Methods

Doses: natalizumab from either manufacturing process, 3.0 mg/kg

Species/strain: *Macaca fasicularis* (cynomolgus monkey); purpose-bred [redacted], and experimentally naïve to treatment

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

Route, formulation, volume, and infusion rate: intravenous infusion; natalizumab formulated in 10 mM sodium phosphate-buffered saline, plus 0.02% polysorbate 80, pH 6.2; 10 ml/kg infused; infusion rate 20 ml/kg/h

Sampling times for pharmacokinetics: Blood samples (approximately 1 ml per time point) for measurement of natalizumab serum concentrations were collected into glass tubes with no added anticoagulant from all monkeys prior to dosing, and obtained from treated animals on this study before dosing (0 hr), at 10 and 20 min during the infusion period, at the end of the infusion (30 min), and at 35, 45, 60, 120, and 390 min and 24 hours after start of the infusion, and on Study Days 3, 4, 5, 6, 7, 10, 14, and 17 after the initial day of dosing. Serum samples for evaluation of anti-natalizumab antibody titers were obtained from all animals prior to dosing on Study Day 1, then on Study Days 7 and 17 only.

Age: young, adult female animals, approximately 2.8 – 5.0 years old

Weight (nonrodents only): 2.5 – 3.4 kg

Unique study design or methodology (if any):

[redacted]

Comment: The single-dose toxicology data obtained from this study were not submitted to the toxicology section of the [redacted] BLA, and were not included in the toxicology review. The toxicology findings are included in the results for this study, below.

Observation Times and Results (includes pharmacokinetic, immunogenicity, and toxicology results)

Mortality, Clinical Observations, and Clinical Pathology: Cageside observations were performed on all monkeys twice daily, for general health and behavioral or other clinical signs of overt toxicity, beginning Study Days -7/-8 and continuing until Study Day 17. Body weights were determined following an overnight fast once at Study Day -2 prior to dosing, then weekly thereafter until study termination on d 17. Food consumption was qualitatively assessed daily for each animal as part of the morning cageside observations, the number of biscuits remaining from the previous feeding evaluated, and a notation was made when less than approximately half of the rations had been consumed. After the last sample collection at study termination, all animals were returned to the colony without necropsy.

All monkeys survived for the entire study duration, and both natalizumab BG00002B (commercial process material) and BG00002A (Biogen "resupply" material) were well-tolerated after a single, i/v infusion. There were no overt toxicities noted after treatment with either preparation of natalizumab; several animals had episodes of soft or liquid stool which were not considered treatment-related since they had also been observed in individual monkeys prior to study initiation. Minor lacerations, bruising, and/or swelling at the site of blood collection were observed in most animals at various time points during the 17 day observation period, and were not considered related to the test article. No adverse effects of AN100226 on body weights or food consumption over the duration of the study were observed.

Blood samples for evaluation of hematology and serum biochemistry profiles were obtained from all animals following an overnight fast prior to the initiation of dosing on Study Day 1, then on Study Days 7, 14, and 17. The hematology parameters evaluated included hematocrit, red cell count, hemoglobin, red blood cell morphology, mean red cell hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, white blood cell count (total, absolute, and percent differential), platelet counts, and abnormal blood cell morphology. Serum biochemistry and urinalysis profiles were not evaluated in this experiment.

Mild decreases in erythrocyte parameters, including red cell counts, hemoglobin, and hematocrit ranging from < 10% to approximately 16% from baseline values were noted in both groups of AN100226-treated monkeys, and are not considered related to natalizumab, but rather to the amount of blood sampling performed for the pharmacokinetic evaluations. A slight increase ($\leq$ 10%) in group mean platelet counts from pre-study values was observed at Study Day 7 in both groups of monkeys treated with natalizumab produced by either process, which resolved to baseline by Study Day 17. As previously observed in this species, the pharmacodynamic evaluation of leukocyte counts showed both groups of AN100226-treated monkeys with approximate 2-fold elevations from baseline values in both total peripheral blood leukocyte counts, and lymphocyte differential counts (both absolute and percentages) on Study Day 7. In this particular experiment, absolute numbers of monocytes, eosinophils, and basophils were also increased at Study Day 7, although to a much lesser degree than the lymphocyte counts. Corresponding decreases in both the differential and absolute numbers of neutrophilic granulocytes were also observed. These changes were partially reversed by the end of the observation period on Study Day 17, but had not fully resolved to pre-study values at that time. There were no differences noted in pharmacodynamic response between the two groups, following treatment with the two different scale preparations of natalizumab. The data for the means and standard deviations for the total leukocyte and absolute lymphocyte counts in each

dose group at Study Day 17 are represented in Table 36 below, and were derived from the data presented by the sponsor in the final study report.

Table 36. Pharmacodynamic effect of AN100226 produced by — commercial process (BG00002B) or by Biogen "resupply" material (BG00002A) on peripheral blood leukocyte counts in cynomolgus monkeys

Source of Natalizumab	Group Mean Total Leukocyte Counts ($10^3/\mu l$), ± S.D.			
	Pre-Study	Study Day 7	Study Day 14	Study Day 17
BG00002B ("commercial")	10.5 ± 2.8	22.8 ± 7.3	14.6 ± 2.9	11.0 ± 1.9
BG00002A ("resupply")	11.6 ± 5.1	22.8 ± 7.3	14.3 ± 3.3	10.2 ± 3.5
	Group Mean Absolute Lymphocyte Counts ($10^3/\mu l$), ± S.D.			
	Pre-Study	Study Day 7	Study Day 14	Study Day 17
BG00002B ("commercial")	5.8 ± 1.2	16.4 ± 5.5	9.7 ± 2.3	7.3 ± 2.3
BG00002A ("resupply")	7.3 ± 4.6	16.7 ± 5.9	9.0 ± 2.7	6.8 ± 3.0

Pharmacokinetics: Serum AN100226 levels were evaluated by non-parametric methods, using the WinNonlin software package. In both groups of animals, natalizumab concentrations in serum were below the limits of quantitation of the assay prior to infusion of the biologic. Maximal serum levels (C_{max}) of either preparation of natalizumab were detected immediately after, or within 30 min from the completion of infusion. The calculated values for C_{max}, Tmax, half-life, $AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$, area under the moment curve (AUMC), initial and steady state volumes of distribution (V_z and V_{dss}, respectively), and clearance (Cl) for AN100226 produced by — at the —— scale processes are presented in Table 37 below, and were calculated from the individual animal data for each parameter provided in the sponsor's final study report.

Table 37. Comparative pharmacokinetic profiles of natalizumab (AN100226) produced at two different processes in cynomolgus monkeys after a single, 3 mg/kg i/v infusion

Pharmacokinetic Parameter	Mean Value, ± S.D. (n = 8/group)	
	BG00002B-01	BG00002A
C_{max} (µg/ml)	74 ± 13	74.0 ± 24
t½ (h)	61.0 ± 19.6	67.7 ± 18.3
$AUC_{0\text{-last}}$ (µg*h/ml)	3672 ± 777	3293 ± 909
$AUC_{0\text{-inf}}$ (µg*h/ml)	3894 ± 945	3574 ± 939
V_z (ml/kg)	70.3 ± 23.5	84.7 ± 22.9
Cl (ml/h/kg)	0.81 ± 0.2	0.89 ± 0.22

No formal analysis of bioequivalence was performed for the BG00002 material produced by either the — commercial process, or the Biogen "resupply" process. However, the sponsor conducted a one-way ANOVA to statistically compare the mean values for each of the derived pharmacokinetic parameters. There were no statistically significant differences between the different pharmacokinetic parameters derived from serum concentrations of natalizumab from monkeys treated with AN100226 produced by either manufacturing process. Therefore, the sponsor has concluded that the natalizumab product produced by the —— commercial process is comparable to the reference "resupply" product produced by Biogen, and used in the phase 3 clinical studies.

Comment: Independent statistical analysis of the pharmacokinetic parameters derived for the two different groups of AN100226 treated monkeys was performed as part of this review, using a 2-tailed, Student's *t* test, as originally specified in the protocol, and confirmed that there were no statistically significant differences ($p > 0.05$) in C_{max}, $AUC_{0\text{-last}}$, or $AUC_{0\text{-inf}}$.

Comment: A human pharmacokinetics study to determine the bioequivalence of natalizumab produced by the — commercial scale process with that manufactured by Biogen "resupply" process was subsequently conducted, using a randomized, 2-way cross-over design in 80 healthy volunteer subjects (Clinical Study #C-1806). The lots of product used in this study differed from those used for the cynomolgus monkeys; the Biogen "resupply" material BG00002A used was from lot #H23001A, and the — commercial supply material BG00002B was used from lot #G68001AB. In this clinical study all criteria for bioequivalence, based on C_{max}, $AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$ were met, and the two products were deemed comparable.

Pharmacodynamics: In addition to the leukocyte counts, the pharmacodynamic activity of natalizumab BG00002B produced at commercial scale and the BG00002A Biogen "resupply" product were compared by flow cytometric evaluation of the percentage of α4-integrin receptor saturation on monkey peripheral blood lymphocytes by. Table 38 below, which contains the data provided by the sponsor in the final study report shows that there were no statistically significant differences in the percent receptor saturation, and therefore the AN100226 binding to α4-integrin receptor between the two products at any time point on study. As anticipated, receptor binding by natalizumab is maximal on Study Day 7, and decreases to less than the pre-study values in both groups by Study Day 17. The differences between receptor saturation in monkey lymphocytes following treatment with BG00002A or BG00002B were not statistically significant ($p = 0.74$, ANOVA, as performed by the sponsor).

Table 38. Pharmacodynamic effect of AN100226 produced by — commercial process (BG00002B-01) or by Biogen "resupply" material (BG00002A) on peripheral blood leukocyte α4-integrin receptor saturation in cynomolgus monkeys

Source of Natalizumab	Percent saturation of α4-integrin receptor on PBL, ± S.D.			
	Pre-Study	Study Day 7	Study Day 14	Study Day 17
BG00002B ("commercial")	12.5 ± 5.2	35.2 ± 5.1	20.8± 11.9	8.7 ± 5.4
BG00002A ("resupply")	13.5 ± 5.0	29.8 ± 4.2	21.8 ± 12.3	8.0 ± 4.4

Immunogenicity: Anti-natalizumab antibodies were not detectable in any AN100226 treated monkeys at either the pre-study or Study Day 7 time points. At Study Day 17, all animals in both dose groups had detectable antibody against AN100226 present; however, the magnitude of the total anti-natalizumab antibody responses was highly variable between animals within the two groups treated with AN100226, ranging from 29.9 µg/ml to 884.2 µg/ml for monkeys treated with the BG00002A product manufactured by the Biogen "resupply" process, to 20.7 µg/ml to 709.6 µg/ml for animals dosed with AN100226 manufactured by the — commercial scale process. The mean values for the anti-natalizumab antibody response were 241 ± 296 µg/ml and 238 ± 264 mg/ml for the BG00002A and BG00002B-01 products, respectively.

Study Conclusion: Intravenous infusion of a single dose of AN100226 produced by either the Biogen "resupply" process or by the — commercial process was well-tolerated in cynomolgus macaques, with no clinical, hematologic, or serum biochemical evidence of toxicity. Elevations in both total and differential leukocyte counts were observed in both groups at Study Day 7, and

are an expected pharmacodynamic effect of natalizumab in a responsive species. The pharmacokinetics of natalizumab after a single, intravenous infusion in monkeys demonstrated that the AN100226 product produced by the — commercial process was comparable to the Biogen "resupply" reference material, although a formal analysis of bioequivalence was not performed. All animals developed antibody against AN100226, with no remarkable differences in anti-natalizumab antibody response detected between monkeys treated with AN100226 produced by either manufacturing process.

Study title: Pharmacokinetic report PD039. Biogen studies #P00002-02-02, #P00002-02-03, #P00002-02-04, and #P00002-03-02.

Key study findings: Dose-related increases in AN100226 (BG00002) serum trough levels were observed in female nude mice and SCID mice following repeated injection with a 10 mg/kg loading dose of natalizumab i/p, and subsequent, twice weekly dosing with 5 mg/kg/dose until Study Day 25. Trough levels were maintained at > 20 μg/ml in nude mice, and >32 μg/ml in SCID mice for the duration of the study. Taken together, these data support the dosing regimen for use in future studies to evaluate the potential tumor growth promoting and metastasis effects of natalizumab in human tumor xenograft models in these two species.
Study no.: #PD03-09 (Biogen studies #P00002-02-02, #P00002-02-03, #P00002-02-04, and #P00002-03-02)
Volume #, and page #: EDR files: BLA 125104\000\module4\pharmacokinetics\pd03-09.pdf
Conducting laboratory and location: Biogen, Inc.
14 Cambridge Center
Cambridge, MA 02142
Date of study initiation: not specified in the final report
GLP compliance: No
QAU statement: yes ( ) no (X)
Drug, lot #, and % purity: BG00002 (AN100226, natalizumab), lot #F23014 (all four studies); concentration, percent purity not specified in the final study report

Methods:
A series of four, non-GLP studies were conducted by the sponsor, for the purpose of developing an optimal dosing regimen in nude and SCID mice that would allow for sustained trough levels of natalizumab of ≥ 20 mg/ml, in support of future studies to evaluate the effects of natalizumab on the promotion of human tumor xenograft growth. Briefly, mice were injected with single i/v or i/p injection, or twice weekly i/p injections for 25 days. In the single dose study (Study #P00002-02-02), female nude mice received 10 mg/kg AN100226 by either i/v or i/p injection on Study Day 1. In the repeat-dose studies #P00002-02-03 and #P00002-02-04, female nude mice were injected with a loading dose of either 3.7 or 10 mg/kg natalizumab i/p on Study Day 1, followed by twice weekly i/p injection with 1.5 or 5 mg/kg AN100226, respectively on Study Days 4, 8, 11, 15, 18, 22, and 25. The same dosing schedule and duration of treatment were used in Study #P00002-03-02, however, female SCID mice were treated with only the 10 mg/kg loading dose, followed by repeat injections of 5 mg/kg AN10226. Blood was obtained from three animals per time point by retro-orbital bleed (Study #P00002-02-04) or cardiac puncture (terminal bleeds) prior to dosing, and at 15, 30, and 45 min and 1, 2, 6, 8, 24, 48, 72, 96, 120, 168, 192, and 240 hours after treatment in the single dose studies, and prior to dosing on days 1, 4, 11, 18, and 25 and 8 hours after the final injection in the repeat dose studies. Serum samples were prepared, stored at -70°C until analysis, and evaluated for serum natalizumab concentrations by ELISA as

previously described. The lower limit of quantitation for the ELISA assay was [redacted] AN100226/ml. The mean serum concentration values were determined for each time point, and used to obtain an overall pharmacokinetic profile of natalizumab for each study. A one-compartment, i/v bolus model with first-order elimination, and a one compartment model with first order input and elimination, as provided in the WinNonlin software package were used to evaluate the serum concentration data from the single dose i/v, and single and repeat-dose, i/p natalizumab treated groups.

