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APPLICATION NUMBER:

125057/0

PHARMACOLOGY REVIEW(S)

Pharmacology/Toxicology Review

BLA: 125057
Sponsor: Abbott Laboratories, Abbott Park, Illinois
Product: Adalimumab (LU 200134), Anti-Tumor Necrosis Factor α (TNF-α IgG1 monoclonal antibody)
Formulation: Each single use vial contains the following:

[redacted]	[redacted]	[redacted]
Adalimumab	40	Active substance
Sodium chloride	4.93	
Monobasic sodium phosphate dihydrate	0.69	
Dibasic sodium phosphate dihydrate	1.22	
Sodium citrate	0.24	
Citric acid monohydrate	1.04	
Mannitol	9.6	
Polysorbate 80	[redacted]	
Water for injection	---	

Indication: The proposed indication for adalimumab is for reducing the signs and symptoms and inhibiting the progression of structural damage in adult patients with moderately to severely active rheumatoid arthritis (RA) who have an inadequate response to one or more DMARDS.

Introduction: TNF-α is a cytokine produced mainly by mononuclear phagocytes that has many effects in the immune response. Its effects include the following: [1] stimulating the recruitment of neutrophils and monocytes to sites of infection and activating them to eliminate microbes, [2] stimulating vascular endothelial cells to express adhesion molecules, [3] stimulating endothelial cells and macrophages to secrete cytokines [4] increasing hepatic synthesis of acute phase proteins, and [5] acting on the hypothalamus to induce fever. TNF-α is one of several cytokines referred to as pro-inflammatory. There are two distinct receptors for TNF-α, TNF-R1 (55 kD) and TNF-R2 (75 kd). These receptors are found on almost all cell types.

In addition to playing a beneficial role in the normal function of the immune system, TNF-α has detrimental effects. RA is a chronic systemic inflammatory disease, which principally affects joints, leading to synovitis. A number of inflammatory mediators, including TNF-α have been implicated in this disease process. TNF-α and other cytokines can be detected in the synovial fluid of joints affected by rheumatoid arthritis. Based on its role in the inflammatory process, TNF-α has been considered a good therapeutic target in the treatment of RA. To date there are two biotechnology-derived, anti-TNF-α products approved for the treatment of RA, Remicade® (infliximab), a chimeric anti-TNFα monoclonal antibody, and Enbrel®, a p75 TNF-R Fc fusion protein.

Adalimumab differs from Remicade® in that it is human derived (based on human sequences) as opposed to being chimeric. Abbott developed adalimumab under IND 7627, and they submitted the BLA in March 2002. The types of studies that Abbott submitted to support the safety of adalimumab from the pharmacology/toxicology

perspective include the following: relevant publications from the open scientific literature, and reports of pharmacology, pharmacokinetics, and toxicology (general toxicology, reproductive toxicology, and genotoxicity) studies conducted with adalimumab. In this review of these studies adalimumab will be referred to LU 200134.

During the course of development, the manufacturing process for LU 200134 was modified. As shown in the table below, there were four major phases in the manufacturing process for adalimumab.

LU 200134 Batch #	Process Name
AFP603 to AFP707	
AFP750 to AFP808	
AFP809 to AFP15B	
AFP02C to current	

This review is divided into three sections, pharmacology, pharmacokinetics, and toxicology.

PHARMACOLOGY

This section of this review is divided into four parts, [1] Primary Pharmacodynamics [2] Secondary Pharmacodynamics, [3] Efficacy in Animal Models of Arthritis, [4] Safety Pharmacology, [5] Tissue Cross-Reactivity, and [6] Immunogenicity. The study reports that the sponsor submitted to address each of these areas are listed and summarized/reviewed below.

►Primary Pharmacodynamics

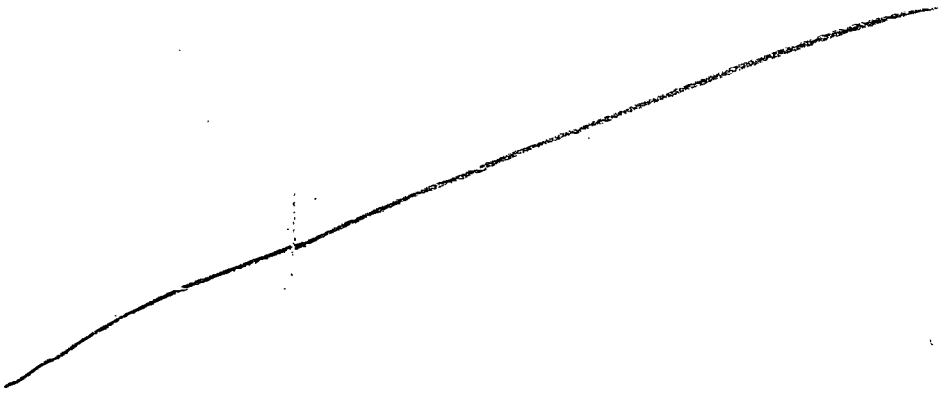
All of the studies listed below, except for the last study, BBC/M 0001, were conducted with process development batches of LU 200134. Material from these batches was not used in the clinical trials. Study BBC/M 0001 was conducted using two different production batches of LU 200134. Additional information on these two batches is provided below in the discussion of this study.

BBC/I 9602	Characterization of LU 200134: Recombinant human TNFα interaction kinetic rate constant by
BBC/I 9603	Inhibition of ^{125}rh TNFα binding to TNFα receptors by LU 200134
BBC/I 9606	Neutralization of rhTNFα in murine — bioassay by LU 200134
BBC/I 9605	Inhibition of recombinant TNFα induced expression of adhesion molecules on human umbilical vein endothelial cells by LU 200134
BBC/I 9609	Determination of TNF species specificity of LU 200134 in the — cytotoxicity assay
BBC/I 9604	Determination of the cytokine specificity of LU 200134 by a competitive binding assay
BBC/I 9608	Neutralization of rhTNFα induced lethality in D-galctosamine sensitized C57BL/6 mice by LU 200134
BBC/I 9612	Inhibition of recombinant human-induced rabbit pyrexia by LU 200134
BBC/M 0001	Comparison of LU 200134 — and —

	batches in preclinical characterization assays and animal models

Report N°: BBC/I 9602
Title: Characterization of LU 200134: Recombinant human TNFα interaction kinetic rate constant by
Nonclinical Review Volume: 3/15
Adalimumab Review Volume: 23/986
Study Facility: BASF Bioresearch Corporation, Immunology Department, Worcester, MA
Batch N°: AF504A-STD
Report Date: October 8, 1997

1. **Methods:** In this study, molecular kinetics interaction between LU 200134 and rhTNFα were quantitatively analyzed using technology, which



2. **Results:** The mean k_d and k_a were 8.81×10^{-5} and 1.91×10^{5}, resulting in a K_d of $6.09 \times ^{-10}$M.

Report N°: BBC/I 9603
Title: Inhibition of ^{125}rh TNFα binding to TNFα receptors by LU 200134
Nonclinical Review Volume: 3/15
Adalimumab Review Volume: 23/986
Study Facility: BASF Bioresearch Corporation, Worcester, MA
Batch N°: AF504A-STD
Report Date: October 8, 1997

1. **Methods:** The ability of LU 200134 to inhibit the binding of ^{125}I-rhTNFα to TNFα receptors on the surface of human cells was investigated using the cell line. The cells were incubated with a constant amount of ^{125}I TNFα with or without serial dilutions of LU 200134, human IgG_1, or rhTNFα. At the end of the incubation period, the mixtures were centrifuged. The free ^{125}I-rhTNFα was removed and that contained in the pellet analyzed for radioactivity using a gamma counter.

2. **Results:** LU 200134 inhibited the binding of ^{125}I-rhTNFα to receptors on human — cells with an IC_{50} of 1.56 ± 0.12 e – 10.

Report N°: BBC/I 9606
Title: Neutralization of rhTNFα in murine — bioassay by LU 200134
Nonclinical Review Volume: 3/15
Adalimumab Review Volume: 23/986
Study Facility: BASF Bioresearch Corporation, Worcester, MA
Batch N°: AF504A-STD
Report Date: October 8, 1997

1. **Methods:** L929 cells were incubated with 500 pg/mL of rhTNFα in the presence and absence of varying concentrations of LU 200134. Human IgG1 served as a negative control. The experiment was conducted three times to determine the IC_{50} value.

2. **Results:** In all three assays, LU 200134 inhibited the rhTNF-α-induced cytotoxicity in a dose dependent manner. The IC_{50} value for the three experiments combined was 1.25 ± 0.01 e-10.

Report N°: BBC/I 9605
Title: Inhibition of recombinant TNFα induced expression of adhesion molecules on human umbilical vein endothelial cells by LU 200134
Nonclinical Review Volume: 3/15
Adalimumab Review Volume: 23/986
Study Facility: BASF Bioresearch Corporation, Worcester, MA
Batch N°: AF504-STD
Report Date: October 8, 1997

1. **Methods:** The ability of LU 200134 to inhibit rhTNFα-induced expression of ELAM-1, VCAM-1, and ICAM-1 was investigated in human umbilical vein endothelial cells. The quantity of expressed adhesion molecules was measured using mouse anti-ELAM-1, VCAM-1, or ICAM-1 followed by ^{125}I-labeled sheep anti-mouse antibody. Unrelated human IgG1 was used as a control.

2. **Results:** The IC_{50} values obtained in this study are shown in the table below. LU 200134 inhibited TNFα – induced expression of all 3 adhesion molecules in a dose dependent manner.

1.85 ± 0.14 e – 10	2.17 ± 0.46 e – 10	1.01 ± 0.01 e – 10

Report N°: BBC/I 9609
Title: Determination of TNF species specificity of LU 200134 in the — cytotoxicity assay
Nonclinical Review Volume: 3/15

Adalimumab Review Volume: 23/986
Study Facility: BASF Bioresearch Corporation, Worcester, MA
Batch N°: AF504A-STD
Report Date: October 8, 1997

1. **Methods:** The purpose of this study was to test the ability of LU 200134 to neutralize TNFα-induced cytotoxicity *in vitro* using — cells. TNFα was obtained from nine species: baboon, canine, chimpanzee, cynomolgus monkey, human, marmoset, porcine, and rhesus monkey. The TNFα from the different species was either recombinant or produced from LPS-stimulated peripheral blood monocytes or whole blood. Cell viability was quantified by measuring optical density after MTT dye was added to the cells.

2. **Results:** The IC_{50} values of LU 200134 for TNFαs from the various species are shown in the table below.

Species	TNFα Source	LU 200134
Baboon	Recombinant	6.0 e-11
Canine	LPS-stimulated WBC	2.2 e-10
Chimpanzee	LPS-stimulated PBMC	5.5 e-11
Cynomolgus monkey	LPS-stimulated PBMC	8.0 e-11
Human	Recombinant	1.3e-10
Marmoset	LPS-stimulated PBMC	4.0 e-10
Murine	Recombinant	> 2.0 e-7
Porcine	Recombinant	> 1.0 e-7
Rhesus monkey	LPS-stimulated PBMC	4.0 e-11

3. **Conclusion:** The results of this study support the sponsor's selection of the cynomolgus monkey as a relevant model for the toxicology studies.

Report N°: BBC/I 9604
Title: Determination of the cytokine specificity of LU 200134 by a competitive TNF binding assay
Nonclinical Review Volume: 3/15
Adalimumab Review Volume: 23/986
Study Facility: BASF Research Corporation, Worcester, MA
Batch N°: AF501X
Report Date: October 8, 1997

1. **Methods:** In this study, the specificity of LU 200134 for — TNFα was assessed in a competitive binding assay utilizing a panel of recombinant cytokines — using — technology. The cytokines were tested in a 21:1 molar ratio (cytokine:LU 200134).

