CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:
BLA 125160/0

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D.
         OND IO
BLA: 125160
Submission date: April 30, 2007
Drug: certolizumab pegol (CIMZIA)
Sponsor: UCB, Inc.
Indication: patients with active Crohn's disease
Reviewing Division: Division of Gastroenterology Products

Comments:
I concur with the Division pharmacology/toxicology recommendation that the non-clinical studies submitted to this BLA provide sufficient information to support the use of CIMZIA for the treatment of patients with active Crohn's disease.

The sponsor proposed a pregnancy labeling category of B and the Division agreed with this category although some minor changes in specific wording describing the relevant studies were suggested by the pharm/tox reviewer. The human antibody is not active in rodent species. A rodent surrogate antibody was tested in rat reproductive and developmental toxicity studies. Essentially no adverse effects were observed. Pregnancy category C is generally appropriate when no animal reproductive toxicology data are available on a drug product. In this case, although the human product has not been tested in animal studies, the surrogate antibody has been tested and found to be nonteratogenic. Assignment of pregnancy category B to CIMZIA is consistent with other therapeutic agents of the same class (antiTNFα) that have a similar set of supporting data. Therefore, I concur with the pregnancy category of B and with the description of the findings in labeling.

Section 13.2 of the labeling describes ———————————————— .ncluding these findings in the labeling does not appear to be critical to the safe use of the product. The issues in section 13.2 appear to be adequately addressed in other portions of the labeling that discuss human information. It is my opinion that section 13.2 of the labeling could be deleted.

Paul C. Brown · 4-18-08

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

PHARMACOLOGIST'S REVIEW OF BLA 125160 (Complete Response to the Complete Response Letter dated December 21, 2006).

Sponsor and Address: UCB, Inc., 1950 Lake Park Drive, Smyrna, GA.

Date of Submission: April 30, 2007

Date of HFD-180 Receipt: April 30, 2007 (electronic submission)

Date of Review: April 2, 2008

Drug: Certolizumab Pegol (CIMZIA)

Category: Recombinant humanized antibody Fab' fragment, with specificity for human TNFα

Submission contents: Complete response to the Complete Response Letter.

Background: Certolizumab pegol is a recombinant humanized antibody Fab' fragment, with specificity for human tumor necrosis alpha (TNFα). The sponsor submitted BLA 125160 on February 28, 2006 for marketing CIMZIA for treatment of patients with Crohn's disease. The BLA was not approved based on the lack of substantial evidence of efficacy. The current submission is a complete response to the Division's Complete Response Letter. The sponsor did not submit any new nonclinical studies under the current submission. All nonclinical studies submitted under the original BLA application were reviewed earlier (October 31, 2006).

Summary and Evaluation:

Under the current submission, no new nonclinical studies with certolizumab pegol were submitted. Nonclinical studies submitted under the original BLA were reviewed earlier, and no safety issues were identified. From a nonclinical standpoint, the BLA application was approvable.

For the labeling of the nonclinical section, the following changes are recommended.

_____ Page(s) Withheld

_____ Trade Secret / Confidential

_____ Draft Labeling

_____ Deliberative Process

Sushanta _for_ _Christine_ _____ 4/2/08

Sushanta Chakder, Ph. D.   Date
Pharmacologist, HFD-180

cc.
BLA
HFD-180
HFD-189/RPM
HFD-180/SChakder
OND IO/AJacobs

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER: 125160
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: February 26, 2006, Electronic Submission
PRODUCT: **Certolizumab pegol (CIMZIA)**
INTENDED CLINICAL POPULATION: **Patients with active Crohn's disease**
SPONSOR: **UCB Inc., 1950 Lake Park Drive, Smyrna, Georgia 30080**

DOCUMENTS REVIEWED: **Electronic submission of the BLA**
REVIEW DIVISION: **Division of Gastroenterology Products (HFD-180)**

PHARM/TOX REVIEWER: **Sushanta Chakder, Ph.D.**
PHARM/TOX SUPERVISOR: **Jasti B. Choudary, B.V. Sc., Ph.D.**
DIVISION DIRECTOR: **Brian Harvey, M.D., Ph.D.**
PROJECT MANAGER: **Marlene Swider**

Date of review submission to Division File System (DFS): 10/31/06

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APPENDIX/ATTACHMENTS ....

EXECUTIVE SUMMARY

I.   Recommendations

A. **Recommendation on approvability:** The sponsor conducted adequate preclinical studies to determine the safety of CDP870. Thus, from a preclinical standpoint, the BLA application is approvable.

B. **Recommendation for nonclinical studies:** None

C. **Recommendations on labeling:** Included in the labeling section of the review.

II.   Summary of nonclinical findings

A.   **Pharmacology:** There is considerable evidence that excessive tumor necrosis factor alpha (TNFα) activity is involved in the pathogenesis of inflammatory bowel disease (IBD). In humans, TNFα is strongly expressed in bowel wall in areas affected by Crohn's disease, and fecal concentrations of TNFα have been shown to reflect clinical severity of the disease. CDP870 (certolizumab pegol) is a recombinant, humanized antibody Fab' fragment with specificity for human TNFα. CDP870 binds to recombinant human TNFα with high affinity (K_d, about 90 pM). It also binds to cynomolgus monkey TNFα with a low affinity (about $1/40^{th}$ of that of human TNFα), but does not bind to rat, mouse, guinea pig and rabbit TNFα. The binding affinity of CDP870 for human TNFα is higher than that of infliximab and adalimumab and lower than that of etanercept. It neutralizes soluble and membrane TNFα, inhibits binding of TNFα to human TNF receptors and inhibits LPS-induced cytokine production in human monocytes. In addition, CDP870 neutralizes the biological activity of human TNFα *in vivo*, in animals in which human TNFα was the physiologically active molecule. It inhibits human TNFα-induced neutrophil accumulation in the peritoneal cavity of mice and pyrexia in rabbits, and chronic inflammatory polyarthritis in transgenic mice. CDP870 does not mediate antibody-dependent cell-mediated cytotoxicity; it is therefore not cytotoxic to TNF-expressing cells. However, no studies were conducted to examine the effects of CDP870 on any inflammatory bowel disease (IBD) animal model.

B. **ADME:** PEGylation of HTNF 40 IgG with the 40 KD PEG increased the elimination half-life ($t_{1/2\alpha}$) of the compound in monkeys following a single i.v. dosing. The plasma exposure level for the 40 KD PEGylated IgG (CDP870) was higher than that for the — KD PEGylated IgG (78% and 30% of the non-PEGylated IgG, respectively). PEGylation also decreased the immunogenicity of the antibody. In rats also, CDP-870 also had longer half-life and higher plasma exposure levels than that of Fab' following an i.v. dose. Following subcutaneous administration of a single dose (3 and 31 mg/kg) to monkeys, plasma concentrations of CDP870 increased with increasing dose, and the maximum plasma concentrations were reached between 24 and 48 hours with a $t_{1/2}$ of about 200 hours (8.4 days). The estimated bioavailability in rats following s.c. administration was 23.5% in males and 33.8% in females. Tissue distribution of CDP870 in rats following an i.v. dose was similar to that of the non-PEGylated form. At 3 hours following administration, the highest level was found in the kidneys, followed by lung, liver and spleen. CDP870 was not an inhibitor of P-glycoprotein. In humans, following subcutaneous (up to 800 mg) or intravenous (up to 10 mg) administration, the C_{max} and AUC values increased with increasing dose, and the peak CDP870 concentrations were attained between 54 and 171 hours following s.c. administration. The terminal elimination half-life ($t_{1/2}$) was approximately 14 days for all doses tested. Following s.c. administration to humans for 12 weeks, anti-CDP870

antibodies were detected in 5% (at 800 mg/4 week dose) to 67% (at 50 mg/4 week dose) of the subjects, depending on the doses administered. The presence of the antibody decreased the C_{max} and AUC by more than 50%.

C. Toxicology: Toxicology studies with CDP870 were conducted in monkeys following administration of single and multiple doses. Intravenous administration of single doses up to 870 mg/kg was well-tolerated without any treatment-related adverse effects. In a 28-day i.v. toxicity study in monkeys, decreased hemoglobin, RBC and packed cell volume was observed immediately after administration of 50, 100 and 400 mg/kg weekly doses. Increased WBC levels were observed in male and female monkeys receiving 10 and 100 mg/kg subcutaneous doses for 13 and 26 weeks. Hematological parameters returned to their normal levels following a 13-week treatment-free recovery period. Histiocytic vacuolation in the hemolymphoreticular tissues (splenic red pulp, medullary sinuses of the mandibular and mesenteric lymph nodes, bone marrow, thymus) were observed in animals treated with the 400 mg/kg dose for 28 days. Vacuolation (foamy macrophages) was also observed in different organs (choroid plexus, adrenals, mesenteric and mandibular lymph nodes, lamina propria of the urinary bladder and endometrial stromal mucosa of the uterus and spleen) in monkeys receiving a 100 mg/kg weekly dose for 13, 26 or 52 weeks. Vacuolation of the hemolymphoreticular tissues may be related to the pharmacological effects of the drug. An increase in the activated partial thromboplastin time (APTT) was observed in monkeys receiving 50 and 100 mg/kg doses. A similar increase in the APTT was also observed in an *ex vivo* study with monkey blood. However, no effects on APTT were observed in animals receiving the drug for 13 or 26 weeks.

The genotoxic potential for CDP870 was assessed in the bacterial reverse mutation assay (Ames assay), the human peripheral blood lymphocyte chromosomal aberration assay and the mouse bone marrow micronucleus assay. CDP870 was not genotoxic in any of these assays.

CDP870 binds to human TNFα with high affinity and cross-reacts with TNFα from non-human primates. However, CDP870 does not recognize TNFα from rodents. So, instead of using CDP870, the sponsor conducted Segment I (fertility and early embryonic development), Segment II (teratogenicity) and Segment III (pre- and post- natal development) using a   ⌐——⌐   anti-TNF antibody (cTN3 —. In the Segment I fertility and early embryonic development study in rats, male and female animals were treated with 20 and 100 mg/kg i.v. doses of cTN3, administered twice weekly. cTN3 had no effects on the fertility and early embryonic development in rats. In the segment II teratogenicity study with cTN3 in rats, the test agent was administered intravenously at 20 and 100 mg/kg doses on gestation days 1 and 4, or gestation days 6, 9, 13 and 16. cTN3 PF was not teratogenic in rats at the doses examined. cTN3 PF had no adverse effects on the pre- and post-natal development in rats at i.v. doses up to 100 mg/kg (bi-weekly).

D. **Nonclinical safety issues relevant to clinical use:** Toxicology studies conducted with CDP870 in cynomolgus monkeys did not reveal any nonclinical safety issues relevant to clinical use of the drug.

PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1   INTRODUCTION AND DRUG HISTORY

BLA number: 125160
Review number: 01
Sequence number/date/type of submission: 000/February 28, 2006/Original.
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: UCB Inc., 1950 Lake Park Drive, Smyrna, GA
Manufacturer for drug substance:

Reviewer name: Sushanta Chakder, Ph.D.
Division name: Division of Gastroenterology Products
HFD #: 180
Review completion date:

Drug:

      Trade name: CYMZIA
      Generic name: Certolizumab pegol
      Code name: CDP870, PHA738144
      Chemical name: gHTNF40Fab' 40 kDa PEG. Certolizumab pegol is a recombinant
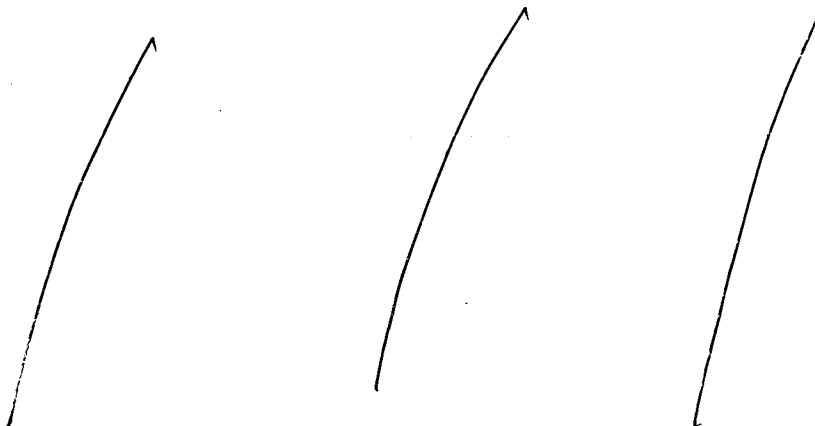humanized antibody Fab' fragment, with specificity for human tumor necrosis factor alpha (TNFα)
      CAS registry number:
      Molecular weight: 90, 000 Da
      . **Structure**:

Figure 2.4:2:1 Diagram of humanized HTNF40   Figure 2.4:2:2 CDP870
IgG and CDP870 Fab'.

Relevant INDs/NDAs/DMFs:
IND 11, 197, Certolizumab pegol, for treatment of Crohn's disease; UCB Inc., Smyrna, GA.

Drug class: TNFα monoclonal antibody.
Intended clinical population: Patients with active Crohn's disease.
Clinical formulation: CIMZIA is supplied as a sterile, white lyophilized powder for reconstitution and subcutaneous administration. Each single-use vial contains approximately 200 mg certolizumab pegol, 100 mg sucrose, 0.9 mg lactic acid, and 0.1 mg polysorbate.
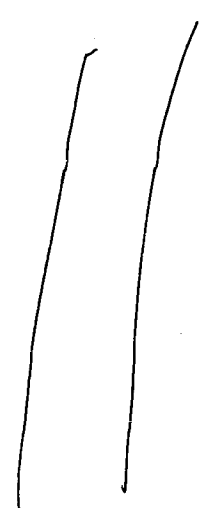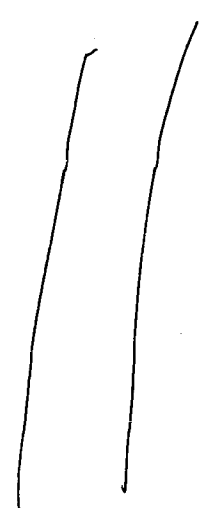
Route of administration: Subcutaneous

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

Studies reviewed within this submission:

STUDY	STUDY #	LOT #	TESTING LAB	PAGE # OF REVIEW
Pharmacology				9
Pharmacokinetics/Toxicokinetics **Absorption** Single i.v. dose pharmacokinetic study in male Cynomolgus monkeys	4000824	--	--	45
Single i.v. dose pharmacokinetic study in male Cynomolgus monkeys	4000825	--	--	46
Single dose subcutaneous pharmacokinetic study in monkeys	40000998 40001080	--	--	49
Distribution Biodistribution of ^{125}I-labelled CDP870 in rats	40000836	--	--	50
Other Pharmacokinetic Studies Pharmacokinetics of ^{125}I-CDP870 in rats	40000881	--	--	53
Pharmacokinetics of CDP870 in rats following i.v. or subcutaneous administration	40001360	--	--	54
Pharmacokinetics of CDP870 in rats following multiple subcutaneous daily administration	40001359	--	--	56

Pharmacokinetic compatibility of CDP870 stages 1,2 and 3 following single s.c. administration to male monkeys	40001393	--	--	58
Pharmacokinetics of PEG-related substances in rat after i.v. administration of CDP870	40001512	--	--	60
Excretion of PEG-containing substances in rat urine after i.v. administration of CDP870	40001513	--	--	61
Inhibition of p-glycoprotein by CDP870 in Caco-2 cells and MDCK cells transfected with ⎯⎯	40001358	--	--	62
Acute Toxicity Study:				
Single i.v. tolerance/toxicity study in the monkey	40000922 40000966	--		66
Repeat-Dose Toxicity Study:				
28-day i.v. toxicity study in the Monkey	40001074 40001020	10012116/ 26		68
13- and 26-week s.c. toxicity study in the monkey	40001545 40001077	98HM464- 00S-CON		74
52-week s.c. toxicity study in monkeys to examine the effects on hematology and morphological parameters	40001535	PCE003		82
Genetic Toxicology Study				
Bacterial reverse mutation (Ames) assay	40000945	97LM202P		88
Chromosomal aberration assay in cultured human peripheral blood lymphocytes	40000980	97LM202P		92
Mouse bone marrow micronucleus assay	40000982	97LM202P		98
Reproductive and Developmental Toxicology:				
Segment I (i.v.) fertility and reproductive performance study in rats with anti-rat TNFα	40001216	10014367/ 53		100

monoclonal antibody equivalent to CDP870				
Segment II (i.v.) embryotoxicity study in rats with anti-rat TNFα monoclonal antibody equivalent to CDP870	40001119	10014166/ 75		105
Segment III (i.v.) pre- and post-natal development study in rats with anti-rat TNFα monoclonal antibody equivalent to CDP870	40001534	10016740/ 05		110
Local Tolerance Single-dose s.c. local tolerance study in the rat	40000999	12842/35		119
Single-dose s.c. local tolerance study in the rat	40001362	⎯ 19964		122
Other Studies: Single-dose range finding i.v toxicity study in rats	40000905	--	--	123
5-day i.v. range-finding toxicity study in the rat	1084/66- D6154	--	--	124

Studies not reviewed within this submission:

Studies related to the validation of ELISA methods for determinations of CDP870 and anti-CDP870 antibodies in biological fluids were not reviewed.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Tumor necrosis factor alpha (TNFα) is a multifunctional cytokine involved in the normal physiological response to infection. There is considerable evidence that excessive TNFα activity is involved in the pathogenesis of inflammatory bowel disease (IBD). In humans, TNFα is strongly expressed in bowel wall in areas affected by Crohn's disease, and fecal concentrations of TNFα have been shown to reflect clinical severity of the disease. CDP870 (certolizumab pegol) is a recombinant, humanized antibody Fab' fragment with specificity for human TNFα. CDP870 was found to bind to recombinant human TNFα with high affinity (K_d, about 90 pM). It was also found bind to cynomolgus monkey TNFα with a low affinity (about 1/40th of that of human TNFα), but did not bind to rat, mouse, guinea pig and rabbit TNFα. The binding affinity of CDP870 for human TNFα was higher than that of infliximab and adalimumab and lower than that

of etanercept. It neutralized soluble and membrane TNFα, inhibited binding of TNFα to human TNF receptors p55 and p75 (types I and II) and inhibited LPS-induced cytokine production in human monocytes. In addition, CDP870 neutralized the biological activity of human TNFα *in vivo*, in animals in which human TNFα was the physiologically active molecule. Intravenously administered CDP870 inhibited human TNFα-induced neutrophil accumulation in the peritoneal cavity of mice and pyrexia in rabbits, and inhibited chronic inflammatory polyarthritis in transgenic mice.

2.6.2.2 Primary pharmacodynamics

—— Analysis of the Affinity of Binding of CDP870 to Recombinant Cynomolgus Monkey TNF-α (Report #40001507):

The affinities of CDP870 for recombinant cynomolgus monkey tumor necrosis factor alpha (cyno TNF-α) and recombinant human TNF-α were determined using a ——— assay. ———
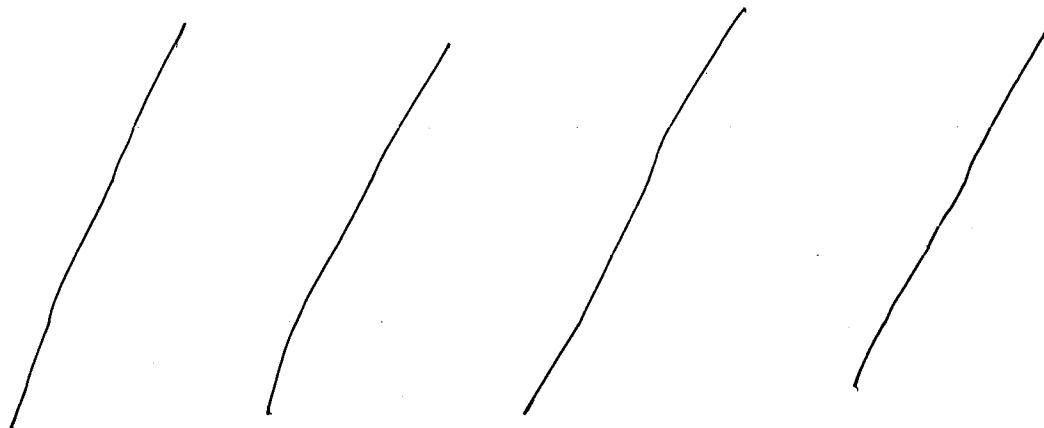
The affinities were measured at or below TNF-α concentration of 7.5 nM, and this concentration was determined from a kinetics study. The affinity of binding of CDP870 to cyno TNF-α was estimated to be 3590 pM, compared to the affinity to human TNF-α of 80 pM to 103 pM. The kinetics of binding of CDP870 to cyno TNF-α showed very fast association (6.5 times faster than human TNF-α) and dissociation rates. The affinities of CDP870 for cyno TNF-α and human TNF-α are shown in the Table below.

Table 11:1 Affinity of CDP870 for cyno TNF-α and human TNF-α

Reference	k_a (M^{-1}s^{-1})	k_d (s^{-1})	K_d (M)	K_d (pM)
human TNF-α	7.84E+05	6.27E-05	7.99E-11	80
cyno TNF-α	4.98E+06	1.79E-02	3.59E-9	3590
human TNF-α	7.49E+05	7.68E-05	1.03E-10	103

Thus, the affinity of CDP870 for recombinant cyno TNF-α was about 1/40th (estimated to be 3590 pM) of that for human TNF-α (approximately 90 pM) using a ——' assay.

__Analysis of the Affinity of CDP870 for Human TNF-α (Report #400001479)__

The affinities of CDP870 for recombinant human TNF-α were determined using a

The affinities of CDP870 for recombinant human TNF-α were determined at human TNF-α concentrations at or below 9.37 nM. The affinity values of CDP for human TNF-α ranged from 71.6 to 102 pM with a mean of 90.2 ± 14.3 pM, which is similar to that found in previous studies. The affinity of CDP870 for recombinant human TNF-α is summarized in the Table below.

TABLE 1: Affinity of CDP870 for human TNFα .

Reference	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_d (M)	K_d pM
10016489/59	1.35E+06	9.65E-05	7.16E-11	71.6
10016489/60	1.15E+06	9.98E-05	8.65E-11	86.5
10016489/62	1.15E+06	1.16E-04	1.01E-10	101
10016489/63	1.22E+06	1.25E-04	1.02E-10	102

__Analytical Characterization of Liquid/Lyophilised Formulations (Report #40001396)__

The study was conducted to determine the characteristics of the original (a liquid   ) and the new lyophilized formulation (SP19973), with respect to functionality (binding TNF-α and/or inhibition of TNF-α binding to human receptors) or higher order structure.