Comment: The sponsor states in the final report that "Post-dose BG00002 serum levels reported as below the limit of quantitation [redacted] were not included in the mean pharmacokinetic profile." It is unclear as to how many missing values were obtained and at what time points, and how the mean values were determined when data were below the limits of the assay detection.

Results:

Study #P00002-02-02 evaluated the pharmacokinetic profiles of AN100226 after a single i/v or i/p administration of 10 mg/kg. Similar serum levels and exposures were obtained for the two routes of administration, with mean C_{max} values of 170 µg/ml and 91 µg/ml, and $AUC_{0\text{-last}}$ values of 11728 µg*hr/ml and 10482 µg*hr/ml after i/v, or i/p injection, respectively. Mean elimination half-lives were approximately 77 h after i/v exposure, and 92 h after i/p natalizumab injection. Systemic clearance of AN100226 (not adjusted for bioavailability) was approximately equal following either route of administration, at about 900 ml/hr/kg. Estimated bioavailability following i/p injection was 89%, based on the ratio of the calculated $AUC_{0\text{-last}}$ exposures.

Following repeat administration of AN100226, trough serum levels of natalizumab were below the targeted 20 µg/ml level in 9/12 mice in Study #P00002-02-03 and 8/12 mice in Study #P00002-02-04 for the groups treated with 3.7 mg/kg on Study Day 1 and 1.5 mg/kg AN100226 on Study Days 4, 8, 11, 15, 18, 22, and 25 (data not shown in this review). In both studies, the loading dose of 10 mg/kg AN100226 followed by twice weekly repeat i/p injection with 5 mg/kg resulted in trough serum values of natalizumab greater than the 20 mg/ml targeted level in 23/24 mice. Figure 27 was provided by the sponsor in the final study report, and shows the predicted and observed mean serum concentrations of BG00002 (natalizumab) for the two studies, combined.

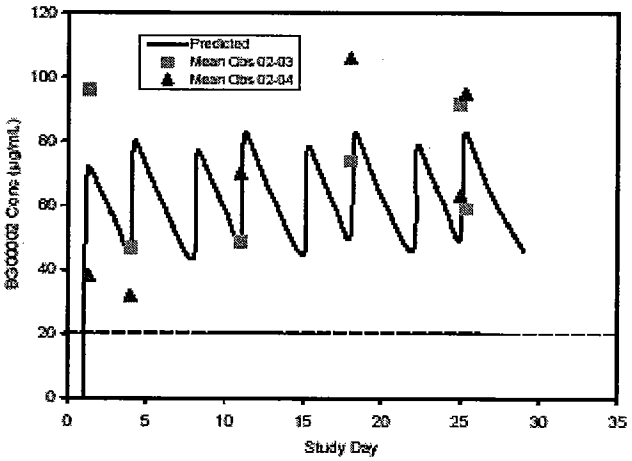
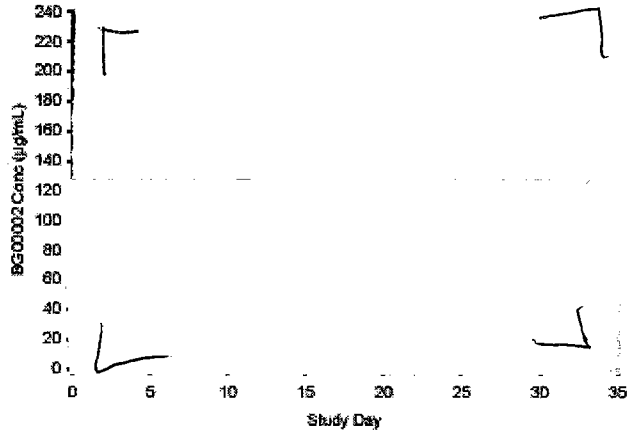


Figure 27. Predicted and observed mean serum concentrations in nude mice following repeat i/p administration of natalizumab. Loading dose of 10 mg/kg AN100226 injected on Study Day 1, followed by 5 mg/kg/dose on Study Days 4, 8, 11, 15, 18, 22, and 25.

Similar trough serum concentrations of AN100226 were obtained in SCID mice following twice weekly i/p injection at the 10/5 mg/kg/dose regimen and schedule in Study #P00002-03-02. In this study, the mean serum concentrations of natalizumab achieved were higher than those obtained following repeat dosing in nude mice, with trough serum concentrations approximately 2 to 3-fold greater than predicted from the single dose studies in nude mice. Additionally, all animals maintained serum trough levels at or above 32 µg/ml following Study Day 8, and mean values calculated from this point onward suggested that there was evidence of accumulation of AN100226 in SCID mice after repeat administration under the conditions of this study. Figure 28, which was provided by the sponsor in the final study report shows the predicted and observed serum trough natalizumab concentrations for individual animals (panel A), and the mean values for each time point (panel B).

A. Individual animal values



B. Mean values (n = 3/time point)

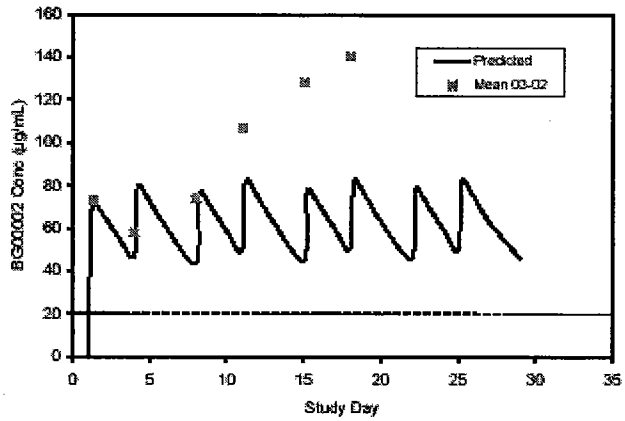


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Figure 28. Predicted and observed individual animal (panel A) and mean (panel B) serum concentrations in SCID mice following repeat i/p administration of natalizumab. Loading dose of 10 mg/kg AN100226 injected on Study Day 1, followed by 5 mg/kg/dose on Study Days 4, 8, 11, 15, 18, 22, and 25.

Study Conclusion: Pharmacokinetic profiles of natalizumab (BG00002, AN100226) were comparable after a single i/v or i/p injection of 10 mg/kg in nude mice. Bioavailability was approximately 89% after i/p dosing; therefore this route of administration was used for the subsequent repeat-dose studies in nude and SCID mice. To obtain sustained serum trough levels of greater than 20 mg/ml, mice were treated with a 10 mg/kg loading dose of AN100226 on Study

Day 1, followed by twice weekly injections of 5 mg/kg/dose until Study Day 25. A lower dose regimen tested (3.7 mg/kg loading dose, and 1.5 mg/kg/dose repeated injection) was not able to achieve the targeted concentration range. Taken together, these data suggest that exposure to natalizumab will be continuous after the 10/5 mg/kg dosing regimen, and support its use in future tumor xenograft studies in these species.

2.6.4.4 Distribution

Six studies were conducted to determine the potential for AN100226 or AN100226m cross-reactivity with human and animal tissues. Natalizumab (both humanized and the parental murine antibody) was found to bind to α4-integrin on the surface of only human and cynomolgus monkey lymphoid tissues, including submucosal tissue in the large and small intestines, spleen, tonsil, thymus, lymph node, bone marrow, and prostate..

A review of each of the individual studies is provided, below.

Study title: Cross reactivity of mouse monoclonal antibody 21-6 (AN100,226M) with human tissues.
Key findings: Localization of immunohistochemical staining with the parental mouse monoclonal antibody AN100226m (21-6) in human tissue sections was limited to the spleen, lymph node, thymus, and tonsil, and to gut-associated lymphoid tissue in the esophagus, stomach, and large and small intestines. The intensity of AN100226m staining of lymphocytes present in these organs was moderate to intense, predominantly present at the cell surface, and the distribution was consistent with the known localization of mature lymphocytes in these organs.
Study #: IM050
Volume #, and page #: EDR files: BLA 125104\000\module4\toxicology\other\im050.pdf
Conducting laboratory and location:

Date of study initiation: September 10, 1993 (final report signed March 16, 1995)
GLP compliance: yes
QAU statement: yes (X) no ( )
Drug, lot #, and % purity: AN100226 (natalizumab), 5.07 mg/ml; lot #25644PC; purity, IgG, SDS-PAGE (non-reduced), IgG, SDS-PAGE (reduced), monomeric IgG by HPLC (a Certificate of Analysis from the sponsor was included in the final study report)

Methods: The binding of the parental mouse monoclonal antibody AN100226m to a panel of frozen tissue sections from human samples was evaluated by immunohistochemical staining, to determine the pattern of distribution of α4 integrin in normal human tissues, and identify any potential target organs of natalizumab. Frozen human tissue samples that had obtained previously via autopsy or surgical biopsy were sectioned serially at 5 microns, fixed in acetone, and stained with AN100226m as the primary antibody, or an irrelevant mouse monoclonal IgG1k antibody as an isotype-matched control to detect any non-specific binding. Three dilutions of AN100226m antibody were prepared from the stock concentration of 8.4 mg/ml, for final primary antibody concentrations of 42, 4.2, and 0.42 µg/ml respectively. The control antibody was used at concentration of 4.2 µg/ml. Primary antibody was omitted from the incubation as a control for the assay itself. The positive control tissue sample for this experiment consisted of a containing the human Jurkat T cell line that expresses α4β1 integrin, and the human myeloid leukemia cell line K563, that does not

express α4 integrin, but expressed β2 in association with α5 (α5β1 integrin). Following incubation with either primary antibody, sections were incubated with a biotinylated, ——— Colored immunoreactive product was developed using diaminobenzidine (DAB) as a capture reagent, in the presence of hydrogen peroxide. All samples were counterstained with hematoxylin and evaluated for staining using light microscopy.

Results: The positive control human Jurkat T lymphoid cells bound AN100226m at all three concentrations tested, with an intensity of 1^+-2^+ at the 0.42 μg/ml concentration, and at a staining intensity of 3^+ at the two higher antibody levels. Reaction product was concentrated at the cell surface, with α4 integrin expressed on the cell membrane. There was no detectable binding of AN100226m to the human K562 cell line, and no specific staining in any tissue in the absence of primary antibody (assay control), or when ——— was used as an isotype-matched, control antibody. Peripheral blood lymphocytes showed only weak cross-reactivity with AN100226m (staining intensity 1^+). Tissue sections of human lymphoid organs, including thymus, lymph nodes, spleen, superficial lymphocytes in the lamina propria of the esophagus, stomach, and large and small intestines, and tonsil stained positively with AN100226m. Staining intensities of lymphocytes in these tissues ranged from 1^+ to 3^+, with the germinal centers of the spleen and tonsil, scattered lymphocytes in the lymph node cortex, and at the corticomedullary junction in the thymus, and interstitial lymphocytes in the esophagus and stomach showing the strongest binding of AN100226m. Occasional lymphocytes were present in the interstitial region of breast, lung, parathyroid, salivary gland, and thymus tissue samples, and when present, were intensely stained (2^+-3^+) with AN100226m. There was no detectable AN100226m staining of brain tissue (cerebrum, cerebellum, and medulla oblongata), eye, or peripheral nerve in this experiment. Other tissues with no detectable AN100226m staining included adrenal glands, peripheral blood monocytes, neutrophils, eosinophils, and platelets, vascular endothelium in all examined tissues, bone marrow, mammary glandular and duct tissue, heart, kidney, liver, lung parenchyma, ovary, pancreas, parathyroid, pituitary, prostate, skin, skeletal (striated) muscle, testis, thyroid, or urinary bladder. In one of two samples of uterine tissue, the basilar endometrial cells demonstrated intense to marked (3^+ to 4^+) staining at all three test concentrations of antibody, however, the remainder of the uterine tissue was negative. The results from the tissues showing positive reactivity with AN100226m are presented in Table 39 below, which was derived from the data included in the final study report.

Table 39: Cross-Reactivity of AN100226m with Normal Human Tissues			
Tissue Sample	Concentration of AN100226m Tested		
	0.42 µg/ml	4.2 µg/ml	42 µg/ml
Human Jurkat T cells	1^+ - 2^{+a}	3^+	3^+
Blood			
Neutrophils	(-)	(-)	(-)
Lymphocytes	1^+	1^+	1^+
Monocytes	(-)	(-)	(-)
Eosinophils	(-)	(-)	(-)
Platelets	(-)	(-)	(-)
Brain (cerebrum)	(-)	(-)	(-)
Brain (cerebellum)	(-)	(-)	(-)
Brain (medulla oblongata)	(-)	(-)	(-)
Breast			
glands and ducts	(-)	(-)	(-)
Interstitial cells	2^+ (1/2)[b]	3^{+b}	3^{+b}
Esophagus			
submucosal lymphs	(-)	±	1^+
other elements	(-)	(-)	(-)
Large intestine			
lymphocytes (rare)	2^+	2^+-3^+	3^+
other elements	(-)	(-)	(-)
Small Intestine			
lymphocytes (rare)	± - 2^+	1^+ - 3^+	1^+ - 3^+
other elements	(-)	(-)	(-)
Lung			
interstitial cells (rare)	2^{+b}	(-)[b]	3^{+b}
other elements	(-)	(-)	(-)
Parathyroid			
interstitial cells (rare)	(-)[c]	2^{+c}	2^{+c}
other elements	(-)	(-)	(-)
Salivary gland			
interstitial cells (rare)	2^{+b}	2^{+b}	2^{+b}
other elements	(-)	(-)	(-)
Spleen			
lymphocytes	(-)[b]	1^{+b}	2^{+b}
other elements	(-)	(-)	(-)
Thymus			
lymphocytes (rare)	1^+	1^+ - 3^+	3^+
interstitial cells (rare)	1^{+b}	1^{+b}	1^{+b}
other elements	(-)	(-)	(-)
Tonsil			
lymphocytes (germinal centers, interfollicular regions)	2^+	3^+	3^+
other elements	(-)	(-)	(-)
Uterus			
basilar endometria	3^+	3^+ - 4^+	3^+ - 4^+
other elements	(-)	(-)	(-)

[a] (-) = negative staining; ± = equivocal staining; 1^+ = weak; 2^+ = moderate; 3^+ = strong; 4^+ = intense; N.A. = not applicable; N.D. = not done

[b] positive in only one of two donor samples

[c] only one donor sample evaluated

Comment: The distribution pattern and intensity of AN100226m staining of lymphocytes present in human spleen, lymph node, thymus, and tonsil is consistent with the expected localization of mature lymphoid cells in these tissues. The cross-reactivity with uterine basilar endometrial cells is not unexpected; there are multiple reports in the literature, several of which

were provided by the sponsor in the BLA submission that demonstrate differing patterns of α4β1 and other integrin expression over the duration of the menstrual cycle[3].

