

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

125104

**CLINICAL PHARMACOLOGY AND
BIOPHARMACEUTICS REVIEW(S)**

This Clinical Review for BLA 125104
begins on page 7.

There are no pages missing or withdrawn
from this review.

Summary

Pharmacokinetics:

In the first human study, natalizumab was given as IV infusion to healthy volunteers as a single dose ranging from 0.03 to 3.0 mg/kg (study #1). The C_{max} increased with the dose and was linear between 0.3 to 3 mg/kg dose, however, AUC did not increase proportionally with dose. The half-life ranged from 49 (0.3 mg/kg dose) to 202 hours (3 mg/kg dose). The clearance of natalizumab decreased with increasing doses. The clearance of natalizumab at 0.3 mg/kg dose was 1.01 mL/hr/kg and at 3 mg/kg dose it was 0.26 mL/hr/kg. The healthy volunteers also received a fixed dose of 300 mg natalizumab. The C_{max} , clearance, and half-life were 100 mcg/mL, 0.20 mL/hr/kg, and 250 hours, respectively (study #3).

In two bioequivalence studies, natalizumab produced by Biogen's commercial process and by _____' clinical process (study #2) as well as natalizumab produced by Biogen's commercial process and by Biogen's clinical process (study #3) were found to be bioequivalent.

In patients with MS, the single dose pharmacokinetics of natalizumab followed the same pattern as in healthy subjects (study #4). The C_{max} increased with the dose and was linear between 0.3 to 3 mg/kg dose, however, AUC did not increase proportionally with dose. The half-life ranged from 37 (0.3 mg/kg dose) to 108 hours (3 mg/kg dose). The clearance of natalizumab decreased with the increasing doses. The clearance of natalizumab at 0.3 mg/kg dose was 1.45 mL/hr/kg and at 3 mg/kg dose it was 0.31 mL/hr/kg. In patients with MS, the half-life of natalizumab was shorter than the healthy volunteers but the clearance was almost similar in both groups at 3 mg/kg dose. Following multiple dosing (3 and 6 mg/kg monthly for six doses) of natalizumab in patients with MS, C_{max} , half-life and AUC(0-inf) or AUC(0-tau) were comparable between the first and the sixth dose (study #9). No accumulation of natalizumab following multiple dosing was noted in this study. Following a 300 mg fixed dose of natalizumab given every 4 weeks to patients with MS, C_{max} , half-life and AUC(0-inf) or AUC(0-tau) were comparable between the first and the sixth dose (study #10).

The effect of AVONEX (interferon beta-1a) on the pharmacokinetics of natalizumab was assessed in the patients with MS (study #11). There was an increase in the C_{max} and half-life of natalizumab by 20% and 70%, respectively, following the administration of AVONEX. The clearance of natalizumab decreased by 35% in the presence of AVONEX, an indication that AVONEX may alter the pharmacokinetics of natalizumab.

In study 6 (Table 6.2), it appears that there is no effect of gender on the pharmacokinetics of antegen. It should be however, noted that the sample size is unbalanced. The number of female subjects in the study is almost 4 times than the male subjects. Therefore, the study may not reflect the true impact of gender on the pharmacokinetics of antegen.

Pharmacodynamics:

Pharmacodynamic markers such as elevated peripheral WBC and lymphocyte counts and the determination of $\alpha 4$ -integrin saturation on peripheral blood mononuclear cells (PBMC) were measured throughout the clinical development program.

In Studies C-1805 and C-1806 (studies 2 and 3 in this review), a 300 mg fixed dose of natalizumab was administered IV to healthy male and female volunteers. $\alpha 4$ -integrin saturation levels were measured by flow-cytometric techniques. In both studies, mean saturation levels demonstrated an immediate rise from baseline at two hours post-infusion followed by a plateau up to 14 days post-infusion, after which there was a decline over time up to 28 days. In both preparations, saturation of the $\alpha 4$ receptor was maintained at a level of greater than 80% over the 28-day dosing interval. The total lymphocyte counts also demonstrated a steep rise from baseline to 7 days post-infusion followed by a slow decline. At 42 days post-infusion, mean counts remained above baseline. Although elevated, the mean lymphocyte counts remained within the normal range ($0.91-4.28 \times 10^9/L$) throughout the observation period.

In MS231 (study #9 in this review), doses of 3 mg/kg and 6 mg/kg natalizumab resulted almost complete saturation of $\alpha 4$ -integrin immediately following the initial infusion. Monthly dosing of 3 or 6 mg/kg resulted in sustained $\alpha 4$ -integrin saturation levels of approximately 75% and 80%, respectively. The administration of 300 mg natalizumab resulted in a similar $\alpha 4$ -integrin saturation profile. Mean saturation levels were in excess of 90% immediately post-infusion and resulted in sustained $\alpha 4$ -integrin saturation levels of approximately 70%.

Elevations in absolute lymphocyte counts were relatively consistent across studies. Lymphocyte counts in the natalizumab-treated subjects were elevated to approximately 3.0×10^9 cells/L throughout the treatment periods. Following the cessation of dosing in MS231, lymphocyte counts remained elevated through month 8 in the 3 mg/kg dose group and month 9 in the 6 mg/kg dose group.

Justification for 300 mg fixed dose:

The Sponsor justified the selection of a fixed 300 mg dose in MS patients in phase III studies based on subject demographics, PK/PD and efficacy profile of natalizumab. The PK/PD data from phase II study were used.

A population PK approach was taken to evaluate the effect of weight on the clearance of natalizumab. Data were pooled from two studies (CD202 and MS231) and clearance values in individual subjects were generated by post-hoc (Bayes estimates). There was no relationship between body weight and clearance.

Individual subject concentration and percent saturation data from study MS231 were analyzed for a PD model. An Emax model with a baseline was used to generate PD parameters

(E_0 , EC_{50} , and E_{max}). Based on the model, natalizumab serum concentrations of approximately 2.5-3 mcg/mL would be required to maintain a minimum $\alpha 4$ -integrin saturation of 80%. Mean \pm SD trough serum concentrations for the 3 and 6 mg/kg dose groups in Study MS231 were 4.6 ± 2.2 and 16.8 ± 5.6 mcg/mL, respectively. Approximately 90% of the subjects in both dose groups had natalizumab serum concentrations in excess of 2.5 mcg/mL four weeks following the last infusion.

In Study MS231, the mean baseline body weights were 72 and 70 kg, respectively, in the 3 and 6 mg/kg dose groups. These mean body weights correspond to an average administered dose of 216 and 420 mg, respectively. Since both doses resulted in similar efficacy, safety, and tolerability, a 300mg fixed dose was chosen for Phase 3. This dose was considered appropriate because it would provide an intermediate exposure between the average 3 and 6 mg/kg doses. In addition, a 300 mg fixed dose would not exceed a dose of 6 mg/kg in subjects greater than or equal to 50 kg or be less than 3 mg/kg in subjects less than or equal to 100 kg.

Immunogenicity:

All subjects were screened for the development of anti-natalizumab antibodies. The assay methodology changed from a fluorometric to a colorimetric for the Phase 3 studies in order to improve assay sensitivity in whole serum. A screening ELISA assay was used to initially detect the presence of anti-natalizumab antibodies. This was followed by a cell-based assay to determine if the antibodies blocked the ability of natalizumab to bind to $\alpha 4$ -integrin. All subjects who had detectable screening antibodies were also positive for blocking antibodies.

In single dose studies (MS200, MS202, MS221, and MS224) in patients with MS the incidence rate of antibody-positive subjects ranged from 10% to 25% across studies and antibodies were generally detected within 5 weeks of dosing. The combined antibody positive rate was approximately 21 %. Antibodies were detected in subjects receiving natalizumab doses ranging from 1 to 6 mg/kg and there was no apparent relationship between dose and immunogenicity.

In multiple dose studies (MS201 and MS231) in patients with MS antibodies were detected in subjects from all dose groups and the incidence rate of antibody-positive subjects ranged from 11 % to 19% across studies. Antibodies were generally detected within 4 weeks of dosing. The combined antibody positive rate was approximately 12%.

Impact of antibody formation on the PK of natalizumab:

From the current available data it is not possible to evaluate if the formation of antibody has any impact on the pharmacokinetics of natalizumab. Data are available only from three subjects who were identified as antibody positive. The pharmacokinetics of natalizumab were evaluated

after the first and the sixth dose in these subjects. The pharmacokinetic data for these three subjects are summarized in the following Table.

Subject ID	C _{max} (mcg/mL)	AUC (mcg*h/mL)	Clearance (mL/h)	T _{1/2} (hrs)
126-021				
Dose 1	—	20421	14.69	239
Dose 6		12627	24.88	165
111-004	✓			
Dose 1		13981	21.46	248
Dose 6	✓	54	5562	24
16-1110				
Dose 1	✓	9935	21.14	65
Dose 6	✓	529	399	NA

From the Table it appears that antibody formation may increase the clearance of natalizumab but in order to evaluate this, a systematic study with adequate sample size is warranted.

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Study #1

Title: A phase I, double-blind, placebo-controlled, ascending single intravenous dose, safety, tolerability, pharmacokinetic, immunogenicity and potency study in healthy male volunteers (AN100226-101).

This was a phase I study in healthy male volunteers aged 18 to 55 years. The subjects received either placebo (n =9) or 0.03, 0.1, 0.3, 1.0, and 3.0 mg/kg active treatment. There were 4 subjects each in the dose groups of 0.03 and 0.1 mg/kg, and 6 subjects each in the dose groups of 0.3, 1.0, and 3.0 mg/kg. The drug was administered by intravenous infusion over 30 minutes. Blood samples were taken at time 0, 30, 35, 40, 50 min and at 1, 1.5, 3, 6, 12, 18 and 24 h and on Days 3; 4, 5, 6, 7, 8, 11, 15, 22, 29 and 36 for the pharmacokinetic study of AN100226. Blood samples were also taken for:

Immunogenicity assay: pre-dose and at days 8, 11, 15, 22 and 92

Neutralizing antibody assay: pre-dose and days 11, 15, and 22

Blood samples were also collected at regular intervals till day 36 for $\alpha 4$ integrin saturation.

The concentrations of AN100226 were measured by ELISA. The LOQ was _____ and reproducibility was in the _____ range. Anti-AN100226 antibodies in serum were determined using a validated ELISA assay. The LOQ was _____ $\alpha 4$ integrin saturation was measured by a direct fluorometric method which assessed the relative saturation by AN100226 of the $\alpha 4$ integrin receptor on lymphocytes. The results of the pharmacokinetic study are summarized in the following Table.

TABLE 1.1
Mean pharmacokinetic parameters of AN100226 in healthy volunteers

Parameters	0.3 mg/kg	1 mg/kg	3 mg/kg
C_{max} (mcg/mL)	7.6 ± 1.9	22.4 ± 3.8	71.8 ± 4.8
T_{max} (hrs)	0.61 ± 0.14	0.95 ± 0.31	0.78 ± 0.37
$AUC_{(0-inf)}$ mcg*hr/mL	349 ± 146	2276 ± 1848	3968 ± 543
CL (mL/min/kg)	0.0169 ± 0.0083	0.0074 ± 0.0015	0.0043 ± 0.0007
$T_{1/2}$ (hrs)	49 ± 13	118 ± 23	202 ± 38
Vss (mL/kg)	88 ± 30	63 ± 8	53 ± 8

The results of the study indicate that the C_{max} follows the linear kinetics over the dose range of 0.3 to 3 mg/kg, whereas the AUC indicates a nonlinearity of AN10026 over this dose range. The clearance of AN10026 decreases and the half-life increases with the increasing dose. No anti-natalizumab antibodies were detected in the serum of any subject through day 22 post dosing.

