Table 11. Cross-reactivity of AN100226m with α4 and α4β1, but not β2 or β7 integrins (experiment #1)

	Control Transfected L Cell	Human $\alpha_4\beta_1$ Integrin Transfected L Cell	— Cells
IgG_1 (Negative control antibody)	8	9	3
TS2/16 (control antibody against β_1 integrin)	12	326	3
AN100226m	9	386	301
IOT18 (control antibody against β_2 integrin)	9	10	163
ACT-1 (control antibody against β_7 integrin	9	10	300

Table 12. Cross-reactivity of AN100226m with α4 and α4β1, but not β1 integrins (experiment #2)

Primary Antibody	JY Cells	K562	Ramos
None (Negative control)	3	3	2
HP2/1 (control antibody against α_4 integrin)	57	7	68
AN100226m	56	13	75
TS2/16 (control antibody against β_1 integrin)	9	84	86
84H10 (control antibody against ICAM-1)	112	63	88

In both experiments, AN100226m bound to α4 integrin expressing cell lines, including the transfected L cells, human Ramos T lymphoid cells that also express α4β1 integrin complex, and human JY and — B lymphoid cells that express the α4 subunit, but not β1 integrin. There was no detectable binding of AN100226m to human K562 myeloid leukemia cells, which express the β1 subunit in the absence of α4 integrin. Taken together, these data support the conclusion that AN100226m recognizes the α4 subunit of the α4β1 integrin complex independently of the β1 integrin.

Competition studies of HP2/1 anti-α4 integrin antibody binding by AN100226m antibody are shown in Table 13 below, which was derived from data presented in the sponsor's final study report. Both HP2/1 and AN100226m could effectively compete with the binding of FITC-labeled

HP2/1 to the surface of U937 cells, suggesting that they recognize the same epitope on α4 integrin. The [redacted] antibodies directed against the β1 integrin had no effect on FITC-labeled HP2/1 binding.

Table 13. AN100226m competition of control anti-α4 integrin antibody binding on human U937 monocytic cells

Antibody Pre-Treatment	Secondary Antibody	Mean Channel Fluorescence
None	IgG1-FITCa	4
None	HP2/1-FITC	76
HP2/1	HP2/1-FITC	6
AN100226m	HP2/1-FITC	17
[redacted] (anti-human β1 integrin)	HP2/1-FITC	75
[redacted] (anti-human β1 integrin)	HP2/1-FITC	73

Immunoprecipitation studies with AN100226m on U937 cell lysates confirmed the binding of AN100226m to the same molecular weight proteins as recognized by the HP2/1 and L25 anti-α4 integrin antibodies. U937 cells express α4 integrin as a 150 kD protein, which is largely cleaved on the cell surface to two fragments of [redacted]. U 937 cells also express low levels of a [redacted] modified variant of α4 integrin, and a [redacted] β1 integrin. Figure 19, which was provided by the sponsor in the final study report shows the immunoprecipitation analysis of AN100226m binding to U937 cell lysates.

[redacted]

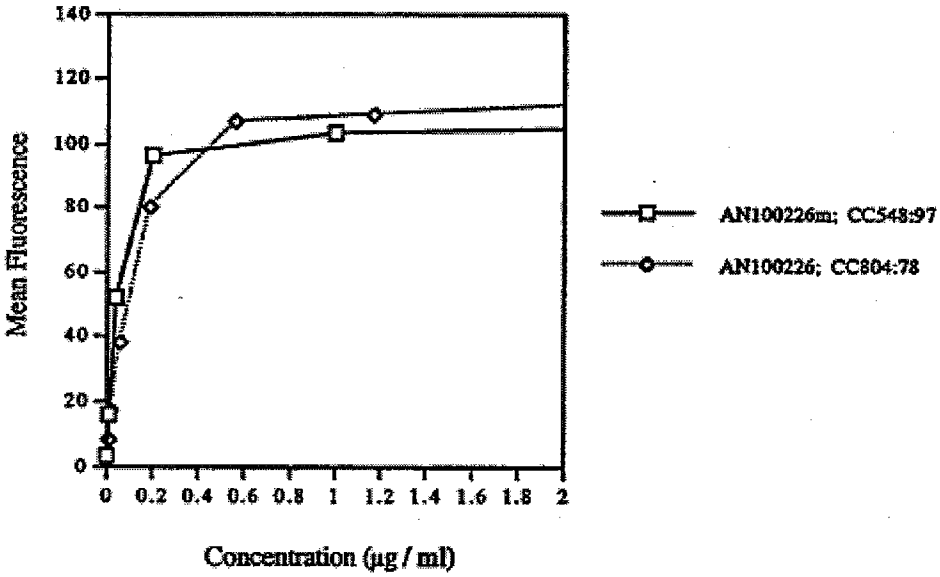
Figure 19. Immunoprecipitation analysis of AN100226 binding to U 937 cell lysates.

Both L25 and HP2/1 control antibodies directed against α4 integrin precipitated bands corresponding to the expected [redacted] fragments, as well as a band at [redacted] corresponding to the β1 subunit of the α4β1 integrin complex. These two antibodies also detected minor bands at [redacted] (uncleaved α4 integrin) and [redacted] (the modified, variant form of α4 integrin). An identical pattern of staining of both the major and minor α4 integrin bands was detected by immunoprecipitation using AN100226m, confirming its reactivity with the α4 integrin. The antibody K20 directed against the β1 integrin precipitated a [redacted] band as expected, as well as bands at [redacted] consistent with its interaction with α4 to form the α4β1 integrin complex. However, this antibody also precipitated a number of other bands with molecular weights ranging between [redacted] suggesting that it also interacts with other

α-chain integrins present on the surface of U937 cells. The anti-β2 integrin antibody GG5/1 precipitated two bands at [redacted] corresponding to the α_L and β2 integrin subunits, respectively, of the leukocyte adhesion molecule LFA-1. The irrelevant mouse IgG_1 control antibody [redacted] did not immunoprecipitate any bands from U937 cell lysates.

Studies to compare the affinity of the humanized AN100226 antibody natalizumab, with the parent murine monoclonal antibody AN100226m were conducted with human Jurkat T lymphoid and guinea pig peripheral blood mononuclear cells. Figure 20, from the sponsor's final study report shows the reactivity of both AN100226m and natalizumab with the human (panel A) and guinea pig (panel B) cells.

A. human Jurkat T lymphoid cells



B. guinea pig peripheral blood mononuclear cells

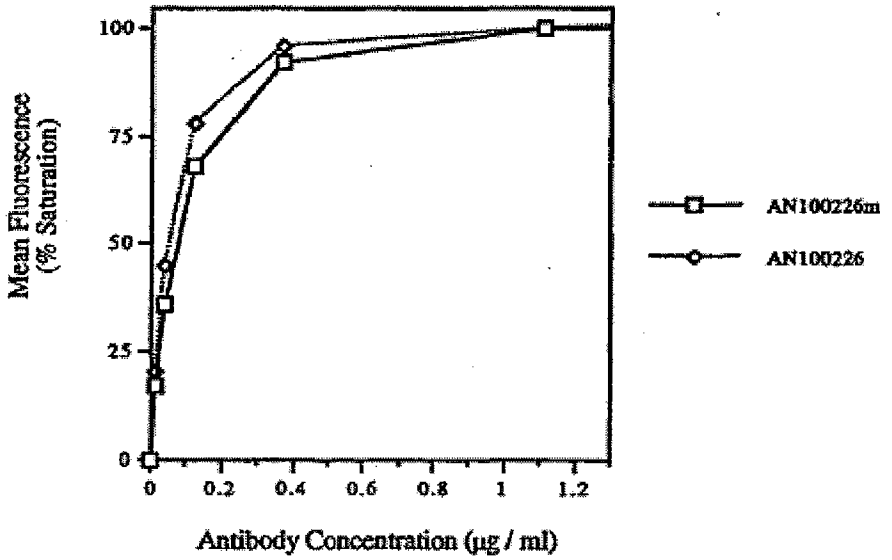


Figure 20. AN100226m and AN100226 (natalizumab) reactivity with human Jurkat T lymphoid cells (panel A), and mononuclear cells from guinea pig peripheral blood (panel B).

Natalizumab demonstrated nearly identical affinity and saturation of binding with AN100226m on both Jurkat and guinea pig mononuclear cells. The reported concentration for saturation of human lymphocyte binding by both AN100226m and natalizumab was approximately 0.2 μg/ml (K_d = 0.05 μg/ml or 0.3 nM). Both AN100226m and natalizumab bound guinea pig cells at saturating concentrations of 0.38 μg/ml. With the guinea pig lymphocytes, half-maximal saturation was reported at a concentration of 0.05 μg/ml, indicating that these antibodies exhibit approximately the same affinity for α4 integrin expressed on guinea pig lymphocytes (K_d = 0.3 nM) as they do for α4 integrin on human cells.

Comment: The level of saturation of Jurkat cell binding at 0.2 μg/ml appears to only be about 80%, not 100%. Raw data for these experiments were not provided in the final study report, so independent calculation and confirmation of the reported K_d values cannot be performed.

The ability of the humanized AN100226 antibody natalizumab to inhibit human T cell adhesion was examined using mouse L-cell fibroblasts that had been transfected to express human VCAM-1. Figure 21 below was provided by the sponsor in the final study report, and shows that both antibodies can effectively inhibit adhesion of Jurkat T lymphoid cells to VCAM-1 with comparable potency. Half-maximal inhibition (IC_{50}) was achieved after pre-incubation with 0.3 μg/ml (2 nM) of either AN100226m or natalizumab, with complete inhibition of binding observed at 3 μg/ml.

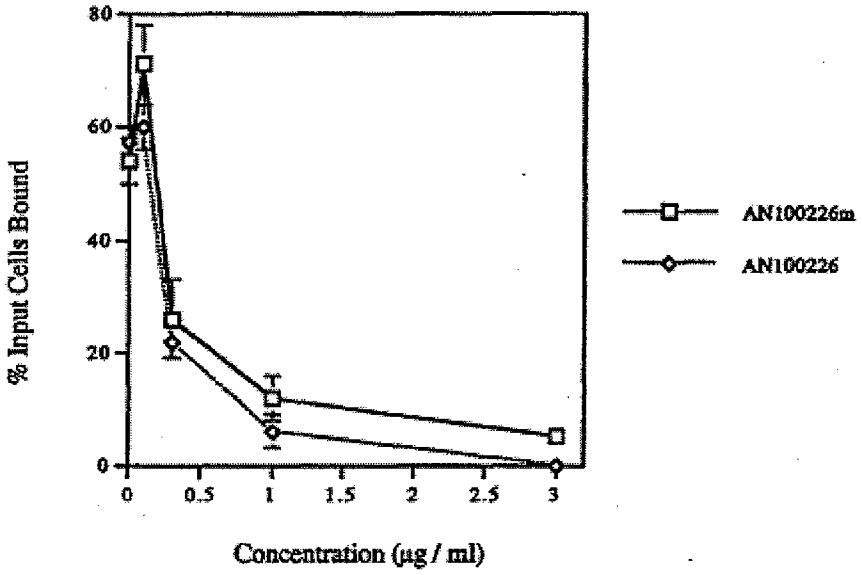


Figure 21. Inhibition of Jurkat T cell adhesion to VCAM-1 transfected mouse L-cells by AN100226m and natalizumab (AN100226).

Comment: The final study report states that complete inhibition of human Jurkat T cell adhesion to mouse L-cells expressing human VCAM-1 was achieved at a concentration of 1 μg/ml, while the data presented in Figure MM above suggest that complete inhibition of binding does not occur until Jurkat cells are exposed to 3 μg/ml of either antibody. No raw data were included in the final study report, so an independent confirmation of the reported values is not possible.

Study conclusion: Adhesion of human T lymphocyte cell lines to activated rat brain EC, HUVEC, or a murine L-cell fibroblast cell line expressing human α4β1 integrin or of human U937 monocytes to EAE rat brain endothelium was significantly inhibited by pre-treatment with AN100226m. Flow cytometric studies demonstrated cross-reactivity of AN100226m with α4

integrin expressed on peripheral blood monocytes and lymphocytes, but not neutrophils from human subjects. Cross-reactivity of AN100226m was demonstrated with lymphocytes from pig, dog, guinea pig, ferret, and Rhesus and cynomolgus macaques, but not marmoset, rat, hamster, rabbit, or gerbil. Specificity of AN100226m for the α4 subunit of the α4β1 integrin complex was confirmed by flow cytometric evaluation of human T lymphoid and monocytic cell lines expressing various combinations of α4 and β integrin family members, and by immunoprecipitation studies with U937 human monocytic cell lysates. The humanized anti-α4 integrin antibody AN100226 (natalizumab) bound both human Jurkat T cells and guinea pig peripheral blood cells with approximately equal affinity, and could inhibit adhesion of Jurkat cells to mouse L-cells expressing VCAM-1. Saturation concentrations for guinea pig and human T cell natalizumab cell surface binding, and the IC_{50} values for inhibition of T cell adhesion were approximately equal to those obtained for AN100226m, showing the comparability of the humanized version to the parent, murine monoclonal antibody.

Study title: Expression of α4 integrin on lymphocytes isolated from healthy volunteers and from patients with multiple sclerosis.

Key findings: No remarkable differences were noted in the affinity of the murine anti-α4 integrin antibody AN100226m for α4 integrin expressed on the surface of lymphocytes from healthy human subjects, as compared to patients with multiple sclerosis.

Study #: PC100

Volume # and page #: EDR file: STN BLA 125104\000\module4\primarypharm\pc100.pdf
Conducting laboratory and location: Athena Neurosciences, 800 Gateway Boulevard, South San Francisco, CA 94080
Date of study initiation: not specified (final report dated August 23, 1996)
GLP compliance: no
QAU statement: yes ( ) no (X)
Drug, lot #, and % purity: AN100226m (murine, parent monoclonal antibody) and AN100226 (humanized version; natalizumab), lot number, concentration, formulation, and percent purity not specified in final study report

Methods: The expression of α4 integrin on the surface of lymphocytes from healthy human subjects, as compared to patients with multiple sclerosis was determined by flow cytometry. Briefly, peripheral blood samples from 10 healthy volunteer subjects and from 18 patients with multiple sclerosis were obtained and incubated for 30 minutes at 4°C with 50 µl of a 5 µg/ml of the murine anti-α4 integrin antibody AN100226m, diluted in phosphate buffered saline containing . [redacted] Mouse IgG_1 (5 µg/ml) was used as an isotype-matched control for evaluation of non-specific staining. Following incubation in primary antibody the cells were washed with [redacted] incubated on ice for an additional 30 minutes. Red cells were then lysed for 15 min at room temperature using [redacted] lysing solution, washed, and resuspended in [redacted] Lymphocytes were evaluated for AN100226m staining using a . [redacted] flow cytometer, and results are reported as the mean fluorescent intensity for each sample.

Competition studies were also performed at the same time using the humanized form of natalizumab, AN100226. Lymphocytes from selected healthy volunteer and multiple sclerosis patient donors were labeled with increasing concentrations of AN100226 (0.02 to 3 µg/ml) as described above, washed, then labeled with saturating concentrations (50 µl of 5 µg/ml solution)

of the murine anti-α4 integrin antibody AN100226m, or 5 μg/ml mouse IgG_1 as an isotype-matched control. Following incubation in primary antibody the cells were washed, resuspended in phycoerythrin-conjugated, [redacted], and evaluated for fluorescence as described above. Results were expressed as the percentage of AN100226m lymphocyte binding in the presence of humanized AN100226 as compared to control (no AN100226 added), and the concentration at which AN100226m competed 50% of the AN100226 binding from control (IC_{50}) was calculated.