2. **Results:** Binding of LU 200134 to — rhTNFα resulted in a sensogram typical of an antibody binding to antigen. Pre-incubation of LU 200134 with rhTNFα decreased the magnitude of the signal. In contrast, the other cytokines tested did not

impact LU 200134 binding.

Report N°: BBC/I 9608
Title: Neutralization of rhTNFα induced lethality in D-galactosamine sensitized C57BL/6 mice by LU 200134
Nonclinical Review Volume: 3/15
Adalimumab Review Volume: 23/986
Study Facility: BASF Bioresearch Corporation, Worcester, MA
Batch N°: AF50466-4-126A
Report Date: October 8, 1997

1. **Methods:** The objective of this study was to demonstrate the ability of LU 200134 to inhibit the lethal effects of rhTNFα in a D-galactosamine sensitized murine *in vivo* model. Groups of 7 female C57BL/6 mice were treated intraperitoneally with 1, 2.6, 5.2, and 26 ug of LU 200134 followed 30 minutes later by a 20 mg ip injection of rhTNFα + D-galactosamine mixture. Three additional groups received no antibody, a human antibody control, or LU 200134 + galactosamine. The mice were observed 24 and 48 hours after treatment, and the percent survival was determined.

2. **Results:** The percent survival at 24 hours after treatment is shown in the table below. Treatment with LU 200134 resulted in a dose dependent increase in survival.

[illegible]	[illegible]	[illegible]
No LU 200134	0/7	0
1 ug LU 200134	1/7	14
2.6 ug LU 200134	5/7	71
5.2 ug LU 200134	6/7	86
26 ug LU 200134	6/7	86
26 ug LU 200134 (no rhTNF)	7/7	100
25 ug Hu IgG1	1/7	14

Report N°: BBC/I 9612
Title: Inhibition of recombinant human TNF-α-induced rabbit pyrexia by LU 200134
Nonclinical Review Volume: 3/15
Adalimumab Review Volume: 23/986
Study Facility: BASF Research Corporation, Worcester, MA
Batch N°: 50466-5-3 (preliminary experiment and experiment 1) AF504E-EX (experiments 3 and 4)
Report Date: October 8, 1997

1. **Methods:** Human TNFα is pyrogenic in rabbits. Additionally, preformed immune complexes of anti-rhTNFα antibodies + rhTNFα have been shown to elicit pyrexia in rabbits. The aim of this study was to evaluate the ability of LU 200134 to neutralize TNFα by assessing its ability to inhibit TNFα-induced pyrexia in rabbits and to investigate the ability of LU 200134 + rhTNFα to induce pyrexia. The treatments are shown in the table below. All treatments were administered iv. The treatments were administered first followed 15-20 minutes later by rhTNFα administered iv. Rectal

temperatures were recorded every minute for up to 4 hours.

Preliminary	3 3	138 (LU 200134) 0	0 5
1	3 3 3	137 (LU 200134) 14 (LU 200134) 0	
2	3 3	24 (LU 200134) 0	5 5
3	3 3 3	48 (LU 200134) 30 (human IgG) 0	5 5 5
4	3 3 3	792 (LU 200134) 792 (LU 200134) + 5 (rhTNFα) 0	5 5 5

2. **Results:** rhTNFα induced time-dependent pyrexia, with peak temperatures occurring 40 – 60 minutes after injection. LU 200134 alone had no effect on temperature. As shown in the table below, treatment with LU 200134 resulted in a dose dependent inhibition of rhTNFα-induced pyrexia.

14	53
24	72
48	94
137	100
792	100

Pre-formed immune complexes of LU 200134 + rhTNFα did not elicit any temperature rise in rabbits.

Report N°: BBC/M 0001
Title: Comparison of LU 200134 [redacted] and [redacted] batches in preclinical characterization assays and animal models
Nonclinical Review Volume: 3/15
Adalimumab Review Volume: 23/986
Study Facility: Abbott Bioresearch Center, Worcester, MA
Batch N°: [redacted] = AFP704; [redacted] = AFP810
Report Date: December 11, 2001

1. **Methods:** In order to [redacted] the manufacturing process and the [redacted] producing LU 200134 were changed. The antibody produced by the new process/ [redacted] is referred to as [redacted] and the previous process referred to as [redacted] The sponsor conducted a series of *in vivo* and *in vitro* studies to compare the two antibodies. A brief description of the studies is shown in the table below.

Determination of kinetic rate constants	As described above for BBC/I 9602 except LU 200134 was [redacted] instead of TNFα
[redacted] cell cytotoxicity assay	Neutralizing potencies of LU 200134 [redacted] were tested in murin[redacted] cytotoxicity assay *in vitro*.
Determination of antigen specificity	Antigen specificity was determined using [redacted] technology. [redacted] The amount of bound LU 200134 was recorded for each LU 200134/cytokine mixture.
Binding to pro-TNF	The ability of [redacted] and [redacted] to bind pro-TNFα was assessed in [redacted] cells. The assay included pro-TNF transfected cells and nontransfected cells. Cell binding was evaluated on a [redacted] analyzer.
Complement dependent cytotoxicity assay	[redacted] cells expressing [redacted] on their cellular membranes were incubated with LU 200134 [redacted] and [redacted] in the presence of a fresh complement source. Complement dependent cytotoxicity was assessed by measuring LDH release into the supernatant.
Fc receptor binding assay	The ability of [redacted] LU 200134 to bind to Fc receptors was evaluated in [redacted] cells treated with one of four anti-human antibody blocking solutions (mouse anti-human FcRI, FcRII, FcRIII, or human IgG1. Fc receptor binding was assessed using a [redacted]
Prevention of huTNF mediated lethality in mice	Female C57BL/6J mice were injected ip with 0.6 ug/kg to 10 ug/kg of LU 200134. At 30 minutes post-treatment, the mice were challenged with 0.7 ug/mouse of rhTNFα. The mice were monitored for survival 24 and 48 hours later.
Testing for cytokine release from human blood cells	Fresh blood samples were incubated with 20 ug/mL and 200 ug/mL of LU 200134, and 1 ug/mL of LPS (positive control). Cell free supernatants were obtained and analyzed for [redacted]

2. **Results:** In the assays describe above, the results for [redacted] and [redacted] were essentially the same.

3. **Conclusion:** LU 200134 [redacted] and LU 200134 [redacted] are functionally equivalent.

►Secondary Pharmacodynamics

The studies were conducted with process development batches.

BBC/I9601	Cytokine release by human whole blood incubated with LU 200134 ex vivo
MPF/FM 9606E	In vitro pharmacodynamic studies on the receptor binding profile of LU 200134
MPF/FM 9605	In vitro effect of LU 200134 on unstimulated and mitogen-stimulated murine spleen cells

Report N°: BBC/I 9601
Title: Cytokine release by human whole blood incubated with LU 200134
Nonclinical Review Volume: 3/15
Adalimumab Review Volume: 23/986
Study Facility: BASF Research Corporation, Worcester, MA
Batch N°: AF504A-STD and AF504DEX
Report Date: November 21, 1996

1. **Methods:** LU 200134 was incubated with diluted whole blood from three human volunteers at concentrations up to — µg/mL for 24 hours. A lipopolysaccharide positive control was included in the experiment. Following the incubation period, the supernatants were harvested and analyzed for —

2. **Results:** Under the conditions of this study, LU 200134 did not elicit the release of cytokines or cell surface markers.

Report N°: MPF/FM 9606E
Title: In vitro pharmacodymanic studies on the receptor binding profile of LU 200134
Nonclinical Review Volume: 4/15
Adalimumab Review Volume: 24/986
Study Facility: [redacted]
Batch N°: AF 601-EX
Report Date: October 1996

1. **Methods:** The ability of LU 200134 (4uM) and human IgG1 (3uM) to inhibit the binding of radiolabeled ligands for 68 different receptors, including TNFα, was investigated.

2. LU 200134 inhibited the binding of radiolabeled TNFα by 137%. In the case of the other receptors, TNFα was without effect or resulted in a decrease of < 50%. In general, with the noted exception of TNFα, the degree of inhibition observed with LU 200134 was ≤ that observed with IgG1.

Report N°: MPF/FM 9605
Title: In vitro effect of LU 200134 on unstimulated and mitogen-stimulated murine spleen cells
Nonclinical Review Volume: 4/15
Adalimumab Review Volume: 24/986
Study Facility: Knoll AG, Research and Development, Ludwigshafen, Germany
Batch N°: AF 601-EX
GLP Compliance: No
Report Date: September 30, 1996

1. **Methods:** The *in vitro* effect of LU 200134 on the proliferation of unstimulated or mitogen-stimulated murine spleen cells were determined using [redacted] after incubation with or without LU 200134 and/or mitogens. Splenocytes were stimulated with lipopolysaccharide (LPS;B-cell subsets) or staphylococcal enterotoxin b (SEB) or anti-CD3 (T-cell subsets). Doxorubicin, cyclosporine, and MAK 195 F, the murine anti-TNFα monoclonal antibody, were used as reference compounds. LU 200134 concentrations ranged from 7.9×10^{-6} to 7.9×10^{-11}M. The isolated splenocytes were obtained from Balb/c mice.

2. **Results:** LU 200134 showed no mitogenic effect. At the higher concentrations, LU 200134 inhibited the growth of LPS-, SEB-, and anti-CD3-stimulated, with the effect being more pronounced in the case of the latter two. These inhibitory effects were considered extensions of the pharmacological activity of LU 200134.

►*In vivo* Efficacy in Animal Models of Arthritis

	Title
BBC/I 9610E	Prevention of polyarthritis in [redacted] Transgenic mice by LU 200134
BBC/I 9611E	Prevention of polyarthritis in [redacted] Transgenic mice by LU 200134: Determination of a dose response
BBC/M 0005E	Prevention of polyarthritis in [redacted] transgenic [redacted] mice by chimeric D2E7 (cD2E7): Comparison of cD2E7 and CD2E71-IgG2a isotypes
ABC/H 0106E	Prevention of polyarthritis by D2E7 in human [redacted] transgenic mice: Comparison of [redacted] vs [redacted] batches of D2E7
BBC/M 0004E	D2E7 (LU200134) and methotrexate combination therapy of polyarthritis in human [redacted] transgenic [redacted] mice

Report N°: BBC/I 9610E
Title: Prevention of polyarthritis in [redacted] transgenic mice by LU 200134
Nonclinical Review Volume: 3/15
Adalimumab Review Volume: 23/986
Study Facility: [redacted]

Batch N°: AF504D-EX
Report Date: January 30, 1998

1. **Methods:** [redacted] mice are transgenic animals [redacted] These animals [redacted] and develop a progressive polyarthritis. Details relating to the animals and their treatment are shown in the table below. All treatments were administered by ip injection three times per week beginning at 1 week of age for 10 weeks.

Group	Animals	N	Treatment	Dose (mg/kg)
1	[redacted]	8 (3♂ + 5♀)	None	---
2	[redacted]	8 (2♂ + 6♀)	PBS	---
3	[redacted]	9 (2♂ + 7♀)	LU 200134	2.3
4	[redacted]	8 (2♂ + 6♀)	LU 200134	23.2

5	[redacted]	9 (5♂ + 4♀)	LU 200134	46.5
6	[redacted]	9 (5♂ + 4♀)	Control Human IgG1	30
7	Non-transgenic	5 (3♂ + 2♀)	None	---

The endpoints measured are as follows: [1] Body weight (weekly), [2] Arthritic scores (at the end of the treatment period), and [3] Histopathology (in all groups but group 7 at the end of the treatment period).

