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APPLICATION NUMBER:

125057/0

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Adalimumab (40 mg subcutaneous administration)

STN# 125057/0

Abbott Laboratories, Abbott Park, Illinois 60064

Indication: Rheumatoid Arthritis

Pharmacology & Toxicology Branch, OTRR (HFD-579)

Reviewer: Iftekhar Mahmood, Ph. D.

Branch Chief: Martin David Green, Ph. D.

Summary

Adalimumab (also called D2E7, LU 200134) is a fully human recombinant monoclonal IgGI antibody directed against TNF alpha, to which it binds with high affinity. TNF is a naturally occurring cytokine and plays an important role in pathologic inflammation and the destruction of the joints. Adalimumab by virtue of its binding to TNF, neutralizes the biological function of TNF by blocking its interaction with the p55 and p57 cell surface TNF receptors. In the patients with rheumatoid arthritis (RA), the TNF levels are increased in the synovial fluid. Adalimumab consists of 133 amino acids and the approximate molecular weight of adalimumab is 148 kDa. The recommended dose of adalimumab to the patients with RA is 40 mg subcutaneously every other week. The patients can continue with methotrexate, glucocorticoids, salicylates, nonsteroidal anti-inflammatory drugs or other analgesics. Those patients who are not taking concomitant methotrexate may take 40 mg adalimumab every week.

Following a single intravenous dose, ranging from 0.5 to 10 mg/kg, given over 3 to 5 minutes, both Cmax and AUC increased proportionally with dose indicating that the pharmacokinetics of adalimumab was linear over this dose range. Volume of distribution at steady state (Vss) was 4.9 $\pm$ 1.5 L. The clearance and half-life ranged from 11.5 to 17.4 mL/hr and 10 to 13 days, respectively.

Following a single subcutaneous dose of adalimumab (0.1, 0.3, 1.0 mg/kg), the Cmax ranged from [redacted] microgram/mL and the time to reach the maximum concentration (Tmax) ranged from 4 to 7 days. The apparent clearance of adalimumab was about 21 mL/min and the half-life ranged from 11 to 18 days across the doses. The absolute bioavailability (1 mg/kg SC and IV) of Adalimumab was 52%. When Tween-80

was added (both IV and SC) to the formulation the absolute bioavailability of subcutaneously administered adalimumab at 40 mg dose was 78%.

Following 40 mg SC adalimumab dose, the clearance and half-life were about 13 mL/hr and 16 days, respectively. However, across doses, the average clearance of adalimumab was about 20 mL/hr and the mean half-life was about 20 days. The steady state clearance of adalimumab following 40 mg SC dose ranged from 17 to 25 mL/hr.

MTX decreases adalimumab clearance as high as 70%. For example at 1 mg/kg dose the mean clearance of adalimumab was 17.4 mL/hr but in the presence of MTX the mean clearance of adalimumab was 12 mL/hr (a decrease of 31%). In a population PK study, the mean clearance of adalimumab was 24.6 mL/h and 13.8 mL/hr in the presence of MTX (a decrease of 44%). The clearance of adalimumab in MTX(-) patients with and without HAHA formation was 58.8 and 22.3 mL/hr, respectively (a decrease of 63%) and in MTX(+) patients was 43.2 and 13.7 mL/h, respectively (a decrease of 68%).

A population PK study (adalimumab dose range:0.25 to 5 mg/kg), indicated that adalimumab has also an impact on the CL of MTX. The MTX clearance with and without adalimumab was 6.16 and 4.39 l/hr, respectively. In the presence of adalimumab, the MTX clearance increased by 40%.

The steady-state clearance of adalimumab appears to be same as a single dose. However, in study DE018 (study #13 in this review) the steady state clearance was almost 45% higher than observed in study DE009X (study #10 in this review). The accumulation ratio of adalimumab was 1.69-3.29 for every other week and 2.76-6.04 for weekly dosing.

Body weight between 40 to 82 kg may not have any impact on the clearance of adalimumab but clearance of adalimumab increased by 25% over 82 kg body weight. The clearance of adalimumab decreases with increasing age (<40 years = 26.7 mL/hr; >70 years = 17.8 mL/hr; decreased by 33%). The levels of adalimumab appeared to be lower in the females but when adjusted based on the body weight the difference in clearance between the two genders disappeared (males = 0.33 $\pm$ 0.22 mL/hr*kg (n = 299); females =0.33 $\pm$ 0.25 mL/hr*kg (n = 1009). It is highly unlikely that rheumatism will alter the pharmacokinetics of adalimumab, nevertheless, from the submitted studies it is difficult to make any such conclusion.

The overall HAHA positivity rate while on treatment was low (5.5%). For 40 mg every other week, the rate was 5.7%. With MTX, the rate was very low and it is difficult to make any meaningful assessment. Since the current assay method cannot quantify adalimumab-HAHA complexes, their elimination rate from the body cannot be estimated.

From the clinical trial data, the Sponsor attempted to establish concentration-effect relationship. The primary endpoint was based on the American College of Rheumatology (ACR) disease scoring system (ACR20, ACR50 and ACR70). Attempt was made to relate the mean ACR20% with the mean trough adalimumab concentrations. A sigmoidal Emax model was found to be suitable to describe the concentration-effect relationship of adalimumab.

The ACR classifications, however, are not especially well suited for traditional pharmacokinetic-pharmacodynamic modeling. The data are binary, therefore, a logistic regression approach is more suitable to relate plasma concentrations with effect. On the surface, the Sponsor's Emax model appears reasonable, yet its predictive performance may be poor as this model has not been validated on a separate data set. Furthermore, there are only few data points and these points are also mean values. In short, this pharmacodynamic model may be good for some exploratory purposes but the regular use of this model to predict effect against known concentrations in a given individual is inappropriate.

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Study #1

Title: Pharmacokinetics and safety of single subcutaneous and intravenous administration of adalimumab (Lu 200134, D2E7) in healthy male Caucasians: A double-blind placebo-controlled parallel-group study (DE024).

This was a single center, double-blind, randomized, placebo-controlled, parallel-group, ascending single-dose study in healthy male volunteers. There were 80 subjects (60 active and 20 placebo) in the study. The subjects (19 to 45 years; weight = 61 to 106 kg) received a single intravenous dose of 1 mg/kg of adalimumab over 2 to 3 minutes or 0.1, 0.3 and 1.0 mg/kg adalimumab dose subcutaneously. Five mL of venous blood were drawn at time 0, 0.3, 1, 4, 8, 12, 24, 48, 72, 96, 168, 336, 504, 672, 840, 1008, 1176 and 1344 (day 57) hours. Drug concentrations were measured in serum using a validated — enzyme-linked immunosorbent assay (ELISA) method. The lower limit of quantification of adalimumab in serum was — in undiluted serum. Human anti-human antibodies (HAHA) were measured from 96 hours to 1344 hours. HAHA samples were also taken 4 months after dosing from as many subjects as possible. The LOQ for the HAHA assay was — in undiluted serum.

Following subcutaneous administration of adalimumab the mean Cmax and AUC (0-inf) increased with increasing dose though not necessarily linearly. Time to reach the maximum plasma concentration ranged from 48 to 336 hours. The mean absolute bioavailability of adalimumab was 52% (1 mg/kg SC vs 1 mg/kg IV). The mean systemic and apparent clearances were 11 mL/hr and 22 mL/hr (mean of 0.3 and 1.0 mg/kg SC), respectively. The mean half-lives ranged from 284 to 433 hours across doses. The pharmacokinetic parameters of adalimumab have been summarized in the following Table (next page).

Conclusions: The Cmax and AUC of adalimumab increased proportionally with dose only at doses of 0.3 and 1 mg/kg. Dose of 0.1 mg/kg is probably too low for appropriate characterization of pharmacokineic parameters. Furthermore, this is a parallel design

Study #3

Title: A randomized parallel-group, open label bioequivalence study with the adalimumab (D2E7) clinical trial formulation and the intended market formulation in healthy adult subjects (DE029).

This was a phase I, randomized parallel-group, open label single dose study in which 120 (60 in each group) healthy male and female subjects (19 to 45 years) received two formulations of adalimumab (40 mg) subcutaneously. The objective of this study was to assess the bioequivalence of the new adalimumab formulation, intended for future clinical trials and marketing with the present formulation used in phase I, II, and III clinical trials. Venous blood samples were taken at regular intervals till 360 hours (day 15). Drug concentrations were measured in serum using a validated enzyme-linked immunosorbent assay (ELISA) method. The lower limit of quantification of adalimumab in serum was Human anti-human antibodies (HAHAs) were measured before dosing, at 360 hours and at 1344 hours. The LOQ for the HAHA assay was

Mean Tmax, Cmax and AUC (0-360) of adalimumab were comparable between the two formulations. The 90% confidence interval on log transformed Cmax (0.857-1.041) and AUC (0.850-1.014) was within 80 % to 125%, indicating that the two formulations are comparable (please see comments). The pharmacokinetic parameters of adalimumab have been summarized in the following Table.

Table 11.5a Mean ± SD Pharmacokinetic Parameters of Adalimumab

Pharmacokinetic Parameters		Treatments A: 25 mg/mL adalimumab (Reference)	Treatments B: 50 mg/mL adalimumab (Test)
N		60	59
T_{max}	(h)	137.9 ± 59.3	130.6 ± 55.6
C_{max}	(µg/mL)	4.964 ± 1.516	4.717 ± 1.555
AUC_{0-360}	(µg·h/mL)	1311.3 ± 352.3*	1221.7 ± 338.6

* For AUC_{0-360} N = 59, excluding Subject 1116
Cross references: Tables 14.2__1.1 and 14.2__1.2

Figure 11.5b Mean Serum Adalimumab Concentration – Time Profiles, Log-Linear Scale

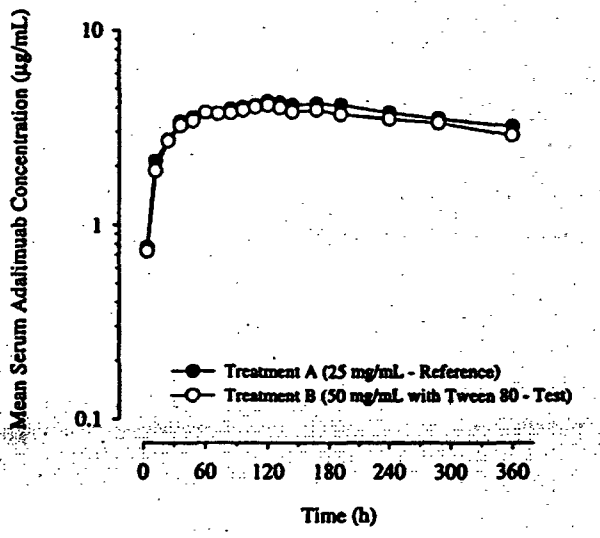


Table 11.5c Bioequivalence Assessment and Relative Bioavailability

Treatments	Pharmacokinetic	Estimate of Population Central Value*		Relative Bioavailability Point	Relative Bioavailability 90% Confidence
Test *vs.* Reference	Parameter	Test	Reference	Estimate+	Interval
B *vs.* A	C_{max}	4.478	4.741	0.944	0.857 – 1.041
	AUC_{0-360}	1173.0	1263.2	0.929	0.850 – 1.014

* Antilogarithm of the means for logarithms.
\+ Antilogarithm of the difference (test minus reference) of the means for logarithms.
Treatment A: 40 mg/1.6 mL of 25 mg/mL adalimumab in citrate/phosphate buffer (Reference)
Treatment B: 40 mg/0.8 mL of 50 mg/mL adalimumab in citrate/phosphate buffer with Tween-80 (Test)
Cross reference: Appendix 16.1_9.2

Table 11.5b Total Variability

Parameter	Variability (%CV)	
	Treatment A (Reference)	Treatment B (Test)
C_{max} (µg/mL)	30.5	33.0
AUC_{0-360} (µg·h/mL)	26.9	27.7

14.4 Formulation Composition and Components

Table 14.4__1.1 Quantitative Composition of the Test and Reference Adalimumab Formulations

Component	40 mg/1.6 mL	40 mg/0.8 mL
	mg	
LU 200134 Concentrate	40.00	40.00‡
Mannitol		9.60
Polysorbate 80		0.80
Citric Acid Monohydrate		1.04
Sodium Citrate		0.24
Sodium Chloride		4.93
Sodium Hydroxide		QS†
Water for Injection		

† As needed to adjust pH buffer vehicle prior to dilution of drug substance to final concentration.

Comments:

1. In the literature it has been advocated that for long half-life drugs, one can use truncated area under the curve (generally till 72 hours) for bioequivalence studies. The selection of 72-hour sampling time is however, arbitrary. The half-life of adalimumab is over 300 hours. In this study blood samples were taken till 360 hours which is approximately one half-life of adalimumab. The 90% confidence interval has been applied on the truncated area of the curve, in this case only till one half-life. The impact of the truncated AUC up to only one half-life on the 90% confidence interval in bioequivalence study is not known and may not be appropriate for bioequivalence study. Therefore, Sponsor's conclusion that the two formulations are bioequivalent is questionable.

2. There is a discrepancy in the Cmax and AUC values shown in Table 11.5a and 11.5c. The reason(s) for this discrepancy is not clear.

3. The study population consists of 50% males and 50% females. The Sponsor should evaluate the effect of gender on the pharmacokinetics of adalimumab from this study also.