Results: Mean fluorescent intensities for AN100226m binding to lymphocytes from healthy human volunteer donors and multiple sclerosis patients are shown in Figure 22 below, which was provided by the sponsor in the final study report. There were no significant differences noted in anti-α4 integrin staining between the two populations, suggesting that there is no increase in the expression of the α4 integrin in patients with multiple sclerosis as compared to healthy subjects.

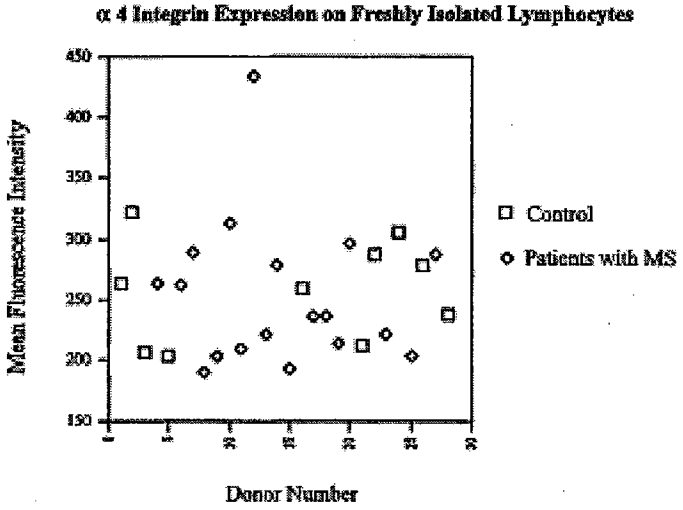


Figure 22. Expression of α4 integrin on freshly isolated human lymphocytes from healthy volunteer donors and from multiple sclerosis (MS) patients.

Competition studies with the humanized anti-α4 integrin antibody AN100226 did not show any remarkable differences in binding over the range in doses tested (Figure 23, from the sponsor's final study report, below). The calculated IC_{50} values for AN100226m competition of AN100226 binding were not remarkably different between lymphocytes from multiple sclerosis patients and healthy volunteer subjects, suggesting that the affinity of natalizumab for α4 integrin does not vary between the normal and diseased stated. The IC_{50} values, as calculated by the sponsor are presented in Table 14, below.

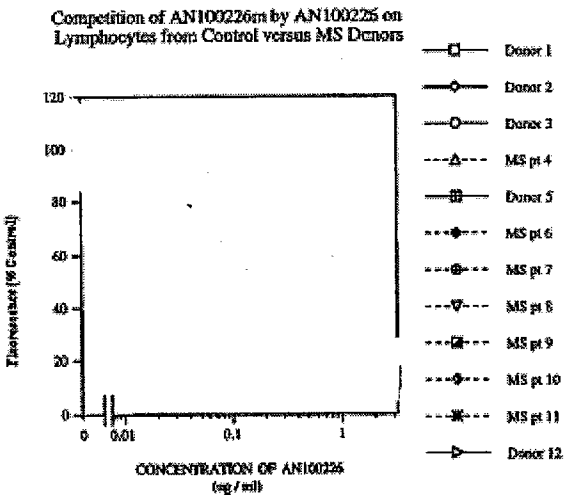


Figure 23. Competition curves of AN100226m and AN100226 for α4 integrin expressed on lymphocytes from healthy human donors and multiple sclerosis (MS) patients.

Table 14. IC_{50} values for AN100226m inhibition of AN100226 binding to lymphocytes isolated from healthy donors and multiple sclerosis patients	
Calculated IC_{50} Values (μg/ml)	
MS Patient Lymphocytes	**Healthy Donor Lymphocytes**
0.041	0.040
0.060	0.061
0.044	0.030
0.067	0.040
0.031	0.045
0.036	
0.051	
Mean ± S.D.	**Mean ± S.D.**
0.047 ± 0.013	0.045 ± 0.011

Study conclusion: There were no remarkable differences detected in the affinity of the murine anti-α4 integrin antibody AN100226 for α4 integrin expressed on the surface of peripheral blood lymphocytes from healthy human volunteer donors, and patients with multiple sclerosis. Binding of the humanized antibody AN100226 (natalizumab) to α4 integrin on either cell population was competed by the AN100226m antibody, confirming that the two monoclonal antibodies recognize the same epitope on α4 integrin. No remarkable differences in the IC_{50} concentrations between the two subject populations were noted. Taken together, these data demonstrate that natalizumab binds to α4 integrin on lymphocytes from MS patients in an identical manner as lymphocytes from healthy donor subjects, and suggest that expression of the α4 integrins on effector lymphocytes is not increased in multiple sclerosis.

2.6.2.3 Secondary pharmacodynamics

A single study was conducted to evaluate the immune effects of natalizumab (AN100226) on human lymphocyte function *in vitro*, and is reviewed, below.

Study title: *In vitro* evaluation of the immunomodulatory potential of anti-α4 integrin antibody.

Key findings: By itself, natalizumab had no stimulatory effects on human peripheral blood cell proliferation *in vitro*. There were no remarkable effects of co-incubation of 0.01 to 100 µg/ml AN100226 on lymphocyte proliferation in response to either mitogen, or T cell receptor stimulation. No effects of natalizumab treatment at these same concentrations were observed for cytokine release by T lymphocytes in response to anti-CD3 monoclonal antibody, or by monocytes in response to stimulation by bacterial lipopolysaccharide. Neither innate, nor interleukin-2 stimulated NK cell function, or anti-CD3 stimulated T lymphocyte cytotoxic function were affected by the presence of natalizumab, at concentrations of 0.01 to 100 µg/ml.

Study number: Study #PC028 (

Volume # and page #: EDR file: STN BLA 125104\000\module4\secondarypharm\PC028.pdf

Conducting laboratory and location:

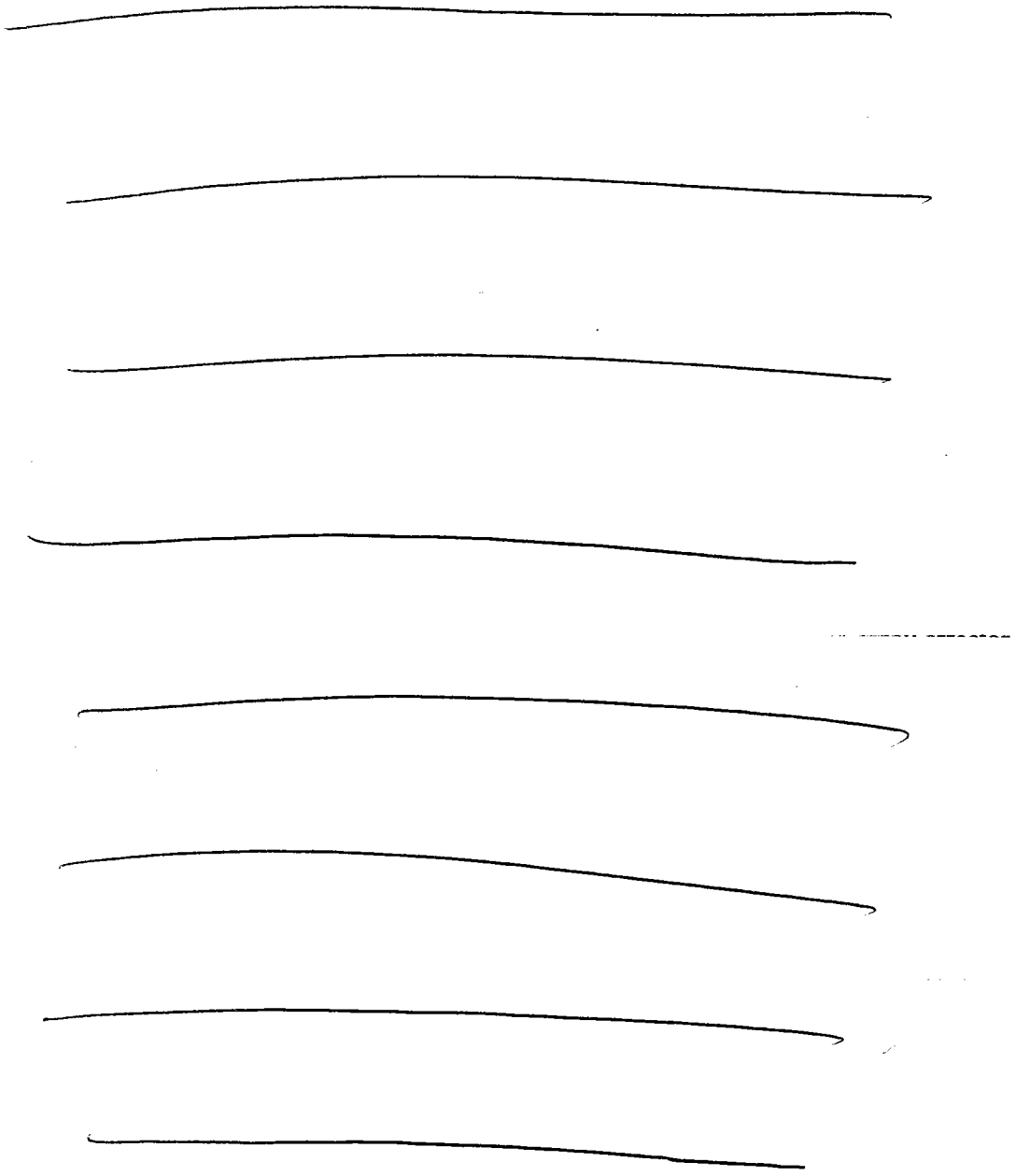
Date of study initiation: not specified (final report dated March, 1995)

GLP compliance: no

QAU statement: yes ( ) no (X)

Drug, lot #, and % purity: AN100226 (natalizumab), 4.23 mg/ml formulated in 150 mM NaCl buffered with 50 mM L-histidine, pH 6.0; lot #S502/56; purity as determined by scanning densitometry of reduced SDS-PAGE gel (analytical report including % purity was included as an appendix to the final study report)

Methods:



Results: Exposure of peripheral blood mononuclear cells to natalizumab resulted in a slight, apparent dose-related increase in cellular proliferation in response to the antibody alone, as shown in Figure 24 from the sponsor's final study report, below. However, none of the proliferative responses of the treated cultures were statistically different from the response in the absence of natalizumab.

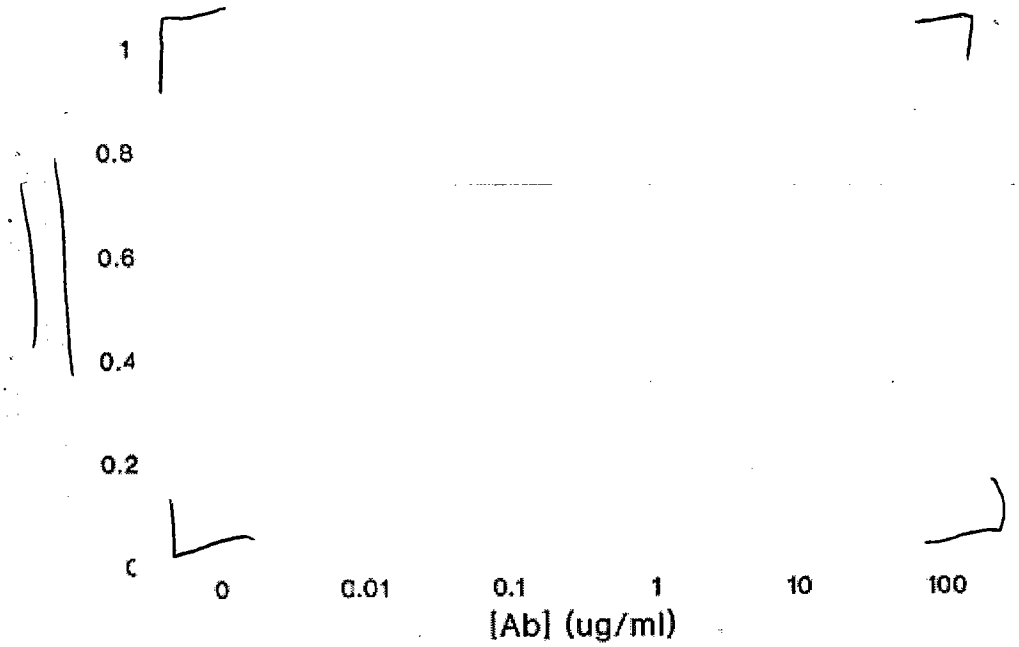


Figure 24. Proliferation of human peripheral blood mononuclear cells following co-culture with AN100226 in the absence of any mitogenic stimulus.

There were no remarkable effects of AN100226 treatment on the proliferative response to T cells to stimulation by the anti-CD3 monoclonal antibody. Slight increases in response to stimulation by increasing concentrations of PHA were observed when lymphocytes were co-cultured with 100 µg/ml AN100226 as compared to cells without added antibody; however, this effect was only statistically significant for lymphocytes stimulated with 0.5 µg/ml PHA. Tables 15a and 15b below, which were provided by the sponsor in the final study report show the results of both of these studies.

Table 15a. Proliferation of human peripheral blood lymphocytes stimulated with PHA, in the presence of natalizumab.

[AN100226] (µg/ml)	[PHYTOHEMAGGLUTININ] (µg/ml)			
	0	0.5	1	5
0	0.795 ± 0.094[a]	0.849 ± 0.026	1.033 ± 0.043	1.352 ± 0.098
0.01	0.805 ± 0.083	0.798 ± 0.051	0.980 ± 0.042	1.250 ± 0.088
0.1	0.834 ± 0.103	0.812 ± 0.034	0.946 ± 0.039	1.257 ± 0.093
1	0.794 ± 0.093	0.799 ± 0.016	0.942 ± 0.043	1.244 ± 0.113
10	0.816 ± 0.113	0.799 ± 0.022	0.937 ± 0.029	1.246 ± 0.086
100	0.988 ± 0.104	0.984 ± 0.026[b]	1.159 ± 0.066	1.331 ± 0.097

[a] Values represent mean (± S.E.M.) absorbance at ~~—~~ of three-day human PBL cultures pulsed four hours previously with XTT/PMS colorimetric indicator reagent. N = 5 individual human samples.

[b] Significantly different from control values at $P \leq 0.05$.

Table 15b. Proliferation of human peripheral blood lymphocytes stimulated with anti-CD3 monoclonal antibody, in the presence of natalizumab.

[AN100226] (µg/ml)	[ANTI-CD3 MONOCLONAL ANTIBODY] (ng/ml)			
	0	10	100	1000
0	0.814 ± 0.084[a]	1.174 ± 0.127	1.022 ± 0.132	0.999 ± 0.122
0.01	0.768 ± 0.086	1.057 ± 0.121	0.962 ± 0.139	0.954 ± 0.126
0.1	0.801 ± 0.088	1.041 ± 0.115	0.931 ± 0.125	0.969 ± 0.132
1	0.827 ± 0.109	1.018 ± 0.110	0.917 ± 0.130	0.934 ± 0.139
10	0.850 ± 0.091	1.049 ± 0.128	0.959 ± 0.118	0.990 ± 0.116
100	0.945 ± 0.098	1.249 ± 0.121	1.140 ± 0.126	1.156 ± 0.106

[a] Values represent mean (± S.E.M.) absorbance at ~~—~~ of three-day human PBL cultures pulsed four hours previously with XTT/PMS colorimetric indicator reagent. N = 5 individual human samples.

No statistically significant differences were observed between control and test groups at any concentration of AN100226 tested in this study.