The kinetics of binding of CDP870 to recombinant human TNF-α was determined using   technology.

Inhibition of TNF-α binding to human receptors type I and type II was assessed by a binding immunoassay using the ____

No differences in the binding to recombinant human TNF-α was observed between the ___ and lyophilized (SP19973) samples, as measured by ELISA. The mean bindings for ___ and SP19973 to recombinant human TNF-α were 99% and 104%, respectively. No significant differences in mean R_{max}, k_a, k_d or KD were observed between the two samples. The R_{max}, ka, kd and KD values for the two samples are shown in the sponsor's Table below.

		Rmax	ka	kd	KD
		RU	1/Ms	1/s	pM
___	Average	163.67	2.34E+05	2.02E-05	86.4
	Standard Deviation	5.6	11,648	1.17E-06	7.4
SP19973	Average	167.83	2.37E+05	2.11E-05	89.0
	Standard Deviation	5.3	8.27E+03	1.38E-06	4.9

The inhibition of TNF-α binding to human receptors I and II, samples were tested in multiple plates, days and plate locations. Both samples caused similar inhibitions of TNF-α binding to human TNF-α I and TNF-α II receptors. The mean relative potencies of the two samples were not significantly different for either receptors ($p>0.05$, t-test). The relative potencies for inhibitions of TNF-α binding to human TNF-α I and TNF-α II receptors are summarized in the Table below.

hR1 Receptor, Inhibition by CDP870, Relative Potency

	___	SP19973
Test 1	104	95
Test 2	92	92
Test 3	101	113
Test 4	88	119
Test 5	98	98
Test 6	91	103
MEAN:	96	103
Standard Deviation	6	11
Upper 95% Confidence Interval	102	115
Lower 95% Confidence Interval	89	92

hR2 Receptor, Inhibition by CDP870, Relative Potency

	___	SP19973
Test 1	98	92
Test 2	98	109
Test 3	101	113
Test 4	108	102
Test 5	95	112
MEAN:	100	105
Standard Deviation:	5	9
Upper 95% Confidence Interval	106	117
Lower 95% Confidence Interval	94	94

Thus, the results show there are no differences in the degree and kinetics of binding, inhibitory properties or higher order structure between the two formulations CDP870.

Analysis of the Affinity of Binding of CDP870 and Other Anti-TNF-α Reagents to Human TNF-α (Report #40001508):

The study was conducted to determine the relative affinities of different therapeutic agents for recombinant human tumor necrosis factor alpha (TNF-α). The affinities of Enbrel (etanercept), Humira (adalimumab) and Remicade (infliximab) for TNF-α were determined using a ˙ assay, and compared with that of CDP870 (measured in a separate experiment; Study Report #40001479). ˝

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. The affinities were determined 3 times in 3 separate experiments.

The affinity of CDP870 for human TNF-α was in the range of 71.6-102 pM (mean, 90.2 ± 14.3 pM). The mean affinities of Enbrel, Humira and Remicade were 33.2 ± 6.2, 158.3 ± 23.1 and 228.7 ± 39.1 pM, respectively. Thus, the rank order of affinities for all the therapeutic agents was as follows: Enbrel>CDP870>Humira>Remicade.
The affinities of Enbrel, Humira, Remicade and CDP870 for human TNF-α are shown in the Table below.

Anti-TNF reagent	Affinity (range) pM	Affinity (mean ± SD) pM
Enbrel	28.5-40.3	33.2 ± 6.2
Humira	144.0-185.0	158.3 ± 23.1
Remicade	194.0-271.0	228.7 ± 39.1
CDP870*	71.6-102.0	90.2 ± 14.3

Binding Studies Using ———— to Evaluate CDP870 ———— with Anti-CDP870 Antibodies and TNF-α (Study Report #40001520)

Stability analysis of the liquid formulation of CDP870 has shown that after 1 year, up to of the product could exist as an ———————— In order to determine whether this impacts either affinity of the product for TNF-α, or whether patients were more likely to produce antibodies to ˙ degraded —— CDP870 preparations were subjected to forced degradation studies. Degraded samples were then examined for affinity binding to TNF-α and to patient anti-CDP870 antibodies. Purified CDP870, ———————— CDP870 were compared for binding to TNF-α or anti-CDP870 antibodies using the ———— Binding kinetics with the CDP870 ———— were evaluated with the ————

used in the ELISA assays for measuring circulating levels of CDP870 and with anti-CDP positive plasma samples from the clinical trial (Study #CDP870-004). Samples of CDP870, ⸺

The results showed that ⸺ f CDP870 had similar binding characteristics for TNF-α, suggesting that all ⸺ ; CDP870 were equivalent with respect to binding kinetics with the TNF-α target. The K_{aff} (affinity constant) values of the CDP870 ⸺ and the CDP870 ⸺ detection antibody reagents were all in the 10^{-8} (nanomolar) range for CDP870, ⸺. This indicates that all ⸺ CDP870 may be detected with either reagent. The binding of TNF-α and the anti-CDP870 antibodies specific for the ⸺ of CDP870 with CDP870, the ⸺, are shown in the Table below.

Table 1 Binding of TNFα, the Anti-CDP870 Antibodies specific for the ⸺ s on CDP870, with CDP870 ⸺

Binding Molecule	Ka			Kd			KD			Kaff		
	CDP 870	⸺		CDP 870	⸺		CDP 870	⸺		CDP 870	⸺	
⸺	8.86E+04	8.25E+04	1.26E+05	2.36E-04	8.86E-05	1.36E-04	2.66E-09	1.07E-09	1.08E-09	3.76E+08	9.35E+08	9.26E+08
⸺	1.93E+03	1.43E+04	1.93E+04	1.39E-04	1.54E+04	1.88E-04	7.23E-09	1.07E-08	9.73E-09	1.38E+08	9.35E+07	1.03E+08
TNFa	1.65E+06	1.74E+06	1.23E+06	2.63E-05	5.82E-05	4.98E-05	1.59E-11	3.34E-11	4.04E-11	6.29E+10	2.99E+10	2.48E+10

The results with the antibody positive patient samples showed that the K_{aff} for ⸺ CDP870 were in the same order of magnitude for each sample. Samples from patients treated with the placebo did not show any binding. The anti-CDP870 antibody development in each of the positive patients showed a relatively constant binding affinity for CDP870, ⸺ with time.

Thus, all ⸺ f CDP870 (CDP870, ⸺) had similar binding potencies for human TNF-α. The anti-CDP870 antibody development in positive patients showed a relatively constant binding affinity for all ⸺ CDP870 with time.

In Vitro Bio-assay Evaluation and Analysis of CDP870 with Respect to Neutralization of Human and Non-Human TNF-α (Study Report #40000904)

The ability of CDP870 and hTNF40 (murine monoclonal antibody to TNK-α) to neutralize *in vitro* the TNF-α produced in different animal species were determined to demonstrate species cross reactivity. The species tested were the rat, guinea pig, rabbit, dog, marmoset, baboon, cynomolgus monkey and rhesus monkey. Five (5) ml of whole blood was collected from each species to which lipopolysaccharide (LPS) was added at a final concentration of 1 μg/ml (to produce TNF-α). The blood samples were incubated at room temperature for 4-6 hours and the serum fractions containing TNF-α was collected. For L-929 cell assay, L-929 cell monolayers

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were treated with TNF-α from animal blood to a final concentration of 100 pg/ml in the presence of 1 μg/ml Actinomycin D. Antibodies were added in a dilution series depending on their potency in TNF-α neutralization. Following incubation for 18 hours at 37^0C, cytotoxicity was determined by the uptake of crystal violet.

The changes necessary to produce CDP870 from its parent hTNF40 resulted in no loss of *in vitro* TNF-α neutralizing activity. The IC_{90} values for hTNF40 and CDP870 in neutralizing TNF-α in the L929 assay were 7-10 ng/ml and 4 ng/ml, respectively. Thus, CDP870, (humanized form) had higher TNF-α neutralizing capacity than hTNF40 (murine form) in this assay. The TNF-α neutralizing potencies of CDP870, hTNF40 and CDP571 (a TNF-α antagonist) are shown in the Figure below.
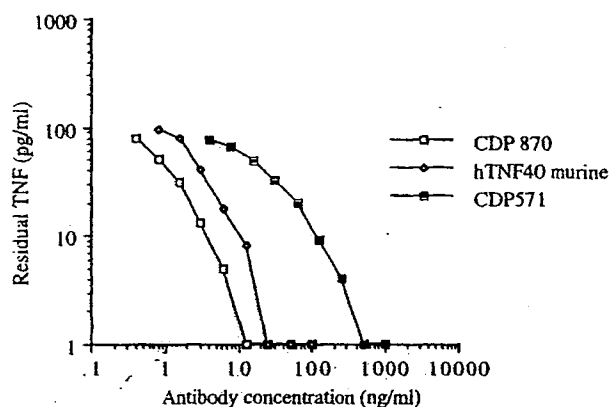
CDP870 was found to cross react weakly with primate TNF-α. The IC_{90} values against TNF-α from rhesus monkey, baboon and cynomolgus monkeys were 3800, 12000 and 10000 ng/ml, respectively. It was about 1000, 3000 and 2500-fold less potent against rhesus monkey, baboon and cynomolgus monkey TNF-α, respectively, when compared with that of human TNF-α. CDP870 showed no cross reactivity with TNF-α produced in rat, guinea pig and rabbit, while only week cross reactivity was seen with the dog TNF-α. The IC_{90} values for CDP870, hTNF40 and CDP571 for human, rhesus monkey, baboon and cynomolgus TNF-α are shown in the Table below.

IC90 for TNFα from Various Species (ng/ml)			
MOLECULE	**HUMAN**	**RHESUS**	**BABOON**	**CYNOMOLGUS MONKEY**
CDP870	4	3800	12000	10000**
HTNF40 murine	9	30000*	23000	50000**
CDP571	100	190	190	100

Table 1. Inhibitory concentration 90 values, calculated
from figures 3, 4 and 5, for CDP870, murine hTNF40 and
CDP571 against TNFα from humans, rhesus monkey,
baboon and cynomolgus monkey. When compared with
human TNFα, CDP870 is 1000, 3000 and 2500 times
less potent against Rhesus, Baboon and Cynomolgus
monkey TNFα respectively.

Thus, CDP870 had weak cross reactivity with cynomolgus monkey, rhesus monkey and baboon TNF-α, less with dog TNF-α, and no cross reactivity with rat, guinea pig and rabbit TNF-α. The week affinity of CDP870 for non-human primate TNF-α suggests that *in vivo* non-human primate efficacy models were not practical for CDP870. However, at high plasma concentrations, sufficient TNF-α-neutralization would occur to use primate as a suitable species for toxicology studies.

Neutralization of Recombinant TNF from Cynomolgus Monkey, Mouse and Rat by CDP870 (Study Report # 40001506)

The ability of CDP870 to neutralize the biological effects of recombinant TNF-α from mouse, rat and cynomolgus monkeys was assessed in a cell-based assay. Cynomolgus monkey TNF-α᾽

Thus, the actual concentration of recombinant cynomolgus monkey TNF-α was not known. Recombinant murine and rat TNF-α was purchased from a commercial source. The assay utilized L929 mouse fibroblast cells that are sensitive to killing by TNF-α. L929 cells were incubated in duplicate in a 96 well plate with TNF-α in the presence of 1 μg/ml actinomycin D and 5 mg/ml methylthiazoletetrazolium (MTT). Each plate contained a titration of CDP870 and a positive control antibody (CDP571). At the end of the incubation, the cells were treated with a solubilization buffer and incubated overnight to dissolve the dye before the plates were read on a plate reader. The data were analyzed using the Genesis software package.

Two identical experiments, each consisting of three plates with the respective TNF-α and anti- TNF-α samples were conducted. CDP870 did not show any detectable inhibition of mouse or rat TNF-α at concentrations up to 500 μg/ml. CDP870 neutralized the biological properties of

recombinant cynomolgus monkey TNF-α with an IC_{90} value of approximately 100 μg/ml. The positive control, CDP571 was about 300 times more potent than CDP870 on cynomolgus monkey TNF-α with an IC_{90} of 300 ng/ml. The neutralization of recombinant cynomolgus monkey TNF-α in the two assays is shown in the figures below.

Figure 12:2 Neutralization of recombinant cynomolgus monkey TNFα measured in a L929 bioassay -Experiment AN1091
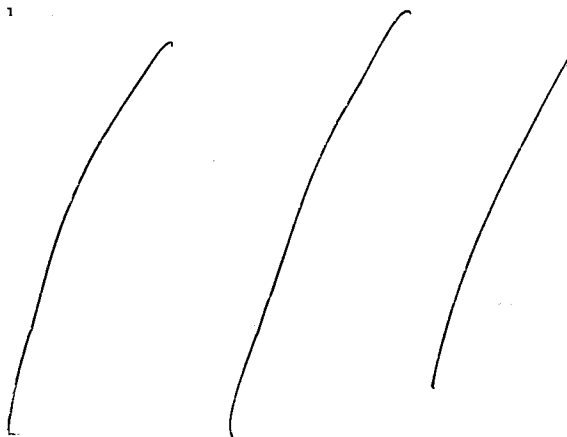
Figure 12:5 Neutralization of recombinant cynomolgus monkey TNFα in a L929 bioassay - Experiment AN1092

    In summary, CDP870 did not neutralize recombinant mouse and rat TNF-α in this assay system, but it neutralized recombinant cynomolgus monkey TNF-α. Thus, rats and mice were not suitable animal species for toxicological evaluation of potential neutralization of TNF-α by CDP870.

Comparison of the Ability of CDP870 and Other Commercially Available Anti-TNF Treatment to Neutralize Soluble TNF-α *In Vitro* (Study Report # 40001532).

The ability of CDP870 to neutralize soluble TNF-α was compared with that of three other agents, Enbrel (etanercept), Humira (adalimumab) and Remicade (infliximab) in the L-929 bioassay system. The TNF-α was produced from an ⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯, and was therefore from a mammalian source.

Enbrel was the most potent followed by CDP870 and Humira and Remicade (both had similar potency) in neutralizing soluble mammalian TNF-α. The concentrations required to neutralize 90% of the bioactivity (IC_{90}) of approximately 100 pg of TNF-α were 0.7, 3.0 and 9.0 and 9.0 ng/ml for Enbrel, CDP870, Humira and Remicade, respectively. The TNF-α neutralizing potencies of different agents are shown in the Figure below.

Figure 12:1 Neutralisation of soluble TNFα by CDP870, Enbrel, Humira and Remicade in the L929 bioassay. Experiment AN1096

Measured soluble TNFα concentration 118.8 pg/mL
Results are plotted as the mean of 3 replicates (± SD)
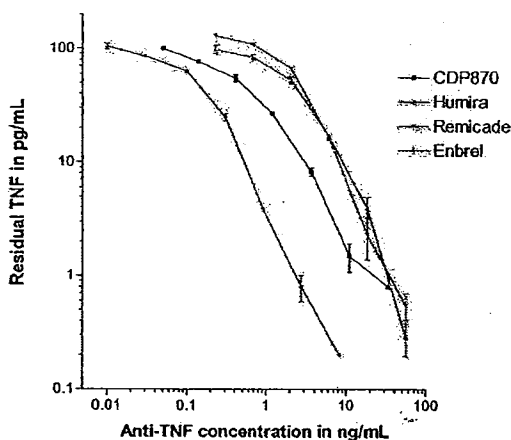
Thus, CDP870 was a potent neutralizer of soluble human TNF-α in this assay system.

Neutralizing Activity of CDP870 and HTNF40 against Recombinant Human TNF-α and Lymphotoxin (Study Report # 40001145)

Five experiments were performed to measure neutralization of lymphotoxin (LT) by CDP870 using the L929 bioassay. L929 mouse fibroblast cells are killed by low concentrations of both TNF-α and LT in the presence of actinomycin D. CDP870 was tested at concentrations up to 1 mg/ml (5 times the maximal concentration expected in the plasma of patients administered CDP870).

In three of five experiments, no neutralization of lymphotoxin by CDP870 was observed, while in two experiments, weak neutralization was observed at CDP870 concentrations of 30 and 100 µg/ml only. The level of CDP870 neutralization of LT in these two experiments was >100,000 times weaker than the equivalent CDP870 neutralization of TNF-α. It was not due to binding specificity of the antibody or the PEG attached to the antibody as there was no neutralization observed in any experiment by the parent murine antibody HTNF40 or the pegylated Fab' controls at concentrations up to 1 mg/ml. The parent murine antibody, HTNF40 and pegylated Fab' controls with differing binding specifications did not neutralize LT at concentrations up to 1 mg/ml. CDP870 showed potent neutralization of human TNF-α with an IC_{90} value of approximately 1.0 ng/ml.

Thus, CDP870 did not neutralize lymphotoxin at clinically relevant concentrations.

Binding to and Neutralization of Membrane TNF-α by CDP870 and Other Anti-TNF Molecules (Study Report # 40001505)

The ability of Enbrel (etanercept), Humira (adalimumab), Remicade (infliximab) and CDP870 to bind to membrane TNF-α was determined by ⎯⎯⎯⎯⎯⎯
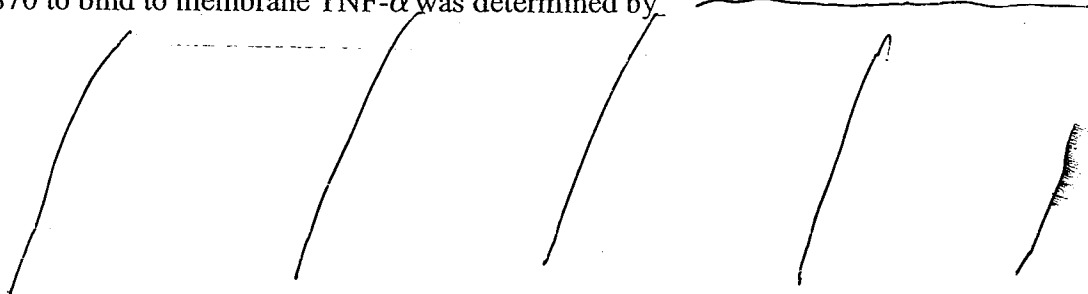
All four anti-TNF agents showed significant binding to membrane TNF-α as determined by ⎯⎯ analysis. For all four agents, a concentration-dependent inhibition of upregulation of luciferase activity resulting from stimulation of membrane TNF-α was observed. No clear differences in the potencies between Remicade, Enbrel, Humira or CDP870 were observed. The binding of the anti-TNF-α agents to ⎯⎯⎯ cells is shown in the Figure below.

Figure 11:10 Binding of the anti-TNFα agents to ——— cells – Experiment AMN1108
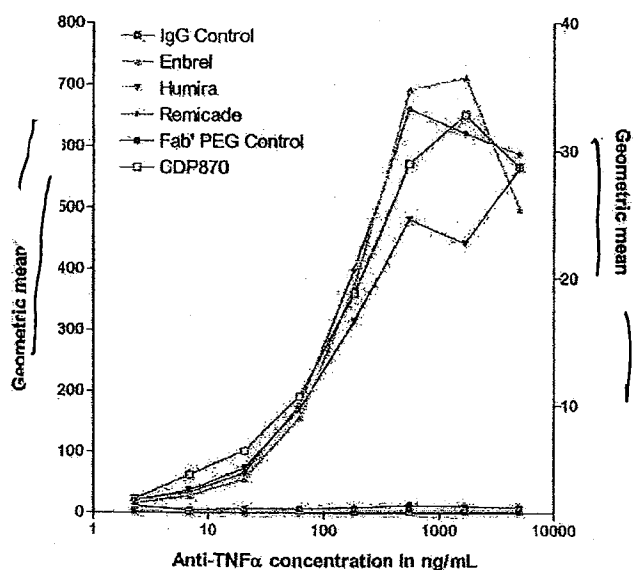
The inhibition of luciferase activity in ——— cells by the anti-TNF agents are shown in the Figure below.

Figure 11:15 Inhibition of luciferase activity from ——— cells mediated by membrane TNFα-expressing cells by anti-TNF agents (Experiment R200534)
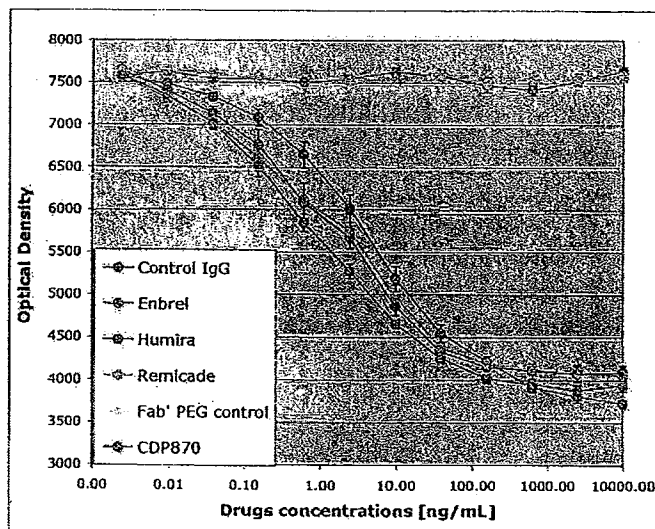
The results suggest that CDP870 effectively neutralizes the biological effects of membrane TNF-α in the assay system used.

Effect of CDP870 and Other Commercially Available Anti-TNF therapies on LPS-induced Cytokine Production by the Human Cell-line Mono Mac 6 (Study Report # 40001533)

The effects of CDP870 on lipopolysaccharide (LPS)-induced cytokines (TNF-α and IL-1β) production was examined in ——— cell line, ———————. The effects of three commercially available anti-TNF agents, Remicade, Humira and Enbrel on LPS-induced cytokines production were also examined. ———— cell cultures were incubated at 37^{0}C in a 96 well plate with CDP870, Remicade, Humira, Enbrel or relevant controls in quadruplicate at a concentration of 100 µg/ml. Following 60 minutes incubation, the cells were washed and incubated with LPS (100 ng/ml) for 4 hours. The cells were then washed, incubated for another 4 hours and the supernatants collected for measurements of TNF-α and IL-1β using enzyme-linked immunosorbent assay (ELISA).

The levels of TNF-α and IL-1β in unstimulated cells were below or close to the limit of detection. TNF-α levels in LPS-stimulated —— cells after incubation with control IgG or control Fab' PEG were approximately 10 ng/ml. The IL-1β levels in LPS-stimulated _——— cells after incubation with control reagents were approximately 4 ng/ml. Pre-incubation of —— cells with CDP870, Remicade and Humira appeared to cause complete inhibitions of the LPS-stimulated production of TNF-α by ——— cells. In contrast, Enbrel was less effective, and exhibited only a partial inhibition of the cytokines production (40-50%). Identical results were observed in two replicate experiments. The mean TNF-α and IL-1β concentrations for the two experiments are shown in the Tables below.