Study #4

Title: A randomized, parallel-group, open-label study to assess the absolute and relative bioavailability of three injectable formulations of human anti-TNF antibody D2E7 (LU 200134) administered to healthy adult subjects (protocol DE015).

This was an open-label, randomized, parallel-group, single-dose study in healthy male and female volunteers (18 to 45 years of age). There were four treatment arms (3 subcutaneous and 1 intravenous). There were 12 subjects in SC group and 16 in IV group. The subjects received 40 mg SC or IV dose. The following is the description of the study drug.

Table 1 D2E7 treatments administered during the study

Treatment group	Planned no. of subjects	Route of administration (Total body dose)	D2E7 Concentration	Formulation	
A	16	sc (40 mg)	25 mg/mL	Clinical (Phase I/II/III)	None
B	16	sc (40 mg)	50 mg/mL	Market candidate	Tween-80
C	16	sc (40 mg)	50 mg/mL	Market candidate	None
D	12	iv (40 mg)	50 mg/mL	Market candidate	Tween-80

Two of the four treatments were reference treatments, the other two were the test treatments, as follows:

Treatment A: 25 mg/mL D2E7, without Tween-80, sc injection, was utilized in early clinical trials and was used here as the reference to estimate relative bioavailability.

Treatment B: 50 mg/mL D2E7, with Tween-80, sc injection, was being tested for use in Phase III trials and as a possible market candidate.

Treatment C: 50 mg/mL D2E7, without Tween-80, sc injection,

Treatment D: 50 mg/mL D2E7, with Tween-80, iv injection, was the reference used to estimate absolute bioavailability of the sc route.

To obtain a 40-mg dose from 25 mg/mL D2E7 prepared as 50 mg in a 2-mL volume (Treatment A), 1.6 mL of study drug was withdrawn from the appropriate vial into a 3-mL syringe. To obtain a 40-mg dose from 50 mg/mL D2E7 with Tween-80 prepared as 60 mg in a 1.2-mL volume (Treatments B and D), 0.8 mL of study drug was withdrawn from the appropriate vial into a 3-mL syringe. To obtain a 40-mg dose from 50 mg/mL D2E7 without Tween-80 prepared as 60 mg in a 1.2-mL volume (Treatment C), 0.8 mL of study drug was withdrawn from the appropriate vial into a 3-mL syringe. Treatments A, B, and C were subcutaneously injected over 3 to 5 seconds using a 25-gauge needle; one injection was administered to the left or right thigh. Treatment D was intravenously injected using a 20-gauge needle by slow iv push over 2 to 5 minutes to the left or right antecubital vein; the injection was immediately followed with a saline flush.

Blood samples were drawn at time 0, 0.25, 0.5, 1, 1.5, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192, 360, 528, 696, 864, 1032, 1200 and 1536 (day 65) after IV administration and at time 0, 1, 4, 8, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 144, 168, 192, 360, 528, 696, 864, 1032, 1200 and 1536 (day 65) after SC administration. Drug concentrations were measured in serum using a validate → enzyme-linked immunosorbent assay (ELISA) method. The lower limit of quantification of adalimumab in serum was —, in undiluted serum. Human anti-human antibodies (HAHAs) were also analyzed in this study. The LOQ for the HAHA assay was —, in undiluted serum.

The mean Cmax and Tmax for the three formulations of D2E7 following SC administration were comparable. Due to the formation of HAHA beyond 360 hours, the Sponsor considered the $AUC_{(0\text{-}360)}$ hours as reasonable for this parameter. Tween 80 did not appear to have any significant impact on the Cmax, Tmax, AUC, and clearance of the three formulations of D2E7. The absolute bioavailability of D2E7 with Tween 80 was about 78%. The relative bioavailability was over 95% for D2E7 without Tween 80 when compared with D2E7 with Tween 80. The following Tables summarize the results of the study.

Table 8 Mean ± SD pharmacokinetic parameters following a single 40 mg D2E7 subcutaneous or intravenous administration in healthy adult subjects by treatment group

PK Parameter	Treatment A	Treatment B	Treatment C	Treatment D
Cmax (µg/mL)	5.21 ± 1.19	5.16 ± 1.65	4.72 ± 1.01	15.07 ± 4.25
Tmax (h)	126 ± 89	130 ± 118	112 ± 55	0.7 ± 0.8[a]
$AUC_{0\text{-}336h}$ (µg•h/mL)	1301 ± 225	1381 ± 346	1256 ± 256	1884 ± 374
$AUC_{0\text{-}T}$ (µg•h/mL)[b]	2494 ± 881	2782 ± 694	2206 ± 807	3581 ± 1168
$AUC_{0\text{-}T}$ (µg•h/mL)[c]	2906 ± 742	3023 ± 637	2707 ± 649	3879 ± 1167
$AUC_{0\text{-}\infty}$ (µg•h/mL)	3311 ± 1046	3541 ± 287	2981 ± 586	4509 ± 1653
K_{el} (1/h)	0.0021 ± 0.0009	0.0016 ± 0.0004	0.0021 ± 0.0009	0.0015 ± 0.0004
t½ (h)	382 ± 164	464 ± 115	385 ± 151	484 ± 128
CL/F (mL/min)	0.22 ± 0.09	0.19 ± 0.01	0.23 ± 0.06	—
CL_s	—	—	—	0.16 ± 0.05
V_z (L)	6.6 ± 1.5	7.5 ± 1.7	7.3 ± 2.1	6.6 ± 2.1
V_{ss}	—	—	—	6.2 ± 1.7

[a] Average time of first blood sample post-dose
[b] $AUC_{0\text{-}T}$ for all subjects
[c] $AUC_{0\text{-}T}$ for subjects with non-measurable HAHAs

Treatment A: sc, 25 mg/mL D2E7
Treatment B: sc, 50 mg/mL D2E7, with Tween-80
Treatment C: sc, 50 mg/mL D2E7
Treatment D: iv, 50 mg/mL D2E7, with Tween-80

Note: Treatment A was the early clinical formulation, used as the reference for relative bioavailability calculations; Treatment D was used as the reference for absolute bioavailability calculations.

Table 9 Mean (%) absolute and relative bioavailability of the four treatments using non-transformed data

PK Parameter	Absolute Bioavailability (F_{abs} %)		
	Trt A/Trt D (N/N)	Trt B/Trt D (N/N)	Trt C/Trt D (N/N)
$AUC_{0\text{-}T}$	69.7 (15/11)	77.7 (15/11)	61.6 (16/11)
$AUC_{0\text{-}\infty}$	73.4 (9/7)	78.5 (8/7)	66.1 (7/7)

	Relative Bioavailability (F_{rel} %)	
	Trt B/Trt A (N/N)	Trt C/Trt A (N/N)
$AUC_{0\text{-}336h}$	104.6 (14/15)	96.5 (16/15)
$AUC_{0\text{-}T}$	111.5 (15/15)	88.4 (16/15)
$AUC_{0\text{-}\infty}$	107.0 (6/9)	90.0 (7/9)

Treatment A: sc, 25 mg/mL D2E7
Treatment B: sc, 50 mg/mL D2E7, with Tween-80
Treatment C: sc, 50 mg/mL D2E7
Treatment D: iv, 50 mg/mL D2E7, with Tween-80

Data source: Section 9.2, Table 9.2.3A

Table 10 Bioavailability comparisons using log-transformed pharmacokinetic parameters

PK Parameter	Statistic	Treatment Comparison (Test vs. Reference)				
		Absolute Bioavailability			Relative Bioavailability	
		A vs. D	B vs. D	C vs. D	B vs. A	C vs. A
ln(Cmax)	Ratio	34.8	33.8	31.6	97.0	90.9
	CI	29.5-41.0	28.7-39.8	26.9-37.2	83.4-112.9	78.3-105.4
	n_T, n_R	15, 11	15, 11	16, 11	15, 15	16, 15
ln(AUC_{0-360h})	Ratio	69.3	71.4	66.6	103.0	96.2
	CI	60.3-79.5	62.0-82.1	58.1-76.3	90.5-117.2	84.9-109.0
	n_T, n_R	15, 11	14, 11	16, 11	14, 15	16, 15
ln(AUC_{0-T})	Ratio	68.9	78.9	60.3	114.6	87.6
	CI	55.2-85.8	63.3-98.3	48.5-74.9	93.5-140.3	71.7-106.9
	n_T, n_R	15, 11	15, 11	16, 11	15, 15	16, 15
ln(AUC_{0-T}) (subjects with non-measurable HAHA)	Ratio	75.3	79.1	70.5	105.0	93.6
	CI	61.4-92.4	64.8-96.6	57.2-86.9	87.0-126.7	76.8-114.1
	n_T, n_R	10, 8	11, 8	9, 8	11,10	9, 10
ln($AUC_{0-\infty}$)	Ratio	73.3	82.3	68.2	112.2	92.9
	CI	57.8-93.1	63.2-107.0	52.9-87.8	87.4-144.0	73.2-118.0
	n_T, n_R	9, 7	8, 7	7, 7	8, 9	7, 9

Ratio = ratio of the means; CI = 90% confidence interval based on two one-sided t-tests; n_T = number of subjects who received the test treatment; n_R = number of subjects who received the reference treatment
Treatment A: sc, 25 mg/mL D2E7 Treatment C: sc, 50 mg/mL D2E7
Treatment B: sc, 50 mg/mL D2E7, withTween-80 Treatment D: iv, 50 mg/mL D2E7, with Tween-80

Note: Treatment A was the early clinical formulation, used as the reference for relative bioavailability calculations; Treatment D was used as the reference for absolute bioavailability calculations.

Comments:

1. Based on the relative bioavailability, it appears that Tween 80 has no impact on the bioavailability of D2E7. A small difference in AUC between the three SC formulations of D2E7 may be due to the parallel design study (there is almost 11% difference in AUC(0-inf) between treatments A and C (without Tween 80)). The Sponsor, however, advocates the 50 mg/mL formulation with Tween-80 as the best candidate for a market formulation.
2. The FDA's guidance on the use of truncated AUC for bioequivalence studies requires further investigation. It is the opinion of this reviewer that in order to detect the true difference between two formulations, the truncated AUC must be obtained at least for three half-lives.

Study #5

Title: A pharmacokinetics asseeement of a multicenter randomized placebo-controlled phase I study of the human anti-TNF antibody adalimumab administered as weekly subcutaneous injection of 0.5 mg/kg in patients with rheumatoid arthritis (protocol DE004).

This was a multicenter, open-label, randomized, placebo-controlled study in patients with rheumatoid arthritis. There were 24 patients (18 active and 6 placebo; 6 males and 12 females in active group) in the study. The patients (28 to 75 years; weight = 54 to 93 kg) received a single subcutaneous dose of 0.5 mg/kg adalimumab weekly for 2.5 years. Blood samples were drawn before the first injection of adalimumab, and then at 15 minutes, 1, 4, 8, 12, and 24 hours after the first injection. Thereafter, blood samples were taken before each dose till 35 months. Drug concentrations were measured in serum using a [redacted] method. The lower limit of quantification of adalimumab in serum was [redacted]. Human anti-human antibodies (HAHAs) were measured at baseline, day 8, and thereafter at all subsequent visits. HAHA concentrations were only measured if adalimumab concentrations were <2.0 microgram/mL. The LOQ for the HAHA assay was [redacted] in undiluted serum.

Plasma concentrations vs time data were analyzed by a population pharmacokinetic approach using NONMEM. The inclusion of POSTHOC in NONMEM analysis provided the PK parameters for individual subjects. The mean clearance (CL/F) following POSTHOC estimation was 19.08 $\pm$ 9.79 mL/hr. The elimination half-life was reported to be 22.4 days. In this study, age, weight and gender did not produce any effect on the PK of adalimumab. Only one patient had HAHA concentration ([redacted] ng/mL) above BLQ. The pharmacokinetic parameters of adalimumab have been summarized in the following Table.

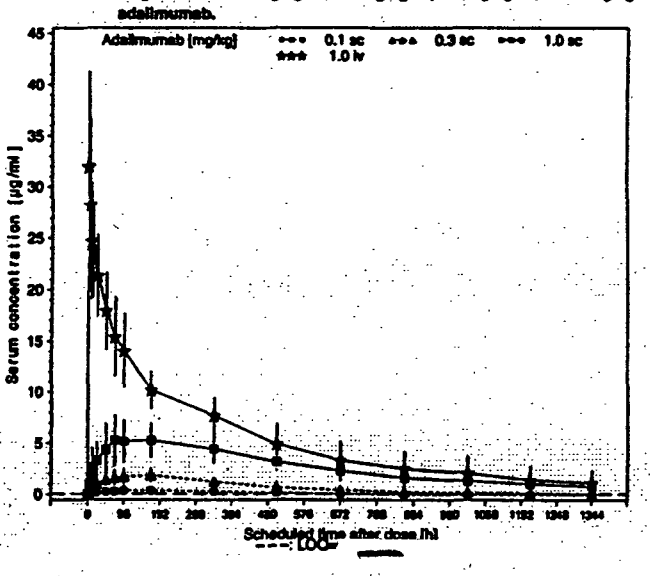
study that may increase the variability in the pharmacokinetic parameters as compared to crossover design that in turn may have impact on dose linearity.