Co-incubation of peripheral blood cells or enriched monocytes with AN100226 did not further enhance release of the various cytokines over what was observed in response to a stimulatory concentration of either anti-CD3 antibody or bacterial LPS alone. The results of these experiments are shown in Tables 16 and 17, from the sponsor's final study report, below.

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Table 16. Cytokine release by human peripheral blood lymphocytes stimulated with anti-CD3 monoclonal antibody (100 ng/ml), in the presence of natalizumab.

[AN100226] (µg/ml)	[IL-1β] (pg/ml)[b]	[IL-2] (pg/ml)[c]	[IL-6] (pg/ml)[d]	[IL-10] (pg/ml)[e]	[TNF-β] (pg/ml)[f]
0	574.66 ± 117.76	421.70 ± 112.23	177.35 ± 57.00	680.50 ± 118.18	468.84 ± 89.63
0.01	556.44 ± 117.42	280.36 ± 75.37	178.48 ± 43.29	582.12 ± 126.03	415.94 ± 85.11
0.10	482.90 ± 111.68	182.15 ± 59.31	142.75 ± 35.35	527.92 ± 122.96	387.28 ± 79.48
1.00	486.92 ± 114.67	214.32 ± 69.13	146.96 ± 31.63	499.40 ± 116.03	370.70 ± 70.55
10.00	454.20 ± 111.10	262.44 ± 64.00	156.72 ± 35.71	543.00 ± 100.24	394.10 ± 73.72
100.00	562.34 ± 122.42	481.08 ± 113.29	216.80 ± 38.61	627.48 ± 129.80	491.02 ± 90.83

[a] Human PBL were cultured for 48 h in the presence of anti-CD3 MAb and various concentrations of AN100226. The culture supernatant fluids were subsequently harvested and cytokine production was quantitated in duplicate by ELISA. N = 5 human blood samples.

[b] Values represent mean (± S.E.M.) concentration of human IL-1β extrapolated from an internal reference curve generated with recombinant cytokine according to kit directions. Correlation coefficient for reference curve was 1.000.

[c] Values represent mean (± S.E.M.) concentration of human IL-2 extrapolated from an internal reference curve generated with recombinant cytokine according to kit directions. Correlation coefficient for reference curve was 0.999.

[d] Values represent mean (± S.E.M.) concentration of human IL-6 extrapolated from an internal reference curve generated with recombinant cytokine according to kit directions. Correlation coefficient for reference curve was 0.999.

[e] Values represent mean (± S.E.M.) concentration of human IL-10 extrapolated from an internal reference curve generated with recombinant cytokine according to kit directions. Correlation coefficient for reference curve was 1.000.

[f] Values represent mean (± S.E.M.) concentration of human IL-TNFβ extrapolated from an internal reference curve generated with recombinant cytokine according to kit directions. Correlation coefficient for reference curve was 0.996.

No statistically significant differences were observed between control and test groups at any concentration of AN100226 tested in this study.

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Table 17. Cytokine release by human peripheral blood monocytes stimulated with bacterial LPS (100 ng/ml), in the presence of natalizumab.

[AN100226] (μg/ml)	[IL-1α] (pg/ml)	[TNF-α] (pg/ml)
0	381.08 ± 16.50[b]	1061.94 ± 89.94[c]
0.01	354.14 ± 22.66	1062.76 ± 99.56
0.10	356.38 ± 26.81	1069.50 ± 96.93
1.00	342.22 ± 32.44	1070.78 ± 93.00
10.00	348.12 ± 32.68	1086.08 ± 98.18
100.00	376.52 ± 28.73	1060.92 ± 58.45

[a] Human monocytes were cultured for 48 h in the presence of LPS and various concentrations of AN100226. The culture supernatant fluids were subsequently harvested and cytokine production was quantitated in duplicate by ELISA. N = 6 human blood samples.

[b] Values represent mean (± S.E.M.) concentration of human IL-1α extrapolated from an internal reference curve generated with recombinant cytokine according to kit directions. Correlation coefficient for reference curve was 0.996.

[c] Values represent mean (± S.E.M.) concentration of human IL-TNFα extrapolated from an internal reference curve generated with recombinant cytokine according to kit directions. Correlation coefficient for reference curve was 0.997.

No statistically significant differences were observed between control and test groups at any concentration of AN100226 tested in this study.

Table 18, which was provided by the sponsor in the final study report, demonstrates that there were no inhibitory or stimulatory effects of natalizumab observed on the cytolytic effector function of anti-CD3 stimulated T lymphocytes. Similarly, there were no effects of natalizumab pretreatment on basal NK cell cytolytic activity, as shown in Table 19A, below. Co-incubation of NK cells with IL-2 resulted in an increase in cytolytic effector activity as shown in Table 19B; however, there was no additional augmentation of cytolysis in the presence of natalizumab. Both Tables 19A and B were provided by the sponsor in the final study report.

Table 18. Effects of natalizumab on cytotoxic T lymphocyte activity.

[AN100226] (μg/ml)	EFFECTOR:TARGET RATIO		
	100:1	50:1	25:1
0	43.59 ± 6.98[a]	41.68 ± 4.63	26.43 ± 3.11
0.01	66.54 ± 9.06	50.75 ± 6.83	29.65 ± 3.80
0.10	55.58 ± 8.12	37.64 ± 6.69	29.92 ± 4.84
1.00	53.19 ± 8.22	41.52 ± 6.66	36.52 ± 5.23
10.00	51.13 ± 8.22	40.01 ± 10.30	23.21 ± 4.03
100.00	42.21 ± 10.18	32.20 ± 4.94	18.49 ± 3.70

[a] Values represent mean (± S.E.M.) percent lysis of radiolabeled OKT3 tumor target cells following an overnight incubation. N = 5 individual human samples.

No statistically significant differences were observed between control and test groups at any concentration of AN100226 tested in this study.

Table 19. Effects of natalizumab on basal and IL-2 stimulated NK cytolytic activity.

A. Basal effector function

[AN100226] (µg/ml)	EFFECTOR:TARGET CELL RATIO		
	100:1	33:1	11:1
0	59.56 ± 8.09*	37.15 ± 11.18	15.84 ± 6.32
0.01	65.97 ± 7.48	43.10 ± 12.58	18.85 ± 9.01
0.10	57.80 ± 6.69	32.55 ± 8.68	17.02 ± 9.25
1.00	51.62 ± 11.26	39.99 ± 12.31	14.05 ± 8.30
10.00	47.46 ± 9.30	24.30 ± 10.48	11.18 ± 6.53
100.00	41.90 ± 6.37	35.47 ± 14.21	20.85 ± 14.60

* Values represent mean (± S.E.M.) percent lysis of radiolabeled K562 tumor target cells in a 4-hr cytotoxicity assay. N = 5 individual human samples.

No statistically significant differences were observed between control and test groups at any concentration of AN100226 tested in this study.

B. IL-2 Stimulated effector function

[AN100226] (µg/ml)	EFFECTOR:TARGET CELL RATIO		
	100:1	33:1	11:1
0	77.66 ± 6.87*	56.86 ± 10.14	28.97 ± 8.64
0.01	80.52 ± 4.49	62.78 ± 10.57	30.10 ± 9.40
0.10	67.29 ± 5.30	51.65 ± 8.61	27.02 ± 10.22
1.00	70.85 ± 5.87	49.84 ± 10.41	23.78 ± 9.52
10.00	68.65 ± 8.16	40.72 ± 10.44	21.32 ± 9.19
100.00	59.13 ± 4.45	48.73 ± 8.57	27.26 ± 11.65

* Values represent mean (± S.E.M.) percent lysis of radiolabeled K562 tumor target cells in a 4-hr cytotoxicity assay. N = 5 individual human samples.

No statistically significant differences were observed between control and test groups at any concentration of AN100226 tested in this study.

Study conclusion: There were no remarkable effects of natalizumab exposure at concentrations of 0.01 to 100 µg/ml on immune cell effector functions, including proliferative and cytokine responses to T cell receptor or mitogen stimulation, or basal or stimulated NK and T lymphocyte cytolytic effector functions. Taken together, these data demonstrate that there are either no inhibitory or stimulatory effects of natalizumab on innate immune function at plasma concentrations that are likely to be present in the treated patient population.

2.6.2.4 Safety pharmacology

A single study to evaluate the cardiovascular safety pharmacology of the humanized AN100226 anti α4-integrin monoclonal antibody was conducted in the conscious beagle dog, and is reviewed, below. Cardiovascular effects of AN100226 were also evaluated in cynomolgus monkeys by ECG evaluation as part of the single- and repeat-dose toxicology studies; these results are included in the review of the toxicology data, by Barbara J. Wilcox, Ph.D.

Study title: A cardiovascular profile study following a single intravenous infusion of AN100226 in the conscious beagle dog.

Comment: Lymphocytes from dog peripheral blood were previously found to bind AN100226m with similar affinity and receptor density as human lymphocytes by flow cytometric evaluation (Table 10, Study #PC032, above). However, the affinity of the humanized version of natalizumab, AN100226 for dog lymphocytes was not evaluated. Bridging studies of AN100226 and AN100226m binding by flow cytometry show comparable degrees of fluorescent intensity with either antibody using peripheral blood lymphocytes. Therefore it is assumed that the humanized version AN100226 will be pharmacologically active in the dog similar to the murine parent monoclonal antibody, AN100226m.

Key findings: Transient although marked decreases in systolic and left ventricular pressures, slight decreases in cardiac output, and increased heart rates and total peripheral resistance were observed in unanesthetized dogs during a 30 min intravenous administration of 3.0 or 30 mg/kg AN100226. These effects were related to infusion of natalizumab in both incidence and severity, and had resolved to baseline values within 5 min after completion of the infusion. There were no definitive effects of natalizumab treatment on ECG profiles, tidal volume, or respiratory rate.

Study number: Study #AL107
Volume # and page #: EDR file: STN BLA 125104\000\module4\safetypharm\AL107.pdf
Conducting laboratory and location:

Date of study initiation: March 29, 1995 (final report dated December 15, 1995)
GLP compliance: Yes
QAU statement: yes (X) no ( )
Drug, lot #, and % purity: AN100226 vehicle (150 mM NaCl, buffered with 50 mM L-histidine, pH not specified), lot #4T36QT; natalizumab, lot #AN-100226-002; analytical report including % purity was not included in the final study report

Methods

Doses: vehicle, 0.5, 1.0% morphine sulfate (positive control), natalizumab (AN100226, lot #AN-100226-0002), 0.3, 3.0, 30 mg/kg
Species/strain: *Canis familiaris* (beagle dogs), **female only**; purpose-bred

Number/sex/group or time point (main study): 3 females/group
Route, formulation, volume, and infusion rate: intravenous infusion; natalizumab formulated in 150 mM saline and 50 mM L-histidine; 10 ml/kg infused; infusion rate 20 ml/kg/h
Satellite groups used for toxicokinetics or recovery: not included in the present study.

Age: approximately 5 to 6 months
Weight (nonrodents only): 5.6 – 7.1 kg
Unique study design or methodology (if any): one day prior to initiation of testing, all animals were implanted with medical grade catheters in the left ventricle via the carotid artery for monitoring of left ventricular parameters, and a ——— thermal catheter in the pulmonary artery, via the femoral vein and right atrium and ventricle. An additional catheter was inserted into the femoral artery and its tip placed in the abdominal aorta at approximately the level of the kidneys, to determine mean arterial pressure.

Animals were acclimated to a restraining sling prior to study initiation. On the day of dosing, the dogs were restrained in the sling for approximately 1 hour prior to dosing, then administered the vehicle for natalizumab by a 30 min infusion through a catheter placed in the saphenous vein. Following at least a 30 min washout period, the test article (0.3, 3.0, or 30 mg/kg natalizumab) was administered by 30 minute infusion, and various cardiovascular and respiratory parameters were recorded (please see description, below)

Comment: One female dog died from complications post-surgery, but prior to treatment with AN100266. This animal was replaced with an additional dog, and treated on the following day.

Results:
Neurological effects: Not tested in this system.

Cardiovascular effects: Electrocardiograms were measured off limb leads I, II, III, aVR, aVL, and aVF 3 times prior to administration of the control article (vehicle), and at 5, 15, 30, and 60 minutes after injection of either vehicle or natalizumab, and hourly for up to 3 hours following AN100226 dosing. Cardiac output measurements were collected at the same time points, using a cardiac output computer connected to the ——— catheter, and stroke volume and peripheral resistance were calculated for each occasion of cardiac output measurement. At least a 30 min washout period was allowed between vehicle and natalizumab dosing. Heart rate, systolic, diastolic, and mean arterial blood pressures, peak systolic left ventricular pressure, and maximal rate of left ventricular contraction (+dp/dt), relaxation (-dp/dt), and cardiac contractile index (+dp/dt/P) were determined from the catheter situated within the left ventricle, and recorded continuously using a hemodynamic computer. Pulmonary artery systolic, diastolic, and mean pressures were determined from the ——— catheter implanted in the pulmonary artery, and recorded continuously for the duration of the study. At termination of the observation period after natalizumab dosing, each dog was euthanized by overdose with sodium pentobarbital followed by exsanguination, and subjected to a complete external and internal gross pathological evaluation.

Ventricular extrasystoles were noted on ECG readings from all animals, and one dog had evidence of occasional premature atrial beats; however, these findings were present prior to initiation of treatment with either the vehicle control article or natalizumab, and are considered by the consulting veterinary cardiologist to be related to the catheter placement. There were no definitive, treatment-related effects of natalizumab at any dose level on heart rate, systemic blood pressure, pulmonary artery or left ventricular pressures, cardiac output, stroke volume, or total peripheral resistance; however, sporadic changes these parameters were observed in individual animals. One dog (animal #153) in the 0.3 mg/kg dose group exhibited minor fluctuations in mean cardiac output, and corresponding changes in stroke volume and peripheral resistance 30 minutes after infusion of the control article, and at 5 and 60 minutes after AN100226 dosing. No other changes in hemodynamic parameters were observed in this animal; therefore, these findings

are considered unrelated to natalizumab. Transient, but marked (approximately 35 – 90 mm Hg from baseline) decreases in systolic blood and left ventricular pressure, were observed in 1/3 dogs treated with 3.0 mg/kg AN100226, and in 2/3 dogs receiving 30 mg/kg natalizumab beginning 5 to 10 min into the 30 minute infusion period. Ventricular contraction and relaxation rates, contractile index, and mean cardiac output were also decreased, while changes in heart rate and pulmonary arterial pressure were more variable. All three dogs showed partial recovery of hemodynamic parameters towards baseline values by the completion of the 30 min infusion period. All changes observed in animal #252, treated with 3.0 mg/kg natalizumab had resolved completely to baseline values by 15 minutes after the infusion was terminated. In the two dogs treated with 30 mg/kg natalizumab that demonstrated changes in hemodynamic parameters, mean cardiac output remained reduced and peripheral resistance increased in animal #351 at 3 hours post-infusion, with no other corresponding changes in any other parameters. All hemodynamic changes had resolved to baseline in the remaining dog (animal #353) in this dose group within 5 minutes after the end of the infusion period.

Comment: The contracting veterinary cardiologist considers these findings related to natalizumab at the 3 and 30 mg/kg dose group, with a greater incidence and severity of response at 30 mg/kg. All findings had resolved to baseline by the end of the infusion period, or within several minutes afterwards, and no lasting effects were observed.

Pulmonary effects: Respiratory rate and tidal volume were measured once prior to treatment with the vehicle control, then at 5, 15, 30, and 60 minutes after infusion of either vehicle or natalizumab, and hourly for 3 hours after completion of the AN100226 infusion. There were no remarkable effects of natalizumab treatment on either tidal volume or respiratory rate.