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Table 10:5 Mean TNFα concentrations for experiments R200525 and R200526

Average experiments R200525-R200526						
	without LPS					
Drugs 100µg/mL	Control IgG	Enbrel	Humira	Remicade	Fab' PEG control	CDP870
TNFα (pg/mL)	0.00	0.00	0.00	0.00	0.00	0.00
SEM	0.00	0.00	0.00	0.00	0.00	0.00
	with LPS					
Drugs 100µg/mL	Control IgG	Enbrel	Humira	Remicade	Fab' PEG control	CDP870
TNFα (pg/mL)	10100.00	5399.90	0.00	0.00	9829.15	0.00
SEM	100.00	179.80	0.00	0.00	270.85	0.00

Table 10:6 Mean IL-1β concentrations for experiments R200525 and R200526

Average experiments R200525-R200526						
	without LPS					
Drugs 100µg/mL	Control IgG	Enbrel	Humira	Remicade	Fab' PEG control	CDP870
IL-1β (pg/mL)	22.37	19.65	19.98	20.25	21.38	18.66
SEM	8.41	6.63	5.93	6.50	6.89	5.68
	with LPS					
Drugs 100µg/mL	Control IgG	Enbrel	Humira	Remicade	Fab' PEG control	CDP870
IL-1β (pg/mL)	3947.84	2518.30	23.66	23.20	3927.52	22.97
SEM	262.43	2.15	8.52	8.68	210.48	7.83

Thus, CDP870, Humira and Remicade completely inhibited the LPS-induced production of TNF-α in cultured   cells, while Enbrel only partially inhibited this effect.

Effect of CDP870 and Other Commercially Available Anti-TNF Therapies on LPS-induced Cytokine Production by Human Peripheral Blood Monocytes (Study Report # 40001552)

The effects of CDP870 on lipopolysaccharide (LPS)-induced cytokines (TNF-α and IL-1β) production was examined in human peripheral blood monocytes, and compared with that of three commercially available anti-TNF agents Remicade, Humira and Enbrel. Venous blood from healthy donors was collected and peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation. Monocytes, purified from the PBMC, were incubated at 37^0C in a 96 well plate with CDP870, Remicade, Humira, Enbrel or relevant controls in quadruplicate at a concentration of 100 µg/ml. In separate experiments to assess the concentration response, concentrations ranging from 100 pg/ml to 100 µg/ml were used. Following 60 minutes incubation, the cells were washed and incubated with LPS (100 ng/ml) for 4 hours. The cells were then washed, incubated for another 4 hours and the supernatants collected for measurements of TNF-α and IL-1β using enzyme-linked immunosorbent assay (ELISA).

The levels of TNF-α in unstimulated cells were below 250 pg/ml. The TNF-α concentrations in unstimulated cells pre-treated with CDP870, Remicade or Humira were below 70 pg/ml, which suggests that the basal TNF-α production in the monocytes is inhibited by these agents. The concentrations of IL-1β in unstimulated cells were below 100 pg/ml. TNF-α levels in LPS-stimulated monocytes after incubation with control IgG or control Fab' PEG were approximately 12 ng/ml. The IL-1β levels in LPS-stimulated monocytes after incubation with control reagents were approximately 3 ng/ml. Pre-incubation of the monocytes with CDP870, Remicade and Humira caused almost complete inhibitions of the LPS-stimulated production of TNF-α and IL-1β. In contrast, Enbrel was less effective, and exhibited only a partial inhibition of the cytokines production (17.5% and 30% inhibitions of TNF-α and IL-1β, respectively). The mean IC$_{50}$ values for CDP870, Humira and Remicade were 0.09, 12.5 and 32.5 ng/ml for TNF-α inhibition, and 0.19, 16.5 and 41.25 ng/ml for IL-1β inhibition, respectively. CDP870 was more potent than Humira and Remicade in inhibiting LPS-induced cytokines activation in human peripheral monocytes this experiment. The mean TNF-α and IL-1β concentrations in the monocytes in the absence or presence of different agents are shown in the Tables below.

Table 11:7 Mean TNFα concentrations for experiments R200527, R200528 and R200529

Drugs 100µg/mL	Average TNFα production from monocytes without LPS					
	Control IgG	Enbrel	Humira	Remicade	Fab' PEG control	CDP870
pg/mL	204.3	164.3	44.0	34.6	168.3	28.5
SEM	14.4	8.8	1.4	1.20	22.1	1.8
Drugs 100µg/mL	Average TNFα production from monocytes with LPS					
	Control IgG	Enbrel	Humira	Remicade	Fab' PEG control	CDP870
pg/mL	12103.7	8547.4	38.5	44.8	12133.9	59.6
SEM	161.0	847.9	4.2	0.6	432.9	2.7

Table 11:8 Mean IL-1β concentrations for experiments R200527, R200528 and R200529

Drugs 100µg/mL	Average IL-1β production from monocytes without LPS					
	Control IgG	Enbrel	Humira	Remicade	Fab' PEG control	CDP870
pg/mL	59.3	67.5	57.1	60.0	59.4	43.5
SEM	3.9	3.8	2.0	1.4	6.7	7.5
Drugs 100µg/mL	Average IL-1β production from monocytes with LPS					
	Control IgG	Enbrel	Humira	Remicade	Fab' PEG control	CDP870
pg/mL	2933.2	2330.0	90.7	85.0	2728.1	96.1
SEM	219.6	224.0	1.1	2.1	94.4	3.8

Thus, CDP870, Humira and Remicade almost completely inhibited the LPS-induced TNF-α and IL-1β production in human peripheral blood monocytes, whereas Enbrel only partially inhibited the cytokines production. CDP870 was the most potent among all the anti-TNF agents examined.

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In Vivo Studies:

Comparison of CDP870 Neutralization Potency from Liquid and Lyophilized Formulations Using an *In Vivo* Bioassay in Mice (Study Report # 40001372)

The neutralization potency of three different batches of CDP870 was compared with reference CDP870 using an *in vivo* bioassay in mice. The assay measured the ability of CDP870 (i.v.) to inhibit neutrophil recruitment stimulated by intraperitoneal (i.p.) human recombinant TNF-α. The stimulation of neutrophil accumulation in the peritoneal cavity of male Balb/c mice pre-treated with i.v. CDP870 (3 hr) was determined by flow cytometry at 2 hr following i.p. administration of TNF-α. The optimal dose of human TNF-α for stimulation of neutrophil accumulation in the peritoneal cavity was determined, and the dose of CDP870 to cause 50% inhibition (ED$_{50}$) of the TNF-α-stimulated neutrophil accumulation was determined. The ED$_{50}$ values for CDP870 from different manufacturing processes were compared with that of a reference CDP870 (liquid formulation). Two reference formulations were compared with three test formulations (two lyophilized and one liquid formulation). The manufacturers and the batch numbers of the CDP870 formulations are given in the Table below.

Key Material/ Reagent	Manufacturer	Vial label/ Batch Number	Formulation (manufacturing process)	Storage Condition
Reference CDP870		GDS-15982-117 Lot# BC071403	Liquid formulation	-70°C
Reference CDP870		GDS-17210-030-A-050 Lot#BR042701	Liquid formulation	-70°C
Test CDP870		Vial 0004;A02570-005L01 SP20193	Lyophilised formulation	+4°C
Test CDP870		⎯	Liquid formulation (stage 1)	-70°C
Test CDP870		SP19973	Lyophilised formulation (stage 2)	-70°C

Intravenous CDP870 dose-dependently inhibited human TNF-α-induced neutrophil accumulation in the peritoneal cavity of mice. The mean ED$_{50}$ for 3 separate experiments was 0.052 mg/kg (minimal, 0.003-0.03 mg/kg; maximal, 3 mg/kg). Based on these findings, doses ranging from 0.003 to 3 mg/kg, were used for dose response curves for reference and test CDP870. No significant differences in potencies for inhibition of the TNF-α-induced neutrophil accumulation in the peritoneal cavity were observed between the reference CDP870 and three different batches of test CDP870. ED$_{50}$ comparisons for different test formulations of CDP870 are shown in the Table below.

Table 6. Summary table – comparison of the *in vivo* neutralisation potency of reference CDP870 with 3 batches of test CDP870

Laboratory notebook reference (date)	CDP870 Batches	ED50 (mg/kg)	logED50 (mg/kg)	Residuals normality test	ED50 ratio (Test:Ref)	ED50 ratio 95% CI (upper-lower)	Significant difference between ED50
10016074/58 (18.11.03)	Ref BC071403 Test-A02570-005L01	0.076 0.056	-1.12 -1.254	Pass Pass	1 : 1.36	2.22 - 0.83	No
10016074/69 (24.11.03)	Ref BC071403 Test-A02570-005L01	0.050 0.058	-1.3 -1.238	Pass Pass	1 : 0.87	1.72 - 0.44	No
10016074/80 (27.11.03)	Ref BR042701 Test-/ —	0.052 0.12	-1.281 -0.93	Pass Pass	1 : 0.45	1.3 - 0.15	No
10016074/92 (11.12.03)	Ref BR042701 Test-SP19973	0.041 0.015	-1.387 -1.824	Pass Pass	1 : 2.74	12.9 - 0.58	No

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The comparisons of the test CDP870 with the reference sample are shown in the Figures below.

Figure 3. 10016074/58. Comparison of reference CDP870 (BC071403, liquid) with CDP870 (A02570-005L01, lyophilised).
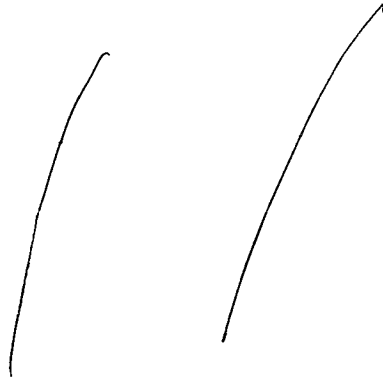
Figure 4. 10016074/69. Comparison of reference CDP870 (BC071403, liquid) with CDP870 (A02570-005L01, lyophilised).
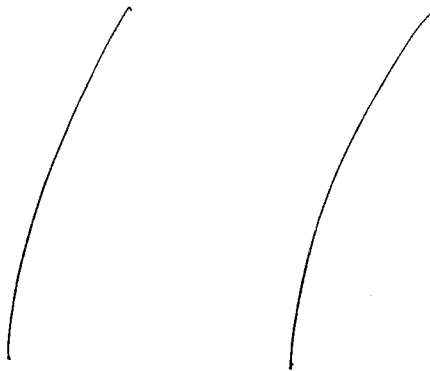
Figure 5. 10016074/80. Comparison of reference CDP870 (BR042701, liquid) with test CDP870 ⌐   ⌐ i, liquid).
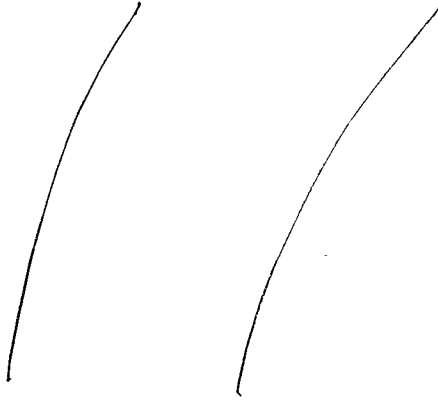
Figure 6. 10016074/92. Comparison of reference CDP870 (BR042701, liquid) with test CDP870 (SP19973, lyophilised).
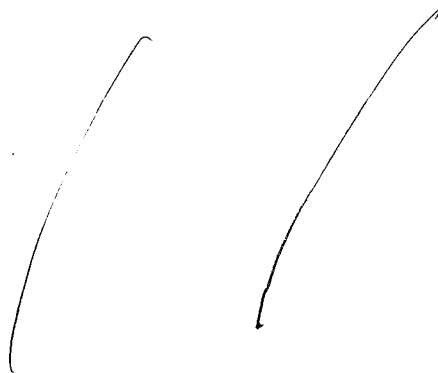
Comparison of Liquid and Lyophilized Formulation of CDP870 Potency in an *In Vivo* Bioassay in Mice Using Formulation ⌐ as a Diluent (Study Report # 40001373)

The neutralization potency (ED_{50}) for a liquid formulation (diluted in ⌐ , of CDP870 and a lyophilized formulation (diluted in ⌐ containing ⌐ sucrose) of CDP870 administered in relevant formulation ⌐ was compared using an *in vivo* bioassay in mice. The inhibition by i.v. CDP870 of neutrophil recruitment stimulated by intraperitoneal recombinant human TNF-α was determined in male Balb/c mice using a flow cytometry method. The doses of CDP870 used in this study were 0.003, 0.03, 0.1, 0.3 and 3 mg/kg.

No significant differences were observed between the ED_{50} of a liquid formulation of CDP870 diluted in ⎯⎯⎯⎯⎯⎯⎯⎯ ⎯ ⎯) and that of a lyophilized formulation diluted in ⎯⎯⎯ containing ⎯ sucrose (⎯⎯ ⎯ng/kg). The mean ED_{50} values for the liquid formulation and the lyophilized formulation of CDP870 were 0.020 and 0.029 mg/kg, respectively. The potencies of the liquid and the lyophilized formulation of CDP870 in causing an inhibition of TNF-α-induced neutrophil recruitment are compared in the Figure below.

Figure 1.   Comparison of liquid formulation CDP870 with lyophilised formulation CDP870.
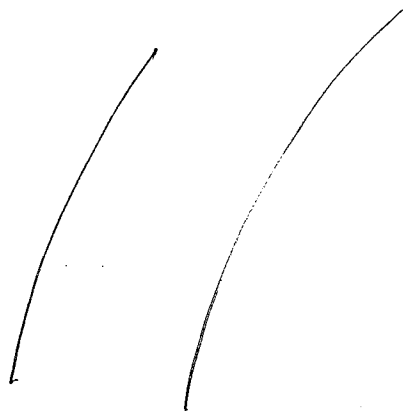
Prevention of Chronic Inflammatory Polyarthritis in Tg197 HuTNF Transgenic Mice by HTNF40.4 and CDP870 (Study Report # 40000902)

The effect of CDP870 in a transgenic mouse model ⎯⎯⎯⎯⎯⎯⎯⎯⎯) was examined following repeated intraperitoneal administration of the drug. In this mouse model, the pathology of the disease is dependent on human TNF-α. The ⎯⎯⎯⎯⎯⎯⎯ mice ⎯⎯ ⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯⎯ develops a severe polyarthritis characterized by marked inflammatory cell infiltration of the joints with cartilage loss and bone erosion. The animals (n=6) were treated from the first week after birth with saline only, CDP870 (10 mg/kg or 30 mg/kg i.p. twice weekly), HTNF40.4 (10 mg/kg i.p. twice weekly; a humanized IgG with same specificity as CDP870). The animals were scored weekly for joint appearance and mobility, and following sacrifice at week 9, the joints were scored histologically.

Animals treated with saline only developed severe arthritis with all attaining a maximum arthritic score of 3 by week 8. Histological examinations showed that 5 of 6 animals had a maximum score of 3 and 1 of 6 animals had a score of 2.5. Animals treated with CDP870 showed no signs of arthritis during the study (score of 0 in both dose groups), and histological examinations showed only minor signs of joint inflammation in some animals. The mean arthritic

scores of untreated mice, and those treated with hTNF40.4 (10 µg/kg) and CDP870 (10 and 30 µg/kg) are shown in the Table below.

Arthritic score

Dose Group	Age (Weeks)								
	1	2	3	4	5	6	7	8	9
Control	0	0	0	0.5	1	2.3	2.8	3	3
CDP870 (10 µg/kg)	0	0	0	0	0	0	0	0	0
CDP870 (30 µg/kg)	0	0	0	0	0	0	0	0	0
hTNF40.4 (10 µg/kg)	0	0	0	0	0	0	0	0	0.16

Thus, repeated intraperitoneal treatment with CDP870 can prevent disease development in this mouse model of polyarthritis.

Effect of CDP870 in the Conscious Rabbit Pyrexia Model (Study Report # 40001391)

The effects of CDP870 and another anti-human TNF-α monoclonal antibody, CDP571 were studied in a conscious rabbit pyrexia model. In this model, conscious rabbits are given an i.v. bolus of recombinant human TNF-α which causes a transient rise in rectal temperature. CDP870 and CDP571 were administered intravenously at a dose range of 1 to 100 µg/kg. Two doses of the anti-TNF agents were administered at 15 min intervals. Rectal temperature of the animals was measured for 45 minutes before administration and then continuously for 5 hours. Blood samples were collected for measurement of plasma TNF-α concentrations by an ELISA method.

There were no effects of either CDP870 or CDP571 alone on the general behavior or rectal temperature of the animals. Both agents ameliorated the recombinant human (rh) TNF-induced pyrexia and neutralize the rhTNF present in the plasma in a dose-dependent manner. No differences in the antipyretic effects were observed between CDP870 and CDP571 in this animal model. CDP870 was about 1.9-fold more potent than CDP571 in neutralizing the TNF in the plasma of rabbits. The effect of CDP870 on rh-TNF-induced pyrexia in rabbits is shown in the Figure below.

Effect of CDP870 on rhTNF-induced pyrexia in conscious rabbits
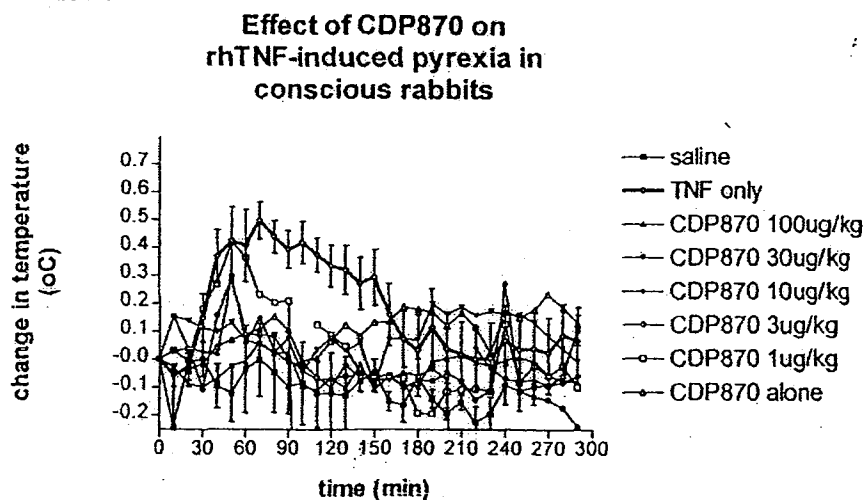
Thus, CDP870 caused a dose-dependent inhibition of rhTNF-induced rise in rectal temperature in rabbits.

Effects of CDP870 in the Rabbit Pyrexia Model (Study Report # 40001392)

The ability of CDP870 from three different sources to neutralize the pyrogenic effects of recombinant human TNF-α was examined in New Zealand white rabbits. Male Hra:NZW)SPF rabbits were randomly assigned to 20 dosage groups (2-10 animals/group). CDP870 Stage 1, Stage 2 or Stage 3, or the vehicle (0.9% sodium chloride) was administered via i.v. bolus injection on day 1 at doses of 0, 1, 3, 10, 30 or 100 mg/kg (1 ml/kg). Dosage of one of the test articles or the vehicle occurred approximately 45 minutes into a 60 min observation period during which the body temperatures were recorded every 10 minutes. The vehicle or recombinant human TNF-α (rh TNF-α; 1.0 µg/kg) was administered 15 minutes following administration of the test article or the vehicle. The animals were observed for clinical signs before dosing and every 10 minutes after administration. Body temperatures were recorded every 10 minutes for 1 hr before administration and then every 10 minutes after administration for approximately 5 hours. Blood samples were collected for measurement of plasma rh TNF-α and plasma CDP870 levels immediately after the second treatment, and then hourly for 5 hours.

There were no deaths or clinical observations related to the treatment of the test articles or the vehicle. Recombinant human TNF-α, at a dose of 1.0 µg/kg, caused an expected rise (maximum rise, 71^{0}C, at 40 minutes after administration) in body temperature. CDP870 Stage 1 was effective in neutralizing the pyrogenic effects of rh TNF-α at doses of 10, 30 and 100 mg/kg. CDP870, at doses of 1 and 3 mg/kg, when administered in conjunction with rh TNF-α caused increases in rectal temperatures in an inverse dose-dependent manner (0.67^{0}C at 1 mg/kg; 0.57^{0}C at 3 mg/kg) that persisted longer than that produced by TNF-α alone. Thus, CDP870 Stage 1 did not neutralize the pyrogenic effects of rh TNF-α.

CDP870 Stage 2 was effective at neutralizing the pyrogenic effects of TNF-α at doses of 10 and 30 mg/kg. CDP870 Stage 2, at concentrations of 1 and 3 mg/kg, did not appear to neutralize the effects 1 µg/kg rhTNF-α, and the temperatures increased at these concentrations when used in conjunction with TNF-α. Increases in rectal temperatures were also observed at 100 mg/kg CDP870 Stage 2. However, the increases in the temperatures were not dose-dependent (increases in body temperatures were 0.43^{0}C, 0.85^{0}C and 0.38^{0}C at 1, 3 and 100 mg/kg doses, respectively).

CDP870 Stage 3 neutralized the pyrogenic effects of rh TNF-α at a dose of 30 mg/kg. Temperatures were increased in rabbits administered 1, 3 and 10 mg/kg doses of CDP870 in conjunction with TNF-α. Thus, the 1, 3 and 10 mg/kg doses did not neutralize the pyrogenic effects of rh TNF-α. Additionally, increased body temperatures were also observed at 100 mg/kg CDP870 Stage 3 in conjunction with rh TNF-α. A rise in the body temperature also occurred when this dose was administered alone (0.20 to 0.40^{0}C).