Table 6 Summary of pharmacokinetic parameters following a single subcutaneous or intravenous administration of adalimumab in healthy adult subjects

PK Parameter	0.1 mg/kg sc	0.3 mg/kg sc	1.0 mg/kg sc	1.0 mg/kg iv
Cmax [µg/mL]	[N = 15]	[N = 15]	[N = 15]	[N = 15]
Mean ± SD	0.5 ± 0.1	2.0 ± 0.7	6.1 ± 2.5	34. 6 ± 8.8
(Min. – Max.)				
Tmax [h]	[N = 15]	[N = 15]	[N = 15]	[N = 15]
Median	168.0	168.0	96.0	1.0
(Min. – Max.)				
AUC(0-336h) [µg•h/mL]	[N = 15]	[N = 14]	[N = 15]	[N = 15]
Mean ± SD	119.3 ± 38.8	483.5 ± 101.0	1585.6 ± 561.7	4178.2 ± 646.2
(Min. – Max.)				
AUC(0-tn) [µg•h/mL]	[N = 15]	[N = 15]	[N = 15]	[N = 15]
Mean ± SD	186.3 ± 86.9	863.5 ± 420.2	3596.4 ± 961.9	7220.8 ± 1621.8
(Min. – Max.)				
AUC(0-∞) [µg•h/mL]	[N = 10]	[N = 11]	[N = 15]	[N = 15]
Mean ± SD	291.8 ± 94.7	1164.3 ± 415.3	4248.0 ± 1217.1	8153.9 ± 2793.3
(Min. – Max.)				
t½ [h]	[N = 10]	[N = 11]	[N = 15]	[N = 15]
Mean ± SD	284.3 ± 118.1	268.2 ± 175.4	433.1 ± 194.0	357.9 ± 218.1
(Min. – Max.)				
CL/F [mL/h]	[N = 10]	[N = 11]	[N = 15]	-
Mean ± SD	31.2 ± 11.8	23.8 ± 11.9	20.5 ± 7.1	-
(Min. – Max.)				
CL [mL/h]	-	-	-	[N = 15]
Mean ± SD	-	-	-	11.0 ± 3.0
(Min. – Max.)	-	-	-	
Vss [L]	-	-	-	[N = 15]
Mean ± SD	-	-	-	4.9 ± 1.5
(Min. – Max.)	-	-	-	

Data source: Section 9.2, Tables 9.2.5 through 9.2.8

Figure 1 Mean (± SD) serum adalimumab concentration-time profiles (linear scale) after a single dose of 0.1 mg/kg sc, 0.3 mg/kg sc, 1.0 mg/kg sc, or 1.0 mg/kg iv adalimumab.



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Study #2

Title: A multicenter, placebo controlled ascending dose phase I study of the human anti-TNF antibody D2E7 administered as an intravenous injection in patients with rheumatoid arthritis (DE001).

This is a multicenter, double-blind, randomized, placebo-controlled, parallel, ascending single-dose study in patients with rheumatoid arthritis (RA). There were 24 patients (6 placebo and 18 treated, a total of 43 males and 77 females) in each dosing group. The patients (24 to 80 years) received a single intravenous dose of 0.5, 1.0, 3.0, 5.0, and 10 mg/kg dose of D2E7 over 3 to 5 minutes. Five mL of venous blood were drawn at time 0, 0.08, 0.25, 1, 4, 12 and 24 hours and then weekly through day 29. Synovial fluid samples were also collected from 5 patients receiving either drug or placebo. Drug concentrations were measured in serum and synovial fluid using a validated enzyme-linked immunosorbent assay (ELISA) method. The lower limit of quantification of D2E7 in serum and synovial fluid was Human anti-human antibodies (HAHA) were measured up to 6 months post dose. In order to establish a dose-response relationship, responses such as Disease Activity Score (DAS) and the American College of Rheumatology for 20% (ACR 20) and 50% (ACR 50) improvements were measured. Noncompartmental analysis was used to estimate the pharmacokinetic parameters of D2E7.

Mean serum D2E7 concentrations increased with increasing dose. The mean Cmax ranged from 24.6 mcg/mL (0.5 mg/kg) to 283.7 mcg/mL (10 mg/kg). The Cmax was linear over the dose range of 3 to 10 mg/kg dose. The mean AUC ranged from 2729 mcg*hr/mL (0.5 mg/kg) to 67115 mcg*hr/mL (10 mg/kg). The clearance slightly decreased with increasing dose. This, however, is not an outright indication that D2E7 is nonlinear over the dose range of 0.5 to 10 mg/kg. The half-life of D2E7 ranged from 242 to 326 hours. The pharmacokinetic parameters of this study have been summarized in the following Table.

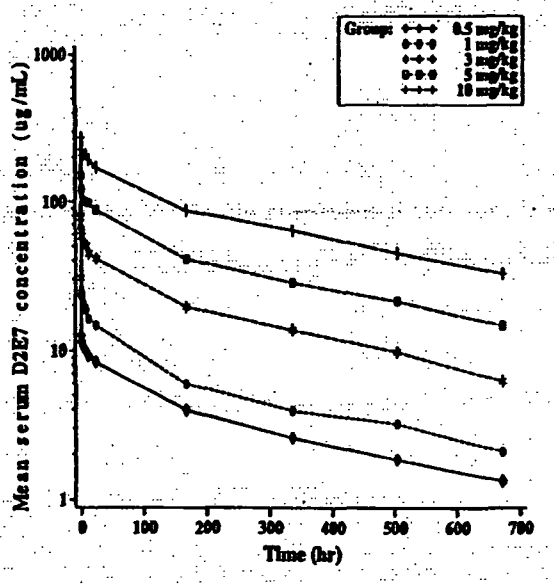
Table 3 Mean PK parameters for each D2E7 dose level cohort

PK Parameters	Mean ± SD (CV%) of PK Parameters by D2E7 Dose Group				
	0.5 mg/kg	1.0 mg/kg	3.0 mg/kg	5.0 mg/kg	10.0 mg/kg
Cmax (µg/mL)	24.8 ± 21.5 (87.4%)	67.5 ± 75.9 (112.5%)	78.0 ± 27.9 (35.8%)	143.7 ± 46.4 (32.3%)	283.7 ± 74.0 (26.1%)
$AUC_{0-\infty}$ (µg•h/mL)	2729 ± 707 (26%)	4363 ± 1807 (41%)	14229 ± 3703 (26%)	32963 ± 11558 (35%)	67115 ± 17385 (26%)
t½ (h)	284.4 ± 118.7 (41.7%)	241.5 ± 169.7 (70.3%)	266.9 ± 89.7 (33.6%)	325.9 ± 129.3 (39.7%)	320.6 ± 116.6 (36.4%)
CL (mL/h)	15.0 ± 5.6 (37.1%)	17.4 ± 6.3 (36.2%)	14.9 ± 3.6 (24.0%)	11.8 ± 4.0 (34.3%)	11.5 ± 2.9 (25.6%)
Vss (L)	5.3 ± 1.4 (26.9%)	5.0 ± 1.3 (26.5%)	5.5 ± 1.4 (25.0%)	4.7 ± 1.0 (21.1%)	4.7 ± 1.3 (28.9%)

Data source: Section 7.2, Table 7.2.2

Figure 7.2.1
Mean serum D2E7 concentration vs. time profiles following a single D2E7 iv injection (with ± SD bars on original scales)

Semi-log scale



The linear regression of dose vs AUC ($r^2 = 0.86$) indicated that the slope was significantly different from zero whereas intercept was not different than zero indicating the linearity of D2E7 over the dose range of 0.5 to 10 mg/kg (Sponsor's claim).

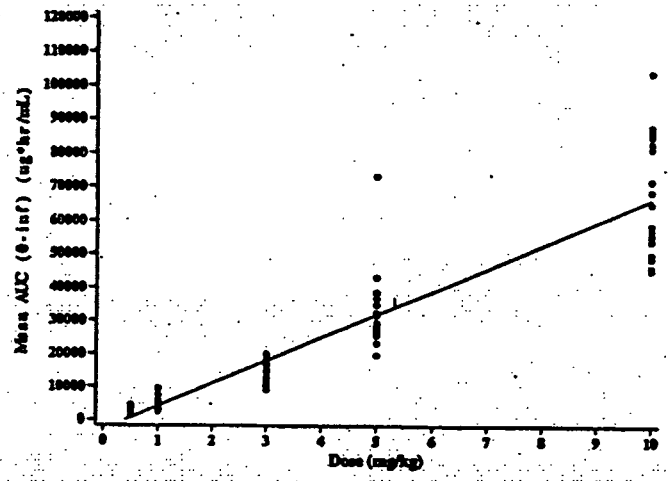


Figure 3 Individual patient AUC$_{0-\infty}$ vs dose following a single D2E7 intravenous injection

Weight and age showed no impact on the clearance and volume of D2E7 but gender seems to have an impact on the clearance of D2E7. The males had higher clearance of D2E7 than the females.

Measurable D2E7 concentrations (microgram/mL) were observed in 11 synovial fluid samples collected from 5 subjects and the concentrations appeared to be dose dependent. D2E7 appeared to persist for more than 2 months in the synovial fluid after a single IV dose. No HAHAs were detected in any patient in the first four weeks of the study. Only one patient had a detectable HAHA level at 3 months after dosing.

A plot of Area Under the Effect Curve (AUEC) of DAS vs AUC indicated slight increase in response with increasing D2E7 exposure. Plots of AUEC of DAS vs dose and AUEC of ACR20 vs mean AUC of D2E7 for each dose indicated that the response increased at dose from 0.5 to 1.0 mg/kg. Beyond this dose no meaningful increase in the response was noted. However a plot of AUEC of ACR50 vs mean AUC of D2E7 for each dose indicated that the response increased at dose from 0.5 to 3.0 mg/kg. The plot also indicates that as the AUC of D2E7 increases (at doses 5 and 10 mg/kg), the AUEC of ACR50% decreases. The reason of this decrease in response is not obvious.

Figure 7.3.3
Plot of AUEC(0-29d) of DAS vs Dose

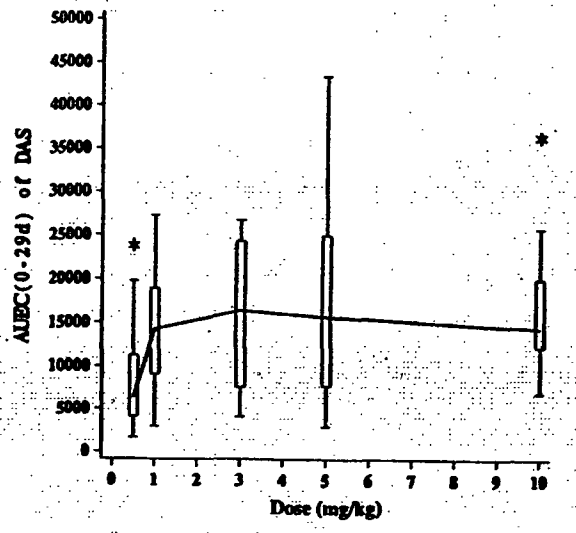
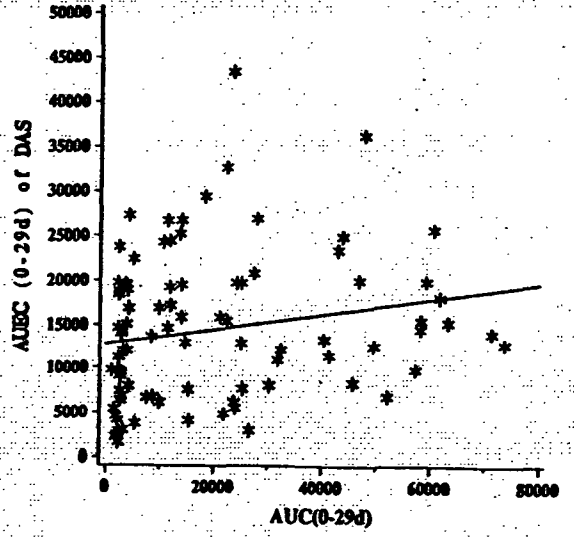


Figure 7.3.2
Plot of AUEC(0-29d) of DAS vs AUC(0-29d)



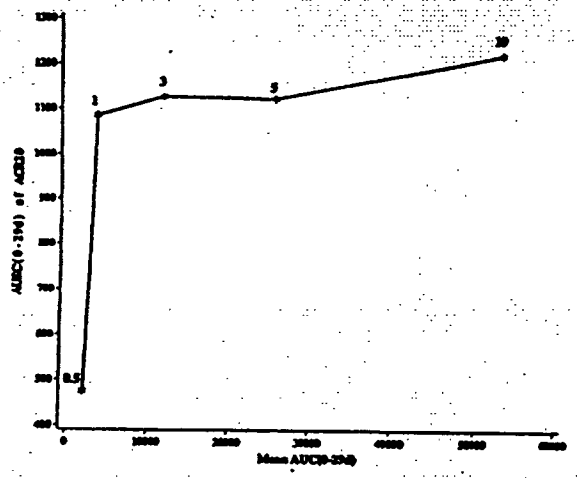
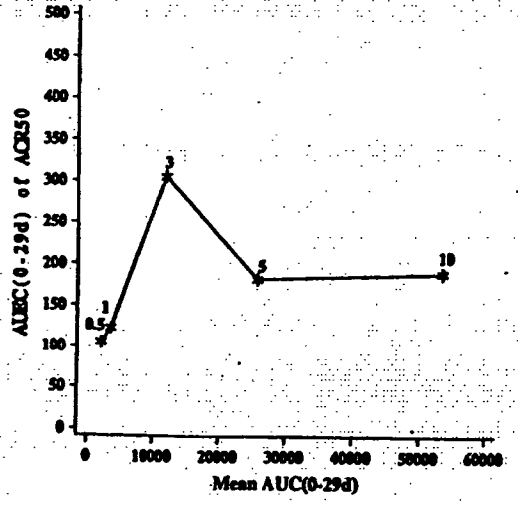


Figure 4 AUEC of ACR50 vs mean AUC for each dose level following a single D2E7 intravenous injection

Figure 7.3.5
Plot of AUEC(0-29d) of ACR50 vs mean AUC(0-29) of each treatment group



Conclusions: The pharmacokinetics of D2E7 based on AUC appear to be linear over the dose range of 0.5 to 10 mg/kg. Though the correlation between AUC and dose is not very strong ($r^2 = 0.86$) yet it is unrealistic to conclude that D2E7 is nonlinear over this dose range. Lack of a strong correlation between dose and AUC may be due to parallel design study. Covariates like body weight and age do not have any impact on the clearance of D2E7 but females have lower levels (the difference between males and females in plasma concentrations of D2E7 has not been provided by the Sponsor) of drug than males. The Sponsor concluded that comparatively lower levels of D2E7 in females than males may not be of any clinical significance.