Renal effects: Not tested in this system.

Gastrointestinal effects: Not tested in this system.

Abuse liability: Not tested in this system.

Other: Necropsy evaluation of the natalizumab treated dogs was performed at study termination. Occasional findings, including dark areas present in the heart and/or lungs were present in several animals in all dose groups, and were considered by the consulting pathologist to be related to placement of the catheters and unrelated to natalizumab treatment.

Study Conclusion: Intravenous administration of 0.3, 3.0, or 30 mg/kg AN100226 by 30 min infusion to conscious beagle dogs had no definitive effects on ECG profiles, tidal volume, or respiratory rate. Transient, although marked systolic and left ventricular hypotension, slight decreases in cardiac output, and increased heart rates and total peripheral resistance were observed in 1/3 animals treated with 3.0 mg/kg AN100226, and in 2/3 dogs treated with 30 mg/kg during the infusion period, but had generally resolved within 5 minutes following completion of the infusion period. These increases were not statistically significantly different between the dose groups, and were not considered to be biologically relevant.

2.6.2.5 Pharmacodynamic drug interactions

The pharmacodynamic interactions of AN100226 with the IFN-β AVONEX® were evaluated in Rhesus monkeys, and are reviewed as part of the pharmacokinetic substudy in Section 2.6.4.8, below.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

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

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Single-dose pharmacokinetic studies of AN100226m, AN100226, or natalizumab manufactured at different stages of product development showed dose-related, although proportionally non-linear increases in C_{max}, $AUC_{0\text{-last}}$, mean residence time, and elimination half-lives, and decreased clearance values that were suggestive of a saturable clearance mechanism(s) in both cynomolgus macaques and male and pregnant and non-pregnant female guinea pigs. Mean elimination half-life values for natalizumab (± S.D.) following a single, intravenous dose of 0.3, 3.0, or 30 mg/kg in cynomolgus monkeys were 8.2 ± 3.8, 58.0 ± 24.2, and 73.8 ± 23.4 hours, respectively. In the guinea pig, the mean elimination half-lives were 18.8 ± 5.3, 36.9 ± 7.9, and 73.0 ±24.1 hours following a single, intracardiac injection of 1, 3, or 8 mg/kg respectively, of AN100226. Both species showed comparable elimination half-life values after a dose of 3.0 mg/kg natalizumab, with a range of 58.0 to 80.1 hours in the monkey, and a mean value of 36.9 ± 7.9 hours in the guinea pig after intra-cardiac injection. Combination treatment of Rhesus macaques with weekly doses of 30 or 60 mg/kg AN100226 and 30 µg/animal AVONEX® had no remarkable effects on the pharmacodynamic, pharmacokinetic, or immunogenic profiles of either product, as compared to animals receiving either agent alone. Antibodies to natalizumab were detected in all test species in both single and repeat-dose studies, in an inverse relationship for both incidence and titer to the dose of AN100226 or AN100226m administered. Anti-natalizumab antibody levels in serum tended to decrease with longer duration of treatment-free recovery periods.

The distribution of natalizumab tissue binding was evaluated in a series of studies using a panel of frozen sections from adult and fetal human and cynomolgus monkey tissues, and to fetal Rhesus monkey tissue sections. Tissue binding studies revealed that natalizumab staining was localized to tissues of lymphoid origin, with the most intense staining present (2^+-3^+) in the lymph nodes, spleen, thymus, and germinal centers of tonsillar tissue, in the gut-associated lymphoid tissue present in the small and large intestines, and in occasional interstitial lymphocytes present in mammary, lung, parathyroid, and stomach samples. The pattern of distribution of AN100226 or AN100226m tissue binding in the cynomolgus monkey was similar to that observed in the human tissues, with two exceptions. One of two samples of human uterus showed strong (3^+) staining localized to the basilar endometrial cell layer, which was not observed in any of the cynomolgus or Rhesus monkey samples. In cynomolgus monkeys, weak but detectable natalizumab binding (± to 1^+) was observed in samples of prostate tissue, with no comparative activity noted in the human samples.

2.6.4.2 Methods of Analysis

[please see under individual study reviews]

2.6.4.3 Absorption

Comment: Using the common technical document format for submission of the licensing application, the sponsor has included pharmacokinetic studies of natalizumab under the section for "Absorption." However, no specific studies to determine the extent of AN100226 absorption were conducted in support of this application. The proposed indication is for intravenous administration in multiple sclerosis patients; therefore, 100% of the drug is expected to be available for binding to the receptor.

A total of nine studies evaluating the pharmacokinetic profiles of either AN100226m or natalizumab, and to determine the comparability of AN100226 produced during scale-up of manufacturing were included in the BLA application. Toxicokinetic evaluations of natalizumab were also included in the single- and repeat-dose, and reproductive and developmental toxicology studies and were reviewed by Barbara J. Wilcox, Ph.D. as part of the toxicology data for this submission.

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

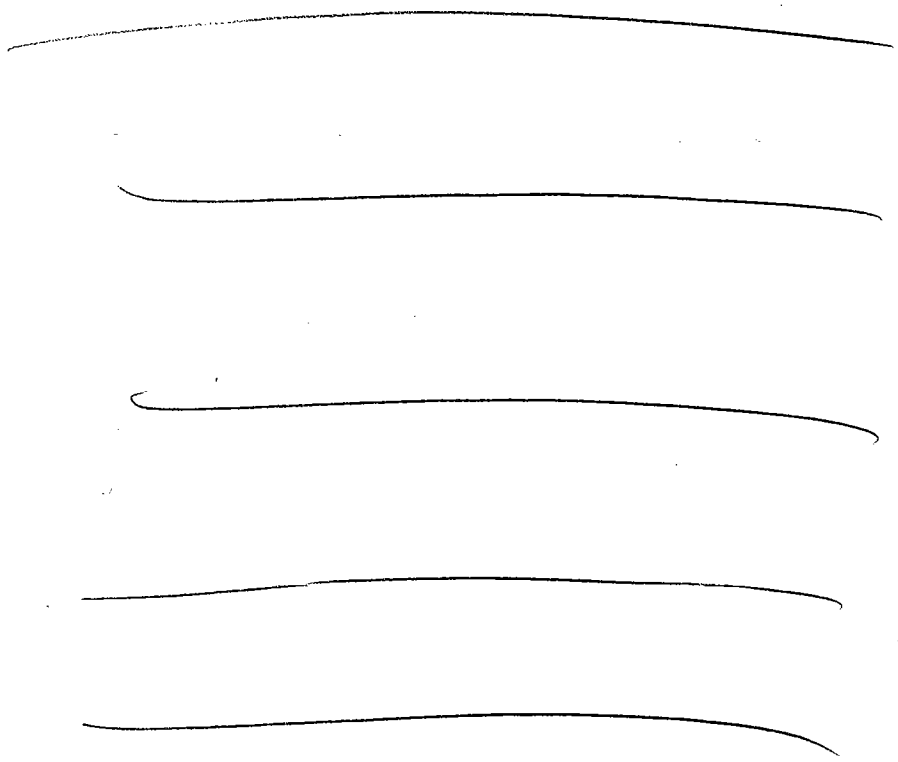
Study title: A single-dose pharmacokinetic intravenous infusion study of Antegren in cynomolgus monkey – comparison of — and Biogen processes.
Key study findings: The criteria for bioequivalence, based on evaluation of $AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$ for AN100226 produced at — compared to commercial material produced at Biogen were not met for the 80%-125% rule; however, C_{max} did pass the test for bioequivalence. There were no detectable differences in either total anti-natalizumab antibody response, or pharmacodynamic response to products manufactured at either site.
Study no.: #0309-003-01 (———— , #1112-93)
Volume #, and page #: EDR files: BLA 125104\000\module4\pharmacokinetics\309-003-01.pdf
Conducting laboratory and location: ————

Date of study initiation: May 30, 2001 (final report dated September 7, 2001)
GLP compliance: Yes
QAU statement: yes (X) no ( )
Drug, lot #, and % purity: natalizumab (AN100226) manufactured by —— control #E23001, item # 600062, 20 mg/ml, purity —, SDS-PAGE (reduced), and — monomeric IgG (———— HPLC); and natalizumab manufactured by Biogen, lot #MFG1330124, 20 mg/ml; purity by SDS-PAGE and ———— HPLC were not reported;(copy of sponsor's Certificate of Analysis for each product was included as an Appendix to the final study protocol)

Methods

Doses: natalizumab from either manufacturing process, 3.0 mg/kg
Species/strain: *Macaca fasicularis* (cynomolgus monkey); purpose-bred (————
Number/sex/group or time point (main study): 15 female/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 6.0; 10 ml/kg infused; infusion rate 20 ml/kg/h
Sampling times for pharmacokinetics: Blood samples for measurement of natalizumab serum concentrations were 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, 17, 21, 24, and 28 after dosing.
Age: 3 to 8 years old
Weight (nonrodents only): 2.2 – 3.0 kg
Unique study design or methodology (if any): Natalizumab concentrations in



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: All monkeys were observed twice daily for general health and behavioral or other clinical signs of overt toxicity. Body weights were determined prior to dosing on study d 1, then weekly thereafter until study termination on d 28. At study termination, all animals were returned to the colony without necropsy. There were no overt toxicities noted after treatment with either preparation of natalizumab; all monkeys survived for the entire study duration, and no adverse effects of AN100226 on body weights over the duration of the study were observed.

Blood samples for evaluation of hematology and coagulation profiles, and serum biochemistry were obtained twice pre-study, then on Study Day 8. Decreases in erythrocyte parameters 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. There were no other treatment-related findings on serum

biochemistry or coagulation parameters. As expected from the pharmacodynamic mechanism of natalizumab, both groups of AN100226-treated monkeys showed approximate 2 to 3-fold elevations in peripheral blood leukocytes from baseline on Study Day 8. There were no differences noted between the two groups, either before or following treatment with the two preparations of natalizumab. These data are represented graphically in Figure 25, below, which was provided by the sponsor in the final study report.

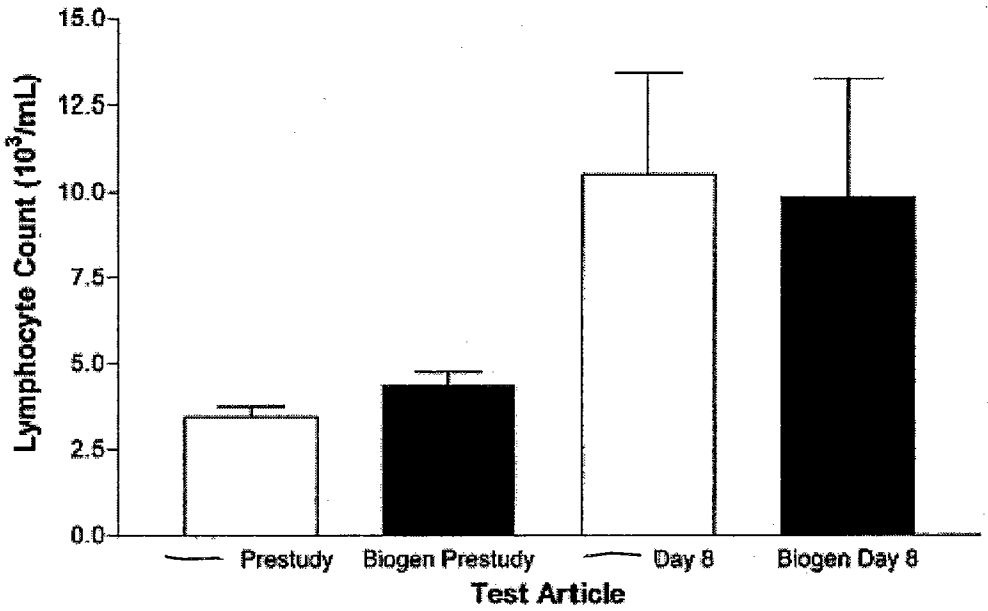


Figure 25. Pharmacodynamic effect of AN100226 produced by —— (open bars) or Biogen (filled bars) manufacturing methods on lymphocyte counts in cynomolgus monkeys. Mean lymphocyte counts ± S.D. are shown for each group (n = 15/group) pre-study (average of two samples), and Study Day 8.

Pharmacokinetics: 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 either at 5 min after, or immediately upon completion of infusion and decreased over time so that by Study Day 14 they were below the lower limit of quantitation of the assay for both groups. The calculated values for C_{max}, $AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$ for AN100226 produced by either manufacturer, over the first 24 hours after infusion are presented in Table 20, below.

Table 20. Pharmacokinetic and bioequivalence analyses of AN100226 (natalizumab) manufactured by —— or Biogen processes

Pharmacokinetic Parameter	Mean Value ± S.D.		Geometric Mean Ratio (%)	90% Confidence Interval (%)
	—— Process	Biogen Process		
C_{max} (µg/ml)	85.3 ± 15.6	87.1 ± 15.1	102.4	91.9 – 114.0
$AUC_{0\text{-last}}$ (µg*hr/ml)	2067 ± 4177	1691 ± 363	82.6	71.5 – 95.4
$AUC_{0\text{-inf}}$ (µg*hr/ml)	2308 ± 566	1948 ± 570	83.4	71.0 – 97.9

Bioequivalence analyses for C_{max}, $AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$ were computed as the difference of the means in the natural log scale with accompanying 90% confidence intervals, then converted back to the original scale to obtain the estimated ratio of geometric means and confidence interval

(Table 20, above). The data derived from the serum concentrations of natalizumab produced by the two different methods did not meet the criteria for bioequivalence for $AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$, but did pass the criteria for bioequivalence based on C_{max}. The initial ELISA assays had been performed by two separate analysts, who evaluated samples from separate animals in each group. One analyst was found to have performed these assays with greater precision than the second analyst. Therefore, to rule out that the difference in assay precision was responsible for the failure to meet the criteria for bioequivalence, each analyst completed the assays for the remaining samples in each group, and the results were re-evaluated for bioequivalence. Once again, the values for natalizumab $AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$ with the Biogen material were slightly lower than those obtained for product manufactured by —— regardless of analyst. Under this secondary analysis, C_{max} again met the criteria for bioequivalence of the two products, while $AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$ did not pass the test for bioequivalence.

Comment: A human pharmacokinetics study to determine the bioequivalence of natalizumab produced at —— with that manufactured by Biogen was subsequently conducted, using a randomized, 2-way cross-over design in 80 healthy volunteer subjects (Clinical Study #C-——). The lots of product used in this study differed from those used for the cynomolgus monkeys; the Biogen material used was from lot #G83001B, and the —— material was used from lot #G23005A. 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. The difference in results from the animal and human studies may relate to differences in either the lots of product used, or to the interaction with, and/or affinity of natalizumab for non-human primate α4-integrin, as compared to the human protein expressed on circulating lymphocytes.

Immunogenicity: All animals on study developed antibody responses to natalizumab, beginning on Study Day 14 with the exception of one monkey in the Biogen product group (animal #FN17637F), where the anti-natalizumab antibody response was first detectable on Study Day 17. Mean antibody titers, ± S.D. are shown in Table 21 below. There were no statistically significant differences in the titer of total anti-natalizumab antibody developed against material produced by either Biogen or —— processes, at any time point tested. Neutralizing antibody directed against natalizumab was not measured in this study.