Saturation of $\alpha 4$ integrin was dose-dependent and correlated with serum AN100226 concentrations. All doses resulted in nearly 100% saturation immediately post infusion. Greater than 80% saturation was maintained up to 11 days post administration of 1.0 mg/kg and up to 22 days after 3.0 mg/kg.

There were positive AN100226 assay results found for some serum samples in the placebo treatment group and for some pre-dose serum samples. There was no obvious reason for these false positives. These positive values suggested that the bottom two dose groups (0.03 mg/kg and 0.1 mg/kg) had unreliable serum AN100226 data and these results were excluded from the analysis.

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Study #2

Title: A randomized, double-blind, crossover study in healthy volunteers comparing the pharmacokinetic properties of natalizumab produced by Biogen's commercial process and by [redacted] clinical process (C-1805).

The primary objective of this study was to establish the bioequivalence of single doses of BGNCOM (natalizumab commercial material produced by the Biogen [redacted] scale process and [redacted] (natalizumab clinical material produced by [redacted] scale process.

This was a single center, randomized, double-blind, 2-period crossover study in healthy volunteers. Subjects were randomized in a 1: 1 ratio to 1 of 2 sequences (approximately 40 subjects each) of 2 preparations of natalizumab ([redacted] and BGNCOM). Subjects received a 300 mg IV infusion (over 60 minutes) of either [redacted] or BGNCOM. There was a 8-week washout period between the administration of the two formulations. Healthy male (n = 44) and female (n = 45) volunteers between the ages of 18 and 47 years and weighing between 52 to 90 kg were included in the study. Blood samples (7 mL) were collected at time 0 and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 12, 24, 48, and 96 hours after dosing and at days 7, 14, 28, 35, and 42 post-dosing. The concentrations of natalizumab were measured by ELISA. Blood samples were also collected at time 0 and 2 hours, and days 7, 14, 21, and 28 post-dose for pharmacodynamic assessment.

Fifty five subjects received both formulations but bioequivalence analysis was conducted using 47 subjects' pharmacokinetic data. Seven subjects developed antibodies and one subjects received partial dose. Out of 89 subjects, 27 subjects had detectable antibodies at least 1 time point during the study. The following table summarizes the pharmacokinetic parameters and the 90% confidence interval (CI) on log transformed data obtained from 27 subjects. Figure 2.1 shows the plasma concentrations vs time profiles for two formulations of antegen (taken from the sponsor).

TABLE 2.1

Mean ± SD pharmacokinetic parameters of two natalizumab formulations

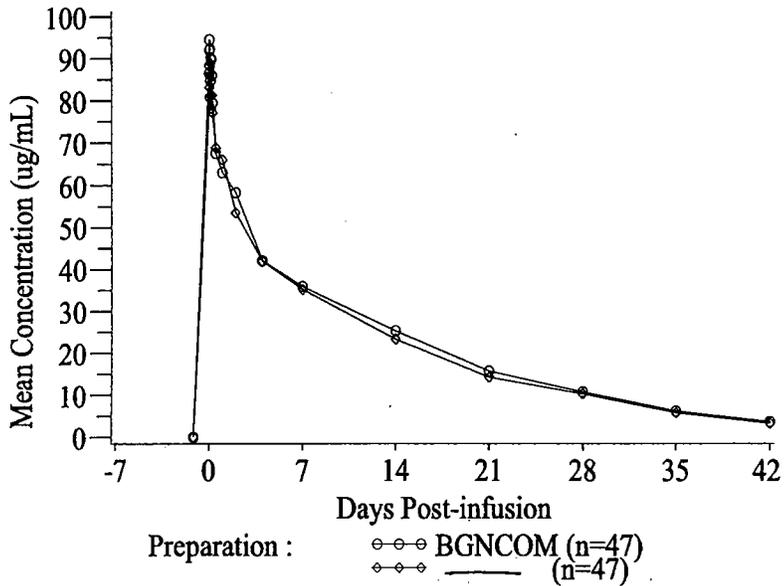
Parameters	BGNCOM	[redacted]	90% CI
C_{max} (mcg/mL)	120.4 ± 44.2	114.3 ± 39.4	97-110
$AUC_{(0-inf)}$ (mcg*hr/mL)	22155 ± 7743	21057 ± 6237	97-107
T_{max} (hrs)	6.2 ± 10.1	4.4 ± 4.6	NA
$T_{1/2}$ (hrs)	230 ± 86	235 ± 159	NA

NA = Not applicable

Based on the confidence interval, the two formulations of natalizumab are bioequivalent.

Figure 2.1

Mean serum concentrations of two formulations of natalizumab



There was an immediate rise in $\alpha 4$ integrin percentage saturation from baseline at 2 hours post-infusion followed by a shallow decline over time up to 28 days. The pattern was similar in both preparations. In both groups, saturation of the $\alpha 4$ receptor was maintained at a level of greater than 80% over the 28-day dosing interval. Mean WBC count and mean total lymphocyte count rose from baseline to 7 days post-infusion, followed by a slow decline. At 42 days post-infusion, mean count levels were still above baseline. At no point did the mean WBC count rise above the upper limit of normal. The pattern was similar for both preparations.

Conclusion: Natalizumab commercial material produced by Biogen (BGNCOM) is bioequivalent to natalizumab clinical material produced by _____

Immunogenicity:

Antibodies were detected 28 days following drug administration. Antibodies were detected in 10 of 43 subjects following the administration of AN100226 and in 5 of 43 subjects following the administration of BG00002-B.

Study #3

Title: A randomized, double-blind, crossover study in healthy volunteers comparing the pharmacokinetic properties of natalizumab produced by Biogen's commercial process and by Biogen's clinical process (C-1806).

The primary objective of this study was to establish the bioequivalence of single doses of BGNCOM (natalizumab commercial material produced by the Biogen scale process and BGNRSH (natalizumab clinical material produced by Biogen scale process.

This was a single center, randomized, double-blind, 2-period crossover study in healthy volunteers. Subjects were randomized in a 1: 1 ratio to 1 of 2 sequences (approximately 40 subjects each) of 2 preparations of natalizumab (BGNRSH and BGNCOM). Subjects received a 300 mg IV infusion (over 60 minutes) of either BGNRSH or BGNCOM. There was a 8-week washout period between the administration of the two formulations. Healthy male (n = 41) and female (n = 45) volunteers between the ages of 19 and 50 years and weighing between 51 to 88 kg were included in the study. Blood samples (7 mL) were collected at time 0 and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 12, 24, 48, and 96 hours after dosing and at days 7, 14, 28, 35, and 42 post-dosing. The concentrations of natalizumab were measured by ELISA. Blood samples were also collected at time 0 and 2 hours, and days 7, 14, 21, and 28 post-dose for pharmacodynamic assessment.

Fifty five subjects' data were included in the bioequivalence analysis. The following table summarizes the pharmacokinetic parameters and the 90% confidence interval (CI) on log transformed data obtained from 27 subjects. Figure 3.1 shows the plasma concentrations vs time profiles for two formulations of antegren (taken from the sponsor).

TABLE 3.1

Mean + SD pharmacokinetic parameters of two natalizumab formulations

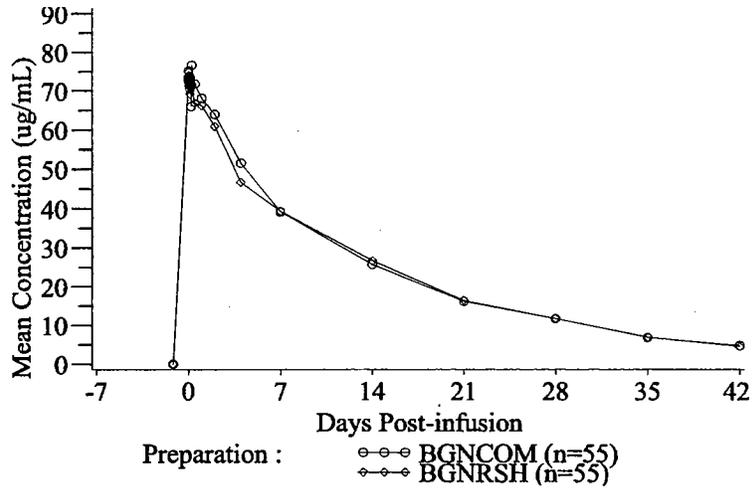
Parameters	BGNCOM	BGNRSH	90% CI
C _{max} (mcg/mL)	100.3 ± 30.3	99.73 ± 29.2	96-106
AUC _(0-inf) (mcg*hr/mL)	25646 ± 9393	25288 ± 10469	100-109
T _{max} (hrs)	11.2 ± 20.3	5.0 ± 7.7	NA
T _{1/2} (hrs)	263 ± 127	266 ± 154	NA

NA = Not applicable

Based on the confidence interval, the two formulations of natalizumab are bioequivalent.

Figure 3.1

Mean serum concentrations of two formulations of natalizumab



There was an immediate rise in $\alpha 4$ integrin percentage saturation from baseline at 2 hours post-infusion followed by a shallow decline over time up to 28 days. The pattern was similar in both preparations. In both groups, saturation of the $\alpha 4$ receptor was maintained at a level of greater than 80% over the 28-day dosing interval. Mean WBC count and mean total lymphocyte count rose from baseline to 7 days post-infusion, followed by a slow decline. At 42 days post-infusion, mean count levels were still above baseline. At no point did the mean WBC count rise above the upper limit of normal. The pattern was similar for both preparations.

Conclusion: Natalizumab commercial material produced by Biogen (BGNCOM) is bioequivalent to natalizumab clinical material produced by Biogen (BGNRSH).

Immunogenicity:

Like study C-1805, antibodies were detected 28 days following drug administration. Antibodies were detected in 5 of 43 subjects following the administration of BG00002-A and in 7 of 43 subjects following the administration of BG00002-B.

Study #4

Title: A placebo-controlled, safety, tolerability, dose escalation, pharmacokinetic study of various doses of intravenous antegren in patients with multiple sclerosis (MS200).

This was a two-center, placebo-controlled, outpatient pharmacokinetic study in male and female patients (n = 28) with multiple sclerosis (MS). There were 4 patients (3 active and 1 placebo) each in the dose groups of 0.03, 0.1, and 0.3 mg/kg and 8 patients (6 active and 2 placebo) each in the dose groups of 1.0 and 3.0 mg/kg. The drug was administered by intravenous infusion over 45 minutes. Blood samples were taken at time 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, and 24 hours and on weeks 1, 2, 3, and 4 for all dose groups and an additional two samples on weeks 6 and 8 for 3.0 mg/kg dose. Blood samples for $\alpha 4$ saturation were collected at time 0, 6, and 24 hours and on weeks 1, 2, 3, and 4 for all dose groups and an additional two samples on weeks 6 and 8 for 3.0 mg/kg dose. For immunogenicity assay, blood samples were collected on day 0, at week 4 for 0.03, 0.1, 0.3, and 1.0 mg/kg and at week 8 for 3.0 mg/kg dose. Neutralizing antibody assay: pre-dose and days 11, 15, and 22

The concentrations of AN100226 were measured by ELISA. The LOQ was — and reproducibility was in the — range. Anti-AN100226 antibodies in serum were determined using a validated ELISA assay. The LOQ was — $\alpha 4$ integrin saturation was measured by a direct fluorometric method which assessed the relative saturation by AN100226 of the $\alpha 4$ integrin receptor on lymphocytes. The results of the pharmacokinetic study are summarized in the following Table.