Study conclusion: The murine monoclonal antibody AN100226m was capable of detecting cell surface α4 integrin expression in tissue sections from human spleen, lymph node, tonsil, thymus, and in lymphoid tissue present in the esophagus, stomach, and large and small intestines. The results were comparable to, although less intense than those obtained using sections of human Jurkat T cells as a positive control. Cross-reactivity of AN100226m with human tissue samples was optimal at a final incubation with 4.2 mg/ml. The pattern and intensity of AN100226m distribution in human tissues is consistent with the localization of mature and developing lymphocytes in these tissues.

Study title: Cross-reactivity of humanized monoclonal antibody AN100226 with human tissues.
Key findings: The humanized monoclonal antibody AN100226 demonstrated cross-reactivity with cell surface α4 integrin present on lymphoid cells in tissue sections from human spleen, lymph node, tonsil, thymus, and in lymphoid tissue present in the large and small intestines. The results were comparable to, although less intense than those obtained using the parental murine monoclonal antibody AN100226m (Study #IM050, above), and to sections of human Jurkat T cells stained at the same concentrations of antibody as a positive control.
Study #: PC002 (— Study #IM106)
Volume #, and page #: EDR files: BLA 125104\000\module4\toxicology\other\pc002.pdf
Conducting laboratory and location: —

Date of study initiation: December 21, 1994 (final report signed April 19, 1995)
GLP compliance: No
QA statement: yes ( ) no (X)
Drug, lot #, and % purity: AN100226 (natalizumab), 5.07 mg/ml; lot #25644PC; purity, — IgG, SDS-PAGE (non-reduced), — IgG, SDS-PAGE (reduced), — monomeric IgG by — HPLC (a Certificate of Analysis from the sponsor was included in the final study report)

Methods: Natalizumab (humanized monoclonal antibody AN100226) cross-reactivity to a panel of frozen tissue sections from human samples was evaluated by immunohistochemical staining, to determine the pattern of distribution of α4 integrin in normal human tissues, and whether the staining was similar to that previously observed with the murine parental antibody, AN100226m. Five micron sections of frozen human tissue samples from autopsy or surgical biopsy specimens, as well as selected tissues from multiple sclerosis patients (heart, kidney, liver, lung, and skeletal muscle) were prepared and fixed in absolute acetone, and stained with AN100226 at concentrations of 0.8 and 8 μg/ml. Three separate donor samples were evaluated for each tissue type, where possible. Cryosections of pelleted, human Jurkat T lymphoid cell line that expresses α4β1 integrin were used as a positive control; no negative control cell line was evaluated in this study. An isotype-matched, irrelevant human IgG4 antibody was used as a

[3] Tabibzadeh, S. 1992. Patterns of expression of integrin molecules in human endometrium throughout the menstrual cycle. *Human Reprod.*, 7:876-882.

control for non-specific binding of the primary AN100226 antibody, and primary antibody was omitted from the initial incubation step as an assay control. An additional series of tissue samples was incubated with a peroxidase conjugated, rabbit polyclonal antibody directed against β2-microglobulin, as a control to ensure that each tissue would demonstrate reactivity in this assay. After incubation with the primary antibodies, sections were then exposed for 30 min to biotinylated mouse anti-human IgG4 antibody (5 μg/ml), washed, and further incubated with the

Colored immunoreactive product was developed using diaminobenzidine (DAB) as a capture reagent, in the presence of hydrogen peroxide. All samples were counterstained with hematoxylin and evaluated for staining using light microscopy.

Results: Binding of natalizumab to the positive control human Jurkat T lymphoid cells was detected at both test concentrations, with a staining intensity of $\pm$-3^+ at the 0.8 μg/ml concentration, and at a staining intensity of 2^+-3^+ at the 8.0 mg/ml concentration. Staining was localized predominantly at the cell surface, with α4 integrin expressed on the cell membrane. There was no detectable binding of the irrelevant, human IgG4 isotype-matched antibody control, and no specific staining in any tissue in the absence of primary antibody (assay control). All tissues tested stained positively with the rabbit polyclonal antibody directed against β2-microglobulin, confirming that the immunoperoxidase methods not affect the ability to detect reactivity under the conditions of this assay (data not shown in this review).

No detectable natalizumab staining was observed in tissue samples of heart, liver, kidney, lung, or skeletal muscle obtained from multiple sclerosis patients (data not shown). There was no detectable AN100226 reaction product in samples of peripheral blood leukocytes, including neutrophils, monocytes, lymphocytes, eosinophils, and platelets. Bone marrow sections from one of three donors showed only weak cross-reactivity of AN100226 (staining intensity 1^+) in occasional lymphoid cells after staining with 8.0 μg/ml natalizumab. Staining with AN100226 was detected primarily in lymphoid organs, including the spleen, lymph nodes, thymus, and occasional lymphocytes in the lamina propria of the large and small intestines. The intensity of natalizumab staining in these tissues ranged from 1^+ to 3^+, with lymphoid cells in the marginal follicular and paracortical regions of the lymph node, and in the germinal centers of the spleen showing the strongest binding of AN100226, and occasional positive lymphocytes in the outer cortex of the thymus, with a staining intensity of $\pm$ to 2^+. In this series of experiments, human tonsil stained positively, although at low intensity with AN100226 in 1/3 donors; high levels of non-specific binding of the IgG4 isotype-matched antibody control precluded determination of more specific staining of natalizumab. Occasional lymphocytes were present in the interstitial region of prostate, salivary gland, and in the propria mucosa of the ureter, and when present, were weak to intensely stained (1^+-3^+) with AN100226. All three samples of uterine tissue showed moderate natalizumab staining of the basilar endometrial cells, with an intensity ranging from $\pm$ to 2+, with no detectable staining in the remaining uterine tissue. The results from the tissues showing positive reactivity with AN100226 are presented in Table 40 below, which was derived from the data included in the final study report.

Table 40: Cross-Reactivity of AN100226 (natalizumab) with Normal Human Tissues		
Tissue Sample	Concentration of AN100226 Tested	
	0.8 µg/ml	8.0 µg/ml
Human Jurkat T cells	$\pm^{+}$ - 3^{+a}	3^{+}
Blood		
Neutrophils	(-)	(-)
Lymphocytes	1^{+}	1^{+}
Monocytes	(-)	(-)
Eosinophils	(-)	(-)
Platelets	(-)	(-)
Brain (cerebrum)	(-)	(-)
Brain (cerebellum)	(-)	(-)
Brain (medulla oblongata)	(-)	(-)
Large intestine lymphocytes	$\pm$ - 2^{+}	1^{+} - 3^{+}
other elements	(-)	(-)
Small Intestine lymphocytes	$\pm$ - 2^{+}	$\pm$ - 3^{+}
other elements	(-)	(-)
Lymph node marginal follicular lymphoid cells	(-) - 2^{+b}	1^{+} - 3^{+b}
paracortical lymphoid cells	(-) – 2^{+b}	1^{+} - 3^{+b}
medullary lymphoid/ mononuclear cells	(-) – 2^{+b}	1^{+} - 3^{+b}
other elements	(-)	(-)
Prostate interstitial lymphs	1^{+c}	2^{+} - 3^{+c}
other elements	(-)	(-)
Salivary gland interstitial cells (rare)	$\pm$ - 1^{+c}	1^{+} - 2^{+c}
ductal epithelia (rare)	$\pm$ - 1^{+c}	1^{+} - 2^{+c}
other elements	(-)	(-)
Spleen follicular center lymphoid cells	3^{+c}	3^{+c}
marginal follicular lymphoid cells	$\pm$ - 1^{+b}	1^{+} - 2^{+b}
parafollicular lymphoid cells	$\pm$ - 1^{+b}	1^{+} - 2^{+}
other elements	(-)	(-)
Thymus outer cortical lymphocytes (rare)	$\pm$ - 1^{+b}	$\pm$ - 2^{+b}
other elements	(-)	(-)
Tonsil marginal follicular lymphoid cells	$\pm$ - 1^{+c}	$\pm 1^{+c}$
other elements	(-)	(-)
Ureter lymphoid cells in propria mucosa	1^{+}	1^{+} - 2^{+}
other elements	(-)	(-)
Uterus basilar endometria	2^{+d}	2^{+d}
other elements	(-)	(-)

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[a] (-) = negative staining; $\pm$ = equivocal staining; 1^{+} = weak; 2^{+} = moderate; 3^{+} = strong; 4^{+} = intense; N.A. = not applicable; N.D. = not done

[b] positive in two of three donor samples

[c] positive in only one of three donor samples
[d] 1/3 donor samples had equivocal to weak staining at the high concentration of AN100226, and the samples were negative at the lower concentration

Comment: The distribution pattern and intensity of AN100226 staining of lymphocytes present in human spleen, lymph node, thymus, and tonsil is consistent with the expected localization of mature lymphoid cells in these tissues. Additionally, the staining of lymphocytes in the tissues by natalizumab is comparable to that previously detected using the murine, parental monoclonal antibody AN100226m.

There was no detectable AN100226 cross-reactivity with brain tissue (cerebrum, cerebellum, and medulla oblongata), eye, spinal cord, or peripheral nerve in this experiment. Other tissues with no detectable natalizumab staining included adrenal glands, peripheral blood cells, vascular endothelium in all examined tissues, bone marrow, breast, heart, kidney, liver, lung parenchyma, ovary, pancreas, parathyroid, pituitary, skin, skeletal (striated) muscle, testis, thyroid, or urinary bladder.

Study conclusion: The humanized monoclonal antibody AN100226 (natalizumab) was capable of detecting cell surface α4 integrin expression in tissue sections from human spleen, lymph node, thymus, and in lymphoid tissue present in the large and small intestines. The results were comparable to, although less intense than those obtained using sections of human Jurkat T cells as a positive control. Cross-reactivity of AN100226 with human tissue samples was similar to that observed using the murine parental monoclonal antibody AN100226m. The pattern and intensity of natalizumab distribution in human tissues is consistent with the localization of mature and developing lymphocytes in these tissues.

Study title: Cross reactivity of humanized monoclonal antibody AN100226 with cynomolgus monkey tissues and guinea pig heart.
Key findings: Natalizumab binding to α4 integrin was detected in cynomolgus monkey tissues, including lymph node, spleen, thymus, tonsil, and gut-associated lymphoid cells in the large and small intestines. The most intense staining was present in the follicular centers of the lymph node, spleen, and tonsil (3^+-4^+), with less intense staining present in the marginal and parafollicular zones of these organs. Occasional, interstitial lymphoid cells in the bone marrow, salivary gland, and prostate also stained with natalizumab, but at much lower intensity (± to 2^+). Weak, but detectable staining was present in rare basilar endometrial cells and occasional stromal or periglandular round cells in the uterus, at low intensity (± to 2^+). There was no detectable binding of AN100226 to frozen tissue samples of cynomolgus brain (cerebrum and cerebellum), eye, peripheral nerve, or to guinea pig heart.
Study #: PC001 (Study #IM179)
Volume #, and page #: EDR files: BLA 125104\000\module4\toxicology\other\pc001.pdf
Conducting laboratory and location:

Date of study initiation: January 31, 1995 (final report not dated)
GLP compliance: No
QA statement: yes ( ) no (X)
Drug, lot #, and % purity: AN100226 (natalizumab), 5.07 mg/ml; lot #25644PC; purity, IgG, SDS-PAGE (non-reduced), IgG, SDS-PAGE (reduced), monomeric IgG

by [redacted] HPLC (a Certificate of Analysis from the sponsor was included in the final study report) [redacted]

Methods: The purpose of this study was to evaluate the binding of the humanized AN100226 antibody to a panel of frozen tissue sections from normal cynomolgus monkeys, to determine whether natalizumab can cross-react with the α4β1 integrin of this species and therefore identify the cynomolgus macaque as a relevant species for additional toxicology and pharmacology testing. Guinea pig heart sections were also evaluated for AN100226 binding, since toxicity studies were being conducted in this species by the intra-cardiac route of injection. Five micron sections of either frozen cynomolgus monkey tissues or guinea pig heart were prepared and fixed in absolute acetone, then labeled with biotinylated natalizumab (AN100226-B, lot #25644PC) at concentrations of [redacted]. Cryosections of pelleted, human Jurkat T lymphoid cell line that expresses α4β1 integrin were used as a positive control; no negative control cell line was evaluated in this study. An isotype-matched, irrelevant human IgG4 antibody was used as a control for non-specific binding of the primary AN100226 antibody, and primary antibody was omitted from the initial incubation step as an assay control. After incubation with either AN100226 or the isotype-matched, IgG4 control, sections were then exposed for 30 min to biotinylated mouse anti-human IgG4 antibody (5 µg/ml), washed, and further incubated with the [redacted]

Colored immunoreactive product was developed using diaminobenzidine (DAB) as a capture reagent, in the presence of hydrogen peroxide. All samples were counterstained with hematoxylin and evaluated for staining using light microscopy.

Results: The AN100226 antibody stained human Jurkat T lymphoid cells at both the [redacted] concentrations, with an intensity of 2^+-4^+ at the lower concentration, as compared to 3^+-4^+ at the higher antibody level. The localization of the reaction product was at the cell surface, indicating reactivity with α4β1 integrin expressed on the cell membrane. Tissue sections of cynomolgus monkey lymphoid organs, including thymus, lymph nodes, spleen, submucosal lymphoid tissue in the gut, and tonsil stained positively with AN100226, with intensities ranging from 1^+ to 3^+ for spleen and small intestine, 2^+ to 4^+ for tonsil and peripheral lymph nodes, and 2+ for thymus. Of interest, the detected staining with natalizumab in the spleen, tonsil, and lymph nodes was most intense in the regions of more mature lymphocyte development in these organs, specifically, the follicular centers and the marginal regions, with less intense although still detectable staining at the parafollicular and subcapsular regions. Occasional, infiltrating lymphocytes staining with natalizumab at an intensity of ± to 1^+ were detected in samples from salivary gland, and in prostate at a staining intensity of 2^+-3^+ (one male animal only). Lymphoid cells in the bone marrow, although rare, also stained positively with AN100226, at a staining intensity of 2^+. Localization of the signal in these tissues was highly specific for the cellular surface. No staining of these tissues or of the Jurkat cell line was observed when the primary antibody was omitted from the procedure. These results are presented in Table 41 below, which was derived from the data included in the sponsor's final study report.