Table 21. Anti-natalizumab total antibody titers following a single dose of AN100226 manufactured by either —— or Biogen

AN100226 Produced by	Mean Anti-Natalizumab Antibody Titer				
	Day 14	Day 17	Day 21	Day 24	Day 28
——	79.0 ± 61.5	79.0 ± 61.5	79.0 ± 61.5	79.0 ± 61.5	79.0 ± 61.5
Biogen	79.0 ± 61.5	79.0 ± 61.5	79.0 ± 61.5	79.0 ± 61.5	79.0 ± 61.5

Study Conclusion: Intravenous infusion of a single dose of AN100226 produced by either —— or Biogen was well-tolerated in cynomolgus macaques, with no clinical, hematologic, or serum biochemical evidence of toxicity. Elevated lymphocyte counts were observed in both groups at Study Day 8, and are an expected pharmacodynamic effect of natalizumab in a responsive species. The pharmacokinetics of natalizumab after a single, intravenous infusion in monkeys demonstrated comparable C_{max} between the Biogen and —— products, as determined by the confidence interval of the geometric mean ratio of the two materials (geometric mean ratio Biogen: —— 102.4%, 90% confidence interval 91.9 – 114.0%). The $AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$ for the two products did not meet the prespecified bioequivalence criteria. There were no differences in the total anti-natalizumab antibody response detected between monkeys treated with AN100226 produced by either manufacturing site, under the conditions of this study.

Comment: The sponsor has stated in the final study report that since both intra-individual and inter-individual variability in serum concentrations and in assay variation are greater for protein therapeutics (like natalizumab) than for small molecules, that a less stringent bioequivalence criterion of 30% (confidence intervals of 70% - 143%) might be more appropriate. The values derived here for both $AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$ for the [redacted] and Biogen materials would meet these less stringent criteria; however, no consensus has been reached with FDA regarding the applicability of less stringent standards for bioequivalence for protein therapeutics.

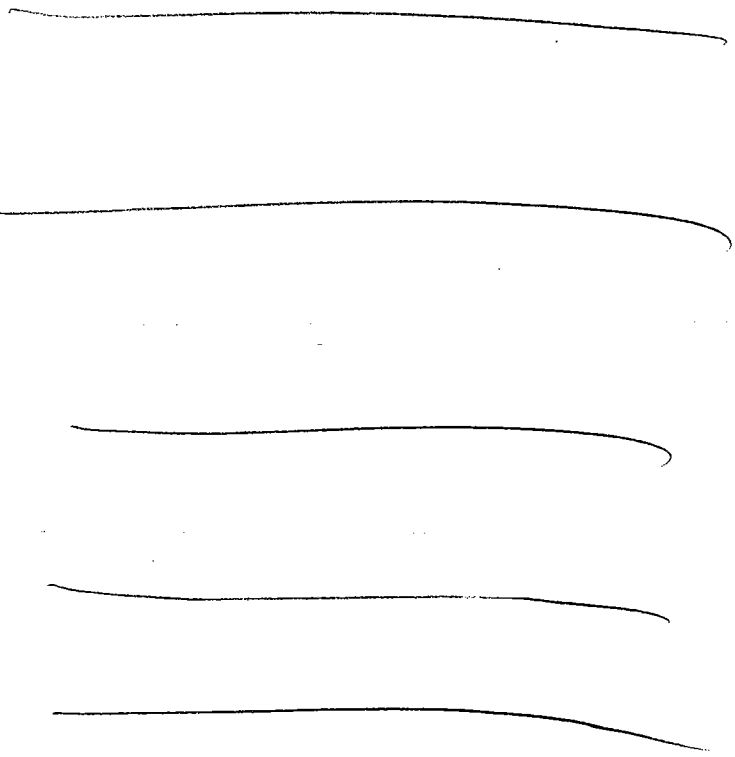
Study title: Pharmacokinetic study of Antegren (AN100226, natalizumab) in guinea pigs.
Key study findings: Dose-related, although non-linear increases in C_{max} and AUC_{last} were observed in guinea pigs following repeated i/v administration of 3, 10, or 30 mg/kg natalizumab, every other day for up to 60 days. Anti-AN100226 antibody titers were detectable in the serum from all groups of guinea pigs treated with 3 mg/kg/dose, but in only one female guinea pig after repeat administration of 30 mg/kg/dose natalizumab.
Study no.: #0309-010-01 ([redacted] #1147-112)
Volume #, and page #: EDR files: BLA 125104\000\module4\pharmacokinetics\309-010-01.pdf
Conducting laboratory and location: [redacted]
Date of study initiation: September 24, 2001 (final report dated July 8, 2003)
GLP compliance: Yes
QAU statement: yes (X) no ( )
Drug, lot #, and % purity: natalizumab, lot #F23001; 22 mg/ml; purity, [redacted] SDS-PAGE, reduced, [redacted] monomeric IgG, [redacted] HPLC, and lot #F23007, 22 mg/ml; purity, [redacted] SDS-PAGE, reduced, [redacted] monomeric IgG, [redacted] HPLC (copy of sponsor's Certificate of Analysis for each lot was included as Appendices to the final study protocol)

Methods

Doses: natalizumab 3, 10, 30 mg/kg/dose, every other day for Study Days 1-65 for male guinea pigs, GD4 – GD32 for early gestation females, and GD30 to approximately 32 days post-partum for late gestation females
Species/strain: guinea pig, Hartley strain
Number/sex/group or time point (main study): 6/sex/dose group, males and presumed pregnant, early (GD4) and late (GD30) females
Route, formulation, volume, and infusion rate: intravenous injection; natalizumab formulated in phosphate buffered saline; 1.5 ml/kg injected
Sampling times for pharmacokinetics: Blood samples for measurement of natalizumab serum concentrations were obtained from treated animals on this study by retro-orbital puncture, under light isoflurane anesthesia. Samples were collected from male animals prior to dosing (0 hr), and at 5 min, 2, 6, 12, and 48 h for doses administered on Study Days 1, 9, 17, 25, 33, 41, 49, 57, and 65. Blood samples from presumed pregnant, early gestation females were collected pre-dose, and at approximately 5 min and 2, 6, 12, and 48 h after dosing on GD 4 (initial dose), 12, 20, and 28. A single blood sample was collected from each animal on GD36, GD44, GD52, and GD60. Samples for pharmacokinetic analysis of natalizumab from late gestation females were collected at pre-dose, 5 min, 2, 6, 12, and 48 post-dose on GD30, GD38, GD54, GD63, GD70, GD78, GD86, and GD94.

Comment: The contracting laboratory states in the final study report that for the late gestation, presumed pregnant animals "These females delivered their litters on GD58-71, but for dosing purposes the subsequent days [post-partum] were recorded as GD days."

Age: ≥ 16 weeks old (males); ≥ 12 weeks old (presumed pregnant, early and late gestation females)
Weight (nonrodents only): 793 – 913 g (males), 798 – 988 g (presumed pregnant, early gestation females), and 712 – 1181 g (presumed pregnant, late gestation females)
Unique study design or methodology (if any): Natalizumab concentrations in guinea



Observation times and results

Mortality and Clinical Observations: All animals were observed twice daily for general health, mortality, moribundity, and clinical signs of toxicity. Individual animal body weights were recorded at randomization, on the first day of dosing and then weekly throughout the treatment period, and prior to terminal sacrifice. The day of littering and the number of pups were recorded for females in the late gestation group. All pups were allowed to remain with their sow for 14 days post-littering, then euthanized and discarded without necropsy. All parental female and male guinea pigs were euthanized following the final collection of blood samples, and discarded without necropsy evaluation.

There were a number of mortalities in all groups of animals treated with natalizumab on this study. One male guinea pig in the 10 mg/kg dose group (animal #25569) was found dead on Study Day 27. Prior to death, clinical observations for this guinea pig had included signs of dyspnea, decreased activity (reported by the contracting study laboratory as "languid behavior"),

thin appearance, and rough hair coat. A second male guinea pig in the 30 mg/kg/dose group (animal #25589) was found dead on Study Day 19, with no prior abnormal observations reported. Clinical observations for the surviving males in these dose groups included hunched and thin appearance, dyspnea, and evidence of rales.

There were a total of four early gestation females either found dead or sacrificed moribund on this study. Female #25561 in the 3 mg/kg/dose group was euthanized moribund on GD12 after clinical observations of decreased activity and thin appearance. One early gestation female in the 10 mg/kg/dose group (animal #25580) was found dead on GD59, after reported observations of dyspnea and thin appearance. One 30 mg/kg/dose early gestation female (animal #25594) was found dead on GD12. Prior to death, this animal had reported observations of decreased activity, diarrhea, and large, approximately 2.1 to 5 cm tissue masses present in both the right and left axilla. A second female (animal #25595) in this same dose group was euthanized as moribund on GD31, following eye trauma due to the blood collection procedure. Surviving, early gestation females in all three dose groups had reported observations of decreased activity, thin appearance, rough haircoat, dyspnea and/or rales, and "small, movable" or "large, movable" tissue masses in the axillary regions over the duration of the study period.

Five female guinea pigs in the late gestation group did not survive until scheduled study termination. Two female guinea pigs in the 3 mg/kg/dose group were found dead, one (animal #25563) on GD57 after having spontaneously aborted, and the second (animal #25568) on GD46 with no prior observed clinical abnormalities. One 10 mg/kg/dose female (animal #25584) was euthanized as moribund on GD85 following trauma from the retro-orbital bleeding procedures. Female #25603 in the 30 mg/kg/dose group was found dead on GD83 after reported observations of dyspnea, and a second late gestation female (animal #25602) was sacrificed moribund on GD59 after clinical observations of thin appearance, soft feces, rough haircoat, dyspnea and rales, decreased activity, lethargy, and prostration were noted. Recorded clinical observations in the surviving females in the late gestation treatment groups included decreased activity, lethargy, prostration, dyspnea, rales, and ptosis, head tilt, a cloudy eye lens, discolored feces, rough haircoat, thin appearance, and spontaneous abortions. There were no apparent relationships of either incidence or severity, or duration of the clinical observations to the dose of AN100226 administered.

Comment: The contracting laboratory states in the final report that the clinical observations and unscheduled deaths were comparable across all natalizumab treatment groups, and attributed these findings to repeated anesthesia and blood collection procedures. However, the presence of dyspnea, rales, decreased activity, and especially the enlarged masses present in the axillary regions of these animals suggests that the observed clinical signs of morbidity and the early mortalities may be secondary to induction of hypersensitivity responses to natalizumab, which is a humanized protein. Guinea pigs are known to be highly susceptible to anaphylaxis following exposure to foreign proteins, and these findings may represent early changes associated with development of hypersensitivity to the product.

Animals in all three AN100226 dose groups lost body weight over the first four weeks of dosing, but by the end of the treatment period most had either recovered to baseline values, or gained weight. However, the body weight gain at end of study for the presumed pregnant, early and late gestation females was less than would be anticipated for these animals in the absence of natalizumab treatment. The contracting laboratory did not perform statistical evaluation of the body weight changes, since no control groups were included in the study design.

Comment: Independent calculation by this reviewer of mean and standard deviation values for each of the AN100226 dose groups did not show statistically significant differences either between the treatment groups ($p > 0.05$, one-way ANOVA), or for the individual treatment groups at study termination as compared to baseline values ($p > 0.05$, paired Student's *t* test).

Spontaneous abortions occurred in one late gestation female each in the 3 mg/kg/dose and 30 mg/kg/dose groups, at GD56 and GD43, respectively, as described in the clinical observations, above. All remaining, presumed pregnant females, 50%, 50%, and 20% in the 3, 10, and 30 mg natalizumab/kg/dose groups, respectively, delivered live litters between GD58-GD71. Dams #25566 and #25567 in the 3 mg/kg/dose group had 2 pups each per litter, on GD58 and GD67, respectively. Dams #25582, #25584, and #25586 in the 10 mg/kg dose groups had litter sizes of 3, 8, and 1 pups on GD69, GD66, and GD71, respectively. One pup in the litter from dam #25584 was dead at the time of littering. The one female guinea pig in the 30 mg/kg/dose group produced a litter of 4 live pups on GD68. No further evaluation of litter parameters (*e.g.*, crown-rump length, pup weight, skeletal or visceral abnormalities) was performed.

Pharmacokinetics: On Study Day 1, or GD4 or GD30 for the early and late gestation females, respectively, natalizumab concentrations in serum were below the limits of quantitation of the assay prior to treatment with AN100226. Dose-related increases in both C_{max} and AUC were observed in both male and presumed pregnant females at this time point, and were approximately proportional to the dose of natalizumab administered. Beginning on Study Day 9 in the male guinea pigs, and on GD12 and GD38 in the early and late gestation females, respectively, AN100226 was detectable in serum from both male and presumed pregnant females prior to dosing. At these time points, both C_{max} and AUC for AN100226 in all three groups of animals were increased by approximately 2-fold over the same values for the initial dose. However, the increases in AUC and C_{max} observed between the dose groups were less than proportional to the increase in dose, suggesting that the pharmacokinetics of natalizumab in this species are non-linear. On repeated administration of natalizumab, values for AUC and C_{max} remained elevated as compared to following the initial dose; however, the mean values began to decrease in all three dose groups of male and pregnant female animals, and then remain at a plateau for the remainder of the study. The mean data for C_{max} and AUC for the male, early gestation presumed pregnant female, and late gestation presumed pregnant females are presented below in Tables 22, 23, and 24, respectively, which were abstracted directly from the sponsor's final study report.

Table 22. Mean Cmax (μg/ml) and AUC (μg*hr/ml) for AN100226 in male guinea pigs after repeat-dosing every other day for 65 days

Day	3 mg/kg		10 mg/kg		30 mg/kg	
	Cmax	AUC	Cmax	AUC	Cmax	AUC
1	72	1694	213	4458	700	17457
9	98	1453	550	14734	1320	34142
17	70	1119	478	13977	1833	47976
25	18	168	452	11368	1497	40692
33	9	124	356	12426	1598	48852
41	22	350	498	16259	1662	55995
49	44	785	492	16212	1558	55695
57	63	1456	1042	33295	1543	55653
65	52	1419	679	23272	1806	61047

N=5-6 animals/group at each time point

Table 23. Mean Cmax (μg/ml) and AUC (μg*hr/ml) for AN100226 in presumed pregnant, female guinea pigs after repeat-dosing every other day from GD4 – GD30

Day	3 mg/kg		10 mg/kg		30 mg/kg	
	Cmax	AUC	Cmax	AUC	Cmax	AUC
GD 4	53	1424	292	7290	532	16804
GD 12	137	2828	571	15644	1770	52696
GD 20	56	846	385	8894	1294	37568
GD 28	21	411	306	5947	1246	40436

N=4-6 animals/group at each time point

Table 24. Mean Cmax (μg/ml) and AUC (μg*hr/ml) for AN100226 in presumed pregnant, female guinea pigs after repeat-dosing every other day from GD30 –GD94

Day	3 mg/kg		10 mg/kg		30 mg/kg	
	Cmax	AUC	Cmax	AUC	Cmax	AUC
GD 30	59	1505	258	6169	629	15104
GD 38	147	4571	447	13488	1229	36293
GD 46	105	3229	250	7639	1039	32088
GD 54	63	1915	241	6663	1356	38887
GD 62	102	3029	536	16452	1243	33600
GD 70	91	2142	681	23410	1393	46318
GD 78	91	2574	584	20584	1816	66177
GD 86	78	2247	733	25913	1515	55109
GD 94	98	2896	421	14766	1559	61003

N=4-6 animals/group at each time point

Female guinea pigs in the early gestation, presumed pregnant group were also evaluated for serum AN100226 levels following completion of dosing on GD30. Serum levels of natalizumab remained detectable at GD60 in 3/4 surviving animals treated with 30 mg/kg AN100226 from GD4 to GD30. While serum levels of AN100226 were undetectable in the lower dose groups at this time point, the early gestation females in the 10 mg/kg dose group still had detectable natalizumab present in 5/6 animals at GD35, in 3/6 animals at GD44, and in 2/6 animals at GD52. Natalizumab was detectable in only 1/5 surviving early gestation females in the 3 mg/kg/dose group at GD36, and was undetectable at all remaining time points out to GD60.