Administration of CDP870 to the rabbits caused a dose-dependent increase in the plasma exposures, with no significant differences due to the source of CDP870. There were no detectable levels of rh TNF-α in the plasma of rabbits administered 30 and 100 mg/kg CDP870 in combination with TNF-α, suggesting that a neutralization of TNF-α occurred at these
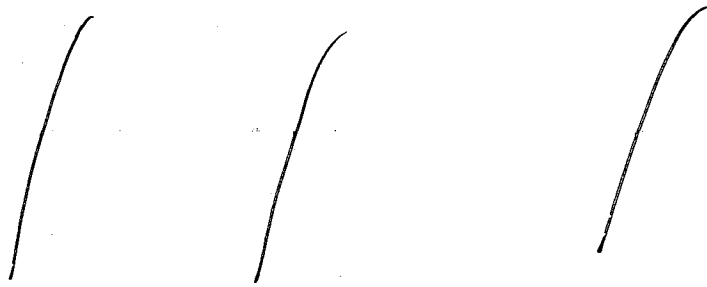
CDP870 did not mediate cell killing by either CDC or ADCC. On the other hand, Remicade, Humira and Enbrel all mediated cell killing by both CDC and ADCC. All three agents showed comparable cell killing by CDC, with approximately 75% of the maximal release of LDH and 60% of maximal number of cells   Only small amounts of LDH release was observed at concentrations at or below 0.01 μg/ml, and the maximal LDH release of about 80% was observed at 10 μg/ml. No further increases in the LDH release were observed at a concentration of 100 μg/ml. Remicade, Humira and Enbrel induced concentration-dependent cytoxicity, as measured by an   The abilities of these agents to mediate antibody-mediated cell killing were different. Remicade and Humira mediated ADCC to a similar extent with approximately 50% of the maximum numbers of cells   while the cell killing by Enbrel with mediation ADCC was lower (approximately 35%) than that of Remicade or Humira. The complement-dependent cytotoxicities of controls, CDP870, Remicade, Humira and Enbrel, measured by LDH release and PI uptake are shown in the Figures below.

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ON ORIGINAL

Figure 11:2 Complement-dependent cytotoxicity of ⸺ cells determined by LDH release (R20058)
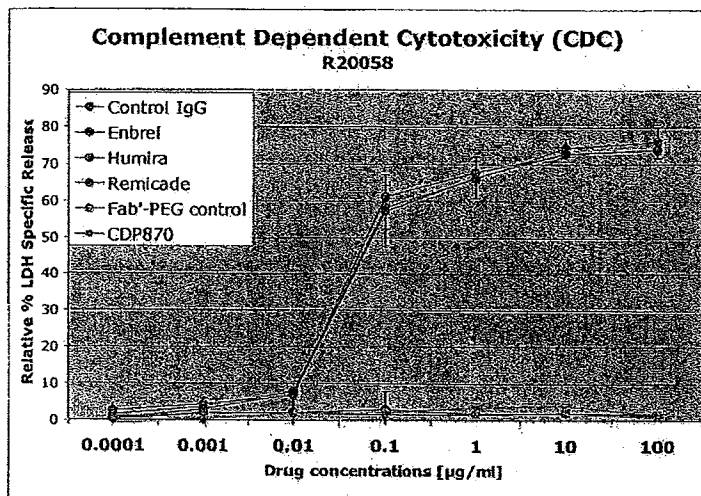
Figure 11:2 Complement-dependent cytotoxicity of ⸺ cells determined by LDH release (R20058)
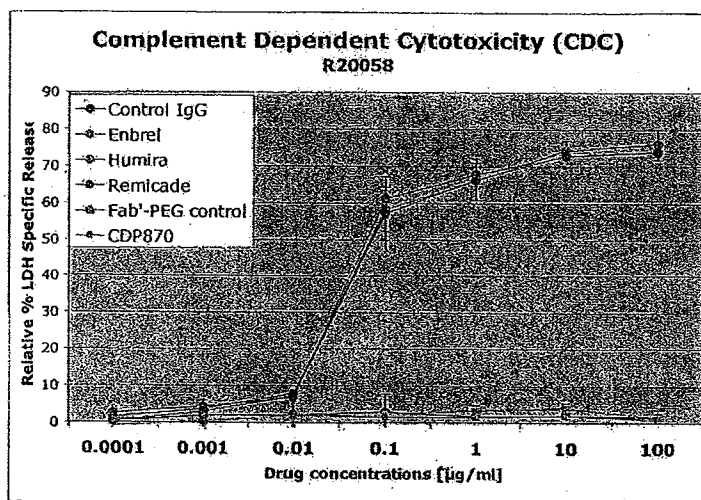
The antibody dependent cell mediated cytotoxicity (ADCC) of ⸺ cells, detemined by ⸺ , for controls and the anti-TNF agents is shown in the Figure below.

33

Figure 11:14 Antibody-dependent cell cytotoxicity of ⌐ cells determined by ⌐ (R200521)

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)
R200521
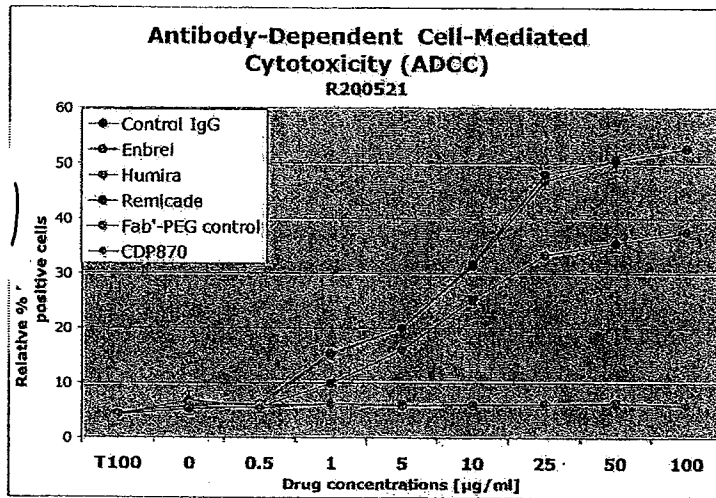
Thus, CDP870 did not mediate cell killing by CDC or ADCC in this experimental system, while the three commercially available anti-TNF agents, Remicade, Humira and Enbrel killed cells by both mechanisms. These differences may be due to the structural difference of CDP870 as compared with the other anti-TNF agents.

Effects of CDP870 and Other Anti-TNF Products on Apoptosis of T-Cells and Monocytes (Study Report # 40001503)

The ability of CDP870 to induce apoptosis in human peripheral blood-derived lymphocytes and monocytes was compared with that of the available anti-TNF agents, Remicade, Humira and Enbrel. The levels of apoptosis were assessed by

34

_____1_____ Page(s) Withheld

_____✓_____ Trade Secret / Confidential

_____ Draft Labeling

_____ Deliberative Process
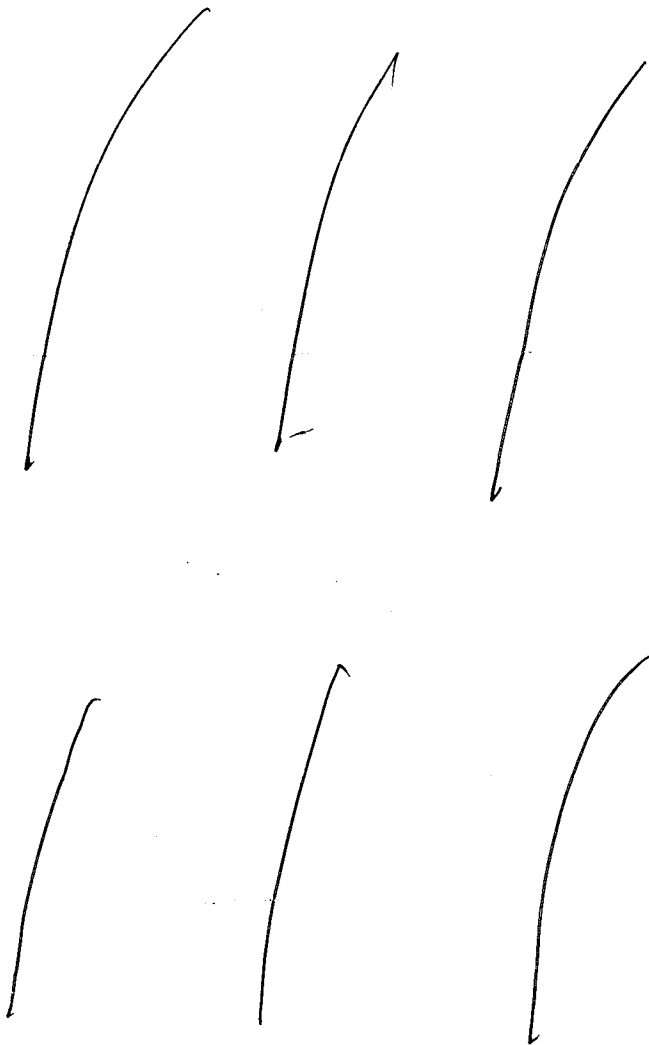
Thus, CDP870 did not show any apoptosis (measured by _____ in human peripheral blood-derived lymphocytes and monocytes in this *in vitro* assay. On the other hand, other available anti-TNF agents, Remicade, Humira and Enbrel, all induced concentration-dependent increases in the percentage of cells exhibiting an apoptotic phenotype.

Degranulation of Neutrophils Following Anti-TNF Binding (Study Report # 40001504)

The effect of CDP870 on *in vitro* neutrophil survival was compared with those of currently marketed anti-TNF therapies, Remicade, Humira and Enbrel. Neutrophils were isolated from human peripheral blood, and the level of degranulation induced by different anti-TNF agents was determined by _____

integrity. Three repeat experiments were conducted each for assessment of
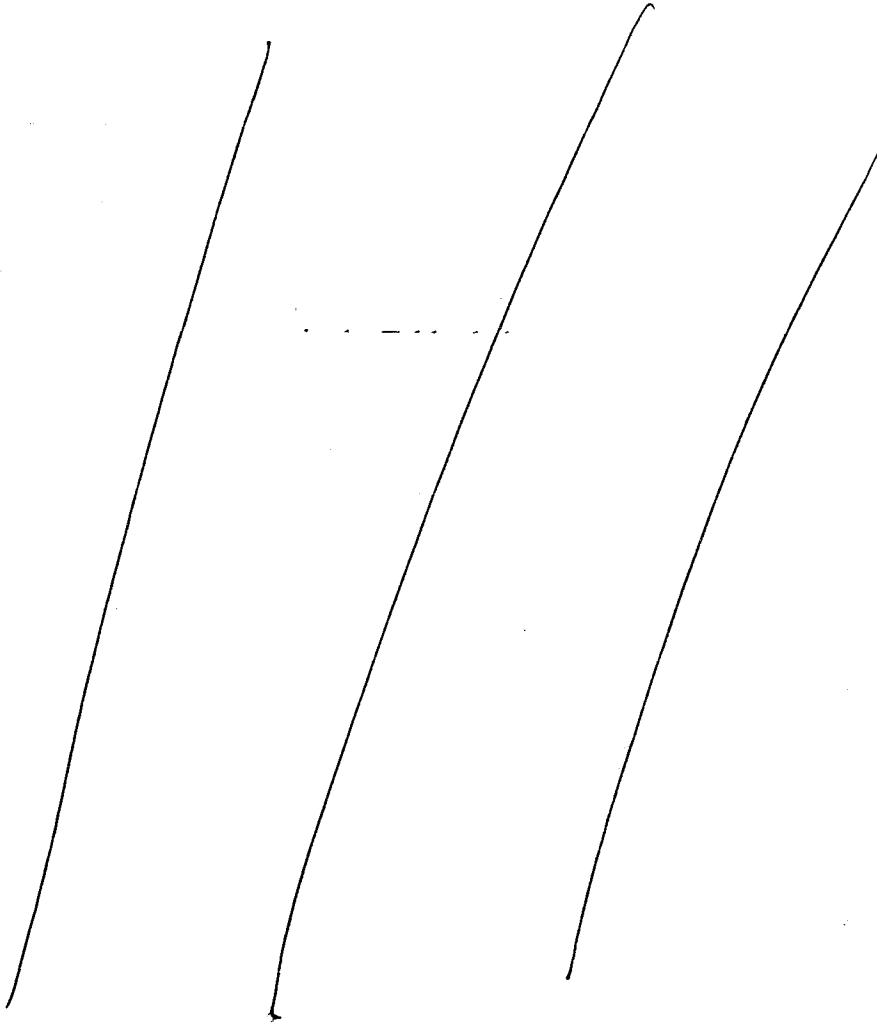
Thus, CDP870 did not induce neutrophil degranulation in this *in vitro* assay, while the currently available anti-TNF agents, Remicade, Humira and Enbrel induced significant neutrophil degranulation, as measured by ———————————————— Thus, CDP870 may work differently than the available anti-TNF agents in modifying the biological effects of TNF-α.

2.6.2.4 Safety pharmacology

Cross-Reactivity Study of CDP870, Polyethylene Glycol-Conjugated Human Monoclonal Fab antibody with Normal Human tissues (Study #PAI IM444; Study Report # 40000903)

The objective of the study was to evaluate potential cross-reactivity of CDP870 with cryosections of normal human tissues. In order to detect binding, polyethylene glycol-conjugated CDP870 was applied to 5 μm human tissue sections (at least two and, where possible three sources per tissue) at concentrations of 3 and 10 μg/ml. _____ _____ cells were used as positive controls. _____ cells were used as negative controls. Other controls were produced by omission of the test antibody (assay control) or substitution of a human _____ antibody of the same immunoglobulin subclass ' _____ but different antigenic specificity for CDP870 (negative control antibody, designated as _____ . The cross-reactivity was examined by a _____ _____ technique. As a tissue staining control, separate cryosections from each tissue were stained in parallel for the expression of _____ _____ . The following human tissues were used in the cross-reactivity study:
Adrenal, blood (neutrophils, lymphocytes, eosinophils, monocytes, platelets), blood vessel, bone marrow, cerebellum, cerebrum, cervix, esophagus, eye, heart, kidney, large intestine, liver, lung, lymph node, mammary gland, ovary, fallopian tube, pancreas, parathyroid, peripheral nerve, pituitary, placenta, prostate, salivary gland, skin, small intestine, spinal cord, spleen, stomach, striated muscle, testes, thymus, thyroid, tonsil, ureter, urinary bladder and uterus.

CDP870 specifically stained the positive control, human _____ _____ cells. Test article reactivity was intense at both concentrations examined (3 and 10 μg/ml). CDP870 did not react with _____ cells, the negative control. An isotype matched negative control antibody did not specifically bind to either the positive or the negative control cells. To determine cross-reactivity, CDP870 was also applied to a panel of 37 normal human tissues at concentrations of 3 and 10 μg/ml. No cross-reactivity was observed with any human test tissue. Nonspecific background staining of some tissues was apparent as weak to moderate labeling of parenchyma, collagen, occasional dendritic cells and/or smooth muscle at one or both concentrations of both test and control antibodies. This was considered nonspecific because both the control and test antibodies stained the same tissues and the staining intensity varied within and between sections. Expression of _____ used as a tissue staining control) was observed in vascular endothelia and variety of cells in all tissues, and in platelets and occasional intravascular lymphoreticular cells.

To determine tissue cross-reactivity, CDP870 was applied to a panel of 37 normal human tissues at concentrations of 3 and 10 μg/ml. CDP870 did not show cross-reactivity with any human tissue examined in this study.

CDP870 – Blood Compatibility Study (Study Report #40001254)

The compatibility of CDP870 with whole blood, plasma or serum from humans was evaluated *in vitro*. Saline (0.9%) and water for injection were used as negative and positive controls, respectively. The test article, vehicle and the negative and positive controls were mixed with whole blood from healthy human volunteers in a 1:5 ratio. Erythrocyte clumping was scored immediately after mixing. The mixture was centrifuged approximately 1 hr (at room temperature) after preparation for evaluation of hemolysis (optical density at ⸺) in the supernatant. In addition, each test solution was mixed in a ratio of 1:5 with the plasma or serum of each donor to score for possible precipitate formation. The study was carried out in 2 steps. In the first step, CDP870 was tested at a concentration of 33.33 mg/ml by mixing with the plasma or serum from 8 donors (4 males, 4 females). In the second step, the tests were carried out in two independent sets (3 males and 3 females) of donors, one for the evaluation of erythrocyte clumping and possible precipitate formation in plasma and the other for precipitate formation in serum. These additional studies were conducted at CDP 870 final concentrations of 0.003, 0.033, 0.333, 3.333 and 33.33 mg/ml. Hemolysis, erythrocyte clumping, and precipitate were scored visually using an arbitrary qualitative scale (ranging from 0 to 4).

As expected, water for injection caused slight hemolysis. No hemolytic effects related to the presence of the test article or the vehicle (acetate buffer) were observed in any sample. The pH and osmolarity values of the 1:5 test article or vehicle/plasma mixtures were similar to the concurrent control mixture. Strong erythrocyte clumping and opalescence of the plasma or serum were observed in the presence of the CDP870 at a concentration of 33.33 mg/ml.
At concentrations below 33.33 mg/ml, no opalescence was observed either in the plasma or serum. Erythrocyte clumping was also observed at 0.33 mg/ml and higher concentrations in step 2 of the study. In addition, trace erythrocyte clumping was also observed at lower concentrations. Erythrocyte clumping in the control samples and those in the presence of different concentrations of CDP870 is summarized in the Table below.

HUMANS	Final CDP870 conc. in the mixture (mg/ml)	Visual Index
Group 1: vehicle	0	0
Group 2: 200 mg/ml CDP870 solution in the vehicle	33.33	4
Group 3: 20 mg/ml CDP870 solution in the vehicle	3.333	3
Group 4: 2 mg/ml CDP870 solution in the vehicle	0.333	2
Group 5: 0.2 mg/ml CDP870 solution in the vehicle	0.033	1
Group 6: 0.02 mg/ml CDP870 solution in the vehicle	0.003	1
Group 7: 0.9% NaCl solution	0	1

0 = No observed reaction (neg.)

1 = Trace positive reaction (T)
2 = Weak positive reaction (+)
3 = Medium positive reaction (++)
4 = Strong positive reaction (+++)

Thus, CDP870 did not show any *in vitro* hemolytic effect in human whole blood at concentrations up to 33.33 mg/ml. Erythrocyte clumping was observed at 0.33 mg/ml and higher concentrations of CDP870. Trace erythrocyte clumping was also observed at concentrations lower than 0.33 mg/ml. The maximal expected concentration of CDP in the blood of humans following subcutaneous administration of the therapeutic dose is in the range of 0.05-0.1 mg/ml.

Assessment of Changes in Activated Partial Thromboplastin Time (APTT) after ex vivo Addition of CDP870 in Cynomolgus Monkeys (Study #259283)
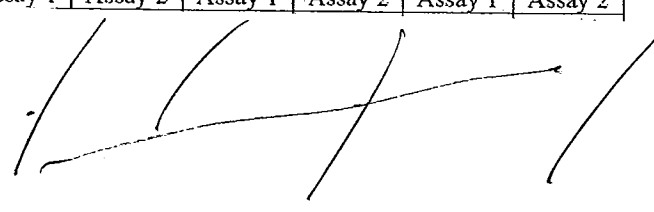
In *in vivo* animal studies with CDP870, slight increases in APTT were observed. This study was conducted to assess the changes in APTT *ex vivo* in monkeys. Blood was collected from three cynomolgus monkeys into citrated vacutainers, and the plasma from each animal was used to prepare the following analytical aliquots:

a. Plasma control: 1 ml plasma
b. Dilution control: 0.9 ml plasma and 0.1 ml vehicle
c. CDP870 200 µg/ml: 0.9 ml plasma and 0.1 ml 2 mg/ml CDP870 solution
d. CDP870 600 µg/ml: 0.9 ml plasma and 0.1 ml 6 mg/ml CDP870 solution
e. CDP870 2000 µg/ml: 0.9 ml plasma and 0.1 ml 20 mg/ml CDP870 solution
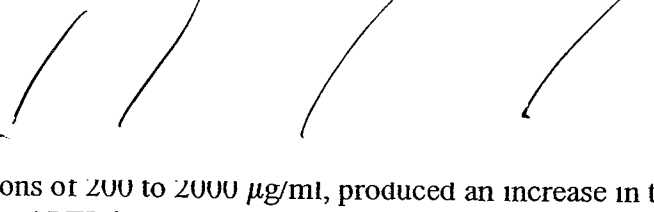
Each aliquot was analyzed in duplicate on a coagulation analyzer using APTT setting within 2 hr of aliquot preparation. All aliquots were stored at 4^0C for 24 hr and APTT assessed in duplicate.

Monkey plasma treated with CDP870 produced an increase in APTT (about 30%) which was not concentration related. Comparison of the dilution controls to the plasma controls showed that plasma dilution did not affect APTT. Storage at 4^0C for 24 hours slightly increased the APTT for all quality control and test aliquots but did not affect the increase produced by CDP870 spiked aliquots. The effect of CDP870 on APTT in monkey plasma is shown in the Tables below.

Table 1   *Activated Partial Thromboplastin Time Results for CDP870 Spiked Plasma within 2 Hours of Dilution*

Aliquot	APTT (seconds)					
	Animal No 1		Animal No 2		Animal No 3	
	Assay 1	Assay 2	Assay 1	Assay 2	Assay 1	Assay 2
Plasma Control						
Dilution Control						
CDP870 200 µg/mL						
CDP870 600 µg/mL						
CDP870 2000 µg/mL						

Table 2   *Activated Partial Thromboplastin Time Results for CDP870 Spiked Plasma after 24 hours Storage at 4^0C*

Aliquot	APTT (seconds)					
	Animal No 1		Animal No 2		Animal No 3	
	Assay 1	Assay 2	Assay 1	Assay 2	Assay 1	Assay 2
Plasma Control						
Dilution Control						
CDP870 200 µg/mL						
CDP870 600 µg/mL						
CDP870 2000 µg/mL						

Thus, CDP870, at concentrations of 200 to 2000 µg/ml, produced an increase in the APTT in monkey plasma. However, the APTT increase was not dose-dependent.

Characterization of the Antibody Response to CDP870: Supplementary Research Report;

The antibody response to CDP870 was assessed in cynomolgus monkeys and healthy human volunteers following administration of the drug. Antibody responses were assessed using a screening ELISA assay using a CDP870-coated microtiter plate. Samples from subjects from three studies of CDP870, positive in this screening assay were further characterized to determine the epitope recognized and the class of the antibody produced. Samples were tested from two toxicology studies in monkeys, a single dose, 28-day follow-up study and an intermittent dose study of 4 weekly intravenous doses with a 28-day follow-up, both at 50, 100 and 400 mg/kg CDP870.

One of 12 animals in the single dose study and 4 of 30 animals in the intermittent dosing studies were positive for antibodies to CDP870. The antibodies mapped to ─────────── ─────── . CDP870. Anti-CDP870 was of the IgM type at the earlier time points (single dose study) and switched to IgG at later time points (1 and 2 months). The levels of IgM and IgG in the single dose and intermittent dose monkey studies are summarized in the Table below.

Table 6.6: Levels of IgM and IgG in single dose monkey 8; Pre-dose, 13 days and 27 days.

Dilution	IgM			IgG		
	Pre-dose	Day 13	Day27	Pre-dose	Day 13	Day 27
0.1	0.04	0.73	0.33			
0.03	0.02	0.35	0.16	0.00	0.01	0.68
0.01	0.01	0.14	0.07	0.00	0.01	0.48
0.003	0.00	0.06	0.03	0.00	0.00	0.25
0.001				0.00	0.00	0.09

Results are OD at . ──── reference──────.