The mathematical relationship between AUC and AUEC is not a simple linear process rather it is a complex process and has not been clearly established. Therefore, the Sponsor's conclusion that the relationship between clinical response (AUEC of DAS) and AUC in individual patients was unclear from this study due to high variability may not be a correct explanation.

Comment:

The Sponsor should provide the data on males and females from this study to explain the lack of gender effect on the pharmacokinetics of adalimimab.

Table 9.2a Population Pharmacokinetic Parameter Estimates

Parameter	Units	Estimate	SE	Eta
CL/F	L/days	0.365 (15.21 mL/h)	0.0424	0.187
V1/F	L	1.32	0.824	0.542
Q	L/days	0.336 (14.0 mL/h)	0.392	—
V2/F	L	5.32	3.26	—

Allowing etas for Q, and V2 / F did not improve the value of objective function, therefore only population means were estimated for these parameters.

Table 9.2b POSTHOC Estimates of PK Parameters

Variable	N	Mean	SD	CV [%]	Min	Median	Max	Geo. Mean
CL/F [mL/h]	23	19.081	9.788	51.3		16.074		17.228
CL/F [mL/h*kg]	23	0.274	0.143	52.3		0.256		0.2498
V_{ss}/F [L]	23	6.783	0.718	10.6		6.664		6.7484
V_{ss}/F [L/kg]	23	0.100	0.024	23.6		0.098		0.0979
V [L]	23	1.460	0.718	49.2		1.341		1.289
$t_{1/2}$ [days]	23	22.399	5.893	26.3		21.795		21.747

Source Data: Table 3 of Appendix 13.1__4

Comments:

1. In this study, the method of estimation of half-life is not clear. Please provide this information.

2. In the individual serum concentrations Table (11.2_1.2), what does it mean by the term no value reported (NVR)? Was plasma concentration not measured or was concentration below the detection limit? Please clarify.

Study #6

Title: A multicenter, placebo-controlled, double blind phase I study of the human anti-TNF antibody D2E7 administered as an intravenous injection in patients with rheumatoid arthritis treated with methotrexate (protocol DE005).

This was a multicenter, double-blind, placebo-controlled, ascending single-dose study in patients with rheumatoid arthritis. There were 60 subjects (45 active and 15 placebo) in the study. There were 12 females and 3 males in the placebo group and 35 females and 10 males in treatment group. The subjects (24 to 73 years; weight = 43.4 to 716 kg) received a single intravenous (infusion) dose of 0.25, 0.5, 1, 3, or 5 mg/kg of adalimumab Patients also received methotrexate weekly at doses >12.5 and <25 mg by any route of administration. Some patients received methotrexate as low as 10 to 12.5 mg/week if they could not tolerate high dose of methotrexate. Five mL of venous blood were drawn at time 0, 15 minutes after end of infusion and at 2, 6, 12, and 24 hours (in fact blood samples were taken till 673 hours). Blood samples at 2 and 6 hours were also used to measure methotrexate concentrations (only two samples). Drug concentrations were measured in serum using a validated [redacted] enzyme-linked immunosorbent assay (ELISA) method. The lower limit of quantification of adalimumab in serum was [redacted]. Human anti-human antibodies (HAHAs) were measured at baseline, day 8, and thereafter at all subsequent visits. HAHA concentrations were only measured if adalimumab concentrations were <2.0 microgram/mL. The LOQ for the HAHA assay was [redacted] in undiluted serum. Methotrexate concentrations were measured using [redacted] The lower limit of quantification was [redacted]

Plasma concentrations vs time data were analyzed by a population pharmacokinetic approach using NONMEM (and also by noncomparmental analysis as claimed by the Sponsor). The inclusion of POSTHOC in NONMEM analysis provided the PK parameters for individual subjects. Across the dose group, the mean clearance (CL/F) following POSTHOC estimation ranged from 0.009 to 0.012 L/hr (9-12 mL/hr). The elimination half-life ranged from 353-464 hours. The age did not appear to have any

impact on the pharmacokinetics of adalimumab. There was a linear increase in serum clearance with an increase in total body weight but the relationship between body weight and clearance was not very strong ($r^2 = 0.14$). The males had a 30% higher clearance of adalimumab than the females but the weight based CL was not different between the two genders. The pharmacokinetic parameters of D2E7 have been summarized in the following Tables.

Methotrexate concentrations were measured at 2 and 6 hours following oral (n = 21), subcutaneous (n=2) and intramuscular (n=6) administration. In the presence of D2E7, the concentrations of methotrexate following oral administration decreased both at 2 (64%) and 6 (41%) hours sampling time. Following subcutaneous and intramuscular administration, the concentrations of MTX remained almost unchanged in the presence and absence of D2E7 (probably the difference could not be detected due to small sample size). The population pharmacokinetic study indicated that D2E7 increased the clearance of MTX by 40% (4.39 l/hr vs 6.16 l/hr).

Table 5 Summary of dose normalized MTX concentrations, measured at 2 and 6 h after administration (planned times). Effect of administration route on MTX concentration at timepoint 2 or 6 h is analyzed with a Wilcoxon Rank Sum test

D2E7 comedication	Time (h)	Route	n	Concentration Median (µg/L)	p (oral = control)	p (s.c. = control)
Yes	2	oral	21	10.9	-	-
		s.c.	2	25.8	< 0.05 (oral vs. s.c.)	-
		i.m.	6	22.9	< 0.05 (oral vs. i.m.)	> 0.05 (s.c. vs. i.m.)
Yes	6	oral	19	3.9	-	-
		s.c.	2	8.4	< 0.05 (oral vs. s.c.)	-
		i.m.	5	7.3	< 0.05 (oral vs. i.m.)	> 0.05 (s.c. vs. i.m.)
D2E7 comedication	Time (h)	Route	n	Concentration Median (µg/L)	p (oral = control)	p (s.c. = control)
no	2	oral	21	17.9	-	-
		s.c.	2	22.4	> 0.05 (oral vs. s.c.)	-
		i.m.	6	24.7	< 0.05 (oral vs. i.m.)	> 0.05 (s.c. vs. i.m.)
no	6	oral	21	5.5	-	-
		s.c.	2	11.8	< 0.05 (oral vs. s.c.)	-
		i.m.	6	8.9	< 0.05 (oral vs. i.m.)	> 0.05 (s.c. vs. i.m.)

Summary Table: Pharmacokinetic parameters (2-Compartment model fit) following a single i.v. injection of D2E7 in patients with rheumatoid arthritis treated concomitantly with methotrexate					
Pharmacokinetic Parameters	0.25 mg/kg (Mean±SD)	0.5 mg/kg (Mean±SD)	1 mg/kg (Mean±SD)	3 mg/kg (Mean±SD)	5 mg/kg (Mean±SD)
CL (l/h)	0.010±0.003	0.011±0.002	0.012±0.003	0.009±0.003	0.009±0.003
V1 (l)	3.00±0.67	3.23±0.87	3.22±1.05	2.98±0.66	3.01±0.40
Q (l/h)	0.03±0.01	0.03±0.01	0.09±0.16	0.04±0.02	0.04±02
V2 (l)	2.53±0.51	2.49±0.37	2.53±0.56	2.12±0.32	2.43+0.23
t_{α} (h)	32.8±14.2	29.4±9.5	24.6±15.1	30.9±23.3	28.6±8.1
$t_{1/2\beta}$ (h)	422.8±120.5	389.0±71.0	353.4±74.6	454.8±139.7	464.1±122.5
AUC(0-inf) (mg*h/L)	1884±600	3169±514	5880+1305	24775±7295	37964±11585
Cmax (mg/L)	7.38±2.47	11.97±2.39	25.37±9.03	75.61+18.18	116.78±19.71
Vss (l)	5.53±0.80	5.72±0.94	5.75±1.00	5.10+0.94	5.44±0.38
AUC(0-inf)/dose [(mg*h/L)/mg]	101.32±26.75	92.46±21.92	85.24±21.12	122.52±39.51	117.19+32.13
Cmax/dose [(mg/L)/mg)]	0.40±0.15	0.35±0.10	0.38±0.18	0.38±0.12	0.36±0.06
Vss (l/kg)	0.075±0.013	0.082±0.012	0.082±0.009	0.075±0.014	0.085±0.010
CL [(mL/h)/kg]	0.144±0.041	0.162±0.027	0.177±0.035	0.132±0.044	0.143±0.048

Summary Table: 2-Compartment model fit PK parameters following a single injection of D2E7 in patients concomitantly treated with methotrexate by gender		
Pharmacokinetic Parameters	Males (Mean±SD)	Females (Mean±SD)
CL (l./h)	0.013±0.002	0.010±0.003
Vss (l)	6.22±0.88	5.30±0.71
CL (mL/h)/kg	0.16±0.03	0.15±0.04
Vss (l./kg)	0.076±0.010	0.081±0.014
Weight (kg)	82.06±7.30	66.73±12.79
Age (years)	56.70±11.03	52.46±13.49

Summary Table: Effect of D2E7 on MTX pharmacokinetic parameters		
	Without D2E7 (Mean±SD)	With D2E7 (Mean±SD)
Dose (µg)	16724±3726	17241±3858
CL (l./h)	4.39±2.34	6.16±3.29
V* (l)	10.90	10.90
Q* (l./h)	6.70	6.70
Vss* (l)	52.20	52.20
*due to the limited data, only the population mean value with and without D2E7 is estimated for V, Q, and Vss		

Comment:

The Sponsor mentions that in this study, a noncomparmental analysis was also performed but no data have been provided.

Study #7

Title: Pharmacokinetic assessment from: A multicenter, phase I continuation study of the human anti-TNF antibody D2E7 administered as an intravenous injection in patients with rheumatoid arthritis treated with methotrexate (protocol DE005X).

This study was continuation of the previous study (study #6) where patients with rheumatoid were given D2E7 with methotrexate as a single IV ascending dose. This study was also an ascending dose study given biweekly to patients with rheumatoid arthritis along with methotrexate. There were 60 subjects in the study. Data from 43 subjects were evaluated in the PK study (34 females and 9 males). The subjects (30 to 73 years; weight = 50 to 94 kg) received multiple (biweekly) intravenous (infusion) dose of 0.25, 0.5, 1, 3, or 5 mg/kg of adalimumab over 3 to 5 minutess.

Study DE005X became open-label with administration of the third dose. Patients were assigned to the same dose level to which they were assigned during Study DEO05. The dose levels explored were 0.25 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 3 mg/kg and 5 mg/kg and placebo for adalimumab. Those patients receiving placebo were switched to adalimumab at the time of the third dose. Though adalimumab doses were administered via intravenous (iv) injection over a period of 3 to 5 minutes, later, patients were allowed to switch to a 40 mg dose of adalimumab administered *via* the subcutaneous (sc) or iv route. Patients were to receive doses of adalimumab biweekly for the first 24 weeks of treatment. If a patient registered an ACR of 50 or better (ACR 50), the dosing interval was allowed to be extended to a monthly schedule, provided that consent was obtained from the investigator, the medical monitor and the patient. The maximum treatment duration for a patient was approximately 26 months (counted from the first dose administered during Study DE005). Patients who maintained an ACR 50 response or better indication of improvement in disease status were allowed to taper their corticosteroid dose after 24 weeks of adalimumab therapy at the rate of 1mg/month, provided they continued to demonstrate at least a 50% improvement in disease status compared to study entry in DE005. A patient's methotrexate dose was to remain unchanged during the study. However, at the discretion of the investigator and with the

approval of the medical monitor, patients exhibiting signs or symptoms associated with methotrexate toxicity were allowed to reduce their dose by 2.5-5.0 mg weekly. No patient was allowed to have their methotrexate dose tapered below 7.5 mg weekly; any patients who required such a reduction were to be withdrawn from the study. Additionally, the methotrexate dose was not to be tapered concomitantly with corticosteroid dose tapering.

Blood samples for the measurement of adalimumab concentrations were collected at 0.25, 2, 6 and 24 hours after the end of injection administered at the month 5 visit (till week 72. HAHA concentrations were measured at months 6, 12 and 18. Blood samples at 2, 6, and 24 hours dosing were also used to measure methotrexate concentrations. Drug concentrations were measured in serum using a validated [redacted] enzyme-linked immunosorbent assay (ELISA) method. The lower limit of quantification of adalimumab in serum was [redacted]. The LOQ for the HAHA assay was [redacted] in undiluted serum. Methotrexate concentrations were measured using [redacted] The lower limit of quantification was [redacted].