TABLE 4.1
Mean pharmacokinetic parameters of antegren in patients with MS

Parameters	0.3 mg/kg	1 mg/kg	3 mg/kg
C_{max} (mcg/mL)	4.3 ± 0.46	13.8 ± 2.5	52.58 ± 12.0
T_{max} (hrs)	0.72 ± 0.21	2.8 ± 2.0	2.0 ± 1.2
$AUC_{(0-inf)}$ mcg*hr/mL	217 ± 54	1657 ± 386	9899 ± 1284
CL (mL/hr/kg)	1.45 ± 0.42	0.64 ± 0.17	0.31 ± 0.04
$T_{1/2}$ (hrs)	37 ± 4	92 ± 28	108 ± 30
Vss (mL/kg)	75 ± 12	81 ± 10	67 ± 17

Following IV infusion of antegren, the maximum concentrations were achieved within 0.75 to 2.5 hours. The pharmacokinetics of Antegren were nonlinear over the dose range of 0.3 - 3.0 mg/kg. C_{max} and $AUC_{(0-inf)}$ increased with dose over the 0.03 - 3.0 mg/kg dose range. However, both C_{max} and $AUC_{(0-inf)}$ showed a greater than proportional increase with dose, indicating a nonlinear pharmacokinetics. In some patients there was a sudden drop in plasma concentrations at the end

of the terminal phase (approximately two to eight weeks after dosing). This decrease may be due to the production of anti-antegren antibodies. Two of three patients with measurable levels of anti-Antegren antibodies at Week 8 showed a pronounced drop in plasma concentrations. Systemic clearance decreased with increasing dose, ranging from 1.45 - 0.31 mL/hr per kg. Half-life increased with increasing dose, ranging from 37 - 108 hours. Vss ranged from 67 - 81 mL/kg, suggesting a distribution limited to plasma volume. No notable differences in pharmacokinetics were observed between males and females.

The extent and duration of $\alpha 4$ receptor saturation appear to be dose related. The long duration of occupancy observed following the 1.0 and 3.0 mg/kg doses suggests that occupancy is related to plasma antegren concentrations. However, when anti-antegren antibodies are present, the $\alpha 4$ saturation assay used in this study may incorrectly conclude occupancy of the receptor.

There were 3 patients who were positive for anti-natalizumab antibodies. These patients were in 3.0 mg/kg dose group. Antibody concentrations ranged from _____ Two subjects were positive from weeks 8 through 14 and one subject was positive at weeks 8 and 11. Figures 4.1 and 4.2 show the plasma concentrations vs time profiles for antegren (taken from the sponsor).

FIGURE 4.1

SEMI-LOGARITHMIC PLOT OF THE MEAN PLASMA CONCENTRATION-TIME PROFILES FOR ANTEGREN BY DOSE GROUP

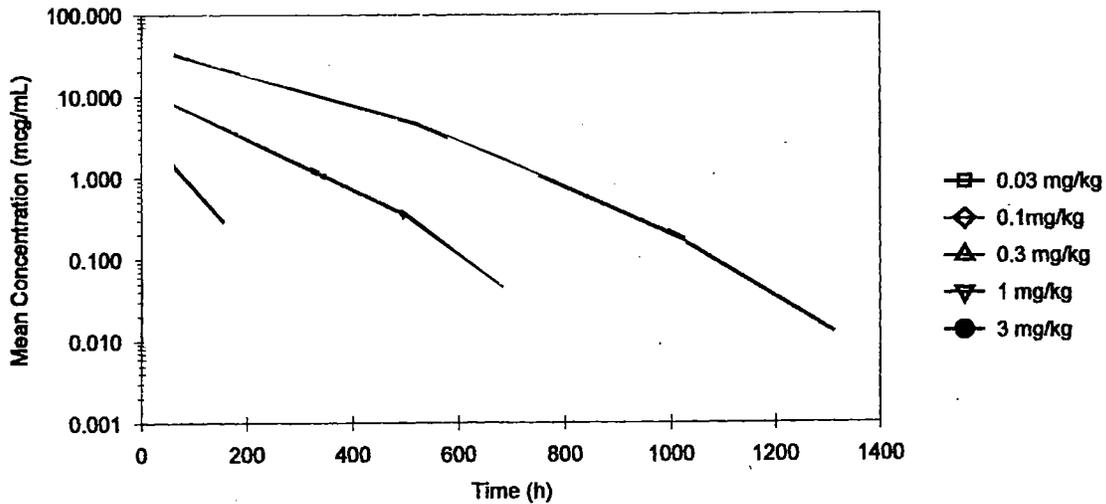
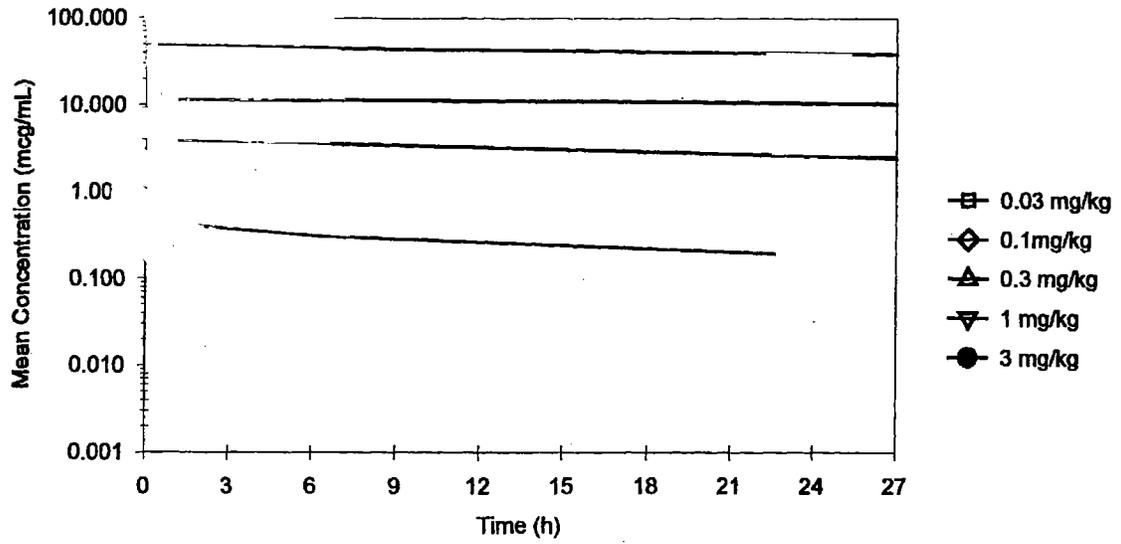


FIGURE 4.2

SEMI-LOGARITHMIC PLOT OF THE MEAN PLASMA CONCENTRATION-TIME PROFILES FOR ANTEGREN BY DOSE GROUP - FIRST 24 HOURS



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Study #5

Title: A placebo-controlled, pharmacodynamic, pharmacokinetic tolerability, and safety study of three doses of intravenous antegren in patients with multiple sclerosis (MS221).

This was a randomized, double-blind, placebo-controlled, parallel-group, dose ranging study in male and female relapsing-remitting or secondary-progressive MS patients following a 45-minute IV infusion. Doses of 1.0, 3.0, and 6.0 mg/kg were evaluated. A total of 9 subjects received 1.0 mg/kg, 10 received 3.0 mg/kg, and 10 received 6.0 mg/kg of natalizumab. A total of 10 subjects received placebo (5 males and 5 females). There were 14 males and 15 females in the drug group. The age of the patients ranged from 24 to 58 years. Blood samples were taken at time 0, 0.33, 0.75, 2, 4, and 24 hours and on weeks 1, 3, 4, 5, 6, 7, 8, 10, and 14 weeks post-infusion. Blood samples for $\alpha 4$ saturation were collected at time 0, 2, and 24 hours and on 1, 3, 4, 5, 6, 7, 8, 10, and 14 weeks post-infusion. For immunogenicity analysis, blood samples were collected at baseline and at weeks 3, 4, 5, 6, 7, 8, 10, and 14.

Mean serum natalizumab concentrations were detected through Weeks 4, 8, and 10, respectively, in the 1.0, 3.0, and 6.0 mg/kg dose groups. Mean C_{max} values were dose-proportional. Mean clearance decreased with increasing dose indicating non-linear pharmacokinetics over the dose range of 1 to 6 mg/kg. The half-life was 129 hours and 143 hours for 3 and 6 mg/kg dose, respectively. The half-life in the 1.0 mg/kg dose group could not be estimated. The mean pharmacokinetic parameters are summarized in Table 5.1 (sponsor's Table). Mean natalizumab plasma concentrations vs time profile is shown in Figure 1 (sponsor's figure).

TABLE 5.1
Mean \pm SD pharmacokinetic parameters of antegren for all dose groups

	Antegren		
	1.0 mg/kg N = 9	3.0 mg/kg N = 9 ^a	6.0 mg/kg N = 10
C_{max} ($\mu\text{g/mL}$)	24.22 (5.02)	70.65 (18.56)	151.93 (31.68)
T_{max} (hr)	1.85 (1.03)	1.55 (1.12)	2.08 (0.79)
$t_{1/2}$ (hr) ^b	---	129.22 (46.79)	142.56 (32.22)
AUC_{0-24} ($\mu\text{g}\cdot\text{hr/mL}$)	1793.15 (896.50)	8726.20 (3492.92)	22746.19 (6293.46)
AUC_{∞} ($\mu\text{g}\cdot\text{hr/mL}$)	1992.04 (826.97)	8819.75 (3461.79)	22907.70 (6293.08)
Cl (mL per hr/kg)	0.6244 (0.3761)	0.3859 (0.1403)	0.2813 (0.0798)
V_{ss} (mL/kg)	72.00 (30.75)	67.33 (20.29)	73.05 (20.98)

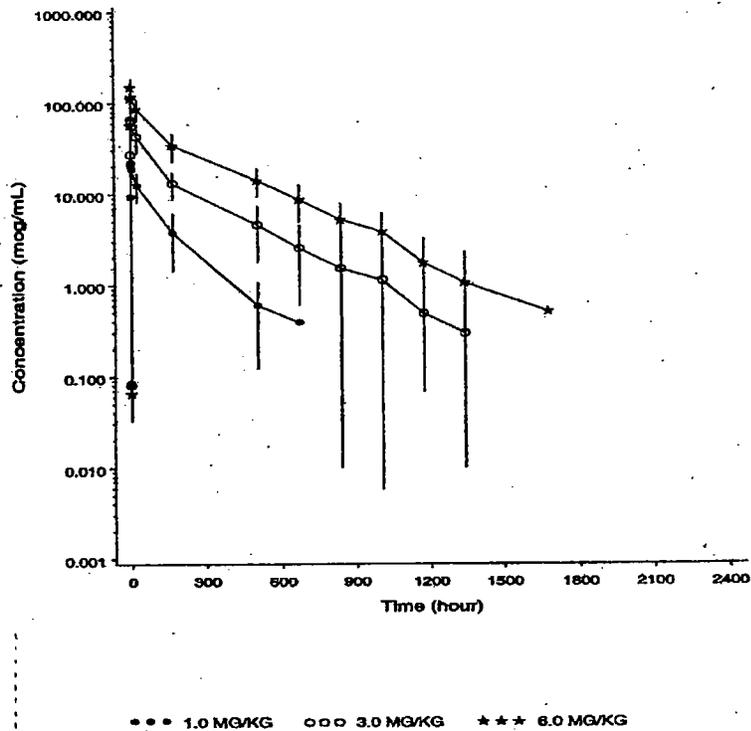
Reference: Section 15.3.2

^a Patient 20 was not included due to corticosteroid use on Day 6.

