fetal development and on pre- and postnatal development, including maternal function in cynomolgus monkeys by twice weekly subcutaneous administration (T-2004-009)

No genotoxicity studies were conducted with ustekinumab. However, genotoxicity studies are not recommended for monoclonal antibodies due to their large size and inability to reach the target of interest (i.e., DNA). The sponsor did not conduct any studies to address the carcinogenic potential of ustekinumab. The sponsor provided literature references to address the carcinogenic potential of ustekinumab. The literature data demonstrated that administration of murine IL-12 had an anti-tumor effect in mice that contained transplanted tumors. In addition, the literature data suggested that host defense to neoplasia decreased in IL-12/IL-23p40 knockout mice or in mice treated with anti-IL-12/IL-23p40 antibody. The overall body of literature data available to address the potential carcinogenic risk associated with inhibition of IL-12/IL-23 indicates that there may be an increased carcinogenic risk associated with chronic use of ustekinumab.

Conclusion:

The literature information along with the nonclinical studies conducted by the sponsor are adequate to support the systemic safety of ustekinumab, from a pharmacology and toxicology perspective. The labeling of ustekinumab should use the information from the nonclinical studies conducted by the sponsor and from the literature as outlined in the review by Dr. Jiaqin Yao. The literature data does suggest that a potential increased carcinogenic risk may be associated with the chronic use of ustekinumab in psoriasis patients. It is essential that the information from the literature that addresses the carcinogenic potential of ustekinumab be incorporated into the label per Dr. Jiaqin Yao's review.



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER: 125261
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 11/28/07
DRUG NAME: Ustekinumab (CNTO 1275)
INDICATION: Moderate to severe plaque psoriasis
SPONSOR: Centocor
REVIEW DIVISION: Dermatology and Dental Products
PHARM/TOX REVIEWER: Jiaqin Yao *Jiaqin Yao* 7-28-2008
PHARM/TOX SUPERVISOR: Barbara Hill *Barbara Hill* 7-28-08
DIVISION DIRECTOR: Susan Walker
PROJECT MANAGER: Maria Walsh

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

The NDA is approvable from a pharmacology/toxicology perspective.

B. Recommendation for nonclinical studies

None

C. Recommendations on labeling

8.1 Pregnancy

Teratogenic Effects: Pregnancy Category B:

There are no [REDACTED] studies with TRADENAME in pregnant women. No teratogenic effects were observed in the developmental and reproductive toxicology studies performed in cynomolgus monkeys at doses up to 45 mg/kg ustekinumab, which is 45 times (based on mg/kg) the highest intended clinical dose in psoriasis patients (approximately 1 mg/kg based on administration of a 90 mg dose to a 90 kg psoriasis patient). TRADENAME should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

b(4)

Ustekinumab was tested in two embryo-fetal development toxicity studies.

[REDACTED] No significant adverse developmental effects were noted in either study.

b(4)

In an embryo-fetal development and pre- and post-natal development toxicity study, three groups of 20 pregnant cynomolgus monkeys were administered subcutaneous doses of 0, 22.5, or 45 mg/kg ustekinumab twice weekly from [REDACTED] delivery. There were no treatment-related effects on mortality, clinical signs, body weight, food consumption, hematology, or serum biochemistry in dams. Fetal losses occurred in six control monkeys, six 22.5 mg/kg-treated monkeys, and five 45 mg/kg-treated monkeys. Neonatal deaths occurred in one 22.5 mg/kg-treated monkey and in one 45 mg/kg-treated monkey. No ustekinumab-related abnormalities were observed in the neonates from birth through six months of age in clinical signs, body weight, hematology, or serum biochemistry. There were no treatment-related effects on functional development until weaning, functional development after weaning, morphological development, immunological development, and gross and histopathological examinations of offsprings by the age of 6 months.

b(4)

8.3 Nursing Mothers

Ustekinumab is excreted in the milk of lactating monkeys administered TRADENAME. It is not known if ustekinumab is absorbed systemically after ingestion.

b(4)

b(4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Animal studies have not been conducted to evaluate the carcinogenic or mutagenic potential of [TRADENAME]. Published literature showed that administration of murine IL-12 caused an anti-tumor effect in mice that contained transplanted tumors and IL-12/IL-23p40 knockout mice or mice treated with anti-IL-12/IL-23p40 antibody had decreased host defense to tumors.

A male fertility study was conducted with only 6 male monkeys per group administered subcutaneous doses of 0, 22.5, or 45 mg/kg ustekinumab twice weekly prior to mating and during the mating period for 13 weeks, followed by a 13-week treatment-free period. Although fertility and pregnancy outcomes were not evaluated in mated females, there were no treatment-related effects on parental toxicity or male fertility parameters.

A female fertility study was conducted in mice using an analogous IL-12/IL-23p40 antibody by subcutaneous administration at doses up to 50 mg/kg, twice weekly, beginning 15 days before cohabitation and continuing through GD 7. There were no treatment-related effects on maternal toxicity or female fertility parameters.

13.2 Animal Toxicology

In a 26-week toxicology study, one out of 10 monkeys subcutaneously administered 45 mg/kg ustekinumab twice weekly for 26 weeks had bacteria infection.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Ustekinumab (CNTO 1275, a human monoclonal antibody against the p40 subunit of IL-12 and IL-23) has been tested in cynomolgus monkeys at doses up to 45 mg/kg by intravenous administration weekly for up to 1 month or by subcutaneous administration twice weekly for up to 6 months. No significant adverse effects were noted in these studies, except that in the 26-week subcutaneous study, one out of 10 monkeys treated with 45 mg/kg CNTO 1275 twice weekly for 26 weeks exhibited signs of bacterial enteritis.

Genetic toxicology and nonclinical carcinogenicity studies have not been conducted with CNTO 1275. No tumors or histopathological evidence of pre-neoplastic changes were observed in organs or tissues examined following subcutaneous administration of CNTO 1275 to monkeys at doses up to 45 mg/kg twice weekly for 6 months followed by a 3-month post-dose observation period. However, literature data showed that administration of murine IL-12 had an anti-tumor effect in mice that contained transplanted tumors and host defense to neoplasia decreased in IL-12/IL-23p40 knockout mice or in mice treated with anti-IL-12/IL-23p40 antibody.

A male fertility study, two embryo-fetal development toxicity studies, and an embryo-fetal development and pre- and postnatal development toxicity study have been conducted in cynomolgus monkeys at doses up to 45 mg/kg CNTO 1275 by subcutaneous or intravenous administration. A female fertility study was conducted in mice using an analogous IL-12/IL-23 p40 antibody. No significant adverse effects were noted in these studies.

Toxicokinetic evaluations confirmed high CNTO 1275 exposure of monkeys in the toxicity studies. Following single and multiple subcutaneous administrations, CNTO 1275 was absorbed into the systemic circulation with a mean T_{max} ranging from 2 to 7 days. Mean C_{max} and AUC values increased in an approximately dose-proportional manner. The mean $t_{1/2}$ values ranged from 2 - 3 weeks following multiple subcutaneous injections of CNTO 1275 in monkeys, which were similar to that observed in psoriasis patients. With repeated dosing CNTO 1275 showed 5 to 10-fold accumulation of drug and steady state was achieved in about 13 weeks following twice weekly subcutaneous dosing. The mean steady-state C_{max} value following 45 mg/kg twice-weekly subcutaneous dosing in monkeys (2347 $\mu\text{g/mL}$) was over 100-fold higher than the median C_{max} value following 4-weekly 90 mg subcutaneous doses in subjects with psoriasis (20.3 $\mu\text{g/mL}$). The serum concentrations attained were well in excess of those required for complete inhibition of IL-12/23 activity based on in vitro activity evaluations and reported serum concentrations of IL-12/IL-23p40 in monkeys and humans.

B. Pharmacologic activity

CNTO 1275 binds to the shared p40 protein subunit of human IL-12 and IL-23 and inhibits IL-12 and IL-23 bioactivity by preventing their interaction with their cell surface IL-12R β 1 receptor protein. Through this mechanism of action, CNTO 1275 neutralizes IL-12 and IL-23-mediated cellular responses. CNTO 1275 was shown to have comparable binding and neutralization activity against human and cynomolgus monkey IL-12 and IL-23. No unexpected binding of CNTO 1275 to normal human tissues was observed in tissue cross-reactivity studies. Pharmacodynamic activity studies indicated that cynomolgus monkey was the pharmacologically relevant toxicology species for CNTO 1275.

C. Nonclinical safety issues relevant to clinical use

Based on mechanism of action and roles of IL-12 and IL-23, immunotoxicity, infection, and malignancy are safety concerns for patients chronically treated with CNTO 1275. Although nonclinical studies did not show an association between CNTO 1275 treatment and immunotoxicity or immunosuppression in monkeys, one out of 10 monkeys subcutaneously administered 45 mg/kg CNTO 1275 twice weekly for 26 weeks had bacteria infection. The dose of 45 mg/kg in monkeys is 45 times (based on mg/kg) the highest intended clinical dose in psoriasis patients (approximately 1 mg/kg based on administration of a 90 mg dose to a 90 kg psoriasis patient). In addition, published literature reported that human subjects with genetic deficiencies in IL-12 signaling were susceptible to infections, presumably due to immunosuppression. Immunosuppressive agents have the potential to increase the risk of malignancy. Published literature further showed that administration of murine IL-12 caused an anti-tumor effect in mice that

contained transplanted tumors and IL-12/IL-23p40 knockout mice or mice treated with anti-IL-12/IL-23p40 antibody had decreased host defense to tumors. Adequate labeling on nonclinical information and post-marketing patient monitoring of infection and malignancy are necessary.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

BLA number: 125261

Review number: 1

Sequence number/date/type of submission: 000 / 11-28-2007 / Original submission

Information to sponsor: Yes (X) No ()

Sponsor and/or agent: Centocor, Inc.

Manufacturer for drug substance: Centocor Biologics., LLC, 4777 LeBourget Drive,
St. Louis, MO

Reviewer name: Jiaqin Yao

Division name: Dermatology and Dental Products

HFD #: 540

Review completion date: 7-28-2008

Drug:

Trade name: STELARA

Generic name: Human anti-IL-23/IL-23p40 IgG1 monoclonal antibody

Code name: CNTO 1275, 12B75

CAS name: 815610-63-0

USAN name: Ustekinumab

CAS registry number: NA

Molecular formula/molecular weight: 1326 Amino acids / 148.6 KD

Structure: The amino acid sequence of CNTO 1275 deduced from the DNA sequence for the molecule is shown below for the heavy and light chains, respectively.

Heavy Chain Amino Acid Sequence

b(4)

Light Chain Amino Acid Sequence

[Redacted]

b(4)

Relevant INDs/NDAs/DMFs: BB-IND 9590 [Redacted]

Drug class: Interleukin receptor inhibitor, monoclonal antibody

Indication: Moderate to severe plaque psoriasis

Clinical formulation:

Table 1 Composition of CNTO 1275 90 mg and 45 mg FVP

<u>Component</u>	<u>90 mg Dose Amount Per Dose (mg)</u>	<u>45 mg Dose Amount Per Dose (mg)</u>	<u>Concentration</u>
CNTO 1275	90	45	[Redacted]
Sucrose	76	38	[Redacted]
L-histidine	1.0	0.5	[Redacted]
Polysorbate 80	0.04	0.02	[Redacted]

b(4)

Route of administration: Subcutaneous injection

Proposed use: The human recommended dose is 45 mg initially and 4 weeks later, followed by dosing every 12 weeks. Alternatively, 90 mg may be used in patients with a body weight greater than 100 kg.

Disclaimer: Tabular and graphical information are duplicated from the sponsor unless cited otherwise.

Studies reviewed within this submission:

Pharmacology:

1. Binding of CNTO 1275 to human IL-12 and IL-23 (DIS.RES.DRR.005.jb.doc)

2. **Mechanism of action of CNTO 1275 mediated neutralization of human IL-12 and IL-23 (DIS.RES.DRR.006.jb.doc)**
3. **Functional effects of CNTO 1275 neutralization of human IL-12 and IL-23 (DIS RES DRR.007.jb.doc)**
4. **Species binding and activity for CNTO 1275 and the anti-mouse IL-12/23p40 antibody CNTO 3913 (DIS RES DRR.014.jb.doc)**
5. **Efficacy of CNTO 1275 in a humanized mouse model of psoriasis (DIS RES DRR.008.jb.doc)**

Pharmacokinetics/Toxicokinetics:

1. **A single subcutaneous dose pharmacokinetic study in cynomolgus monkeys with 12B75 (anti-human IL-12 monoclonal antibody) (P-2000-005)**
2. **Single intravenous dose pharmacokinetic study in cynomolgus monkeys with 12B75 (P-099-001)**

General toxicology:

1. **Pilot intravenous dose tolerance study in cynomolgus monkeys with 12B75 (T-099-003)**
2. **Multiple intravenous dose toxicity study with 12B75 in cynomolgus monkeys (T-099-004)**
3. **26-Week subcutaneous dose toxicity and toxicokinetic study with CNTO 1275 in cynomolgus monkeys with a 12-week recovery period (T-2001-004)**

Reproductive toxicology:

1. **CNTO 1275: Male fertility study in cynomolgus monkeys following twice-weekly subcutaneous injections (T-2005-015)**
2. **CNTO3913: Mouse subcutaneous female fertility and general reproduction toxicity study of a mouse anti-IL-12/23p40 monoclonal antibody (T-2007-003)**
3. **A study for the effect of CNTO 1275 on embryo-fetal development and on pre- and postnatal development, including maternal function in cynomolgus monkeys by twice weekly subcutaneous administration (T-2004-009)**

Local tolerance:

1. **Pharmacokinetics and injection site irritation study of multiple subcutaneous doses of 12B75 mAb in monkeys (T-2001-003)**

Special toxicology:

1. **Cross-reactivity of biotinylated 12B75 (anti-human IL-12 monoclonal antibody) with selected normal human tissues (T-098-005)**
2. **Cross-reactivity of biotinylated 12B75 (anti-human IL-12 monoclonal antibody) with normal human tissues (T-099-002)**
3. **Pilot study of 12B75 in an acute model of asthma in cynomolgus monkeys (T-099-006)**
4. **A study of 12B75 in a model of asthma in cynomolgus monkeys (T-2000-001)**

Studies not reviewed within this submission:

The following 2 studies were reviewed by Dr. Andrea B. Weir in BB-IND 9590.

1. **A study of the effects of subcutaneous administration of CNTO 1275 on embryo-fetal development in cynomolgus monkeys (T-2002-005)**
2. **A study of the effects of 12B75 on embryo-fetal development in cynomolgus monkeys (T-2001-001)**

The following 2 studies have been reviewed by Laurie Graham (see CMC review of this BLA):

1. **Single subcutaneous dose pharmacokinetic study in cynomolgus monkeys with CNTO 1275 (anti-IL-12 mAb) produced by cell lines _____ (P-2003-009)**
2. **Single intravenous dose pharmacokinetic study in cynomolgus monkeys with CNTO 1275 (Phase II lyophilized formulation) and CNTO 1275 (Phase III liquid formulation) anti-IL-12 monoclonal antibodies (P-2005-004)**

b(4)

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

IL-12 and IL-23 cytokines are comprised of a shared p40 subunit and a subunit unique to each cytokine, p36 for IL-12 and p19 for IL-23. Inappropriate expression of IL-12 and IL-23 might be linked to immune-mediated diseases including psoriasis, making the cytokines as targets for immunotherapy for psoriasis. Ustekinumab (CNTO 1275) is a fully human IgG1 monoclonal antibody against the p40 subunit of IL-12 and IL-23. Ustekinumab binds to the shared p40 protein subunit of human IL-12 and IL-23 and inhibits IL-12 and IL-23 bioactivity by preventing their interaction with their cell surface IL-12R β 1 receptor protein. Through this mechanism of action, ustekinumab neutralizes IL-12 and IL-23-mediated cellular responses.