2. **Results:**

2.1. Body weight: In the [redacted] mice receiving no treatment, PBS, or control human IgG1, body weight was decreased 25% - 35% relative to that of the non-transgenic animals at the end of the treatment period. In contrast, the body weights of the [redacted] mice receiving LU 200134 were essentially the same as the non-transgenic animals.

2.2. Macroscopic changes in joint morphology: The arthritic scores (0 → +++) in the [redacted] mice receiving no treatment, PBS, or control human IgG1 were "+++". In contrast, those of the [redacted] mice receiving LU 200134 were the same as non-transgenic animals ("0").

2.3. Histopathology: The histopathology scores (0 → 3) in the [redacted] mice receiving no treatment, PBS, or control human IgG1 were "3". In contrast, those of the [redacted] mice receiving LU 200134 were the same as non-transgenic animals ("0"), with the exception of one mouse each from the 2.3 ug/gm and 23 ug/gm groups which had a score of "1".

3. **Conclusion:** Under the conditions of this study, treatment with LU 200134 prevented the development of polyarthritis due to the expression of human TNFα.

Report N°: BBC/I 9611E
Title: Prevention of polyarthritis in [redacted] transgenic mice by LU 200134: Determination of a dose response
Nonclinical Review Volume: 3/15
Adalimumab Review Volume: 23/986
Study Facility: [redacted], and BASF Bioresearch Corporation, Immunology Department, Worcester, MA
Batch N°: AFP-606 [redacted]
Report Date: March 1, 1999

1. **Methods:** Details relating to the animals and their treatment are shown in the table below. All treatments were administered by ip injection three times per week beginning at 1 week of age for 8 weeks.

1		8	LU 200134	10
2		8	LU 200134	1
3		8	LU 200134	0.5
4		8	LU 200134	0.1
5		8	LU 200134	0.01
6		8	PBS	0

The endpoints measured in this study are shown in the table below.

Body weight	Weekly
Macroscopic changes in joint morphology	Weekly
Histopathology of ankle joints	One week after the last treatment
Serum levels of LU 200134	Weeks 5 and 9
Serum levels of mouse anti-human antibodies (MAHA)	Weeks 5 and 9

2. Results

2.1. Body weight: As shown in the table below, mean body weight increased in a dose dependent manner. Data below were obtained at Week 9.

16.9 g	17.5 g	18.7 g	20.3 g	21.3 g	23.5 g

2.2. Macroscopic changes in joint morphology: Mice treated with 0.5, 1 or 10 ug/g of LU 200134 exhibited few if any signs of arthritis. In contrast, mice in the 0.01 ug/g, 0.1 ug/g, or PBS group exhibited progressive signs of arthritis in their ankle joints. The mean arthritic scores for these groups were essentially the same at the end of the experiment.

2.3. Histopathology of ankle joints: As shown in the table below, the mice exhibited a dose dependent decrease in histopathological signs of arthritis. The ED_{50} for this effect was 0.48 ug/g.

0			1/8	7/8
0.01			2/8	6/8
0.1			3/8	5/8
0.5	3/8	2/8	3/8	
1.0	8/8			
10.0	8/8			

2.4. Serum levels of LU 200134 and MAHA: According to the study report, serum levels of LU 200134 greater than 100 ug/mL are inhibitory in the MAHA assay. In the high dose group, serum levels of LU 200134 exceeded 100 ug/mL in all animals at both time points; no MAHAs were detected at either time point. In all other treatment groups, serum levels of LU 2000134 were extremely low;

MAHA were detected in almost all animals.

3. **Conclusion:** Under the conditions of this study, treatment with LU 200134 inhibited the development of arthritis in a dose dependent manner.

Report N°: BBC/M 0005E
Title: Prevention of polyarthritis in [redacted] transgenic [redacted] mice by chimeric D2E7 (cD2E7): comparison of CD2E7-IgG1 and cD2E7-IgG2a isotypes
Nonclinical Review Volume: 3/15
Adalimumab Review Volume: 23/986
Study Facility: [redacted]

Report Date: December 2001

1. **Methods:** [redacted] and develops arthritis similar to human rheumatoid arthritis. The chimeric D2E7s (LU 200134) were comprised of human variable regions and murine constant regions. Complement-fixing IgG2a and complement non-fixing IgG1 were tested to evaluate the importance of effector functions in preventing arthritis in this animal model.

   The cD2E7 IgG1 and the cD2E7 IgG2a groups received 1, 5, 10 and 20 ug of antibody/gm body weight. Each treatment group consisted of 8 animals (♂ and ♀ in varying ratios); the control consisted of 16 mice (♂ and ♀). Treatments were administered three times weekly. Treatment was initiated beginning at 10 to 14 days of age and continued through 10 weeks of age.

   The following endpoints were monitored in this study: [1] body weight (weekly), [2] macroscopic changes in joint morphology (weekly), and [3] histopathology of ankle joints (at the end of the study).

2. **Results:** cD2E7-IgG1 and cD2E7-IgG2a were equally effective in preventing the development of arthritis in [redacted] mice.

3. **Conclusions:** The results of this study indicate that complement activation is not involved in LU 200134-induced prevention of polyarthritis.

Report N°: ABC/H 0106E
Title: Prevention of polyarthritis by D2E7 in [redacted] transgenic [redacted] mice: Comparison of [redacted] vs [redacted] batches of D2E7
Nonclinical Review Volume: 3/15
Adalimumab Review Volume: 23/986
Study Facility: [redacted]

Analytical Facility: Abbott Bioresearch Corporation, Worcester, MA
Batch N°: AFP-704 [redacted] and AFP-810 [redacted]

1. **Methods:** The purpose of this study was to compare the *in vivo* efficacy of LU 200134 manufactured using the [redacted] process to that manufactured using the [redacted] process in the [redacted] transgenic murine model of rheumatoid arthritis. LU 200134 was administered weekly for 10 weeks using the ip route of administration. The animals were sacrificed at the end of the treatment period. The treatment groups are shown in the table below.

Group	Dose (mg/kg)	N
Untreated	0	4/sex
[redacted]	0.01	4/sex
	0.1	"
	0.5	"
	1.0	"
	10	"
[redacted]	0.01	4/sex
	0.1	"
	0.5	"
	1.0	"

The following endpoints were assessed in this study.

Endpoint	Timing
Body weight	Weekly
Macroscopic changes in joint morphology	Weekly
Histopathology of ankle joint sections	At termination
Serum levels of LU 200134	Week 5 and termination
Serum levels of TNFα	Week 5 and termination
Serum levels of mouse anti-human antibody (MAHA)	Week 5 and termination

2. **Results:**

2.1. Body weight: At the end of the treatment period, body weight in the mice receiving 10 mg/kg, 1 mg/kg, and 0.5 mg/kg of [redacted] and 1.0 mg/kg and 0.5 mg/kg of [redacted] was increased 15% - 24% relative to the untreated control group. There was no difference between the [redacted] and [redacted] groups that received the same dose.

2.2. Macroscopic changes in joint morphology: Mice receiving 10 mg/kg, 1 mg/kg, and 0.5 mg/kg of [redacted] and 1.0 mg/kg and 0.5 mg/kg of [redacted] exhibited essentially no evidence of arthritis. In contrast, the arthritic score of the mice receiving the lower doses of LU 200134 were essentially the same as for the untreated group. There were no differences between the [redacted] and [redacted] groups.

2.3. Histopathology of ankle joints: The histopathology scores are shown in the table below. The results for the [redacted] and [redacted] groups were essentially the same. Both batches resulted in a dose dependent decrease in the

severity of the joint findings.

Treatment Group	Histopathology Score (Mean ± S.E.)
Untreated	2.6 ± 0.5
10 mg/kg	0 ± 0
1 mg/kg	0 ± 0
0.5 mg/kg	0.4 ± 0.5
0.1 mg/kg	2.9 ± 0.4
0.01 mg/kg	2.8 ± 0.5
1 mg/kg	0 ± 0
0.5 mg/kg	0.1 ± 0.4
0.1 mg/kg	3.0 ± 0.0
0.01 mg/kg	2.9 ± 0.4

2.4. Serum levels of LU 2000134: In general, serum levels of LU 200134 were comparable between the [redacted] and [redacted] groups.

2.5. Serum Levels of TNFα: Serum levels of TNFα were measured using a kit that detects anti-body bound TNFα. [redacted], no TNFα was detected in the untreated animals. A dose dependent increase in TNFα was detected in the treated mice at all time points, with the values for the [redacted] and [redacted] being generally comparable.

2.6. Serum levels of mouse anti-human antibodies (MAHA): Treatment with [redacted] and [redacted] batches resulted in MAHA production at all doses. There was considerable variation in measurements, with no apparent difference between the [redacted] and [redacted] groups.

3. **Conclusion:** Under the conditions of this study, [redacted] and [redacted] were biologically comparable.

Report N°: BBC/M 0004E
Title: D2E7 (LU 200134) and methotrexate combination therapy of polyarthritis in human [redacted] transgenic [redacted] mice
Nonclinical Review Volume: 4/15
Adalimumab Review Volume: 24/986
Study Facility: [redacted]

Batch N°: AFP810
Report Date: September 24, 1998

1. **Methods:** Groups of 8 mice (♂ and ♀) received the treatment shown in the table below.

Group	LU 200134 mg/kg	LU 200134 Treatment Regimen	Methotrexate mg/kg	Methotrexate Treatment Regimen
1	0.01	ip, weekly, starting at	0	NA

		Week 1 of age thru Week 10 of age		
2	0.1	"	0	NA
3	0.5	"	0	NA
4	1.0	"	0	NA
5	0.01	"	0	ip, 3X weekly, starting at Week 2.5 of age thru Week 10 of age
6	0.1	"	1	"
7	0.5	"	1	"
8	1.0	"	1	"
9	0	NA	1	"
10	0	NA	0	NA

The endpoints measured in this study were as follows: [1] body weight (weekly), [2] macroscopic changes in joint morphology (weekly), and [3] histopathology of ankle joints (at the end of the study).

2. **Results:** The results of this study indicate that LU 200134 administered alone is as effective in preventing the development of polyarthritis as a combination of LU 200134 and methotrexate.

►Safety Pharmacology

The safety pharmacology studies that were conducted with LU 200134 are listed in the table below.