^b λ_z was estimable for zero patients in the 1.0 mg/kg dose group and six patients in the 3.0 mg/kg dose group, only these patients were used to calculate the mean and standard deviation. The mean half-life (138 hours) derived from patients with an estimable λ_z was used to calculate (AUC_{∞} and Cl) for patients in whom λ_z was not estimable.

FIGURE 5.1

Mean plasma concentrations \pm SD vs time plot for natalizumab



α 4-integrin saturation levels were measured for all evaluable subjects using a flow cytometric immunoassay. The results of this assay indicated that the extent and duration of α 4-integrin saturation appear to be related to dose. Maximum saturation levels were generally observed at the first post-infusion timepoint (2 hrs). Mean maximum percent saturation levels were 97%, 97%, and 105%, respectively, in the 1.0, 3.0, and 6.0 mg/kg dose groups. Saturation levels in individual subjects generally returned to baseline levels by Weeks 3-8 in the 1.0 mg/kg dose group, Weeks 7-14 in the 3.0 mg/kg dose group, and Weeks 8-14 in the 6.0 mg/kg dose group. Mean α 4-integrin saturation for all dose groups are shown in Table 5.2 (sponsor's Table) and figure 5.2 (sponsor's figure).

Additional analyses using  an antibody specific for α 4-integrin that does not compete with natalizumab, demonstrated that there was a reduction in the expression of α 4-integrin following administration of natalizumab. The change in receptor expression was attributed to natalizumab binding to the receptors on the cell surface and down-modulation as a result of capping and internalization. Receptor expression returned to baseline in a timeframe consistent with the clearance of natalizumab.

TABLE 5.2

Mean + SD percent α 4-integrin saturation levels for all dose groups

Percent Saturation	Antegren		
	1.0 mg/kg N = 9	3.0 mg/kg N = 10	6.0 mg/kg N = 10
Baseline	6.5 (5.0) n= 9	5.5 (5.0) n=10	5.3 (4.0) n= 9
Hour 24	84.4 (17.2) n= 9	94.9 (21.3) n=10	93.2 (26.7) n= 9
Week 1	59.5 (35.0) n= 9	82.6 (12.3) n= 9	87.1 (13.7) n=10
Week 3	27.7 (24.4) n= 9	63.3 (18.8) n= 9	75.7 (14.4) n= 8
Week 4	15.8 (10.9) n= 9	50.4 (18.9) n=10	68.7 (11.2) n=10
Week 6	4.1 (2.6) n= 9	21.1 (13.8) n= 8	48.4 (22.3) n= 9
Week 8	4.2 (2.0) n= 8	8.1 (8.2) n=10	22.4 (13.3) n=10
Week 10	3.5 (1.0) n= 7	4.3 (1.4) n= 9	10.4 (8.6) n= 9
Week 12	4.4 (1.5) n= 8	6.1 (4.4) n= 8	4.4 (2.3) n=10
Week 14	3.0 (1.3) n= 9	4.2 (3.7) n= 9	4.6 (2.8) n= 9

Immunogenicity was assessed in all subjects throughout the duration of the study. A total of 3 of 29 (10%) subjects were positive for anti-natalizumab antibodies at one or more timepoints over the course of the study. All three subjects were in the 1.0 mg/kg dose group. An additional three subjects (two in the 1.0 mg/kg group and one in the 3.0 mg/kg group) had positive anti-natalizumab antibody values, but their titers were below the cutoff value for the assay and they were not included in the analysis. No antibody-positive subjects were observed in the 6.0 mg/kg dose group. Antibodies were detected by Weeks 5-6 post-infusion. In Table 5.3, anti-antegren antibody response is shown (sponsor's Table).

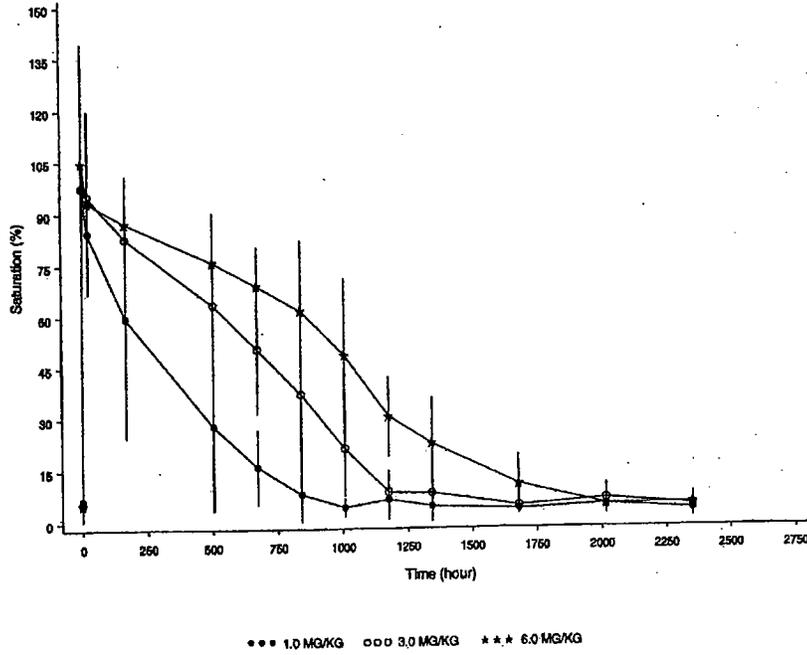
TABLE 5.3

Summary of anti-antegren antibody response for all dose groups

Patients With Presence of Antibodies	Antegren		
	1.0 mg/kg N = 9	3.0 mg/kg N = 10	6.0 mg/kg N = 10
Anti-Antibody (Total)			
Time to Response (Days)			
N	3	No Antibody response	No Antibody response
Mean	40		
Median	41		
Standard Deviation	3		
Range (Min-Max)			
Maximum Antibody Concentration			
N	3	No Antibody response	No Antibody response
Mean	12.9		
Median	15.2		
Standard Deviation	5.9		
Range (Min-Max)			

Figure 5.2

**MEAN (SD) PERCENT SATURATION ANTEGREN FOR ALL DOSE GROUPS
USING THE FLOW CYTOMETRY ASSAY**



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Study #6

Title: A double-blind, placebo-controlled, dose-determination, safety, tolerability and efficacy study of intravenous antegren in patients with multiple sclerosis during an acute exacerbation (MS202).

This was a double-blind, placebo-controlled, study in male and female patients with MS following a 45-minute IV infusion at 20 sites. The patients received doses of 1.0 and 3.0 mg/kg antegren. There were 180 patients (63 placebo, 57 received 1.0 mg/kg antegren, and 60 received 3.0 mg/kg antegren) in the study. Blood samples for measurement of Antegren concentrations were collected on Day 1 (predose), immediately following completion of study drug administration (45 minutes post-IV), 72 hours, weeks 1, 2, 3, 4, 6, 8, and 14. Blood samples for anti-natalizumab and anti-idiotypic of idiotypic antibody determinations were collected at screening, weeks 3, 4, 6, 8, and 14 (and at rescue and In-Study Exacerbation Visits if necessary). At a subset of sites, blood samples for $\alpha 4$ saturation assay were collected on Day 1 (predose), 72 hours, weeks 1, 2, 3, 4, 6, 8, and 14.

Sporadic antegren concentrations were measurable up to 14 weeks following both 1 and 3.0 mg/kg antegren doses but in most subjects serum natalizumab concentrations were almost undetectable after week 4 in 1.0 mg/kg group and after week 6 for the 3.0 mg/kg group. Mean C_{max} values were dose-proportional but AUC increased disproportionately (a four-fold increase with a three-fold increase in dose). Mean clearance decreased with the increasing dose. The half-life in the 1.0 mg/kg dose was 94 hours and 145 hours in the 3.0 mg/kg dose group. The mean pharmacokinetic parameters are summarized in Tables 6.1 and 6.2.

TABLE 6.1
Mean pharmacokinetic parameters of antegren in all patients with MS

Parameters	1 mg/kg	3 mg/kg
C_{max} (mcg/mL)	23.5 ± 10.1 (n = 56)	70 ± 27 (n = 60)
T_{max} (hrs)	2.5 ± 12.6 (n = 55)	5.8 ± 29.6 (n = 60)
$AUC_{(0-inf)}$ mcg*hr/mL	2209 ± 878 (n = 45)	9310 ± 2976 (n = 57)
CL (mL/hr/kg)	0.57 ± 0.46 (n = 45)	0.35 ± 0.11 (n = 57)
$T_{1/2}$ (hrs)	94 ± 30 (n = 45)	145 ± 49 (n = 57)
V_{ss} (mL/kg)	74 ± 91 (n = 45)	75 ± 30 (n = 57)

$\alpha 4$ -integrin saturation was determined on a subset of 18 subjects in the 1.0 mg/kg dose group and 15 subjects in the 3.0 mg/kg dose group using a flow cytometric immunoassay. The results indicated that the extent and duration of $\alpha 4$ -integrin saturation is dose related. Maximum saturation levels were generally observed at the first post-infusion time point (72 hours). Mean

maximum percent saturation levels were 78% and 91%, respectively, in the 1.0 and 3.0 mg/kg dose groups. Saturation levels in individual subjects generally returned to baseline levels by Weeks 6-8 in the 1.0 mg/kg dose group and 8-14 weeks in the 3.0 mg/kg dose group.

TABLE 6.2

Mean pharmacokinetic parameters of antegen in patients with MS by dose and gender

Parameters	1 mg/kg		3 mg/kg	
	Females (n = 36)	Males (n = 9)	Females (n = 45)	Males (n = 12)
C _{max} (mcg/mL)*	23.2 ± 10.8	24.5 ± 5.6	69.6 ± 25.9	71.8 ± 32.2
T _{max} (hrs)*	2.8 ± 13.9	0.76 ± 0.02	7.2 ± 33.4	0.77 ± 0.04
AUC _(0-inf) mcg*hr/mL	2258 ± 954	2014 ± 457	9209 ± 3032	9687 ± 2853
CL (mL/hr/kg)	0.58 ± 0.51	0.52 ± 0.14	0.36 ± 0.11	0.34 ± 0.10
T _{1/2} (hrs)	92 ± 31	101 ± 24	141 ± 47	156 ± 55
V _{ss} (mL/kg)	76 ± 101	68 ± 17	75 ± 32	71 ± 22

*The C_{max} and T_{max} values are in 46 and 45 females in 1.0 mg/kg dose group and for 47 females in 3.0 mg/kg dose group. The C_{max} and T_{max} values are in 10 males in 1.0 mg/kg dose group and for 13 males in 3.0 mg/kg dose group.