Table 41: Study #PC001. Cross-Reactivity of AN100226 (natalizumab) with Normal Cynomolgus Monkey Tissues		
Tissue Sample	**Concentration of AN100226 Used**	
	1.6 µg/ml	**16 µg/ml**
Human TM (Jurkat) cell line (positive control)	2^+ - 4^{+a}	3^+ - 4^+
Blood Vessel	(-)	(-)
Bone marrow (occasional lymphoid cells)	2^{+b}	2^{+b}
Brain (cerebrum)	(-)[a]	(-)
Brain (cerebellum)	(-)[c]	(-)
Eye	(-)	(-)
Large intestine (colon) lymphoid cells in lamina propria	± - 1	± - 1
Large intestine (other elements)	(-)	(-)
Small Intestine lymphoid cells in lamina propria	2^+ - 3^+	2^+ - 3^+
Small intestine (other elements)	(-)	(-)
Lymph node marginal and follicular center lymphoid cells	2^+ - 4^+	2^+ - 4^+
Lymph node paracortical lymphoid cells	1^+ - 2^+	1^+ - 2^+
Lymph node medullary lymphoid/mononuclear cells	1^+	1^+
Lymph node other elements	(-)	(-)
Prostate occasional lymphoid cells in interstitium	2^+ (rare)	3^+
Prostate lymphoid nodule in interstitium	±	1^+
Prostate (other elements)	(-)	(-)
Salivary gland occasional interstitial cells	1^{+b}	1^+ - 2^+
Salivary gland occasional ductal epithelial cells	(-)	± – 1^{+b}
Salivary gland (other elements)	(-)	(-)
Spleen Follicular center lymphoid cells	1^+	(-) - ±
Spleen marginal follicular lymphoid cells	1^+	(-)
Spleen parafollicular and interfollicular lymphoid cells	2^+ - 3^+	(-)
Spleen lymphoreticular cells (trabeculae/subcapsular)	1^+ - 3^+	± - 1^+
Spleen (other elements)	(-)	(-)
Thymus occasional outer cortical lymphoid cells	2^+	2^+
Thymus occasional lymphocytes at corticomedullary jct	2^+	2^+
Thymus (other elements)	(-)	(-)
Tonsil marginal follicular lymphoid cells	1^+ - 2^{+b}	2^+ - 3^{+b}
Tonsil follicular center lymphoid cells	2^+ - 4^+	3^+ - 4^+
Tonsil parafollicular and interfollicular lymphoid cells	2^+ - 4^+	2^+ - 4^+
Tonsil (other elements)	(-)	(-)
Uterus basilar endometrial cells	(-) - 1^+ (rare)	1^+ (rare)
Uterus occasional stromal/periglandular round cells	1^+ - 2^+ (rare)[b]	1^+ - 2^{+b}
Uterus (other elements)	(-)	(-)

[a] (-) = negative staining; ± = equivocal staining; 1^+ = weak; 2^+ = moderate; 3^+ = strong; 4^+ = intense; N.A. = not applicable; N.D. = not done

[b] positive in only one of two donor samples

The following cynomolgus monkey tissue samples were also evaluated in this study, with no cross-reactivity with natalizumab detected by immunohistochemical staining. These included endothelial cells in blood vessels in all tissues examined, brain (cerebrum and cerebellum) and peripheral nerve, adrenals, breast (mammary gland), heart, kidney, liver, lung, pancreas, parathyroid, skeletal muscle, skin, stomach, ovary, testes, and thyroid (data not shown in review).

Both tissue samples of guinea pig heart were negative for cross-reactivity to natalizumab, after incubation with either [redacted] of the monoclonal antibody. The positive control human T cell line Jurkat stained with AN100226 at the [redacted] concentration at an intensity of 2^+ - 3^+ (Table 41, above). The Jurkat sample stained with the higher concentration of [redacted] natalizumab was not interpretable, due to a low number of cells present in the sample examined.

Study conclusion: The humanized monoclonal antibody natalizumab was capable of detecting cell surface α4 integrin expression on lymphoid cells present in tissue sections of spleen, lymph node, tonsil, bone marrow, thymus, and in the lamina propria of the large and small intestines from cynomolgus monkeys. The results were comparable to, although less intense than those obtained using sections of human Jurkat T cells as a positive control. Cross-reactivity of AN100226 was similar in both distribution and intensity to that previously observed with human tissue sections, while binding of natalizumab to guinea pig heart was not detected under these assay conditions. Therefore, the cynomolgus macaque is identified as a pharmacologically relevant species in which to further study the toxicity of AN100226.

Study title: Cross-reactivity of Antegren™ (natalizumab) with fetal and perinatal tissues and fetal Rhesus and cynomolgus monkey tissues.
Key findings: Similar patterns of natalizumab binding to α4 integrin were detected in fetal and early neonatal human, and fetal Rhesus and cynomolgus monkey tissues, spleen, bone marrow, stomach, and lamina propria in the small intestine. Both the intensity and the frequency of tissue cross-reactivity differed with developmental age, with increased staining detected in lymphoid organs with increasing age. Notable differences between adult and fetal tissues included a lack of AN100226 cross-reactivity with follicular cells in the lymph node, spleen, and thymus, the presence of weak, but specific staining on the cell surface of vascular endothelium and in various organs in fetal, but not adult tissues from both species, and the localization of natalizumab staining in samples of fetal human liver (hematopoietic tissue), and in developing Rhesus monkey papillary dermis, skeletal muscle, and brain vasculature.
Study #: IM342
Volume #, and page #: EDR files: BLA 125104\000\module4\toxicology\other\im342.pdf
Conducting laboratory and location: [redacted]

Date of study initiation: February 27, 1997 (final report signed January 21, 1998)
GLP compliance: Yes
QA statement: yes (X) no ( )
Drug, lot #, and % purity: AN100226 (natalizumab), 5.0 mg/ml; lot #1450-65-1, clinical formulation in sodium phosphate buffered saline, plus 0.02% polysorbate 80, pH 6.0; purity not specified (no Certificate of Analysis from the sponsor was included in the final study report)

Methods: This study was performed to evaluate the potential cross-reactivity with a panel of tissues from human and fetal Rhesus and cynomolgus monkeys. One to three samples for each fetal and/or perinatal human tissue were obtained from autopsy or surgically-derived biopsy material. The ages of the donors ranged from approximately 20 weeks gestational age, to neonates 6 days post-partum. The fetal and perinatal monkey samples were from two Rhesus monkeys (gestation days 71 and 97), and from two cynomolgus monkeys (gestation days 46 and 66). Human and Rhesus monkey tissues were provided as individual tissues; cynomolgus monkey tissues were provided as intact fetuses which were multiply sectioned at 5 micron thickness for evaluation. Previously fixed and frozen human or monkey tissue sections were incubated with natalizumab as the primary antibody, at concentrations of 1.0 and 10.0 µg/ml. Immunohistochemical staining was carried out as previously described (please see Studies #PC002 and #PC001, above), and samples were evaluated microscopically for evidence of colored reaction product. Cryosections of pelleted human Jurkat T cells were used as a positive control for natalizumab staining, and human K562 myeloid leukemia cells, which do not express $\alpha 4\beta 1$ integrin, were evaluated for AN100226 staining as a negative control. An irrelevant, unconjugated human IgG4 was used as an isotype-matched antibody control to evaluate non-specific staining with the primary antibody. In this experiment, a parallel series of slides was stained with a polyclonal rabbit antibody directed against human β2-microglobulin, as a tissue staining control.

Results: No specific staining was detected in any tissue following incubation with the human, myeloma-derived IgG4 isotype-matched control antibody, and no reaction product was present when the primary antibody was omitted from the incubation. All human tissues stained positively with the rabbit polyclonal antibody against β2-microglobulin.

There was no staining of human K562 myeloid leukemia cells detected after incubation with either 1 or 10 µg/ml AN100226. By contrast, natalizumab staining was present at the cell surface of human Jurkat T cells at moderate to marked intensity (2^+-4^+). Staining of both human and non-human fetal and perinatal tissues with AN100226 was detected in various lymphoid tissues, and in some additional tissues that had not shown cross-reactivity previously in adult organs.

In the human tissues, natalizumab staining was detected specifically at the cell surface of mononuclear cells present in developing thymus, spleen, in the lamina propria of the stomach and large intestine, and in bone marrow and fetal liver, which is a site for developing hematopoietic cells. Endothelial cells in developing blood vessels, including the retina of the eye, peripheral nerve, spleen, small intestine, and stomach in human fetal and perinatal tissue samples also showed specific staining with natalizumab, in contrast to the samples from adult tissues, which were negative in all organs evaluated. Additionally, occasional positively-stained mononuclear cells (presumed lymphocytes or monocytes) were detected in the interstitial areas of the pancreas and parathyroid glands. Specific staining was also observed in interstitial stromal cells in the adrenal (1/3 donors), bone marrow (2/3 donors), kidney (1/3 donors), and ovary (2/2 donors), and in undifferentiated mesenchymal cells present in the full-term placenta (1/3 donors). Developing smooth muscle cells in the small intestine (3/3 donors) and in the urinary bladder (2/3) donors also showed specific staining at the cell surface with natalizumab, although the intensity of the staining was variable. The results from the samples stained with 10 µg/ml AN100226 are presented in Table 42 below, which was derived from data included in the submitted final study report.

A similar distribution of AN100226 tissue cross-reactivity was observed in isolated tissue sections from fetal Rhesus monkeys, at gestational ages of 71 and 97 days. However, no

natalizumab staining was detected in fetal thymus, lymph node, or liver in the Rhesus monkey samples, as compared to the human tissue samples. Rhesus monkey tissues also showed more cytoplasmic staining than the human tissue samples, which may reflect a difference in the localization of $\alpha 4\beta 1$ integrin during the different stages of fetal development in monkey versus humans. The results of the samples stained with 10 µg/ml of AN100226 are presented in Table 42, below.

Table 42: Study #PC001. Cross-Reactivity of AN100226 (natalizumab) with Fetal Human and Rhesus Monkey Tissues

Tissue Sample	Test Species	
	Human	Rhesus monkey
Human TM (Jurkat) cell line (positive control)	2^+ - 4^{+a}	2^+ - 4^+
Human K563 leukemia cell line (negative control)	(-)	(-)
Adrenal		
interstitial stromal cells	$\pm^b$	(-)
other elements	(-)	(-)
Bone marrow		
interstitial stromal cells	$\pm^c$, 2^+ - 3^{+b}	(-)
mononuclear cells	2^+ - 3^{+b}	$\pm$ - 1^{+i}
other elements	(-)	(-)
Brain (cerebrum)		
developing vessels	(-)	1+ - 2^{+i}, $\pm$ - 1^{+i}
other elements	(-)	(-)
Brain (cerebellum)		
developing vessels	(-)	$\pm$ - 1^+
other elements	(-)	(-)
Eye		
developing vessels in retina	$\pm^c$	$\pm$ - 1^{+i}
other elements	(-)	(-)
Heart		
interstitial stromal cells	$\pm^b$	(-)
other elements	(-)	(-)
Kidney		
interstitial stromal cells	1^{+b}	1^{+i}
mononuclear cells	(-)	$\pm$ - 1^{+i}
other elements	(-)	(-)
Large intestine (colon)		
lymphoid cells in lamina propria	$\pm$ - 2^{+d}	(-)
other elements	(-)	(-)
Liver		
mononuclear cells	1^{+d}	(-)
other elements	(-)	(-)
Lymph node	not tested	(-)[j]
Ovary		
interstitial stromal cells	1^{+e}	(-)[i]
other elements	(-)	(-)
Pancreas		
interstitial stromal cells	1^+ - 2^{+b}	(-)
mononuclear cells	1^+ - 2^{+f}	$\pm$[i]
other elements	(-)	(-)
Parathyroid		
mononuclear cells	1^{+b}	not tested
other elements	(-)	
Peripheral nerve		
developing vessels	$\pm$ - 1^{+b}	not tested
other elements	(-)	
Placenta		
mesenchymal cells	$\pm^g$	(-)
other elements	(-)	(-)
Prostate		
interstitial stromal cells (periductal)	not tested	1^{+i}
other elements		(-)
Skeletal muscle		
skeletal myofibers	(-)	$\pm$ - 1^{+i} (focal)

other elements	(-)	(-)
Skin		
papillary dermal fibrils	(-)	1^+ - 2^{+i}
other elements	(-)	(-)
Small Intestine		
developing blood vessels	1^+ - 3^+	(-)
smooth muscle cells in lamina propria	1^+ - 3^+	(-)
mononuclear cells in lamina propria	(-)	1^{+i}
other elements	(-)	(-)
Spleen		
developing vessels	$\pm$ - 1^{+f}	(-)
mononuclear cells	$\pm$ - 2^+	$\pm$ - 1^{+i}
interstitial stromal cells	(-)	$\pm^j$
other elements	(-)	(-)
Stomach		
mononuclear cells in lamina propria	$\pm$ - 2^+	$\pm$ - 1^{+i}
developing vessels	$\pm$ - 1^+	$\pm$ - 1^{+i}
other elements	(-)	(-)
Thymus		
mononuclear cells in cortex	$\pm$ - 2^+	(-)
mononuclear cells in medulla	$\pm$ - 2^+	(-)
epithelial cells/macrophages	$\pm$ - 2^+	(-)
other elements	(-)	(-)
Thyroid		
mononuclear cells in interstitium	(-)	$\pm$ - 1^{+i}
other elements	(-)	(-)
Umbilical cord		
occasional lymphocytes at corticomedullary jct	(-)	not tested
other elements	(-)	
Urinary bladder		
smooth muscle cells in tunica media	$\pm$ - 2^{+h}	(-)
other elements	(-)	(-)
Uterus (other elements)	not tested	(-)

[a] (-) = negative staining; $\pm$ = equivocal staining; 1^+ = weak; 2^+ = moderate; 3^+ = strong; 4^+ = intense; N.A. = not applicable; N.D. = not done
[b] 1/3 donor samples, 22 weeks gestational age
[c] 1/3 donor samples, neonatal tissue
[d] 3/3 donors positive, 22-25 weeks gestational age and neonate
[e] 2/2 donors positive, 22, 26 weeks gestational age
[f] 1/3 donor samples, 23 weeks gestational age
[g] 1/2 donor samples, full-term
[h] 2/3 donor samples, 23, 23 weeks gestational age
[i] GD97 fetus
[j] GD71 fetus

Fetal tissues that were completely negative for AN100226 cross-reactivity in both humans and Rhesus monkey included adrenal, heart, liver parenchyma, lung, pituitary, spinal cord, testis, ureter, and uterus (data not shown). In the human, negative cross-reactivity of natalizumab was also demonstrated for developing brain (cerebrum and cerebellum), diaphragm, esophagus, skin, skeletal muscle, thyroid, and umbilical cord. In the Rhesus monkey samples, natalizumab staining was not detected in large intestine, liver, ovary, thymus, or urinary bladder, while these tissues showed some degree of specific staining in the human samples.