The pharmacokinetic parameters of D2E7 have been summarized in the following Table.

Comments:

1. The method to calculate AUC from time 0-336 is not clear especially when blood samples were collected till 24 hours. Therefore, the estimated CL values of adalimumab across doses appear to be ambiguous.
2. The estimated AUC for MTX based on only two samples are unreliable and cannot be used for any conclusion.

Summary of Results:

Pharmacokinetic Results: The Month 5 adalimumab pharmacokinetic parameters are summarized in the following table.

Pharmacokinetic Parameters	Dose Group				
	0.25 mg/kg adalimumab (N = 3)	0.5 mg/kg adalimumab (N = 7)	1 mg/kg adalimumab (N = 13)	3 mg/kg adalimumab (N = 6)	5 mg/kg adalimumab (N = 9)
C_{pre} (µg/mL)	4.1 ± 3.7	11.2 ± 7.9	14.6 ± 7.0	74.6 ± 52.7	100.8 ± 23
C_{max} (µg/mL)	18.6 ± 11.6	27.5 ± 12.1	44.8 ± 13.9	159.5 ± 35.7	268.5 ± 41.5
T_{max} (h)	0.8 ± 1.0	1.8 ± 2.0	3.9 ± 6.4	2.1 ± 2.1	2.1 ± 2.4
AUC_{0-336} (µg•h/mL)	2407 ± 1052	4988 ± 2043	8109 ± 2888	33646 ± 13561	50067 ± 8436
CL (mL/h)	9.63 ± 5.89	9.42 ± 5.43	10.01 ± 5.15	6.94 ± 3.17	6.76 ± 0.78

Mean ± SD.

The methotrexate AUC_{2-6} values are summarized in the following table.

Pharmacokinetic Parameter	Study		
	Screening DE005 (N = 21)	Day 1 DE005 (N = 21)	Month 5 DE005X (N = 21)
AUC_{2-6} (µg•h/L)	996 ± 363 (464 – 2045)	829 ± 467 (318 – 1977)	918 ± 429 (318 – 2018)

Mean ± SD (Range).

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Study #8

Title: A multicenter randomized placebo-controlled phase II study of the human anti-TNF antibody D2E7 comparing three dose levels of D2E7 and placebo administered over 12 weeks as weekly subcutaneous injections in patients with rheumatoid arthritis followed by a continuation period with long term treatment with D2E7 (protocol DE007).

This was a phase II, multicenter, randomized, placebo-controlled study in patients with rheumatoid arthritis. For the 284 patients (59 males and 225 females) who were included in the pharmacokinetic study, mean age and weight were 52 years (22 to 83 years) and 68.3 kg (42 to 100 kg), respectively. The patients received either placebo or adalimumab as 20, 40, and 80 mg dose subcutaneously weekly for 12 weeks. Patients who received adalimumab during the placebo-controlled period continued to receive the same weekly dose for an additional 40 weeks (blinded continuation). Those patients who received placebo were switched to receive 40 mg adalimumab for the next 40 weeks (blinded continuation). Patients who completed the blinded continuation period continued to receive adalimumab at a dose of 40 mg per week for another year followed by a six month follow up (open label continuation period). Blood samples for the determination of serum adalimumab concentrations and HAHA were collected prior to dosing at baseline, and at 2, 4, 6, 8, 10, and 12 weeks and then every four weeks till 104 weeks. Additional samples for HAHA concentrations were collected at 1, 2, 3, and 6 months after the last dose of adalimumab. If a patient had a positive HAHA, then additional samples were collected at 9 and 12 months after the lat dose of adalimumab. Drug concentrations were measured in serum using a [redacted] method. The lower limit of quantification of adalimumab in diluted serum was [redacted] The LOQ for the HAHA assay was [redacted] in undiluted serum.

The mean adalimumab serum trough concentration between week 28 and 52 was 5.38, 10.73, and 25.24 ng/mL, which followed the linear pharmacokinetics. The mean adalimumab serum trough concentrations reached steady-state by week 16 and stayed stable thereafter (Sponsor's claim). Thirty-four patients were found to have at least one

serum sample with a HAHA positive signal during the treatment period. The results of the study have been summarized in the following Table.

Table 9.2a Summary Statistics of Steady-State Serum Adalimumab Trough Concentrations Observed Between Week 28 and Week 52

Dose (mg)	N	Mean (µg/mL)	SD (µg/mL)	Median (µg/mL)	Minimum (µg/mL)	Maximum (µg/mL)
20	27	5.38	3.31	5.00		
40	30	10.73	5.25	9.62		
80	30	25.24	11.92	26.98		

Data Source: Table 11.2__1.11

Figure 9.2d Mean Serum Adalimumab Trough Concentration – Time Profiles for Patients who were on Stable Weekly Doses During the Placebo Controlled and Blinded Continuation Periods

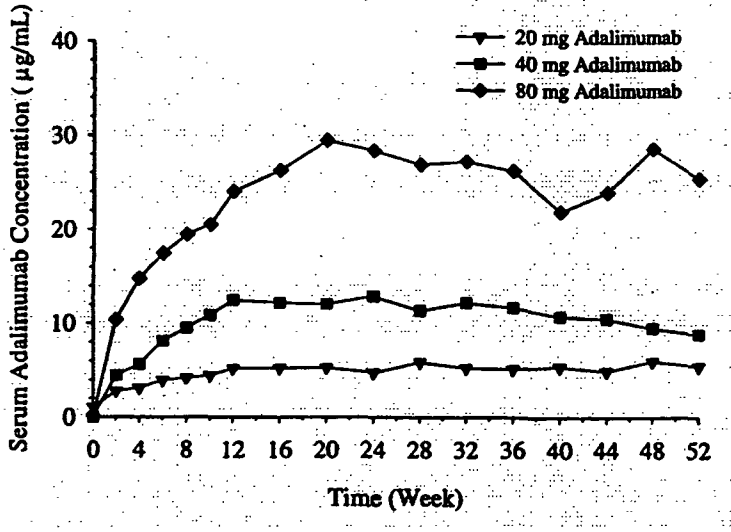
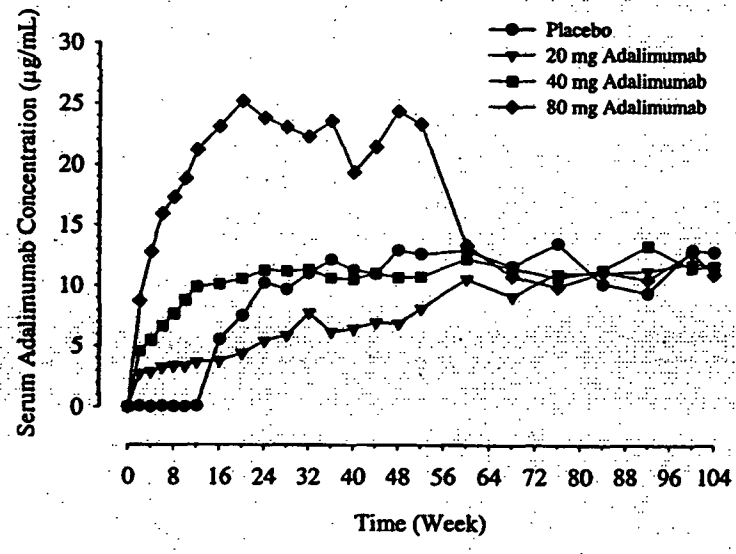


Figure 9.2c Mean Serum Adalimumab Trough Concentration – Time Profiles Following Weekly sc Dosing During the Placebo Controlled, Blinded Continuation, and Open Label Periods



Commenst:

1. A glance at the plot (9.2c) contradicts the Sponsor's claim that the steady state has reached by week 16 and remains stable thereafter. This reviewer concludes that the steady-state has not reached till 104 weeks.

2. In Table 9.2a, the data have been reported for 87 subjects, whereas in the study reports the Sponsor claims that 284 subjects were evaluated for pharmacokinetics. The Sponsor should explain this discrepancy.

3. The Sponsor has many trough samples from a given subject. A population pharmacokinetic approach would have helped to determine pharmacokinetic parameters that may be more relevant to draw pharmacokinetic conclusions.

Study #9

Title: A multicenter randomized placebo-controlled phase II study of the human anti-TNF antibody D2E7 administered as subcutaneous injections in rheumatoid arthritis patients treated with methotrexate (protocol DE009).

This was a phase II, multicenter, randomized, placebo-controlled study in patients with rheumatoid arthritis. For the 271 patients (63 males and 208 females), the mean age and weight were 56 years (28 to 84 years) and 74.5 kg (42.3 to 114.4 kg), respectively. The patients with rheumatoid arthritis who had insufficient efficacy with methotrexate received either placebo or adalimumab as 20, 40, and 80 mg dose subcutaneously every two weeks for 24 weeks. Blood samples for the determination of serum adalimumab concentrations and HAHA were collected at time 0, 4, 8, 12, 16, and 24 weeks. Drug concentrations were measured in serum using a [redacted] method. The lower limit of quantification of adalimumab in 10% human serum was [redacted] The LOQ for the HAHA assay was [redacted] in undiluted serum.

The mean adalimumab serum trough concentration increased with increasing dose and followed the linear pharmacokinetics. Eight patients were found to have at least one serum sample with a HAHA positive signal during the treatment period. The results of the study have been summarized in the following Table.

Table 9.1a Mean ± SD Serum Adalimumab Pre-Dose Concentrations

	Adalimumab Pre-dose Concentrations (µg/mL)					
	20 mg adalimumab		40 mg adalimumab		80 mg adalimumab	
Week	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
0	0.00 ± 0.00	68	0.00 ± 0.00	66	0.00 ± 0.00	73
4	2.70 ± 1.64	69	5.88 ± 3.95	65	10.01 ± 4.70	69
8	2.74 ± 1.59	65	6.11 ± 2.95	61	10.86 ± 4.69	67
12	3.10 ± 1.73	69	6.43 ± 3.33	66	12.10 ± 5.28	71
16	3.51 ± 2.33	61	8.03 ± 4.10	62	13.79 ± 6.19	67
24	3.63 ± 2.36	39	7.92 ± 3.87	48	13.15 ± 6.92	53

Comment:

1. In this study no attempt has been made by the Sponsor to evaluate the impact of methotrexate on the pharmacokinetics of adalimumab. A historical comparison could have been made by comparing the trough values of adlimumab in the presence and absence of methotrexate.

2. A population PK study using trough levels could have provided the estimates of pharmacokinetic parameters. An approach that could have been used to evaluate the impact of methotrexate on the pharmacokinetics of adalimumab.

Study #10

Title: A multicenter phase II continuation study of the human anti-TNF antibody D2E7 administered as subcutaneous injections in rheumatoid arthritis patients treated with methotrexate (protocol DE009X).

This was a phase II, multicenter, open-label study in patients with rheumatoid arthritis who were previously participated in study DE009 (study #9). Patients receive 40 mg dose every other week for a period of 6 or 8 months. There were 250 patients who were enrolled in the study of which 23 patients took part in the pharmacokinetic study. For the 23 patients (10 males and 13 females), the mean age and weight were 60 years (41 to 77 years) and 73.9 kg (50.9 to 111.1 kg), respectively. Blood samples for the determination of serum adalimumab concentrations were collected over a 14-day dosing interval and were drawn at time 0, 24, 48, 72, 96, 120, 168, 216 and 312 hours. Blood samples for HAHA were collected at time 0, and at 3, 6, and 8 months.. Drug concentrations were measured in serum using a [redacted] method. The lower limit of quantification of adalimumab was [redacted] in undiluted serum. The LOQ for the HAHA assay was [redacted] in undiluted serum.

The pharmacokinetic parameters were estimated with and without investigator 7 (debarred by the FDA). Investigator 7 provided the data for 8 patients. The only notable difference in the pharmacokinetic parameters with and without investigator 7 was in the clearance. Two subjects in investigator 7's group had very high clearance (124 and 202 mL/hr) which resulted in high mean clearance as compared to the mean clearance in 15 patients (without investigator 7). The mean apparent clearance at steady state was 20 mL/hr. Ten patients had HAHA signals greater than 20 ng/mL during treatment that could be suppressed by human serum by more than 50%. The results of the study have been summarized in the following Table.

Table 9.1a Mean ± SD Pharmacokinetic Parameters of Adalimumab (Investigator 7 Omitted)

Pharmacokinetic Parameters (units)		N	Adalimumab Treatment 40 mg dose every 14 days
C_{pre}	(µg/mL)	15	7.95 ± 6.38
C_{max}	(µg/mL)	15	9.97 ± 5.58
C_{min}	(µg/mL)	15	5.83 ± 3.52
T_{max}	(hours (h))	15	83.2 ± 62.8
$AUC_{0-\tau}$	(µg•h/mL)	15	2563.2 ± 1367.4
CL_{ss}/F	(mL/h)	15	19.60 ± 9.11

Cross-reference: Table 11.2__1.2.1.