2.6.2.2 Primary pharmacodynamics

1. **Binding of CNTO 1275 to human IL-12 and IL-23 (DIS.RES.DRR.005.jb.doc):** The study showed in vitro that CNTO 1275 bound to the p40 subunit of human IL-12 and human IL-23, but did not bind to the structurally related proteins IL-6, IL-6sR, CNTFR, or IL-11R using ELISA and nitrocellulose membrane binding analysis. Crystal structure and mutational analysis elucidated the molecular binding interactions as discontinuous residues located in the D1 domain of IL-12/IL-23 p40, spatially distant from the p35 subunit. The surface plasmon resonance and isothermal titration calorimetry analysis showed that CNTO 1275 bound human IL-12 and IL-23 with high affinity and the expected ligand/mAb 2:1 stoichiometry ratio. These data indicated CNTO 1275 binds with high affinity and specificity to the p40 subunit of human IL-12 and IL-23.
2. **Mechanism of action of CNTO 1275 mediated neutralization of human IL-12 and IL-23 (DIS.RES.DRR.006.jb.doc):** CNTO 1275 was shown in vitro to prevent IL-12 or

IL-23 binding to the IL-12R β 1 receptor chain of IL-12 and IL-23 receptor complexes. CNTO 1275 inhibited binding of IL-12 or IL-23 to IL-12R β 1 expressed on the cell surface either alone or in functional IL-12R β 1/ β 2 and IL-12R β 1/23R dual receptor complexes. CNTO 1275 cannot bind to IL-12 or IL-23 that was pre-bound to cell surface receptors or contribute to complement dependent cytotoxicity of human PHA activated lymphocytes. These data indicated inhibition of IL-12 or IL-23 binding to IL-12R β 1 might be the molecular mechanism of action of CNTO 1275.

3. Functional effects of CNTO 1275 neutralization of human IL-12 and IL-23 (DIS RES DRR.007.jb.doc): CNTO 1275 neutralized IL-12 mediated intracellular phosphorylation of STAT4 and STAT6, cell surface expression of CD95 (Fas), cutaneous lymphocyte antigen (CLA), and IL-12R β 2, NK and lymphokine killer cell (LAK) lytic activities, and IFN γ cytokine production from primary human T cell blasts and a human NK cell line. IFN γ neutralization was achieved with intact CNTO 1275 IgG or with a CNTO 1275 Fab fragment. CNTO 1275 was also shown to neutralize IL-23 mediated intracellular STAT3 phosphorylation and protein production of IL-17A, IL-17F, IL-22, and IL-10. These data indicated that CNTO 1275 could neutralize human IL-12 and IL-23 cellular responses.

4. Species binding and activity for CNTO 1275 and the anti-mouse IL-12/23p40 antibody CNTO 3913 (DIS RES DRR.014.jb.doc): Human and cynomolgus monkey IL-12 and IL-23 subunits have greater than 92% amino acid homology. The key IL-12/IL-23p40 amino acid residues required for CNTO 1275 binding are identical between human and cynomolgus monkey IL-12/IL-23p40. CNTO 1275 bound and neutralized only human and non-human primate native IL-12 and IL-23, but showed either partial or no activity to native mouse, rat, dog, or rabbit IL-12 or IL-23 (see the next table).

Table 1: Summary of species cross-reactivity of CNTO 1275 with native IL-12 and IL-23.

Species	IL-12 Binding Intracellular Flow Cytometry	IL-12 Neutralization IFN γ mRNA [†]	IL-12 Neutralization IFN γ Protein [†]	IL-23 Neutralization IL-17A Protein [†]
Human	+++	+++	+++	+++
Chimpanzee	ND [§]	ND	+++	+++
Baboon	+++	+++	+++	+++
Cynomolgus	+++	+++	+++	+++
Marmoset	*	++	+++	ND
Rhesus	ND	ND	+++	+++
Mouse	--	ND	--	--
Rat	--	ND	--	--
Rabbit	*	*	+/-	*
Dog	--	*	+/-	++

PBMC were stimulated with LPS + IFN γ to induce native IL-12 and IL-23 production. Cells were permeabilized and analyzed by flow cytometry for intracellular binding of biotinylated CNTO 1275. Supernatants were used with or without CNTO 1275 to elicit IFN γ mRNA expression, or protein production of IFN γ or IL-17A. [†] = bioactivity assay; ND[§] = not done; * = results were inconclusive; -- = no binding or neutralization observed.

CNTO 1275 was shown to have comparable binding to recombinant human and cynomolgus monkey IL-12 and IL-23, but no binding to recombinant mouse IL-12 or IL-23. CNTO 1275 had comparable neutralization of recombinant human versus recombinant cynomolgus monkey IL-12 and IL-23 bioactivity, but showed no neutralization of recombinant mouse IL-12 or IL-23 bioactivity.

CNTO 3913 is a chimeric monoclonal antibody (mAb) constructed using the heavy and light chain variable regions from a neutralizing rat anti-mouse IL-12/IL-23p40 mAb and heavy and light chain constant regions from a mouse IgG2a kappa mAb. CNTO 3913 was shown in vitro to have comparable binding to recombinant mouse IL-12 and IL-23, with reduced binding to rat IL-12 and IL-23. CNTO 3913 also neutralized mouse, but not rat, IL-12 and IL-23 bioactivity. In vitro, CNTO 3913 neutralization potency against mouse IL-12 was comparable to CNTO 1275 neutralization potency against human IL-12. However, CNTO 3913 neutralization potency against mouse IL-23 is approximately 10 fold lower than CNTO 1275 neutralization potency against human IL-23.

In addition, the sponsor further estimated that the range of serum CNTO 1275 concentrations to achieve complete ligand neutralization were [redacted] in normal humans and [redacted] in normal cynomolgus monkeys and the range of serum CNTO 3913 concentrations to achieve complete ligand neutralization was [redacted] in normal mice (see the next table).

Table 5: Estimated serum CNTO 1275 or CNTO 3913 concentrations for complete ligand neutralization

Species & Ligand	Reported IL-12/23p40 serum levels	Estimated serum mAb concentration for complete ligand neutralization ¹
Human IL-12	[redacted]	/
Human IL-23	[redacted]	
Cynomolgus IL-12	[redacted]	
Cynomolgus IL-23	[redacted]	
Mouse IL-12	[redacted]	
Mouse IL-23	[redacted]	

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¹ (mAb concentration for 100% neutralization in bioassay / ligand concentration in bioassay) x reported IL-12/23p40 serum levels = estimated serum mAb concentration for complete ligand neutralization

² Quantikine Human IL-12/23p40 immunoassay #DP400; ³ Herzyk, 2002; ⁴ Quantikine Mouse IL-12/23p40 immunoassay #MP400

These data along with others support that cynomolgus macaque and mice are pharmacologically relevant toxicology species for CNTO 1275 and CNTO 3913, respectively.

5. Efficacy of CNTO 1275 in a humanized mouse model of psoriasis (DIS RES DRR.008.jb.doc): CNTO 1275 was evaluated for efficacy in a humanized mouse model of psoriasis. Non-lesional skin from human psoriasis donors was transplanted onto immunodeficient BNX mice and the psoriatic process was triggered by the intradermal

injection of autologous activated T cells after acceptance of the grafts. Five groups of 6 or 7 mice were treated intraperitoneally once weekly with 0 (PBS), 0.25, 2, or 10 mg/kg of CNTO 1275, or 20 mg/kg of Cyclosporin A for 3 weeks, starting 1 day prior to T cell transfer. The transplanted skin biopsies were then evaluated for psoriasis pathologies including epidermal thickness and expression of cytokeratin 16, HLA-DR, and Ki-67 molecules. Compared to vehicle, CNTO 1275 at doses of 2 and 10 mg/kg CNTO 1275 was effective in inhibiting epidermal thickening (49% and 29%, respectively) and keratinocyte proliferation (Ki-67 staining positive cells). CNTO 1275 treatment also slightly reduced cytokeratin 16 expression. Neither CNTO 1275 nor Cyclosporin A altered expression of HLA-DR. These data suggested that CNTO 1275 might ameliorate human psoriasis through inhibition of keratinocyte proliferation and epidermal thickening.

2.6.2.3 Safety pharmacology

Some safety pharmacology endpoints have been examined in toxicology studies in monkeys (see the Toxicology section).

2.6.3 PHARMACOLOGY TABULATED SUMMARY

NA

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

1. A single subcutaneous dose pharmacokinetic study in cynomolgus monkeys with 12B75 (anti-human IL-12 monoclonal antibody) (P-2000-005): Three (3) monkeys were administered 12B75 subcutaneously at a dose of 0.9 mg/kg (Lot 052189). Blood samples were collected at pre-dose, 0.5, 2, 6, and 24 hours and 2, 3, 7, 14, 21, 28, 35 and 42 days post-dose for TK analysis. The mean T_{max} was 4.33 days, the mean AUC was 131.184 $\mu\text{g}/\text{mL}\cdot\text{day}$, with a mean C_{max} of 8.163 $\mu\text{g}/\text{mL}$, the mean V_d/F was 42.52 mL/kg, and the mean half-life ($t_{1/2}$) was 4.71 days. The individual animal $t_{1/2}$ was 1.97, 2.06, and 10.10 days, and the two monkeys with short $t_{1/2}$ (~2 days) developed antibodies against 12B75.

2. Single intravenous dose pharmacokinetic study in cynomolgus monkeys with 12B75 (P-099-001): Two groups of 3 monkeys received a single intravenous dose of 0.9 or 9 mg/kg 12B75 (Lot JG101398) in Dulbecco's Phosphate Buffered Saline (DPS). Blood samples were collected at pre-dose, 5, 15, and 30 minutes, 1, 3, 6, and 24 hours, and 2, 3, 4, 7, 14, 21, 28, 42, 56, 70, and 84 days after dosing. The TK parameters were shown as the following tables

Pharmacokinetic Parameters For Monkeys Given 0.9 mg/kg 12B75

	T_{max} (hours)	AUC ($\mu\text{g}/\text{mL}\cdot\text{days}$)	C_{max} ($\mu\text{g}/\text{mL}$)	V_d (mL)	CL (mL/kg/day)	$T_{1/2}$ (days)
Mean	2.333	138.24	25.57	70.1	6.7	7.583
Standard Deviation	3.175	27.02	5.80	19.0	1.5	1.677

Pharmacokinetic Parameters For Monkeys Given 9 mg/kg 12B75

	Tmax (hours)	AUC (µg/mL X days)	Cmax (µg/mL)	Vd (mL)	CL (mL/kg/day)	T1/2 (days)
Mean	1.667	2452.50	436.50	60.4	3.73	12.10
Standard Deviation	1.155	396.54	48.36	13.2	0.6	1.326

3. Results from other pharmacokinetic/toxicokinetic studies included in the next table can be found in the Toxicology section.

Table 1 Pharmacokinetic/Toxicokinetic Studies with CNTO 1275

Study Type/ Duration	Study Number ^a	Pharmacokinetic/ Toxicokinetic Report Number(s)	ROA	GLP Compliance	Species	CNTO 1275		
						Cell Line	Lot Number Formulation ^b	
Absorption								
Repeated SC dose toxicity and toxicokinetic study* (26 weeks)	T-2001-004	CP2002T-091 CP2002T-091-A1 CP2002T-091-A2	SC	Yes	<i>macaca fascicularis</i>	5380.39	Liquid in vial	
Repeated SC dosing - male fertility* (6 months**)	T-2005-015	CP2006T-060 CP2006T-060-A1	SC	Yes	<i>macaca fascicularis</i>		05CS043	Liquid in pre-filled syringe
Injection site irritation study (1 month)	T-2001-003	CP2001T-022 CP2001T-022-A1	SC	Yes	<i>macaca fascicularis</i>		4841-89	Liquid in vial
Single SC dosing	P-2000-005	ClinPharm204tr.as/aaf ClinPharm204tr.as/aaf-A1 ClinPharm204tr.as/aaf-A2 ClinPharm204tr.as/aaf-A3	SC	No	<i>macaca fascicularis</i>		052189	Liquid in vial
						CNTO 1275		
Study Type/ Duration	Study Number ^a	Pharmacokinetic/ Toxicokinetic Report Number(s)	ROA	GLP Compliance	Species	Lot Number	Formulation ^b	
Single IV dose study	P-099-001	CP2005T-054 ^d CP2005T- 054-A1 ^d	IV	No ^e	<i>macaca fascicularis</i>	JG101398	Liquid	
Pilot multiple dose IV dose tolerance study (1 month)	T-099-003	REStr136.as CP2006T-011	IV	No ^e	<i>macaca fascicularis</i>	052189	Liquid in vial	
Repeated IV dose toxicity study (1 month)	T-099-004	REStr161.as REStr161.as-A1	IV	Yes ^e	<i>macaca fascicularis</i>	052189	Liquid in vial	

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Distribution							
Embryo-fetal development study Embryofetal Development* (3 months)	T-2002-005	CP2003T-017 CP2003T-017-A1	SC	Yes	macaca fascicularis	b(4)	5380.39; 4841-90 D01PJ7094 Lyophilized
Embryofetal Development/Pre and Postnatal Development* (6 months)	T-2004-009	CP2007T-002	SC	Yes	macaca fascicularis		D03PM7322 Lyophilized
Embryofetal Development (5 weeks)	T-2001-001	CP2002T-007 CP2002T-007-A1	IV	Yes	macaca fascicularis		4841-66 Liquid

* Primary studies for CNTO 1275

** 13 weeks of treatment followed by 13 weeks treatment-free

a "T" for Toxicology studies and "P" for Pharmacokinetic studies

b See Table 2.6.5.1 for formulation components

c Noncompliance with regard to data archival and retrieval

d The original pharmacokinetic report for P-099-001, "Single Intravenous Dose Pharmacokinetic Study in Cynomolgus Monkeys with 12B75", did not have a report number. The report numbers listed here are for amendments to this original report.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

NA

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: CNTO 1275 has been tested in cynomolgus monkeys at doses up to 45 mg/kg by intravenous administration weekly for up to 1 month or by subcutaneous administration twice weekly for up to 6 months. No significant adverse effects were noted in these studies, except that in the 26-week subcutaneous study, one out of 10 monkeys administered 45 mg/kg CNTO 1275 for 26 weeks exhibited signs of bacterial enteritis.

Genetic toxicology: No genetic toxicology studies have been conducted with CNTO 1275.

Carcinogenicity: Nonclinical carcinogenicity studies have not been conducted with CNTO 1275. No tumors or histopathological evidence of pre-neoplastic changes were observed in organs or tissues examined following subcutaneous administration of ustekinumab to monkeys at dose levels up to 45 mg/kg twice weekly for 6 months followed by a 3-month post-dose observation period. However, literature data showed that administration of murine IL-12 had an anti-tumor effect in mice that contained transplanted tumors and host defense to neoplasia decreased in IL-12/IL-23p40 knockout mice or in mice treated with anti-IL-12/IL-23p40 antibody.

Reproductive toxicology: A male fertility study, two embryo-fetal development toxicity studies, and an embryo-fetal development and pre- and postnatal development toxicity study have been conducted in cynomolgus monkeys at doses up to 45 mg/kg CNTO 1275

via subcutaneous or intravenous administration. A female fertility study was conducted in mice using an analogous IL-12/IL23 p40 antibody. No significant adverse effects were noted in these studies.

Special toxicology: No unexpected binding of CNTO 1275 to normal human tissues was observed in tissue cross-reactivity studies. Intravenous administration of CNTO 1275 at 45 mg/kg on two occasions did not affect or exacerbate asthmatic responses in the monkey asthma model studies.

2.6.6.2 General toxicity

1. Pilot intravenous dose tolerance study in cynomolgus monkeys with 12B75 (T-099-003): Three groups of 2 male and 2 female monkeys received intravenous doses of 0 (saline), 9, or 45 mg/kg 12B75 once weekly for 4 weeks. There were no treatment-related effects on mortality, clinical observations, body weights, food consumption, physical examinations (heart rate, respiratory rate, capillary refill time, and body temperature), cardiovascular parameters (ECG, heart rate, and blood pressure), macroscopic findings, or organ weights.

The mean serum 12B75 concentrations observed 2 h post dosing increased from 388 to 950 µg/mL and from 1159 to 2138 µg/mL between the first and fourth dose at 9 and 45 mg/kg, respectively, suggesting accumulation of drug with repeated dosing. Measurement of monkey antibodies against 12B75 was not performed in this study.

2. Multiple intravenous dose toxicity study with 12B75 in cynomolgus monkeys (T-099-004): Three groups of 5 male and 5 female cynomolgus monkeys received intravenous doses of 0 (saline), 9, or 45 mg/kg 12B75 (lot 052189) on Days 1, 8, 15, and 22. The animals were euthanized on Day 29 (three/sex/group) or Day 59/60 (two/sex/group). There were no treatment-related effects on mortality, clinical signs, body weight, food consumption, body temperature, indirect blood pressure, electrocardiograms, physical and ophthalmic evaluations, coagulation, serum chemistry, organ weight, and macroscopic or histopathologic evaluations.