Immunogenicity: Anti-natalizumab antibody titers in male guinea pigs dosed with AN100226 were below the limits of quantitation of the assay until Study Day 25, at which point low titers were present in 2/6 males (animals #25551 and #25553) in the 3 mg/kg dose group only. Antibody titers in these two animals continued to increase at Study Day 33, by which time an additional male in this group (animal #25555) had developed a low titer of anti-natalizumab activity (approximately 4 µg/ml anti-AN100226). Anti-natalizumab titers in these three animals showed some variation over the remainder of the study, but remained at approximately plateau levels until termination of dosing at Study Day 65. The values for absorbance for anti-AN100226 antibody in the groups of male guinea pigs treated with 10 or 30 mg/kg/dose remained below the limit of quantitation of the assay for the entire study duration.

In the early gestation female guinea pigs treated with AN100226, no detectable anti-natalizumab antibodies were present in any animal prior to initiation of dosing on GD4. A single presumed pregnant female (animal #25562) in the 3 mg/kg dose group had a low-level (1.1 µg/ml) anti-natalizumab titer present at 48 h after dosing on GD12, which increased to 18, 186, and 265 µg/ml 48 h after dosing on GD20, GD28, and GD36, respectively. A second female in this dose group (animal #25559) developed a low titer (2.5 µg/ml) of anti-natalizumab antibody at 48 h after dosing on GD 20, which increased to 62 µg/ml and 228 µg/ml on GD28 and GD36, respectively. By GD 36, all 5 surviving females in the 3 mg natalizumab/kg/dose group had developed anti-AN100226 titers, which ranged from 4.8 to 265 mg/ml. Following discontinuation of dosing, anti-natalizumab antibody titers decreased slightly in all animals in this group, but remained above the limit of detection of this assay until study termination at GD60.

Anti-natalizumab serum antibody titers in all presumed pregnant, early gestation females treated with 30 mg/kg/dose AN100226 remained below the limit of quantitation of the ELISA assay for the duration of the study, with the exception of a single titer of 1.7 µg/ml detected in female #25593 at GD60. Antibody titers directed against AN100226 were first detectable in 3/6 early gestation females in the 10 mg/kg/dose group at 48 hours after dosing on GD 28, with levels ranging from 0.6 to 12 µg/ml. On GD36 and GD44, anti-AN100226 titers in these three animals had increased to ranges of 1.5 to 59 µg/ml and 6.9 to 142 µg/ml, respectively. An additional animal in this dose group developed an anti-natalizumab titer of 2.4 µg/ml on GD53, which had increased to a titer of 9.7 µg/ml on GD60, at the termination of the study.

Comment: The presumed pregnant, early gestation females were reported in the study protocol as being treated with AN100226 every other day from GD4 to GD30. However, the results in the immunogenicity section of the final report are for serum samples obtained 48 hours after dosing on GD35. Animal #25562 in the 3 mg/kg/dose group had relatively high titers of anti-natalizumab at this time point. No reason was provided by either the sponsor, or the contracting study laboratory for this apparent discrepancy in the timing of the samples.

All presumed pregnant, late gestation female guinea pigs were negative for serum levels of anti-natalizumab from the time of initiation of treatment at GD30 until GD54, at which point a single animal (female #25565) in the 3 mg/kg/dose group developed an anti-AN100225 antibody titer which reached 14 µg/ml, at 48 h after dosing. Antibody titers in this animal continued to increase to 89 µg/ml pre-infusion, and 109 µg/ml 48 h after treatment on GD62, 140 mg/ml on GD70, and 165 mg/ml on GD86 prior to treatment with AN100226. At the end of the dosing period 48 h after the last injection of natalizumab on GD95, anti-AN100226 antibody titers in this animal remained at approximately 163 µg/ml. Anti-natalizumab antibody levels were below the limits of quantitation of the assay for all other animals in this dose group, and for all late gestation female guinea pigs in the 10 and 30 mg AN100226/kg/dose groups at all time points evaluated.

Study Conclusion: The pharmacokinetics of natalizumab after repeated, intravenous injection in male and presumed pregnant female guinea pigs demonstrated dose-related, although not dose-proportional increases in C_{max} and AUC_{last}. Mean values for both C_{max} and AUC_{last} increased with increasing doses until the third time point evaluated, at which time they tended to remain at relatively constant, plateau levels until termination of dosing. In presumed pregnant females treated early in gestation (GD4-GD30) with natalizumab, serum AN100226 levels were still detectable at GD60, approximately 2 months after the final dose. Both male and presumed pregnant female guinea pigs treated with repeat injections of 3 mg/kg/dose AN100226 developed anti-natalizumab antibody titers; however, this response was inversely related to the dose of AN100226, since very few animals treated with 10 mg/kg/dose, and only one female guinea pig treated with 30 mg/kg/dose natalizumab developed detectable titers by study termination.

Study title: A single-dose pharmacokinetics study with intravenous infusion of Antegren in female cynomolgus monkeys – comparison of [redacted] processes.
Key study findings: Natalizumab produced by the [redacted] scaleup process was bioequivalent to product manufactured by [redacted] at a [redacted] scale, as determined by pharmacokinetic analysis of C_{max}, AUC_{last}, and $AUC_{0\text{-}inf}$ in serum samples from monkeys treated with 3 mg/kg, i/v of AN100226 produced by either process. There were no treatment-related toxicities observed, although 2 to 3-fold increases in total and differential leukocytes from baseline were detected on Study Day 8 after treatment as an expected, pharmacodynamic effect. All monkeys developed both binding and anti-idiotype antibody directed against natalizumab by Study Day 14 after treatment, with no significant differences in antibody response observed between the groups treated with AN100226 produced by either scale process.
Study no.: #723-004-98 [redacted] Study #0621-78)
Volume #, and page #: EDR files: BLA 125104\000\module4\pharmacokinetics\723-004-98.pdf
Conducting laboratory and location: [redacted]

Date of study initiation: March 18, 1998 (final report dated July 9, 1998)
GLP compliance: Yes
QAU statement: yes (X) no ( )
Drug, lot #, and % purity: natalizumab (AN100226) manufactured by [redacted] lot #A010A (Athena Neurosciences lot #A007, [redacted] scale; 1.7 mg/ml, purity [redacted] SDS-PAGE (reduced), and [redacted] monomeric IgG [redacted] HPLC); and natalizumab manufactured by [redacted] scale, lot #C0237, 5.4 mg/ml; purity [redacted] IgG by SDS-PAGE, and [redacted] monomeric IgG by [redacted] HPLC (copy of sponsor's Certificate of Analysis for each product was included as an Appendix to the final study protocol)

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 with monoclonal antibodies or other immunoglobulin products

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 6.0; 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 24.5 hours after start of the infusion, and on Study Days 3, 4, 5, 6, 7, 10, 14, 17, 21, 24, and 28 after the initial day of dosing.

Age: young, adult female animals (age not specified)

Weight (nonrodents only): 2.3 – 3.6 kg

Unique study design or methodology (if any): Natalizumab concentrations in [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: All monkeys were observed twice daily for general health and behavioral or other clinical signs of overt toxicity, beginning 3 days prior to dosing and continuing until Study Day 28. Body weights were determined once during the week prior to dosing, within 24 h of initiation of treatment on Study Day 1, then weekly thereafter until study termination on d 28. Food consumption was qualitatively assessed daily for each animal, 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 AN100226 produced at either the [redacted] scale was 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 bruising at the site of blood collection was observed in most animals at various time points during the 28 day observation period, and was 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 within one week pre-study, then on Study Day 8. 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 samples from AN100226-treated cynomolgus monkeys were analyzed for glucose, hepatic transaminases, γ-GT, alkaline phosphatase (ALP) and total bilirubin, BUN, creatinine, and electrolyte levels, and total protein, albumin, globulin, and A:G ratios. Urine samples were collected in specially designed cage pans, and analyzed for color, pH, specific gravity, and for levels of nitrite, urobilinogen, leukocyte esterase, protein, glucose, ketones, bilirubin, and occult blood. Microscopic examination of samples was only performed if any abnormalities in appearance, leukocyte esterase, protein, blood or nitrites were observed.

Mild decreases in erythrocyte parameters, ranging from 11% to 14% 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. As expected from the pharmacodynamic mechanism of natalizumab, both groups of AN100226-treated monkeys showed approximate 2 to 3-fold elevations from baseline values in both total and differential peripheral blood leukocyte counts on Study Day 8. In this particular experiment, all leukocyte populations, including lymphocytes, monocytes, eosinophils, basophils, and neutrophilic granulocytes were affected. There were no differences noted in pharmacodynamic response between the two groups, following treatment with the two different scale preparations of natalizumab. These data are represented in Table 25, which was abstracted from the final study report, below.

Table 25. Pharmacodynamic effect of AN100226 produced by — at . — scale on peripheral blood leukocyte counts in cynomolgus monkeys

Cell Type	% Increase of Group Means on Day 8 (Compared to Prestudy Values)	
	Group 1 (200L)	Group 2 (2000L)
Total White Blood Cell Count	85%	81%
Polysegmented Neutrophils*	26%	30%
Lymphocytes	110%	98%
Monocytes	191%	122%
Eosinophils	312%	173%
Basophils	100%	109%

*Band cells were increased slightly on Day 8 to — in Group 1 and Group 2, respectively, compared to none present prestudy.

Serum biochemistry and urinalysis profiles were not remarkably different between the groups of monkeys treated with AN100226 produced at either the — scale. There were no definitive, treatment-related effects of natalizumab in either group; however, individual animals in both groups had elevations in serum ALT at Study Day 8 as compared to baseline. Animal #F7555F in the group treated with the — process material had a baseline ALT value of 45 U/ml, that increased by > 4-fold to 207 U/ml at Study Day 8. A second monkey in this dose group (animal #F7570F) had an initial ALT of 47 U/ml at baseline, that increased by > 2-fold on Study Day 8 to 122 U/ml. In the group of monkeys treated with AN100226 produced at the — scale, an elevation in ALT from a baseline value of 77 U/ml to a final value of 116 U/ml on Study Day 8 was observed for animal #F7572F. There were no other changes noted in other serum enzyme or analyte levels between baseline and Study Day 8, or between animals in the groups treated with the two different preparations of natalizumab. The biological relevance of the increases in ALT is not known at the present time. There were no remarkable findings on urinalysis in either group of monkeys treated with the — scale AN10026, either prior to dosing, or on Study Day 8.

Pharmacokinetics: 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 — and — scale processes are presented in Table 26 below, which was derived from the sponsor's final study report.

Table 26. Comparative pharmacokinetic profiles of natalizumab (AN100226) produced at two different scale processes in cynomolgus monkeys after a single, 3 mg/kg i/v infusion		
Pharmacokinetic Parameter	**Mean Value, ± S.D. (n = 8/group)**	
	— Process	**— Process**
C_{max} (µg/ml)	67.3 ± 2.4	64.0 ± 1.6
T_{max} (h)	0.74 ± 0.07	0.65 ± 0.06
t½ (h)	68.3 ± 4.4	61.8 ± 4.0
$AUC_{0\text{-last}}$ (µg*h/ml)	1150 ± 42	1085 ± 42
$AUC_{0\text{-inf}}$ (µg*h/ml)	1217 ± 49	1120 ± 36
AUMC (µg/h^2/ml)	67087 ± 6143	54085 ± 4398
MRT (h)	54.5 ± 3.5	48.3 ± 3.7
V_z (ml/kg)	244 ± 15	241 ± 19
V_{dss} (ml/kg)	134 ± 7	131 ± 12
Cl (ml/h/kg)	2.5 ± 0.1	2.7 ± 0.1

Bioequivalence analyses for C_{max}, $AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$ were computed as the difference of the means in the natural log scale with accompanying 90% confidence intervals, then converted back to the original scale to obtain the estimated ratio of geometric means and confidence intervals. The results of these analyses are represented in Table 27 below, which was derived from data included in the sponsor's final study report.

Table 27. Bioequivalence analysis of pharmacokinetic profiles of AN100226 produced at — scale in cynomolgus monkeys			
Pharmacokinetic Parameter	**Mean Value ± S.D.**		**90% Confidence Interval (%)**
	— Process	**— Process**	
C_{max} (µg/ml)	67.3 ± 2.4	64.0 ± 1.6	88.6 – 102.4
$AUC_{0\text{-last}}$ (µg*hr/ml)	1150 ± 42	1085 ± 42	86.8 – 102.4
$AUC_{0\text{-inf}}$ (µg*hr/ml)	1217 ± 49	1120 ± 36	84.7 – 100.3

The 90% confidence intervals derived from the pharmacokinetic profiles of natalizumab produced by the two different scale methods met the criteria for bioequivalence for all three parameters. Therefore it can be concluded that the AN100226 product produced at the — scale is comparable (bioequivalent) to the reference product produced at the — scale.

Immunogenicity: All animals on study developed antibody responses to natalizumab, beginning on Study Day 14. At Study Day 17, the magnitude of the total anti-natalizumab antibody responses was highly variable between animals within the two groups treated with AN100226, ranging from 15.4 µg/ml to 253.8 µg/ml for monkeys treated with natalizumab produced at the — scale, to 21.0 µg/ml to 703.3 µg/ml for animals dosed with AN100226 manufactured by the — process. Similar findings, although at much lower magnitude were observed for the anti-idiotype anti-natalizumab antibody response. The mean values for each of the different antibody responses for the animals treated with either product are shown in Table 28, below. Although the apparent mean values were much higher for the group receiving the — material than those treated with the — product, there were no statistically significant differences in the titer of either total or anti-idiotype, anti-natalizumab antibody developed against material produced by either process, at any time point tested. Neutralizing antibody directed against natalizumab was not measured in this study.

Table 28. Anti-natalizumab total and anti-idiotype antibody titers in cynomolgus monkeys following a single dose of AN100226 manufactured by —— at either —— scale					
AN100226 Produced by	Mean Anti-Natalizumab Total Antibody Titer (µg/ml), ± S.D. (n = 8/group)				
	Day 14	Day 17	Day 21	Day 24	Day 28
—	86.4 ± 91.5	85.5 ± 82.3	64.1 ± 62.4	54.1 ± 44.8	39.0 ± 24.9
—	139.8 ± 193.5	151.7 ± 227.8	122.3 ± 183.7	91.1 ± 117.1	72.7 ± 68.8
AN100226 Produced by	Mean Anti-Natalizumab Anti-Idiotype Ab Titer (µg/ml), ± S.D. (n = 8/group)				
	Day 14	Day 17	Day 21	Day 25	Day 28
—	8.6 ± 7.7	6.5 ± 6.3	5.2 ± 2.5	8.6 ± 5.9	11.4 ± 6.2
—	13.2 ± 14.0	12.6 ± 23.5	16.7 ± 23.2	18.8 ± 17.5	20.0 ± 16.6

Study Conclusion: Intravenous infusion of a single dose of AN100226 produced by either —— or Biogen 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 8, 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 —— scaleup process was comparable to the —— reference material, with respect to C_{max}, AUC0-last, and AUC0-inf. 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: A single-dose pharmacokinetic study with intravenous infusion of Antegren™ in female cynomolgus monkeys.