MPF/N 9610	Observational assessment of the effect of LU 200134 on behavioral and physiological parameters (Irwin) in the mouse after a single intravenous administration
MPF/N 9728	Observational assessment of the effect of LU 200134 on behavioral and physiological parameters (Irwin) in the mouse after single intravenous administration of 786 mg/kg
MPF/N 9611	Test on various CNS effects (sedation, stimulation, pro- or anti-convulsant effects) of LU 200134 in mice after single intravenous administration
MPF/N 9729	Test on various CNS effects (sedation, stimulation, pro- or anti-convulsant effects) of LU 200134 in mice after single intravenous administration of 786 mg/kg
MPF/N 9706	Further tests on various CNS effects (antinociceptive and anticonvulsant effects) of LU 200134 in mice after single intravenous administration
MPF/N 9707	Interaction of LU 200134 with hexobarbital after single intravenous administration in mice
MPF/N 9730	Interaction of LU 200134 with hexobarbital after single intravenous administration of 786 mg/kg in mice
MPF/HPK 9611	The effect of LU 200134 on cardiovascular and respiratory parameters after single intravenous administration in conscious, normotensive dogs
MPF/HPK 9702	The effect of LU 200134 on histamine-, acetylcholine-, and $BaCl_2$-induced spasms on the isolated guinea pig ileum
MPF/ET 9742	Effect on the gastrointestinal transit time of charcoal in mice at single

	intravenous administration
MPF/HPA 9706	Effect of LU 200134 on the intestinal passage rate of coal particles in conscious rats after intravenous bolus administration
MPF/HPA 9710	Effect of anti-TNF antibody LU 200134 in gastric acid secretion in conscious rats after single intravenous bolus administration
MPF/ET 9658E	General pharmacology of LU 200134 effect on isolated uterus of female rats
MPF/ET 9734	LU 200134-Effect on urine production and renal protein and electrolyte excretion in mice at single intravenous administration
MPF/HPK 9609	Influence of LU 20134 on urine and electrolyte excretion in rats after single intravenous administration (general pharmacodynamics study)
MPF/HPA 9616	Influence of the anti-TNF antibody LU 200134 on human blood (hemolysis) and on coagulation parameters (activated partial thromboplastin time, prothrombin time, thrombin time) in plasma of healthy human volunteers
MPF/N 9702	Study on the potential local anesthetic effect of LU 200134 in the guinea pig wheal test
MPF/FG 9806	Study on the potential local anesthetic effect of LU 200134 in the guinea pig wheal test: additional investigations using higher drug concentrations
BBC/M 0004E	D2E7 (LU200134) and methotrexate combination therapy of polyarthritis in [redacted] transgenic [redacted] mice

These studies were not addressed in detail in this section of the review because it was decided, based on the high specificity of LU 200134 for TNFα, that safety pharmacology endpoints were adequately addressed in the repeat-dose toxicology studies. This rationale is consistent with ICH Guidance S7A, *Safety Pharmacology Studies of Human Pharmaceuticals.*

In the studies conducted in mice (MPF/N 9610, 9728, 9611, 9729, 9706, 9707, and 9730), LU 200134 was administered a single iv dose. The doses used ranged from ~ 90 mg/kg to 786 mg/kg). These studies did not reveal any biologically relevant effects.

In the study conducted in dogs (MPF/HPK 9611), LU 200134 was administered as a single 46.4 mg/kg iv, bolus dose. Treatment with LU 200134 did not result in any biologically relevant effects.

In the studies conducted in rats (MPF/HPA 9706 and 9710, and MPF/HPK 9609), LU 200134 was administered as single iv doses of ~ 90 mg/kg to 786 mg/kg. The animals did not exhibit any relevant effects.

In the studies conducted in guinea pigs (MPF/N 9720 and MPF/FG 9806), LU 200134 did not result in any relevant effects when administered intracutaneously at concentrations of 10 mg/mL to 78 mg/mL.

LU 200134 was without effect in the two *in vitro* studies (MPF/HPK 9702, MPF/ET 9658E, and MPF/HPA 9616).

▶Tissue Cross-Reactivity

BBC/M 9918 E	Preliminary studies of cross reactivity screening of an unconjugated human monoclonal antibody D2E7
BBC/M 9917 E	Cross-reactivity study of unconjugated D2E7 human monoclonal antibody with normal human tissues
BBC/M 0003	Cross-reactivity study of unconjugated D2E7 — human monoclonal IgG1 antibody with normal tissue

The first study conducted was a preliminary study intended to establish the most appropriate methods. The two subsequent studies were definitive studies. One was conducted with the LU 200134 manufactured using the — process (Batch AFP701) and the other with the LU 200134 manufactured using the — process (Batch AFP810).

Report N°: BBC/M 9918
Title: Preliminary studies of cross reactivity screening of an unconjugated human monoclonal antibody D2E7
Nonclinical Review Volume: 3/15
Adalimumab Review Volume: 23/986
Study Facility: —
Batch N°: AFP701
GLP Compliance: No
Report Date: March 31, 1999

1. **Methods:** The purpose of this study was to determine the appropriate positive controls, concentration of antibody, and the fixation and staining conditions to be used in a subsequent full human tissue cross reactivity. The study was conducted with various human and nonhuman tissues, tissue culture cells, and —

2. **Results:** A potential cross-reactivity was observed with the filamentous structures in the cytoplasm of vascular smooth muscle in multiple tissues. Reactivity was prominent with medium sized muscular arterioles and venules and was less prominent on smaller arterioles and venules. Tissues from marmosets, cynomolgus and rhesus monkeys, rat, baboon, and a chimpanzee were examined to determine whether the cross-reactivity was limited to human tissues. Similar reactivity was seen with the chimpanzee but not with the other species.

Report N°: BBC/M 9917 E
Title: Cross-reactivity study of unconjugated D2E7 human monoclonal IgG1 antibody with normal human tissues
Nonclinical Review Volume: 3/15
Adalimumab Review Volume: 23/986
Study Facility: —
Batch N°: AFP701

GLP Compliance: Yes
Report Date: March 31, 1999

1. **Methods:** The objective of this study was to evaluate the potential cross-reactivity of LU 200134 with cryosections of the human tissues. The tissue panel included all of the tissues recommended in CBER's "Points o Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use". In order to assess binding, unconjugated LU 200134, at concentrations of 2 and 10 ug/mL, was applied to at least two, and where possible, three sources per tissue. rhTNFα — and TNF-expressing alveolar macrophages in human lung cryosections served as positive controls. Human IgG1 with different antigen specificity than LU 200134 served as the negative control. Immunoperoxidase staining was performed according to — SOPs.

2. **Results:** Weak to intense reactivity of both concentrations of unconjugated LU 200134 was observed with the vascular and intrinsic smooth muscle of multiple tissues, with the reactivity at 10 ug/mL often being more intense than at 2 ug/mL. LU 200134 did not react with vascular endothelium or capillaries.

   LU 200134 also reacted with [1] filamentous structures in the cytoplasm of intrinsic smooth muscle in many tissues (large intestine, small intestine, stomach, lung, oviduct, uterus, testes, and ureter), [2] cytoplasmic filamentous structures in other cells with contractile properties (glomerular mesangium in the kidney and myoepithelium in the mammary gland), [3] capsular or extrinsic smooth muscle in the adrenal, testis, and mammary gland, and [4] rare Kupffer cells in the liver of 1/3 donors.

Report N°: BBC/M 0003 E
Title: Cross-reactivity study of unconjugated D2E7- — human monoclonal IgG1 antibody with normal human tissues
Nonclinical Review Volume: 4/15
Adalimumab Review Volume: 24/986
Study Facility: —
Batch N°: AFP810
GLP Compliance: Yes
Report Date: May 15, 2000

1. **Method:** The methods were the same as described for study BBC/M 9917 E, which was reported in March 1999, except a difference in the low concentration of LU 200134. In the March 1999 study, the low concentration was 2ug/mL. In the current study, it was 1 ug/mL.

2. **Results:** The results were essentially the same as observed for the previous study.

PHARMACOKINETICS

The pharmacokinetics studies that the sponsor submitted are shown below. Reports addressing the same study are included in the same row of the table.

MPF/EB 9636	Pharmacokinetic study in male cynomolgus monkeys after single intravenous and single subcutaneous dose
SBL 36-56	A pharmacokinetic study in cynomolgus monkeys after single subcutaneous and intravenous administrations of D2E7
MPF/DDB 9903	Determination and pharmacokinetic evaluation of LU 200134 concentrations in serum samples from cynomolgus monkeys after subcutaneous administration of LU 200134 with and without Tween from the study MPF/DDK 9907E
MPF/DDK 9907E	Comparison of pharmacokinetics in cynomolgus monkeys for subcutaneous administration of LU 200134 with and without Tween
SBL 36-55	A bridging study in cynomolgus monkeys after single intravenous administration of D2E7.
MPF/EBB 9711	Determination and pharmacokinetics evaluation of LU 200134 concentrations in mouse serum samples after single dose iv administration from kinetic study MPF/ET 9654E.

Because TNF binding studies showed that non-human primates are the most relevant species, most of the pharmacokinetics studies were conducted in cynomolgus monkeys. In addition to providing pharmacokinetics information in a relevant animal model, these studies address three key issues. First, all of the toxicology studies were conducted using iv administration as opposed to the clinical route of administration, sc. Second, the clinical formulation contains polysorbate 80 (Tween) [redacted] [redacted]. Third, during the course of drug development, the manufacturing process for the drug substance was changed. Therefore, nonclinical pharmacokinetics studies were conducted to [1] establish comparability between the subcutaneous and intravenous routes of administration, [2] verify that the addition of Tween to the formulation did not affect pharmacokinetics, and [3] verify that changes in the manufacturing process did not affect pharmacokinetics.

►Pharmacokinetics Studies in Cynomolgus Monkeys

Report N°: MPF/EB 9636
Title: Pharmacokinetic study in male cynomolgus monkeys after single intravenous and single subcutaneous dose administration of LU 200134
Nonclinical Review Volume: 14/15
Adalimumab Review Volume: 34/986
Study Facility: The animals were treated and the samples were collected at [redacted] The samples were analyzed at Knoll AG Research and Development, Department of Biochemistry, Knollstrabe 50, 67061 Ludwigshafen/Germany
Batch N°: AF 504D-EX
GLP Compliance: The animals were treated and the samples collected in compliance

with GLP. Sample analysis was not conducted in compliance with GLP.
Report Date: January 7, 1997

1. **Methods:** The data presented in this report were obtained following the first dose of a 6-month study designed to evaluate the pharmacokinetics and immunogenicity of LU 200134 following 6 monthly iv or sc treatments. The results for other parts of this study are presented in reports BBC/I 9802 E and ABC/H 0101, both of which are located in volume 11/15 of the nonclinical review section of this submission (volume 31/986 for the BLA).

   The treatment protocol and the sampling times for pharmacokinetics endpoints following the first dose are shown in the table below.

1	15.5	4 ♂	iv	Blood samples were obtained at the following times after treatment 0 (predose) 5, 15, 30, and 60 min 2, 4, 8, and 24 hr 3, 5, 8, 12, 16, 21, 26, and 30 days
2	15.5	4 ♂	sc	Blood samples were obtained at the following times after treatment 0 (predose) 10, 15, 30, and 60 min 2, 4, 8, and 24 hr 3, 5, 8, 12, 16, 21, 26, and 30 days

   The samples were analyzed for LU 200134 using a [redacted] ELISA (enzyme linked immunosorbent assay).

   The first immunogenicity blood sample was not collected until Day 36, 6 days after the last sample was collected for the single dose segment of the study and 5 days after the second monthly injection was administered. PAHA (primate anti-human antibodies) were measured using an ELISA.

2. **Results:**
   The pharmacokinetics results (expressed as mean ± S.D.) are shown in the table below. Absolute bioavailability following subcutaneous administration was ~ 96%.

iv	42,400 ± 7643	0.37 ± 0.07	9.2 ± 0.77	55 ± 3.5	---	---
sc	40,821 ± 16,734	0.43 ± 0.18	12.4 ± 3.5	----	48 ±	89±

   On Day 36, PAHAs were detected in 2/4 and 3/4 animals in the iv and sc groups, respectively.

3. **Conclusions:** Under the conditions of this study, the pharmacokinetics parameters of adalimumab following iv administration were comparable to those obtained after sc administration.

Report N°: SBL 36-56
Title: A pharmacokinetic study in cynomolgus monkeys after single subcutaneous and intravenous administrations of D2E7
Nonclinical Review Volume: 14/15
Adalimumab Review Volume: 34/986
Study Facility: The animals were treated and the samples analyzed for LU 200134 at

Pharmacokinetics parameters were calculated at Knoll AG. Primate anti-human antibodies (PAHAs) were measured at Knoll AG.
Lot N°: AFP908
GLP: Yes
Study Initiation: October 19, 1999
Report Date: December 12, 2001

1. **Methods:** This study was conducted to determine serum levels of LU 200134 and anti-LU200134 antibodies in cynomolgus monkeys.

   The treatment of the animals and the sampling times following the first dose are shown in the table below.