At Day 28, when compared to the control group, the relative lymphocyte counts were significantly decreased (Table 8. *The actually doses of 12B75 tested in this study were 0, 9, and 45 mg/kg in the tables.*) and the absolute and relative (Table 9) polymorphonuclear leukocytes were significantly increased in the 45 mg/kg female group. In addition, the two 45 mg/kg female recovery monkeys (Nos. 3104 and 3105) had increases in their relative lymphocyte values and decreases in their polymorphonuclear leukocyte values compared to their Day 28 values, suggesting that the potential effect to the lymphocytes and polymorphonuclear leukocytes observed on Day 28 did not worsen and had recovered. Immunotoxicity evaluations revealed no 12B75-related differences across groups in lymphoproliferative responses to T-cell mitogen stimulation (Con A and PHA). In addition, lymphocyte subset analyses (CD2+, CD4+, CD8+, CD20+, and CD14+) were performed on frozen PBMCs collected on Day

28 from all study animals. All lymphocyte subsets for all dose groups were considered to be within normal ranges observed for cynomolgus monkeys.

Text Table 8
Summary of Relative Lymphocyte Counts in Females (%)

Dose	Animal No.	Day -15	Day 9	Day 28	Day 58
0 mg/kg	1101	50	64 (+14)	75 (+25)	
	1102	41	67 (+26)	51 (+10)	
	1103	65	63 (-2)	63 (-2)	
	1104	42	61 (+19)	46 (+4)	55
	1105	46	56 (+10)	55 (+9)	43
	Mean	48.8	62.2 (+13.4)	58.0 (+9.2)	
10 mg/kg	2101	47	72 (+25)	63 (+16)	
	2102	54	69 (+15)	53 (-1)	
	2103	34	40 (+6)	27 (-7)	
	2104	65	66 (+1)	61 (-4)	64
	2105	56	44 (-12)	34 (-22)	54
	Mean	51.20	58.20 (+7)	47.6 (-3.6)	
50 mg/kg	3101	55	59 (+4)	36 (-19)	
	3102	42	37 (-5)	7 (-35)	
	3103	29	CS	31 (+2)	
	3104	50	55 (+5)	35 (-15)	41 (-9)
	3105	28	43 (+15)	25 (-3)	69 (+41)
	Mean	40.8	48.5 (+4.75)	↓ 26.80 (-14)	

() = change from pretest Day -15
 CS = clotted sample
 ↓ = significant decrease compared to controls $p \leq 0.05$

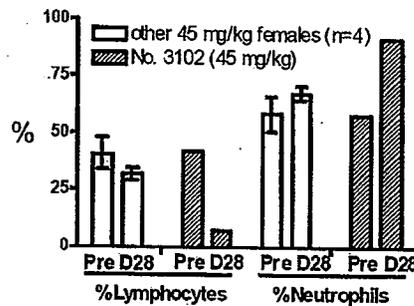
Text Table 9
Summary of Relative Polymorphonuclear Leukocyte Counts in Females (%)

Dose	Animal #	Day -15	Day 9	Day 28	Day 58
0 mg/kg	1101	49	33	24	
	1102	57	29	47	
	1103	31	33	31	
	1104	58	35	48	54
	1105	53	41	35	49
	Mean	49.60	34.20	37.00	
10 mg/kg	2101	53	27	33	
	2102	45	28	40	
	2103	66	60	70	
	2104	30	30	38	33
	2105	43	54	66	44
	Mean	47.40	39.80	49.40	
50 mg/kg	3101	42	37	63	
	3102	58	59	91	
	3103	71	CS	68	
	3104	48	43	63	55
	3105	72	57	75	31
	Mean	58.20	49.00	↑ 72.00	

CS = clotted sample
 ↑ = significant increase compared to controls $p \leq 0.05$

As shown in the next figure, the sponsor further stated that only one female monkey (No. 3102) showed a marked decrease in the percentage of lymphocytes and increases in neutrophil percentages in the Day 28 differentials relative to pre-dose values;

the differentials were relatively unchanged from pre-dose values for the other females in the 45 mg/kg group. No increases in immature cells of neutrophil lineage (band neutrophils, myelocytes, or metamyelocytes) were observed in any animal of this group. No corresponding changes were observed in the male monkeys dosed at 45 mg/kg at any time during the study. Lymphocyte and PMN values in the 45 mg/kg females at Day 9 (2 days after the second dose) and in the 2 recovery animals on Day 58 were comparable to pre-dose values. No changes were noted in the 45 mg/kg females in lymphocyte subset analyses, lymphoproliferative responses to the T-cell mitogens, lymphoid organ weights or histopathology. The sponsor stated that the peripheral blood differential changes observed in female monkeys dosed at 45 mg/kg were considered likely to be related to stress-induced margination of the circulating leukocytes.



Toxicokinetic analyses revealed that 12B75 accumulated in the sera with repeated weekly dosing at 9 or 45 mg/kg. The mean maximum serum concentrations observed in the 9 and 45 mg/kg groups were approximately 540 µg/mL and 3600 µg/mL, respectively in samples obtained 2 h following the last dose. Measurement of monkey antibodies to 12B75 was not performed in this study, due to the high serum concentrations of 12B75.

3. Study title: 26-Week subcutaneous dose toxicity and toxicokinetic study with CNTO 1275 in cynomolgus monkeys with a 12-week recovery period

Key study findings: Treatment at doses of 22.5 or 45 mg/kg CNTO 1275 twice weekly for 26 weeks by subcutaneous injection in monkeys did not cause treatment-related effects on survival, clinical signs, body weight, food consumption, blood pressure, physical, ophthalmic, and electrocardiographic examinations, clinical pathology, macroscopic observations, organ weights, and histopathological examinations. There were no treatment-related differences in histomorphology or CD3 and CD20 immunohistostaining of the lymphoid organs, and no treatment-related effects on functional immune responses measured by KLH analysis and circulating lymphocyte subpopulation. No delayed signs of toxicity were observed in the 12-week recovery groups monkeys. However, one out of 10 monkeys given 45 mg/kg CNTO 1275 for 26 weeks exhibited signs of bacterial enteritis.

Study no.: T-2001-004

Conducting laboratory and location: _____

Date of study initiation: 12-14-2001

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GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: CNTO 1275, Lot No. 5380-39, 95.9-103.1 mg/mL in 0.01M sodium phosphate, 8.5% sucrose, and 0.001% Tween 80, pH 6.0.

Methods

Doses: 0, 22.5, and 45 mg/kg, twice weekly

Species/strain: Cynomolgus monkeys

Number/sex/group or time point (main study): 8/sex/group

Route, formulation, volume, and infusion rate: Subcutaneous injection; in 0.01M sodium phosphate, 8.5% sucrose, and 0.001% Tween 80, pH 6.0; 25 or 50 mL/kg

Satellite groups used for toxicokinetics or recovery: None

Age: 2- 3 Years

Weight (nonrodents only): Males 2.3 - 3.3 kg; females 2.4 - 2.8 kg

Unique study design or methodology (if any): *The actual dose levels were 0, 22.5 and 45 mg/kg in the following table.*

GROUP DESIGNATION AND DOSE LEVELS

Group	No. of Animals ^a		Dose Level	Dose Concentration	Dose Volume
	Male	Female	(mg/kg)	(mg/mL)	(mL/kg)
1 (Control) ^b	8	8	0	0	0.50
2 (CNTO 1275)	8	8	25	100	0.25
3 (CNTO 1275)	8	8	50	100	0.50

a Three animals/sex/group will be sacrificed after 13 weeks of treatment, three animals/sex/group will be sacrificed after 26 weeks of treatment, and two animals/sex/group will be placed on recovery for 12 weeks after 26 weeks of treatment.

b Animals in Group 1 will receive the control article only.

Observation times and results

Mortality: Twice daily. All animals survived to their scheduled sacrifice.

Clinical signs: Cage-side observations were done once daily; detailed observations were done at least once before initiation of treatment, weekly during treatment, and on the day of sacrifice. There were no treatment-related effects.

Physical examinations: Conducted during Weeks -1, 5, 12, 25, 30, and 37. There were no treatment-related effects on heart rates, rectal body temperatures, and respiration rates.

Body weights: Prior to treatment, the first day of treatment, and weekly thereafter. There were no treatment-related effects.

Food consumption: Qualitatively assessed daily. There were no treatment-related effects.

Ophthalmoscopy: Prior to treatment and during Weeks 4, 12, 26, and 37. There were no treatment-related effects.

EKG: Once prior to initiation of treatment and during Weeks 5, 12, 25, 30, and 37. There were no treatment-related effects

Hematology: Blood was collected from all animals during Weeks -3, -2, 4, 13, 26, 30, and 38. Marked, transient lymphocytosis was observed only at Week 4 for several animals, including the controls. The cause of the lymphocytosis was not determined.

Clinical chemistry: Blood was collected from all animals during Weeks -3, -2, 4, 13, 26, 30, and 38. There were no treatment-related effects. Although alanine aminotransferase at Week 13 for females given 45 mg/kg was statistically higher than that for the control group, it was not considered to be caused by CNTO 1275, because these 45 mg/kg CNTO 1275-treated females had notably high values before treatment was initiated, their mean value at Week 13 was only 36% higher than at Week -3, males given 45 mg/kg were not similarly affected, and there were no histopathologic correlates.

Urinalysis: Urine was collected at the time of each scheduled sacrifice. The sponsor stated that because of insufficient sample volume, some tests could not be completed for a few animals. No summary data were included in the study report. No significant adverse effects were noted.

Gross pathology: No CNTO 1275-related macroscopic observations were present at Week 14, 27, or 38. There were no treatment-related effects.

Organ weights: There were no treatment-related effects on organ weights.

Histopathology: There were no treatment-related microscopic findings seen at Weeks 14, 27, and 38. However, one male treated with 45 mg/kg CNTO 1275 had hyperplasia of myeloid cells in the bone marrow (scarified at Week 27). This animal also had acute inflammation in the ileum. This animal had stool abnormalities and lost a substantial amount of body weight during Week 26 just prior to the terminal necropsy. Results from the bacterial culture collected at Week 27 from this animal suggest that this animal had a bacterial enteritis, which is consistent with the weight loss, diarrhea, elevated white cell count, hyperplasia of the bone marrow and inflammation in the gastrointestinal tract. Bacterial culture results from this animal indicated heavy gram-positive staining and mild to moderate gram-negative staining. Additionally, the culture from this animal produced heavy, mixed bacteria, with gram-positive organisms predominating. The Sponsor stated that "The adverse clinical signs observed in this one 45 mg/kg animal were possibly related to CNTO 1275 administration, but were considered unlikely related to treatment with CNTO 1275 because spontaneous repeated bouts of diarrhea are often observed in control and stock cynomolgus monkeys."

There were no treatment-related differences in histomorphology or CD3 and CD20 immunohistostaining of the lymphoid organs between animals in the control group and those administered 22.5 mg/kg or 45 mg/kg CNTO 1275 at any of the time points (interim sacrifice following 13 weeks of treatment, terminal sacrifice following 26 weeks

of treatment, and recovery sacrifice following 26 weeks of treatment and 12 weeks of recovery).

Toxicokinetics: Blood was collected from all animals on Day 1 (pre-dose and approximately 2, 6, 24, 48, and 72 hours post-dose), from all animals pre-dose and approximately 48 hours after the second, third, fourth, and fifth doses (Days 5, 8, 12, and 15, respectively), from all animals during Weeks 11 and 12 pre-dose and approximately 48 hours after the twenty-first, twenty-second, twenty-third, and twenty-fourth doses (Days 71, 75, 78, and 82, respectively), from animals selected for interim sacrifice during Week 13 approximately 2, 6, 24, 48, and 72 hours after the twenty-sixth dose (Day 89), from all animals during Weeks 23 and 24 pre-dose and approximately 48 hours after the forty-fifth, forty-sixth, forty-seventh, and forty-eighth doses (Days 155, 159, 162, and 166, respectively), from animals selected for terminal sacrifice during Week 26 approximately 2, 6, 24, 48, and 72 hours after the fifty-second dose (Day 180), and from all animals weekly from Weeks 27 through 38.

All CNTO 1275 treated animals received extensive exposure to CNTO 1275 throughout the dosing period (see the next table). No CNTO 1275 was detected in animals in the control group. CNTO 1275 exposure (mean C_{max} and $AUC_{(0-t)}$) appeared to increase with the dose over the dose range of 22.5 to 45 mg/kg (see the next table, adapted from the sponsor). A 2-fold increase in dose resulted in an approximately 1.7-fold increase in both mean values of C_{max} and $AUC_{(0-t)}$. Steady state was reached by Week 13. The accumulation ratios after twice weekly dosing of 22.5 and 45 mg/kg CNTO 1275 for 26 weeks were approximately 5.27 and 5.87 for C_{max} and $AUC_{(0-t)}$, respectively. The individual terminal half-life ranged from approximately 9.24 to 17.04 days with a mean value of 12.43 days.

Table 3 Summary of exposure levels (C_{max} and AUC) in cynomolgus macaques following twice weekly SC injections for 13 to 26 weeks

Time	PK Parameter	Dose CNTO 1275	
		22.5 mg/kg	45 mg/kg
First Dose-Day 1	C_{max} ($\mu\text{g/mL}$)	370 \pm 198	673 \pm 367
	$AUC_{(0-72h)}$ ($\mu\text{g}\cdot\text{day/mL}$)	765 \pm 437	1424 \pm 701
26 th Dose-Week 13	C_{max} ($\mu\text{g/mL}$)	1517 \pm 617	2239 \pm 328
	$AUC_{(0-72h)}$ ($\mu\text{g}\cdot\text{day/mL}$)	4108 \pm 798	5976 \pm 966
52 nd Dose-Week 26	C_{max} ($\mu\text{g/mL}$)	1419 \pm 494	2347 \pm 660
	$AUC_{(0-72h)}$ ($\mu\text{g}\cdot\text{day/mL}$)	3661 \pm 1061	6186 \pm 1500

Other:

1. Blood pressure: Conducted during Weeks -3, 5, 12, 25, 30, and 37. There were no treatment-related effects.
2. Monkey anti-CNTO 1275 antibody: Blood was collected from all animals once prior to initiation of treatment and during Weeks 13, 26, 30, 34, and 38. Antibodies to CNTO 1275 were not detected in sera from any monkeys treated with subcutaneous injections of 22.5 or 45 mg/kg/CNTO 1275. Excessive concentrations of circulating serum CNTO 1275 are capable of interfering with the detection of antibodies to CNTO 1275 and the

12-week post-dose recovery observation period did not permit complete washout of CNTO 1275 due to the long half-life of CNTO 1275. Therefore, the result was inconclusive.

For some unknown reason(s), fifteen of 16 monkeys in the saline treatment group appeared to develop antibodies to CNTO 1275. No antibodies to CNTO 1275 were detected in Day 1 pre-treatment sera, whereas antibodies to CNTO 1275 were indicated in all subsequent sera prepared from these animals. The greatest titer observed for each animal ranged from 10 to 1600 with a median peak titer of 320. Titers were low on Day 92 (range of 10 to 200, n=5) appeared to peak at Week 30 (range of 800 to 1600, n=4), and declined through Week 38 (range of 400 to 800, n=4).

3. Flow cytometry: Blood was collected from all animals three times prior to initiation of treatment and during Weeks 1, 4, 13, 26, 30, and 38. Although there were statistically significant changes (increase or decrease) in the percentages of leukocytes staining positive for total lymphocytes (CD45+), for Natural Killer cells (CD16+), and for CD2+, the percentages were within the range of values measured during the pre-treatment period and not dose-dependent. These changes were small and not considered to be related to the treatment of CNTO 1275.

4. Keyhole Limpet Hemocyanin (KLH) Analysis: Conducted prior to initiation of treatment, Day 27, and during Weeks 8, 13, 26, 30, and 38. Positive anti-KLH antibody titers (as defined in the assay validation of 3 SD above the mean OD value of the 1:100 dilution of pre-immune serum) were detected in all study animals. **Marked animal to animal variation in anti-KLH antibody response was observed.** The median anti-KLH titers for all treatment groups in both male and females at Day 27 (13 days after the first KLH injection) were similar (see the next Figure). The median titer for all groups showed a maximum level of anti-KLH at Week 8 [26 days after the second (boost) injection of KLH], for both male and female animals. Center-point titers at Week 8 for female animals were similar for all three dose groups, while titers for male animals showed a decreasing trend from Group 1 through Group 3. The sponsor stated that the lower values for males in Groups 2 and 3 appeared not to be related to the CNTO 1275 dose level due to the variability in measurements within the three groups. Median anti-KLH titers decreased after Week 8 (with the exception of Week 13) for all groups to a similar degree as compared to their Week 8 values. In female animals, the center-point value at Week 13 was similar to the value at Week 8 for Group 1, while values for Week 13 for Groups 2 and 3, were approximately half of the value for Week 8. In males, center-point values at Week 13 were approximately half the values at Week 8 for all groups.