Immunogenicity was assessed in all subjects throughout the duration of the study. A total of 29 of 117 (25%) subjects were positive for anti-natalizumab antibodies at one or more time points over the course of the study. An additional nine subjects (five in the 1.0 mg/kg dose group and four in the 3.0 mg/kg dose group) had positive anti-natalizumab antibody values, but their titers were below the cutoff value for the assay and they were not included in the analysis. Antibody positive subjects were fairly evenly distributed between dose groups with 13 of 57 (23%) in the 1.0 mg/kg dose group and 16 of 60 (27%) in the 3.0 mg/kg dose group. Antibodies were detected by Week 6 post-infusion in the majority of subjects. Antibody concentrations ranged from —

Comment:

Although from Table 6.2 it appears that there is no effect of gender on the pharmacokinetics of antegen, it should be noted that the sample size is unbalanced. The number of female subjects in the study is almost 4 times than the male subjects. Therefore, the study may not reflect the true impact of gender on the pharmacokinetics of antegen.

Study #7

Title: An open-label safety and pharmacokinetic drug interaction study of intravenous antegren (natalizumab) injection and intramuscular interferon β -1a in subjects with multiple sclerosis (MS224).

This was an open-label, multicenter study in male and female patients with MS (18 to 65 years of age) to evaluate safety, pharmacokinetics, pharmacodynamics, and immunogenicity of interferon β -1a (INF β -1a) prior to and following a single IV infusion of natalizumab. A total of 38 patients were administered once weekly IM injections of INF β -1a (30 mcg) for three months prior to natalizumab treatment. Fifteen subjects received a 3.0 mg/kg IV infusion of natalizumab and 23 subjects received a 6.0 mg/kg infusion. Subjects received antegren 3.0 mg/kg or 6.0 mg/kg infusions at the study site on Week 0 (Day 0), and were instructed to self-administer INF β -1a at home at Weeks 6, 8, 9, 11, 12, and 13 using their standard technique. Table 7.1 (sponsor's Table) summarizes the dosing schedule.

TABLE 7.1

Dosing schedule of INF β -1a and antegren

Dose Group	Week -1	Week 0 (Day 0) ¹	Weeks 1-14
Antegren 3.0 mg/kg	INF β -1a (30 μ g IM injection)	INF β -1a (30 μ g IM injection) + Antegren (3.0 mg/kg IV infusion)	INF β -1a (30 μ g IM injection/week) ← →
Antegren 6.0 mg/kg	INF β -1a (30 μ g IM injection)	INF β -1a (30 μ g IM injection) + Antegren (6.0 mg/kg IV infusion)	INF β -1a (30 μ g IM injection/week) ← →

¹INF β -1a was administered one hour prior to the initiation of Antegren IV infusion.

Blood samples were collected for INF β -1a pharmacokinetic and pharmacodynamic (neopterin and P2-microglobulin) evaluations prior to INF β -1a injections at Weeks -1, 0, 1, 2, and at specified timepoints up to 96 hours postdose at Weeks -1, 0, and 2 visits. Additional blood samples were also drawn pre-dose during Weeks 3, 4, 5, 7, 10, 14. For the antegren pharmacokinetic evaluations, blood samples were collected prior to the INF β -1a injection and the antegren infusion at Week 0 (Day 0), and additional sampling was done at selected timepoints up to 24 hours after the start of the antegren infusion. For anti- INF β -1a antibodies, blood samples were collected at screening and at Weeks 5, 10, 14. Blood samples were also collected for anti-

antegren antibody tests prior to INFβ-1a injections and/or antegren infusion at Weeks 0, 2, 7, and 14. However, samples collected for antegren concentrations were also analyzed for anti-antegren antibodies at Weeks 0, 2, 3, 4, 5, 7, 10, and 14.

Mean serum natalizumab concentrations were detected in majority of subjects through week 7. Mean C_{max} values were 174.3 mcg/mL and were observed approximately 1 hour following the end of the infusion. The mean t_{max} was 175 hours. INFβ-1a pharmacokinetic parameter estimates for seven subjects in 6.0 mg/kg were compared across treatment periods at Weeks -1 (baseline), 0, and 2. Statistical comparisons of parameter estimates yielded p-values greater than 0.05 indicating that a single infusion of 6.0 mg/kg natalizumab does not significantly affect the pharmacokinetics of INFβ-1a. The pharmacokinetic parameters of natalizumab and INFβ-1a are summarized in Tables 7.2 and 7.3 (sponsor's Tables)

TABLE 7.2
Mean ± SD pharmacokinetic parameters of antegren

Parameter	Mean ± SD N=21
AUC _{0-inf} (μg•hr/mL)	31,516.2 (8767.71)*
AUC _{0-last} (μg•hr/mL)	30,429.2 (9371.6)
C _{max} (μg/mL)	174.2 (44.92)
T _{max} (hr)	2.4 (1.74)
k _{el} (1/hr)	0.0043 (0.00114)*
t _½ (hr)	175.29 (56.956)*

*n = 20

Pharmacodynamic parameters for INFβ-1a were calculated in the 6.0 mg/kg dose group only. No natalizumab-specific pharmacodynamic endpoints were evaluated in this study. β₂-microglobulin and neopterin exposures were compared across treatment periods at Weeks -1 (baseline), 0, and 2. Mean maximum β₂-microglobulin serum concentrations and AUC values ranged from 1.5 to 1.7 mcg/mL and 213 to 224 mcg•hr/mL, respectively (Table 7.4, sponsor's Table). Mean maximum neopterin serum concentrations and AUC values ranged from 3.6 to 3.9 ng/mL and 407 to 434 ng•hr/mL, respectively (Table 7.5, sponsor's Table). Statistical comparisons of parameter estimates yielded p-values greater than 0.05 indicating that a single infusion of 6.0 mg/kg natalizumab does not significantly affect the pharmacodynamics of INFβ-1a.

TABLE 7.3
Mean ± SD pharmacokinetic parameters of INFβ-1a

Parameter	Week -1 Mean (±SD) N = 21	Week 0 Mean (±SD) N = 21	Week 2 Mean (±SD) N = 20	P-Value ^a	P-Value ^b
AUC _τ (IU•hr/mL)	4093 (7193.47) N = 21	3978.6 (7021.41) N = 21	3481.5 (7197.72) N = 20	0.7705	0.0506
C _{max} (IU/mL)	26.9 (47.09) N = 21	26.1 (44.84) N = 21	24.1 (48.03) N = 20	0.4187	0.1438
T _{max} (hrs)	28.7 (53.31) N = 9	36.4 (53.83) N = 10	28.9 22.97 N = 6	0.9606	0.9177
C _{min} (IU/mL)	22.6 (40.28) N = 21	22.1 (39.24) N = 21	17.4 (39.51) N = 20	—	—
FLUCT (%)	19.4 (13.62) N = 7	17.7 (9.65) N = 7	21.4 (9.39) N = 5	—	—
C _{ss} (IU/mL)	24.4 (42.82) N = 21	23.7 (41.79) N = 21	20.7 (42.84) N = 20	—	—

^aTreatment comparison: Week 0 vs Week -1

^bTreatment comparison: Week 2 vs Week 0

Immunogenicity of natalizumab was assessed in all subjects throughout the duration of the study. A total of 8 of 38 (21 %) subjects were positive for anti-natalizumab antibodies at one or more timepoints over the course of the study. No anti-natalizumab antibodies were detected in the serum of any subject in the 3 mg/kg dose group. A total of 5 of 23 (22%) subjects in the 6 mg/kg dose group were positive for anti-natalizumab antibody titers in the screening assay. Antibody concentrations ranged from ————. A total of 8 of 23 (35%) subjects in the 6 mg/kg dose group were positive for anti-natalizumab antibody titers in the blocking assay. Antibody concentrations ranged from ————. Antibodies were detected from Week 5 onwards with most subjects becoming antibody positive at Week 10 post-infusion.

TABLE 7.5
Mean ± SD serum microglobulin parameters of INFβ-1a

Parameter	Week -1 Mean (±SD) N = 21	Week 0 Mean (±SD) N = 21	Week 2 Mean (±SD) N = 20	P-Value ^a	P-Value ^b
C _{max} (µg/mL)	1.7 (0.38) N = 21	1.6 (0.51) N = 21	1.5 (0.49) N = 20	0.1821	0.0728
AUC _{0-last} (µg•hr/mL)	224.1 (60.06) N = 21	221.7 (71.26) N = 21	212.6 (66.44) N = 20	0.4413	0.1489
T _{max} (hrs)	38.6 (26.3) N = 21	45.8 (24.04) N = 21	48.3 (32.94) N = 20	0.4425	0.2961

^aTreatment comparison Week 0 vs Week -1

^bTreatment comparison Week 2 vs Week -1

TABLE 7.6
Mean ± SD serum neopterin parameters of INFβ-1a

Parameter	Week -1 Mean (± SD) N = 21	Week 0 Mean (± SD) N = 21	Week 2 Mean (± SD) N = 20	P-Value ^a	P-Value ^b
C _{max} (ng/mL)	3.9 (1.57) N = 21	3.6 (1.46) N = 21	3.6 (1.3) N = 20	0.1522	0.1843
AUC _{0-last} (ng•hr/mL)	434.2 (137.95) N = 21	415.8 (124.95) N = 21	406.5 (115.64) N = 20	0.2761	0.0893
T _{max} (hrs)	44.9 (15.56) N = 21	38.9 (11.96) N = 21	50.6 (31.65) N = 20	0.3687	0.308

^aTreatment comparison Week 0 vs Week -1

^bTreatment comparison Week 2 vs Week -1

Immunogenicity of INFβ-1a was assessed in all subjects throughout the duration of the study. Anti- INFβ-1a antibodies were detected in a total of 4 of 38 (11 %) subjects, two in each dose

group. Antibody concentrations ranged from ~~_____~~ Antibodies were detected from Weeks 5-14 in one subject in the 3 mg/kg natalizumab dose group. All other subjects were antibody-positive at a single timepoint.

Comment: This study is not adequate to conclude that natalizumab has no effect on the PK of INF β -1a. A single dose of natalizumab may not have any impact on the PK of INF β -1a but under steady state condition of natalizumab this conclusion may not hold true.

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Study #8

Title: A preliminary study of the effect of intravenous antegren on brain lesion activity detected by magnetic resonance imaging in patients with multiple sclerosis (MS201).

This was a randomized, double-blind, placebo-controlled, parallel-group repeat-infusion study in male and female patients (24.6 to 52.1 years of age) with MS (relapsing-remitting or secondary-progressive type). A total of 37 subjects (12 males and 25 females) received natalizumab and 35 (14 males and 21 females) subjects received placebo. A 3.0 mg/kg dose of natalizumab was given as IV infusion over 30 to 45 minutes at week 0 and a second dose was given at week 4. The patients were followed up for 24 weeks. Two pharmacokinetic sampling schemes were employed for this study. Seventeen subjects underwent intensive sampling. Blood samples in this group were drawn at 0, 30, 35, 40, 50, and 60 minutes, 1.5, 3, 6, 12, and 24 hours from the start of infusion. Additional samples were collected at weeks 1, 2, and 4 after the first dose. Following the second dose, blood samples were collected at baseline (week 4) and 30, 35, 40, 50, and 60 minutes, 1.5, 3, 6, 12, and 24 hours from the start of second infusion. Additional samples were collected at weeks 6, 8, 12, 16, 20, and 24. Twenty subjects were enrolled in a sparse sampling cohort in which samples were taken at predose, 1 hour, weeks 1, 2, and 4 following the first dose and similarly after the second dose of antegren with additional samplings at 12, 16, 20, and 24. Antegren concentrations were measured by ELISA and the lower limit of quantitation for antegren was ————. Pharmacokinetic parameters were estimated by model independent method.