Cynomolgus monkey samples were evaluated as whole body sections from two fetuses, obtained at GD46 and GD66, and the tissues were examined *in situ* in multiple serial sections. Where AN100226 cross-reactivity was detected, the staining was present predominantly within the cytoplasm, with some reaction product detected at the cell surface of mononuclear cells present within the sections. Staining intensity with natalizumab was variable between the two donors of

differing gestational age, but specific staining was detected in the cranial bone marrow of the GD66 fetus, and in the mesenchymal tissues of the placenta in the GD46 fetus. Spindloid cells, and primitive skeletal myofibers in developing skeletal muscle, and mesenchymal cells in developing stratified squamous epithelium in the papillary dermis showed specific staining with AN100226 in both the 46 and 66 gestational age fetuses. Specific natalizumab cross-reactivity was also detected in undifferentiated mesenchymal cells in head sections and in the cores of the fetal placental villi from the GD46 fetus, and in sections of head, thorax, liver, peritoneal cavity, and pelvis from the GD66 fetus. Developing blood vessels in the head (GD46 fetus), anterior thorax, lower abdomen, and pelvic limb (GD66 fetus only) demonstrated specific natalizumab staining in presumed developing smooth muscle cells. The results of this study are shown in Table 43 on the following page, as provided in the sponsor's final study report.

The distribution and intensity of natalizumab cross-reactivity with fetal tissues from human and non-human primate donors demonstrated specific patterns as the age of the fetus increased. A qualitative evaluation in human and non-human primate tissues was provided by the contracting laboratory in the final study report, which is presented in Table 44, on the following pages below. AN100226 staining of mononuclear or lymphoid cells increased in both frequency and intensity as the age of the fetuses increased, while the expression of $\alpha 4\beta 1$ integrin in developing smooth and skeletal muscle was more frequent in the younger fetuses, particularly in the cynomolgus monkey tissue sections. Positive staining in vascular endothelial cells and developing smooth muscle decreased with developing age, while positive staining of interstitial cells in the retina of human and Rhesus monkeys was only detected at late gestation/neonatal development. These findings are consistent with previously published reports of the distribution of $\alpha 4$-integrins in fetal development and organogenesis[4].

[4] Arroyo, A.G. *et al.*, 1996. Differential requirements for a4 integrins during fetal and adult hematopoiesis. *Cell*, **85**:997-1008; Liakka, K.A. 1994. The integrin subunits $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 6$, αV, $\beta 1$ and $\beta 3$ in fetal, infant, and human spleen as detected by immunohistochemistry. *Differentiation*, **56**:183-190; Sheppard, A.M. *et al.*, 1994. Expanding roles for a4 integrin and its ligand in development. *Cell. Adhesion. Comm.*, **2**:27-43; Yang, J.T. *et al.*, 1995. Cell adhesion events mediated by $\alpha 4$ integrins are essential in placental and cardiac development. *Development*, **121**:549-560.

Table 43. Cross-Reactivity of AN100226 (natalizumab) with Fetal Cynomolgus Monkey Tissues.

TISSUE	SOURCE (Gestational Age)	TEST ARTICLE ANTIBODY CONCENTRATION 10 µg/mL	TEST ARTICLE ANTIBODY CONCENTRATION 1 µg/mL	NEGATIVE CONTROL HUMAN IgG4 10 µg/mL	NEGATIVE CONTROL HUMAN IgG4 1 µg/mL	ASSAY CONTROL
Positive Control						
α4β1 integrin-expressing lymphoid cells	TM (Jurkat)	2+ to 4+	2+ to 4+	Neg	Neg	Neg
Negative Control						
α4β1 integrin-negative leukemia cells s	K562	Neg	Neg	Neg	Neg	Neg
Head Section 1	CynM (46 days)					
Subepithelial embryonal mesenchyme (including papillary dermal fibrils)		1+ to 2+	1+ to 2+	Neg	Neg	Neg
Developing vessels		1+ to 2+	1+ to 2+	Neg	Neg	Neg
Mesenchymal cells		1+	± to 1+	Neg	Neg	Neg
Other elements (cartilage anlagen, developing muscle)		Neg	Neg	Neg	Neg	Neg
Head Section 2	CynM (46 days)					
Subepithelial embryonal mesenchyme (including papillary dermal fibrils)		2+	1+ to 2+	Neg	Neg	Neg
Other elements (optic cup, tongue, cartilage anlagen, brain)		Neg	Neg	Neg	Neg	Neg
Body Section 1	CynM (46 days)					
Developing skeletal muscle (myotomes)		1+ to 3+ (focal)	1+ to 2+ (focal)	Neg	Neg	Neg
Other elements (vertebral cartilage anlagen, primordial internal organs)		Neg	Neg	Neg	Neg	Neg
Body Section 2	CynM (46 days)					
Developing skeletal muscle (myotomes)		1+ to 2+	1+ to 2+	Neg	Neg	Neg
Other elements (vertebral and sternal cartilage anlagen, developing kidney, liver, gastro-intestinal tract, heart, and lung		Neg	Neg	Neg	Neg	Neg

± = equivocal, 1+ = weak, 2+ = moderate, 3+ = strong, 4+ = intense, Neg = Negative, Pos = Positive, M= Missing (insufficient tissue for evaluation), focal = stained cells located in a single site or restricted number of sites, rare = very few stained cells noted, occas. = occasional stained cells noted singly or in clusters; CynM = cynomolgus monkey

Table 44. Relationship Between Fetal Age and Distribution of AN100226 (natalizumab) Cross-Reactivity in Human and Rhesus and Cynomolgus Monkey Tissues

Age	Donor Species/ Number	Histologic Distribution of Antegren-Reactive Cells/Fibers
46 Days	Cynomolgus Monkey	Subepithelial Embryonal Mesenchyme /Papillary Dermal Fibrils 1+ to 2+ (Head) Developing Vessels 1+ to 2+ (Head) Mesenchymal Cells ± to 2+ (Head, Placenta) Developing Skeletal Muscle 1+ to 3+ (Body)
66 Days	Cynomolgus Monkey	Mononuclear Cells ± to 1+ (Head Posterior [Bone Marrow]) Subepithelial Embryonal Mesenchyme /Papillary Dermal Fibrils ± to 4+ (Head, Thorax, Peritoneal Cavity, Pelvis, Leg) Developing Vessels ± to 2+ (Head, Thorax, Peritoneal Cavity [Kidney and Urachus]), Leg) Developing Skeletal Muscle ± to 3+ (Head, Thorax, Leg) Embryonic Scleral Tissues ± to 2+ (Eyes) Interstitial Stromal Cells ± to 1+ (Thorax, Pelvis) Hepatocytes ± to 1+ (Peritoneal Cavity)
71 Days	Rhesus Monkey 19484	Papillary Dermal Fibrils 1+ to 2+ (Skin) Developing Vessels 1+ to 2+ (Cerebellum) Mononuclear Cells ± to 1+ (Kidney) Interstitial Stromal Cells ± to 1+ (Kidney, Prostate, Spleen) Skeletal Myofibers ± to 1+ (Striated [Skeletal] Muscle)
97 Days	Rhesus Monkey 19469	Mononuclear Cells ± to 1+ (Bone Marrow, Pancreas, Small Intestine [Lamina Propria], Spleen, Stomach [Lamina Propria], Thyroid) Developing Vessels ± to 2+ (Cerebrum, Cerebellum, Eye [Retina], Stomach)
20 Weeks (140 Days)	Human	Mononuclear Cells 1+ to 2+ (Stomach [Lamina Propria]) Developing Vessels 1+ to 2+ (Small Intestine, Stomach) Smooth Muscle Cells 1+ to 2+ (Small Intestine)
22 Weeks (154 Days)	Human	Mononuclear Cells 1+ (Liver) Interstitial Stromal Cells ± to 1+ (Kidney)
22 Weeks (154 Days)	Human	Mononuclear Cells 1+ to 2+ (Spleen) Developing Vessels 1+ to 3+ (Small Intestine) Smooth Muscle Cells 1+ to 3+ (Small Intestine, Urinary Bladder) Interstitial Stromal Cells ± to 2+ (Heart, Pancreas)
22 weeks (154 Days)	Human	Mononuclear Cells 1+ to 3+ (Bone Marrow) Interstitial Stromal Cells 2+ to 3+ (Bone Marrow)
22 weeks (154 Days)	Human	Mononuclear Cells ± to 1+ (Large Intestine [Lamina Propria], Stomach [Lamina Propria], Parathyroid) Developing Vessels ± to 1+ (Peripheral Nerve, Small Intestine, Stomach) Smooth Muscle Cells 1+ to 3+ (Small Intestine)
22 weeks (154 Days)	Human	Interstitial Stromal Cells 1+ (Ovary)

Severity values given in Table 5 reflect the values for both high and low antibody concentrations

Study conclusion: The distribution of natalizumab staining in fetal and neonatal human, and Rhesus and cynomolgus monkey tissues is similar to that observed in adult tissue samples, with the notable exception of staining of developing vasculature and muscle cells. Although several tissues in fetal Rhesus and/or cynomolgus macaques including skin, skeletal myofibers, and developing vascular endothelium in the brain demonstrated cross-reactivity with AN100226 as compared to human tissues, overall there were no unexpected tissue localization results obtained. Taken together, these data demonstrate that fetal tissues from both humans and non-human primates express $\alpha 4\beta 1$ integrin receptor as demonstrated by positive tissue staining with AN100226, and confirm that the cynomolgus or Rhesus monkey are appropriate models to study the reproductive and developmental toxicity of natalizumab.

2.6.4.5 Metabolism

No studies of this type were included in the present submission.

2.6.4.6 Excretion

No studies of this type were included in the present submission.

2.6.4.7 Pharmacokinetic drug interactions

A single toxicology study to determine the toxicity, pharmacodynamic, and toxicokinetic profiles of natalizumab when given in combination with weekly doses of AVONEX® (recombinant, human interferon-β 1a) for 4 weeks was conducted in Rhesus macaques. The toxicokinetics portion of this study, evaluating the immunogenicity, pharmacokinetic and pharmacodynamic interactions between the two biologics is reviewed below.

Study title: Avonex®/Antegren: A four-week combination study of Antegren administered intravenously and Avonex® administered intramuscularly in the Rhesus monkey, followed by an eight-week recovery.

Key study findings: Weekly i/v infusion of natalizumab in Rhesus monkeys at doses of either 30 or 60 mg/kg in combination with 30 μg AVONEX®, i/m (recombinant, human interferon-β 1a, IFN-β) had no effect on serum IFN-β pharmacokinetic, pharmacodynamic, or immunogenicity profiles of either agent, when compared to values obtained in animals treated with AN100226 or AVONEX® alone. Both AVONEX® and natalizumab were well-tolerated at these doses in Rhesus macaques, with no evidence of toxicity. Flow cytometric evaluation revealed that weekly treatment of monkeys with AVONEX® had no effect of the saturation of the α4β1 integrin receptor on peripheral blood mononuclear cells by AN100226.
Study no.: #P00002-01-01 (— Study #1164-87)
Volume #, and page #: EDR files: BLA 125104\000\module4\toxicology\repeat-dose\P00002-01-01.pdf
Conducting laboratory and location: [redacted]

Date of study initiation: June 5, 2001 (final report dated July 31, 2002)
GLP compliance: Yes
QAU statement: yes (X) no ( )
Drug, lot #, and % purity: natalizumab (AN100226; BG00002B-01) manufactured by Biogen, formulated in 10 mM sodium phosphate-buffered saline 0.02% polysorbate 80, lot #MFG-133-01-31, 20 mg/ml; AVONEX® (commercially available material), lot #727-00-0158, 15.0 mg/ml; percent purity of either product was not reported in the sponsor's Certificates of Analysis included as Appendix C to the final study report

Methods

Doses: natalizumab, 30, 60 mg/kg, alone or in combination with AVONEX®, 30 μg (fixed dose) as described in Table 45 below, which was obtained from the sponsor's final study report

Table 45. Treatment Scheme for Combination Toxicity Study of AVONEX® and Antegren in Rhesus Macaques.

Group No.	No. of Animals (M / F)	Treatment	Route	Dose Level	Dose Conc. (mg/mL)	Dose Volume	Number Sacrificed on:	
							Day 29 (M / F)	Day 85 (M / F)
1	5 / 5	Avonex® Vehicle	IM[1]	0	0	1.0 mL	3 / 3	2 / 2
		Antegren Vehicle	IV[2]	0	0	10 mL/kg		
2	5 / 5	Avonex®	IM[1]	30 µg	0.030	1.0 mL	3 / 3	2 / 2
3	5 / 5	Antegren	IV[2]	30 mg/kg	3.0	10 mL/kg	3 / 3	2 / 2
4	5 / 5	Antegren	IV[2]	60 mg/kg	6.0	10 mL/kg	3 / 3	2 / 2
5	5 / 5	Avonex®	IM[1]	30 µg	0.030	1.0 mL	3 / 3	2 / 2
		Antegren	IV[2]	60 mg/kg	6.0	10 mL/kg		
6	5 / 5	Avonex®	IM[1]	30 µg	0.030	1.0 mL	3 / 3	2 / 2
		Antegren	IV[2]	30 mg/kg	3.0	10 mL/kg		

[1] IM = Intramuscular injection administered on Days 1, 8, 15 and 22
[2] IV = 30-minute intravenous infusion administered on Days 1, 8, 15 and 22

Species/strain: *Macaca mulatta* (Rhesus monkey); purpose-bred (_____ and experimentally naïve to treatment

Number/sex/group or time point (main study): 5/sex/dose group (pharmacokinetic portion of study; for the toxicology portion of the study, 3 animals/sex/dose group were used for the main study, and 2/sex/group were dosed and held for the 8-week recovery period)

Route, formulation, volume, and infusion rate: natalizumab, intravenous infusion; natalizumab formulated in 10 mM sodium phosphate-buffered saline, plus 0.02% polysorbate 80, pH 6.2; 10 ml/kg infused; infusion rate 20 ml/kg/h; AVONEX®, commercial product formulated in buffer containing 1.5% human serum albumin, i/m once weekly as bolus injection on Study Days 1, 8, 15, and 22

Sampling times for pharmacokinetics: Blood samples (approximately 6 ml per time point) for measurement of natalizumab and AVONEX® serum concentrations were collected into glass tubes with no added anticoagulant from all monkeys prior to and approximately 2 hours after completion of AN100226 infusion (approximately 2-4 hours after AVONEX® dosing) on Study Days 1, 8, 15, and 22, and on Study Day 28 for evaluation of pharmacokinetic profiles. Additional serum samples for evaluation of anti-natalizumab antibody titers were obtained from all animals prior to dosing on Study Days 1, 15, and 22, then on Study Days 28, 56, and 84 during the treatment-free recovery period. Whole blood samples (approximately 2 ml) for the pharmacodynamic evaluation of AN100226 binding were collected approximately 2 hours following completion of the natalizumab infusion on Study Day 22, and on Study Days 29, 56, and 84 of the recovery period and analyzed by flow cytometry for receptor saturation on peripheral blood lymphocytes.