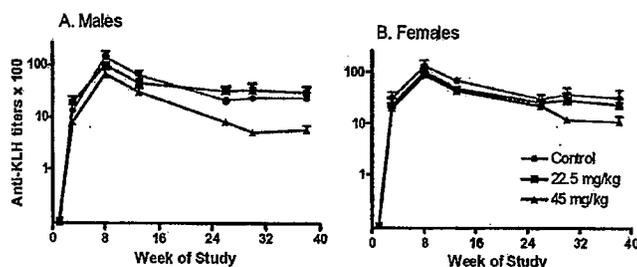


Figure 1: Effect of SC 1275 treatment on neoantigen responses of cynomolgus monkeys to KLH in 26 week toxicity study

5. Bacterial Cultures: Collected from the duodenal fluid of two animals/sex in Groups 1 and 3 during Week 27. No pathogenic colonies were evident in bacterial cultures derived from samples from two control animals/sex and two females and one male given 45 mg/kg, except that bacterial culture results from one male given 45 mg/kg indicated heavy gram-positive staining and mild to moderate gram-negative staining and the culture from this animal produced heavy, mixed bacteria, with gram-positive organisms predominating.

Histopathology inventory (optional)

Study	T-2001-004	
Species	Monkey	
Adrenals	*	X
Aorta		X
Bone Marrow smear		X
Bone (femur)		X
Brain	*	X
Cecum		X
Cervix		
Colon		X
Duodenum		X
Epididymis		X
Esophagus		X
Eye		X
Fallopian tube		
Gall bladder		X
Gross lesions		X
Harderian gland		
Heart	*	X
Ileum		X
Injection site		X
Jejunum		X
Kidneys	*	X
Lachrymal gland		
Larynx		
Liver	*	X

Lungs	*	X
Lymph nodes, axillary		X
Lymph nodes, inguinal		X
Lymph nodes mandibular		X
Lymph nodes, mesenteric		X
Mammary Gland		X
Nasal cavity		
Optic nerves		
Ovaries	*	X
Pancreas		X
Parathyroid		X
Peripheral nerve		
Peyer's patches		X
Pharynx		
Pituitary	*	X
Prostate	*	X
Rectum		X
Salivary gland		X
Sciatic nerve		X
Seminal vesicles		X
Skeletal muscle		X
Skin		X
Spinal cord		X
Spleen	*	X
Sternum		X
Stomach		X
Testes	*	X
Thymus	*	X
Thyroid	*	X
Tongue		X
Tonsils		X
Trachea		X
Urinary bladder		X
Uterus		X
Vagina		X
Zymbal gland		

X, histopathology performed

*, organ weight obtained

6.6.6.3 Genetic toxicology

No genetic toxicology studies have been conducted with CNTO 1275.

2.6.6.4 Carcinogenicity

Nonclinical carcinogenicity studies have not been conducted with CNTO 1275. No tumors or histopathological evidence of pre-neoplastic changes were observed in organs or tissues examined following subcutaneous administration of CNTO 1275 to monkeys at dose levels up to 45 mg/kg twice weekly for 6 months followed by a 3-month post-dose observation period. The sponsor proposed to monitor malignancy in psoriasis patients administered CNTO 1275 as a part of a comprehensive Risk Management Plan for CNTO 1275.

The risk of malignancy in patients is a safety concern for immunosuppressive drugs. CNTO 1275, an antibody to IL-12/IL-23p40, is a selective immunosuppressant. It presumably inhibits the bioactivity of human IL-12 and IL-23 by preventing these cytokines from binding to their IL-12R β 1 receptor protein expressed on the surface of immune cells, which further prevents IL-12 and IL-23 contributions to NK cell activation and CD4+ T cell differentiation and activation. CNTO 1275 is believed to interrupt signaling and cytokine cascades that are central to psoriasis pathology. Since psoriasis is a chronic disease, psoriasis patients may be under treatment for long periods of time. Long term use of CNTO 1275 may lead to increased risk of tumor development in psoriasis patients, particularly in those who have been exposed to other therapies which could increase the risk of tumor development, such as UVB, photodynamic therapy, and other immunosuppressive agents. Although published literature (Fieschi and Casanova, 2003) reported an observation that 73 subjects with genetic deficiencies in IL-12 signaling had not developed cancer, the information available on these subjects is not sufficient to determine whether the subjects have a different cancer risk than the general population. The number of subjects is relatively small and many of the subjects appear to be very young. It is not clear that these subjects were followed for a sufficient duration, given the long latency of malignancy development. In addition, the fact that these subjects were susceptible to infections, presumably due to immunosuppression, may indicate a potential for increased cancer risk.

CNTO 1275 can not be tested in a traditional 2-year rodent study to evaluate its carcinogenic potential, due to its species specific binding to humans and non-human primates. A mouse carcinogenicity study with an analogous antibody to mouse IL-12 may be an approach for carcinogenicity risk evaluation. Available scientific literature on animal studies suggests a potential malignancy hazard is associated IL-12/IL-23p40 antagonism. IL-12 has been shown to play a critical role in tumor surveillance and host defense in rodents. Recent publications further demonstrated the association between IL-12 deficiency and the susceptibility to UV-induced skin tumors in mice.

Published studies showed that administration of murine IL-12 exerted an anti-tumor effect in mice, which was associated with enhanced anti-tumor activities of T cells and NK cells, induction of IFN- γ production and other cytokines induced-by IL-12, and potential secondary anti-angiogenic activities. In mice, intraperitoneal injection of murine IL-12 five times per week for 3 weeks reduced experimental pulmonary metastases of B16F10 melanoma cells, inhibited subcutaneous growth of established melanoma, reticular, and renal cell carcinomas, and increased survival time of tumor bearing mice in these models (Brunda et al, 1993). Sixteen weekly injections of IL-12 blocked carcinogenic progression in a mouse HER-2/neu oncogene-dependent mammary carcinogenesis model with established atypical hyperplastic mammary glands (Cifaldi et al, 2001). Intraperitoneal injection of murine IL-12 five days a week with an injection schedule of 3 weeks on and 1 week off for 18 weeks delayed tumor appearance and reduced tumor incidence in mice administered 3-methyl-cholanthrene (MCA) in a mouse MCA tumor promotion model (Noguchi et al., 1996). In addition, modest anti-tumor

activity was noted in clinical trials for human IL-12 at doses which was over 10-fold lower than those tested in mice (Columbo and Trinchieri, 2002).

Data from IL-12/IL-23 knockout (KO) mice and data from studies in which IL-12/IL-23 activity is inhibited using neutralizing antibodies provide further evidence that IL-12/IL-23 contributes to endogenous host defense to neoplasia. Compared to the wild-type mice, IL-12/IL-23p40 KO mice developed UV-induced tumors earlier and more frequently, and tumors generated in IL-12/IL-23p40 knockout mice grew faster *in vivo* and had greater intrinsic invasion potential (Maeda et al, 2006). Similarly, the development of UV-induced tumors was more rapid and the tumor multiplicity and tumor size were significantly greater in IL-12p35 KO mice than the wild-type mice; the incidence of malignant transformation of UVB-induced papillomas to carcinomas was higher in IL-12p35 KO mice in terms of carcinoma incidence; UVB-induced DNA damage in the form of cyclobutane pyrimidine dimers was removed or repaired more rapidly in the wild-type mice than the IL-12p35 KO mice (Meeran et al, 2006). In contrast, one published paper (Langowski et al, 2006) showed that IL-12/23p40 KO mice were resistant to 9,10-dimethyl-1,2-benzanthracene (DMBA)-induced papillomas, although IL-12p35 KO mice showed earlier appearance and developed significantly increased numbers of papillomas induced by DMBA, compared to the wild-type mice. This paper further suggested that "Genetic deletion or antibody-mediated elimination of IL-23 leads to increased infiltration of cytotoxic T cells into the transformed tissue, rendering a protective effect against chemically induced carcinogenesis." However, a dramatically increased tumor incidence was seen in the IL-12p35 or IL-12/IL-23p40 KO mice, compared to the wild-type or IL-23p19 KO mice, after the mice were challenged intradermally with PDV squamous carcinoma cells (Langowski et al, 2006). Furthermore, mice treated with a neutralizing antibody to mouse IL-12/IL-23p40 to deplete both IL-12 and IL-23 had a significantly increased tumor incidence and developed larger, faster-growing tumors after challenge with PDV squamous carcinoma cells; treatment with anti-IL-12/IL-23p40 antibody also led to a marked increase of tumor growth and an increase of metastasis formation in mice bearing tumors formed by EP2 breast cancer cells (Langowski et al, 2006). Consistently, in rats bearing spontaneously regressing AK-5 rat histiocytoma cells, treatment with anti-IL-12 antibody caused the tumor size to be much larger and the animal life span was shorter, compared to the control animals (Rao et al, 1997). Although results from one literature paper suggested that "neutralizing antibodies to IL-12 did not inhibit antitumor efficacy of low dose melphalan in BALB/c mice bearing large MOPC-315 plasmacytoma burden" (Gorelick and Moky, 1995), which may be due to that anti-IL-12 antibody and melphalan work on different pathways, this reviewer believes that additional studies are needed to understand the role(s) of anti-IL-12 antibody on this observation.

In summary, based on a weight of evidence approach, published literature suggests that a potential malignancy hazard is associated with antagonism of IL-12/IL-23p40 in rodents. At this time, this reviewer does not believe that another carcinogenicity study with IL-12/IL23p40-depleted mice will be very informative. Adequate labeling on animal data from literature and post-marketing patient monitoring of malignancy may be sufficient at this time.

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2.6.6.5 Reproductive and developmental toxicology

1. Study title: CNTO 1275: Male Fertility Study in Cynomolgus Monkeys Following Twice-Weekly Subcutaneous Injections

Key study findings: Three groups of 6 male monkeys were administered subcutaneous doses of 0, 22.5, and 45 mg/kg CNTO 1275 twice weekly prior to mating and during the mating period for 13 weeks, followed by a 13-week treatment-free period. No mortality or CNTO 1275-related effects on clinical observations, body weight or food consumption were observed. There were no treatment-related effects on semen color or volume; sperm counts, viability, activity, and morphology; mating behavior based on evaluations of time elapsed until mounting, number of mountings, mounting positions, and ejaculation; and serum inhibin B or testosterone levels. Toxicokinetic analyses confirmed that the expected high levels of systemic CNTO 1275 exposure were attained.

(Reviewer comments: The animal number/group in this study is too small and should have included at least 12 male monkeys/group)

Study no.: T-2005-015 (150-001)

Conducting laboratory and location: _____

Date of study initiation: 10-12-2005

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: CNTO 1275, lot 05CS043 (1090 pre-filled syringes), purity 99.4% by SDS PAGE

Methods

Doses: 0 (0.9% sodium chloride), 22.5, and 45 mg/kg, twice weekly, subcutaneous injection

Species/strain: Cynomolgus monkeys

Number/sex/group: 6 Males of proved fertility/group, 18 females for mating only

Route, formulation, volume, and infusion rate: Subcutaneous injection, 1 mL contained 90 mg CNTO 1275 IgG; 85 mg sucrose, 0.53 mg histidine, 1.37 mg histidine monohydrochloride monohydrate, and 0.04 mg polysorbate 80, pH 6.0; 0.25 or 0.50 mL/kg volume

Satellite groups used for toxicokinetics: None

Study design: Three groups of 6 males were administered 0, 22.5, or 45 mg/kg CNTO 1275 twice weekly by subcutaneous injection for a total of 13-weeks (9 weeks prior to mating and during a 4 week mating period, on Days 0, 3, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, 42, 45, 49, 52, 56, 59, 63, 66, 70, 73, 77, 80, 84, and 87 of dosing, totally 26 times). Upon completion of the dosing phase, study evaluations were continued for an additional 13 weeks (non-treatment/recovery period).

Parameters and endpoints evaluated: Mortality, clinical signs, body weight, food consumption; semen color or volume; sperm counts, viability, activity, and morphology; mating behavior based on evaluations of time elapsed until mounting, number of mountings, mounting positions, and ejaculation; serum inhibin B and testosterone levels.

Results

Mortality: Observed daily. There were no deaths.

Clinical signs: Observed daily. There were no treatment-related effects.

Body weight: Weighed once weekly. There were no treatment-related effects.

Food consumption: Calculated daily. There were no treatment-related effects.

Toxicokinetics: Blood samples were collected from the femoral vein for PK analysis on Day 0 (Pre-dose and 24 hours post-dose), Day 3 (prior to dosing), Day 28 (prior to dosing), Day 56 (prior to dosing), Days 73 (prior to dosing), Day 87 (prior to dosing), 24

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and 72 hours post-dose), and Weeks 1, 2, 3, 6, 8, and 13 after the last dose. Systemic exposure of CNTO 1275 in monkeys increased with dose in the 22.5 and 45 mg/kg groups (see the next table). The increase in C_{max} values was less than dose proportional, 1.48-fold and 1.46-fold following the first and last dose, respectively. At both dose levels, there was an approximately 10-fold increase in the CNTO 1275 C_{max} between the first dosing interval and the last dosing interval. The median terminal elimination half-life of CNTO 1275 was 19.9 and 21.9 days following twice-weekly subcutaneous dosing at 22.5 and 45 mg/kg. Immune response analysis revealed no detectable anti-CNTO 1275 antibodies in the control or CNTO 1275 treated animals.

Table 5. Mean (\pm SD) Pharmacokinetic Parameters Following Subcutaneous Dosing of 22.5 and 45 mg/kg CNTO 1275 to Male Cynomolgus Monkeys

Dose (mg/kg)	C_{max} ($\mu\text{g/mL}$)			T_{max} (day)	AUC(87-178d)		$t_{1/2}$ (day)
	First Dose	Accumulation	Exposure Ratio	Median (Range)	($\mu\text{g}\cdot\text{day/mL}$)	Exposure Ratio	Median (Range)
22.5	262.57 \pm 57.37		N/A	3.00 (1.00, 3.00)			
	2598.54 \pm 702.50	9.90	N/A	1.00 (1.00, 3.00)	65174.02 \pm 34807.11	N/A	19.88 (15.66, 32.49)
45	387.72 \pm 111.96		1.48	3.00 (1.00, 3.00)			
	3781.85 \pm 1322.19	9.75	1.46	1.00 (1.00, 3.00)	86668.58 \pm 37837.40	1.33	21.93 (12.08, 30.79)

Necropsy: No necropsy was performed.

Fertility parameters: Semen analysis was performed a total of 8 times: twice during the acclimation period, during Weeks 4, 9, and 13 of the dosing phase and during Weeks 4, 9, and 13 of the non-treatment phase. There were no CNTO 1275-related changes in semen color or volume, no treatment-related effects on sperm counts, viability (see the next table constructed by this reviewer), activity, and morphology. Mating behavior was evaluated during Weeks 10-13 of dosing; each male was mated with a female for 15 minutes. There were no CNTO 1275 related effects on mating behavior based on evaluations of time elapsed until mounting, number of mountings, mounting positions or ejaculation.

	Time point		Control	22.5 mg/kg CNTO 1275	45 mg/kg CNTO 1275
Sperm viability (%)	Acclimated period	1st time	80.65 \pm 13.59	82.00 \pm 17.71	80.65 \pm 8.21
		2nd time	89.10 \pm 7.14	92.13 \pm 7.54	94.25 \pm 4.79
	Days of dosing	24-27	88.28 \pm 7.05	87.27 \pm 13.57	94.12 \pm 3.07
		59-62	94.91 \pm 1.90	94.23 \pm 2.98	94.62 \pm 3.68
		87-90	90.77 \pm 5.37	93.27 \pm 1.90	90.90 \pm 4.89

	Days of non-treatment	24-27	91.27±7.60	91.73±4.16	92.90±5.72
		59-62	90.70±6.31	90.90±2.35	92.17±3.51
		87-90	88.53±4.31	89.25±4.80	88.53±6.96
Sperm malformation ratio (%)	Acclimated period	1st time	11.98±7.47	11.45±4.62	13.12±3.69
		2nd time	10.85±5.13	9.75±2.18	10.23±4.37
	Days of dosing	24-27	12.52±4.25	9.33±2.74	11.34±3.02
		59-62	11.67±5.38	10.27±1.97	11.98±3.63
		87-90	11.63±4.49	9.90±2.21	9.35±2.57
	Days of non-treatment	24-27	10.10±4.75	11.80±2.05	12.20±6.06
		59-62	12.62±4.38	9.22±6.65	10.70±4.60
		87-90	14.78±6.99	10.63±3.03	13.02±3.60

Others: Blood samples were obtained for measurement of serum testosterone and inhibin B, twice during the acclimation period (Days 0 and 14 of acclimation) and on Days 24, 59, and 87 of dosing, and Weeks 4 (Day 24 of non-treatment), 9 (Day 59 of non-treatment), and 13 (Day 87 of non-treatment) of non-treatment (total: 8 times). CNTO 1275 treatment did not affect serum inhibin B or testosterone levels.