Mean serum natalizumab concentrations were detected through Week 8 in the majority of subjects. A comparison of pharmacokinetic parameters in the intensive sampling group revealed similar profiles following each infusion. Mean C_{max} values were 64.1 mcg/mL and 60.2 mcg/mL following infusion 1 and infusion 2, respectively. Mean CL values were 0.45 and 0.39 mL/hr/kg following each infusion. The $t_{1/2}$ values were 143 and 139 hours, respectively, for infusions 1 and 2. Table 8.1 (sponsor's Table) summarizes the pharmacokinetic parameters of antegren in extensive sampling group. Plasma concentrations vs time profile is shown in Figure 1 (Sponsor's figure)

Mean AUC values in the sparse sampling group were approximately 50% greater than those calculated for the intensive sampling group. The resulting CL values were lower by the same factor. The sparse sampling scheme using a model independent approach did not appear to be suitable for the estimation of pharmacokinetic parameters.

Immunogenicity was assessed in all subjects throughout the duration of the study. A total of 7 of 37 (19%) subjects were positive for anti-natalizumab antibodies at one or more time points over the course of the study. Four subjects had antibodies detected at three or more consecutive

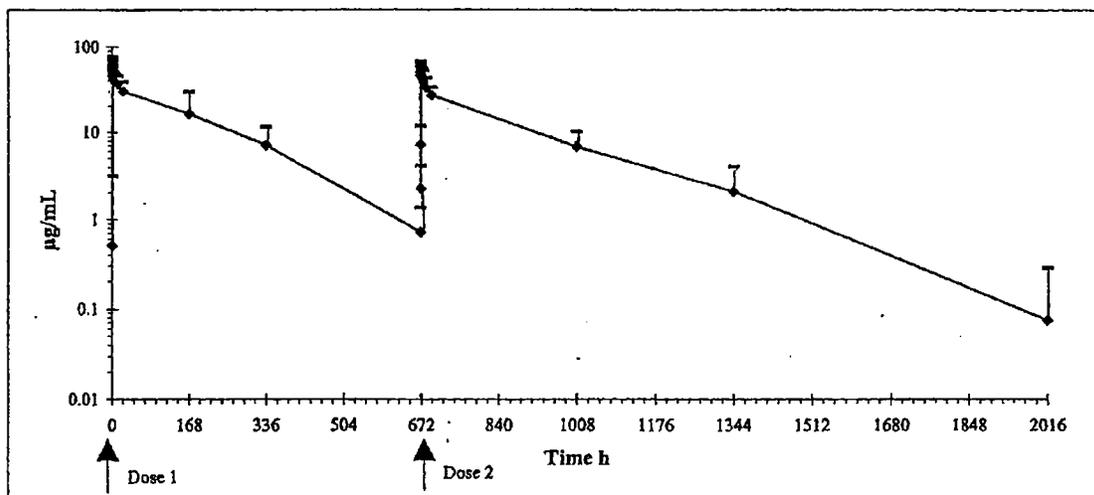
time points, while the remaining three subjects were positive at a single time point. Antibody concentrations ranged from 1. ——— in the screening assay and \leftarrow in the blocking assay. Antibodies were detected from week 4 onwards with most subjects becoming antibody positive at week 12 post-dose.

TABLE 8.1
Mean (SD) Pharmacokinetic Parameters of Antegren in MS Patients
Following 2 doses of 3 mg/kg Dose (N=17)

Parameters	Dose 1	Dose 2
C_{max} ($\mu\text{g/mL}$)	64.1 (18.8)	60.2 (17.5)
T_{max} (h)	1.6 (3.0)	0.8 (0.3)
$T_{1/2}$ (h)	143 (50)	139 (42)
AUC (0-t) ($\mu\text{g.h/mL}$)	6848 (2631)	9496 (5490)
AUC (0- ∞) ($\mu\text{g.h/mL}$)	7895 (3850)	9723 (5644)
V_z (mL/kg)	88 (35)	72 (29)
Cl (mL/h/kg)	0.45 (0.17)	0.39 (17)

Figure 1

Mean (+SD) Antegren serum concentrations after two doses at 3.0 mg/kg i.v. (N=37)
in multiple sclerosis patients
Protocol No. AN100226-201



Study #9

Title: A randomized, multicenter, double-blind, placebo-controlled, safety, tolerability, and dose evaluation study of intravenous antegren (natalizumab) at two dose levels using magnetic resonance imaging in subjects with multiple sclerosis pharmacokinetic study of various doses of in patients with multiple sclerosis (MS231).

This was a randomized multicenter study (26 principal investigators, 15 in the USA, 4 in Canada, 7 in the UK) in male and female patients (22 to 66 years of age) with MS (relapsing-remitting or secondary-progressive type). There were 213 subjects (61 men and 152 women) in the study of which 71 received placebo, 68 received 3 mg/kg natalizumab, and 74 received 6 mg/kg natalizumab. Natalizumab was administered monthly for six months. A subset of 10 patients was selected for pharmacokinetic study in each dose group. Pharmacokinetic parameters were calculated after the first (week 0) and the fifth dose (week 20). Blood samples were collected at time 0, 1, 2, 24 hour, and weeks 1, 2, 3, and months 1, 2, 3, 4, and 5. After the last dose additional blood samples were taken monthly till month 10. Antegren concentrations were measured by ELISA and the lower limit of quantitation for antegren was _____ Pharmacokinetic parameters were estimated by model independent method. α 4-integrin saturation levels were measured for all evaluable subjects using a flow cytometric immunoassay. Blood samples were collected for anti-natalizumab antibody levels prior to each infusion at months 1, 2, 3, 4, and 5 and at months 6, 7, 8, 9, 10, and 12. Antibody concentrations were measured by ELISA and the lower limit of quantitation was _____

Mean serum natalizumab concentrations were detected eight weeks following the last infusion in the 3.0 mg/kg dose group and 12 weeks following the last infusion in the 6.0 mg/kg dose group. Mean C_{max} values were 73.7 and 149.9 mcg/mL following the Week 0 infusion and 64.2 and 142.4 mcg/mL following the Week 20 infusion in the 3.0 mg/kg and 6.0 mg/kg dose groups, respectively. Mean t_{1/2} was approximately 200 hours in the 3.0 mg/kg dose group and 275 hours in the 6.0 mg/kg dose group. Preinfusion natalizumab serum concentrations at Weeks 4, 8, 12, and 16 were used to determine the time to reach steady state. Visual inspection of the trough concentrations suggests that steady state was reached by Week 8. Average trough concentrations were 3.1 mcg/mL and 13.0 mcg/mL in the 3.0 and 6.0 mg/kg dose groups, respectively. The pk parameters of natalizumab are summarized in Table 9.1 (sponsor's Table). Natalizumab plasma concentrations vs time data are shown in Figure 9.1 (sponsor's Figure).

α 4-integrin saturation levels were assessed in the same cohort of subjects and at the same timepoints as the pharmacokinetic samples. Maximum saturation levels in the natalizumab-treated subjects were generally observed at the first post-infusion timepoint (1 hour post-infusion). Mean maximum percent saturations were 99% and 101 % following the Week

0 infusion and 92% and 99% following the Week 20 infusion in the 3.0 mg/kg and 6.0 mg/kg dose groups, respectively. Pre-infusion saturation levels at Weeks 4, 8, 12, and 16 indicated that the mean α 4-integrin saturation remained above 70% in the 3.0 mg/kg dose group and above 83% in the 6.0 mg/kg dose group over the course of the study.

Absolute lymphocyte counts were also assessed as a measure of natalizumab pharmacodynamics. Lymphocyte counts were elevated from a mean pre-infusion baseline of approximately 1.8×10^3 cells/microliter to approximately 3.0×10^3 cells/microliter throughout the treatment course in both dose groups. Counts returned to preinfusion baseline levels by Week 32 (Month 8) in the 3.0 mg/kg group and by Week 36 (Month 9) in the 6.0 mg/kg dose group.

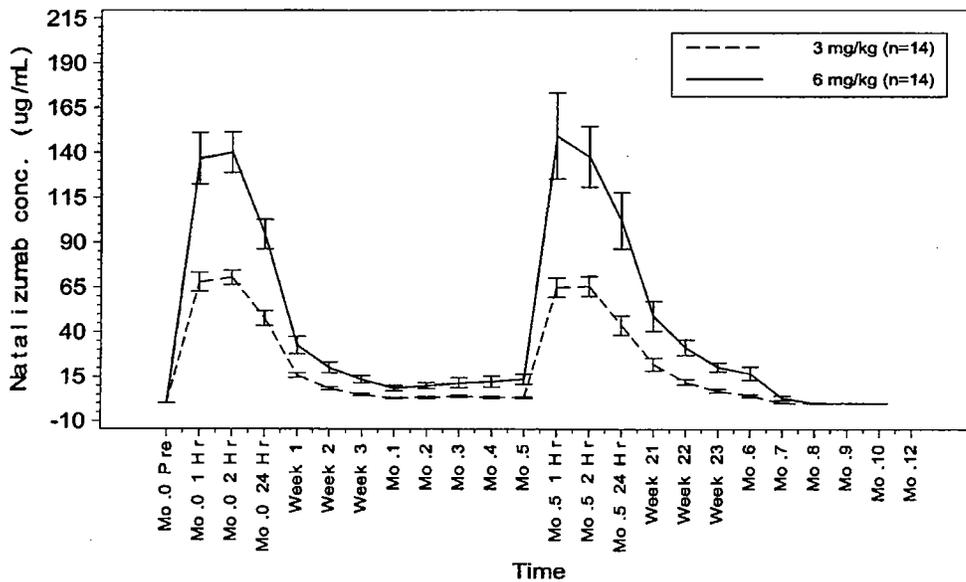
TABLE 9.1
Mean (SD) PK parameters of natalizumab in patients with multiple sclerosis

	3 mg/kg natalizumab	6 mg/kg natalizumab
Single dose PK		
C _{max} (ug/mL)	73.65 (17.293)	149.85 (18.219)
T _{max} (h)	1.81 (0.580)	1.67 (0.525)
AUC last (h.ug/mL)	9870 (2502.6)	20452 (3833.8)
AUC inf (h.ug/mL)	10775 (2684.9)	24603 (4914.7)
Kel (h ⁻¹)	0.00401 (0.002158)	0.00277 (0.001153)
t _{1/2} (h)	203 (69.4)	289 (111.5)
Multiple-dose PK		
C _{max} (ug/mL)	64.17 (25.386)	142.37 (54.949)
T _{max} (h)	1.35 (0.621)	1.37 (0.693)
AUC last (h.ug/mL)	11388 (6251.5)	31721 (13113.3)
AUC tau (h.ug/mL)	10588 (5463.1)	25442 (10135.0)
Kel (h ⁻¹)	0.00362 (0.000764)	0.00271 (0.000445)
t _{1/2} (h)	202 (56.6)	262 (44.3)
C _{avg} (ug/mL)	15.756 (8.1297)	37.861 (15.0818)
C _{min} (ug/mL)	3.11 (2.085)	13.02 (4.301)
% fluctuation	2128 (1276.5)	1054 (558.6)

Immunogenicity was assessed in all subjects throughout the course of the study. A total of 16 of 142 (11 %) subjects were positive for anti-natalizumab antibodies at one or more timepoints over the course of the study. A total of 8 of 68 (12%) subjects in the 3.0 mg/kg dose group were positive for anti-natalizumab antibodies in the screening assay. Antibody concentrations ranged from _____ . A total of 7 of 68 (10%) subjects in the 3.0 mg/kg dose group were positive for anti-natalizumab antibody titers in the blocking assay. Antibody concentrations ranged from _____ . A total of 8 of 74 (11%) subjects in the 6.0 mg/kg dose group were positive for anti-natalizumab antibody titers in the screening assay. Antibody

concentrations ranged from _____ .. A total of 4 of 74 (5%) subjects in the 6.0 mg/kg dose group were positive for anti-natalizumab antibody titers in the blocking assay. Antibody concentrations ranged from _____ Antibodies were detected from Week 4 onwards with most subjects becoming antibody positive at Week 8 post-infusion.