Group	Dose (mg/kg)	M	Route	PK sampling times	Immunogenicity sampling time
1	3	6 ♂	iv	0 (predose) 5, 10, 15, 30, and 60 min 2, 4, 8, and 24 hr 2, 4, 7, 14, 21, 28, 35, 42, 49 and 56 days	 0 7, 14, 21, 28, 35, 42, 49, and 56 days
2	1 3 10	6 ♂ 6 ♂ 6 ♂	sc	0 (predose) 1, 2, 4, 8, and 24 hr 2, 3, 4, 7, 14, 21, 28, 35, 42, 49, and 56 days	 0 7, 14, 21, 28, 35, 42, 49, and 56 days

   The samples were analyzed for LU 200134 and PAHAs using an ELISA. Only samples with LU 200134 < 2 ng/mL were analyzed for PAHA because higher concentrations of LU 200134 interfere with the PAHA assay.

2. **Results:** Pharmacokinetic parameters are shown in the table below

Route	Dose (mg/kg)	AUC (ng·hr/mL)	Parameter		
sc	1		1439 ± 173	76 ± 10	12 ± 1
	3		4167 ± 581	132 ± 39	32 ± 4
	10		13699 ± 2021	104 ± 56	105 ± 13
iv	3	9656 ± 1491	5910 ± 398	----	----

PAHAs were not detected until Day 21, at which time they were detected in one to three animals in each treatment group. On Days 49 and 56, PAHAs were detected in all animals. Sharp declines in LU 200134 levels were observed in some of the animals.

Report N°: MPF/DDB 9903
Title: Determination and pharmacokinetic evaluation of LU 200134 concentrations in serum samples from cynomolgus monkeys after subcutaneous administration of LU 200134 with and without Tween from the study MPF/DDK 9907E
Nonclinical Review Volume: 14/15
Adalimumab Review Volume: 34/986
Study Facility: The animals were treated and the samples collected at [redacted] The samples were analyzed for LU 200134 and anti-LU 200134 antibodies at Knoll AG, Research and Development, Knollstrabe 50, 67061 Ludwigshafen, Germany.
Batch/Lot N°: AFP802/880200A0 (without Tween) and AFP803 and AFP804/880100A0 (with Tween)
GLP: Yes
Study Initiation: January 20, 1999 (Study director signed the protocol)
Report Date: November 28, 1999 (Study director signed the report)

1. **Methods:** This study was conducted to investigate the bioequivalence of LU 200134 when administered subcutaneously in the presence and the absence of Tween. Details for the study are provided in the table below.

1	1	6 ♀	No	0 (predose) 10, 15, 30, and 60 min 2, 4, 8, and 24 hr 3 5, 12, 16, 21, 26, 30, 35, 42, 49 and 56 days	0 (predose) 3, 5, 12, 16, 21, 26, 30, 35, 42, 49, and 56 days
2	1	6 ♀	Yes	"	"

Samples were analyzed for LU 200134 and PAHAs using an ELISA

2. **Results**

The pharmacokinetic parameters are shown in the table below

112 ± 19	7.9 ± 1.9	2254 ± 497	0.46 ± 0.09	88 ± 24	7.9 ± 2.3	2200 ± 532	0.48 ± 0.11

PAHAs were first detected on Day 12 and were detected in all animals in both groups by Day 30. Due to the formation antibodies, serum levels of LU 200134 could not be determined after 500 hours (21 days).

3. **Conclusions:** Under the conditions of this study, the addition of Tween did not affect the pharmacokinetics of LU 200134.

Report N°: SBL 36-55
Title: A bridging study in cynomolgus monkeys after single intravenous administration of D2E7
Nonclinical Review Volume: 15/15
Adalimumab Review Volume: 35/986
Study Facility: The animals were treated and the samples analyzed for LU 200134 at [redacted] The samples were analyzed at [redacted]

Batch/Lot N°: AFP908 (produced by process [redacted] and AFP604 (produced by process [redacted]
GLP: Yes
Study Initiation: October 19, 1999
Report Date: December 12, 2001 (Study director signed the report)

1. **Methods:** The purpose of this study was to compare the pharmacokinetics of two adalimumab batches prepared by different manufacturing processes, [redacted] and [redacted]

   The details of the study are provided in the table below. The treatments were administered intravenously.

Group	Dose (mg/kg)	N	Dosage	PK Sampling Times	Immunogenicity Sampling Times
1	32	6 ♂	[redacted]	0 (predose) 5, 10, 15, and 30 min 1, 2, 4, 8, and 24 hr 2, 4, 7, 14, 21, 28, 35, 42, 49 and 56 days	0 (predose) 7, 14, 21, 28, 35, 42, 49, and 56 days
2	32	6 ♀	[redacted]	"	"

   LU 200134 and primate anti-human antibodies (PAHAs) were measured using an ELISA. Only samples with LU 200134 < 2 ng/mL were analyzed for PAHA because higher concentrations of LU 200134 interfere with the PAHA assay.

2. The pharmacokinetics parameters are shown in the table below. The results are expressed as the mean ± S.D.

168,111 ± 53,354	64 ± 12	0.21 ± 0.07	9.3 ± 5.9	156,380 ± 38,635	54 ± 11	0.22 ± 0.06	6.5 ± 3.6

PAHAs were detected from one animal in each group beginning on Day 28 and continuing through the end of the study. Both of these animals exhibited a rapid decline in LU 200134 levels. One additional animal in the [redacted] group exhibited a sharp decline in LU 200134 levels, but no PAHAs were detected.

3. **Conclusion:** The pharmacokinetics for the two batches were essentially the same.

►Pharmacokinetics Studies in Mice

Report N°: MPF/EBB 9711
Title: Determination and pharmacokinetic evaluation of LU 200134 concentration in mouse serum samples after single dose iv administration from kinetic study MPF/ET 9654 E
Nonclinical Review Volume: 14/15
Adalimumab Review Volume: 34/986
Study Facility: The animals were treated and the samples collected at [redacted] The samples were analyzed at Knoll AG, Research and Development, Germany
Batch/Lot N°: AFP 603/W69853
GLP: Yes
Report Date: August 7, 2000

1. **Methods:** The details of this study are provided in the table below.

Dose	Route	N	Blood Collection
32 mg/kg	iv	5/sex/timepoint	Prior to dosing After dosing 5 and 30 minutes 1, 4, 8, 24, and 96 hours 8, 11, 15, 22, 29, 36, and 43 days

Serum levels of LU 200134 were determined using an ELISA.

2. **Results:** The pharmacokinetic parameters are shown in the table.

Sex	AUC (0-inf) (h*µg/ml)	Cl (ml/h/kg)	t1/2 (h)
M	82,901	0.38	102
F	58,609	0.54	193

TOXICOLOGY

Repeat-dose Toxicology

Report N°: MDF/ET 9732 E
Title: 39-Week Chronic Toxicity Study of LU 200134 (D2E7) by Intravenous Administration to *Cynomolgus* Monkeys – Administration Once Per Week
Nonclinical Review Volumes: 8/15 to 11/15
Adalimumab Review Volumes: 28/986 to 31/986
Study Facility: [redacted]
(unless otherwise specified)
Batch N°: 780100A0, 780200A0, AFP706, AFP752, AFP801
GLP Compliance: Yes
Study Initiation: June 10, 1997 (date on the protocol)
Study Termination: February 21, 2000 (date that the study director signed the report)
In-life Phase: September 24, 1997 to November 19, 1998

1. **Methods:** This study was conducted in purpose-bred cynomolgus monkeys. This monkey was considered the most relevant species based on TNF neutralization potency. The animals received the test article, LU 200134, intravenously, once weekly for 39 weeks. At the end of the treatment period a subset of animals from the control and high dose groups were maintained for a 20-week treatment-free recovery period. The dose levels and related information are shown in the table below.

1 (Control)	5 (3 main + 2 recovery)	0	6
2 (low dose)	3	32	6
3 (mid dose)	3	82.9	6
4 (high dose)	5 (3 main + 2 recovery)	214.8	6

The endpoints measured in this study are shown in the table below:

Clinical signs	Daily on a regular basis
Mortality	2× daily
Body weight	Weekly
Food consumption	Calculated daily
Clinical pathology	Hematology – Before initiation of treatment, Days 92, 183, and 274 of treatment, and Weeks 47 and 60 of the recovery period Lymphocyte subset counts – Day 235 of the study and Week 44 of the recovery period Clinical chemistry – Before initiation of treatment, Days 92, 183, and 274 of treatment, and Weeks 47 and 60 of the recovery period Urinalysis – Before initiation of treatment, Days 92, 183, and 274 of treatment, and Weeks 47 and 60 of the recovery period.
Cardiac and respiratory	ECG, blood pressure, and respiratory rate: Days 1, 92, 183 and 274 (on the

parameters	days of the 1st, 14th, 27th, and 40th treatments), before and 15 minutes (ECG), 10 minutes (blood pressure, and 9 minutes (respiratory rate) after injection
Ophthalmoscopy	Week 39 of the treatment period and week 60 of the recovery period
Macroscopic exam	Main - Day 281 of the treatment period (7 days after the last administration Recovery – At the end of the 20 week recovery period
Organs weights	Adrenal, brain, epididymis, heart, kidney, liver, lungs, ovary, pancreas, pituitary, prostate, seminal vesicle, spleen, testicle, thymus, thyroid, and uterus
Histopathology	Adrenal, aorta, bone marrow, bone, brain (5 levels – frontal lobe, brain stem, hippocampus, paraventricular parts, and cerebellum), epididymis, esophagus, eye with optic nerve, gall bladder, heart (3 levels – left and right ventricle and septum), injection site, intestine (duodenum, jejunum, ileum/cecum, colon, rectum), kidney, lacrimal gland, liver, lungs with mainstem bronchi, lymph node (cervical and mesenteric), mammary gland, nerve (sciatic), ovary, pancreas, pituitary, prostate, ribs, salivary gland, seminal gland, skin, spinal cord, spleen, stomach, synovial membrane, thyroid, tongue, trachea, urinary bladder, uterus (with cervix), vagina, vein (jugular), and gross lesions
Immunohistochemistry	The interaction of LU 200134 with immunocompetent cells in the spleen, thymus, and mesenteric lymph node were investigated using monoclonal human antibodies against T cells (CD2, CD4, and CD8), B cells (CD20 and CD21) and macrophages. (CD68). Immune complex deposition in spleen, liver, kidneys, lungs, skin, mammary gland, and synovial membrane of phalanges using monkey antibodies against IgG, IgM, complement, and LU 200134. This segment of the study was conducted at Knoll AG, Research and Development, Knollstr. 50, 67061 Ludwigshafen, Germany
Toxicokinetics	Main 0, 5 min, and 8, 24, 48, and 96 hours after the 1st administration Immediately before each following injection Immediately before and 5 min, 8, 24, 48, 96, and 168 hours after the last injection Recovery 264, 360, 480, 600, and 696 hours after the last injection
Immunogenicity	Not determined

2. Results:

2.1. Clinical signs: The animals did not exhibit any treatment-related effects.

2.2. Mortality: The animals did not exhibit any treatment-related effects.

2.3. Body weight: Females, but not males, in the high dose group exhibited a decrease in body weight (↓10% to 15% and ↓16% to 20% during Weeks 2 to 21 and Weeks 22 to 39, respectively). During the recovery period, body weight in the high dose females was decreased ~ 10% relative to the control group.

2.4. Food consumption: The animals did not exhibit any treatment-related effects.

2.5. Clinical pathology

2.5.1. Hematology: The females in the high dose group exhibited relatively mild decreases (~15%) in RBC parameters at Weeks 14, 27, and 40, with a reversal during the recovery period.