Table 9.1b Mean ± SD Pharmacokinetic Parameters of Adalimumab

Pharmacokinetic Parameters (units)		N	Adalimumab Treatment 40 mg dose every 14 days
C_{pre}	(µg/mL)	23	6.86 ± 5.89
C_{max}	(µg/mL)	23	10.07 ± 7.89
C_{min}	(µg/mL)	23	5.29 ± 3.83
T_{max}	(hours (h))	23	88.7 ± 60.73
$AUC_{0-\tau}$	(µg•h/mL)	23	2398.5 ± 1607.9
CL_{ss}/F	(mL/h)	23	32.27 ± 43.81

Cross-reference: Table 11.2__1.2.

Study #11

Title: A multicenter randomized placebo-controlled study of the human anti-TNF antibody D2E7 administered as an intravenous or subcutaneous injections in patients with rheumatoid arthritis treated with methotrexate (protocol DE010).

This was a multi-center, randomized, placebo-controlled study conducted in patients with rheumatoid arthritis (RA) who had demonstrated insufficient efficacy or tolerability to MTX alone. After a wash-out period of three weeks in order to clear non-MTX disease modifying anti-rheumatic drugs, 54 patients (18 patients each) were randomized to receive one of the following treatments:
1 mg/kg adalimumab iv, 1 mg/kg adalimumab sc, or placebo for adalimumab.
After a minimum of four weeks (the exact interval depended on the patient's response status as defined by the European League Against Rheumatism [EULER] criteria based on the disease activity score [DAS]), the patients received a second blinded injection of study drug. On the third injection, the study became open-label after which all patients were to be treated with a 1 mg/kg dose of adalimumab administered subcutaneously for up to 2.5 years counted from their first injection. The timing of treatment during the open-label period was determined by the patient's response status. All patients were to receive a stable weekly methotrexate dose between 7.5 and 25 mg throughout the study period.

For the 54 patients (14 males and 40 females), the mean age and weight were 53 years (22 to 71 years) and 73.5 kg (41 to 96 kg), respectively. Blood samples (5 mL) for the determination of serum adalimumab concentrations were collected from each patient at time 0, 0.25, 1, 4,8, 12 and 24 hours and on days 8, 15, 22, and 29 and thereafter at 2, 3, and 6 months (NONMEM data file indicates that blood samples were taken beyond day 900). Blood samples for HAHA were collected at study entry, predose screening and during the follow-up and post-study visit periods. Drug concentrations were measured in serum using a [redacted] method. The lower limit of quantification of adalimumab was [redacted] in diluted serum. The LOQ for the HAHA assay was [redacted] in undiluted serum.

The pharmacokinetic parameters were estimated using non-compartmental as well as population pharmacokinetic approach. The estimated pharmacokinetic parameters were comparable by the two approaches. The following Tables summarize the results of the study.

Table 9.2c Noncompartmental and Population Pharmacokinetic Parameters for Adalimumab

Pharmacokinetic Parameters	1 mg/kg Adalimumab iv (N = 18)	1 mg/kg Adalimumab sc (N = 18)	Population PK Estimates* (N = 54)
C_{max} (µg/mL)	30.84 ± 6.56 (21.3%)	—	—
T_{max} (h)	1.15 ± 1.14 (99.1%)	—	—
$t_{1/2}$ (days)	16.3 ± 6.4 (39%) [391.89 ± 152.7 hours]	20.3 ± 5.9 (29.2%) [486.02 ± 141.96 hours]	19.9 (34.0%)
AUC_{0-t} (µg·h/mL)	5408.72 ± 1750.75 (32.4%)	—	—
AUC_{∞} (µg·h/mL)	7013.67 ± 2598.1 (37.0%)	—	—
CL (mL/h)	11.0 ± 4.2 (38.2%)	—	11.2 (54.6%)
V_{ss} (L)	5.28 ± 1.11 (21.0%)	—	6.0 (16.2%)
F value	—	—	0.58

iv = intravenous, sc = subcutaneous
() = values in parentheses are CV%.
* Using all serum adalimumab concentration data available up to 2.5 years.
Cross references: Tables 11.2__1.5 and 11.2__1.6 and Appendix 13.1__4.1

The population estimates of the PK parameters are presented in Table 9.2a below.

Table 9.2a Population Estimates of Pharmacokinetic Parameters Using NONMEM Pharmacokinetic Software

Parameter	Units	Estimate	SE
CL	L/day	0.237 (9.875 mL/h)	0.0242
V1	L	3.07	0.211
Q	L/day	1.08 (45.00 mL/h)	0.341
V2	L	2.86	0.355
F		0.58	0.0526

Both clearance and half-life were comparable when estimated either by noncompartmental analysis or population PK approach. Two out of 54 patients had HAHA positive signals.

Comments:

1. Despite the fact that the data are available for SC administration of adalimumab, the Sponsor did not calculate the PK parameters as was done with IV administration. Please provide the data.
2. It is not clear how did the Sponsor generate POSTHOC estimation of VSS. In the control stream file Vss does not appear. It appears that Vss has been calculated by adding V1 and V2 that renders all calculations of Vss as incorrect.
3. In the POSTHOC estimation, it is not clear how did Sponsor estimate the half-life.
4. Table 2 is incomplete (P#397 of the study report, V#19). Some subjects' data on PK are missing.
5. There are 54 subjects in the study of which 18 were given placebo. NONMEM data file consists of 54 subjects with plasma concentrations.

Study #12

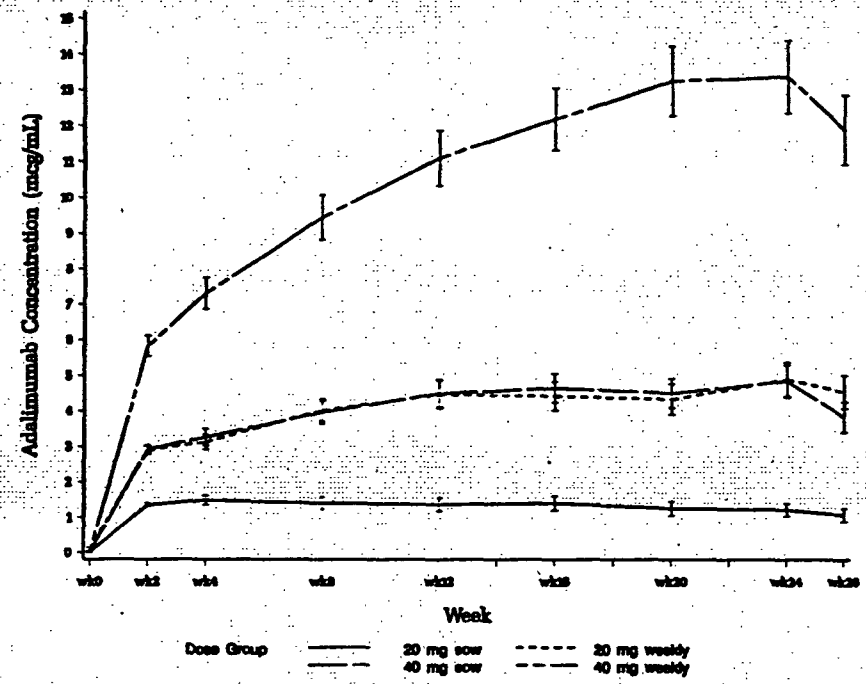
Title: A multicenter randomized placebo-controlled phase III study comparing two doses and two dosing intervals of the fully human anti-TNF antibody adalimumab versus placebo administered over 6 months as subcutaneous injections in patients with rheumatoid arthritis (protocol DE011).

This was a phase III, multicenter, randomized, placebo-controlled study in patients with rheumatoid arthritis. For the 364 patients (79 males and 285 females), the mean age and weight were 52.8 years (19 to 79 years) and 68.7 kg (43 to 102 kg), respectively. The patients received either placebo or adalimumab as 20 or 40 mg dose administered subcutaneously every week or every two weeks for 26 weeks. Blood samples for the determination of serum adalimumab concentrations were collected at time 0, 2, 4, 8, 12, 16, 20, 24, and 26 weeks and at follow up at 1, 2, 3, and 6 months. HAHA levels were determined at weeks 4, 8, 12, 16, 20, 24, 26 and at follow up at 1, 2, 3, and 6 months. Drug concentrations were measured in serum using ELISA. The lower limit of quantification of adalimumab in diluted serum was [redacted]. The LOQ for the HAHA assay was [redacted] in undiluted serum. The administration of adalimumab at both doses and at both intervals resulted in a flat serum concentrations time profiles.

Comment:

In this study, no pharmacokinetic parameter estimates were reported. The Sponsor intends to use the data in a population pharmacokinetic analysis.

Figure 9.1a Mean ± SE Concentrations of Adalimumab for Patients who Completed the Placebo Controlled Study Period



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Study #13

Title: A multicenter open label continuation study with subcutaneous D2E7 for patients with rheumatoid arthritis who completed a preceding clinical study with D2E7 (protocol DE018).

This was a phase III, multicenter, continuation study in patients with rheumatoid arthritis. These patients had previously completed a clinical phase I, II or III study with adalimumab. There were 21 patients of which 6 were taking methotrexate concurrently. For the 21 patients (7 males and 14 females), the mean age and weight were 52.8 years (35 to 66 years) and 68.5 kg (46 to 91 kg), respectively. For the 15 patients (5 males and 10 females) out of 21 patients, who were not concurrently taking methotrexate the mean age and weight were 49.7 years (35 to 65 years) and 68.7 kg (46 to 91 kg), respectively. The patients received 40 mg adalimumab administered subcutaneously every other week for 20 weeks. Blood samples for the determination of serum adalimumab concentrations were collected at time 0, and at 24, 48, 72, 96, 120, 168, 216 and 312 hours after dosing. HAHA levels were determined before dosing and at every 2 months up to 24 months and at 3 and 6 months post study visits. Drug concentrations were measured in serum using a validated — . The lower limit of quantification of adalimumab in diluted serum was — The LOQ for the HAHA assay was — . in undiluted serum. The following Table summarizes the pharmacokinetic parameters obtained in this study.

Pharmacokinetic Results: The mean ± SD pharmacokinetic parameters are listed in the following table.

		Adalimumab Treatment: 40 mg dose every other week	
Pharmacokinetic Parameters	(units)	All Patients (N = 21)	Patients not Concurrently Taking Methotrexate (N = 15)
C_{pre}	(µg/mL)	4.36 ± 2.37	4.16 ± 2.35
C_{min}	(µg/mL)	4.04 ± 2.21	3.80 ± 2.05
T_{max}	(h)	80.0 ± 45.1	89.6 ± 48.4
C_{max}	(µg/mL)	8.15 ± 3.98	7.70 ± 3.38
$AUC_{0-\tau}$	(µg•h/mL)	1931.5 ± 933.7	1831.6 ± 847.4
CLss/F	(mL/h)	27.46 ± 17.99	28.97 ± 19.88

Cross-reference: Table 11.2_1.2

This study confirms the pharmacokinetic observations noted in previous PK studies. However, the steady state clearance of adalimumab appears to be higher (almost 100%) than a single SC dose. No patient had HAHA levels above the limit of quantitation.

Study #14

Title: Pharmacokinetics and safety of single subcutaneous and intravenous administration of adalimumab (Lu 200134, D2E7) in healthy male Japanese: A double-blind placebo-controlled parallel-group study (protocol DE024).

This was planned as double-blind, randomized, placebo-controlled, parallel and four dose group study. Subjects were supposed to receive 0.1 to 1.0 mg/kg SC and 1.0 mg/kg IV adalimumab dose. Instead, the study was terminated after the 0.1 mg/kg SC dose due to the injection site reactions in the adalimumab treated group. The subjects were healthy Japanese males between 20 to 45 years old. There were 16 active and 4 placebo subjects. Blood samples drawn at time 0, 0.3, 1, 4, 8, 12, 24, 48, 72, 96, 168, 336, 504, 672, 840, 1008, 1176 and 1344 (day 57) hours. Drug concentrations were measured in serum using a validated sandwich enzyme-linked immunosorbent assay (ELISA) method. The pharmacokinetic parameters obtained in the Japanese subjects (n =16) were comparable with the pharmacokinetic parameters observed in Caucasian subjects (n =15, a historical comparison). Six subjects were positive for HAHA. The pharmacokinetic parameters of adalimumab have been summarized in the following Table (next page).

Comments:

1. The Sponsor has only provided the preliminary summary of the study without the details of the data (raw data, individual PK parameters and plots).
2. The study raises concern about the administration of adalimumab to the Japanese population as they encountered the injection site reaction. The labeling should indicate this concern.

Pharmacokinetic Results: Pharmacokinetic parameters, reported as mean (SD) for the 0.1 mg/kg subcutaneous dose in Japanese and Caucasian (Protocol DE024) subjects are summarized by in the following table:

Cmax (µg/mL)	Tmax* (h)	AUC(0-336h) (µg-h/mL)	t½** (d)	AUC(0-∞) (µg*hr/mL)
Japanese Subjects (Protocol LU200134-101)				
0.47 (0.14) N = 16	168 — N = 10	118 (37) N = 16	14.6 (3.2) N = 10	349 (106) N=10
Caucasian Subjects (Protocol DE 024)				
0.46 (0.15) N = 15	168 — N = 15	119 (39) N = 15	11.8 (3.2) N = 10	292 (95) N=10

* Median Tmax with range.

** Due to appearance of HAHAs in the terminal elimination time period, t1/2 could not be calculated in 1/3 of subjects and the effect of HAHAs on the estimated t1/2 values was unknown.