Age: young, adult male and female animals, approximately 2 – 6 years old

Weight (nonrodents only): 2.2 – 5.8 kg (males), 2.2 – 4.6 kg (females)

Unique study design or methodology (if any): _____

[redacted]

Comment: The final report for this study was submitted to the toxicology section of the [redacted] BLA, and was not initially identified as having pharmacokinetic and pharmacodynamic drug-interaction data present in the data. These data were only recognized as being part of this report

when Module 2 (summary information and Tables, please see Appendix 4, below) was reviewed, and were subsequently added to this review.

Results (includes pharmacokinetic, immunogenicity, and toxicology results)
Mortality, Clinical Observations, and Clinical Pathology: Twice daily cageside observations did not reveal any clinical signs of general or behavioral toxicity that were related to treatment with either natalizumab or AVONEX®, or the combination of the two agents. All monkeys survived until scheduled sacrifice. Sporadic incidences of decreased food consumption, changes in fecal consistency and frequency, and various bruising and/or lacerations were noted in individual animals in all treatment groups including the vehicle control group, and during the treatment-free recovery period. Rectal prolapse, requiring surgical intervention was noted in two monkeys in the group treated with 30 mg/kg/dose natalizumab, but was considered unrelated to treatment since it was not observed in either the higher AN100226 dose group, or in animals treated with the combination of the two agents at either dose level. Food consumption was reported as decreased in several animals over the duration of both the treatment and recovery phases of the study; however, there were no statistically significant, remarkable effects of treatment with AN100226 or AVONEX®, either alone or in combination on individual or group mean body weights, or body weight gains as compared to the control group. No remarkable effects of treatment with AVONEX® or natalizumab, either alone or in combination were observed on electrocardiograms, rectal body temperature, respiratory and heart rates, or ophthalmologic examination.

Blood samples for evaluation of hematology, coagulation, and serum biochemistry profiles were obtained from all animals at Study Days -14 and Day 1 prior to the initiation of dosing, then on Study Days 2, 16, 28, 56, and 84. Urine was collected overnight at these same intervals and subjected to urinalysis, for evidence of renal pathology. There were no definitive treatment-related, remarkable effects of repeated weekly dosing with AVONEX® or AN100226, either alone or in combination on the serum biochemistry, coagulation, or urinalysis profiles at any time point on study, as compared to values obtained for the corresponding vehicle control group, or to mean pre-study baseline values for the different treatment groups. Plasma fibrinogen, activated partial thromboplastin time and prothrombin times showed occasional, statistically significant differences between the control and the treatment groups in this study; however, these findings were also observed during the treatment-free recovery period, and likely reflect random intergroup differences secondary to individual animal variation.

Slight increases in reticulocyte counts were observed in all groups of monkeys treated with AN100226, either alone or in combination with AVONEX®, and were statistically significantly different from the control group on Study Days 2, 16, and 28 in the groups treated with the combination of 30 or 60 mg/kg/dose natalizumab and IFN-β ($p \leq 0.05$, ANOVA with Dunnett's test). Microscopic evaluation of peripheral blood smears from these animals showed clear increases in the incidence of nucleated red blood cells in these dose groups that were most obvious at Study Day 16, but still present at Study Day 28, and resolved completely by Study Day 56. Lesser increases in the incidence of both anisocytosis and polychromatic erythrocytes were also observed in these samples. These findings may be related to the pharmacodynamic inhibition of a4 integrin receptor by natalizumab, since its counter-ligand VCAM-1 has been

implicated in regulating the normal cell traffic between the bone marrow and peripheral blood compartment[5].

Dose-related increases in both total and differential (lymphocyte) counts were observed in the groups of monkeys treated with either 30 or 60 mg/kg/dose of natalizumab alone or in combination with weekly dosing with 30 μg/animal AVONEX®. These increases were statistically significant as compared to control on Study Days 2 and 16 in the monkeys receiving the 30 mg/kg dose of AN100226, and on Study Days 2, 16, and 28 in the groups of animals treated with 60 mg/kg/dose natalizumab and both groups treated with the combination of the two products ($p \leq 0.05$, ANOVA with Dunnett's test). Monocyte, eosinophil, and basophil differential counts were also increased above pre-study baseline in these four groups, and were statistically significantly different from the vehicle control group ($p \leq 0.05$, ANOVA with Dunnett's test) at differing time points out to Study Day 28. There were no statistically significant differences detected between the vehicle control and the natalizumab or AVONEX® treated groups at Study Days 56 and 84, during the treatment-free recovery period. Increased total and differential leukocyte counts were not observed in the animals treated with AVONEX® alone; therefore, these findings are related to natalizumab, and are expected pharmacodynamic responses to blockade of the α4 integrin subunit by AN100226.

Statistically significant decreases in absolute neutrophils counts were observed on Study Days 2 and 16 for animals treated with 30 mg AVONEX® alone, when compared to both pre-study and vehicle control values ($p \leq 0.05$, ANOVA with Dunnett's test). However, the majority of the individual animal values were still within normal range for this strain of macaques, and these decreases were not observed in the monkeys treated with AVONEX® in combination with either dose of natalizumab. There were no decreases in absolute neutrophils counts noted in any of the groups of monkeys treated with AN100226, either alone or in combination with AVONEX®. Therefore, the findings in the AVONEX® control group are likely related to individual variation between animals, and are not a toxicity of IFN-β.

Sporadic gross lesions were identified at necropsy in animals in all treatment groups including the vehicle control, and were not considered treatment-related. Spleen weights were increased at the Study Day 29 sacrifice in all groups of monkeys administered 30 or 60 mg/kg/dose of natalizumab, either alone or in combination with AVONEX®, and were still slightly elevated over the control group at recovery sacrifice on Study Day 84. Microscopic evaluation of spleen sections revealed areas of lymphoid hyperplasia in all groups of AN100226-treated monkeys at Study Day 29, that were completely resolved by the end of the recovery period. Other, treatment-related microscopic findings at Study Day 29 included B and T lymphocyte hyperplasia in the spleen and lymph nodes, and leukocytosis and leukocytic infiltrates in the liver, which were partially resolved on microscopic evaluation of the tissues at the end of the recovery period on Study Day 84. Once control monkey, and 2/6 monkeys each in the groups treated with AVONEX® alone or in combination with 30 mg/kg/dose of natalizumab had histologic evidence of minimal to mild, myofiber degeneration and necrosis at the i/m injection site for AVONEX® at the Day 29 sacrifice, which was decreased in both incidence and severity at the recovery sacrifice. There were no other microscopic findings that were considered related to treatment with natalizumab, either alone or in combination with weekly doses of AVONEX®.

[5] Kravitz, J.A. *et al.*, 1996. Adhesion receptors on bone marrow stromal cells: *In vivo* expression of vascular cell adhesion molecule-1 by reticular cells and sinusoidal endothelium in normal and gamma-irradiated mice. *Blood,* 87:7342-7347.

Pharmacodynamic effects: The effects of natalizumab treatment either alone or in combination with AVONEX® on lymphocyte subset profiles (T cell and B cell numbers) were evaluated in samples of peripheral blood mononuclear cells by flow cytometry. Total lymphocyte counts, as determined by hematology analysis were elevated in all groups of animals relative to both the control and AVONEX® alone treated monkeys at Study Days 2, 16, and 28. Flow cytometric evaluation of different lymphocyte subsets revealed increases in the absolute numbers of CD20-positive B lymphocytes, and CD3+/CD4+, cytotoxic T lymphocytes; however, these increases were not significantly different from the control groups. AVONEX® treatment alone had no effect on either the lymphocyte numbers, or the absolute counts of the B and T lymphocyte subsets. There were no significant or remarkable differences in lymphocyte subsets in any of the AN100226 or AVONEX®-treated groups at the Study Day 84 evaluation, as compared to the control group.

Saturation of the α4 integrin receptor on the cell surface of peripheral blood monocytes and lymphocytes in treated and control monkeys was evaluated by flow cytometry, following dosing with 30 or 60 mg/kg/dose AN100226, either alone or in combination with 30 mg/dose AVONEX® weekly for 4 weeks. As expected, natalizumab staining was highest following the final dose on Study Day 22, and diminished during the treatment-free recovery period. There was no effect of AVONEX® treatment on the level of either α4β1 integrin receptor binding, or percent saturation after natalizumab treatment. The results from this study are shown in Figures 29 and 30 below, which were provided by the sponsor in the final study report.

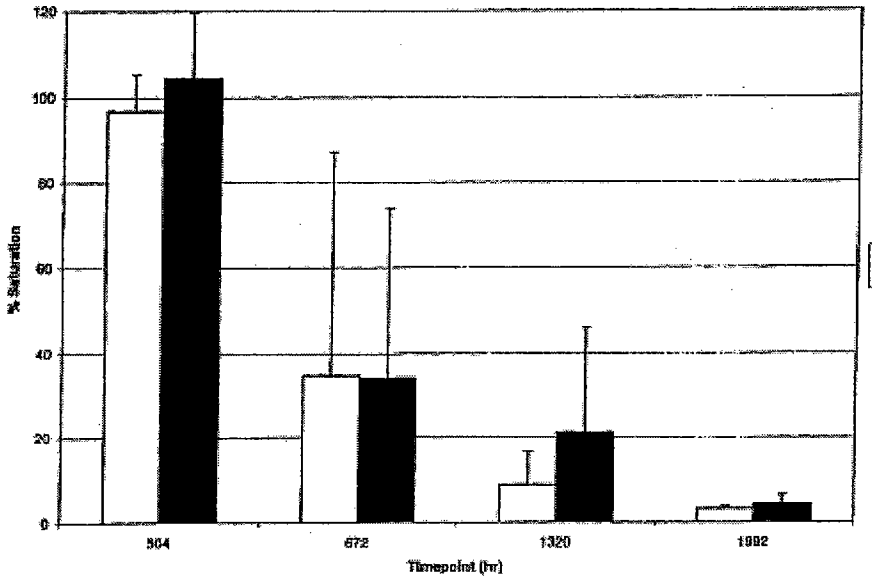

Figure 29. Percent receptor saturation of Rhesus monkey peripheral blood lymphocytes after treatment with 30 mg/kg natalizumab alone (open bars) or in combination with 30 μg AVONEX® (filled bars). Values represent the mean of calculated percent receptor saturation for individual animals in each group ± S.D., at the indicated time points on study (n = 4-10/time point).

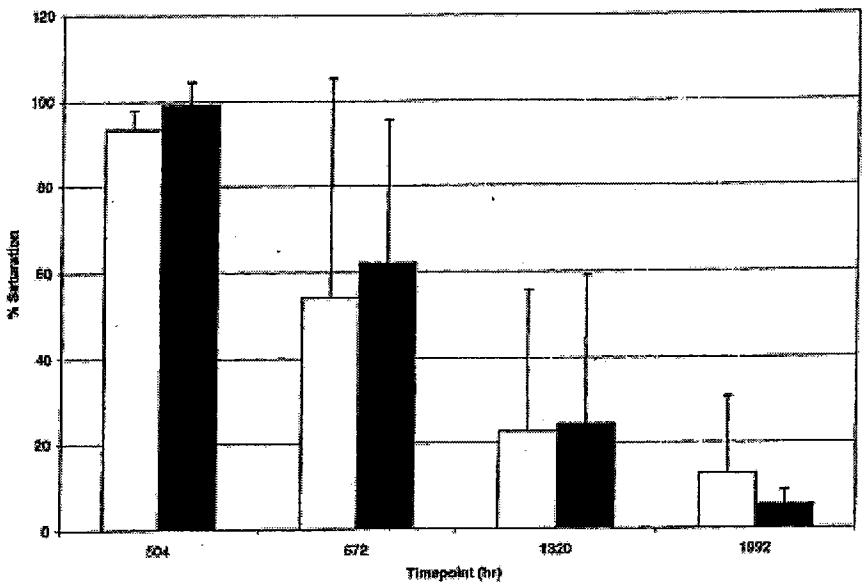

Figure 30. Percent receptor saturation of Rhesus monkey peripheral blood lymphocytes after treatment with 60 mg/kg natalizumab alone (open bars) or in combination with 30 μg AVONEX® (filled bars). Values represent the mean of calculated percent receptor saturation for individual animals in each group ± S.D., at the indicated time points on study (n = 4-10/time point).

Pharmacokinetics: Serum concentrations of both natalizumab and IFN-β were measured at various time points on study as described above in the Methods section. Figures 31 and 32 below were obtained from the sponsor's final study report, and show the peak (C_{max}) and trough (C_{min}) serum levels of AN100226 after dosing with either 30 mg/kg/dose (Figure 31) or 60 mg/kg/dose natalizumab (Figure 32), alone or in combination with 30 μg/week AVONEX®. Figure 33 below, also provided by the sponsor in the final study report, shows the serum IFN-β concentrations following treatment with AVONEX® alone, or in combination with 30 or 60 mg/kg/week natalizumab. Low, but detectable serum levels of AN100226 were present in Rhesus monkeys at each pre-dose time point (C_{min}) prior to weekly natalizumab dosing; however, there were no increases in the mean pre-dose values observed with subsequent doses, indicating that there is no accumulation of AN100226 occurring over the duration of the study (Figures 31 and 32). Pre-dose serum levels of IFN-β (C_{min}) were below the limits of the assay detection ——— at all time points, indicating that there is no accumulation of AVONEX® after weekly i/m administration to Rhesus monkeys for 4 weeks (Figure 33). There were no statistically significant differences in either serum AN100226 or IFN-β C_{max} concentrations in the groups of monkeys treated with the combination of the two agents, as compared to the respective values obtained for the groups treated with either biologic alone (statistical data not shown in this review). An apparent, statistically significant, gender related difference in AN100226 C_{max} concentration was noted between male and female monkeys within the group treated with 60 mg/kg/dose natalizumab alone at 336 and 504 hours after the final doses of natalizumab and AVONEX® (Figure 32, below). Further analysis of the immunogenicity data from this study (please see below) indicated that 3 of 5 female monkeys, and 0/5 male monkeys had anti-natalizumab antibodies present at Study Day 28, which was the likely reason for the apparent, gender related difference at these two time points.