Table 6.7: Levels of IgM and IgG in Intermittent-dose Monkey 26 (50mg/kg) ; Pre-dose and 2 months.

Results are expressed as OD at ── reference ──

IgM Levels			IgG levels		
Dilution	Pre-dose	2 months	Dilution	Pre-dose	2 months
0.1	0.21	0.79	0.03	0.04	1.26
0.03	0.08	0.27	0.01	0.03	1.22
0.01	0.05	0.11	0.003	0.03	1.18
0.003	0.04	0.06	0.001	0.03	1.12

Both IgM and IgG specific antibodies are increased at 2 months over baseline.

During the first study in man, samples were collected from a single dose intravenous study in male volunteers, given 0.3, 1.0, 3.0 and 10 mg/kg (3 per group, 3-month follow-up). Eight of 12 volunteers had detectable antibodies to CDP870, but antibodies decreased in frequency and magnitude with increasing dose. In all samples tested, antibodies were directed only against the CDP870 ──── A switch from IgM to IgG was seen in some individuals by day 28. IgM antibodies peaked within 7 to 14 days, while specific IgG was undetectable at this time. Specific IgG, when occurred, was detectable from 2 weeks onwards. The IgG anti-CDP870 from 2

volunteers was further typed into subclass by detecting with specific anti-IgG isotype antibodies. Both volunteers showed the greatest relative increase in IgG3 isotype (7-10 fold), however specific antibodies of all isotypes were also increased. The IgG subtypes in two volunteers are shown in the Table below.

Table 6.13: IgG subtypes, by OD, in volunteer 3; Pre-dose and 2 months.
        Results are OD at   —   reference   —

IgG subtype	Pre-dose	2 months	Fold Increase
IgG1			4.42
IgG2			5.76
IgG3			7.21
IgG4			3.83

Table 6.14: IgG subtypes, by OD, in volunteer 15; Predose and 3 months.
        Results are OD at   —   reference   __

IgG subtype	Pre-dose	3 months	Fold Increase
IgG1			1.81
IgG2			3.74
IgG3			10.58
IgG4			9.35

Thus, antibodies to CDP870, detected in cynomolgus monkeys and human volunteers, were directed entirely against the   —   CDP870, and could switch from IgM to IgG class at later time points. All four IgG isotypes were detected in the 2 volunteers studied.

Comparison of the Effects of a Control Fab' PEG and CDP870 on Macrophages In Vitro (Study Report *40001521)

The effects of a control Fab' PEG and CDP870 were examined *in vitro* on mouse peritoneal macrophages (isolated from Balb/c mice) and human peripheral blood monocyte-derived macrophages (from blood obtained from healthy donors) following incubations with these agents at concentrations ranging from 0.01 mg/ml up to 10 mg/ml. The presence of intracellular vacuoles was examined histologically. Cells were incubated for 22 hours after which they were _____ to differentially stain the cell nucleus and cytoplasm. The cells were then viewed microscopically and the extent and pattern of macrophage cytoplasmic vacuolation was recorded. The level of vacuolation induced by CDP870 was compared with a control Fab' PEG and relevant — controls — ᵢ lactic acid — sucrose, — ᵤ Tween — ᵗ for CDP870, and _____
) for the control Fab' PEG).

Incubation of human and mouse macrophages with the appropriate concentration of formulation — for CDP870 caused increased vacuolations in both types of cells compared to Fab' PEG — However, it was not usually as extensive as that seen with 10 mg/ml Fab' PEG or CDP870. This may be due to the presence of — sucrose in the CDP870 formulation

Fab' PEG and CDP870, at concentrations up to 1 mg/ml, had no effect on the normal background vacuolation state of human or mouse macrophages in vitro. At 10 mg/ml of either Fab' PEG or CDP870, however, extensive intracellular vacuolation was observed in both human and mouse macrophages that appeared to be comparable for both agents. Under the experimental condition, it was not possible to conclude whether the vacuolation induced by CDP870 was significantly different from that induced by a control Fab' PEG.

Thus, comparable vacuolation was observed in mouse and human macrophages treated with either control Fab' PEG or CDP870.

2.6.2.5 Pharmacodynamic drug interactions
N/A

2.6.3 PHARMACOLOGY TABULATED SUMMARY
N/A

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary:
PEGylation of HTNF 40 IgG with the 40 KD PEG increased the elimination half-life ($t_{1/2\alpha}$) of the compound in monkeys following a single i.v. dosing. The plasma exposure level for the 40 KD PEGylated IgG (CDP870) was higher than that for the ' — , PEGylated IgG (78% and 30% of the non-PEGylated IgG, respectively). PEGylation also decreased the immunogenicity of the antibody. CDP-870 also showed longer half-life and higher plasma exposure levels than that of Fab' in rats following an i.v. dose. Following subcutaneous administration of single doses (3 and 31 mg/kg) of CDP870 to monkeys, plasma concentrations increased with increasing dose, and the maximum plasma concentrations were reached between 24 and 48 hours with a $t_{1/2}$ of about 200 hours (8.4 days). Following subcutaneous administration (10 and 200 mg/kg, twice a week for 4 weeks) in rats, the C_{max} values were reached in 24 hours, and the $t_{1/2}$ value was about 60 hours. The estimated bioavailability in rats following s.c. administration was 23.5% in males and 33.8% in females. Tissue distribution of CDP870 in rats following an i.v. dose was similar to that of the non-PEGylated form. At 3 hours following administration, the highest level was found in the kidneys, followed by lung, liver and spleen. In humans, following subcutaneous (up to 800 mg) or intravenous (up to 10 mg) administration, the C_{max} and AUC values increased with increasing dose, and the peak CDP870 concentrations were attained between 54 and 171 hours following s.c. administration. The terminal elimination half-life ($t_{1/2}$) was approximately 14 days for all doses tested. Following s.c. administration to humans for 12 weeks, anti-CDP870 antibodies were detected in 5% (at 800 mg/4 week dose) to 67% (at 50 mg/4 week dose) of the subjects, depending on the doses administered. The presence of the antibody decreased the C_{max} and AUC by more than 50%. CDP870 was not an inhibitor of P-glycoprotein.

2.6.4.2 Methods of Analysis

CDP-870 and the anti-CDP-870 antibodies in the plasma samples were determined by ELISA methods.

2.6.4.3 Absorption

HTNF 40 and Derivatives: Single Intravenous Dose Pharmacokinetic Study in Male Cynomolgus Monkeys (Study # ARLE05K2604; Study Report #40000824)

Methods: The pharmacokinetic profiles of HTNF 40 IgG and its derivatives HTNF 40 PEGylated Fab' 25 KD and HTNF 40 PEGylated Fab' 40 KD were studied in male cynomolgus monkeys after a single intravenous administration. Two male cynomolgus monkeys each received a single i.v. dose of one of the radiolabeled test substances (^{125}I-labeled; about 0.1 mCi/ml) at a dose of 1 mg/kg. The dosing volume was 10 ml/kg, and the total volume was administered over a period of 1 hour. The animals were observed regularly for treatment-related clinical signs. Blood samples (approximately 1.0 ml) were collected into heparinised tubes on the day of dosing (Day 0) prior to and at the end of infusion and at 0.5, 1, 3, 6, 12 and 24 hours after completion of dosing, and on days 2, 3, 4, 5, 6, 7, 10, 14, 21, 28, 35 and 53. Prior to centrifugation, the radioactivity in the blood samples was measured using a gamma scintillation counter.

Results:

There was an accelerated clearance of radioactivity in whole blood from around Day 10 post-dose in animals receiving HTNF 40 IgG. Clearance of the radioactivity from animals administered HTNF 40 PEGylated Fab' 25 KD or HTNF 4- PEGylated Fab' 40 KD did not show this accelerated clearance. A decrease in blood radioactivity concentrations was observed from day 21 in animals receiving the PEGylated form of the antibody. The accelerated clearance of the radioactivity from animals receiving HTNF 40 IgG may be related to the development of antibodies to this agent.

The levels of radioactivity in the whole blood of animals receiving HTNF 40 IgG (pair 1) and its derivatives HTNF 40 PEGylated Fab' 25 KD (pair 2) and HTNF 40 PEGylated Fab' 40 KD (pair 3) are shown in the Table below.

Concentrations of ^{125}I in whole blood

Results expressed as dpm/ml

Time	Pair 1		Pair 2		Pair 3	
	263M	265M	267M	269M	271M	273M
0 hours						
0.5						
1						
3						
6						
12						
24						
2 days						
3						
4						
5						
6						
7						
10						
14						
21						
28						
35						
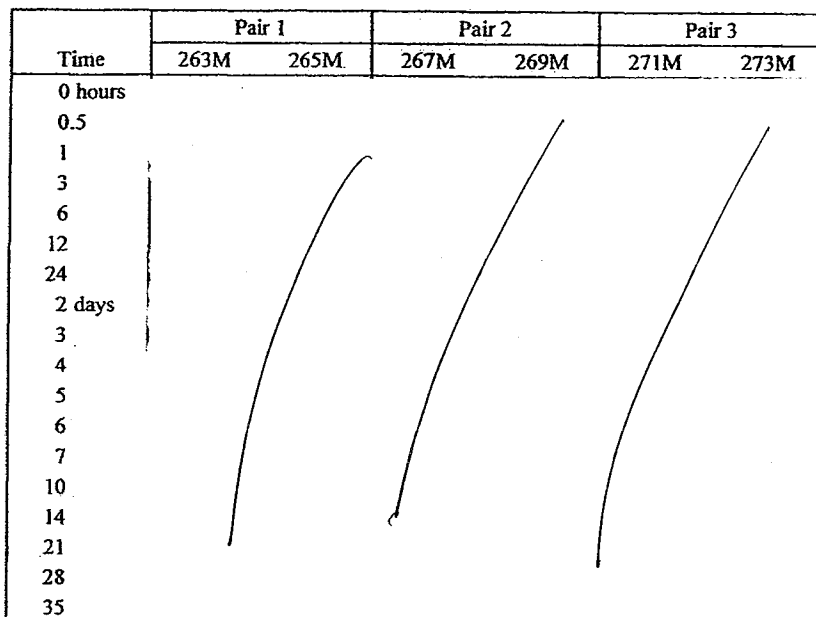
*   0 hours = end of infusion

HTNF 40 and Derivatives: Single Intravenous Dose Pharmacokinetic Study in Male Cynomolgus Monkeys Pharmacokinetics and Immunogenicity (Study Report # 40000825)

Methods:

The pharmacokinetic profiles of HTNF 40 IgG and its derivatives, HTNF 40 PEGylated Fab'   ⸺ KD PEG) and HTNF 40 PEGylated Fab' (40 KD PEG) were studied in male cynomolgus monkeys after single intravenous administration. The test substances were radiolabeled with ^{125}I (approximately 0.1 mCi/ml). Two monkeys each received a single intravenous dose of the test substance at a dose of 1 mg/kg, administered over a one hour period. Blood samples were collected for 35 days after infusion, and the radioactivity in the whole blood was counted. In addition, plasma samples were analyzed by enzyme-linked immunosorbent assay (ELISA) for test substance pharmacokinetics and immunogenicity. ELISA for antibodies to HTNF 40 IgG was conducted in HTNF 40 IgG-coated microtiter plates. Standards were prepared from   ⸺ ·
——————————————/   . Samples were diluted 1/100 directly into wells. Plates were cultivated for 1 hour at room temperature, washed and then incubated for 30 minutes with HRP-streptavidin. The color development was measured using a plate reader.

Results:

From the whole blood radioactivity counts, HTNF 40 IgG had a $t_{1/2\alpha}$ of 4 hours which lasted less than 24 hours and the IgG was then eliminated with a mean $t_{1/2\beta}$ of 145.4 hours (6.1 days) until day 10, after which the antibody was cleared rapidly from the circulation. For the ⸺

KD and 40 KD PEGylated Fab' derivatives of HTNF40, the $t_{1/2\alpha}$ values were 15 and 12 hours, respectively, and lasted for 3 to 4 days. The $t_{1/2\beta}$ values for elimination of these agents were 147.3 and 188.9 hours (6.1 and 7.9 days), respectively. The $AUC_{(0-\infty)}$ calculations indicated that the —. KD PEGylated Fab' AUC was 29.6% of the IgG and the 40 KD PEGylated Fab' AUC was 77.8% of the IgG. The whole blood pharmacokinetics of HTNF 40 IgG and PEGylated fragments (radioactivity) in cynomolgus monkeys is shown in the Figure below.

Pharmacokinetics of HTNF40 IgG and PEGylated
fragments in cynomolgus monkeys
Whole blood counts
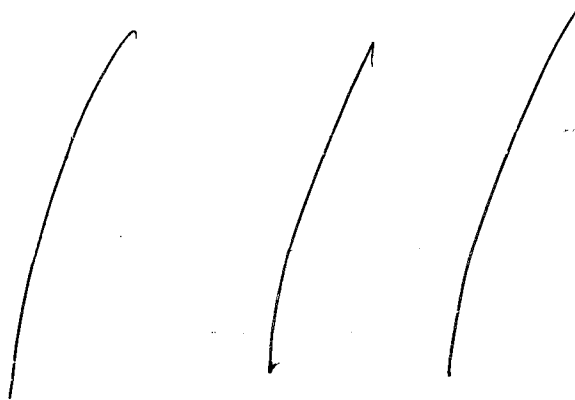
The pharmacokinetic parameters for the total radioactivity for the three agents are summarized in the Table below.

Pharmacokinetic Summary Data

	Animal No.	Points used for terminal phase	α t½ (h)	β t½ (h)	AUC (0-∞) (h.dpm/ml)	AUC (0-∞) (h.µg/ml)	% IgG AUC
IgG	263	8 (24-240 hrs)	4.3	185.4	72323895.7	1085.94	
	265	8 (24-240 hrs)	3.7	105.4	53348656.0	801.0	100
KDPEG	267	9 (96-840 hrs)	15.7	145.9	18832761.5	282.77	
	269	9 (96-840 hrs)	14.8	148.6	18338385.0	275.35	29.6
40KDPEG	271	10 (72-840 hrs)	12.8	172.1	46249965.3	694.44	
	273	10 (72-840 hrs)	11.1	205.7	51530359.5	773.73	77.8

Similar elimination profiles were obtained using the ELISA assays, confirming that the antibodies are still able to bind TNF. PEGylated Fab' 40 KD was cleared more rapidly in the α phage but had a similar β phase. The —KD PEGylated HTNF 40 was cleared more rapidly. The pharmacokinetics of HTNF 40 and derivatives from the ELISA assay are shown in the Figure below.

Pharmacokinetics of HTNF40 and Derivatives
Data from _____ ELISAs
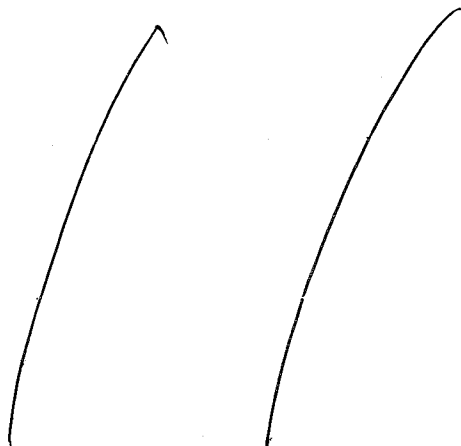
Pretreatment plasma samples and samples collected after day 7 were assayed for the presence of antibodies to HTNF 40 IgG. The baseline antibody levels were 0.10 to 0.29 units/ml. In the 2 animals receiving IgG, there was a progressive increase in antibody levels from day 14 onwards (_____ units/ml, respectively). In animals receiving ⌐ KD PEGylated Fab', there was a slight increase in the antibody levels (_____ units/ml, respectively), while the animals receiving 40 KD PEGylated Fab, did not show any increase in the antibody levels. The anti-HTNF 40 IgG antibody levels for the 3 groups of monkeys at different times are shown in the Table below.

	HTNF40 IgG		Fab'-PEG ⌐ KD		Fab'-PEG 40KD	
Animal Number	263	265	267	269	271	273
Time (days)						
0						
7						
10						
14						
21						
28						
35						

Results are expressed as units/ml where 1unit is equivalent to 1μg/ml .

Thus, HTNF 40 KD PEGylated Fab' had a similar elimination half-life to that of IgG, but it did not appear to be immunogenic in cynomolgus monkeys.

CDP870: Single Dose Subcutaneous Pharmacokinetic Study in the Monkey (Study Report # 40000998 and 40001080):

Methods: The pharmacokinetic profile of CDP870 was determined in cynomolgus monkeys (2 animals/sex/group) following a single subcutaneous administration of 3 and 31 mg/kg doses. The dosing volumes were 16 µl/kg and 165 µl/kg for 3 and 31 mg/kg doses, respectively. The animals were observed daily for clinical signs and mortality for 70 days following administration, and sacrificed on day 71. Blood samples were withdrawn from all animals prior to dosing and at 4, 8, 16, 24, 36 and 48 hours after dosing on day 1, and on study days 4, 5, 6, 8, 11, 15, 22, 29, 60 and 71. Plasma concentrations of CDP870 and antibodies to CDP870 were determined by ELISA.

Results: There were no deaths, or effects on body weight, food consumption or clinical signs. There were no macroscopic or microscopic findings at the injection sites. Plasma concentrations of CDP870 increased with increasing dose in a dose-proportional manner. The maximum plasma concentrations (C_{max}) were reached between 24 and 48 hours after administration. The C_{max} values in monkeys receiving 3 and 31 mg/kg doses ranged from 30.15-45.21 µg/ml and from 267.37-630.41µg/ml, respectively. The $AUC_{(0-\alpha)}$ value at 31 mg/kg was approximately 10 times higher than that at 3 mg/kg. No apparent differences in the C_{max} values were observed between male and female animals. CDP870 was cleared with a half-life of approximately 200 hours (8.4 days) with a range of 137 to 252 hours. The pharmacokinetic parameters of individual animals are shown in the Table below.

INDIVIDUAL PHARMACOKINETIC PARAMETER ESTIMATES – CDP870 PLASMA CONCENTRATION (µg/mL)

DOSE (mg/kg)	MONKEY NUMBER	SEX	Cmax (µg/mL)	tmax (h)	ß (1/h)	t½ (h)	AUC (0-t) (µg.h/mL)	AUC (0-inf.) (µg.h/mL)	MRT (h)
3	1	MALE	30.15	36.00	0.00321	216.24	8530.87	9766.28	225.19
	2	MALE	31.05	48.00	0.00295	235.02	9451.70	11028.37	232.12
	5	FEMALE	45.21	24.00	0.00275	252.39	16813.72	17199.69	317.88
	6	FEMALE	36.72	35.98	0.00425	163.12	9059.36	9739.48	193.27
31	3	MALE	267.37	48.00	0.00309	224.26	94590.13	95107.80	321.83
	4	MALE	421.15	36.00	0.00507	136.67	110857.88	113805.61	199.44
	7	FEMALE	630.41	24.02	0.00389	178.24	112083.03	112553.61	238.86
	8	FEMALE	330.33	48.00	0.00330	209.98	127997.86	128294.74	319.99

The elimination phase of the drug ranged from 672 to 1677 hours in monkeys. The elimination phase in individual animals is summarized in the Table below.

ELIMINATION PHASE SPECIFICATION - CDP870 PLASMA CONCENTRATION (µg/mL)

DOSE (mg/kg)	MONKEY NUMBER	SEX	START OF THE ELIMINATION PHASE (h)	END OF THE ELIMINATION PHASE (h)	NUMBER OF DATA POINTS
3	1	MALE	36.00	672.13	9
	2	MALE	36.00	671.98	10
	5	FEMALE	36.00	1417.40	11
	6	FEMALE	35.98	671.85	10
31	3	MALE	36.00	1677.77	12
	4	MALE	36.00	671.63	10
	7	FEMALE	36.00	1417.08	11
	8	FEMALE	36.00	1677.58	11

Antibodies to CDP870 were detected in one male animal (out of 8 animals) receiving the 31 mg/kg dose. This animal had the shortest plasma half-life of 137 hours.

In summary, following subcutaneous administration of 3 and 31 mg/kg doses of CDP870 to monkeys, plasma concentrations of CDP870 increased in a dose-proportional manner. The C_{max} values were reached between 24 and 48 hours after administration, and ranged from 30.15-45.21 µg/ml and from 267.37-630.41µg/ml in monkeys receiving 3 and 31 mg/kg doses, respectively. No apparent differences in C_{max} values were observed between male and female animals. CDP870 was cleared with a half-life of approximately 200 hours (8.4 days) with a range of 137 to 252 hours. Antibody to CDP870 was detected in one (of 8) animal.

2.6.4.4 Distribution

Biodistribution of ^{125}I-labeled CDP870 in Rats (Study Report # 40000836).

Methods: The tissue distribution of ^{125}I-labeled CDP870 (hTNF 40 Fab;-40K PEG; specific activity, 0.16 µCi/µg) was studied in male Wistar rats following i.v. administration and compared with that of hTNF 40 IgG and Fab'. The same rats were injected with rat serum albumin (RSA) radiolabeled with [131]I (1.09 µCi/µg), 15 minutes prior to termination to determine whether the injection of 40 KD PEG conjugated to Fab' changed the blood distribution in the tissues and there was any evidence of plasma leakage. The animals were killed at various time points (3, 24, 48 and 144 hr post-administration) following i.v. administration of each component and the following tissues were excised: muscle, bone, lung, liver, spleen, kidney, colon and brain. The wet weight of the tissues were obtained, solubilized in KOH (except bone) and dissolved in 5 M HCl. The radioactivities of the samples were counted in a dual channel gamma counter (for Iodine 125 and Iodine 131).

Results: All three molecules were cleared from blood and tissues with time. Fab' levels in blood declined very rapidly and the levels rose in kidneys. CDP870 had similar tissue distribution and clearance to that of IgG. The kidney levels of Fab'-40K PEG were slightly higher than that of IgG at the 3 hour time point (kidney to blood ratios were 0.49 and 0.20, respectively). Besides kidney, all other tissues had similar distribution for these two molecules. More differences became

apparent at 144 hr post-administration due to slower blood clearance of IgG, and at this time period, a number of tissues had higher IgG levels than Fab'-40K PEG. However, at this time point, the actual levels were very low in all tissues. The tissue distribution of ^{125}I hTNF40.4 at different times is shown in the Figure below.