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Study #15

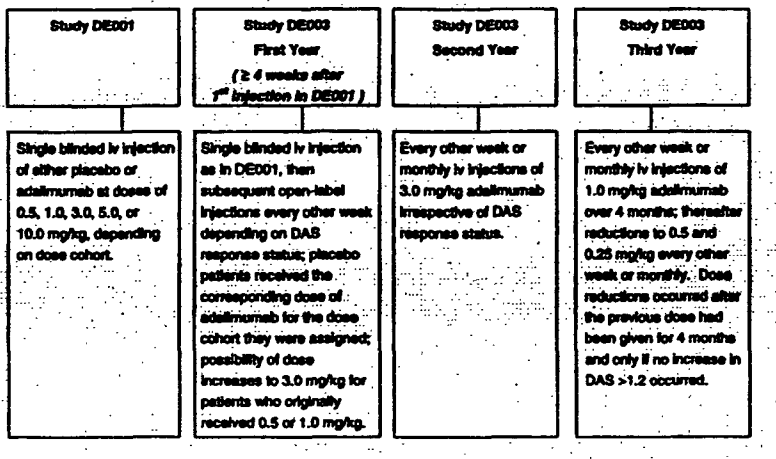
Title: Population pharmacokinetic assessments from a phase I trial: A multi-center phase I continuation study of the human anti-TNF antibody D2E7 administered as an intravenous injection in patients with rheumatoid arthritis (protocol DE003).

This was a multi-center continuation study of the Phase I Study DE00I (originally D2E7HAB 001). Study DE00I was a multi-center, double-blind, randomized, placebo-controlled, ascending single-dose study. The study had five ascending dose cohorts (0.5, 1.0,3.0,5.0, or 10.0 mg/kg) with 24 patients in each cohort. Within each cohort 18 patients received adalimumab and 6 patients received placebo. Adalimumab or placebo was administered via intravenous (iv) injection as a single dose. At the start of this study, all patients remained in the dose cohort (0.5, 1, 3, 5, or 10 mg/kg) they had been assigned to during Study DE001. Patients who received adalimumab in Study DE001 received the same blinded dose of adalimumab for their second iv injection in Study DE003. Placebo patients received placebo a second time. A minimum interval of 4 weeks between the first and second injection was required, and the precise time point of the second administration was determined by the response status of the individual patient as measured by the Disease Activity Score (DAS).

Study DE003 was open-label starting with the third injection, and those patients who had received placebo as their first and second injection were switched to the corresponding dose of adalimumab for the dose cohort they were assigned. The time points for subsequent injections of study drug were also determined by the patient's response status. During the first treatment year in DE003, patients randomized to 0.5 or 1.0 mg/kg could be stepwise titrated to 3.0 mg/kg. During the second treatment year, patients were treated with 3.0 mg/kg every other week irrespective of their responder status. Some patients could have received 3.0 mg/kg once monthly if they had already achieved a good response at that dose during the first year. During the third year, the dose of adalimumab was reduced to 1.0 mg/kg, and then stepwise reduced to 0.5 and 0.25 mg/kg, respectively. Each dose level was given for 4 months before the next lower dose could have been given. Dose reduction only took place if the disease activity was

controlled - defined as no increase in DAS >1.2 (compared to the value observed before the dose was de-escalated to 1.0 mg/kg). The summary of the study scheme was as follows:

Figure 6.1a Study Schematic



There were 119 patients (42 males and 77 females) between 24 to 80 years of age. The patients weight varied from 46 to 100 kg. Five mL venous blood samples were drawn at study entry and at every follow up examination following the second dose. Additional samples were collected at 3 and 6 months after the last dose. Serum samples were used to determine adalimumab concentrations.

The population pharmacokinetics was performed on NONMEM (version 5, Level 1.0). The final model was a two-compartment model with posthoc estimates of individual subject's PK parameters.

To model the wide variety of dosages and regimens between and within patients and to take into account a possible dependence of the adalimumab clearance on the adalimumab dose, the mean biweekly dose (DMEA) was calculated on a three month basis as follows: each patient's observation period was subdivided into cycles of 90 days. For each cycle, the total dose in the cycle (DTOT) was obtained by summing up all individual doses and DMEA was determined according to the formula:

DMEA = (DTOT * 14)/(delta t + 14). Here, delta t = 90 denotes the length of the respective cycle in days. DMEA was categorized into three classes (DMEA $\leq$100 mg, 100 mg < DMEA $\leq$180 mg, DMEA > 180 mg). For each DMEA class a different clearance rate was estimated.

The variable HOL was defined to indicate that a patient had a "drug holiday" (that he received no study drug) of 70 days (approximately 5 half-lives) or more. The time-dependent covariate "HOL" was defined as follows: for each patient, HOL was initially set to zero (HOL = 0). From the first time point on, when a patient received a dose of adalimumab more than 70 days after the previous dose HOL was set to 1. HOL was related to clearance by estimating a basic clearance for patients before a drug holiday (HOL = 0, including patients with no drug holiday at all), and including an additional multiplicative factor after a drug holiday (HOL = 1). Apart from the mean biweekly dose (DMEA) and the drug holidays (HOL), no other covariates were considered.

In addition to the parameters used for model parameterization, the volume of distribution at steady state (Vss) was obtained: Vss = V1+V2. V I, V ss and CL were presented both unadjusted and adjusted by body weight. Parameter estimates ($\pm$standard error) from the final model were as follows:

- V_1 = 3.08 ± 0.193 L,
- CL = 0.271 ± 0.018 L/day = 11.29 ± 0.77 mL/h if DMEA ≤ 100 mg
- CL = 0.261 ± 0.014 L/day = 10.88 ± 0.58 mL/h if 100mg < DMEA ≤ 180 mg
- CL = 0.279 ± 0.013 L/day = 11.63 ± 0.54 mL/h if DMEA > 180 mg
- CL/HOL: 0.257 ± 0.12 (i.e., the population mean CL increases by 25.7% if HOL = 1 compared to HOL = 0)
- V_2 = 5.27 ± 0.341 L,
- Q = 1.69 ± 0.164 L/day = 70.42 ± 6.83 mL/h.

After a drug holiday of 5 half-lives or more, the population mean clearance increased by

approximately 25.7 %. Without a drug holiday, the mean clearance values were in the range of 13.47 to 14.65 mL/h for all three categories, whereas after a drug holiday, the mean clearance ranged from 18.72 to 37.50 mL/h.

	Summary Statistics of Mean Biweekly Dose					
	≤ 100 mg		100 – 180 mg		> 180 mg	
	Drug Holiday		Drug Holiday		Drug Holiday	
	No	Yes	No	Yes	No	Yes
N	86	22	81	13	78	8
Mean	13.47	28.80	13.68	37.50	14.65	18.72
SD	5.50	46.74	5.46	65.64	4.88	5.61
Minimum						
Median	12.50	16.43	12.64	17.32	13.81	17.83
Maximum						
Geo Mean	12.57	19.03	12.78	20.80	13.95	17.99

Geo Mean = Geometric Mean

Comments:

1. No attempt was taken by the Sponsor to evaluate the effect of age, gender and weight on the pharmacokinetics of adalimumab that could have been easily done in this study.
2. The Sponsor's calculation of Vss is incorrect. Vss is not the sum of V1 and V2.
3. The comparison between patients with or without drug holiday may not be appropriate due to fairly unbalanced sample size (245 without drug holiday and 43 with drug holiday).

Study #16

Pharmacokinetic-Pharmacodynamic Relationship of adalimimab

In the clinical trials, attempts were made to establish concentration-effect relationship. The primary endpoint was based on the American College of Rheumatology (ACR) disease scoring system in which a number of variables were examined, and the smallest percentage change is recorded as the ACRNUM variable. For a 6-month endpoint, the fractions of the population with 20%, 50% or 70% changes (binary) were tabulated as the ACR20, ACR50 and ACR70 values, respectively, and these were compared to placebo or responses. The following Table summarizes Phase II and III studies for which trough concentration and ACR values were available.

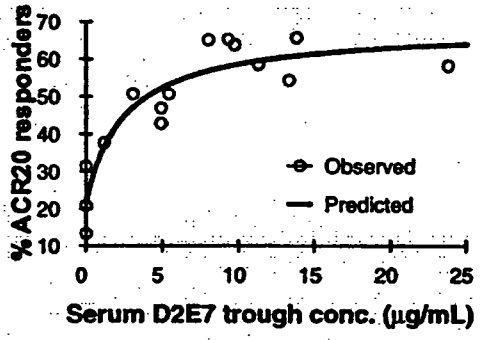
Comparison of Efficacy – Concentration Relationships in Adalimumab Phase II and III Studies

Study	Regimen	Trough Concentration (µg/mL)	Week 24 Efficacy† ACR20 (%)	ACR50 (%)	Mean SJC Decrease
DE007	Placebo	0.0	NA	NA	NA
	20 weekly	5.4	50.7	22.5	8.2
	40 weekly	11.3	58.6	31.4	8.7
	80 weekly	23.8	58.3	27.8	10.0
DE009	Placebo	0.0	13.3	6.7	2.7
	20 eow	3.6	50.7	31.3	7.6
	40 eow	7.9	65.1	52.4	9.7
	80 eow	13.2	65.7	41.4	10.9
DE011+	Placebo	0.0	20.9	8.2	2.4
	20 eow	1.2	37.7	18.9	5.7
	20 weekly	4.9	42.9	22.3	7.1
	40 eow	4.9	46.9	23.0	8.5
	40 weekly	13.3	54.4	36.9	8.3
DE019	Placebo	0.0	31.5	10.5	6.1
	20 weekly	9.7	63.7	42.5	11.9
	40 eow	9.3	65.2	39.6	11.3

NA = Not Applicable; eow = every other week.
† LOCF data from the ISE.
+ = 26-week data.

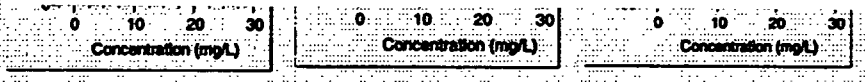
The mean trough concentration and ACR20 values from the above Table were fitted to an Emax model as shown in the figure.

Mean Concentrations and ACR20 Scores from Phase II and III Studies



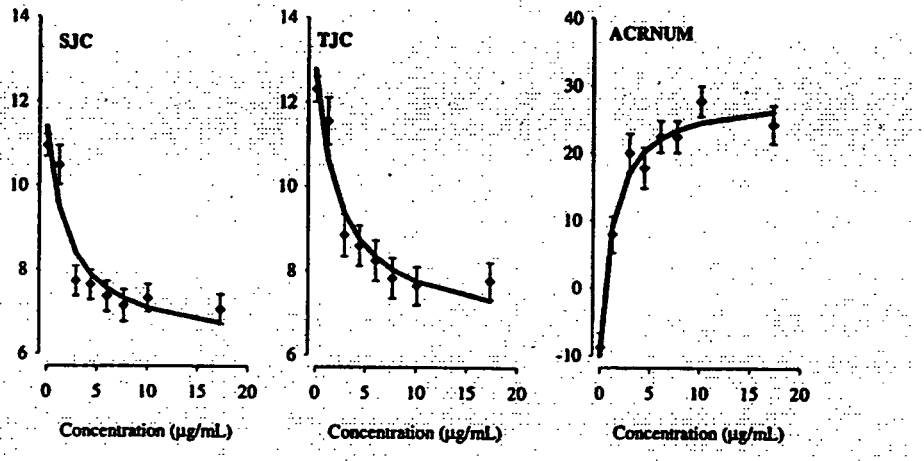
Individual subject data from studies DE007, DE009, DE011 and DE019 were submitted to various SAS analyses to establish relationship between AUC and the corresponding area under the effect curve (AUEC), using linear and Emax models. For each individual, the time-weighted average trough concentration and time-weighted average responses for ACRNUM, swollen joint count (SJC) and tender joint count (TJC) were computed for up to 24 weeks of dosing. The individual data, comprising over 1600 observations, for average trough concentration versus average response revealed a substantial variability across individuals in response. The data for SIC, TJC and ACRNVM with corresponding smoothing spline fits are presented in the following figures.

Concentration – Effect Scatter Plots for Adalimumab in Phase II and III Studies



The vertical dispersion of the data is the greatest near the Y-axis, which mostly represents patients on placebo treatment. With increasing concentration, the dispersion decreases reflecting the drug effect. The above scatter plots were further plotted as deciles. The concentrations were represented as deciles (tenths), and the corresponding time-averaged responses for each decile (approximately160 patients per decile) were computed. These are shown, for SJC, TJC and ACRNUM, for each efficacy metric, in the following figures.

Mean ± SE Response of SJC, TJC and ACRNUM *vs.* Average Adalimumab Concentrations (Studies DE007, DE009, DE011, DE019)



From the figures, which contain placebo data in the first three deciles, it may be seen that the curves resemble those of the hyperbolic Emax with an asymptotic flattening of the relationship when concentrations exceed 5 microgram/mL.