2. Study title: CNTO 3913: Mouse Subcutaneous Female Fertility and General Reproduction Toxicity Study of a Mouse Anti-IL-12/23p40 Monoclonal Antibody

Key study findings: Subcutaneous administration of CNTO 3913 at doses up to 50 mg/kg, twice weekly, did not cause any treatment-related effects on mortality, clinical signs, body weight, estrous cycling, and necropsy in female mice. There were no treatment-related effects on mating parameters (numbers of days in cohabitation, fertility index, mice that mated, and mice with confirmed mating dates during the first or second week of cohabitation), corpora lutea, implantations, viable and nonviable embryos, and percent nonviable embryos per litter. TK analyses confirmed exposure of mice to CNTO 3913 and showed dose proportional increases in exposure and $AUC_{(0-3d)}$ and accumulation of CNTO 3913 following repeated twice weekly subcutaneous dosing. The maternal no-observable-adverse-effect-level (NOAEL) for general toxicity of CNTO 3913 is greater than 50 mg/kg. The NOAEL for CNTO 3913 on fertility in female mice is greater than 50 mg/kg.

Study no.: T-2007-003

Conducting laboratory and location: _____

Date of study initiation: 4-2-2007

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: CNTO 3913 [a rat-mouse chimeric anti-mouse IL-12/23p40 monoclonal antibody (mouse IgG2 α isotype)], lot FV16C07B, 93.5% by SDS-PAGE.

CNTO 1322 [negative control, a chimeric rat-mouse monoclonal antibody (mouse IgG2 α isotype) with no specific reactivity], lot FV16C07A, 94.1% by SDS-PAGE.

Formulation/vehicle: Phosphate buffered saline (PBS), pH 7.2

Methods

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Doses: 0 (PBS), 0 (CNTO 1322), 5, and 50 mg/kg CNTO 3913, twice weekly
Species/strain: Mouse/CD1(ICR)

Number/sex/group: 25 females/group

Route, formulation, volume, and infusion rate: Subcutaneous injection,
formulation was not provided in the study report, 5 mL/kg

Satellite groups used for toxicokinetics: 9 females in the PBS group, 24 females
in the 5 and 50 mg/kg CNTO 3913 groups

Study design: Four groups of females were administered the test article, negative
control article, or the vehicle twice weekly (Monday and Thursday) beginning 15 days
before cohabitation (maximum 21 days) and continuing through gestation day (GD) 7.
Additional satellite groups of females were also treated for toxicokinetics analysis.
Female mice with spermatozoa observed in a smear of the vaginal contents and/or a
copulatory plug in situ were considered to be GD 0 and assigned to individual housing.
All surviving female mice were sacrificed by carbon dioxide asphyxiation on GD 13.

Parameters and endpoints evaluated: Mortality, clinical signs, body weight,
estrous cycling, and necropsy on dams; mating parameters (numbers of days in
cohabitation, fertility index, mice that mated, and mice with confirmed mating dates
during the first or second week of cohabitation), corpora lutea, implantations, viable and
nonviable embryos and percent nonviable embryos per litter.

Results

Mortality: Twice a day. No deaths occurred

Clinical signs: Observed daily before dosage administration and within 60 ±10 minutes
of dosage administration on the first days of dosing and then within two hours after
dosage administration for the rest of the study. Observations were made once daily on
non-dosing days and during the post-dose period and on the day sacrifice occurred. A
bent tail, which is a common clinical observation in this mouse strain, occurred in three
mice in the 5 mg/kg CNTO 3913 group and two mice in the 50 mg/kg CNTO 3913
group. No treatment-related clinical signs were noted.

Body weight: Recorded weekly during the acclimation and pre-dose periods, daily
during the dose and post-dose periods and on the day sacrifice occurred. Compared the
PBS-treated groups, there is a statistically significant increase in body weight or body
weight gain in other groups. However, the increase in body weight is not biologically
significant (up to 5%).

Food consumption: NA

Estrous cycling: Evaluated by examination of vaginal cytology for 14 days before
initiation of dosage administration and for 14 days beginning with the day after the first
administration, and then until spermatozoa were observed in a smear of the vaginal
contents or a copulatory plug was observed in situ during the cohabitation period. The
number of estrous stages per 14 days was comparable among the four dose groups before
the start of administration and during the pre-cohabitation period (see the next table).

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DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG) ^a		0 (PBS CONTROL)	50b	5	50
ESTROUS CYCLING OBSERVATIONS					
MICE EVALUATED	N	25	25	25	25
PREDOSSAGE ESTROUS CYCLING					
ESTROUS STAGES/ 14 DAYS	MEAN±S.D.	2.2 ± 1.1	2.4 ± 0.9	2.2 ± 1.0	2.4 ± 0.9
MICE WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N	7	3	7	6
MICE WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N	1	3	0	3
PRECORRIBITATION ESTROUS CYCLING					
ESTROUS STAGES/ 14 DAYS	MEAN±S.D.	2.5 ± 0.7	2.8 ± 0.7	2.5 ± 0.8	2.8 ± 0.8
MICE WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N	3	1	4	3
MICE WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N	1	2	1	2

a. Dosage occurred twice weekly (Monday and Thursday) beginning 15 days before cohabitation continuing through day 7 of presumed gestation.
 b. Negative control article.

Toxicokinetics: Blood samples were collected from 3 mice treated with PBS at pre-dose, 72 hours after the first dose, and 72 hours after the last dose, from 3 mice treated with 5 or 45 mg/kg CNTO 3913 at pre-dose, 24, 48, and 72 hours after the first dose and at pre-dose, 24, 48, and 72 hours after the last dose. Toxicokinetic analysis confirmed exposure of mice to CNTO 3913. No CNTO 3913 was detected in serum of control mice or mice administered PBS. In mice treated with CNTO 3913, dose-proportional increases in C_{max} and $AUC_{(0-3d)}$ values were observed. There was a 3.7- and 3.3-fold accumulation in CNTO 3913 $AUC_{(0-3d)}$ between the first and last 5 and 50 mg/kg dose, respectively.

Dose mg/kg	C_{max}	T_{max}	$AUC_{(0-3d)}$
	($\mu\text{g}/\text{mL}$)	(day)	($\mu\text{g}\cdot\text{day}/\text{mL}$)
Following First Dose			
5	57.50	2	127.67
50	561.47	3	1259.59
Following Last Dose			
5	169.32	1	476.52
50	1608.52	1	4129.03

Necropsy: There were no treatment-related effects.

Fertility parameters: There were no treatment effects on mating parameters (numbers of days in cohabitation, fertility index, mice that mated, and mice with confirmed mating dates during the first or second week of cohabitation. The litter averages for corpora lutea, implantations, viable and nonviable embryos, and percent nonviable embryos per litter were comparable among the four dose groups. One dam in the 50 mg/kg CNTO 3913 group had a litter consisting of only nonviable embryos.

DOSAGE GROUP DOSAGE (MG/KG) ^a		I 0 (PBS CONTROL)	II 50 ^b	III 5	IV 50
MATING OBSERVATIONS					
MICE IN COHABITATION	N	25	25	25	25
DAYS IN COHABITATION ^c	MEANS ± S.D.	2.9 ± 1.1	3.4 ± 3.3 [23]	2.4 ± 1.2 [23]	3.1 ± 2.7 [23]
MICE THAT MATED	N(%)	25(100.0)	24(96.0)	25(100.0)	25(100.0)
FERTILITY INDEX ^d	N/N (%)	24/25 (96.0)	21/24 (87.5)	23/25 (92.0)	25/25 (100.0)
MICE WITH CONFIRMED MATING DATES	N	25	23	23	23
MATED BY FIRST MALE ^e					
DAYS 1-7	N(%)	25(100.0)	22(95.6)	23(100.0)	22(95.6)
DAYS 8-14	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.3)
MATED BY SECOND MALE ^e					
DAYS 15-21	N(%)	0(0.0)	1(4.3)	0(0.0)	0(0.0)
MICE PREGNANT/MICE IN COHABITATION	N/N (%)	24/25 (96.0)	21/25 (84.0)	23/25 (92.0)	25/25 (100.0)

[] = NUMBER OF VALUES AVERAGED

a. Dosage occurred twice weekly (Monday and Thursday) beginning 15 days before cohabitation continuing through day 7 of presumed gestation.

b. Negative control article.

c. Restricted to mice with a confirmed mating date and mice that did not mate.

d. Number of pregnancies/number of mice that mated.

e. Restricted to mice with a confirmed mating date.

DOSAGE GROUP DOSAGE (MG/KG) ^a		I 0 (PBS CONTROL)	II 50 ^b	III 5	IV 50
MICE TESTED	N	25	25	25	25
PREGNANT	N(%)	24(96.0)	21(84.0)	23(92.0)	25(100.0)
MICE PREGNANT AND CAESAREAN-SECTIONED ON DAY 13 OF GESTATION	N	24	21 ^c	23 ^c	25 ^c
CORPORA LUTEA	MEANS ± S.D.	13.9 ± 1.7	14.5 ± 2.0	14.0 ± 1.7	13.1 ± 2.1
IMPLANTATIONS	MEANS ± S.D.	13.4 ± 1.8	14.4 ± 2.0	13.8 ± 1.5	12.2 ± 4.0
VIABLE EMBRYOS	N MEANS ± S.D.	305 12.7 ± 1.9	288 13.7 ± 2.3	288 12.5 ± 2.7	296 11.8 ± 4.0
NONVIABLE EMBRYOS	N MEANS ± S.D.	18 0.8 ± 1.0	14 0.7 ± 0.9	29 1.3 ± 1.9	8 0.3 ± 0.6
MICE WITH ANY NONVIABLE EMBRYOS	N(%)	11(45.8)	9(42.8)	9(39.1)	7(28.0)
MICE WITH ALL NONVIABLE EMBRYOS	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
MICE WITH VIABLE EMBRYOS	N(%)	24(100.0)	21(100.0)	23(100.0)	24(96.0)
PLACENTAE APPEARED NORMAL	N(%)	24(100.0)	21(100.0)	23(100.0)	24(100.0)
% NONVIABLE EMBRYOS/LITTER	MEANS ± S.D.	5.6 ± 7.4	4.8 ± 6.3	9.6 ± 14.5	6.1 ± 20.0

a. Dosage occurred twice weekly (Monday and Thursday) beginning 15 days before cohabitation continuing through day 7 of presumed gestation.

b. Negative control article.

c. Includes values for mice that did not have a confirmed mating date.

3. Study title: A Study for the Effect of CNTO 1275 on Embryo-fetal Development and on Pre- and Postnatal Development, including Maternal Function in Cynomolgus Monkeys by Twice Weekly Subcutaneous Administration

Key study findings: Three groups of 20 pregnant cynomolgus monkeys were administered subcutaneous injections of 0, 22.5, or 45 mg/kg CNTO 1275 twice weekly from gestation day (GD) 20 to Day 33 after delivery, to evaluate potential adverse effects of CNTO 1275 on the pregnant/lactating female and on development of the conceptus. No dam died during this study and no CNTO 1275-related abnormalities were observed in dams in clinical signs, body weight, food consumption, hematology, or serum biochemistry. Fetal losses occurred in six control animals, six 22.5 mg/kg-treated

animals, and in five 45 mg/kg-treated animals. Neonatal deaths occurred in one 22.5 mg/kg-treated animal and in one 45 mg/kg-treated animal. No CNTO 1275-related abnormalities were observed in the neonates from birth through six months of age in clinical signs, body weight, hematology, or serum biochemistry. There were no treatment-related effects on functional development until weaning, functional development after weaning, morphological development, immunological development, and gross and histopathological examinations of F₁ animals.

Maternal and fetal CNTO 1275 exposure increased with dose from 22.5 to 45 mg/kg following twice-weekly subcutaneous administration to the dams from Day 20 of gestation to Day 33 of lactation. C_{max} values in the dams were 1591 and 3048 µg/mL in the 22.5 and 45 mg/kg groups, respectively. Terminal half-life values in the dams and the fetuses were comparable in both dose groups (18 to 19 days). Steady-state conditions were reached in the dams by Day 76 to Day 104 of gestation. CNTO 1275 was present at low concentrations in breast milk on Days 14 and 24 of lactation and concentration increased with dose (1.43 to 1.64 µg/mL and 3.12 to 3.18 µg/mL in the 22.5 and 45 mg/kg groups, respectively). None of the CNTO 1275-treated dams (treated with 22.5 and 45 mg/kg) were detected as positive for antibodies to CNTO 1275.

Study no.: T-2004-009 / — 27-15)

Conducting laboratory and location: _____

Date of study initiation: 11-5-2004

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: CNTO1275, Lot D03PM7322, 99.1% by SEC-HPLC

Methods

Doses: 0 (PBS), 22.5, and 45 mg/kg, twice weekly

Species/strain: Cynomolgus monkeys

Number/sex/group: 20 Females/group

Route, formulation, volume, and infusion rate: Subcutaneous injection, clinical formulation, 0.25 or 0.5 mL/kg.

Satellite groups used for toxicokinetics: None

Study design: Three groups of 20 pregnant monkeys subcutaneously received 0 (0.9% sodium chloride), 22.5, or 45 mg/kg CNTO 1275 twice weekly from Day 20 of gestation to Day 33 after delivery (on GD 20, 23, 27, 30, 34, 37, 41, 44, 48, 51, 55, 58, 62, 65, 69, 72, 76, 79, 83, 86, 90, 93, 97, 100, 104, 107, 111, 114, 118, 121, 125, 128, 132, 135, 139, 142, 146, 149, 153*, 156*, 160*, 163*, 167* and 170*, * if gestation continues, and LD 1, 5, 8, 12, 15, 19, 22, 26, 29, and 33). After delivery, nursing was observed daily until Day 180 of lactation.

Group Assignment					
Group	Test and Control Article	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)	Dose Volume ^{a)} (mL/kg/day)	Number of Dams (Animal No.)
1	Control	0	0	0.50	20 (10001 – 10020)
2	CNT01275	22.5	90	0.25	20 (10101 – 10120)
3	CNT01275	45	90	0.50	20 (10201 – 10220)

a) Administration volume was calculated on the basis of the most recent individual body weight. The dose volume was calculated to the nearest 0.01 mL.

Parameters and endpoints evaluated: In the F₀ (maternal) generation: viability, clinical signs, body weight, food consumption, clinical pathology (hematology and serum biochemistry), duration of gestation, litter size, pup sex and viability, maternal behavior, toxicokinetics, immune response, and monkey chorionic gonadotropin (mCG), milk analysis. In the F₁ generation: viability, clinical signs of toxicity, body weight, clinical pathology, immunological examination, functional development until weaning (pupil reflex, Preyer reflex, pain reflex, and grip strength), functional development after weaning (electrocardiographic recordings and ophthalmology examinations), morphological development, immunological development, toxicokinetics and immune response, skeletal examinations, organ weight, and gross and histopathological examinations.

Results

F₀ in-life: No dam died during this study. Abortion or premature delivery (stillbirth) occurred in three dams in the saline control group (on Days 27, 54, and 123 of gestation), three dams in the 22.5 mg/kg group (on Days 31, 40, and 48 of gestation), and three dams in the 45 mg/kg group (on Days 27, 30, and 70 of gestation). Fetal death occurred in one dam in the 22.5 mg/kg group (on Day 90 of gestation) and one dam in the 45 mg/kg group (on Day 142 of gestation). Stillbirth occurred in three dams in the saline control group (on Days 155, 157, and 172 of gestation), two dams in the 22.5 mg/kg group (on Days 143 and 154 of gestation) and one dam in the 45 mg/kg group (on Day 162 of gestation).

Placental signs (genital bleeding) were observed in 16, 14, and 16 dams in the control, 22.5, and 45 mg/kg groups, respectively, for 2 to 20 days between Days 12 and 38 of gestation. External genital bleeding was observed in one, two, and two dams in the control, 22.5, and 45 mg/kg groups, respectively, coupled with abortion or fetal death. No animals showed difficulty at delivery in any group. Abnormal nursing behavior was observed in one dam in the saline control group (tip of the tail of the F₁ was bitten off by dam on Day 17 after delivery) and one dam in the 45 mg/kg group (abandoned nursing and fingertips of the F₁ bitten off by the dam on Day 1 after delivery). The lengths of the gestation periods were 142 to 174 days in the saline control group, 146 to 166 days in the 22.5 mg/kg group, and 141 to 170 days in the 45 mg/kg group.