Figure 9.1
Mean (SD) serum natalizumab concentrations following 3 mg/kg and 6 mg/kg natalizumab administration to patients with MS



The impact of the presence of anti-natalizumab antibodies on the pharmacokinetics and pharmacodynamics of natalizumab could not be definitively determined in this study. One subject (#16-1110, 3.0 mg/kg dose group) was positive for anti-natalizumab antibodies. In this subject, anti-natalizumab antibodies were detected in both the screening and blocking assays during Weeks 12-36 of the study. A comparison of the pharmacokinetic profiles at Weeks 0 and 20 showed that these antibodies were correlated with a reduction in natalizumab serum concentrations. Cmax values were _____ following the Week 0 infusion and _____ following the Week 20 infusion. Corresponding AUClast values were 9916 mcg \cdot hr/mL and 324 mcg \cdot hr/mL, respectively. In addition, pre-infusion natalizumab serum concentrations at Weeks 4, 8, 12, and 16 were all below the limit of quantitation _____. α -integrin receptor saturation was also reduced in this subject.

Study #10

Title: A randomized, double-blind, placebo-controlled, parallel group, multicenter study to determine safety and efficacy of natalizumab in subjects with relapsing-remitting multiple sclerosis (C-1801).

This is ongoing study which will be conducted for 2 years. The results of 1 year study have been presented in this submission. The primary objective of 1 year study was to determine whether natalizumab, when compared with placebo, was effective in reducing the rate of clinical relapses. This was a multicenter, randomized, double-blind, placebo-controlled, parallel-group study. Subjects were randomized in a 2: 1 ratio to 1 of 2 treatment groups: placebo (approximately 315 subjects) or 300 mg natalizumab (approximately 627 subjects). The patient population included male and female subjects aged between 18 to 50 years. Subjects received study drug by intravenous (IV) infusion once every 4 weeks for up to 116 weeks and are to be followed-up for an additional 12 weeks. For 1-year study clinical pharmacology data were analyzed for 48 weeks.

For the PK study, there were 31 subjects of which 20 were in active treatment group. Blood samples were taken at week 0 (dose 1) and week 20 (dose 6). Samples were taken at pre-infusion, 2, and 24 hours, and weeks 1, 2, 3, and 4 weeks following first and the sixth dose. The PK parameters are summarized in Table 10.1. The PK parameters after the first dose and the last dose appear to be similar. The C_{max} , clearance, and half-life essentially remain unchanged between the first and the sixth dose. Mean trough levels at weeks 12, 24, 36, and 48 were 15.3, 21.6, 24.6, and 23.0 mcg/mL, respectively.

TABLE 10.1
Mean pharmacokinetic parameters of natalizumab in patients with MS

Parameters	Dose 1 (n = 20)	Dose 6 (n = 16)
C_{max} (mcg/mL)	84.8 ± 22.3	94.7 ± 34.2
T_{max} (hrs)	11.2 ± 37.7	5.2 ± 8.2
C_{avg} (mcg/mL)	-	29.5 ± 8.7
$AUC_{(0-inf)}$ mcg*hr/mL	22823 ± 17231	24700 ± 9480
CL (mL/hr)	17.6 ± 9.9	16.6 ± 5.4
$T_{1/2}$ (hrs)	250 ± 105	265 ± 98
V _{ss} (mL)	5394 ± 2064	5659 ± 1795

α 4-integrin saturation was determined using a flow cytometric immunoassay. Mean saturation levels in the placebo subjects remained below 4% throughout the sampling period.

Receptor saturation levels correlated with natalizumab serum concentrations. Maximum saturation levels were observed at 1 hour post-infusion and decreased during the period prior to the next infusion. The mean maximum percent saturation was 99% following the Week 0 infusion and remained in excess of 86% 4 weeks post-infusion. The corresponding mean trough natalizumab concentration was approximately 10 mcg/mL. Mean saturation levels prior to the Week 20 infusion remained in excess of 70%. The mean trough natalizumab concentration at Week 20 associated with this level of α 4-integrin saturation was approximately 20 mcg/mL. Following the Week 20 infusion, mean maximum percent saturation was 93%. The mean α 4-integrin saturation levels remained in excess of 77% 4 weeks post-infusion. The corresponding mean trough natalizumab concentration was approximately 14 mcg/mL.

Absolute lymphocyte counts and WBC counts were also assessed as a measure of natalizumab pharmacodynamics. Samples were taken prior to dosing and every 12 weeks throughout the treatment period. Mean counts were identical in the two groups at baseline. Lymphocyte and WBC counts in the placebo group remained consistent with pre-infusion baseline levels throughout the treatment period. Lymphocyte counts in the natalizumab-treated subjects were elevated from a mean pre-infusion baseline of 2.1×10^9 cells/L to approximately 3.6×10^9 cells/L throughout the treatment period. Although elevated, the mean lymphocyte counts remained within the normal range (0.91 - 4.28×10^9 cells/L) throughout the observation period. WBC counts in the natalizumab-treated subjects were elevated from a mean pre-infusion baseline of 7.2×10^9 cells/L to approximately 9.3×10^9 cells/L throughout the treatment period. WBC counts also remained within the normal range (3.8 - 10.7×10^9 cells/L).

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Study #11

Title: A randomized, double-blind, placebo-controlled, parallel group, multicenter study to determine the safety and efficacy of natalizumab, when added to AVONEX (interferon beta-1a), in subjects with relapsing-remitting multiple sclerosis (C-1802).

This is ongoing study which will be conducted over the span of 2 years. The results of 1 year study have been presented in this submission. The primary objective of 1 year study was to determine whether adding natalizumab to the standard regimen of AVONEX when compared with placebo, was effective in reducing the rate of clinical relapses. This was a multicenter, randomized, double-blind, placebo-controlled, parallel-group study. Subjects were randomized in a 1: 1 ratio to 1 of 2 treatment groups: placebo (proximately 600 subjects each). The patients received a fixed-infusion of 300 mg natalizumab monthly for two years. All subjects in the study continue to self-administer 30 mcg AVONEX by 1M injection once weekly throughout the study. A total of 589 subjects received natalizumab plus AVONEX and 582 subjects were randomized to receive placebo plus AVONEX. Clinical pharmacology data were analyzed through Week 36 for inclusion in the study C-1802.

Natalizumab pharmacokinetic parameters were calculated for a subset of 16 subjects in the intensive sampling cohort following the first (Week 0) and sixth (Week 20) infusions. Blood samples were taken at pre-infusion, immediately post-infusion, 2 and 24, and weeks 1, 2, 3, 4 hours post infusion (week 4 before the next infusion). Pharmacokinetic parameter estimates following the Week 20 infusion were consistent with those following the Week 0 dosing, although some accumulation was observed. The pharmacokinetic parameters of natalizumab are summarized in Table 11.1. Mean serum natalizumab concentrations following dose 1 and dose 6 are shown in Figure 11.1 (Sponsor's figure).

TABLE 11.1
Mean pharmacokinetic parameters of natalizumab in patients with MS

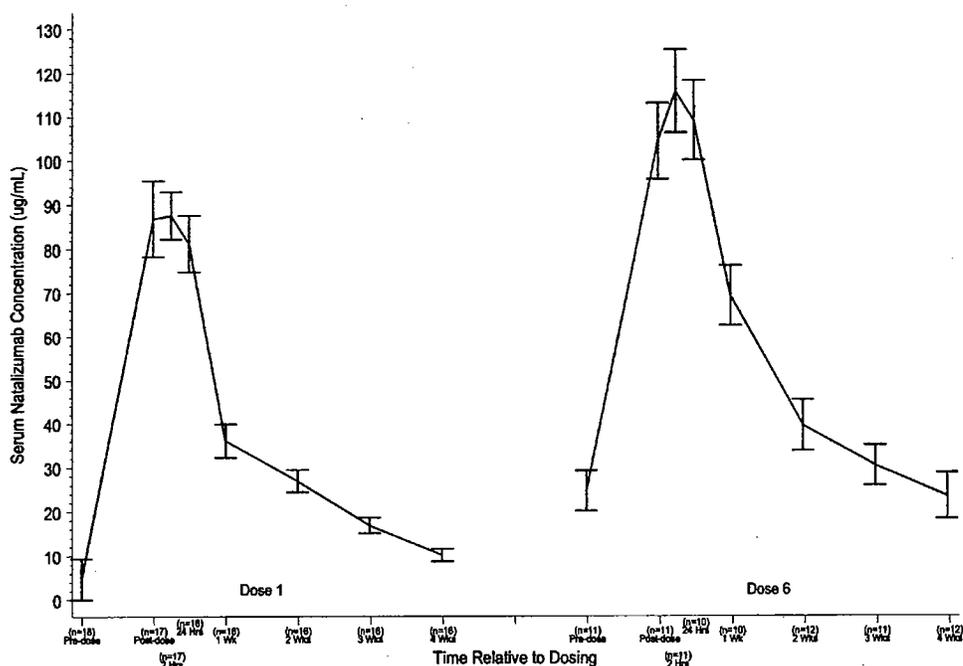
Parameters	Dose 1 (n = 16)	Dose 6 (n = 10)
C _{max} (mcg/mL)	104 ± 21	125 ± 30
T _{max} (hrs)	2.9 ± 5.7	6.7 ± 9.5
C _{avg} (mcg/mL)	-	54.9 ± 15.1
AUC _(0-inf) mcg*hr/mL	25808 ± 9192	54563 ± 22228
CL (mL/hr)	13.2 ± 5.1	8.6 ± 2.1
T _{1/2} (hrs)	240 ± 76	407 ± 208
V _{ss} (mL)	4151 ± 1547	4517 ± 1935

Pre-infusion trough natalizumab samples were taken in all subjects at 12 week intervals throughout the dosing period. Mean trough levels at Weeks 12, 24, and 36 were 17.7, 23.2, and 25.9 mcg/mL, respectively. No AVONEX pharmacokinetic samples were taken in this study.

The results of the study indicate that after the sixth dose, the clearance of natalizumab decreased by 34% and the half-life increased by 70% as compared to the first dose. This change in clearance and half-life may be due to the presence of AVONEX.