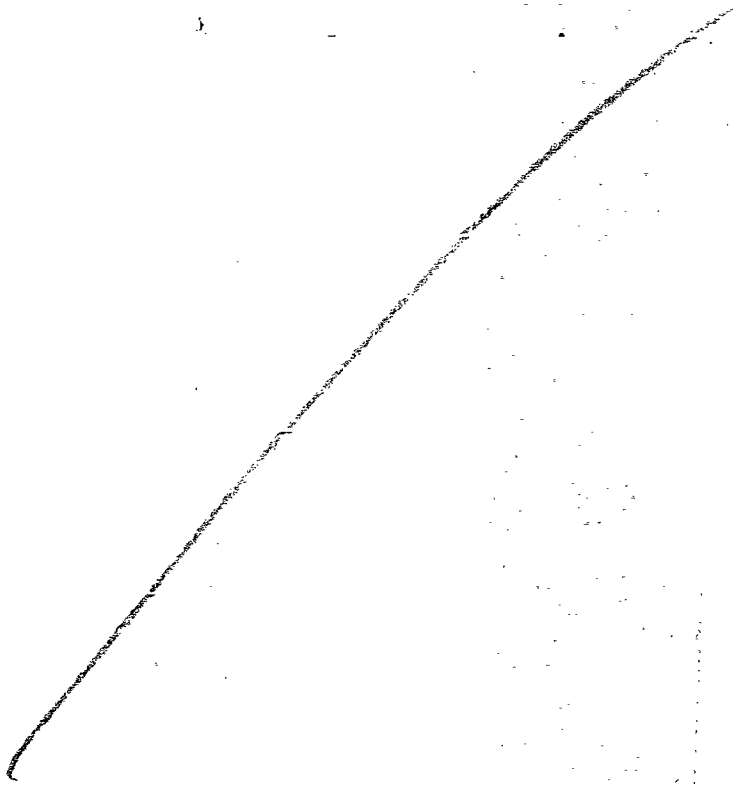
The mean ± SE EC50 for SJC, TJC and ACRNUM were 1.35 ± 0.43, 1.35 ± 0.49, 0.81 ± 0.37 microgram/mL.

Comment:

The ACR classifications are not especially well suited for traditional pharmacokinetic-pharmacodynamic modeling. The data are binary, therefore, a logistic regression approach is more suitable to relate plasma concentrations with effect. On the surface, the Sponsor's Emax model appears reasonable, yet its predictive performance may be poor as this model has not been validated on a separate data set. Furthermore, there are only few data points and these points are also mean values. Univariate regression based on Emax model, which incorporated duration of dosing, HAHA formation and presence of MTX, provided a poor correlation ($R^2 = 0.204$). However, a better correlation was obtained with SJC ($R^2 = 0.511$) and TJC ($R^2 = 0.500$). In short, these pharmacodynamic models may be good for exploratory purposes but the regular use of these models to predict effect in a given individual will be inappropriate.

Analytical Method

A specific and sensitive assay was developed to measure adalimumab concentrations in serum for the human pharmacokinetic studies. The adalimumab assay was originally developed and validated at Knoll in Ludwigshafen, Germany, where a portion of the serum samples were assayed for adalimumab concentrations. Additional assay work was performed using the same assay at [redacted]. The identical assay was validated at [redacted] and cross-validated among the three laboratories. The assay



Sensitivity and the Range of Quantification:

The original assay method utilized a [redacted] dilution of the serum sample. The range of quantification, which reliably produced a mean precision of coefficient of variation (CV)

and a mean accuracy of , was . The lower limit of quantification (LLQ) was established at in diluted serum, which is equivalent to in original (undiluted) serum sample. During the initial method experiments, the working concentration range was set at However, routine analyses of serum samples from rheumatoid arthritis patients revealed non-linearity in some of the samples in the working range above The working range was therefore reduced to . However, the calibration range of and QC concentration range of was not changed as a result of this observation. For the high sensitivity assay with a diluted serum, the working concentration range was set at . The LLQ was established at , in original (undiluted) serum sample.

Accuracy, inter and intra-assay precision:

Under routine conditions, inter-assay accuracy ranged from of nominal concentration for both the original assay (dilution) as well as using the dilution. Inter-assay precision, under routine conditions was CV for both the original, dilution as well as the dilution assay procedure. For the original (dilution) assay, intra-assay precision was evaluated in two different tests using replicate aliquots per concentration within each single test. Maximum imprecision was < for replicates and for single analysis. For the high sensitivity (dilution) assay, within the working range of the average intra-assay precision was CV.

Stability:

Adalimumab in human serum samples was very stable and easily tolerated freeze- thaw cycles, a storage in the refrigerator or a short-term incubation at 37°C. Stability of samples was established for long-term storage of at least weeks at approximately -80°C or , at approximately -20°C. Adalimumab in human study serum samples stored at approximately -80 °c for up to in either original serum or diluted aliquot (1:10) was also found to be stable under these conditions.

Determination of Anti-Adalimumab Antibodies:

An immunoassay, referred to here as the HAHA assay, was developed to determine anti-adalimumab antibodies in sera of subjects and laboratory animals (mice and monkeys) exposed to adalimumab. The HAHA assay is intended to detect all antibodies directed against epitopes on the adalimumab molecule. It is a [redacted] ELISA that relies on the [redacted] technique. [redacted]

[redacted]).

[redacted]

The LLQ was set at [redacted] in [redacted] diluted serum, which is equivalent to [redacted] in the original, undiluted, serum. The precision and accuracy at the LLQ were [redacted] of the nominal value, respectively.

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Attachment D-1. Adalimumab Analytical Method Summary

Study	Matrix	Method	Calibration Curve Range		LOQ†	Analytical Site*
			Lower (ng/mL)	Upper (ng/mL)	(ng/mL)	
DE001	Serum	ELISA				Knoll
	Synovial Fluid	ELISA				Knoll
DE003	Serum	ELISA				
	Synovial Fluid	ELISA				
DE004	Serum	ELISA				Knoll
DE005	Serum	ELISA				
DE005X	Serum	ELISA				
DE007	Serum	ELISA				
DE009	Serum	ELISA***				Knoll
DE009X	Serum	ELISA***				Knoll

Abbreviations: ELISA = enzyme-linked immunosorbent assay; LOQ = Lower limit of quantification.

* Knoll = Knoll GmbH, Ludwigshafen, Germany;

** Upper limit of quantification was

*** High sensitivity assay.

† In undiluted serum.

Attachment D-1. Adalimumab Analytical Method Summary (Cont.)

Study	Matrix	Method	Calibration Curve Range		LOQ†	Analytical Site*
			Lower (ng/mL)	Upper (µg/mL)	(ng/mL)	
DE010	Serum	ELISA				
DE011	Serum	ELISA				
DE015	Serum	ELISA				
DE018	Serum	ELISA				
DE019	Serum	ELISA				
DE024 Caucasian	Serum	ELISA**				Knoll
DE024 Japanese	Serum	ELISA**				Knoll
DE029	Serum	ELISA				

Abbreviations: ELISA = enzyme-linked immunosorbent assay; LOQ = Lower limit of quantification.

* Knoll = Knoll GmbH, Ludwigshafen, Germany.

** High sensitivity assay.

† In undiluted serum.

Formulation

During the course of the clinical development of adalimumab, the drug product formulation did not change significantly. For the clinical trials the injectable solution was a [redacted] containing mannitol and sodium chloride. Although the chemical composition of the [redacted] did not change, the amounts of each component were changed from the earliest batches to the later batches. Tween 80 (polysorbate 80) was not added to the product used in the pivotal clinical trials, but has been been assessed in Studies DE015, DE018, DE020 and DE029. Product stability testing established that pH 5.2 provided the best stability characteristics. In order to accommodate blinded doses in the clinical trials, the drug product was prepared with three concentrations: 25 mg adalimumab/mL, 12.5 mg adalimumab/mL, and 6.25 mg adalimumab/mL.

The proposed route of administration for market is *via* subcutaneous injection. Administration of 1.6 mL of the 25 mg/mL product, injected subcutaneously, is required for the therapeutic dose of 40 mg. To facilitate the injection process, a more concentrated product was developed to reduce the injected volume to obtain the therapeutic dose. A 50 mg adalimumab/mL product was developed for this purpose. During the development testing, it was established that the addition of [redacted] polysorbate 80 to the solution [redacted]

[redacted] The product, which is the subject of this Biologics License Application (BLA), is contained in 2 mL vials, in a volume sufficient to remove 0.8 mL, the formulation of which is presented in the following table. The formulation is also provided in a prefilled syringe that is designed to inject 0.8 mL.

The drug substance (bulk drug) production underwent several changes during drug development [redacted] Significant improvements included [redacted]

[redacted] The major phases of the drug manufacturing process development are given in the following table.

Major Phases of Drug Manufacturing Process Development

Drug Substance Batch Numbers	Campaign	Process Name
AFP603 to AFP707	1996-1997	
AFP750 to AFP808	1997-1998	
AFP809 to AFP15B	1998-1999	
AFP02C to current batches	1999-2002	

For each production of adalimumab, a drug substance batch number was assigned. A drug substance campaign was defined by the period in yearly dates during which a specific production process was used to prepare adalimumab.

The early Phase I studies were performed with the drug substance from the process. The later Phase I and the Phase II studies used material from the process. Phase III studies and the pivotal bioequivalence, bioavailability, and pharmacokinetic studies used drug substance from the process. The drug substance was used for the final product formulation proposed for marketing, which was also used in the pivotal bioequivalence study, as well as in the open-label clinical extension studies. Characterizations of the drug substances from the various processes have been compared with a number of testing methods and found to be equivalent.

Final Formulation Composition

Name of Ingredient	Quantity (mg)/mL
Active Substance:	
Adalimumab	
Excipients:	
Mannitol	
Citric acid monohydrate	
Sodium citrate	
Sodium monohydrogen phosphate dihydrate	
Sodium dihydrogen phosphate dihydrate	
Sodium chloride	
Polysorbate 80 (Tween 80)	
Water for injection	
Sodium hydroxide	q.s.

q.s. = quantity sufficient.

Drug Formulations and the Process of the Drug Substance Used in Clinical Studies

Clinical Study Number	Dosage Forms Solution for Injection	Drug Substance Process
DE001	25 mg/mL, 2 mL vial	
	25 mg/mL, 10 mL vial	
DE003	25 mg/mL, 2 mL vial	
	25 mg/mL, 10 mL	
DE004	25 mg/mL 2 mL	
DE005	25 mg/mL, 2 mL vial	
	25 mg/mL, 10 mL vial	
DE005X	25 mg/mL, 2 mL vial	
	25 mg/mL, 10 mL vial	
DE007	25 mg/mL, 1.6 mL	
	12.5 mg/mL, 1.6 mL	
	6.25 mg/mL, 1.6 mL	
DE009	25 mg/mL, 1.6 mL	
	12.5 mg/mL, 1.6 mL	
	6.25 mg/mL, 1.6 mL	
DE009X	25 mg/mL, 1.6 mL	
DE010	25 mg/mL, 2 mL vial	
DE011	25 mg/mL, 1.6 mL	
	12.5 mg/mL, 1.6 mL	
DE015	25 mg/mL, 2 mL vial	
	50 mg/mL, 1.2 mL, with Tween 80	
	50 mg/mL, 1.2 mL	
DE018	25 mg/mL, 1.6 mL	
	50 mg/mL, 0.8 mL, with Tween 80	
DE019	25 mg/mL, 1.6 mL	
	12.5 mg/mL, 1.6 mL	
DE020	25 mg/mL, 1.6 mL	
	50 mg/mL, 0.8 mL with Tween 80	
DE024 (Caucasian)	25 mg/mL, 1.6 mL	
DE024 (Japanese)	25 mg/mL, 1.6 mL	
DE029	25 mg/mL, 1.6 mL	
	50 mg/mL, 0.8 mL with Tween 80	
DE031	25 mg/mL, 1.6 mL	

4 Draft Labeling Page(s) Withheld

Comments to the Sponsor

1. Study DE001 states that the concentrations of adalimumab were lower in females than males. Please provide the data on males and females to explain the lack of gender effect on the pharmacokinetics of adalimimab. In fact there are several studies from which data can be pooled to evaluate the effect of gender on the pharmacokinetics of adalimimab.

2. Please provide the method of calculation of half-life in population pharmacokinetic studies using POSTHOC estimation.

3. In the individual serum concentration Tables, what does it mean by the term no value reported (NVR)? Was plasma concentration not measured or was concentration below the detection limit? Please clarify.

4. In study DE005, it has been mentioned that a noncomparmental analysis was performed but no data was provided. Please provide the data of this analysis.

5. In study DE005X, the method to calculate AUC from time 0-336 is not clear especially when blood samples were collected till 24 hours. Therefore, the estimated CL values of adalimumab across doses appear to be ambiguous. Please provide appropriate explaination.

6. Please note that in the same study (DE005X), the estimated AUCs for MTX based on only two samples are unreliable and should not be used for any conclusion or interpretation of data.

7. In study DE007, in Table 9.2a, the data have been reported for 87 subjects, whereas the study report mentions that 284 subjects were evaluated for pharmacokinetics. Please explain this discrepancy.

8. Study DE010:

a. Please calculate the PK parameters for SC administration of adalimumab as done with IV administration.

b. It is not clear how was the POSTHOC estimation of VSS generated? In the control stream file Vss does not appear. It appears that Vss has been calculated by adding V1 and V2 that renders all calculations of Vss as incorrect.

c. In the POSTHOC estimation, it is not clear how was the half-life calculated?

d. Table 2 is incomplete (P#397 of the study report, V#19). Some subjects' data on PK are missing.

e. There are 54 subjects in the study of which 18 were given placebo. NONMEM data file consists of 54 subjects with plasma concentrations. Please clarify.

Study DE003:

1. Vss is not the sum of V1 and V2.

Recommendation:

The pharmacokinetic conclusion drawn from the studies conducted in this BLA are acceptable. The Sponsor's response to the comments is also satisfactory and acceptable.

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