2.5.2. Lymphocyte subsets: The animals did not exhibit any effects.

2.5.3. Clinical Chemistry: The animals did not exhibit any relevant effects.

2.5.4. Urinalysis: The animals did not exhibit any treatment-related effects.

2.6. Cardiac and respiratory parameters: The animals did not exhibit any treatment-related effects.

2.7. Ophthalmoscopy: The animals did not exhibit any treatment-related effects.

2.8. Macroscopic exam: The animals did not exhibit any treatment-related effects.

2.9. Organ weights: Males in the mid and high dose group and females in the mid dose group exhibited a decrease in absolute and relative thymus weight. The data for relative organ weights (gm/kg body wt) obtained at the end of the treatment period are shown in the table below. In the male high dose group, the effect persisted through the recovery period.

Individual data						
Mean ± s.d.	1.13 ± 0.37	0.39 ± 0.21	0.59 ± 0.17	1.04 ± 0.55	0.55 ± 0.09	1.06 ± 0.16

2.10. Histopathology: The histopathology findings identified at the end of the treatment period are shown in the table below. Males in the high dose group exhibited a reduced activation in the lymphoid follicles of the spleen. Males and females receiving the high dose exhibited decreased cellularity in the follicular center of the spleen. Males and females receiving ≥ 82.9 mg/kg exhibited an increase in age-related involution of the thymus with cystic transformation.

Spleen								
Activation, lymph follicle	3/3	3/3	3/3	1/3	3/3	2/3	3/3	3/3
Minimal	-	-	1	1	-	-	-	-
Mild	3	3	2	-	3	2	2	2
Moderate	-	-	-	-	-	-	1	1

Decreased cellularity, follicular center	0/3	0/3	0/3	2/3	0/3	0/3	0/3	2/3
Mild	-	-	-	-	-	-	-	2
Moderate	-	-	-	2	-	-	-	-
Thymus								
Involution	2/3	3/3	3/3	3/3	2/3	3/3	3/3	3/3
Minimal	2	3	-	-	1	1	-	1
Mild	-	-	1	1	1	2	2	1
Moderate	-	-	2	-	-	-	1	1
Marked	-	-	-	2	-	-	-	-
Cystic transformation	1/3	2/3	2/3	3/3	0/3	1/3	1/3	2/3
Minimal	-	2	-	2	-	1	-	1
Mild	1	-	1	1	-	-	1	1
Moderate	-		1		-	-	-	-

The changes in the spleen were reversible. However, at the end of the recovery period, the changes in the thymus were still increased slightly relative to the control group.

2.11. Immunohistochemistry:

2.11.1. Effects on immunocompetent cells: Findings are summarized in the table below. The effects in the spleen were reversible, but those in the thymus were not.

	Spleen	Thymus
♂ 32	↓ cellularity, follicular dendritic cells (3/3 minimal to slight)	No effect
♂82.9	↓ cellularity, follicular dendritic cells (3/3 minimal to slight) ↓ CD20 cellularity, follicular germinal centers, (1/3 slight)	↓ CD2, CD4, and CD8 cellularity, cortex mainly 2/3 (slight to marked)
♂214.8	↓ CD20 cellularity, follicular germinal centers 3/3 (slight to marked) ↓ cellularity, follicular dendritic cells 3/3 (slight to moderate) ↓ CD8 in periarteriolar lymphocyte sheath 3/3 (slight)	↓ CD2, CD4, and CD8 cellularity, cortex mainly 3/3 (moderate to marked)
♀ 32	No effect	No effect
♀ 82.9	↓ cellularity, follicular dendritic cells 1/3 (minimal)	No effect
♀ 214.8	↓ cellularity, follicular dendritic cells 3/3 (slight)	No effect

2.11.2. **Immune complex formation:** Most monkeys in the 214.8 mg/kg group exhibited a co-expression of monkey IgG, complement, and anti-LU 200 134 in single glomerula. In addition, the expression of IgG was linear along the capillary wall as opposed to being in a slight, granular pattern.

2.12. **Toxicokinetics:** The toxicokinetic parameters for serum levels of LU 200134 after the last dose are shown in the table below. There were no gender differences.

32	304744 ± 74634	2731 ± 467	1231 ± 324	2.3 ± 0.5	1814 ± 444	0.11 ± 0.04
82.9	617368 ± 233959	6527 ± 2450	2720 ± 1438	2.7 ± 0.8	3675 ± 1393	0.16 ± 0.07
214.8	1299965 ± 228114	13563 ± 1740	5875 ± 1151	2.4 ± 0.4	7738 ± 1358	0.17 ± 0.03

3. **Conclusion:** A NOAEL was not identified for this study based on the decrease in follicular dendritic cells observed in the spleen at al doses.

Report N°: MPF/ET 9624 E
Title: 4-week Subacute Toxicity Study of LU 200134 (D2E7) by Intravenous Administration to Cynomolgus Monkeys – Administration Once Per Week
Nonclinical Review Volume: 7/15 and 8/15
Adalimumab Review Volume: 27/986 and 28/986
Study Facility: [redacted]

Batch N°: AFP 603/W 69853
GLP Compliance: Yes
Study Initiation: July 12, 1996 (date n protocol)
Study Termination: May 6, 1997 (date that the study director signed the study report)
In-life Phase: July 30, 1996 to September 25, 1996

1. **Methods:** This study was conducted in purpose-bred cynomolgus monkeys. The monkey was considered to be the most relevant species based on TNF neutralization potency. The animals received the test article, LY 200134, intravenously in phosphate buffered saline, once weekly for 4 weeks (5 treatments/animal, Days 1, 8, 15, 22, and 29). The main study animals were sacrificed 24 hours after the last treatment. A subset of animals from the control and high dose group were maintained for a 4-week treatment-free recovery period. The dose levels and related information are shown in the table below.

1 (Control)	5 (3 main + 2 recovery)	0	2
2 (low dose)	3	32	2
3 (mid dose)	3	70.9	2

4 (high dose)	5 (3 main + 2 recovery)	157.2	2

The endpoints measured in this study are shown in the table below.

Endpoint	Description
Clinical signs	Daily on a regular basis
Mortality	2× daily
Body weight	Weekly
Food consumption	Calculated daily
Clinical pathology	Hematology, clinical chemistry, and urinalysis– Before initiation of treatment, 21(urinalysis) or 24(hematology and clinical chemistry) hours after the 5th treatment (test day 30), and at the end of the recovery period.
Cardiac and respiratory parameters	ECG, blood pressure, and respiratory rate: Days 1 and 29 before and 5 minutes (ECG), or 10 minutes (blood pressure and respiratory rate) after treatment, and at the end of the recovery period.
Ophthalmoscopy	After the 5th treatment and at the end of the recovery period
Macroscopic exam	At the end of the treatment and recovery periods
Organs weights	Adrenal, brain, heart, kidney, liver, lungs, ovary, pancreas, pituitary, prostate, spleen, testicle, thymus, thyroid, and uterus
Histopathology	Adrenal, aorta, bone marrow, bone, brain (5 levels – frontal lobe, brain stem, hippocampus, paraventricular parts, and cerebellum), epididymis, esophagus, eye with optic nerve, gall bladder, heart (3 levels – left and right ventricle and septum), injection site, intestine (duodenum, jejunum, ileum/cecum, colon, rectum), kidney, lacrimal gland, liver, lungs with mainstem bronchi, lymph node (cervical and mesenteric), mammary gland, nerve (sciatic), ovary, pancreas, pituitary, prostate, ribs, salivary gland, seminal vesicle, skin, spinal cord, spleen, stomach, thyroid, tongue, trachea, urinary bladder, uterus (with cervix), vagina, vein (jugular), and gross lesions
Immunohistochemistry	The interaction of LU 200134 with immunocompetent cells in the spleen, thymus, skin, skeletal muscle, Peyer's patches, lymph nodes (popliteus, iliacus medialis, and cervicalis superficialis), liver, kidney, heart, and bone marrow. were investigated using monoclonal human antibodies against the following antigens: Common leukocyte (CD45), T cells (CD2 and CD4), B cells (CD20 and CD21) leukocyte functional antigen (LFA1), MHC1 (W6/32), and MHC2 (HLA-DR). Immune complex deposition in the tissues listed above using monkey antibodies against IgG, IgM, complement, and LU 200134. This segment of the study was conducted at Knoll AG, Research and Development, Knollstr. 50, 67061 Ludwigshafen, Germany
Toxicokinetics	0, 5 min, and 8, 24, 48, and 96 hours after the 1st administration Immediately before and 5 minutes after the 2nd and 3rd administration each following injection Immediately before and 5 min, 8, 24, 48, 96, and 168 hours after the last injection
Immunogenicity	Prior to initiation of treatment and immediately before the 5th administration

2. **Results**

It should be noted that the results for immunohistochemistry were provided in a separate report, MPF-ET 9660 (volume 8/15 of the nonclinical section of the BLA). Toxicokinetics and immunogenicity results were provided in report MPF/EBB 9612 (Volume 13/15 of the nonclinical section of the BLA).

Treatment had no effect on any of the endpoints with the exception of immunohistochemistry. At the terminal sacrifice, males receiving ≥ 70.9 mg/kg exhibited a minimal decrease in cellularity of B-cells (CD21) in the splenic follicles, especially the germinal centers. Additionally, most animals at all dose levels exhibited minimally to slightly decreased immunostaining in the germinal centers of the splenic follicles. The reduction in CD21 cells was completely reversible. However, the decrease in IgG and IgM expression was only partially reversible.

No PAHAs were detected in any of the monkeys receiving LU 200134. However, 7/10 control monkeys had low levels of PAHAs. It was suggested that PAHAs might have formed in response to exposure to low levels of LU 200134 present in the animals' cage or treatment facility.

Report N°: MPF/ET 1896 E
Title: 4-Week preliminary study of LU 200134 (D2E7) by intravenous (bolus) injection to cynomolgus monkeys
Nonclinical Review Volume: 6/15
Adalimumab Review Volume: 26/986
Study Facility: [redacted]
Batch N°: AF601-EX Pool
GLP Compliance: Yes
Report Date: February 26, 1997

1. **Methods:** The purpose of this study was to select doses for a final 4-week toxicity study.

1	60.8	Weekly x 4 weeks	1/sex	iv	Observation (2x daily), body weight (weekly), food consumption (daily), clinical pathology (prior to the 1st dose and in Week 4), pathology (gross, microscopic, and immunohistochemistry; Day 30), toxicokinetics (after the 1st and 5th treatment), immunogenicity (before the 1st and after the 5th administration), TNF levels (before and after the 1st and 5th treatments)
2	179.6		1/sex	iv	

2. **Results:** The animals did not exhibit any relevant effects. It should be noted that the immunohistochemistry results were provided in a separate report, MPT/ET 2697

(Volume 6/15 of the nonclinical section of the BLA). Additionally, it should be noted that serum samples were not analyzed for LU 200134, antibody formation, or levels of TNFα because the need for these data was superseded by the final 4-week toxicity study in monkeys (MPF/ET 9624 E).

Report N°: MPF/ET 1796 E
Title: 14-Day preliminary study of LU 200134 (D2E7) by intravenous (bolus) injection to cynomolgus monkeys
Nonclinical Review Volume: 6/15
Adalimumab Review Volume: 26/986
Study Facility: [redacted]
Batch N°: AF601-EX Pool
GLP Compliance: Yes
Report Date: February 26, 1997

1. **Methods:** The purpose of this study was to select doses for a 4-week study. The details of this study are provided in the table below.