There were no treatment-related effects on abnormalities, body weight, food consumption, hematology, and serum biochemistry (on Days 10 and 149 of gestation).

After ultrasound diagnosis was positive, serum concentration of monkey chorionic gonadotropin (mCG) on Days 18, 20, 21, 23, and 27 of gestation was measured and all animals in all groups showed concentrations greater than the detection limit at least 1 point.

F₀ toxicokinetics and milk analysis: Exposure of the dams to CNTO 1275 was determined by measuring the maternal serum concentration of CNTO 1275 on GD 20 (pre-dose), 21 (24 hours post dosing), 23 (prior to Dose 2), 34 (prior to Dose 5), 48 (prior to Dose 9), 76 (prior to Dose 17), 104 (prior to Dose 25), 118 (prior to Dose 29), 132 (prior to Dose 33), 146 (prior to Dose 37), and on LD 33 (prior to the last dose), 34, 60, 90, 120 and 180 after delivery. Exposure in the dams was also determined in the case of abortion, embryo-fetal death, or at unscheduled necropsy.

The mean \pm SD CNTO 1275 values in the dams following the last 22.5 and 45 mg/kg subcutaneous doses were 1590.52 ± 387.92 and 3048.08 ± 900.33 $\mu\text{g/mL}$, respectively (see Table 12 at the end of this study). Dose proportionality in the dams was determined by comparing trough serum concentrations (on Days 23, 34, 48, 76, 104, 118, 132, 146 of gestation and Day 33 of lactation) between the two dose groups. The ratios of the trough concentrations ranged from 1.78 to 3.05. The mean terminal half-life values were 18.22 ± 3.03 and 19.03 ± 4.23 days, following the last 22.5 and 45 mg/kg subcutaneous doses, respectively. Steady-state conditions appear to have been reached in the dams by GD 76-104 (4-5 times the half-life of CNTO 1275). Additionally, CNTO 1275 was detected in the pretreatment serum samples of two animals in the 22.5 mg/kg group and one animal in the 45 mg/kg group.

Milk samples were collected from all dams in all groups on Days 14 and 28 of lactation (2 days after dosing). CNTO 1275 was present at low levels in breast milk. The mean \pm SD concentrations of CNTO 1275 measured in breast milk were 1.43 ± 0.45 and 1.64 ± 0.48 $\mu\text{g/mL}$ on Days 14 and 28 of lactation, respectively for the 22.5 mg/kg dose group and were 3.12 ± 1.66 and 3.18 ± 1.71 $\mu\text{g/mL}$, respectively for the 45 mg/kg dose group. Compared with serum concentrations in the dams, the CNTO 1275 concentrations in the milk were low.

None of the CNTO 1275 treated animals were detected as positive for antibodies to CNTO 1275. However, three dams in the control group treated with 0.9% sodium chloride were positive for antibodies to CNTO 1275, and the remaining 17 dams were negative for antibodies to CNTO 1275. Among the anti-CNTO 1275 antibody positive animals in this group, the titers of the peak samples were 160, 160, and 1280.

F₀ necropsy: NA

F₁ physical development: Neonatal deaths occurred in one 22.5 mg/kg-treated animal (Day 6 after birth) and in one 45 mg/kg-treated animal (Day 1 after birth). No CNTO

1275-related abnormalities were observed in the neonates from birth through six months of age in clinical signs, body weight, hematology, or serum biochemistry.

Morphological development was assessed in all F₁ animals by measuring head width, distance between the eyes, crown-rump length, tail length, chest circumference, paw length, foot length, and anogenital distance on Days 30, 90, and 180 after birth. There were no CNTO 1275 treatment-related changes.

All surviving F₁ animals were euthanized on Day 180. Necropsies were performed; limited tissues were collected; organ weights and macroscopic evaluations were recorded. Histopathological examination was performed for limited tissues including adrenal glands, brain (cerebrum; diencephalon, parietal lobe, temporal lobe; cerebellum, pons, medulla oblongata), bone marrow (left femur), heart, kidneys, liver, lungs, Peyer's patches (ileum), ovaries, spleen, testes, thymus, uterus, lymph nodes (mesenteric and submandibular), vagina and mammary gland. Gross and histopathological examinations did not reveal CNTO 1275 treatment-related changes.

F₁ functional development: There were no treatment-related effects on functional development until weaning, as measured by pupil reflex, Preyer reflex, pain response, and grip strength evaluated in F₁ animals between Day 30 and Day 40 after birth.

After weaning, functional development was assessed in ophthalmology examinations and in cardiovascular evaluations that were performed between Days 150 and 170 after birth. Cardiovascular evaluations consisted of heart rate measurements and electrocardiography exams in which PR-interval, QRS duration, QT-interval and QTc derived from the wave of lead II. There were no treatment-related effects.

F₁ reproduction: NA

F₁ immunological development: Development of the immune system in the F₁ generation was evaluated by immunophenotyping of peripheral blood lymphocytes; characterizing the distribution of T- and B-cells in bone marrow and lymphoid organs following immunohistochemical staining and by assessing humoral immune responses. Lymphocyte counts were performed and CD3, CD4, CD8 and CD20 positive lymphocytes were enumerated by flow cytometry on Days 62, 92 and 180 after birth. The distribution of T-cells (CD3) and B-cells (CD20) in bone marrow, thymus, spleen, Peyer's patches and lymph nodes from six F₁ animals terminated on Day 180 was characterized by immunohistochemical staining and microscopic evaluation. Humoral immune competence was evaluated by injecting the F₁ animals with 2 mg/kg KLH subcutaneously and 6 Lf (limit of flocculation) tetanus toxoid (TTX) intramuscularly on postpartum Days 140 and measuring anti-KLH and anti-TTX antibodies in blood collected on Days 140, 147, 154, 161 and 168. Cellular immune competence was evaluated by evaluating delayed type hypersensitivity responses to TTX. F₁ animals were sensitized with intracutaneous injections of TTX (50 µL TTX per injection site; 12 injection sites) to the shaved dorsal region on Day 140 and the animals were challenged

on Day 168 with intracutaneous injections of TTX (10 μ L/site at doses of 10, 3, 1 and 0 Lf/mL; 3 sites per dose level into the shaved thoracic region). Challenge sites were observed approximately 24 and 48 hours after challenge, and skin reactions were evaluated based on the size of the edema.

Lymphocyte subsets, and T and B-cell distribution in lymphoid tissues of F₁ monkeys showed no differences across groups. “Evaluations of neoantigen response to KLH showed no adverse effects of CNTO 1275 treatments (see Figure 3)”, and “development of IgG and IgM antibodies to tetanus toxoid were not impaired (see Figure 4).” The sponsor also stated that no effects on delayed type hypersensitivity responses were observed.

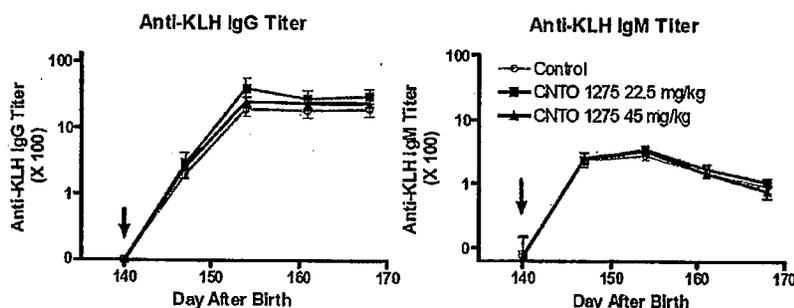


Figure 3: Humoral responses to KLH in F₁ juvenile monkeys from dams treated with CNTO 1275

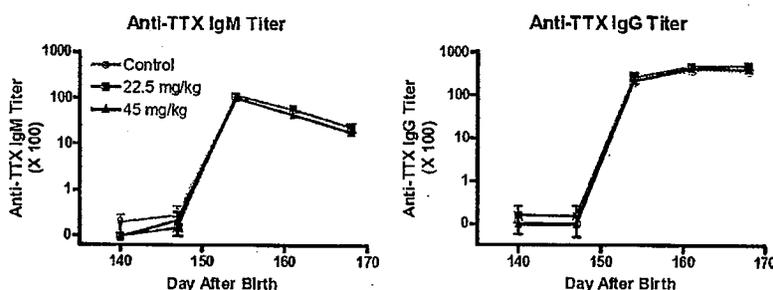


Figure 4: Humoral responses to Tetanus Toxoid in F₁ juvenile monkeys from dams treated with CNTO 1275

F₁ toxicokinetics: Exposure of the respective fetuses to CNTO 1275 was determined by measuring the serum concentration of CNTO 1275 on Day 15, 30, 60, 120, and 180 after birth (AB). The mean serum concentrations of CNTO 1275 appeared to increase with dose in both the dams and fetuses (see the next table). The mean \pm SD concentrations of CNTO 1275 observed in the first serum samples (Day 15 after birth) in the fetuses were 396.35 ± 127.94 and 1230.10 ± 570.24 μ g/mL, respectively for the 22.5 and 45 mg/kg dose groups, respectively. Mean terminal half-life values in the offspring were $19.21 \pm$

2.68 and 19.90 ± 2.75 days, respectively. The sponsor stated that neonates were exposed to maternally derived CNTO 1275 for four to six months after birth.

Table 12: Summary of CNTO 1275 levels ($\mu\text{g/mL}$) in serum of dams and F₁ monkeys and in dam breast milk following treatment of pregnant monkeys from gestation day 50 through lactation day 33

Dose (mg/kg)	Maternal Serum			Breast Milk		Neonatal Serum		
	Cmax* AB 34	AB 60	AB 180	LD 14	LD 28	AB15	AB 30	AB 180
22.5	1590.52 ± 387.92	430.95 ± 111.85	6.2 ± 5.56	1.43 ± 0.45	1.64 ± 0.48	396.35 ± 127.94	235.71 ± 62.20	1.33 ± 0.94
45	3048.08 ± 900.33	1134.80 ± 503.68	20.90 ± 17.96	3.12 ± 1.66	3.18 ± 1.71	1230.10 ± 570.24	588.46 ± 331.76	4.65 ± 4.13

* Following last dose
AB = After Birth Day
LD = Lactation Day

The following was duplicated from the review by Dr. Andrea B. Weir in BB-IND 9590.

“Study T-2002-005. A study of the effects of subcutaneous administration of CNTO 1275 on embryo-fetal development in cynomolgus monkeys [This study was conducted in compliance with GLP at _____”

b(4)

1. Methods: This study was conducted using lot numbers 5380-39, 4841-90, and D01PJ7094. Female cynomolgus monkeys were treated with CNTO-1275 as shown in the table below. The dosing solutions were preformulated in 0.01M sodium phosphate, 8.5% sucrose, and 0.001% Tween 80. The control group received 0.9% saline.

Group	N	Dose		Route	Treatment Days
		mg/kg	mL/kg		
1	12	0	0.5	sc	GD20, 23, 27, 30, 34, 37, 41, 44, 48, and 51
2	12	25	0.25	sc	“
3	12	50	0.5	sc	“

Toxicity was assessed as shown in the table below:

Endpoint	Timing
Clinical signs	GD0 to 19 and GD 51 to cesarean section: 1X daily GD20 to 51: 2X daily
Pregnancy monitoring (fetal heart beat and fetal size were determined by ultrasound examination)	GD25, 30, 40, 50, 60, 70, 80, and 90
Body weight	GD0, 20, 27, 34, 41, 51, 60, 70, 80, 90, and 100
Food consumption	GD7, 14, 19, 20, 23, 27, 30, 34, 37, 41, 44, 48, 51, 55, 58, 62, 69, 76, 83, 90, and 99
Maternal hormones	17 β -estradiol: GD20, 25, 30, 35, 40, 50, 60, 80, and 100 Progesterone: GD20, 23, 26, 29, 32, 35, 40, 50, 60, 80, and 100 Prolactin: GD20, 40, 60, 80, and 100

Endpoint	Timing
Fetal hormones	Luteinizing hormone: GD20, 25, 30, 35, 40, 50, 60, 80, and 100 Cortisol, testosterone, 17B-estradiol, prolactin, luteinizing hormone: At cesarean section (between Days 100 and 102 of gestation)
Maternal immunological examination (CD20, CD3, CD4, and CD8 cells by flow cytometry)	GD20, 35, and 52, and at cesarean section (between Days 100 and 102 of gestation)
Fetal immunological examination (CD20, CD3, CD4, and CD8 cells by flow cytometry)	At cesarean section (between Days 100 and 102 of gestation)
Fetal observation [External observations (Body weight placenta weight, evaluation of external genitalia, and other measurements/observations), visceral examination, organ weights (brain, thymus, heart, lungs, spleen, liver, kidneys, adrenal glands, testes, ovaries, and uterus), skeletal examination, immunohistochemistry (spleen thymus, and mesenteric lymph node were stained for CD20 and CD3)]	At cesarean section (between Days 100 and 102 of gestation)
Maternal toxicokinetics	GD 20 and 51: Prior to treatment and 2 and 24 hours after treatment GD58, 72, and 86: single sample
Fetal toxicokinetics	At cesarean section
Maternal immunogenicity	Pre-dose samples on GD20 and 51 and at cesarean section
Fetal immunogenicity	At cesarean section

2. Results: The number of female fetuses was 1/11, 3/7, and 5/10 for the control, 25 mg/kg and 50 mg/kg groups, respectively. No notable effects were observed for any endpoints other than an increase in abortion/fetal death observed in the low dose during pregnancy monitoring. This finding is discussed below. No abnormalities of external female genitalia were reported.

2.1. Pregnancy monitoring: The incidence of fetal death and abortion is shown in the table below. The historical range for these events at _____ is 0 to 20%. Although the incidence of these events in the 25 mg/kg group exceed the historical range, the sponsor claims that they are not treatment-related because [1] the incidence in the 50 mg/kg group was within the historical control range, and [2] in a previous embryo-fetal developmental study conducted in monkeys with 10 and 50 mg/kg of CNTO 1275 administered iv, the abortion/embryo-fetal death frequencies were within the historical control range.

b(4)

Group	Abortion	Fetal Death	Abortion + Fetal death
0 mg/kg	1/12	0/12	1/12 (8.3%)
25 mg/kg	3/12	2/12	5/12 (41%)
50 mg/kg	0/12	2/12	2/12 (16%)

[Comment: While the sponsor’s reasons for considering the increase in abortion/fetal death to not be treatment-related are valid, they do not allow for a relationship to treatment to be completely dismissed. It is recommended that the finding be included in the Investigator’s Brochure with a statement indicating that a relationship between the finding and treatment is uncertain.]

2.2. Toxicokinetics: Toxicokinetics data are shown in the table below, which was obtained from Centocor’s submission. These data indicate that exposure to CNTO 1275 increased with dose. The terminal half-life, which was not dose dependent, was approximately 9.5 days. CNTO 1275 was detected in maternal and fetal plasma at the time of cesarean section. The fetal plasma levels at cesarean section were 18.40 ± 13.69 and 23.95 ± 11.36 ($\mu\text{g/mL}$) for the 25 mg/kg and 50 mg/kg groups, respectively. The fetal/maternal distribution levels for the 25 and 50 mg/kg groups were 0.47 ± 0.20 and 0.43 ± 0.13 , respectively.

Table 6. Pharmacokinetic Parameters of CNTO 1275 Following 25 and 50 mg/kg SC Doses (Twice Weekly) in Cynomolgus Monkeys (After the Last Dose on Day 51 of Gestation)

	Animal ID	C _{max} ($\mu\text{g/mL}$)	t _{max} (day)	AUC ^a ($\mu\text{g}\cdot\text{day/mL}$)	t _{1/2} (day)	Fetal/Maternal Distribution Ratio
25 mg/kg	10101	1463.00	58.00	36166.79	8.73	0.37
	10103	1465.76	52.00	27045.14	9.50	0.32
	10105	1542.23	52.00	21124.15	8.75	0.52
	10106	1156.68	52.00	15238.66	8.51	NA ^c
	10107	1170.95	52.00	19212.63	9.35	0.35
	10109	1132.56	52.00	14396.14	4.29	0.79
	10112	2476.45	51.00	38949.59	10.76	NA ^c
		N	7	7	7	7
	Mean	1486.80	52.00 ^b	24590.44	8.55	0.47
	SD	468.64	51.00-58.00 ^b	9819.52	2.03	0.20
50 mg/kg	10201	2782.24	52.00	41688.24	8.15	0.49
	10202	3895.93	52.00	56178.67	9.97	NA ^c
	10204	1892.69	52.00	26346.71	8.23	NA ^c
	10205	1708.80	52.00	25458.23	9.68	0.55
	10207	1756.46	51.00	28554.15	9.27	0.24
	10208	3181.31	51.00	51268.55	8.86	NA ^c
	10209	3232.34	51.08	34517.15	9.06	0.54
	10210	2524.87	51.08	37579.35	9.23	NA ^c
	10211	2296.07	52.00	34074.33	8.60	0.35
	10212	2768.66	52.00	35423.33	23.88	NA ^c
		N	10	10	10	10
	Mean	2603.94	52.00 ^b	37108.87	10.49	0.43
	SD	713.58	51.00-52.00 ^b	10159.05	4.74	0.13
Total Mean Value					9.69	0.45

^a AUC was calculated from Day 51.083 to Day 100.