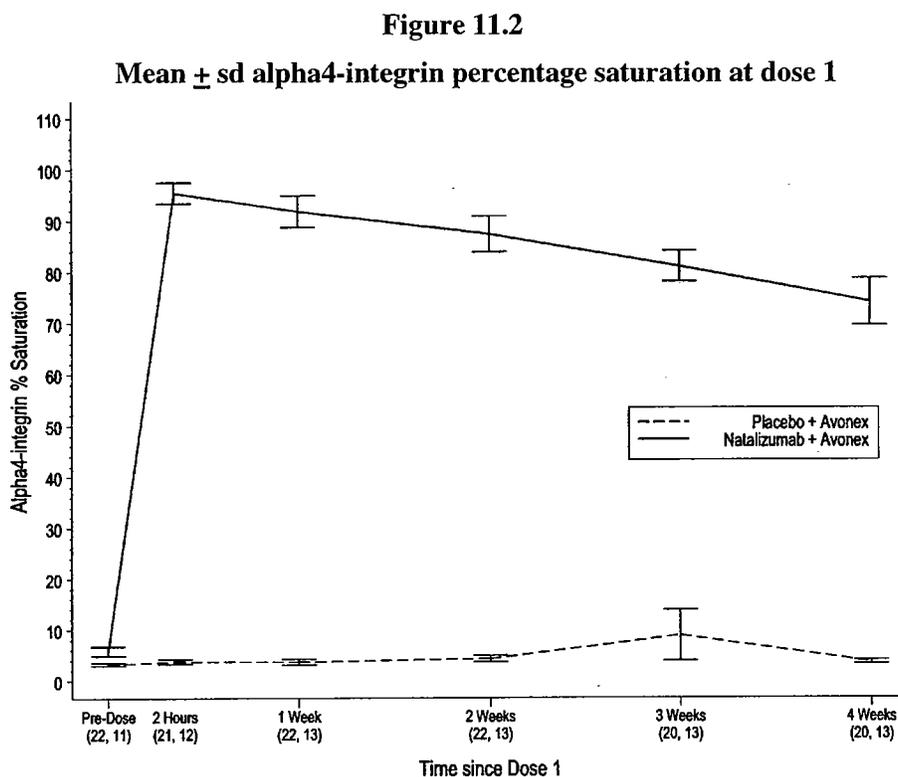
Figure 11.1

Mean \pm sd serum natalizumab concentrations at dose 1 and dose 6



α 4-integrin saturation levels were assessed in the same intensive sampling cohort at the same timepoints as the pharmacokinetic samples. α 4-integrin saturation was determined using a flow cytometric immunoassay. Mean saturation levels in the placebo subjects remained below 8% throughout the sampling period. The maximum saturation levels were observed at the first post-infusion timepoint (1 hour post-infusion) and decreased during the period prior to the next infusion. Mean maximum percent saturation was 95% following the Week 0 infusion and remained in excess of 70% 4 weeks post-infusion. The corresponding mean trough natalizumab concentration was approximately 10 mcg/mL. Mean saturation levels prior to the Week 20 infusion remained in excess of 70%. The mean trough natalizumab concentration at Week 20 associated with this level of α 4-integrin saturation was approximately 24 mcg/mL. Following the

Week 20 infusion, mean maximum percent saturation was 92%. The mean $\alpha 4$ -integrin saturation levels remained in excess of 72% 4 weeks post-infusion (Figures 11.2 & 11.3, Sponsor's figure). The corresponding mean trough natalizumab concentration was approximately 24 mcg/mL.



Absolute lymphocyte counts and WBC counts were also assessed as a measure of natalizumab pharmacodynamics. Samples were taken prior to dosing and every 12 weeks throughout the treatment period. Mean counts were identical in the two groups at baseline. Lymphocyte and WBC counts in the placebo plus AVONEX group remained consistent with pre-infusion baseline levels throughout the treatment period.

Lymphocyte counts in the natalizumab-treated subjects were elevated from a mean pre-infusion baseline of 1.9×10^9 cells/L to approximately 3.2×10^9 cells/L throughout the treatment period (Figure 11.4, sponsor's figure). Although elevated, the mean lymphocyte counts remained within the normal range (0.91 - 4.28×10^9 /L) throughout the observation period. WBC counts in the natalizumab-treated subjects were elevated from a mean pre-infusion baseline of 6.6×10^9 cells/L to approximately 8.2×10^9 cells/L throughout the treatment period. WBC counts also remained within the normal range (3.8 - 10.7×10^9 /L).

Figure 11.3
Mean \pm sd alpha4-integrin percentage saturation at dose 6

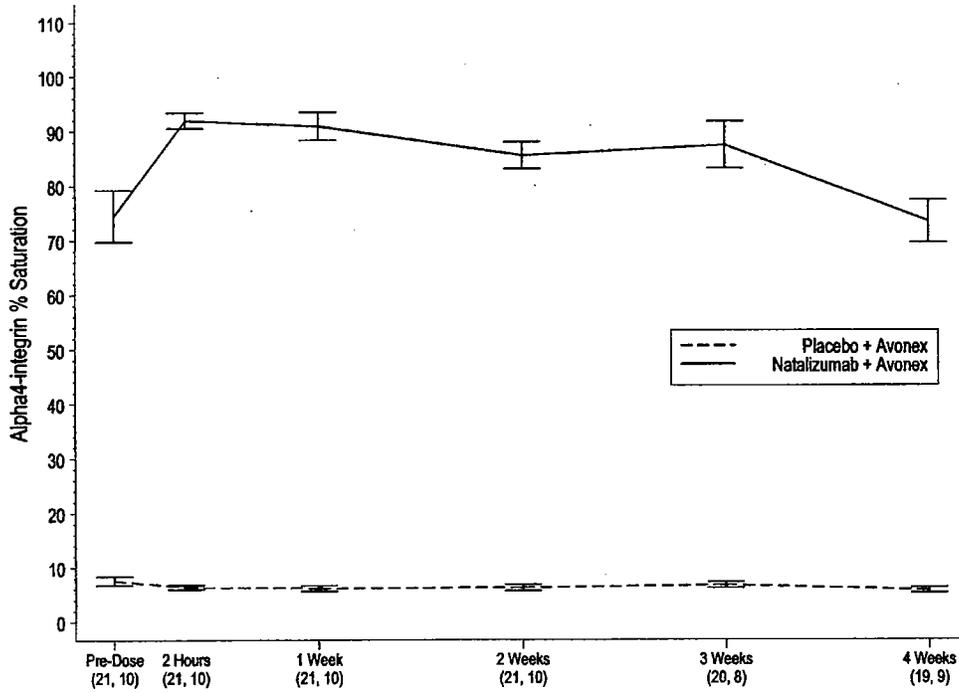
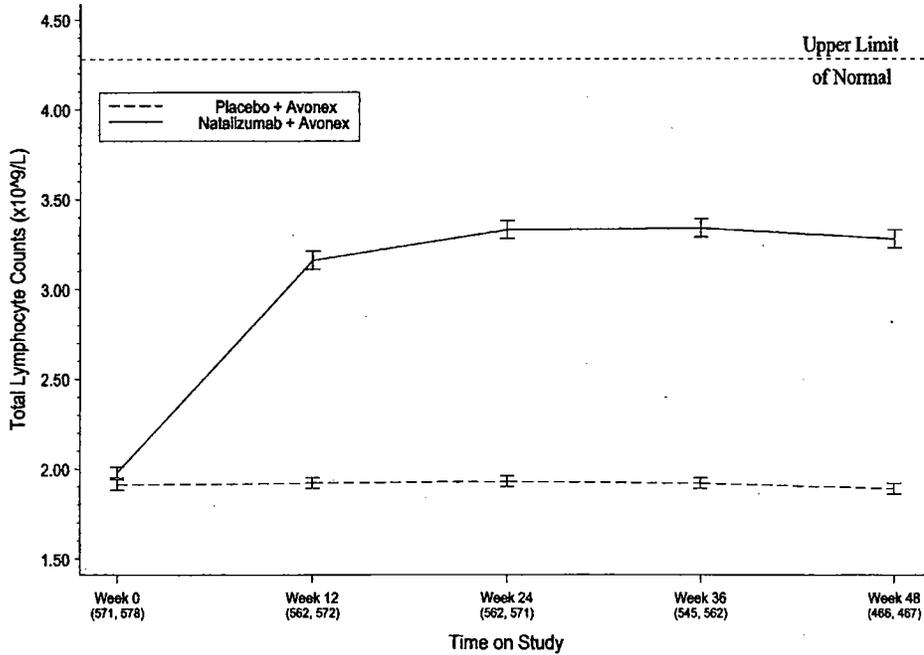


Figure 11.4
Mean \pm sd total lymphocyte counts by visit



Serum neopterin values were evaluated prior to and following IM injections of AVONEX on Week 0 and 20. Similar levels of neopterin were observed in both placebo plus AVONEX subjects and subjects administered both AVONEX and natalizumab. Induction ratios, which were calculated by dividing the neopterin levels 48 hours post AVONEX dosing by the pre-infusion levels were consistent on Week 0 and 20. Week 0 induction ratios for the placebo plus AVONEX and natalizumab-treated subjects were 1.98 and 2.33, respectively. Week 20 induction ratios for the placebo plus AVONEX and natalizumab-treated subjects were 1.89 and 2.61, respectively. These small differences between treatment groups are not thought to be clinically significant.

Natalizumab immunogenicity was assessed in all subjects during the course of the study. Samples were taken prior to dosing and every 12 weeks throughout the treatment period. A total of 68 of 582 (12%) natalizumab-treated subjects had detectable antibody concentrations at one or more timepoints during the study. Of subjects who became antibody positive, 65 of 68 (96%) had detectable antibodies by Week 13, with the remaining subjects becoming antibody positive by Week 24. No subject developed antibodies to natalizumab after Week 24.

Comment: It appears that AVONEX alters the pharmacokinetics of natalizumab. In the previous study (study #10) when patients were not given AVONEX, the pharmacokinetics of natalizumab remained unchanged after the first and the sixth dose. Therefore, the change in PK parameters in this study is not simply due to the drug accumulation rather it is effect of AVONEX on natalizumab pharmacokinetics.

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Study #12

Title: A double-blind, randomized, placebo-controlled, parallel group, safety study of natalizumab in combination with glatiramer acetate (GA) in subjects with relapsing-remitting multiple sclerosis (C-1803).

This is an ongoing randomized, double-blind, placebo-controlled, parallel-group repeat-infusion study in male and female relapsing-remitting MS patients to evaluate the safety and efficacy of natalizumab following IV infusion in combination with GA. A 300 mg fixed-infusion of natalizumab will be administered monthly for six months. Subjects were on a maintenance dose of GA prior to the administration of natalizumab and subjects continue to self-administer 20 mg GA by SC injection daily throughout the study. A total of 55 subjects received natalizumab plus GA and 55 subjects were randomized to receive placebo plus GA. Clinical pharmacology data were analyzed through Week 12 for inclusion in this submission.

Natalizumab pharmacokinetic parameters were calculated for all subjects following the first (Week 0) infusion. The mean C_{max} was 118 mcg/mL. The mean CL was 0.15 mL/hr/kg and the mean half-life was 243 hours. Pre-infusion trough natalizumab samples were taken in all subjects at Week 12. The mean trough level was 18.9 mcg/mL. No pharmacokinetic parameters were estimated for GA.

Comment: The PK parameters were calculated only after the first dose. In order to assess the impact of GA on the PK of natalizumab the sponsor should submit the PK data following the administration of natalizumab on week 12. At this time from this study there is no evidence that GA has no impact on the PK of natalizumab (sponsor's conclusion)

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