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ON ORIGINAL**

Biodistribution of 125-I hTNF40.4 in rats

IgG

Fab'-40K PEG

Fab'
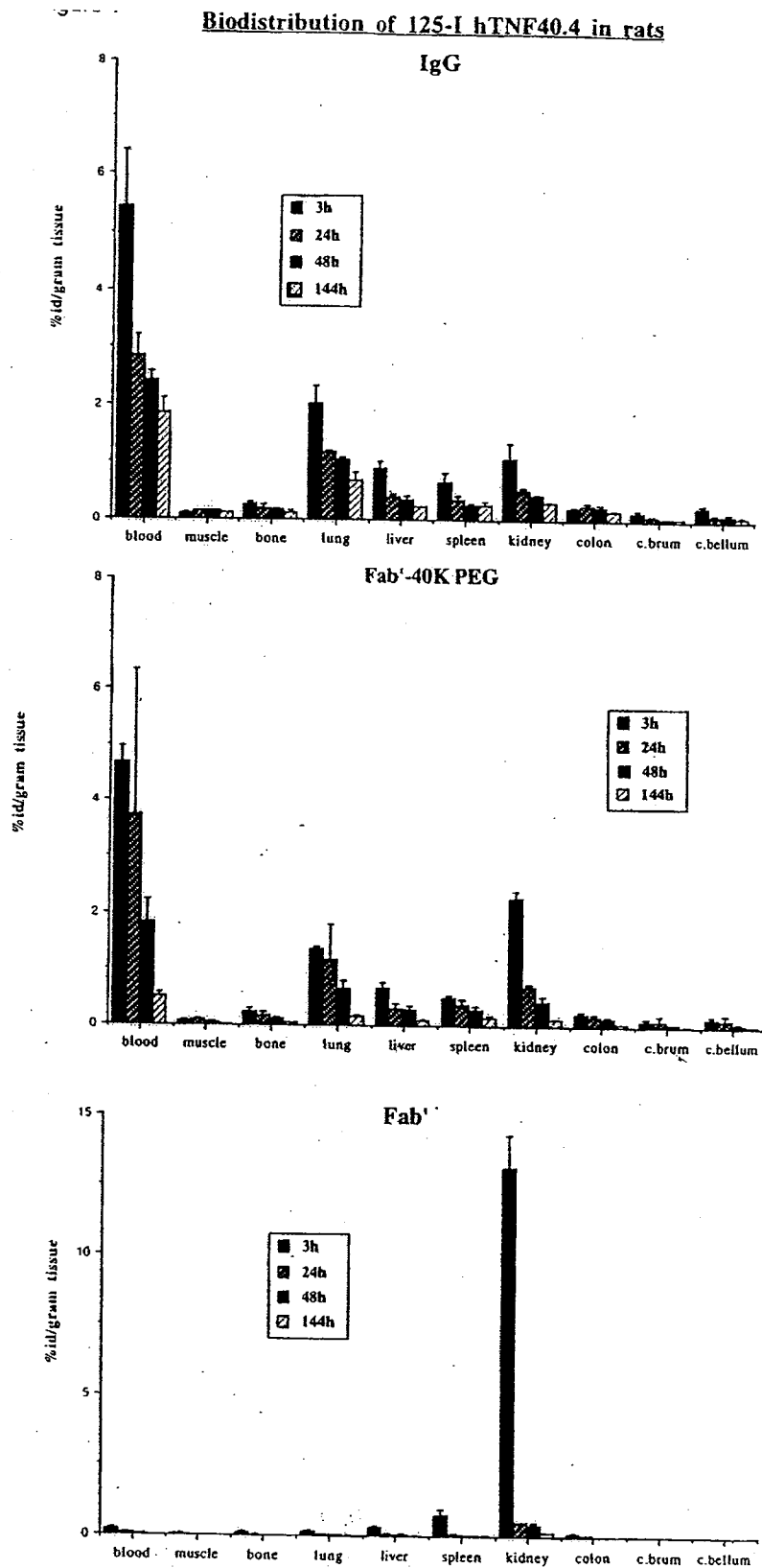
The RSA biodistribution with all three molecules was exactly the same indicating no changes in blood distribution and plasma leakage due to 40 KD PEG. The tissue levels of iodine 125 in the 4 rats that received both iodine 125 and iodine 131 were the same as that of 2 animals that received only iodine 125.

Thus, PEGylation of hTNF 40 Fab' with 40 K PEG did not change the antibody fragment's biodistribution. It created a molecule with a biodistribution similar to that of IgG. The 40 K PEG once conjugated to Fab' did not interfere with blood distribution and did not lead to plasma leakage.

2.6.4.5 Metabolism
No studies on the metabolism of CDP870 were conducted

2.6.4.5 Excretion

No studies to determine the excretion profiles of CDP870 were submitted.

2.6.4.7 Pharmacokinetic drug interactions

N/A

2.6.4.8 Other Pharmacokinetic Studies

Pharmacokinetics of ^{125}I-Labeled CDP870 in Rats (Study Report # 40000881).

Methods: The plasma clearance profile of iodine 125-labled hTNF40 Fab'-40K PEG (CDP870) was studied in rats following i.v. administration, and compared with that of hTNF 40 IgG and Fab'. Male Wistar rats were injected i.v. CDP870, hTNF 40 IgG and Fab' under light anesthesia. Blood samples were collected from the tail artery at 0.5, 2, 4, 6, 24, 48, 72, 144, 240 and 360 hours post-dose. The blood was weighed and the radioactivity was counted in a gamma counter. The results were expressed as % of injected dose per gram of blood.

Results: Following conjugation of 40 K PEG to the Fab' fragment, the plasma exposure (AUC) levels of the conjugated molecule was higher than that of Fab'. The Fab' fragment cleared very rapidly from the blood with $t_{1/2\alpha}$ and $t_{1/2\beta}$ values of 0.33 and 22.7 hr, respectively. When the 40 K PEG was conjugated to the Fab', there was a significant increase in the $t_{1/2}$ value, compared with Fab'. The $t_{1/2}$ and AUC values for Fab', Fab'-40K PEG and IgG are shown in the Table below.

	t1/2α(h)	t1/2β(h)	AUC(0-∞)*	AUC%of IgG
Fab'	0.33	22.7	329	3.7
Fab'-40K PEG	5.60	45.8	4465	51
IgG	5.81	104	8791	100

*the unit for AUC is h%dose

Thus, conjugation of 40 K PEG to Fab' produced a molecule that had higher exposure levels and longer half-lives.

Pharmacokinetics of CDP870 in Male and Female Rats Following Intravenous or Subcutaneous Administration (Study Report # 40001360)

Methods: The pharmacokinetics of CDP870 was determined in male and female rats following a single i.v. administration of 10 mg/kg or a single s.c. administration of 10 or 100 mg/kg doses. Blood samples were collected (3 animals/sex/time point) at 0, 0.33, 0.67, 1, 2, 4, 8, 24, 48, 96, 144, 192, 240 and 360 hr (15 days) following s.c. administration, and 0, 0.08, 0.25, 0.5, 1, 1.5, 2, 4, 8, 24, 48, 96, 144, 192, 240 and 360 hr following i.v. administration. The plasma concentrations of CDP870 were determined using an ELISA method.

Results: Following i.v. administration of a 10 mg/kg dose of CDP870 to rats, the maximum plasma concentrations (238 and 220 μg/ml in males and females, respectively) were reached in 30 minutes and 5 minutes in male and female rats, respectively. The AUC values in males and females were 8990 and 9150 μg.h/ml, with half-lives of 53 hr and 57 hr, respectively following i.v. administration. Thus, no apparent differences in the pharmacokinetic parameters were observed in male and female rats following i.v. administration of CDP870.

Following s.c. administration of a 10 mg/kg dose of CDP870 to rats, the C_{max} values were reached within 24 to 48 hours of dosing, with values of 27.5 and 22.4 μg/ml in males and females, respectively. The corresponding $AUC_{(0-\alpha)}$ values were 2360 and 3060 μg.h/ml, respectively. The estimated bioavailabilities in male and female rats were 26.3% and 33.4%, with half-lives of 71.7 and 71.3 hr, respectively.
Following s.c. administration of a 100 mg/kg dose of CDP870 in rats, the mean C_{max} values of 182 and 283 μg/ml in males and females, respectively, were reached in 24 hr following administration. The corresponding $AUC_{(0-\alpha)}$ values were 17100 and 29500 μg.h/ml, respectively. The bioavailability of CDP870 at this dose was estimated to be 19.0% in males and 32.2% in females. The half-lives were 75.6 and 54.9 hr in males and females, respectively. The mean plasma concentrations in male and female rats at different times following i.v. administration of a 10 mg/kg dose and s.c. administration of 10 and 100 mg/kg doses are shown in the Figures below. Intravenous Dosing:
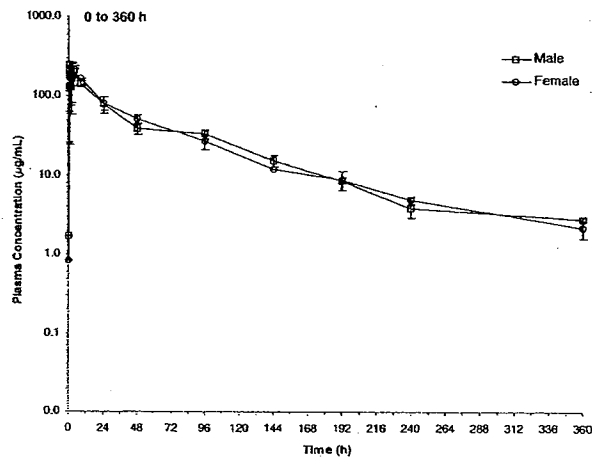
Subcutaneous Dosing:
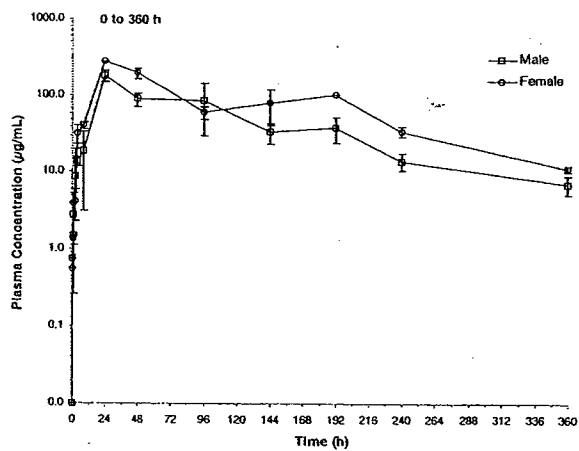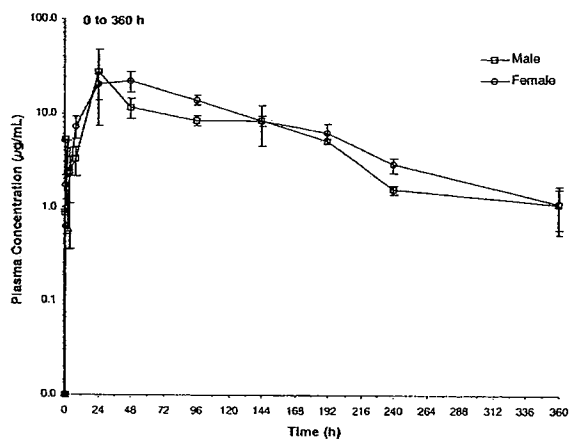
The pharmacokinetic parameters of CDP870 in male and female rats following i.v. and s.c. administration are shown in the Table below.

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Table 5.   Pharmacokinetic Parameters of CDP870 in Rats Following Intravenous or Subcutaneous Administration

PK Parameter	Male	Female	Overall
10 mg/kg Intravenous Administration			
$C_{5\,min}$ (μg/mL)	126	220	173
$T_{1/2}$ (h)	53.0	57.0	53.4
$AUC_{0-360\,h}$ (μg·h/mL)	8780	8980	8870
AUC_{0-inf} (μg·h/mL)	8990	9150	9060
CL (mL/min/kg)	0.0185	0.0182	0.0184
Vd_{ss} (mL/kg)	84.9	78.2	81.6
10 mg/kg Subcutaneous Administration			
C_{max} (μg/mL)	27.5	22.1	23.9
T_{max} (h)	24.0	48.0	24.0
$T_{1/2}$ (h)	71.7	71.3	73.6
$AUC_{0-360\,h}$ (μg·h/mL)	2250	2950	2600
AUC_{0-inf} (μg·h/mL)	2360	3060	2720
Vd_{ss} (mL/kg)	505	383	435
Bioavailibility (%)	26.3	33.4	30.0
100 mg/kg Subcutaneous Administration			
C_{max} (μg/mL)	182	283	223
T_{max} (h)	24.0	24.0	24.0
$T_{1/2}$ (h)	75.6	54.9	62.8
$AUC_{0-360\,h}$ (μg·h/mL)	16400	28700	22000
AUC_{0-inf} (μg·h/mL)	17100	29500	22800
Vd_{ss} (mL/kg)	594	415	529
Bioavailibility (%)	19.0	32.2	25.2

Thus, the s.c. bioavailability of CDP870 in female rats was slightly higher than that in male rats at 10 mg/kg, which was more marked at 100 mg/kg (approximately 1.7 times). Exposure levels of CDP870 in female rats were also higher than that of male rats following i.v. and s.c. administration of the drug.

Pharmacokinetics of CDP870 in Male and Female Rats Following Multiple Subcutaneous Dose Administration (Study Report # 40001359).

**Methods:** The pharmacokinetics of CDP870 was studied in male and female Sprague-Dawley rats following subcutaneous administration of 10 or 200 mg/kg (10 ml/kg) doses twice a week for 4 weeks. The doses were administered on day 1 and day 5 of each week. Blood samples were collected (from 3 animals/sex/time point) up to 96 hours following the first dose, immediately prior to each subsequent dose (days 8, 15, 19, 22 and 26) and up to 360 hours following the final dose administration. The sequence of blood collection from different groups was as follows:

Day 1:   Group A – 0, 8, 96 h post-dose, and Day 15 pre-dose;
               Group B – 2, 24 h post-dose, and Days 8 and 19 pre-dose;
               Group C – 4, 48 h post-dose, and Days 12 and 22 pre-dose.
Day 26:   Group A – 0, 8, 96 and 240 h post-dose;
               Group B – 2, 24, 144 and 360 h post-dose;
               Group C – 4, 48 and 192 h post-dose.

The plasma concentrations of CDP870 and antibodies to CDP870 were determined using ELISA methods.

Results: Following s.c. administration of a 10 mg/kg dose twice a week, plasma concentrations were similar in males and females on days 1 to 22; however, following day 26, the plasma concentrations of males were lower than that of females. The C_{max} values of CDP870 in male and female rats were 21.9 and 19.5 μg/ml on day 1 and 11.2 and 15.0 μg/ml on day 26, respectively. On day 26, the $AUC_{(0-\alpha)}$ values were higher in female rats (3090 μg.h/ml) than in male rats (2110 μg.h/ml). The apparent half-lives were 104 and 155 hr in males and females, respectively on day 26.

Following s.c. administration of a 200 mg/kg dose twice a week for 4 weeks, the C_{max} values were reached at 24 hours after dosing in both males and females on day 1. The C_{max} values in male rats were 405 and 620 μg/ml on day 1 and 248 and 443 μg.h/ml on day 26, respectively. The $AUC_{(0-\alpha)}$ values were 29300 and 37400 μg.h/ml and the $t_{1/2}$ values were 63.6 and 59.3 hr in males and females, respectively, on day 26. The C_{max} and AUC values decreased from day 1 to day 26, possibly because of increased clearance due to formation of anti-CDP870 antibodies on repeated dosing.

The estimated bioavailability of CDP870 following s.c. administration of a 10 mg/kg dose was 23.5% in males and 33.8% in females on day 26. A similarly higher bioavailability in females, as compared to males, was also observed following s.c. administration of a 200 mg/kg dose (16.3% in males and 20.4% in females). The pharmacokinetic parameters of CDP870 in male and female rats following administration of 10 and 200 mg/kg twice a week doses are shown in the Table below.

Table 4. Pharmacokinetic Parameters of CDP870 Following Multiple Subcutaneous Dose Administration

Dose (mg/kg)	PK Parameter	Male	Female	Overall
Study Day 1				
10	C_{max} (μg/mL)	21.9	19.5	20.6
	T_{max} (h)	48	24	48
	AUC_{0-96h} (μg•h/mL)	1580	1450	1520
	AUC_{0-Inf} (μg•h/mL)	3740	3080	3360
	$T_{1/2}$ (h)	124	94.7	107
	Vd_{ss} (mL/kg)	478	473	473
200	C_{max} (μg/mL)	405	620	513
	T_{max} (h)	24	24	24
	AUC_{0-96h} (μg•h/mL)	27900	32500	30200
	AUC_{0-Inf} (μg•h/mL)	47800	38700	40800
	$T_{1/2}$ (h)	68.0	31.8	43.4
	Vd_{ss} (mL/kg)	453	300	363
Study Day 26				
10	C_{max} (μg/mL)	11.2	15.0	12.1
	T_{max} (h)	48	2.0	48
	AUC_{0-360h} (μg•h/mL)	1900	2450	2190
	AUC_{0-Inf} (μg•h/mL)	2110	3090	2700
	$T_{1/2}$ (h)	104	155	139
	Vd_{ss} (mL/kg)	793	703	770
	Bioavailibility (%)	23.5	33.8	29.8
200	C_{max} (μg/mL)	248	443	316
	T_{max} (h)	8.0	24	24
	AUC_{0-360h} (μg•h/mL)	28900	36900	32900
	AUC_{0-Inf} (μg•h/mL)	29300	37400	33300
	$T_{1/2}$ (h)	63.6	59.3	60.5
	Vd_{ss} (mL/kg)	692	466	561
	Bioavailibility (%)	16.3	20.4	18.4

Anti-CDP870 antibodies were not detected in pre-dose samples collected on days 8, 15 and 22. Following the last dose, 7 of 24 samples collected at each dose tested as antibody positive. The anti-CDP870 antibody response seemed to increase following the final dose. In the 10 mg/kg group, males showed a higher incidence of antibody response than females on day 26. The maximum antibody levels in males (day 26, 360 hr) and females (day 26, 192 hr) were 8.08 and 5.05 units/ml, respectively. At 200 mg/kg, the maximum antibody levels in males and females were 1.62 and 5.99 units/ml, respectively.

In summary, following twice weekly s.c. administration of 10 and 200 mg/kg doses of CDP870 to male and female rats for 4 weeks, the bioavailability in females was slightly higher than that in males. On both day 1 and day 26, AUC values increased in a less than dose-proportional manner in male and female rats. The C_{max} values increased in an approximately dose-proportional manner in males, and in a greater than dose-proportional manner in females. The ant-CDP870 antibody response increased following the final dose of the drug.

Pharmacokinetic Compatibility of CDP870 Stages 1, 2 and 3 Materials Following Single Subcutaneous Administration to Male Cynomolgus Monkey (Study Report # 40001393).

Methods: The pharmacokinetic compatibility of CDP870 stages 1, 2 and 3 materials was studied in cynomolgus monkeys following s.c. administration of a single 10 mg/kg dose. Twelve male monkeys were divided into 3 groups and each group was subcutaneously administered a single dose of CDP870 stage 1, stage 2 or stage 3 at a dosing volume of 0.20 ml/kg. Blood samples were collected from a femoral vein of each animal prior to dosing, and at 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 168, 240, 336, 504, 672 and 840 hours post-dose. Plasma concentrations of CDP870 were determined by an ELISA method, and the pharmacokinetic parameters determined.

Results: CDP870 was slowly absorbed in cynomolgus monkeys following subcutaneous administration with mean t_{max} values ranging from 54 to 66 hours. After reaching the C_{max}, the plasma concentrations of the drug declined slowly, and the mean $t_{1/2}$ values ranged from 208 to 225 hours. The mean C_{max} values ranged from 107 to 124 µg/ml and the mean AUC $_{(0-840)}$ values ranged from 40074 to 44724 µg.hr/ml. No differences in the C_{max}, t_{max}, $t_{1/2}$ and AUC values were observed between stage 1, stage 2 or stage 3 preparations of CDP870. The pharmacokinetic parameters of CDP870 in cynomolgus monkeys following single s.c. administration of CDP870 stage 1, stage 2 and stage 3 are summarized in the Table below.

Mean Pharmacokinetic Parameters for CDP-870 in Cynomolgus Monkey Plasma

Dose Level (mg/kg)	Group	Test Substance		C_{max} (µg/mL)	T_{max} (Hours)	AUC_{0-840} (µg·hr/mL)	$AUC_{0-\infty}$ (µg·hr/mL)	$t_{1/2}$ (Hours)
10	1	CDP-870 Stage 1	Mean	107	66.0	40074	43146	208
			SD	10	36.0	3911	4549	24
			N	4	4	4	4	4
	2	CDP-870 Stage 2	Mean	124	54.0	44727	49024	225
			SD	25	23.0	5546	6405	25
			N	4	4	4	4	4
	3	CDP-870 Stage 3	Mean	112	60.0	40253	44003	218
			SD	11	13.9	4430	5794	34
			N	4	4	4	4	4

Plasma concentrations of CDP870 in the three groups of monkeys are shown in the Figure below.

Mean plasma concentrations (µg/mL) of CDP-870 in male cynomolgus monkeys

Legend:
—●— Group 1 (CDP-870, Stage 1)
—○— Group 2 (CDP-870, Stage 2)
—▼— Group 3 (CDP-870, Stage 3)
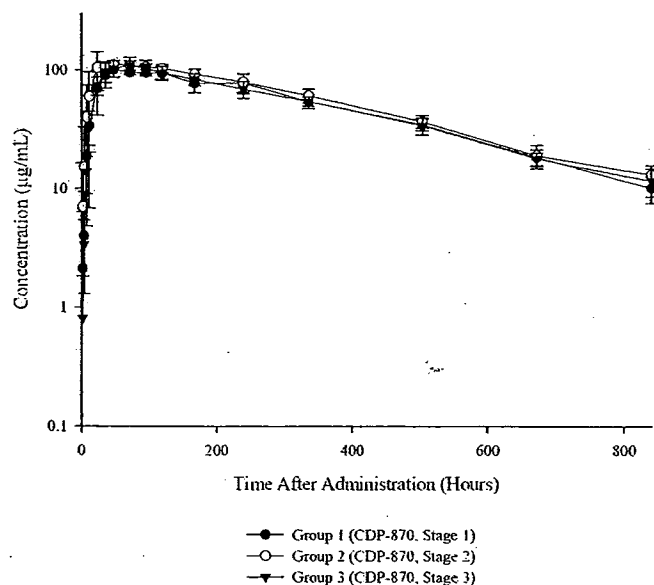
Thus, following s.c. administration of a single 10 mg/kg dose of CDP870 to cynomolgus monkeys, it was slowly absorbed and slowly eliminated from the blood. Pharmacokinetic parameters were comparable among different stages of CDP870 preparation.