^b The Median and Range, instead of Mean and SD, respectively, were calculated for t_{max}.

^c The fetal blood sample was not collected from animals with even numbers.

NA=Not available

2.3. Immunogenicity: No antibodies to CNTO 1275 were detected in the sera from either dams or fetuses. However, according to the study report these results are inconclusive because “antibody responses could have gone undetected due to the persistence of very

high serum concentrations of CNTO 1275". Evaluation of toxicokinetics data did not reveal any evidence of antibody formation.

3. Conclusion: The design of this study is considered adequate. The no-observed-effect level for maternal and embryofetal toxicity is 50 mg/kg."

(Reviewer comments: The sponsor stated that the actual doses of CNTO 1275 were 22.5 and 45 mg/kg. The serum concentrations of CNTO 1275 determined in TK analysis should be multiplied by 0.9).

"Study T-2001-001. A study of the effects of 12B75 on embryo-fetal development in cynomolgus monkeys [This study was conducted in compliance with GLP at _____ The report for this study was provided with the original submission _____]

b(4)

1. Methods: This study was conducted using lot number 4841-66. Female cynomolgus were treated with CNTO-1275 (12B75) as shown in the table below. Sodium chloride (0.9%) was used as the vehicle. The control group received 0.9% saline.

Group	N	Dose		Route	Treatment Days
		mg/kg	mL/kg		
1	12	0	5	iv (3 mL/min)	GD20, 27, 34, 41, and 51
2	12	10	5	iv (3 mL/min)	"
3	12	50	5	iv (3 mL/min)	"

Toxicity was assessed as shown in the table below.

Endpoint	Timing
Clinical signs	GD0 to 19 and GD 51 to cesarean section: 1X daily GD20 to 48: 2X daily
Pregnancy monitoring (fetal heart beat and fetal size were determined by ultrasound examination)	GD25, 30, 40, 50, 60, 70, 80, and 90
Body weight	GD0, 20, 27, 34, 41, 51, 60, 70, 80, 90, and 100
Food consumption	GD7, 14, 19, 20, 23, 27, 30, 34, 37, 41, 44, 48, 51, 55, 58, 62, 69, 76, 83, 90, and 99
Maternal immunological examination (CD20, CD3, CD4, CD8, and CD34 cells by flow cytometry)	GD20, 35, and 52, and at cesarean section (between Days 100 and 102 of gestation)
Fetal immunological examination (CD20, CD3, CD4, and CD8 cells by flow cytometry)	At cesarean section (between Days 100 and 102 of gestation)
Fetal observation [External observations (Body weight placenta weight, evaluation of external genitalia, and other measurements/observations), visceral examination, organ weights (brain, thymus, heart, lungs, spleen, liver, kidneys, adrenal glands, testes, ovaries, uterus, and mesenteric lymph node0),	At cesarean section (between Days 100 and 102 of gestation)

Endpoint	Timing
skeletal examination, immunohistochemistry (spleen thymus, and mesenteric lymph node were stained for CD20 and CD3)	
Maternal toxicokinetics	GD 20 and 48: Prior to treatment and 2 and 24 hours after treatment At cesarean section: single sample
Fetal toxicokinetics	At cesarean section
Maternal immunogenicity	Pre-dose samples on GD20 and 48 and at cesarean section
Fetal immunogenicity	At cesarean section

2. Results: The animals did not exhibit effects. Specifically, the incidence of abortion/embryonic death was similar across treatment groups, and no abnormalities of the external female genitalia were noted. There were 4/10, 3/11, and 6/10 female fetuses in the control, 10 and 25 mg/kg groups respectively.

2.1. Toxicokinetics: Toxicokinetics data are shown in the table below, which was obtained from Centocor's submission, below. Fetal blood levels for the 10 and 50 mg/kg groups were 9.33 ± 4.77 and 21.27 ± 11.83 ug/mL for the fetuses from 10 and 50 mg/kg groups, respectively.

Table 1. Summary of Serum Concentrations (µg/ml) of CNTO 1275 in Pregnant Cynomolgus Monkeys Following Weekly Doses of Either 10 or 50 mg/kg Body Weight

Monkey #	Dose 1			Dose 5			Accumulation Ratio ^a	
	0.00	2.00	24.00	0.00	2.00	24.00	2.00	24.00
10 mg/kg								
10101							1.90	2.94
10102							1.97	10.35
10103							2.26	2.16
10104							2.25	2.71
10105							1.68	1.87
10106							1.95	2.88
10107							2.04	2.72
10108							NA	NA
10109							1.55	2.70
10110							1.31	2.17
10111							1.79	2.90
10112							1.59	3.33
N	12.00	12.00	12.00	11.00	11.00	10.00	11.00	10.00
MEAN	NA	324.21	196.19	255.41	599.03	476.30	1.84	2.84
SD	NA	43.92	42.78	56.89	151.61	53.57	0.30	0.44
50 mg/kg								
10201							1.47	0.49
10202							1.83	2.13
10203							1.01	2.12
10204							2.78	2.58
10205							NA	NA
10206							2.08	2.45
10207							1.74	2.04
10208							1.93	2.25
10209							1.74	2.62
10210							1.47	1.90
10211							1.95	2.55
10212							2.58	1.39
N	12.00	12.00	12.00	11.00	11.00	10.00	12.00	12.00
MEAN	NA	1823.48	1182.69	1334.89	3278.70	2483.20	1.71	2.20
SD	NA	293.55	279.90	289.95	589.63	378.58	0.72	0.38

b(4)

* and * , outliers, are not included in the statistical analysis.
^a Accumulation Ratio is the ratio of Dose 5 over Dose 1.
 NA: Not available.

2.2. Immunogenicity: No antibodies to CNTO 1275 were detected in any of the animals. However, according to the study report, “it has been shown that persistently high circulating levels of CNTO 1275 could have hindered the detection of a low titer immune response in these sera”. Evaluation of toxicokinetics data did not reveal any evidence of antibody formation.

3. Conclusion: The design of this study is considered adequate. The no-observed-effect level for maternal and embryofetal toxicity is 50 mg/kg.”

(Reviewer comments: The sponsor stated that the actual doses of CNTO 1275 were 9 and 45 mg/kg. The serum concentrations of CNTO 1275 determined in TK analysis should be multiplied by 0.9).

2.6.6.6 Local tolerance

1. Study title: Pharmacokinetics and injection site irritation study of multiple subcutaneous doses of 12B75 mAb in monkeys

Key study findings: Subcutaneous injections of 12B75 in monkeys at a dose of 45 mg/kg twice weekly for 3 weeks did not cause treatment-related effects on mortality, clinical observations, physical examination findings, hematology, coagulation, or serum chemistry. All treatment groups (vehicle control, IGIV, and 12B75) had transient minimal to mild macroscopic signs of local irritation, primarily edema during the dosing period of this study. However, histopathological evaluation of the injection sites revealed no findings in the 12B75 and vehicle control groups.

Study no.: T-2001-003

Conducting laboratory and location: _____

Date of study initiation: 7-25-2001

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: 12B75 (CNTO 1275), Lot 4841-89, 97.1±1.2 mg/mL

Formulation/vehicle: 8.5% sucrose, 10 mM sodium phosphate, 0.001% polysorbate 80, pH 6.0

b(4)

Methods:

Doses: 0 (Vehicle control), 0 (Control article), and 45 mg/kg 12B75

Study design: (The actually used dose of 12B75 was 45 mg/kg)

Group Number	Number of Animals		Test Article	Dosage Level (mg/kg)	Dosage Volume (mL/kg)	Dosing Regimen	Route	Study Completion
	Males	Females						
1	2	2	Vehicle Control	0	0.5	Once on Days 1, 4, 8, 11, 15, and 18	SC	Day 46
2	2	2	Control Article (IGIV, Sandoglobulin®)	3	0.1			
3	2	2	12B75	50	0.5			

SC = Subcutaneous

Results:

There were no treatment-related effects on mortality, clinical observations, physical examination findings, hematology, coagulation, or serum chemistry. All treatment groups (vehicle control, IGIV, and 12B75) showed transient minimal to mild macroscopic signs of irritation, primarily edema, at the injection site during the dosing period of this study. However, histopathological evaluation of the injection sites revealed no findings in the 12B75 (Group 3) and vehicle control (Group 1) groups. Minimal subcutaneous chronic inflammation and minimal subcutaneous eosinophil infiltrates were observed in the injection sites from 2 of 4 animals treated with IGIV (Group 2).

No gender difference was observed in the pharmacokinetic profiles except the terminal slopes of the last dose. The highest value of observed mean maximum serum concentration of 12B75 was 2460 µg/mL after the last dose. The lowest value of the observed mean trough serum concentration was 846 µg/mL on Day 7. The mean

terminal half-lives of 12B75 determined after the last dose were 21.78 days for males and 10.84 days for females. Immune antibody response to 12B75 analyses could not be detected because the high 12B75 serum concentrations interfere with the assay for detecting monkey anti-12B75 antibodies.

2.6.6.7 Special toxicology studies

1. Cross-Reactivity of Biotinylated 12B75 (Anti-human IL-12 Monoclonal Antibody) with Selected Normal Human Tissues (T-098-005, Non-GLP): The test article biotinylated 12B75 human monoclonal antibody against IL-12 was applied to a panel of twenty-two human tissues from two donors at concentrations of 11.4 and 1.14 µg/mL. A direct avidin-biotin-peroxidase method was used. The test article reacted intensely with the positive control mononuclear cells that contained IL-12, but not the KYM-1 D4 negative control cells at both concentrations. The negative control antibody did not bind to either control cells. Specific staining was not observed in any of the tissues. However, nonspecific background staining of some tissues was apparent as variable labeling of collagen and tissue mast cells, and globule leukocytes/eosinophils (i.e., cells with endogenous peroxidase or biotin stores).

2. Study title: Cross-Reactivity of Biotinylated 12B75 (Anti-human IL-12 Monoclonal Antibody) with Normal Human Tissues

Key study findings: The test article biotinylated 12B75 reacted with the positive control cells but did not react with any of a battery of normal human tissues at concentrations of 1.14 and 11.4 µg/mL.

Study no.: T-099-002

Conducting laboratory and location: _____

b(4)

Date of study initiation: July 1999

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: Not listed

Formulation/vehicle: Not stated in the report

Methods

Doses: Concentrations of 11.4 and 1.14 µg/mL 12B75 for a panel of human tissues (37) which were recommended in the guidances. In addition, concentrations of 11.4, 114, and 228 µg/mL of 12B75 and control articles were applied to selected tissues with high vascular perfusion (kidney, liver, and spleen).

Study design: Biotinylated 12B75 was applied to acetone fixed cryosections of normal human tissues from three donors at concentrations of 1.14 and 11.4 µg/mL and binding was detected by an indirect avidin-biotin-peroxidase method using 3, 3'-diamino benzidine tetrachloride as substrate for the peroxidase reaction. Higher concentrations of

biotinylated 12B75 (114 and 228 µg/mL) were also evaluated using kidney, liver and spleen tissue to determine the highest concentrations of antibody that did not cause background staining. Cryosections of activated and brefeldin A-treated adherent human peripheral blood mononuclear cells (PBMC) were used as positive control cells. Cryosections of KYM-1D4 human rhabdomyosarcoma cells served as negative control cells. The negative control antibody was a human biotinylated monoclonal antibody of the same isotype (human IgG1) but different antigenic specificity than 12B75. An additional control was included to demonstrate that the tissues would show reactivity in immunohistochemical assays: additional cryosections were stained in parallel using a peroxidase-conjugated polyclonal rabbit antibody to human β2 microglobulin, a ubiquitously expressed protein.

Results:

Cross-reactivity of biotinylated-12B75 was observed on the positive control cells. No staining of the negative control cells by biotinylated 12B75 was observed. No staining of positive or negative controls or of any normal human tissue was observed with the negative control antibody. Reactivity for β2 microglobulin was shown for all tissues.

Specific staining was not observed in any of the tissues at concentrations of 1.14 and 11.4 µg/mL. However, at the high concentrations (114 and 228 µg/mL), staining was observed in renal tubular epithelium with the test and control articles. Staining was observed with the test article only at concentrations of 114 and/or 228 µg/mL on glomerular epithelial and stromal tissues of the kidneys, in hepatocytes, smooth muscle, bile ducts and nerve tissues of the liver, and in smooth muscle and red pulp stroma of the spleen. The staining was cytoplasmic, generally reduced in intensity with decreasing concentration, and was of a diffuse appearance. The sponsor stated that "Staining such as this is considered background and although it was not observed with the negative control antibody, the differences are likely attributable to structural differences in the non-CDR region of the antibody molecules." Nonspecific background staining of some tissues was apparent as variable labeling of collagen and tissue mast cells, and globule leukocytes/eosinophils at the lower antibody concentrations. Staining occurred with both the test and the control articles.

3. Pilot Study of 12B75 in an Acute Model of Asthma in Cynomolgus Monkeys (Study no. T-099-006): The sponsor stated that a single intravenous dose of 9 or 45 mg/kg 12B75 (CNTO 1275) did not affect pulmonary function or bronchoalveolar lavage (BAL) values when administered to allergic cynomolgus monkeys in the pilot study. However, moderate changes in pulmonary function and cellular composition following antigen challenge were observed in 1 out of 2 monkeys treated with CNTO 1275 at 45 mg/kg on two occasions, four weeks apart.

4. A Study of 12B75 in a Model of Asthma in Cynomolgus Monkeys (T-2000-001)

Two groups of 4 monkeys with positive bronchoconstrictor responses to *Ascaris suum* antigen were administered either intravenous infusions of vehicle (0.9% NaCl solution) or CNTO 1275 at 45 mg/kg 1 h prior to aerosol antigen challenge on two occasions, 4 weeks apart. BAL fluid was collected prior to and approximately 24 hours

after each challenge for evaluation of total cell numbers, morphology, and differential in order to assess the degree of pulmonary inflammation. Aerosol *A. suum* antigen challenge in untreated monkeys resulted in a severe bronchoconstrictor response which was associated with marked increases in R_L and decreases in C_{DYN} followed by pulmonary eosinophilia 24 hours post-challenge. Intravenous administration of CNTO 1275 at 45 mg/kg on two occasions did not cause adverse clinical signs, and did not affect or exacerbate the acute bronchoconstrictor response to antigen challenge.

2.6.7 TOXICOLOGY TABULATED SUMMARY

NA

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

IL-12 and IL-23 cytokines are comprised of a shared p40 subunit and a subunit unique to each cytokine, p36 for IL-12 and p19 for IL-23. Inappropriate expression of IL-12 and IL-23 might be linked to immune-mediated diseases including psoriasis, making the cytokines as targets for immunotherapy for psoriasis. Ustekinumab (CNTO 1275) is a fully human IgG1 monoclonal antibody against the p40 subunit of IL-12 and IL-23. CNTO 1275 binds to the shared p40 protein subunit of human IL-12 and IL-23 and inhibits IL-12 and IL-23 bioactivity by preventing their interaction with their cell surface IL-12R β 1 receptor protein. Through this mechanism of action, CNTO 1275 neutralizes IL-12 and IL-23-mediated cellular responses. CNTO 1275 was shown to have comparable binding and neutralization activity against human and cynomolgus monkey IL-12 and IL-23. Pharmacodynamic activity studies indicated that cynomolgus monkey was the pharmacologically relevant toxicology species for CNTO 1275. In addition, data supported that mice were relevant toxicology species for an analogous antibody against mouse IL-12/IL-23p40, CNTO 3913.