Group	Dose (mg/kg)	Duration	M/F	Route	Parameters Evaluated
1	60.8	Daily × 14 days	1/sex	iv	Observations (daily), body weight (weekly), food consumption (weekly), clinical pathology (prior to treatment and on Day 14), histopathology (Day 15), immunohistochemistry (Day 15), and toxicokinetics (after the 13th and 14th treatment), immunogenicity (before the 1st and after the 14th treatment), and TNFα (before and after the 1st and 14th treatment)
2	179.6	Daily × 14 days	1/sex	iv	

2. **Results:** The animals did not exhibit any relevant effects. It should be noted that the immunohistochemistry results were provided in a separate report, MPT/ET 2597 (Volume 6/15 of the nonclinical section of the BLA). Additionally, it should be noted that serum samples were not analyzed for LU 200134, antibody formation, or levels of TNFα because the need for these data was superseded by a 4-week range-finding toxicity study in monkeys (MPF/ET 1896 E).

Report N°: MPF/ET 9635 E
Title: 4-Week intravenous toxicity study with LU 200134 (D2E7) in the mouse followed by a 4-week recovery period
Nonclinical Review Volume: 5/15
Adalimumab Review Volume: 25/986
Study Facility: [redacted]
Batch N°: AFP 603/W69853

GLP Compliance: Yes
Report Date: July 18, 1997

1. **Methods:** This study was conducted in NMRI mice. Details relating to animal treatment are shown in the table below.

Group	Main	Recovery	Satellite	Dose mg/kg	Duration	Route
1	12/sex	18/sex	5/sex	0	Weekly × 4 weeks	iv
2	"	"	30/sex	32		"
3	"	"	"	70.9		"
4	"	"	"	157.2		"

The recovery phase was 4 weeks. The satellite group was used to obtain blood samples for toxicokinetics analysis.

The endpoints measured in this study are shown in the table below.

Endpoint	Description
Clinical signs	1× daily
Mortality	2× daily
Body weight	Weekly
Food consumption	Weekly
Clinical pathology	Days 30 and 57
Macroscopic exam	At the end of the treatment and recovery periods
Organs weights	At the end of the treatment and recovery periods
Histopathology	At the end of the treatment and recovery periods
Immunohistochemistry	At the end of the treatment and recovery periods
Toxicokinetics	Days 1, 8, 15, 22, and 29,
Immunogenicity	After 4 and 8 weeks

2. **Results:** The animals did not exhibit any treatment-related effects.

▶Acute Toxicity

The acute toxicity studies that the sponsor conducted are listed in the table below.

Study	Type
MPF/ET 9626	Single dose toxicity study after intravenous administration in the mouse
MPF/ET 3396	Single dose toxicity study after intravenous administration in the NMRI mouse followed by a 12-week recovery period
MPT/ET 9625	Single dose toxicity study after intravenous administration in the rat

The details of these studies are provided in the table below.

Study	Species	Group	Dose	mg/kg	Route	Additional Comments
MPF/ET 9626	Mouse	1 2	5/sex 5/sex	0 560	iv	**Endpoints:** Observations, body weight, necropsy 14 days after treatment **Findings:** No relevant findings

MPF/ET 3396	Mouse	1 2 3 4	5/sex 5/sex 5/sex 5/sex	0 1.6 16 786	iv	**Endpoints:** Observations, body weight, blood samples for serum levels of LU 200134 and mouse anti-human antibody formation (MAHA) every 2 weeks for 12 weeks after treatment, necropsy and histopathology at the end of the 12 week recovery period. **Findings:** No effects other than MAHA at all dose levels
MPF/ET 9625	Rat	1 2	5/sex 5/sex	0 560	Iv	**Endpoints:** Observations, body weight, necropsy with histopathology 14 days after treatment **Findings:** No relevant findings

▶Local Tolerance

The studies that the sponsor submitted to address local tolerance are shown in the table below. The reports for all of these studies are in volume 11/15 of the nonclinical section of the BLA and 31/986 of the BLA.

MPF/DT 1099	LU 200134 Injection solution with 0.1% Tween 80- Preliminary study to investigate local tolerability after single intravenous, subcutaneous, intramuscular, paravenous, and intraarterial injection in rabbits
MPF/ET 1397	Preliminary study to investigate local tolerability after single intravenous, subcutaneous, intramuscular, paravenous, and intraarterial injection in rabbits
MPR/PT 0105	Study on local tolerance after single intravenous, subcutaneous, intramuscular, paravenous, and intraarterial injection in New Zealand white rabbits

Studies MPT/DT 1099 and MPF/ET 1397 are not relevant to the clinical use of LU 200134 because the concentration of LU 200134 used in these studies, 25 mg/mL, was less than that in the clinical formulation, 50 mg/mL. Study MPR/PT 0105 is not relevant to the proposed clinical used because the test article used in this study did not contain Tween 80.

▶IMMUNOGENICITY

The studies that the sponsor submitted to define immunogenicity in animals are listed in the table below. Reports pertaining to the same study are included in the same row of the table.

MPF/ET 9735 E BBC/1 9906	Immunogenicity study of Lu 200134 (D2E7) in cynomolgus monkeys, effect of route of, dose of and schedule of administration Analysis of samples from immunogenicity study of LU 200134 in cynomolgus monkeys: Effects of route of (iv vs sc), dose of (2 vs 32 mg/kg), and schedule of (weekly vs monthly) administration
BBC/I 9802 E ABC/H 0101	A study to evaluate the pharmacokinetics and immunogenicity of a monocloncal antibody administered intravenously and subcutaneously to cynomolgus monkeys Immunogenicity study of LU 200134 in cynomolgus monkeys following a schedule of six monthly iv or sc administration

Report N°: BBC/1 9906
Title: Analysis of samples from immunogenicity study of LU 200134 in cynomolgus monkeys: Effects of route of (iv vs sc), dose of (2 vs 32 mg/kg), and schedule of (weekly vs monthly) administration
Nonclinical Review Volume: 11/15
Adalimumab Review Volume: 31/986
Study Facility: The animals were treated and the samples collected at [redacted]. The samples were analyzed BASF Bioresearch Corporation, Immunology department, Worcester, MA.
Batch N°: 780100A0
GLP Compliance: The animals were treated and the samples collected in compliance with GLP, but sample analysis was not conducted in compliance with GLP.
Report Date: December 20, 1999

1. **Methods:** The animals were treated and the samples collected as indicated in the table below. The pharmacokinetic assay for LU 200134 was a competitive ELISA. In addition, LU 200134 was quantified using the [redacted] bioassay. Primate anti-human antibodies (PAHA) were measured using a [redacted] ELISA.

Group	No.	Dose (mg/kg)	Route	Treatment Days	Sampling Times (Days)
1	2/sex	2	iv	0,28	0, 7, 14, 21, 28, 31, 25, 38, 42, 45, 49, 52, 56
2	2/sex	32	iv	0,28	0, 7, 14, 21, 28, 31, 35, 38, 42, 45, 49, 2, 56, 59, 63, 66, 70, 73, 77, 80, 84, 87, 91, 94, 98, 101, 105, 108, 112
3	2/sex	2	iv	0,7,14,21,28	0, 7, 14, 21, 28, 31, 25, 38, 42, 45, 49, 52, 56
4	2/sex	32	iv	0,7,14,21,28	0, 7, 14, 21, 28, 31, 35, 38, 42, 45, 49, 2, 56, 59, 63, 66, 70, 73,
5	2/sex	2	sc	0,28	0, 7, 14, 21, 28, 31, 25, 38, 42, 45, 49, 52, 56
6	2/sex	32	sc	0,28	0, 7, 14, 21, 28, 31, 35, 38, 42, 45, 49, 2, 56, 59, 63, 66, 70, 73, 77, 80, 84, 87, 91, 94, 98, 101, 105, 108, 112
7	2/sex	2	sc	0,7,14,21,28	0, 7, 14, 21, 28, 31, 25, 38, 42, 45, 49, 52, 56
8	2/sex	32	sc	0,7,14,21,28	0, 7, 14, 21, 28, 31, 35, 38, 42, 45, 49, 2, 56, 59, 63, 66, 70, 73,

2. **Results:** All 4 groups of monkeys receiving 2 mg/kg developed detectable PAHA. The primary titers were relatively low (2 – 17 ug/mL), but they increased to 22 to 203 ug/mL with repeated administrations. In contrast, only 2/16 monkeys receiving 32 mg/kg developed PAHAs, and the titers were low (7 to 21 ug/mL).

   Within the 2 mg/kg group, the PAHA titers in the monkeys receiving monthly treatments (106 to 203) were higher than those from monkeys receiving weekly treatments (22 to 107 ug/mL). Similarly, titers in monkeys receiving iv treatment (107 to 203 ug/mL) were higher then in monkeys receiving sc treatment (22 – 106 ug/mL).

Report N°: ABC/H 0101
Title: Immunogenicity study of LU 200134 in cynomolgus monkeys following a schedule of six monthly iv or sc administration
Nonclinical Review Volume: 11/15
Adalimumab Review Volume: 31/986
Study Facility: The animals were treated and the samples collected at [redacted] The samples were analyzed BASF Bioresearch Corporation, Immunology department, Worcester, MA.
Batch N°: AF 504D-EX
GLP Compliance: The animals were treated and the samples collected in compliance with GLP, but sample analysis was not conducted in compliance with GLP.
Report Date: December 13, 2001

1. **Methods:** The animals were treated and the samples collected as indicated in the table below.

Group	N	Dose (mg/kg)	Route	Treatment Days	Sampling Times (Days)
1	4♂	15.5	iv	1, 31, 61, 91, 121, 153	Week prior to treatment and on Days 36, 41, 51, 60, 66, 71, 81, 90, 96, 101, 111, 120, 126, 136, 146, and 150
2	4♂	15.5	sc	1, 31, 61, 91, 121, 153	Week prior to treatment and on Days 36, 41, 51, 60, 66, 71, 81, 90, 96, 101, 111, 120, 126, 136, 146, and 150

Primate anti-human antibodies (PAHA) were measured using a [redacted] ELISA. In addition, LU 20014 was measured using a [redacted] ELISA and an [redacted] bioassay. The pharmacokinetics results were provided in report N° MPF/EB 9636, which is addressed on page XX of this review.

2. **Results:** In the intravenous treatment group, PAHAs and increased clearance were detected in 2/4 monkeys. In the subcutaneous treatment group, PAHAs and increased clearance were detected in 3 of 4 monkeys. There was no apparent difference between the response observed following iv administration and that following sc.

►Reproductive Toxicology

Report N°: MPF/DT 9853 E
Title: Development Reproduction Study of LU 200134 (D2E7) by Intravenous Administration (once weekly) to the Cynomolgus Monkey
Nonclinical Review Volume: 12/15
Adalimumab Review Volume: 32/986
Study Facility: [redacted]
GLP Compliance: Yes
Study Initiation: August 7, 1998 (date that the study director signed the protocol)
Study Termination: March 20, 2000 (date that the study director signed the report)
In-life Phase: September 10, 1998 to April 3, 1999

1. **Methods:** This study was conducted in purpose-bred female cynomolgus monkeys. The animals received the test article, LU 200134, intravenously once weekly during Days 20 to 97 *post coitum (pc)*. The dose levels and related information are shown in the table below.

[illegible]	[illegible]	[illegible]	[illegible]
Control/cesarean	5	0	3.1
Control/full term	4	0	3.1
Low dose/cesarean	8	30	3.1
Low dose/full term	5	30	3.1
High dose/cesarean	8	100	3.1
High dose/full term	5	100	3.1
Toxicokinetic	4	100	3.1

The cesarean sections in the control and the two treatment groups were performed on Day 100 pc. The monkeys in the toxicokinetic group underwent cesarean section on Day 104 pc. The study report did not provide an explanation for dividing the animals into cesarean and full term groups.

The endpoints measured in this study are shown in the table below.