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The Pharmacokinetics of Total Polyethylene Glycol (PEG)-Related Substances in Rat Plasma after Intravenous Administration of CDP870 (Study Report # 40001512).

Methods: Male Lewis rats (4/group) were administered intravenously 1, 10 and 100 mg/kg doses of CDP870. Blood samples were taken from the lateral tail vein at intervals of 28 days after administration of the dose, and plasma samples were analyzed by nuclear magnetic resonance (NMR) spectroscopy for the PEG portion of CDP870 and by enzyme-linked immunosorbent assay (ELISA) for the TNF-α binding portion. The NMR method is capable of detecting PEG while bound to the antibody fragment or after processing of the protein.

Results: PEG-related material was detected in all but one of the post-dose plasma collections from rats in the 10 mg/kg and 100 mg/kg groups. The levels in the 1 mg/kg group were low, and samples beyond day 10 from this group were not analyzed. Examination of the plasma samples by NMR showed an approximately bi-exponential profile. The profile of one rat from the 10 mg/kg group was different, and was excluded. Up to day 10, the half-life of PEG in plasma by NMR was close to that obtained from ELISA. However, the plasma concentrations of PEG from the NMR analysis were higher than that by ELISA at all time points (3.5-fold on day 1, 6.9-fold on day 10) and PEG elimination was slower after day 10 with longer half-lives. The pharmacokinetic parameters for PEG analysis by NMR spectroscopy in rat plasma are shown in the Table below.

Pharmacokinetic summary of PEG analysis of rat plasma by NMR quantitation

Dose (mg/kg)	1	10	100
Clearance (CV) (mL/day/kg)	4.8 (38%)	6.2 (11%)	8.1 (11%)
Half-life (CV) (day)	4.5 (70%)	6.6 (27%)	9.5 (23%)
Half-life day 1 to day 10 (CV) (day)	3.3 (74%)	2.9 (2%)	2.7 (7%)
Volume of distribution at steady state (CV) (mL/kg)	28 (67%)	36 (20%)	53 (7%)

Thus, the NMR method used for the detection and quantification of PEG in rat plasma was sensitive and could be applied to quantification of plasma samples from CDP870-administered animals. However, when quantifying PEG in plasma samples by the NMR method, it detects levels of all PEG-related material, and it is not possible to determine if the PEG is still conjugated as intact 40 KD PEG or broken down to smaller PEG units.

The Excretion of Polyethylene Glycol (PEG)-Containing Substances in Rat Urine after Intravenous Administration of CDP870 (Study Report # 40001513).

Methods: The study was conducted in male Lewis rats (4/group) which were administered single i.v. doses of 10 or 100 mg/kg CDP870. The animals were housed in metabolism cages for 28 days and urine and fecal samples were collected. PEG-containing drug-related material in the urine was detected by NMR spectroscopy. The NMR method is capable of detecting PEG both while bound to the antibody fragment and as a free moiety.

Results: The profile of urinary excretion with time was irregular but continued throughout the 28-day period. The mean cumulative excretion of PEG as a proportion of dose after 28 days was 21.0% (range 16.3 – 28.1%) at 10 mg/kg and 10.9% (range 8.8 – 12.5%) at 100 mg/kg. During week 4, the average daily % of doses excreted in the urine were 0.7% and 0.3% at 10 and 100 mg/kg doses, respectively.

The cumulative % dose of PEG excreted in the urine of rats following i.v. administration of 10 and 100 mg/kg doses of CDP870 are shown in the Tables below.

Table 11:1   Cumulative % of dose of PEG in urine after 10mg/kg iv administration CDP870

Animal	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14
Rat 5														
Rat 6														
Rat 7														
Rat 8														
n	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Average	0.2%	1.0%	1.7%	2.2%	3.2%	4.4%	5.4%	6.4%	7.2%	8.1%	8.8%	9.8%	10.6%	11.2%
SD	0.3%	1.5%	1.8%	2.3%	2.3%	2.4%	2.7%	2.8%	3.2%	3.4%	3.1%	2.7%	2.6%	3.0%
%CV	200.0%	144.1%	106.2%	105.8%	72.8%	53.7%	50.6%	44.7%	43.9%	41.6%	35.4%	27.2%	24.4%	26.4%

Animal	14-15	15-16	16-17	17-18	18-19	19-20	20-21	21-22	22-23	23-24	24-25	25-26	26-27	27-28
Rat 5														
Rat 6														
Rat 7														
Rat 8														
n	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Average	11.8%	11.9%	11.9%	13.1%	14.4%	15.4%	16.4%	17.3%	18.1%	18.7%	19.4%	20.1%	20.6%	21.0%
SD	3.0%	3.1%	3.1%	3.2%	3.4%	3.6%	3.7%	3.9%	4.2%	4.0%	4.8%	5.7%	6.0%	5.7%
%CV	25.6%	26.2%	26.2%	24.4%	23.8%	23.2%	22.9%	22.3%	23.2%	21.6%	24.9%	28.3%	29.2%	27.3%

Table 1.   Inhibition of P-gp Transport by CDP870 (FAB), Non-Pegylated Fab' Intermediate (NON), Ketoconazole (KET) and Verapamil (VER) in Caco-2 Cells

	Test Solution	Papp[1] AtoB	Papp[1] BtoA	Pr BtoA/AtoB
1	Digoxin	2.81 ± 0.07	12.0 ± 0.2	4.28 ± 0.06
2	FAB (1 µM)	3.34 ± 0.08	13.3 ± 0.1	3.99 ± 0.14
3	FAB (4 µM)	2.71 ± 0.07	12.4 ± 0.2	4.59 ± 0.09
4	NON (1 µM)	3.03 ± 0.06	12.7 ± 0.2	4.20 ± 0.08
5	NON (4 µM)	2.88 ± 0.01	11.6 ± 0.3	4.01 ± 0.09
6	KET (25 µM)	5.58 ± 0.09	8.63 ± 0.10	1.55 ± 0.04
7	KET (100 µM)	6.19 ± 0.03	6.71 ± 0.33	1.09 ± 0.06
8	VER (25 µM)	6.45 ± 0.01	6.53 ± 0.06	1.01 ± 0.01
9	VER (100 µM)	5.90 ± 0.05	5.66 ± 0.18	0.959 ± 0.029
10	Vinblastine	1.27 ± 0.07	8.13 ± 0.04	6.46 ± 0.41
11	FAB (1 µM)	1.39 ± 0.09	10.1 ± 0.3	7.35 ± 0.25
12	FAB (4 µM)	1.51 ± 0.04	11.4 ± 0.2	7.54 ± 0.11
13	NON (1 µM)	1.56 ± 0.07	9.94 ± 0.29	6.39 ± 0.09
14	NON (4 µM)	2.41 ± 0.05	10.7 ± 0.4	4.43 ± 0.19
15	KET (25 µM)	2.61 ± 0.11	5.77 ± 0.35	2.21 ± 0.08
16	KET (100 µM)	3.43 ± 0.13	5.28 ± 0.04	1.54 ± 0.05
17	VER (25 µM)	4.66 ± 0.44	5.70 ± 0.24	1.24 ± 0.08
18	VER (100 µM)	7.13 ± 0.41	5.53 ± 0.29	0.776 ± 0.014
19	Cyclosporine	1.09 ± 0.12	4.98 ± 0.05	4.73 ± 0.60
20	FAB (1 µM)	1.04 ± 0.13	4.86 ± 0.37	4.73 ± 0.29
21	FAB (4 µM)	0.615 ± 0.025	4.26 ± 0.28	6.92 ± 0.19
22	NON (1 µM)	0.823 ± 0.052	4.53 ± 0.32	5.50 ± 0.19
23	NON (4 µM)	0.721 ± 0.079	3.63 ± 0.16	5.13 ± 0.45
24	KET (25 µM)	0.713 ± 0.071	2.15 ± 0.06	3.09 ± 0.39
25	KET (100 µM)	0.766 ± 0.118	2.06 ± 0.28	2.72 ± 0.18
26	VER (25 µM)	1.19 ± 0.13	2.00 ± 0.20	1.70 ± 0.08
27	VER (100 µM)	1.65 ± 0.21	1.78 ± 0.25	1.08 ± 0.05
28	Mannitol	2.49 ± 0.14	2.71 ± 0.16	1.09 ± 0.00
29	FAB (1 µM)	2.81 ± 0.11	2.16 ± 0.12	0.769 ± 0.036
30	FAB (4 µM)	2.73 ± 0.04	2.32 ± 0.16	0.850 ± 0.059
31	NON (1 µM)	2.63 ± 0.06	2.30 ± 0.01	0.876 ± 0.025
32	NON (4 µM)	2.81 ± 0.06	2.15 ± 0.08	0.766 ± 0.039
33	KET (25 µM)	2.74 ± 0.11	2.13 ± 0.10	0.779 ± 0.058
34	KET (100 µM)	2.68 ± 0.15	2.33 ± 0.14	0.878 ± 0.084
35	VER (25 µM)	2.93 ± 0.10	2.39 ± 0.03	0.816 ± 0.034
36	VER (100 µM)	2.71 ± 0.11	2.50 ± 0.11	0.921 ± 0.018

[1] Papp values expressed in units of x10^{-6} cm/sec.

The inhibition of P-gp transport by CDP870, Non-Pegylated Fab' intermediate, ketoconazole and verapamil in MDCK+ ——— cells is shown in the Table below.

Table 2.   **Inhibition of P-gp Transport by CDP870 (FAB), Non-Pegylated Fab' Intermediate (NON), Ketoconazole (KET) and Verapamil (VER) in MDCK+⁻ Cells**

	Test Solution	P_{app}[1] AtoB	P_{app}[1] BtoA	P_r BtoA/AtoB
1	Digoxin	1.45 ± 0.08	7.83 ± 0.13	5.42 ± 0.26
2	FAB (1 µM)	1.51 ± 0.02	7.92 ± 0.14	5.25 ± 0.10
3	FAB (4 µM)	3.15 ± 0.65	8.36 ± 0.08	2.97 ± 0.75
4	NON (1 µM)	1.61 ± 0.10	7.90 ± 0.11	4.94 ± 0.26
5	NON (4 µM)	2.28 ± 0.33	8.46 ± 0.02	3.88 ± 0.57
6	KET (25 µM)	2.40 ± 0.10	2.77 ± 0.10	1.16 ± 0.03
7	KET (100 µM)	2.36 ± 0.16	2.28 ± 0.05	0.971 ± 0.044
8	VER (25 µM)	2.58 ± 0.36	2.23 ± 0.03	0.892 ± 0.100
9	VER (100 µM)	2.44 ± 0.03	2.20 ± 0.05	0.903 ± 0.020
10	Vinblastine	0.669 ± 0.051	8.39 ± 0.04	12.7 ± 0.9
11	FAB (1 µM)	0.701 ± 0.016	10.8 ± 0.2	15.4 ± 0.1
12	FAB (4 µM)	0.592 ± 0.012	11.1 ± 0.3	18.8 ± 0.9
13	NON (1 µM)	0.561 ± 0.008	8.83 ± 0.27	15.7 ± 0.4
14	NON (4 µM)	0.627 ± 0.033	10.2 ± 0.2	16.3 ± 1.1
15	KET (25 µM)	1.13 ± 0.04	3.21 ± 0.07	2.86 ± 0.14
16	KET (100 µM)	1.39 ± 0.07	2.44 ± 0.02	1.77 ± 0.07
17	VER (25 µM)	1.42 ± 0.03	1.99 ± 0.04	1.40 ± 0.05
18	VER (100 µM)	1.99 ± 0.01	2.63 ± 0.02	1.32 ± 0.01
19	Cyclosporine	0.503 ± 0.067	3.62 ± 0.12	7.38 ± 0.67
20	FAB (1 µM)	0.545 ± 0.065	3.43 ± 0.07	6.45 ± 0.67
21	FAB (4 µM)	0.478 ± 0.049	3.03 ± 0.09	6.47 ± 0.66
22	NON (1 µM)	0.596 ± 0.047	3.24 ± 0.02	5.50 ± 0.43
23	NON (4 µM)	0.764 ± 0.077	2.98 ± 0.08	3.97 ± 0.36
24	KET (25 µM)	0.546 ± 0.031	2.43 ± 0.04	4.48 ± 0.33
25	KET (100 µM)	1.55 ± 0.13	2.32 ± 0.23	1.50 ± 0.07
26	VER (25 µM)	1.48 ± 0.07	1.87 ± 0.04	1.27 ± 0.06
27	VER (100 µM)	1.98 ± 0.02	2.31 ± 0.20	1.17 ± 0.11
28	Mannitol	2.09 ± 0.04	1.73 ± 0.12	0.826 ± 0.052
29	FAB (1 µM)	1.98 ± 0.04	1.78 ± 0.02	0.898 ± 0.013
30	FAB (4 µM)	2.10 ± 0.08	1.58 ± 0.13	0.760 ± 0.081
31	NON (1 µM)	2.03 ± 0.14	1.91 ± 0.10	0.941 ± 0.022
32	NON (4 µM)	2.26 ± 0.36	1.68 ± 0.12	0.790 ± 0.154
33	KET (25 µM)	2.16 ± 0.12	1.61 ± 0.11	0.748 ± 0.050
34	KET (100 µM)	1.80 ± 0.11	2.07 ± 0.20	1.15 ± 0.04
35	VER (25 µM)	2.18 ± 0.06	1.69 ± 0.11	0.782 ± 0.063
36	VER (100 µM)	2.22 ± 0.12	1.73 ± 0.07	0.789 ± 0.072

[1] P_{app} values expressed in units of $\times 10^{-6}$ cm/sec.

The permeability ratios (P_r) for digoxin, vinblastine and cyclosporine in the MDCK +' cell system were significantly reduced by ketoconazole and verapamil at both concentrations. The P-gp mediated transport of digoxin and vinblastin were not affected by CDP870 or the Fab' intermediate at 1 and 4 μM concentrations, indicating that CDP870 and the Fab' intermediate are not P-gp inhibitors in this system. The P-gp antibody, MRK16 caused substantial reduction of P_r

of digoxin and vinblastine, however, there was no substantial change in Pr of cyclosporine in both Caco-2 and MDCK + ⌐ cell systems, suggesting that the cyclosporine assay may not be a good assay for these cell systems.

Thus, CDP870 or its Fab' intermediate did not reduce P-gp mediated transport of digoxin and vinblastine in Caco-2 and MDCK ⌐ cell monolayer systems, suggesting that these compounds are not P-gp inhibitors in these assay systems at the concentrations tested (1 and 4 μM).

2.6.4.9 Discussion and Conclusions

CDP870 (Certolizumab pegol) is a recombinant, humanized antibody Fab' fragment with specificity for human TNFα. It had a long half-life (6.1 days) in monkeys following i.v. administration. Following s.c. administration of a single dose in monkeys, the half-life was 8.4 days. However, the clearance of the drug can be increased in animals in which there is formation of anti-CDP870 antibodies. CDP870 had no specific affinity for any particular tissue. In rats, highest concentrations of the radioactivity were observed in kidneys following administration of a single i.v. dose. In humans, the peak CDP870 concentrations were attained between 54 and 171 hours following s.c. administration. The terminal elimination half-life ($t_{1/2}$) was approximately 14 days for all doses tested. Following s.c. administration to humans for 12 weeks, anti-CDP870 antibodies were detected in 5% (at 800 mg/4 week dose) to 67% (at 50 mg/4 week dose) of the subjects, depending on the doses administered. The presence of the antibody decreased the C_{max} and AUC by more than 50%. It was not a P-gp inhibitor in an *in vitro* assay using Caco cell monolayers. As CDP870 is not metabolized by the CYP450 enzyme system, it has no potential for metabolic drug-drug interactions.

2.6.5   PHARMACOKINETICS TABULATED SUMMARY

N/A

**APPEARS THIS WAY
ON ORIGINAL**

2.6.5   TOXICOLOGY

Study title: CDP870: Single Dose Intravenous Tolerance/Toxicity Study in the Monkey Followed by a 28-Day Observation Period.

Sponsor's ID # ARLE05K2606; Study Report #40000922 and 40000966

Conducting laboratory (and location if not Sponsor): ⸻

Dates of study initiation & completion: February 04, 1998 & June 24, 1998

GLP compliance: Yes

QA Report Yes (X) No ( )

Methods:

Three groups of cynomolgus monkeys (2 animals/sex/group; body weight, 1.75-2.45 kg) were administered CDP870 at a single intravenous dose of 50, 100 and 400 mg/kg. The dosing volume for all groups was 20.9 ml/kg and the administration rate was 4 ml/min via a cephalic vein. The animals were observed daily for clinical signs or overt toxicity. In addition, each animal was given a detailed physical examination once weekly. The body weights of the animals were recorded twice weekly and before necropsy. Bone marrow smears were prepared at necropsy. They were fixed in methanol and visually appraised by a hematologist. Blood samples collected prior to infusion, at the end of infusion, 24 hours after infusion and on days 4, 13 and 27 after infusion were analyzed for lymphocyte subpopulations CD3, CD4, CD8 and CD20. At the end of the 28-day observation period, the animals were sacrificed (Groups 1, 2 and 3 were sacrificed on days 29, 31 and 34, respectively). The weights of the following organs were recorded: Adrenals, brain, heart, kidneys, liver, mandibular lymph nodes, ovaries, pituitary, prostate, spleen, testes with epididymides, thyroids with parathyroids and trachea. Histopathological examinations of the following organs were conducted: Adrenals, brain, cecum, Colon, duodenum, eyes, femur with bone marrow, gall bladder, gross lesions, heart, ileum, injection sites, jejunum, kidneys, liver, lungs, mammary gland, lymph nodes (mesenteric and mandibular), esophagus, ovaries, pancreas, pituitary, prostate, salivary gland, sciatic nerves, skin, spinal cord (cervical, lumber, thoracic), spleen, sternum with bone marrow, stomach, testes with epididymides, thymus, thyroids with parathyroids, trachea, urinary bladder and uterus.

For toxicokinetic analysis, blood samples were withdrawn from each animal prior to infusion, at the end of infusion, and on days 2, 4, 6, 9, 13, 20 and 27 after infusion. Urine samples were collected at 12 to 24 hours after the end of infusion, and on days 6, 13, 20 and 27 after infusion. Levels of CDP870 in the plasma were assessed using a ⸻ ELISA consisting of a ⸻. Levels of antibodies to CDP870 were assessed using a ⸻ ELISA.

Results:

There was no untreated control group in the study. No treatment-related clinical signs were observed in any group and there were no mortalities. Treatment with CDP870 had no adverse effects on the body weights of the animals. The concentrations of the T and B lymphocyte markers CD3, CD4, CD8 and CD20 were reduced in all animals immediately following infusion. Mean CD3, CD4, CD8 and CD20 levels in male and female animals before and after infusion are shown in the Table below.

Dose Group	CD8		CD4		CD3		CD20	
Males								
	Before	After	Before	After	Before	After	Before	After
50 mg/kg	1108	891	1268	1025	2068	1706	1741	1472
100 mg/kg	2094	912	1408	777	2384	1354	721	540
400 mg/kg	3533	1836	4076	2407	6046	3512	1133	667
Females								
	Before	After	Before	After	Before	After	Before	After
50 mg/kg	1685	1324	1508	1156	2264	1967	1220	983
100 mg/kg	809	468	1011	675	1610	1060	677	488
400 mg/kg	2480	1029	2486	1323	3866	1911	1394	735

No treatment-related gross pathological changes were observed in any animal. Only microscopic finding observed was a minor cellulitis at the injection site of a low dose animal.

Toxicokinetics: CDP870 was detected in the plasma of all animals, and the concentrations increased in a dose-proportional manner. Maximum plasma concentrations occurred within 2 hours after the end of infusion, and were 1288.24, 2420.28 and 9008.52 µg/ml for 50, 100 and 400 µg/kg groups, respectively. CDP870 was eliminated with a mean half-life of 7.42 days (range, 6.2 – 9.3 days). There were no apparent differences between male and female animals. Anti-CDP870 antibodies were detected in one female monkey receiving the 50 mg/kg dose. The antibodies peaked on day 14 and then declined. The presence of antibodies did not appear to modify the pharmacokinetics of CDP870. The mean pharmacokinetic parameters are shown in the Table below.

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CDP870 DOSE (mg/kg)		Cmax (μg/mL)	Tmax (h)	T1/2 (hours)	T1/2 (days)	AUC (0-t) (μg.h/mL)	AUC (0-inf.) (μg.h/mL)
50 mg/kg CDP870	MEAN	1288.238	0.683	166.01	6.92	166748.233	178397.854
	SD	36.901	0.592	14.99	0.62	9200.551	7987.636
	MEDIAN	1289.020	0.670	162.19	6.76	164417.047	178241.190
	N	4	4	4	4	4	4
100 mg/kg CDP870	MEAN	2420.283	0.928	185.58	7.73	334072.617	370266.747
	SD	368.064	0.505	26.18	1.09	94376.586	106970.624
	MEDIAN	2331.740	1.180	191.59	7.98	319427.029	358702.548
	N	4	4	4	4	4	4
400 mg/kg CDP870	MEAN	9008.518	0.703	182.66	7.61	1227132.951	1361535.132
	SD	1780.847	0.616	28.84	1.20	306333.150	396752.992
	MEDIAN	9100.215	0.685	176.91	7.37	1140211.464	1226679.994
	N	4	4	4	4	4	4

Thus, a single intravenous administration of CDP870 to cynomolgus monkeys, at doses up to 870 mg/kg, was well tolerated. Treatment-related effects on B and T lymphocytes were observed at all doses. However, no control group was included in the study.

2.6.6.3 Repeat-dose toxicity

Study Title: <u>CDP870: 28-Day Toxicity Study by Intermittent Intravenous Administration in the Monkey, Followed by a 28-Day Treatment-Free Period</u>

Study no. ARLE05K2608 (Study Report # 40001074 and 40001020).

Conducting laboratory (and location if not Sponsor): —————————————————

Dates of study initiation & completion: February 27, 1998 and June 01, 1998.

GLP compliance: Yes

QA Report Yes (X) No ( )

Drug, Lot #, radiolabel (if applicable), and % purity: CDP870, Batch nos. 10012116/26; purity, 100%.

Formulation/vehicle: Acetate buffer was used to dilute the test article solution.

Methods: Groups of cynomolgus monkeys (5/sex/group) were intravenously administered 0, 50, 100 and 400 mg/kg doses of CDP870 once a week (on days 1, 8, 15 and 22). Four animals/sex/group were sacrificed after dosing for 28 days, and 1 animal/sex/group was left untreated for a 28-day recovery at the end of the 28-day treatment period.