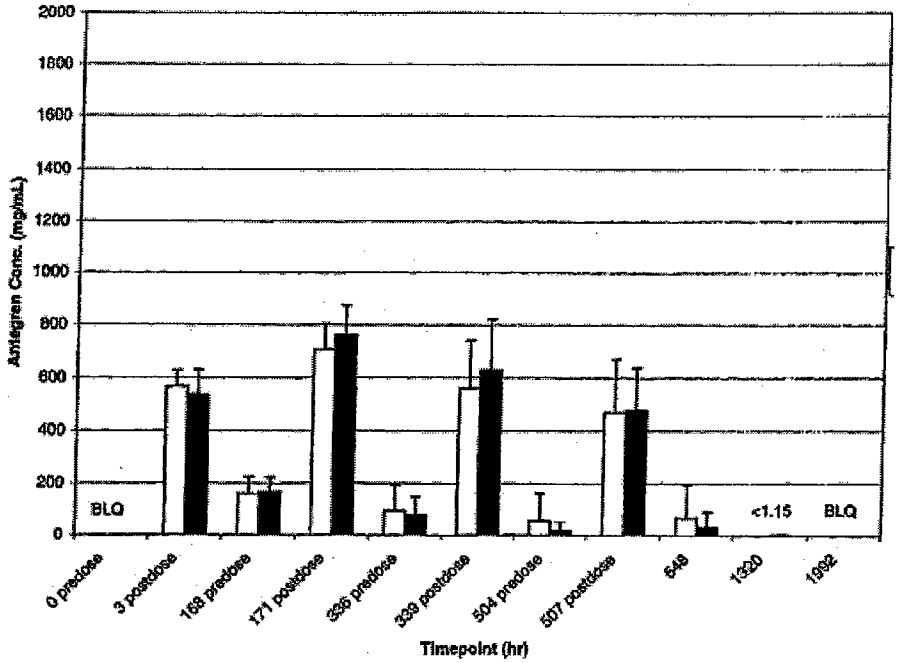

Figure 31. Serum AN100226 peak and trough concentrations Rhesus monkeys treated with 30 mg/kg natalizumab alone (open bars) or in combination with 30 µg AVONEX® (filled bars). Values represent the mean of calculated C_{max} and C_{min} values for individual animals in each group ± S.D., at the indicated time points on study (n = 4-10/time point).

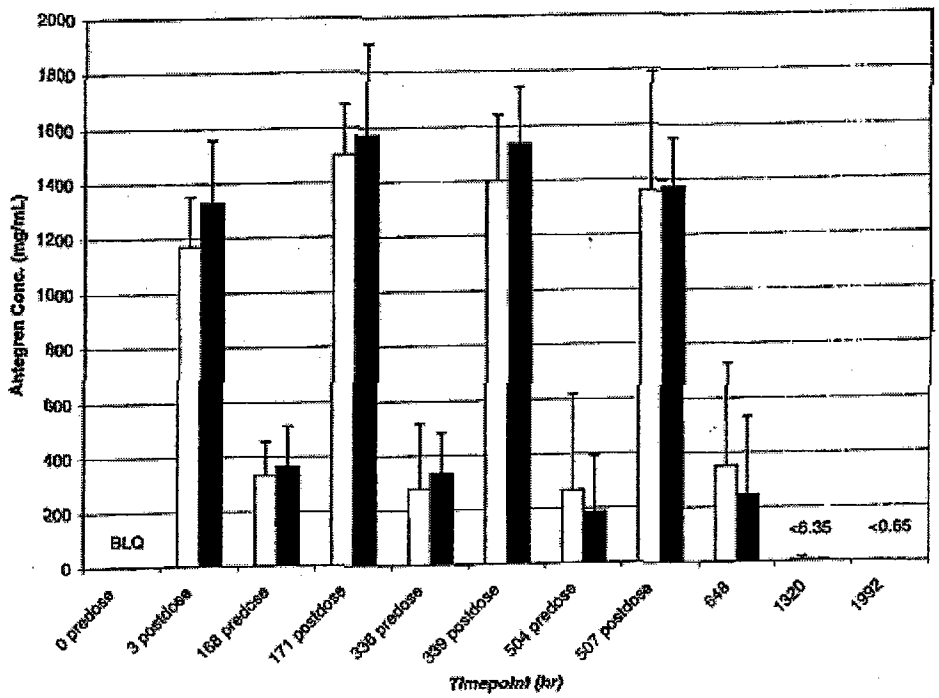

Figure 32. Serum AN100226 peak and trough concentrations Rhesus monkeys treated with 60 mg/kg natalizumab alone (open bars) or in combination with 30 µg AVONEX® (filled bars). Values represent the mean of calculated C_{max} and C_{min} values for individual animals in each group ± S.D., at the indicated time points on study (n = 4-10/time point).

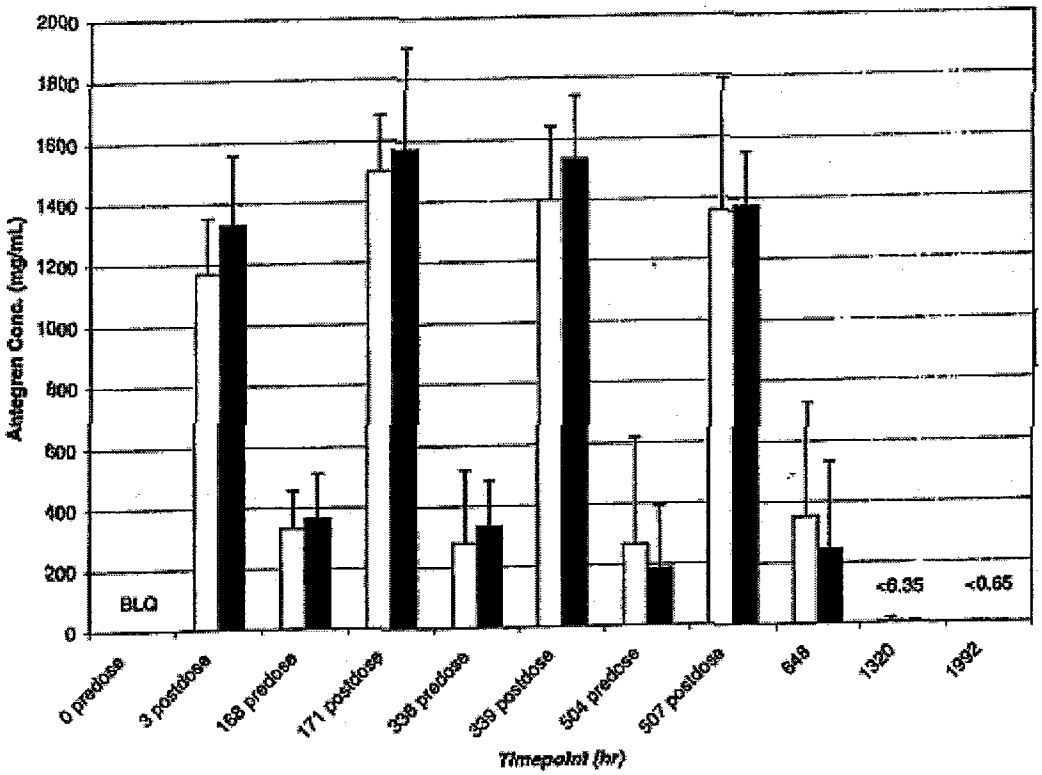

Figure 33. Serum peak and trough IFN-β AVONEX® activity in Rhesus monkeys treated with 30 μg AVONEX® alone (open bars), or in combination with 30 mg/kg/dose natalizumab (filled bars) or 60 mg/kg/dose (hatched bars). Values represent the mean of calculated C_{max} and C_{min} values for individual animals in each group ± S.D., at the indicated time points on study (n = 4-10/time point).

In conclusion, treatment of Rhesus monkeys by i/m injections with 30 μg/dose AVONEX® weekly, for 4 weeks had no remarkable or statistically significant effects on serum natalizumab concentrations, following weekly treatment with 30 or 60 mg AN100226/kg/dose. Conversely, there were no effects of concomitant natalizumab treatment at either dose level on serum IFN-β activity in Rhesus monkeys, as compared to animals treated with AVONEX® alone.

Immunogenicity: Anti-natalizumab antibodies were not detectable in serum samples from any animals treated with either vehicle control, or 30 μg/dose AVONEX® alone, at any time point on study. Low, but detectable anti-AN100226 antibodies were present beginning Study Day 22 in 2/5 male and 4/5 female monkeys treated with 30 mg/kg/dose natalizumab alone, and in 1/5 male and 3/5 female monkeys treated with 60 mg/kg/dose AN100226. Concentrations of anti-natalizumab serum antibodies were inversely related to the dose of AN100226 administered (data not shown in this review). In all recovery animals in these two dose groups, anti-AN100226 antibody levels in serum continued to increase during the treatment-free recovery period, with maximal concentrations of [redacted] at Study Day 84 in the monkeys treated with 30 or 60 mg/kg/dose AN100226, respectively. One male monkey (animal #R16857M) in the 60 mg/kg/dose natalizumab alone dose group remained below the level of quantitation of the assay for anti-AN100226 antibody at all time points on study, including during the recovery phase.

Comment: Monkey #R16857M in the 60 mg/kg natalizumab alone dose group still had low, but detectable serum levels of AN100226 present at terminal sacrifice on Study Day 84, while serum natalizumab levels in all other recovery animals in this dose group were below the level of quantitation of the assay. It cannot be determined from the information included in the final study report whether the presence of natalizumab in the plasma/serum can inhibit detection of anti-product antibody.

Comment: The 60 mg/kg AN100226 alone dose group had an apparent, gender related difference in serum natalizumab concentrations in the pharmacokinetics portion of this study at the Study Day 28 and Day 56 time points (please see Figure 32, above). At Study Day 28, both recovery female monkeys #R169104F and #R16375F were positive for anti-AN100226 antibody, with concentrations of 10.4 µg/ml and 23.4 µg/ml, respectively present in serum. These levels continued to increase following discontinuation of natalizumab dosing, to 45.3 µg/ml and 47.1 µg/ml, respectively on Study Day 56, and 99.1 µg/ml and 82.5 µg/ml, respectively at study termination on Day 84. These high anti-AN100226 antibody levels could result in more rapid clearance of natalizumab from the plasma, and thereby account for the apparent gender difference in C_{max} observed at these time points.

In the two groups of animals treated with natalizumab in combination with weekly dosing with AVONEX®, levels of anti-AN100226 antibody remained below the limits of quantitation of the assay at Study Day 28 in 4/5 male and 2/5 female monkeys treated with IFN-β and 30 mg/kg/dose natalizumab, and in all 10 animals treated with AVONEX® in combination with 60 mg AN100226/kg/dose. Anti-natalizumab antibody serum levels in the remaining animals in the low-dose natalizumab/AVONEX® combination group at Study Day 28 were 16.9 µg/ml in male monkey #R16976M, and 7.2 µg/ml, 7.8 µg/ml, and 18.2 µg/ml in female animals #R169114F, #R16892F, and #R170111F, respectively. Two female monkeys that were antibody positive at Study Day 28 were euthanized as scheduled sacrifices, so no additional data are available regarding anti-natalizumab antibody levels following discontinuation of dosing. In the remaining recovery animals in this dose group, serum anti-AN100226 titers remained below the limits of quantitation of the assay in male monkey #R17187M, and increased to 71.8 µg/ml at Study Day 56, and 88.6 µg/ml at Study Day 84 in monkey #R16976M. Serum anti-natalizumab antibodies also increased with time after completion of dosing in the two female monkeys in the 30 mg/kg/dose natalizumab and AVONEX® dose group, with monkey #R16973F first developing an antibody titer of 26.9 µg/ml on Study Day 56 and increasing to 88.1 µg/ml on Study Day 84, and monkey #R170111F increasing from the Study Day 28 value to 23.1 µg/ml at study termination. Of interest, the Study Day 56 antibody titer in this animal showed a slight decrease to 16.3 µg/ml from the previous value of 18.2 µg/ml on Study Day 28. The biological relevance of this decrease in titer is presently unknown.

A dose-related decrease in both incidence and serum level of anti-AN100226 antibodies was observed for animals treated with 60 mg/kg/dose natalizumab in combination with 30 µg/dose AVONEX® for four weeks. In this group, anti-AN100226 serum antibody levels remained below the limits quantitation for the assay in female #R170118F for the entire study duration, and were not initially detectable in the remaining animals until Study Day 56, at which point titers of 21.6 µg/ml, and 19.7 µg/ml and 21.9 µg/ml were observed in the remaining female monkey #R16962F, and in male monkeys #R17172M and #R15993M, respectively. In all three recovery monkeys with detectable anti-natalizumab antibody, serum concentrations of anti-AN100226 decreased by the completion of the recovery period on Study Day 84, with final titers of 18.1

µg/ml in female monkey #R16962F, and 13.8 µg/ml and 16.9 µg/ml for male monkeys #R17172M and #R15993M, respectively.

In conclusion, there were no apparent effects of AVONEX® treatment on the immunogenicity of natalizumab after 4 weekly doses of either 30 or 60 mg AN100226/kg/dose, as compared to serum anti-AN100226 antibody levels in monkeys treated with either dose of natalizumab alone.

Comment: This study had too few animals in the treatment and recovery groups to adequately determine any statistical differences in anti-AN100226 immunogenicity between the treatment groups, and the relationship of any results obtained to concomitant treatment with 30 µg/dose AVONEX®. However, the effects of AVONEX® on the pharmacokinetics and immunogenicity of natalizumab in multiple sclerosis patients will be evaluated in a future clinical study, as part of the post-marketing commitments from the sponsor.

Both total anti-IFN-β binding and neutralizing antibodies were negative in all control animals at all time points on study, with the exception of monkey #R16963Fin the control group, who had a single, equivocal result at Study Day 56 that was also equivocal on re-test. No anti-IFN-β antibody activity was detected in monkeys treated with 30 mg/kg/dose natalizumab alone at any time point on study. Positive total binding anti-IFN-β antibody was detected in serum samples at a single time point in 2 male monkeys (animals #R16857M at Study Day 56, and #R98E130M at Study Day 28), and beginning prior to treatment and persisting throughout the study for 1 female monkey (animal #R16375F) in the group treated with 60 mg/kg natalizumab alone. There was no detectable neutralizing antibody activity present in these animals, and re-testing of the samples confirmed the original test results.