[illegible]	[illegible]
Morbidity/mortality	2× daily from Day 20 pc until study termination
Clinical signs	2× daily from Day 20 pc until study termination
Body weight	Cesarean groups (including toxicokinetics group) Days 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 90, and 97 pc Day of cesarean section Full term group Days 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 90, 97, 104, 111, 118, 125, 132, 139, 146, 153, 160, 167, 174, and 181 pc, if applicable
Food consumption	Classified as normal or reduced
Ultrasonography	Every 2 weeks from day 30 to day 156 pc, where appropriate
Hematology	Hematocrit was determined as follows: Cesarean groups: Day 97 of gestation (168 and 240 hours treatment for the main study animals and 240 and 336 after treatment for the toxicokinetic animals), day of cesarean section prior to anesthesia, and directly after fetal blood collection for the females in the 30 and 100 mg/kg group. Full term groups: Day 97 of gestation (168 and 240 hours treatment)
Toxicokinetics	▪Blood samples Toxicokinetics subgroup After the 1st dose: 0 and 3min; 8, 24, 48, 56, 96, and 168 hr after dosing After the last dose: 0 and 3min; 8, 24, 48, 56, 96, 168, 240, and 360 hr after dosing Weekly: Pre-dose and ~ 3 min post-dose (peak and trough) Main study (cesarean) Prior to cesarean, directly after fetal blood collection, and 168 and 240 hr

	after the last dose (Day 97 of gestation) Main study (full term) 168 and 240 hr after the last dose (Day 97 of gestation) ▪ Amniotic fluid Toxicokinetics subgroup and Main study (cesarean) At cesarean section
Anti-LU 200134	Not determined
Dead/cesarean section group	
Weight	Fetus and placenta weighed separately
Measurements	Distance from coccyx to cranium, distance from tip of nose to os occipitale, disyance form os frontale to os occipatale, width of head, and distance between the eyes.
Macroscopic and/or stereomacroscopic exam	Body form; symmetry of head; facial form; formation of lower jaw; eyebrows, eyes, and eyelids; hair on head; nipple formation; anus; fingers, toes, and nails, ears, tail, upper and lower extremities, external genitalia, palpation of the vertebral column, umbilical cord, and palate
Necropsy	Full necropsy with visual inspection of organs
Organ weights	Adrenals, brain, eyes, heart, kidneys, liver (including gall bladder, lung, lymph node (mesenteric), ovaries, spleen, testes, thymus, and uterus
Skeletal examination	The carcass and skull of each fetus was processed and stained with Alzarin red for examination
Histopathology	Organs were taken for "possible further histopathological investigation"
Toxicokinetics	Toxicokinetics subgroup At cesarean section Main study (cesarean) At cesarean section
Neonatal/littermate group	
Physical examination	Days 1 and 7 *post partum*
Body weight	Days 1 and 7 *post partum*

2. Results:

2.1. Maternal: The animals did not exhibit any treatment-related effects.

2.2. Fetal: The fetal thymus weight in both treatment groups was decreased 20% relative to the control fetuses. The toxicological significance of this finding cannot be evaluated in the absence of histopathology. The sponsor has been asked to conduct histopathology.

2.3. Toxicokinetics

Maternal toxicokinetic parameters after the first and 12th injections are shown in the table below.

Dose							
1st	3523	1094	---	---	272,787	0.16	9.1

Dose	±875	±44			±39,986	±0.03	±4.9
12th Dose	9077 ± 1990	3160 ± 943	2.8 ± 1.5	2.8 ± 0.80	710,000 ±129,121	0.14 ±0.02	7.6 ±0.5

The fetal serum levels of the fetuses from the TK on Day 104 pc were 684 ± 100 ug/mL. The corresponding amniotic fluid levels were 83 ± 53 ug/mL.

3. **Conclusion:** LU 200134 had no effect on maternal or reproductive parameters. The NOAEL for maternal and developmental toxicity for this compound is 100 mg/kg, the high dose for this study. Based on available information, no NOAEL was established for fetal toxicity.

►Genotoxicity

Report N°: MPF/ET 9621 E
Title: Ames Salmonella/mammalian-microsome nutagenicity test and Escherichia coli/mammalian reverse mutation assay (standard plate test and preincubation test) with LU 200134
Nonclinical Review Volume: 12/15
Adalimumab Review Volume: 32/986
Study Facility: BASF AG, Department of Toxicology, D-67056 Ludwigshafen/Rhein, Germany
Batch N°: AF601-Ex POOL
GLP Compliance: Yes
Report Date: April 15, 1997

FDA/CBER did not request this study. FDA/CBER does not consider genotoxicity studies applicable to biotechnology-derived products.

1. **Methods:** LU 200134 was tested in the presence and absence of S-9 in the following strains: TA 1535, TA 10, TA 1537, TA 98, and E. coli WP2 uvrA. The concentration used were 20 ug to 5,000 ug/plate.

2. LU 200134 was not genotoxic.

Report N°: MPF/ET 9622 E
Title: Cytogenetic study in vivo with LU 200134 in mice micronucleus test single iv administration
Nonclinical Review Volume: 12/15
Adalimumab Review Volume: 32/986
Study Facility: BASF AG, Department of Toxicology, D-67056 Ludwigshafen/Rhein, Germany
Batch N°: AF601-Ex POOL
GLP Compliance: Yes

FDA/CBER did not request this study. FDA/CBER does not consider genotoxicity studies applicable to biotechnology-derived products.

1. **Methods:** — .NMRI BR mice were treated as shown in the table below. All treatments were administered as a single intravenous injection.

Treatment	Dose (mg/kg)	Sacrifice Time (hrs after treatment)	# Animals/Time
Vehicle	0	24 + 48	5
LU 200134	224.5	24	5
	449	24	5
	898	24 + 48	5
Cyclophosphamide (+ control)	20	24	5
Vincristine (+ control)	0.15	24	5

2. LU 200134 was not genotoxic.

SUMMARY and CONCLUSIONS

1. **Pharmacology:** The pharmacology studies that the sponsor submitted support that adalimumab binds specifically to TNF-α and neutralizes its actions. Kinetics studies conducted using — technology revealed that adalimumab has a K_d of 6.09×10^{-10}. In *in vitro* studies, adalimumab was shown to [1] inhibit the binding of ^{125}I-TNF-α to its receptors, [2] neutralize cytotoxic effects of rhTNF-α in — cells, and [3] inhibit rhTNFα-induced expression of adhesion molecules on human umbilical veins. Additionally, *in vitro* studies revealed that the cynomolgus monkey is pharmacologically similar to humans. In *in vivo* studies, adalimumab inhibited rhTNF-α-induced lethality in galactosamine-sensitized mice and rhTNF-α-induced pyrexia in rabbits. As anticipated, based on its ability to bind to and neutralize the effects of TNF-α, adalimumab was shown to be efficacious in preventing the development of polyarthritis in animal models.

2. **Pharmacokinetics:** Because the cynomolgus monkey was shown to the pharmacologically relevant animal model for preclinical studies, most of the preclinical pharmacokinetics studies were conducted in this species. These studies provided pharmacokinetics information in a relevant preclinical model and addressed three issues. First, all of the toxicology studies were conducted using iv administration as opposed to the clinical route of administration, sc. Second, the clinical formulation contains polysorbate 80 (Tween) — which was developed to — Third, during the course of drug development, the manufacturing process for the drug substance was changed. The preclinical pharmacokinetics studies revealed that [1] subcutaneous and intravenous routes of administration were sufficiently comparable, [2] the addition of Tween to the

formulation did not affect pharmacokinetics, and [3] changes in the manufacturing process did not affect pharmacokinetics.

3. **Toxicology:** The sponsor conducted repeat-dose, reproductive, and genotoxicity studies.

3.1. Repeat Dose: The most relevant study that the sponsor conducted was a 39-week study in cynomolgus monkeys. In the study, cynomolgus monkeys received weekly iv injections of adalimumab for 39 weeks. At the end of the treatment period, 3 monkeys/sex were sacrificed. A subset (2/sex) of control and high dose animals were sacrificed after a 20 week treatment free recovery period. The doses used in this study were 0, 32, 82.9, and 214.8 mg/kg/week. These doses are 48, 125, and 325 times the recommended human weekly dose of 40 mg/patient (0.66 mg/g, assuming 60 kg body weight). The following endpoints were monitored in this study: clinical sign/mortality, body weight, food consumption, clinical pathology, cardiac and respiratory parameters, ophthalmoscopy, macroscopic examination, organ weights, histopathology, immunohistochemistry, and toxicokinetics. Primate anti-human antibodies (PAHAs) were not measured because range-finding studies revealed that they are not detected at the relatively high doses used in the toxicology studies. The effects observed in this study are as follows: [1] decreased body weight in the high dose females during Weeks 2 to 21 (↓10 to 15%) and Weeks 22 to 39 (↓16 to 20%), [2] decreased thymus weight in males in the mid dose (↓65%) and high dose group (↓47%) and females in the mid dose group (47%), [3] ↓ activation in splenic lymphoid follicles in the high dose males and ↓ cellularity in the splenic follicular centers on high dose males and females, [4] ↑ thymic involution with cystic transformation and a reduction in lymphocytes in the mid and high dose males and females. The splenic changes were reversible but those in the thymus persisted throughout he recovery period.

Endpoint	Effects			
	32 mg/kg	82.9 mg/kg	214.8 mg/kg	Reversible
Body weight	No effect	No effect	♂: No effect ♀: ↓ during Weeks 2 to 21 (↓10 to 15%) and Weeks 22 to 39 (↓16 to 20%)	Trend towards reversal
Organ weight	No effect	♂: ↓ thymus weight (65%) ♀: ↓ thymus weight (47%),	♂: ↓ thymus weight (47%) ♀: No effect	♂: Not reversible in high dose group ♀: Reversible
Histopathology	No effect	♂ + ♀: ↑ thymic involution with cystic transformation and a ↓ in lymphocytes	♂: ↓ activation in splenic lymphoid follicles ♂ + ♀: ↓ cellularity in the splenic follicular	♂ + ♀: Splenic changes reversible ♂ + ♀: Thymus changes still slightly increased relative to control

			♂ + ♀: ↑ thymic involution with cystic transformation and a ↓ in lymphocytes	
Immunohistochemistry	♂: ↓ cellularity, follicular dendritic cells (spleen) ♀: No effect	♂ + ♀: ↓ cellularity, follicular dendritic cells (spleen) ♂: ↓ CD 20 cellularity, follicular germinal center (spleen) + ↓ CD in periarteriolar sheath ♂: ↓CD2, CD4, and CD8 cellularity (thymus)	♂ + ♀: ↓ cellularity, follicular dendritic cells (spleen) ♂: ↓ CD 20 cellularity, follicular germinal center (spleen) + ↓ CD in periarteriolar sheath ♂: ↓CD2, CD4, and CD8 cellularity (thymus)	Splenic changes were reversible, but thymus changes were not

Based on the effects on follicular dendritic cells, a NOAEL was not identified for this study. However, it should be noted that the splenic change was relatively mild and reversible. It should also be noted that these animals in the study did not exhibit any infections.

Considering that TNF-α stimulates T-cell and B-cell proliferation the pathological changes observed in spleen and thymus are consistent with adalimumab inactivating TNF-α as opposed to a direct toxic effect on these tissues.

3.2. Reproductive toxicity: There were no effects in pregnant female monkeys receiving 30 and 100 mg/kg of adalimumab, iv, during Days 20 to 97 *post coitum*.

3.3. Genotoxicity: The sponsor evaluated the genotoxic potential of adalimumab in the *in vitro* Ames assay and in the *in vivo* mouse micronucleus test. No evidence of genotoxicity was observed in either of these studies. It should be noted that FDA/CBER did not request these studies.

Overall Conclusion: Based on the results of the preclinical pharmacology studies, adalimumab is considered reasonably safe for the proposed indication.

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