Key study findings: The pharmacokinetic profiles of serum from female cynomolgus monkeys treated with natalizumab formulated in 10 mM sodium phosphate-buffered saline and 0.02% polysorbate 80 were comparable to those obtained with the reference material, which was formulated in 50 mM L-histidine buffered saline.

Study no.: #723-012-98, analytical report and calculation of pharmacokinetic profiles; (includes also —— Study #961011, dosing and in-life phase, and Athena Study #AQS-289, analysis of serum samples and retained dosing samples)

Volume #, and page #: EDR files: BLA 125104\000\module4\pharmacokinetics\723-012-98.pdf

Conducting laboratory and location: ——— r, ——— , and Athena Neurosciences, 800 Gateway Boulevard, South San Francisco, CA 94080

Date of study initiation: December 12, 1996 (—— Study ##961011, final report dated February 11, 1998); January 8, 1997 (Athena Study #AQS-289, final report dated February 24, 1998; Athena Study #723-012-98, final report dated January 28, 1998)

GLP compliance: Yes for —— Study #961011, not specified in final study report for Athena Study #723-012-98

QAU statement: yes (X) no ( )

Drug, lot #, and % purity: Antegren™ (natalizumab, AN100226), lot #AN100226-003; 5.0 mg/ml, purity not specified (is cross-referenced back to the Certificate of Analysis for the bulk drug substance, which was not provided in the final report); AN100226 formulation buffer, lot #4T36QT, 50 mM L-histidine, 150 mM NaCl, pH 6.0, ± 0.02% polysorbate 80; purity not

specified; (copy of sponsor's Certificate of Analysis for the test article and vehicle diluent are included as Appendix C to the final study report for —— Study #961011)

Comment: The histidine buffered material is the formulation that was used in all preclinical pharmacology, toxicology, and pharmacokinetic studies in animals, while the product formulated in phosphate buffered saline is the formulation intended for marketing.

Comment: The final study report for —— Study #961011 lists two additional test articles and lot numbers, specified as Antegren™ with 0.02% polysorbate 80, lot #1450-65-1, and AN100226, lot #1450-65-2, without providing additional descriptions or Certificates of Analysis for these products. Appendix C of this same report states that the vehicle consisted of two formulations of histidine-buffered sodium chloride, with and without added polysorbate 80. It is not clear from the information provided in the —— study report whether the lot #1450-65-1 is formulated in phosphate buffered saline with 0.02% polysorbate 80 as stated in the final report for the Athena Study #738-012-98, or in histidine-buffered saline, as implied from the information in the final —— study report. For the purposes of this review, it is assumed that the AN10026 product was formulated in 50 mM L-histidine in 150 mM sodium chloride as the reference standard, and formulated in 10 mM sodium phosphate buffered saline with 0.02% as the test article (referenced to the Athena study report for Study #738-012-98).

Methods

Doses: natalizumab diluted in either vehicle formulation, 3.0 mg/kg

Species/strain: *Macaca fasicularis* (cynomolgus monkey); source (country of origin, purpose-bred vs. wild-caught) not specified in final study report

Number/sex/group or time point (main study): 3 females/dose group

Route, formulation, volume, and infusion rate: intravenous infusion; natalizumab formulated either in 50 mM L-histidine in 150 mM NaCl, pH 6.0, or formulated in 10 mM sodium phosphate-buffered saline, plus 0.02% polysorbate 80, pH 6.0; 10 ml/kg infused; infusion rate 20 ml/kg/h

Sampling times for pharmacokinetics: Blood samples (approximately 1.5 ml per time point) for measurement of natalizumab serum concentrations were collected 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 3, 15, 30 min, and 1.5, 3, 6, and 24 hours after completion of AN100226 infusion. Additional samples were collected daily for 6 days, then weekly until study termination at Study Day 28. Whole blood samples were allowed to clot for at least 30 min after collection, serum was harvested by centrifugation, and aliquots were shipped to the sponsor (Athena Neurosciences) for pharmacokinetic analyses.

Age: adult, female animals, 3.5 – 11 years old

Weight (nonrodents only): 2.48 – 3.46 kg

Unique study design or methodology (if any): AN100226 concentrations and anti-

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: All monkeys were observed twice daily for general health and behavioral or other clinical signs of overt toxicity, beginning 5 days prior to dosing and continuing until Study Day 28. Body weights were determined once prior to dosing, then twice weekly thereafter until study termination on d 28. Food consumption was assessed daily for each animal by counting the number of biscuits remaining from the previous feeding. There were no scheduled sacrifices for this study, and after the last sample collection all animals were returned to the colony without necropsy.

One female monkey dosed with 3 mg/kg AN100226 formulated in L-histidine buffered saline (animal #PR1189) was found dead on Study Day 7. Beginning approximately 4 months prior to study initiation, progressive weight losses were noted for this animal, so that at the time of the pre-dose weighing this monkey had lost 11% of its body weight. In the pre-study period, this animal showed signs of inappetence and decreased water intake, scant feces, and decreased activity. Clinical observations following infusion of AN100226 included hypoactivity and mild dehydration at 1 h post-dosing, which was noted as mild at onset but progressively worsened to moderate dehydration and severe hypoactivity during the next five days. Scant or no feces were noted from Study Days 2 through 5, and epistaxis was observed on Study Days 4, 5, and 6. The animal was found dead on Study Day 7, and a necropsy evaluation was performed. Serum samples obtained on Study Day 5, and stored frozen for pharmacokinetic analyses were subsequently used for evaluation of clinical chemistry, to aid in determining the cause of this animal's death. Clinical chemistry findings on Study Day 5 as compared to pre-study values included mild increases in total cholesterol, moderate elevations in serum glucose and hepatic transaminase levels, moderate decreases in serum calcium and albumin, severe elevations in BUN, creatinine, creatine phosphokinase, and serum triglycerides. At necropsy, the liver in this monkey was noted to be pale and friable, which was associated with moderate vacuolization on microscopic examination. Histopathological evaluation also revealed mild vacuolar changes in the renal tubules. Based on the clinical observations including progressive weight-loss, the serum biochemistry changes, and the histopathological findings of mild to moderate vacuolization in the liver and kidney, the cause of death assigned for this animal was fatal fatty liver-kidney syndrome, and the death was considered unrelated to natalizumab.

Comment: The contracting study laboratory has provided an incident report for this animal, which provides documentation of the clinical observations, serum chemistry, and necropsy and histopathological findings as Appendix E to the final study report.

Comment: The contracting laboratory has provided literature references in the report in Appendix E that document the clinical progression of fatal fatty liver-kidney syndrome in adult

female macaques, and note that the incidence can be as high as 11% in obese animals[2]. The findings observed for animal #PR1189 are consistent with the clinical profile of this disease, although the pathogenesis is not completely understood, since this animal did not appear obese at any time either prior to or following initiation on study.

Comment: The pharmacokinetic samples obtained from this monkey were not analyzed, and a replacement animal was treated with AN100226 formulated in L-histidine buffer, and used for calculation of the pharmacokinetic profiles, below.

All remaining monkeys survived for the entire study duration, and AN100226 formulated in either buffer was generally well-tolerated after a single, 3 mg/kg i/v infusion. There were no clinical toxicities noted for the 4 h observation period immediately after treatment with either formulation of natalizumab. Clinical observations were limited to fecal changes, including scant or no stool which were observed for all animals at various time points on study, and were not considered treatment-related since they had also been observed in individual monkeys prior to study initiation. No adverse effects of either formulation 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 pre-study, then on Study Day 28. Serum samples from AN100226-treated cynomolgus monkeys were analyzed for glucose, hepatic transaminases, γ-GT, alkaline phosphatase (ALP) and total bilirubin, BUN, creatinine, and electrolyte levels, and total protein, albumin, globulin, and A:G ratios. There were no remarkable effects of treatment with either formulation of natalizumab on any of the serum chemistry parameters evaluated, and no differences between the two treatment groups were noted.

Comment: One monkey in each group (animals #PR1187 and #PR1225) had elevated values of creatine phosphokinase of 1451 and 4190 U/ml, respectively at the pre-study sample on Study Day -9. These values were markedly decreased in each animal at Study Day 28 to within normal range for this species, and the elevations are not considered related to treatment with AN100226.

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. Mild decreases in erythrocyte counts, but not in hematocrit or hemoglobin concentration were noted in both groups of AN100226-treated monkeys at Study Day 28 as compared to baseline values, and are not considered related to natalizumab, but rather to the amount of blood sampling performed for the pharmacokinetic evaluations. There were no remarkable changes in either platelet, or reticulocyte counts between pre-study and Study Day 28 values for either group of animals, and no differences noted between the two groups. Total leukocyte counts at Study Day 28 showed slight, although not statistically significant elevations from baseline in both groups of monkeys treated without natalizumab, with no apparent difference between the two groups in the magnitude of the increase noted. A slight, although statistically not significant increase in differential neutrophils counts from pre-study values was observed at study termination in the group of monkeys treated with AN100226 formulated in L-histidine buffered saline, and a similar increase in differential lymphocyte counts was observed in the group treated with natalizumab in phosphate-buffered saline and 0.02% polysorbate 80 (data

[2] Bronson, R.T., *et al.* 1982. Fatal fasting syndrome of obese macaques. *Lab. Anim. Sci.*, **32**:187-192; Laber-Laird, K.E., *et al.*, 1987. Fatal fatty liver-kidney syndrome in obese monkeys. *Lab. Anim. Sci.*, **37**:205-209

not shown). There were no other remarkable effects of either formulation of AN100226 on unsegmented neutrophils, monocyte, eosinophil, or basophil differential counts, erythrocyte morphology, or erythrocyte sedimentation rates at Study Day 28, either between the treatment groups or when compared to pre-study values.

Pharmacokinetics: Serum concentrations of AN100226 were below the limits of quantitation of the assay [redacted] in all animals, prior to infusion of natalizumab and the C_{max} of either formulation was detected immediately after, or within 30 min from the completion of infusion. The calculated values for C_{max}, T_{max}, half-life, $AUC_{0\text{-last}}$, initial and steady state volumes of distribution (V_z and V_{dss}, respectively), and clearance (Cl) for AN100226 formulated in either L-histidine buffered saline or 10 mM sodium phosphate buffered saline plus 0.02% polysorbate 80 are presented in Table 29 below, which was derived from the individual animal values for each parameter included in sponsor's final study report.

Table 29. Comparative pharmacokinetic profiles of natalizumab (AN100226) in two different formulation buffers after a single, 3 mg/kg i/v infusion in cynomolgus monkeys

Pharmacokinetic Parameter	Mean Value, ± S.D. (n = 8/group)	
	50 mM L-histidine	10 mM Sodium Phosphate
C_{max} (µg/ml)	61.7 ± 17.3	43.4 ± 3.4
T_{max} (h)	0.69 ± 0.10	0.78 ± 0.21
t½ (h)	64.3 ± 38.7	59.7 ± 0.6
$AUC_{0\text{-last}}$ (µg*h/ml)	1666 ± 165	1332 ± 303
MRT (h)	69.0 ± 17.3	83.5 ± 2.4
V_z (ml/kg)	162 ± 86	194 ± 42
V_{dss} (ml/kg)	122 ± 25	188 ± 41
Cl (ml/h/kg)	1.8 ± 0.2	1.9 ± 0.5

There were no statistically significant differences in the calculated pharmacokinetic parameters between the two groups of treated animals ($p > 0.05$, two-tailed Student's *t* test). A formal analysis of bioequivalence of the two formulated versions of natalizumab was not performed for this study.

Immunogenicity: All animals on study developed antibody responses to natalizumab, beginning on Study Day 15, with the exception of animal #PR1187 who first had detectable anti-AN100226 activity present on Study Day 11. Peak antibody titers in both groups were observed at Study Day 14, and gradually declined until study termination at day 28. Although the data show an apparent increase in anti-natalizumab antibody titers at all time points in the group of animals treated with AN100226 formulated in 50 mM L-histidine compared to the group treated with the material in phosphate buffer, these differences were due to an exceptionally high antibody response in 1/3 monkeys (animal #PR1187) in this group, and were not statistically significant ($p > 0.05$, ANOVA). The data for the mean anti-AN100226 antibody levels are shown in Table 30, below.

Table 30. Anti-natalizumab antibody titers in cynomolgus monkeys following a single dose of 3 mg/kg AN100226 in 50 mM L-histidine or 10 mM phosphate buffered saline

AN100226 Formulated in	Mean Anti-Natalizumab Antibody Titer (µg/ml), ± S.D. (n = 3/group)				
	Day 14	Day 18	Day 22	Day 25	Day 28
L-histidine	177.1 ± 118.4	109.1 ± 72.0	77.9 ± 40.8	56.7 ± 28.8	57.4 ± 26.8
PBS	106.6 ± 44.9	65.7 ± 36.0	60.5 ± 37.8	45.5 ± 42.9	41.3 ± 16.2

Comment: Only the raw data for the immunogenicity analyses were provided in the final study report, with no further statistical analysis performed by the sponsor or the contracting study laboratory. Calculation of the mean values and standard deviations for each group, as well as statistical analysis were performed as part of the review.

Study Conclusion: There were no differences in any of the calculated pharmacokinetic parameters of AN100226 formulated in 50 mM L-histidine, as compared to natalizumab formulated in 10 mM sodium phosphate buffer plus 0.02% polysorbate 80. The histidine buffered material is the formulation that was used in all preclinical pharmacology, toxicology, and pharmacokinetic studies in animals, while the product formulated in phosphate buffered saline is the formulation intended for marketing.

Study title: Pharmacokinetics of AN100226 in male and female guinea pigs after intracardiac administration.

Key study findings: Dose-related, although non-linear increases in C_{max} and AUC_{last} were observed in guinea pigs following a single intra-cardiac injection of 1, 3, or 8 mg/kg natalizumab. The elimination half-life and mean residence time both increased with dose, while clearance was decreased approximately 3-fold between the lowest and highest dose groups. The volume of distribution of AN100226 was approximately 2-fold greater than the plasma space, suggesting a limited redistribution of natalizumab outside of the vasculature.
Study no.: #AL077
Volume #, and page #: EDR files: BLA 125104\000\module4\pharmacokinetics\al077.pdf
Conducting laboratory and location: Athena Neurosciences, 800 Gateway Boulevard, South San Francisco, CA 94080
Date of study initiation: not specified (final report dated August 10, 1995)
GLP compliance: No
QAU statement: yes ( ) no (X)
Drug, lot #, and % purity: AN100226 (humanized natalizumab), lot #8419/62/1, 3.075 mg/ml, and lot #8502/56, 4.165 mg/ml; percent purity was not specified in the final study report

Methods

Doses: natalizumab 1, 3, or 8 mg/kg
Species/strain: guinea pig, Hartley strain
Number/sex/group or time point (main study): 2-5/sex/dose group
Route, formulation, volume, and infusion rate: intra-cardiac bolus injection; natalizumab formulated in phosphate buffered saline; 1.0 ml/animal injected
Sampling times for pharmacokinetics: Blood samples for measurement of AN100226 serum concentrations were obtained from treated animals on this study via a catheter implanted in the jugular vein prior to study initiation. Samples were collected from all animals prior to dosing (0 hr), and at 0.5, 1, 2, 5, 15, and 30 min, and 1, 2, 4, 8, and 24 h, then once daily after injection for 7 days. Serum samples were harvested, snap-frozen in 2-methylbutane, and stored at -20°C until analysis.
Age: 4-5 weeks old
Weight (nonrodents only): 333 – 405 g (males), 285 – 479 g (females)
Unique study design or methodology (if any):

Results

Pharmacokinetics: Following intra-cardiac injection, natalizumab serum concentrations were highest immediately after dosing, then declined in a biphasic manner, with an initial distribution phase followed by an elimination phase. These results for a single, representative guinea pig in each treatment group are presented graphically in Figure 26 below, as provided by the sponsor in the final study report.