Pharmacokinetic/toxicokinetic studies with CNTO1275 have been conducted in monkeys following single and multiple intravenous or subcutaneous injections at doses ranging from 0.9 to 45 mg/kg. The high dose in toxicity studies with CNTO 1275 is 45 times (based on mg/kg) the highest intended clinical dose in psoriasis patients (approximately 1 mg/kg based on administration of a 90 mg dose to a 90 kg psoriasis patient). Toxicokinetic evaluations confirmed high CNTO 1275 exposure of monkeys in the toxicity studies. Following single and multiple SC administrations, CNTO 1275 was absorbed into the systemic circulation with a mean T_{max} ranging from 2 to 7 days. Mean C_{max} and AUC values increased in an approximately dose-proportional manner. The mean $t_{1/2}$ values ranged from 2 - 3 weeks following multiple subcutaneous injections of CNTO 1275 in monkeys, which were similar to that observed in psoriasis patients. With repeated dosing CNTO 1275 showed 5 to 10-fold accumulation of drug and steady state was achieved in about 13 weeks following twice weekly SC dosing. The mean steady-state C_{max} value following a 45 mg/kg twice-weekly SC dose in monkeys (2347 μ g/mL) was over 100-fold higher than the median C_{max} value following 4-weekly 90 mg SC dosing in subjects with psoriasis (20.3 μ g/mL). Because of the long half-lives, different

treatment regimens, and/or possibly not-enough time-points to measure the serum CNTO 1275 concentrations in monkeys and patients, the comparison between monkeys and patients on systemic exposure levels may not be accurate. However, the serum concentrations attained were well in excess of those required for complete inhibition of IL-12/IL-23 activity based on in vitro activity evaluations and reported serum concentrations of IL-12/IL-23p40 in monkeys and humans.

Anti-CNTO 1275 antibody development was examined in single and repeat dose studies in cynomolgus monkeys. Anti-CNTO 1275 antibodies were detected in single dose studies but not in multiple dose studies, which may have been due to the substantial presence of circulating CNTO 1275 during the observation period or possible immune tolerance induction. However, in the embryo-fetal development and pre- and postnatal development toxicity study, low levels of antibody against CNTO 1275 were detected in 3 out of 20 control (PBS-treated) monkeys and CNTO 1275 was detected in the pretreatment serum samples of 2 out of 20 animals in the 22.5 mg/kg group and 1 out of 20 animals in the 45 mg/kg group.

CNTO 1275 has been tested in cynomolgus monkeys at doses up to 45 mg/kg by intravenous administration weekly for up to 1 month or by subcutaneous administration twice weekly for up to 6 months. Safety pharmacology endpoints were incorporated into the design of toxicology studies. Potential effects on the cardiovascular/respiratory system were evaluated following single and repeated dosing with CNTO 1275 by performing electrocardiograms (ECGs), blood pressure, heart rate, and respiratory rate measurements. Potential effects on the central nervous system were evaluated by daily clinical observations and by measurement of rectal body temperature. There were no treatment-related effects on these safety pharmacology parameters. No adverse findings were noted in ECG recordings in 6-month juvenile monkeys whose respective dams had been exposed to CNTO 1275 during organogenesis resulting in high levels of exposure to CNTO 1275 throughout the 6 month postnatal observation period. No abnormal macroscopic/microscopic cardiac findings were noted in general or developmental toxicity studies.

In the multiple intravenous injection study, three groups of 5 male and 5 female cynomolgus monkeys received intravenous doses of 0 (saline), 9, or 45 mg/kg CNTO 1275 on Days 1, 8, 15, and 22. The animals were euthanized on Day 29 (three/sex/group) or Day 59/60 (two/sex/group). There were no treatment-related effects on mortality, clinical signs, body weight, food consumption, body temperature, indirect blood pressure, electrocardiograms, physical and ophthalmic evaluations, coagulation, serum chemistry, organ weight, and macroscopic or histopathologic evaluations.

In the 26 week study, treatment at doses of 22.5 or 45 mg/kg CNTO 1275 twice weekly for 26 weeks by subcutaneous injection in monkeys did not cause treatment-related effects on survival, clinical signs, body weight, food consumption, blood pressure, physical, ophthalmic, and electrocardiographic examinations, clinical pathology, macroscopic observations, organ weights, and histopathological examinations. There were no treatment-related differences in histomorphology or CD3 and CD20

immunohistostaining of the lymphoid organs, and no treatment-related effects on functional immune responses measured by KLH analysis and circulating lymphocyte subpopulation. No delayed signs of toxicity were observed in the 12-week recovery groups monkeys. However, one out of 10 monkeys administered 45 mg/kg CNTO 1275 for 26 weeks exhibited signs of bacterial enteritis.

No genetic toxicology studies have been conducted with CNTO 1275.

Nonclinical carcinogenicity studies have not been conducted with CNTO 1275. No tumors or histopathological evidence of pre-neoplastic changes were observed in organs or tissues examined following subcutaneous administration of CNTO 1275 to monkeys at doses up to 45 mg/kg twice weekly for 6 months followed by a 3-month post-dose observation period. However, CNTO 1275 is a selective immunosuppressant and the risk of malignancy in patients is a safety concern for immunosuppressive drugs. Immunosuppressive agents have the potential to increase the risk of malignancy. Literature data showed that administration of murine IL-12 exerted an anti-tumor effect in mice that contained transplanted tumors. Literature data furthermore demonstrated that host defense to neoplasia decreased in IL-12/IL-23p40 knockout mice or in mice treated with anti-IL-12/IL-23p40 antibody. Therefore, long-term administration of CNTO 1275 may have the potential to increase the risk of malignancy in patients. At this time, it appears that another 2-year carcinogenicity study with IL-12/IL-23p40-depleted mice will not be very informative. Adequate labeling on animal data from literature and post-marketing patient monitoring of malignancy may be sufficient at this time.

A male fertility study, two embryo-fetal development toxicity studies, and an embryo-fetal development and pre- and post-natal development toxicity study have been conducted in cynomolgus monkeys at doses up to 45 mg/kg CNTO 1275 via subcutaneous or intravenous administration. A female fertility study was conducted in mice using an analogous IL-12/IL-23p40 antibody. In the male fertility study, three groups of only 6 male monkeys (should have included at least 12 male monkeys per group) were administered subcutaneous doses of 0, 22.5, and 45 mg/kg CNTO 1275 twice weekly prior to mating and during the mating period for 13 weeks, followed by a 13-week treatment-free period. No mortality or CNTO 1275-related effects on clinical observations, body weight or food consumption were observed. There were no treatment-related effects on semen color or volume, sperm counts, viability, activity, or morphology, mating behavior based on evaluations of time elapsed until mounting, number of mountings, mounting positions, or ejaculation, and serum inhibin B or testosterone levels. Fertility and pregnancy outcomes were not evaluated in mated females. Toxicokinetic analyses confirmed that the expected high levels of systemic CNTO 1275 exposure were attained.

In the female fertility study in mice using an analogous IL-12/IL-23p40 antibody (CNTO 3913), subcutaneous administration of CNTO 3913 at doses up to 50 mg/kg, twice weekly, beginning 15 days before cohabitation (maximum 21 days) and continuing through gestation day (GD) 7, did not cause any treatment-related effects on mortality, clinical signs, body weight, estrous cycling, and necropsy in female mice. There were no

treatment-related effects on mating parameters (numbers of days in cohabitation, fertility index, mice that mated, and mice with confirmed mating dates during the first or second week of cohabitation), corpora lutea, implantations, viable and nonviable embryos, and percent nonviable embryos per litter. TK analyses confirmed exposure of mice to CNTO 3913 and showed dose proportional increases in exposure and $AUC_{(0-3d)}$ and accumulation of CNTO 3913 following repeated twice weekly subcutaneous dosing.

In the embryo-fetal development and pre- and post-natal development toxicity study, three groups of 20 pregnant cynomolgus monkeys were administered subcutaneous doses of 0, 22.5, or 45 mg/kg CNTO 1275 twice weekly from GD 20 to GD 33 after delivery, to evaluate potential adverse effects of CNTO 1275 on the pregnant/lactating female and on development of the conceptus. No dam died during this study and no CNTO 1275-related abnormalities were observed in dams in clinical signs, body weight, food consumption, hematology, or serum biochemistry. Fetal losses occurred in six control animals (GDs 27, 54, 123, 155, 157, and 172), in six 22.5 mg/kg-treated animals (GDs 31, 40, 48, 90, 143, and 154), and in five 45 mg/kg-treated animals (GDs 27, 30, 70, 142, and 162). Neonatal deaths occurred in one 22.5 mg/kg-treated animal (Day 6 after birth) and in one 45 mg/kg-treated animal (GD 162). The incidence of fetal loss in the treatment groups at the early/middle/late stages of gestation and stillbirth was close to or less than that in the saline control group and within that of historical control values for the test facility, and was therefore considered to be incidental and not related to CNTO 1275 treatment. No CNTO 1275-related abnormalities were observed in the neonates from birth through six months of age in clinical signs, body weight, hematology, or serum biochemistry. Functional development until weaning, as measured by pupil reflex, Preyer reflex, pain response, and grip strength evaluated in F₁ animals between Day 30 and Day 40 after birth, was not affected by CNTO 1275 treatment. Functional development after weaning, as measured by electrocardiographic recordings and ophthalmology examinations conducted at five to six months of age in the F₁ animals, was not affected by CNTO 1275 treatment. Morphological development in the F₁ animals, measured on Days 30, 90, and 180 after birth, showed no CNTO 1275 treatment related changes. Gross and histopathological examinations of F₁ animals at six months of age revealed no CNTO 1275 treatment-related changes. Immunological development in the F₁ generation, as determined by flow cytometry analysis of peripheral blood lymphocytes (CD3, CD4, CD8, and CD20) on Days 62, 92, and 180 after birth; humoral immune competence by antibody response to KLH and tetanus toxoid; and cellular immune competence by delayed-type hypersensitivity reaction to tetanus toxoid between Day 140 and Day 168 after birth, and immunohistological analysis of lymphoid tissues (spleen, thymus, lymph nodes, and bone marrow) on Day 180 after birth showed no CNTO 1275 treatment-related changes.

Maternal and fetal CNTO 1275 exposure increased with dose from 22.5 to 45 mg/kg following twice-weekly subcutaneous administration to the dams from Day 20 of gestation to Day 33 of lactation. C_{max} values in the dams were 1591 and 3048 $\mu\text{g/mL}$ in the 22.5 and 45 mg/kg groups, respectively. Terminal half-life values in the dams and the fetuses were comparable in both dose groups (18 to 19 days). Steady-state conditions were reached in the dams by Day 76 to Day 104 of gestation. CNTO 1275 was present at

low concentrations in breast milk on Days 14 and 24 of lactation and concentration increased with dose (1.43 to 1.64 µg/mL and 3.12 to 3.18 µg/mL in the 22.5 and 45 mg/kg groups, respectively). None of the CNTO 1275-treated dams (treated with 22.5 and 45 mg/kg) were detected as positive for antibodies to CNTO 1275. However, the presence of drug in the samples could have interfered with detection of antibodies to CNTO 1275 in some animals.

CNTO 1275 was also tested in two other embryo-fetal development toxicity studies in which administration of up to 45 mg/kg in pregnant cynomolgus monkeys subcutaneously on GDs 20, 23, 27, 30, 34, 37, 41, 44, 48, and 51 or intravenously on GDs 20, 27, 34, 41, and 51 did not cause any significant adverse developmental effects.

In the local tolerance study, subcutaneous administration of CNTO 1275 in monkeys at a dose of 45 mg/kg twice weekly for 3 weeks did not cause treatment-related effects on mortality, clinical observations, physical examination findings, hematology, coagulation, or serum chemistry. All treatment groups (vehicle control, negative control, and CNTO 1275) had transient minimal to mild macroscopic signs of local irritation, primarily edema during the dosing period of this study. However, histopathological evaluation of the injection sites revealed no findings in the CNTO 1275 and vehicle control groups.

No unexpected binding of CNTO 1275 to normal human tissues was observed in tissue cross-reactivity studies. Intravenous administration of CNTO 1275 at 45 mg/kg on two occasions did not cause adverse clinical signs, and did not affect or exacerbate asthmatic responses in the monkey asthma model studies.

Based on mechanism of action and roles of IL-12/IL23, immunotoxicity evaluations have been incorporated into general toxicity and developmental toxicity studies. However, CNTO 1275 treatment was not associated with immunotoxicity or immunosuppression in monkeys. CNTO 1275 treatment did not cause toxicologically significant effects on functional immune response to a neoantigen or delayed type hypersensitivity responses, did not deplete or otherwise alter lymphocyte subpopulations, and did not reduce ex vivo lymphoproliferative responses to T-cell mitogens. There were no CNTO 1275-related macroscopic observations or adverse effects on organ weights at necropsy, no CNTO 1275-related histopathology findings observed in lymphoid tissues of juvenile, young adult or adult monkeys, and no altered distribution of T and B-lymphocytes in lymphoid tissue.

Unresolved toxicology issues (if any): None

Recommendations:

The following wording is recommended for the labeling on the nonclinical information:

8.1 Pregnancy

Teratogenic Effects: Pregnancy Category B:

There are no adequate and well-controlled studies with TRADENAME in pregnant women. No teratogenic effects were observed in the developmental and reproductive toxicology studies performed in cynomolgus monkeys at doses up to 45 mg/kg ustekinumab, which is 45 times (based on mg/kg) the highest intended clinical dose in psoriasis patients (approximately 1 mg/kg based on administration of a 90 mg dose to a 90 kg psoriasis patient). TRADENAME should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Ustekinumab was tested in two embryo-fetal development toxicity studies.

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No significant adverse developmental effects were noted in either study.

In an embryo-fetal development and pre- and post-natal development toxicity study, three groups of 20 pregnant cynomolgus monkeys were administered subcutaneous doses of 0, 22.5, or 45 mg/kg ustekinumab twice weekly from _____ b(4) delivery. There were no treatment-related effects on mortality, clinical signs, body weight, food consumption, hematology, or serum biochemistry in dams. Fetal losses occurred in six control monkeys, six 22.5 mg/kg-treated monkeys, and five 45 mg/kg-treated monkeys. Neonatal deaths occurred in one 22.5 mg/kg-treated monkey and in one 45 mg/kg-treated monkey. No ustekinumab-related abnormalities were observed in the neonates from birth through six months of age in clinical signs, body weight, hematology, or serum biochemistry. There were no treatment-related effects on functional development until weaning, functional development after weaning, morphological development, immunological development, and gross and histopathological examinations of offsprings by the age of 6 months.

8.3 Nursing Mothers

Ustekinumab is excreted in the milk of lactating monkeys administered TRADENAME. It is not known if ustekinumab is absorbed systemically after ingestion.

_____ b(4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Animal studies have not been conducted to evaluate the carcinogenic or mutagenic potential of [TRADENAME]. Published literature showed that administration of murine IL-12 caused an anti-tumor effect in mice that contained transplanted tumors and IL-12/IL-23p40 knockout mice or mice treated with anti-IL-12/IL-23p40 antibody had decreased host defense to tumors.

A male fertility study was conducted with only 6 male monkeys per group administered subcutaneous doses of 0, 22.5, or 45 mg/kg ustekinumab twice weekly prior to mating

and during the mating period for 13 weeks, followed by a 13-week treatment-free period. Although fertility and pregnancy outcomes were not evaluated in mated females, there were no treatment-related effects on parental toxicity or male fertility parameters.

A female fertility study was conducted in mice using an analogous IL-12/IL-23p40 antibody by subcutaneous administration at doses up to 50 mg/kg, twice weekly, beginning 15 days before cohabitation and continuing through GD 7. There were no treatment-related effects on maternal toxicity or female fertility parameters.

13.2 Animal Toxicology

In a 26-week toxicology study, one out of 10 monkeys subcutaneously administered 45 mg/kg ustekinumab twice weekly for 26 weeks had bacteria infection.

Suggested labeling:

The following wording in the labeling was suggested by the sponsor.

[Redacted]

[Redacted]

b(4)

Reviewer: Jiaqin Yao

BLA No. 125261

b(4)

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

cc list:

APPENDIX/ATTACHMENTS

None

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A
NEW NDA/BLA**

BLA Number: 125261

Applicant: Centocor

Stamp Date: Nov. 28, 2007

Drug Name: CNTO 1275
(Ustekinumab)

NDA/BLA Type: 505(b)(1)

On initial overview of the NDA/BLA application for RTF:

	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		
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		
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 <u>submitted</u> a rationale to justify the alternative route?	X		
7	Has the applicant <u>submitted</u> 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		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A
NEW NDA/BLA**

	Content Parameter	Yes	No	Comment
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.)			NA
11	Has the applicant addressed any abuse potential issues in the submission?			NA
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			NA

**IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION
FILEABLE? Yes**

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

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

Jiaqin Yao

Reviewing Pharmacologist

Jan. 7, 2008

Date

Paul Brown
Team Leader/Supervisor

1-8-08

Date