Dosing:

Species/strain: Cynomolgus monkeys (Macaca fascicularis)

#/sex/group or time point (main study): 5 animals/sex/group were used in the study. Four animals/sex/group were sacrificed on day 28, and the remaining 1 animal/sex/group was allowed to recover for a 28-day treatment-free period.

Satellite groups used for toxicokinetics or recovery: 1 animal/sex was left untreated for a 28-day recovery period.

Weight: males – 1.8 to 2.5 kg.

Doses in administered units: CDP870 was administered at doses of 50, 100 and 400 mg/kg on days 1, 8, 15 and 22.

Route, form, volume and infusion rate: The doses were administered intravenously by means of an infusion pump. The dosing volume was 19 ml/kg.

Times at which Observations were made:

Clinical signs- The animals were observed daily for clinical signs and mortality. In addition, each animal was given a detailed physical examination once a week.

Body weights- Body weights were measured twice a week and before necropsy.

Food and water consumption- Food and water consumption was not measured; only qualitative data on food consumption was recorded.

Ophthalmoscopy: Ophthalmologic examinations were performed on all animals pre-dose and on day 23.

Electrocardiography: ECG recordings were performed on all animals before initiation of dosing and on days 1 and 22. Recordings were taken immediately before, once during and approximately 30 and 60 minutes after the end of the infusion period. Blood pressures were recorded pre-dose and on days 1 and 22.

Respiration rate was monitored visually pre-dose and on days 1 and 22.

Hematology: Blood samples for hematology were collected on days 1, 8, 15 and 22. Blood samples were analyzed for T-lymphocyte subpopulations, CD3, CD4, CD8 and subpopulations of monocyte and B-lymphocyte CD14 and CD20, respectively.

Clinical chemistry- Blood samples for clinical chemistry analyses were collected pre-dose and on days 1, 8, 15 and 22 prior to infusion, immediately after the end of infusion and 24 hours after the end of infusion.

Bone marrow smears were prepared at necropsy, fixed in methanol and examined.

Urinalysis- Urine samples were collected pre-dose and on days 1, 15 and 22.

Gross pathology- At the end of the dosing period, main study group animals were sacrificed on day 29 and the recovery group animals were sacrificed on day 57. A full macroscopic examination was performed and all lesions were recorded.

Organs weighed- The weights of the following organs were recorded.

Adrenals, brain, heart, kidneys, liver, mandibular lymph nodes, ovary, pituitary, prostate, spleen, testes plus epididymides, thyroid with parathyroid and uterus.

Histopathology- Following organs from all animals were fixed and examined microscopically.

Adrenals, brain, cecum, colon, duodenum, eyes with optic nerves, femur with bone marrow, gall bladder, gross lesions, heart, ileum, injection sites, jejunum, kidneys, liver, lungs, mammary gland, mandibular lymph nodes, mesenteric lymph nodes, esophagus, ovaries, pancreas, pituitary, prostate, salivary glands, sciatic nerve, skin, spinal cord (cervical, lumbar and thoracic), spleen, sternum with bone marrow, stomach, testes with epididymides, thymus, thyroids with parathyroids, trachea, urinary bladder and uterus.

Toxicokinetics- Blood samples were withdrawn on days 1, 8, 15 and 22 immediately prior to infusion, at the end of infusion period, 24 hours after end of infusion and on day 29. Levels of CDP870 were assessed using an ELISA. Antibodies to CDP870 were assessed using a ————————— ELISA.

Results:

Clinical signs: No treatment-related clinical signs were observed in any group.

Body weights: The mean body weights of the control male and female animals before initiation of dosing (day -4) were 2.04 ± 0.13 and 1.89 ± 0.07 kg, respectively. The weights of the males and females at the end of the dosing period (day 29) were 2.17 ± 0.14 and 1.98 ± 0.06 kg, respectively. No treatment-related changes in the body weight were observed in any group.

Ophthalmoscopy: No ophthalmologic abnormalities were observed in any of the animals.

Electrocardiography: Treatment with CDP870 had no effects on the heart rate or ECG parameters of animals in any group. No changes in the blood pressure or respiration rate were observed in any animal.

Hematology: Immediately following infusion, hemoglobin concentration, red blood cells count and packed cell volume were reduced in all groups. Twenty four hours after infusion, the values were slightly lower than values observed prior to infusion. The reductions of hemoglobin, red blood cells and packed cell volumes in the treatment group animals at 24 hours after infusion were slightly higher than that of controls. The % differences in these parameters between day 1 prior to infusion and day 22 twenty four hours after infusion are shown in the Table below.

Group and sex	% difference of Day 1 prior to infusion with Day 22 twenty four hours after infusion		
	Haemoglobin concentration	Red blood cell count	Packed cell volume
1M	-10	-10	-7
2M	-11	-11	-7
3M	-15	-16	-11
4M	-16	-17	-13
1F	-8	-9	-5
2F	-13	-14	-9
3F	-12	-14	-11
4F	-22	-22	-20

· The mean hemoglobin (g/dL), RBC (106/cmm) and packed cell volume (fL) levels were 13.3±0.5, 6.78±0.41 and 46.0±1.3 in males and 13.0±1.0, 6.81±0.60 and 54.2±3.0 in females, respectively.

These values returned to pretreatment levels at the end of the recovery period. In all groups, including controls, immediately following infusion, the number and percentage of neutrophils increased and lymphocytes decreased. The neutrophil and lymphocyte levels returned to pretreatment levels at 24 hours after infusion on days 1, 8 and 15. Examination of the bone marrow smears showed foamy macrophages in one high dose female animal.

Clinical chemistry: Slightly decreased alkaline phosphatase (ALP) levels were observed on days 8 (males- control 2022±248 IU/L, 6.3% increase; females – control 2140±708 IU/L, 20.4% increase), 15 (males- control 1941±277 IU/L, 13% increase; females – control 1914±375 IU/L, 20.4% increase) and 22 (males- control 2013±321 IU/L, 17.3% increase; females – control 2315±596 IU/L, 34.2% increase) in males and females receiving the high dose. At the end of the recovery period, ALP levels of all animals returned to pretreatment values.

Urinalysis: No treatment-related changes in the urinalysis parameters were observed in any group.

Gross Pathology: No treatment-related gross pathological changes were observed in any group.

Organ weight: The absolute and relative adrenal weights were higher for males from all treatment groups 35%, 13% and 59% at low, mid and high doses, respectively), and for females from low (59%) and high (45%) dose groups. However, the changes were not dose-dependent. The spleen weight of a high dose male animal was higher than other animals, and this animal showed splenic congestion/hemorrhage.

Histopathology: Histiocytic vacuolation at the injection site was observed in the high dose animals. The finding was characterized by vacuoles of various sizes within the cytoplasm of histiocytes located in perivascular connective tissue. The high and mid dose animals of both sexes had histiocytic vacuolation in the hemolymphoreticular tissue, notably in the splenic red pulp and in the medulary sinuses of the mandibular and mesenteric lymph nodes. Vacuolated histiocytes were also observed to a lesser extent in the bone marrow, thymus, carotid plexus and ovary. Histopathological changes observed in male and female monkeys are shown in the Table below.

Dose Group	Males				Females			
	Control	50 mg/kg	100mg/kg	400mg/kg	Control	50 mg/kg	100mg/kg	400 mg/kg
Spleen								
Histiocyte vacuolation	0/4	0/4	1/4	4/4	0/4	0/4	1/4	4/4
Thymus								
Cyst	1/4	0/4	2/4	1/4	1/4	0/4	2/4	3/4
Histiocyte vacuolation	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4
Mesenteric lymph nodes								
Histiocyte vacuolation	0/4	0/4	3/4	4/4	0/4	0/4	1/4	4/4
Mandibular lymph nodes								
Histiocyte vacuolation	0/4	0/4	2/4	4/4	0/4	1/4	1/4	4/4
Brain								
Histiocyte vacuolation	0/4	0/4	0/4	3/4	0/4	0/4	1/4	2/4
Ovary								
Histiocyte vacuolation	--	--	--	--	0/4	0/4	1/4	4/4

At the end of the treatment period, histiocytic vacuolation was still detectable, but to a lesser extent suggesting a partial recovery during the treatment-free recovery period.

Toxicokinetics: CDP870 was detected in the plasma of all animals receiving the drug, and the plasma concentrations increased in a dose-dependent manner. The mean plasma concentrations at the end of the first infusion were 1032, 1756 and 6924 µg/ml in animals receiving 50, 100 and 400 mg/kg doses, respectively. The mean plasma concentrations at the end of the infusion were 1558, 2800 and 9825 µg/ml, respectively. In 5 of 6 animals retained for a further 28 days, CDP870 was cleared with an elimination half-life of between 6.6 and 9.0 days. In the other animal (50 mg/kg), CDP870 was eliminated with a half-life of 3.0 days, and the increased clearance in this animal coincided with the detection of antibodies in this animal. The plasma concentrations of CDP870 prior to infusion and at different times in animals receiving 50, 100 and 400 mg/kg doses are shown in the Table below.

TABLE 1
PAGE 2 OF 2
CDP870 PLASMA CONCENTRATIONS - SUMMARY STATISTICS (µg/mL)

SCHEDULED TIME AFTER FIRST INFUSION			CDP870 DOSE (mg/kg)		
	CONTROL		50	100	400
14 DAYS POST 1ST INFUSION (END-3RD INFUSION)					
MEAN	0.44		1473.88	2748.92	9434.04
95% CI	0.386,	0.504	1380.984, 1573.015	2434.203, 3104.326	8103.320, 10983.300
N	10		10	10	10
21 DAYS POST 1ST INFUSION (PRE-4TH INFUSION)					
MEAN	0.47		528.96	1043.33	3413.43
95% CI	0.374,	0.585	481.696, 580.856	877.447, 1240.569	2946.637, 3954.165
N	10		10	10	10
21 DAYS POST 1ST INFUSION (END-4TH INFUSION)					
MEAN	0.45		1557.77	2799.97	9824.84
95% CI	0.364,	0.559	1446.404, 1677.705	2496.548, 3140.266	8733.912, 11052.023
N	10		10	10	10
28 DAYS POST 1ST INFUSION (7 DAYS POST 4TH INFUSION)					
MEAN	0.49		534.07	1041.36	3797.31
95% CI	0.410,	0.584	419.802, 679.436	865.146, 1253.470	3049.966, 4727.780
N	10		10	10	10

Note: The geometric mean and 95% confidence interval have been presented.
The limit of quantification was set to _____

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The pharmacokinetic parameters of individual recovery animals are shown in the Table below.

TABLE 2
PAGE 1 OF 1
INDIVIDUAL PHARMACOKINETIC PARAMETER ESTIMATES - CDP870 PLASMA CONCENTRATIONS (µg/mL)

35

CDP870 DOSE (mg/kg)	MONKEY NUMBER	C max (µg/mL)	T max (hours)	ß (h⁻-1)	T1/2 (hours)	T1/2 (days)	AUC (0-t) (µg.h/mL)	AUC (0-inf.) (µg.h/mL)
50	6	1912.76	504.55	0.00439	158.04	6.6	761943.17	771519.28
	26	1582.03	335.97	0.00965	71.82	3.0	613367.67	619033.56
100	11	2518.09	504.15	0.00401	172.83	7.2	932364.06	944429.53
	31	3539.46	504.17	0.00366	189.62	7.9	1846538.18	1890388.84
400	16	9153.71	504.17	0.00360	192.70	8.0	4464817.96	4547290.80
	36	10474.24	335.00	0.00322	215.00	9.0	5619686.26	5774906.52

Note: The limit of quantification was set to ⎯   13NOV98:17:08

Antibodies to CDP870 were detected in three other animals at low levels (<1.0 units/ml) which peaked at 14 to 21 days after the first infusion and then declined. In these 3 animals, the plasma levels or clearance of CDP870 was not affected by the antibodies. The remaining 26 (of 30) monkeys had no detectable antibodies to CDP870. The mean anti-CDP870 antibodies in different groups are summarized in the Table below.

TABLE 4
PAGE 1 OF 1
ANTI-CDP870 IMMUNE RESPONSE - SUMMARY STATISTICS (Units/mL)

SCHEDULED TIME AFTER FIRST INFUSION		CONTROL		CDP870 DOSE (mg/kg)					
				50		100		400	
PRE-1ST INFUSION									
	MEAN	0.30		0.30		0.30		0.30	
	95% CI	0.300,	0.300	0.300,	0.300	0.300,	0.300	0.300,	0.300
	N	10		10		10		10	
7 DAYS POST 1ST INFUSION (PRE-2ND INFUSION)									
	MEAN	0.30		0.30		0.30		0.30	
	95% CI	0.300,	0.300	0.300,	0.300	0.300,	0.300	0.300,	0.300
	N	10		10		10		10	
14 DAYS POST 1ST INFUSION (PRE-3RD INFUSION)									
	MEAN	0.30		0.30		0.35		0.30	
	95% CI	0.300,	0.300	0.300,	0.300	0.276,	0.444	0.300,	0.300
	N	10		10		10		10	
21 DAYS POST 1ST INFUSION (PRE-4TH INFUSION)									
	MEAN	0.30		0.30		0.36		0.30	
	95% CI	0.300,	0.300	0.300,	0.300	0.273,	0.480	0.300,	0.300
	N	10		10		10		10	
28 DAYS POST 1ST INFUSION (7 DAYS POST 4TH INFUSION)									
	MEAN	0.30		0.30		0.33		0.30	
	95% CI	0.300,	0.300	0.300,	0.300	0.287,	0.368	0.300,	0.300
	N	10		10		10		10	

Note: The geometric mean and 95% confidence interval have been presented.
The limit of quantification was set to ⎯ nits/mL.   01DEC98:17:46

Summary: In the 28 –day intravenous toxicity study with CDP870 in cynomolgus monkeys, the drug was administered at weekly intervals at doses of 0, 50, 100 and 400 mg/kg. There were no mortalities or clinical signs at any dose. Treatment group animals had decreased hemoglobin, RBC and packed cell volume immediately following infusion and continued throughout the treatment period. The hematological parameters returned to the pre-treatment values at the end of the recovery period. Vacuolated histiocytes were observed at the injection sites of the high dose animals. The high and mid dose animals of both sexes had histiocytic vacuolation in the hemolymphoreticular tissue, notably in the splenic red pulp and in the medulary sinuses of the mandibular and mesenteric lymph nodes. Vacuolated histiocytes were also observed to a lesser extent in the bone marrow, thymus, carotid plexus and ovary. The 50 mg/kg/week dose was the no effect dose, and the target organs of toxicity were the hemolymphoreticular system, carotid plexus and the ovary.

Study Title: <u>CDP870- 13- and 26- Week Subcutaneous Toxicity Study in the Cynomolgus Monkey Followed by 13-Week Treatment-Free Periods after 13 and 26 Weeks of Treatment</u>

Study no. 767/013 RE (Study Report # 40001545 and 40001077).

Conducting laboratory (and location if not Sponsor):

Dates of study initiation & completion: September 23, 1999 and February 15, 2001

GLP compliance: Yes

QA Report Yes (X) No ( )

Drug, Lot #, radiolabel (if applicable), and % purity: CDP870, Batch nos. 98HM464-00S-CON, CELf10 and CELf008; purity, 100%.

Formulation/vehicle: Acetate buffer was used to dilute the test article solution.

Methods: The study consisted of two phases. Main study group animals were sacrificed following 13 or 26 weeks of dosing, while in both groups there was a 13-week recovery period. The study design is summarized in the Table below.

Group/ Treatment	Dose level	Dose Concentration	Number of animals [1]							
			End of 13 week treatment period		Week 26 End of treatment-free period after 13 weeks of treatment		End of 26 week treatment period		Week 39 End of treatment-free period after 26 weeks of treatment	
	(mg/kg/week)	(mg/ml)	Males	Females	Males	Females	Males	Females	Males	Female
1. Control	0	0	3	3	2	2	3	3	2	2
2. Low dose	10	20	4	5	2	2	4 [2]	4	2	2
3. High dose	100	200	4	4	2	2	4 [2]	4 [2]	2	2

All animals were treated at a dose volume of 0.5 ml/kg/administration.

[1] Number of animals killed at the end of the mentioned periods.

[2] This number does not include the following animals which were killed and necropsied on day 127 (week 19) of the study (after 18 administrations) and all terminal parameters (see sections 5 and 6) were measured.

- Group 2 male no. 2231,
- Group 3 male no. 2257,
- Group 3 female no. 2266.

These animals were killed as a consequence of the limited quantity of remaining test article and were selected based on body weight.

The selection of the animals necropsied after 13 administrations and those continuing to the end of the study (either with or without treatment) was based on the results of the analysis of anti-CDP 870 antibodies after 9 administrations.

- Group 1 animals (control) received the acetate buffer.

Dosing:

Species/strain: Cynomolgus monkeys (Macaca fascicularis)

#/sex/group or time point (main study): 3 animals/sex in the control groups; 4 animals/sex in the treatment groups, with the exception of 5 females in the 10 mg/kg, 13-week treatment group.

Satellite groups used for toxicokinetics or recovery: 2 animals/sex were used for a 13-week recovery period after treatment for 13 or 26 weeks.

Age: 2 to 3 years

Weight: Males – 1.9 to 3.3 kg

Females – 1.7 to 3.3 kg

Doses in administered units: CDP870 was administered at subcutaneous doses of 10 and 100 mg/kg, once a week.

Route, form, volume and infusion rate: The doses were administered subcutaneously at a dosing volume of 0.5 ml/kg.

Times at which Observations were made:

Clinical signs- The animals were observed twice daily for clinical signs and mortality.

Body weights- Body weights were measured once a week.

Food consumption - Food consumption of individual animal was measured daily.

Ophthalmoscopy: Ophthalmologic examinations of all animals were conducted pre-dose and following administration of the doses in weeks 12, 18 and 25.

Electrocardiography: ECG recordings of the animals were performed pre-dose and following administration in weeks 12, 18 and 25.

Hematology- Blood samples for hematology examinations were collected pre-dose, and in weeks 5, 10, 17, 21 and 34.

Clinical chemistry- Blood samples for clinical chemistry analyses were collected pre-dose, and in weeks 5, 10, 17, 21 and 34.

Urinalysis- Urine samples were collected pre-dose, and in weeks 5, 10, 17, 21 and 34.

Gross pathology- Two to three days after the 13th administration (week 13 sacrifice) or after the 26th administration (week 26 sacrifice), the designated animals were sacrificed and necropsies performed. The recovery group animals were necropsied during week 26 or 39.

Organs weighed- The weights of the following organs were recorded.

Adrenals, brain, heart, kidneys, liver, ovaries, pituitary gland, spleen, testes, and thyroid with parathyroid glands.

Histopathology- Histopathological examinations of the following organs from all animals were conducted: adrenal glands, sternum with bone marrow, bronchi, brain, cecum, colon, duodenum, epididymides, eyes, gall bladder, heart, ileum, injection sites, jejunum, kidneys, liver, lungs, lymph nodes (mesenteric, mandibular), mammary gland, esophagus, optic nerves, ovaries, pancreas, pituitary gland, prostate, salivary gland, seminal vesicle, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid gland, trachea, urinary bladder, uterus and all gross lesions.

The histological slides from the control and the high dose animals were peer reviewed by an expert. As a result, the histopathological examination was repeated for several tissues (target organs) from all animals. These organs include injection sites, brain, adrenal, mesenteric and mandibular lymph nodes, uterus, urinary bladder and spleen.

Toxicokinetics- Blood samples for toxicokinetic analysis and measurement of anti-CDP870 antibodies were collected on days 1, 29, 57, 78, 106, 134 and 162 (at the time of administration of 1st, 5th, 9th, 12th, 16th, 20th and 24th administration), during the recovery period (weeks 17, 21, 30 and 34) and just before necropsy. The concentrations of CDP870 in the plasma were measured using an ELISA method. The concentrations of antibodies to CDP870 were measured using a ⸻ ELISA method.

Results:

Clinical signs: No treatment-related clinical signs were observed in any group. There were no unscheduled deaths.

Body weights: The body weights of the control male and female animals before initiation of dosing (day - 6) were 2.49 ± 0.40 and 2.34 ± 0.42 kg respectively. The weights of the males and females at the end of the 13-week dosing period were 2.43 ± 0.40 and 2.46 ± 0.44 kg respectively. Treatment with CDP870 was not associated with any changes in the body weights of the animals.

Food Consumption: the mean food consumptions of the male and female animals on day 1 were 93 ± 14 and 100 ± 1 g/animal/day, respectively. Food consumption was similar in all groups during the dosing and recovery periods.

Ophthalmoscopy: No ophthalmologic abnormalities were observed in any group.

Electrocardiography: No treatment-related effects on the ECG or cardiovascular parameters were observed in any group.

Hematology:

3-month treatment: On day 29/31, six (three 10 mg/kg and three 100 mg/kg) treated animals had higher WBC counts (with increased proportion of neutrophils) compared with concurrent controls (the mean WBC and neutrophil levels of control males on Day 29 were 9.2±1.9 and 3.9±2.3 k/mm^3, respectively; there were 12% and 10% increases in WBC, and 49% and 69% increases in neutrophil levels at 10 and 100 mg/kg, respectively; the mean WBC and neutrophil levels of control females were 10.5±3.6 and 5.4±2.8 k/mm^3, respectively; there were 7.8% increase in WBC and 13% increase in neutrophil at the 100 mg/kg dose). On day 85, the mean platelet count of males treated with the 100 mg/kg dose was slightly higher than that of controls. The mean platelet count of the control males on day 85 was 387±72 k/mm3, and there was an 18% increase in the platelet levels at this time.

On day 29, the mean CD2, CD4 and CD8 cell counts of males treated with the 100 mg/kg dose were lower than that of controls (38%, 37% and 39%, respectively).

At the end of the recovery period, no differences in the hematological parameters were observed between the control and the treatment groups.

6-month treatment: One male receiving 10 mg/kg and another male receiving the 100 mg/kg doses had higher total WBC levels on day 113. One female animal receiving the high dose also had similar changes. The proportion of neutrophils was also elevated in these animals. The mean WBC and neutrophil levels of the control male animals were 11.1±1.1 and 2.1±0.4 k/mm^3 and those of control females were 9.6±3.7 and 4.3±3.5 k/mm^3, respectively, at this time.

Clinical chemistry: No treatment-related changes in the clinical chemistry parameters were observed in any group.

Gross Pathology: Dark areas at the injections sites were observed in the high dose males, and 10 and 100 mg/kg females.

Organ weight: Treatment group males and females had higher weights of the thyroid gland (males, 50% and 49%; females, 25% and 38% at 10 and 100 mg/kg, respectively). Males receiving the 100 mg/kg dose