Total binding antibody to IFN-β was detectable in 10/10 monkeys in the group treated with AVONEX® alone, beginning as early as Study Day 15, and persisting in all 4 recovery animals until terminal sacrifice at Study Day 84. Neutralizing anti-IFN-β antibody titers were more variable in both severity and time to appearance, with 2/5 each male and female monkeys having no detectable titers at Study Day 28. Low titers of neutralizing antibody were detectable in the remainder of the animals, with two male monkeys (animals #R19677M and #R197185M) reaching peak titers of 120 on Study Day 56, and declining to background levels by study termination. In the group of monkeys treated with 30 mg/kg/dose AN100226 in combination with 30 mg/week AVONEX®, serum anti-IFN-β total binding antibodies were detectable in 3/5 each male and female monkeys, beginning at Study Day 22, and persisting in the two male recovery animals until study termination at Day 84. Neutralizing antibody was present at low titer on only a single male monkey (animal #R16976M) in this dose group, with a titer of 4 detected on Study Day 56, and a titer of 84 detected on Study Day 84. Anti-IFN-β binding antibodies were detected in 3/5 each male and female monkeys in the group treated with AVONEX® in combination with 60 mg/kg/dose natalizumab. One female monkey in this dose group (animal #R16998F) had a detectable neutralizing antibody of 3 present at terminal sacrifice on Study Day 28, while a second female monkey (#R170118F) developed an initial neutralizing titer of 120 on Study Day 28, which increased to 1040 on Study Day 56, and declined to a titer of 360 by terminal sacrifice on Study Day 84.

Comment: Due to the small number of animals retained for the recovery period in this study and the high-degree of inter-animal variability in the titers of neutralizing anti-IFN-β antibody developed, it is not possible to determine whether any statistical differences in anti-IFN-β antibody responses exist between the groups treated with AVONEX® alone, and in combination with 30 or 60 mg/kg/dose natalizumab. To determine if natalizumab treatment affects the

immunogenicity of concomitantly administered AVONEX® with adequate power to detect any statistical differences between the groups, a much larger study would need to be conducted. However, the effects of natalizumab on AVONEX® pharmacokinetics and immunogenicity in multiple sclerosis patients will be evaluated in a future clinical study, as part of the post-marketing commitments from the sponsor.

Study Conclusion: In conclusion, treatment of Rhesus monkeys with 30 µg/dose AVONEX®, either alone or combination with 30 mg/kg/dose or 60 mg/kg/dose of natalizumab once weekly for 4 weeks was well-tolerated, with no unexpected clinical, hematologic, serum biochemistry, or microscopic pathologies observed. Increases in total leukocyte counts, and differential lymphocyte, monocyte, and eosinophil counts, and lymphocyte B and T cell subsets were observed in all groups of natalizumab treated monkeys, and are expected pharmacologic effects of the antibody. There were no apparent effects of AVONEX® treatment on AN100226 serum levels, receptor binding and saturation, or immunogenicity of natalizumab at either the 30 or 60 mg/kg/dose levels. Conversely, there was no apparent effect of AN100226 treatment at either dose level on the pharmacokinetic profile and immunogenicity of AVONEX®.

2.6.4.8 Other Pharmacokinetic Studies

No additional pharmacokinetic studies have been submitted to this licensing application.

2.6.4.9 Discussion and Conclusions

Pharmacokinetic studies of natalizumab in cynomolgus monkeys and male and pregnant and non-pregnant female guinea pigs showed dose-related, although non-linear increases in C_{max}, AUC_{0-last}, elimination half-life, and mean residence time, and decreases in systemic clearance. The dose-related increases in half-life, with concomitant decreases in systemic clearance suggest that the mechanism of natalizumab clearance is saturable, resulting in prolonged exposures to the biologic. Tissue distribution studies with either AN100226m or natalizumab showed specific, cell-membrane associated staining present primarily in human and cynomolgus monkey lymphoid organs, including lymph node, spleen, thymus, and tonsil, and gut-associated lymphoid tissue in the large and small intestines, and in occasional interstitial lymphocytes present in the bone marrow, salivary gland, and prostate. Similar patterns of natalizumab staining were observed in fetal and perinatal human tissue samples, and fetal Rhesus and cynomolgus monkey tissues as compared to the adult samples. Treatment of Rhesus monkeys with 30 µg/dose AVONEX®, either alone or combination with 30 mg/kg/dose or 60 mg/kg/dose of natalizumab once weekly for 4 weeks was well-tolerated, with no unexpected clinical, hematologic, serum biochemistry, or microscopic pathologies observed. Increases in total leukocyte counts, and differential lymphocyte, monocyte, and eosinophil counts, and lymphocyte B and T cell subsets were observed in all groups of natalizumab treated monkeys, and are expected pharmacologic effects of the antibody. There were no apparent effects of AVONEX® treatment on AN100226 serum levels, receptor binding and saturation, or immunogenicity of natalizumab at either the 30 or 60 mg/kg/dose levels. Conversely, there was no apparent effect of AN100226 treatment at either dose level on the pharmacokinetic profile and immunogenicity of AVONEX®.

2.6.4.10 Tables and figures to include comparative TK summary

Tables and figures, where obtained from the submitted final sponsor or contracting laboratory study reports, are included in the body of the text in this section, and will not be repeated here.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

A tabulated summary of all preclinical pharmacokinetic studies included in the BLA, as provided by the sponsor in Module 2 of the electronic CTD submission is attached to this review as Appendix 3.

2.6.6 TOXICOLOGY

Toxicology data for single and repeat-dose studies, genetic toxicology and reproductive and developmental toxicology were reviewed separately by Barbara J. Wilcox, Ph.D. and are not included in this review. Toxicology data that were derived from the single dose pharmacokinetic studies of natalizumab conducted in cynomolgus monkeys, and from single and repeat administration studies in the guinea pig were reviewed with their respective studies under the Absorption section of the Pharmacokinetics module (Section 2.6.4.3, above), and did not demonstrate any novel or unexpected adverse events following single dose administration of natalizumab.

2.6.6.1 Overall toxicology summary

General toxicology: Please see review by Barbara J. Wilcox, Ph.D. for this information.

Genetic toxicology: Please see review by Barbara J. Wilcox, Ph.D. for this information.

Carcinogenicity: No studies of this type were included in the present submission.

Reproductive toxicology: Please see review by Barbara J. Wilcox, Ph.D. for this information.

Special toxicology: Please see review by Barbara J. Wilcox, Ph.D. for this information.

2.6.6.2 Single-dose toxicity

Comment: Bioequivalence studies were conducted to evaluate the comparability of AN100226 produced during manufacturing changes to material used previously in clinical and preclinical studies. The primary purpose of these studies was the pharmacokinetic evaluation of exposure levels of AN100226 produced by the two different methods, which was reviewed under Section 2.6.4.3, above. Toxicity data for natalizumab was also collected in the course of these studies, and was reviewed with the respective studies, above and will not be included here.

2.6.6.3 Repeat-dose toxicity

These studies were reviewed separately by Barbara J. Wilcox, Ph.D. and are not included in the present review memorandum.

2.6.6.4 Genetic toxicology

These studies were reviewed separately by Barbara J. Wilcox, Ph.D. and are not included in the present review memorandum.

2.6.6.5 Carcinogenicity

No studies of this type were included in the present BLA submission. Studies to evaluate growth- and tumor promotion potential of AN100226 were conducted both *in vitro* and *in vivo* using human cell lines, and immunodeficient mouse tumor xenografts. These studies were reviewed separately by Barbara J. Wilcox, Ph.D. and are not included in the present pharmacology review.

2.6.6.6 Reproductive and developmental toxicology

These studies were reviewed separately by Barbara J. Wilcox, Ph.D. and are not included in the present review memorandum.

2.6.6.7 Local tolerance

These studies were reviewed separately by Barbara J. Wilcox, Ph.D. and are not included in the present review memorandum.

2.6.6.8 Special toxicology studies

These studies were reviewed separately by Barbara J. Wilcox, Ph.D. and are not included in the present review memorandum.

2.6.6.9 Discussion and Conclusions

Single doses of either AN100226m or natalizumab (AN100226) administered by i/v infusion to cynomolgus monkeys, and repeat administration of AN100226 once weekly for 4 weeks in Rhesus monkeys were generally well-tolerated, with toxicities limited to dose-related increases in both total leukocyte and differential lymphocyte, monocyte, eosinophil, and basophil counts, and histologic evidence of lymphoid proliferation in the spleen and lymph nodes (please see Sections 2.6.4.3, and 2.6.4.8, above). The effects are secondary to exaggeration of the pharmacologic activity of the anti-α4 integrin antibody in inhibiting leukocyte migration across vascular endothelium.

2.6.6.10 Tables and Figures

Not included in this review.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Not included in this review.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Single dose toxicity studies of natalizumab (TYSABRI™) conducted in conjunction with the pharmacokinetic studies showed that doses of up to 30 mg/kg, i/v were well-tolerated, with only leukocytosis observed. Natalizumab was immunogenic in all species tested, including guinea pigs and cynomolgus and Rhesus macaques, even following a single dose administration.

Unresolved toxicology issues (if any): There are no unresolved, preclinical pharmacology issues that need to be addressed by the sponsor at the present time.

Recommendations: Recommendation is for approval of natalizumab (TYSABRI™), based upon an acceptable preclinical pharmacology, pharmacokinetic, and tissue distribution package to support its clinical activity in multiple sclerosis.

Suggested labeling: Recommended changes to the CLINICAL PHARMACOLOGY section of the label are included as Appendix 1 to this review, below.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

APPENDIX 1 – RECOMMENDED CHANGES TO THE SPONSOR LABELING

APPENDIX 2 – LITERATURE REFERENCES IN SUPPORT OF THE PHARMACOLOGIC ACTIVITY OF NATALIZUMAB

APPENDIX 3 - TABULATED SUMMARY OF PRECLINICAL PHARMACOLOGY STUDIES CONDUCTED WITH NATALIZUMAB

APPENDIX 4 – TABULATED SUMMARY OF PRECLINICAL PHARMACOKINETIC STUDIES CONDUCTED IN SUPPORT OF NATALIZUMAB

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2 Page(s) of Draft Labeling have been Withheld from this Portion of the Review.

APPENDIX 2 – LITERATURE REFERENCES IN SUPPORT OF THE PHARMACOLOGIC ACTIVITY OF NATALIZUMAB

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APPENDIX 3 - TABULATED SUMMARY OF PRECLINICAL PHARMACOLOGY STUDIES CONDUCTED WITH NATALIZUMAB (TYSABRI™)

The following tables were copied directly from Module 2, section 2.6.3 of the electronic CTD submission, as provided by the sponsor to the BLA application.

1 Pharmacology

Overview **Test Article:** natalizumab

Type of Study	Test System	Method of Administration	Testing Facility	GLP Compliance	Study Number	Location Section
Primary Pharmacodynamics						
Receptor binding characterization	Human leukocytic cell lines, human peripheral leukocytes	In vitro	Elan Pharmaceuticals	No	PC032	M4.2.1.1
Receptor expression – normal and patient	Human lymphocytes	In vitro	Elan Pharmaceuticals	No	PC100	M4.2.1.1
Receptor expression and binding – toxicology species	Human, guinea pig, cynomolgus monkey, rhesus monkey lymphocytes	In vitro	Elan Pharmaceuticals	No	309-001-03	M4.2.1.1
Efficacy - Experimental Allergic Encephalomyelitis	Guinea Pig	IC	Elan Pharmaceuticals	No	PB554	M4.2.1.1
Efficacy - Experimental Allergic Encephalomyelitis	Guinea Pig	SC	Elan Pharmaceuticals	No	PB555	M4.2.1.1
Efficacy - Experimental Allergic Encephalomyelitis	Guinea Pig	SC	Elan Pharmaceuticals	No	AL078	M4.2.1.1
Efficacy - Experimental Allergic Encephalomyelitis	Guinea Pig	SC	Elan Pharmaceuticals	No	310-1-A	M4.2.1.1

1 **Pharmacology (continued)**

Type of Study	Test System	Method of Administration	Testing Facility	GLP Compliance	Study Number	Location Section
Secondary Pharmacodynamics						
Immunomodulatory Potential	Human peripheral leukocytes	In vitro	[redacted]	No	PC028	M4.2.1.2
Immunomodulatory Potential	Cynomolgus monkey	IV		Yes	AL106	M4.2.3.2
Safety Pharmacology			[redacted]			
Cardiovascular/Respiratory	Beagle dogs	IV		Yes	AL107	M4.2.1.3
Cardiovascular	Cynomolgus monkey	IV		Yes	723-013-98	M4.2.3.2
Cardiovascular	Cynomolgus monkey - juvenile	IV	[redacted]	Yes	309-011-00	M4.2.3.2
Cardiovascular	Rhesus monkey	Avonex® - IM natalizumab - IV		Yes	P00002-01-01	M4.2.3.2
Pharmacodynamic Drug Interactions			[redacted]			
Avonex®/natalizumab	Rhesus monkeys	Avonex® - IM natalizumab - IV	[redacted]	Yes	P00002-01-01	M4.2.3.2
SC = subcutaneous, IV = intravenous, IM = intramuscular						

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2 **Primary Pharmacodynamics**

Overview **Test Article:** natalizumab

Study No.	Test Species	Dose Level (mg/kg)	Study Day	Fold Change in Cell Population Counts Versus Control Animals[a]			Location
				WBC	Lymphocytes	Neutrophils	Section
309-007-01	Guinea pig	3 10 30	37-75	1.33 1.33 1.50	1.38 1.42 1.56	1.17 1.12 1.29	M4.2.3.5.1
309-008-01	Guinea pig	3 10 30	60-95	1.23 1.39 1.26	1.44 1.63 1.65	1.01 1.08 0.80	M4.2.3.5.1
WSRC 940911	Cynomolgus monkey	3 10 30	8	1.13 0.98 1.14	1.49 1.10 1.29	0.67 0.83 0.92	M4.2.3.2
AL106	Cynomolgus monkey	0.3 3 30	8	1.31 1.65 1.48	1.71 2.26 2.28	0.90 1.03 0.74	M4.2.3.2
723-013-98	Cynomolgus monkey	3 10 30 60	22	0.98 1.06 1.42 1.58	1.31 1.48 2.18 2.43	0.61 0.64 0.74 0.70	M4.2.3.2
309-011-00	Cynomolgus monkey - juvenile	10 30 60	23	1.37 1.90 1.82	1.63 2.34 2.28	0.92 1.09 0.98	M4.2.3.2
309-012-00	Cynomolgus monkey	3 10 30	45	1.49 2.13 2.36	1.73 2.53 2.81	0.88 1.16 1.29	M4.2.3.5.2
309-033-01	Cynomolgus monkey	30 (GD20-70) 30 (GD20-term)	70	1.64 1.84	2.18 2.60	1.10 1.08	M4.2.3.5.3

2 **Primary Pharmacodynamics (continued)**

Study No.	Test Species	Dose Level (mg/kg)	Study Day	Fold Change in Cell Population Counts Versus Control Animals*			Location Section
P00002-01-01	Rhesus monkey	30 60	2	1.32 1.41	1.94 2.35	0.89 0.81	M4.2.3.2

* As determined at the first post-dose time point tested in the study. Comparison is to natalizumab vehicle in studies where both saline and natalizumab vehicle controls are present.

WBC = white blood cell

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