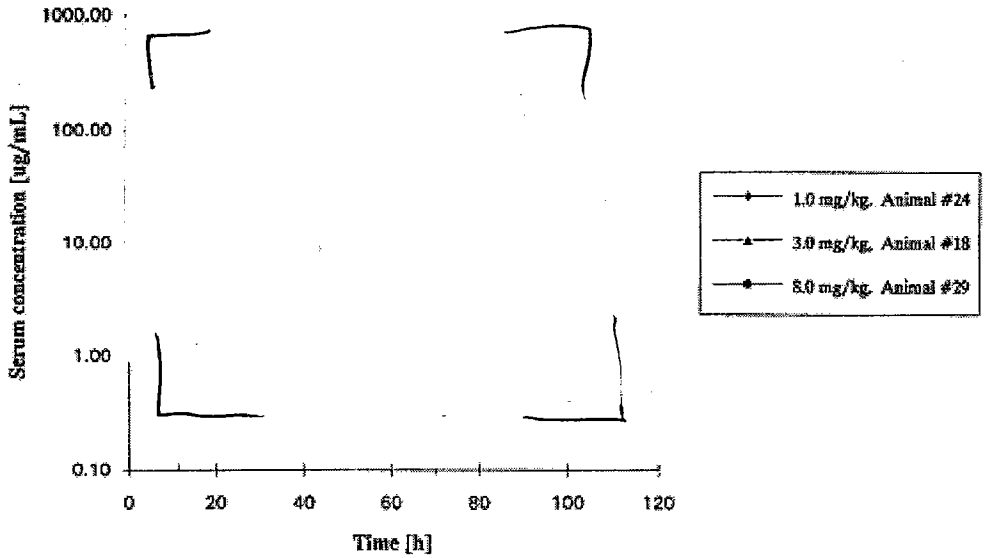


Figure 26. Serum concentration vs. time curve for representative animals treated with 1.0, 3.0, or 8.0 mg/kg AN100226 by intra-cardiac injection.

The calculated values for C_{max} and $AUC_{0\text{-last}}$ were increased in relation to the dose of natalizumab administered, however, these increases were less than proportional to the difference in doses of AN100226. The mean C_{max} values for AN100226 in the groups treated with 1, 3, or 8 mg/kg were 27, 69.8, and 153.8 µg/ml, respectively, which represent an increase of approximately 2.6-fold between the 1 and 3 mg/kg groups, but only a 5.6-fold increase between the values for the 1 and 8 mg/kg dose group. Similarly, the mean values for $AUC_{0\text{-last}}$ increased by only 1.4-fold between the groups treated with 1 and 3 mg/kg natalizumab, and by 3.2-fold between the 1 and 8 mg/kg/dose groups. An 8-fold increase in dose of AN100226 resulted in a 3-fold decrease in the calculated mean values for clearance, and approximately a 4-fold increase for both elimination half-life and mean residence time, as compared to the calculated values in guinea pigs treated with 1 mg/kg natalizumab. Both the initial and the steady state volume of distribution were increased with increasing dose, and exceeded the approximate 40 ml/kg plasma volume expected in guinea pigs, suggesting that AN100226 underwent a limited distribution to the extravascular space. These results are shown in Table 31, below, which was derived from the individual animal data for each parameter, as provided in the sponsor's final study report.

Table 31. Pharmacokinetic profiles of natalizumab (AN100226) after a single, intra-cardiac injection in guinea pigs			
Pharmacokinetic Parameter	**Mean Value, ± S.D.**		
	1 mg/kg[a]	**3 mg/kg[b]**	**8 mg/kg[c]**
C_{max} (µg/ml)	27.0 ± 3.9	69.8 ± 9.4	153.8 ± 28.8
t½α (h)	0.9 ± 0.6	0.7 ± 0.4	n.d.[d]
$t½_{elim}$ (h)	18.8 ± 5.3	36.9 ± 7.9	73.0 ± 24.1
$AUC_{0\text{-last}}$ (µg*h/ml)	366 ± 101	1503 ± 406	9408 ± 3165
AUMC (µg*h^2/ml)	9885 ± 4603	73717 ± 33705	1096393 ± 682327
MRT (h)	26.0 ± 6	47.0 ± 9	111 ± 35
V_z (ml/kg)	76 ± 19	110 ± 27	90 ± 1
V_{dss} (ml/kg)	75 ± 18	97 ± 16	94 ± 2
Cl (ml/h/kg)	2.9 ± 0.9	2.1 ± 0.5	0.9 ± 0.3

[a] n = 4 males, 3 females in this dose group
[b] n = 4 males, 4 females in this dose group
[c] n = 2 females only in this dose group
[b] n.d. = not determined. Not enough samples were collected at the early time points from the animals in the 8.0 mg/kg dose group to be able to calculate t½α.

Comment: The increase in both initial and steady state volume of distribution for natalizumab may reflect binding and saturation of the α4-integrin receptor present on circulating lymphocytes, monocytes, and eosinophils and basophils, rather than distribution of the antibody outside of the vascular compartment. Previous pharmacology studies have demonstrated that AN100226 binds peripheral blood leukocytes in guinea pigs with high affinity, and that this species is pharmacologically responsive to the anti-inflammatory effects of natalizumab in an experimental allergic encephalomyelitis model of multiple sclerosis.

Comment: The final study report does not contain a description of any clinical observations or findings in these animals. In Appendix A to this report containing the individual animal serum concentrations and calculated pharmacokinetic parameters, the values at 96 hours for male #10 in the 1 mg/kg dose group, and at 8 h after treatment for male #6 in the 3 mg/kg dose group are listed as "died," with no further explanation or description in the text.

Comment: The sponsor has performed a statistical analysis of the pharmacokinetic parameters, analyzed by gender, using a one-way ANOVA. The only significant difference between male and female guinea pigs was in the initial volume of distribution (V_z) at the 1 mg/kg dose group (p = 0.0098). Given the small number of animals in this study of either gender, it is unlikely that the results of this analysis are meaningful.

Study Conclusion: The pharmacokinetic profile of natalizumab in guinea pigs after a single, intra-cardiac injection demonstrated a biphasic distribution, with an elimination half-life that increased with increasing dose. Both $AUC_{0\text{-last}}$ and C_{max} were increased with dose, although proportionally less than the differences in dose would have suggested. The apparent volume of AN100226 distribution at steady state was approximately 2-fold greater than plasma volume in the guinea pig, suggesting that a low levels of distribution to the extravascular space occurs. Clearance decreased approximately 3-fold with increasing dose, while $t½_{elim}$ and mean residence time increased proportionally.

Study title: Pharmacokinetics of AN100226 in the cynomolgus monkey.

Key study findings: After a single, i/v infusion of 0.3, 3, or 30 mg/kg AN100226, pharmacokinetic profiles in cynomolgus monkeys showed dose-related, although non-linear increases in C_{max}, MRT, AUMC, and $AUC_{0\text{-last}}$. Mean elimination half-lives were approximately 8, 58, and 74 hours for the groups treated with 0.3, 3, and 30 mg/kg natalizumab, respectively, and clearance was decreased approximately 10-fold between the low and high dose groups. The steady state volume of distribution was approximately were equal to the plasma space. Anti-AN100226 antibody titers were detectable in the serum from all monkeys treated with 3 mg/kg natalizumab, and in the majority of animals treated with 30 mg/kg, and at much lower titers in monkeys treated with 0.3 mg/kg natalizumab.

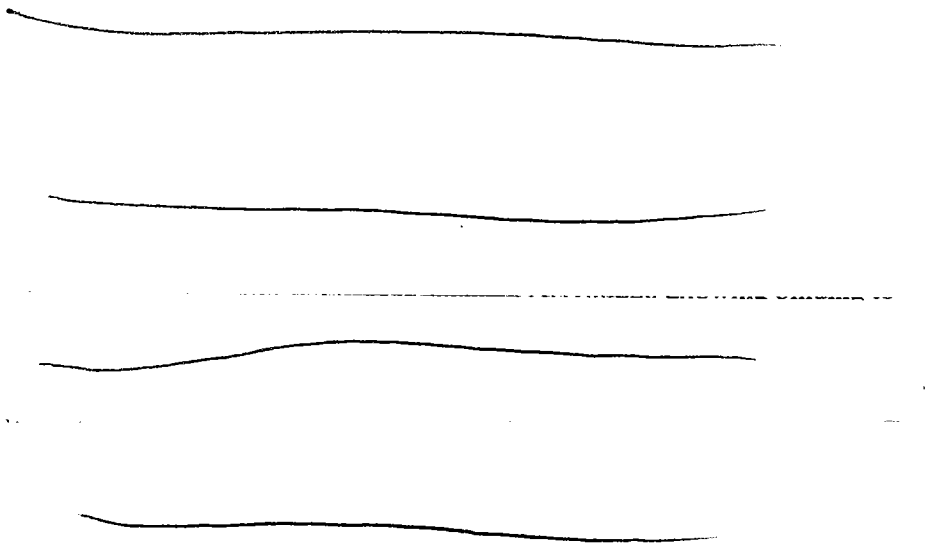
Study no.: #AL1009 (——— ' Study #940811)
Volume #, and page #: EDR files: BLA 125104\000\module4\pharmacokinetics\al109.pdf
Conducting laboratory and location:

Date of study initiation: November 24, 1994 (final report dated September 29, 1995)
GLP compliance: Yes
QAU statement: yes (X) no ( )
Drug, lot #, and % purity: AN100226 (natalizumab), lot #AN10026-0001; 4.0 mg/ml, purity ~, SDS-PAGE, and ~ IgG (——— HPLC); (copy of sponsor's Certificate of Analysis was included as an Appendix to the final study protocol)

Methods

Doses: AN100226, 0.3, 3.0, 30 mg/kg
Species/strain: *Macaca fasicularis* (cynomolgus monkey); experimentally naïve; source not specified
Number/sex/group or time point (main study): 4/sex/dose group
Route, formulation, volume, and infusion rate: intravenous infusion; natalizumab formulated in 50 mM L-histidine in 150 mM, pH 6.08; 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 5, 15, 30, and 90 min, and 3, 6, and 24 hours after the end of the infusion. Additional samples were collected once daily until Study Day 7, then twice weekly for 3 additional weeks.

and methanol, then stored at -70°C until shipped to the sponsor for analysis.
Age: estimated 3.5 – 4 years (males); 3.5 – 8 years (females)
Weight (nonrodents only): 2.56 – 4.11 kg (males); 2.10 – 3.54 kg (females)
Unique study design or methodology (if any):



Comment: The single-dose toxicology data obtained from this study were not submitted to the toxicology section of the —— 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: All monkeys were observed twice daily for general health and behavioral or other clinical signs of overt toxicity, beginning 5 days prior to dosing and continuing until Study Day 28. Additionally, each animal was observed for signs of toxicity immediately after infusion and at hourly intervals until 4 h after completion of the infusion. Body weights were determined once during the baseline period, prior to dosing on Study Day 1, then weekly thereafter until study termination on d 28. Food consumption was qualitatively assessed daily for each animal, as either normal or decreased from expected. At the completion of the study, all animals were returned to the colony without necropsy.

All monkeys survived for the entire study duration, and AN100226 was well-tolerated after a single, i/v infusion. One male and one female monkey in the 0.3 mg/kg dose group exhibited signs of stress from the experimental procedures during the observation period after infusion, with hypoactivity and mydriasis noted in the male monkey #PR1526, and hypoactivity and pallor in the female #PR1323. Both animals had recovered by the following day, and no similar effects were seen in the higher dose groups. There were no other treatment-related toxicities noted after infusion 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. Bloody discharge from the nose was observed sporadically in female #PR 1353 in the 0.3 mg/kg group, and in female #PR1343 in the high dose group, but was 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.

At Study Day 28, there were no remarkable effects of AN100226 treatment of cynomolgus monkeys on levels of serum glucose, hepatic transaminases, γ-GT, ALP, total and direct bilirubin, BUN, creatinine, and electrolyte levels, cholesterol, serum triglycerides, or total protein, albumin, globulin, or A:G ratios, either when compared between dose groups, or when values for individual animals were compared to baseline, pre-study values. One male monkey each in the 0.3 and 30 mg/kg dose groups, and one female monkey each in the 3.0 and 30 mg/kg groups had elevated values for CPK at Study Day 28 that were both outside of the normal range for cynomolgus macaques, and increased at least 2-fold over baseline. However, similar CPK values were obtained in one mid-dose and one high-dose male, and two low-dose, one mid-dose, and one high-dose female prior to study initiation, and these findings were considered incidental to natalizumab treatment. At Study Day 28, serum ALT was elevated $\geq$ 2-fold over baseline value in 3/4 female monkeys treated with 3.0 mg/kg AN100226/kg, and in 1/4 female monkeys in the 30 mg/kg dose group. However, one female in the 0.03 mg/kg dose group (animal #PR1353) had an ALT value pre-study of 160 U/ml, which increased to 183 U/ml on Study Day 28. A second female in the 3.0 mg/kg group had a pre-study value of 131 U ALT/ml, which increased to 265 U/ml at Study Day 28. None of the changes were statistically significant, either between the natalizumab dose groups, or when compared to pre-study values ($p > 0.05$, ANOVA), and were therefore considered unrelated to treatment with AN100226.

Blood samples for evaluation of hematology and serum biochemistry profiles were obtained from all animals following an overnight fast within one week pre-study, then on Study Day 28. 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. Mild decreases in erythrocyte parameters, including red blood cell counts, hematocrit, hemoglobin concentration, MCH, and MCHC were noted in all dose groups of AN100226-treated monkeys at Study Day 28 as compared to baseline. Although an apparent, slight decrease in reticulocyte percentage of total differential counts was noted at Study Day 28 in all three dose groups as compared to baseline, absolute reticulocyte counts were unchanged. These changes were not statistically significant either between the dose groups, or from the pre-study values and are not considered related to natalizumab, but rather to the amount of blood sampling performed for the pharmacokinetic evaluations. Platelet counts were also decreased by 2 to > 40% from pre-study values at Study Day 28 in all groups of natalizumab treated monkeys, without an apparent relationship to the dose of AN100226. The total leukocyte counts were not remarkably different, either between dose groups of natalizumab, or when compared to the baseline values. While dose-related increases in absolute numbers of both segmented neutrophils and lymphocytes were observed in all three natalizumab groups at Study Day 28 as compared to pre-study, the differential counts showed that the percentage of lymphocytes actually decreased, with a corresponding increase in the percentage of neutrophils.

Pharmacokinetics: Pharmacokinetic parameters were calculated by a non-compartmental model, and the area under the serum AN100226 concentration vs. time curve was determined using the linear trapezoidal rule. Peak serum concentrations (C_{max}) were observed within 2 hours after completion of infusion in all monkeys, with the exception of animal #1353F in the 0.3 mg/kg group, in which the C_{max} was reached at 6 h after completion of dosing. Both C_{max} and $AUC_{0\text{-last}}$ were increased with increasing dose of AN100226, although in greater proportion than the difference in the doses administered (mean values are presented in Table 32, below). In all groups of animals, natalizumab concentrations in serum showed a biphasic decline with an initial distribution phase, followed by a longer elimination phase. Elimination half-life decreased with increasing dose of natalizumab; the difference was approximately 10-fold between the 0.3 and 30

mg/kg dose groups, with a corresponding 10-fold increase in MRT between the two respective dose groups (please see Table 32, below). Several animals in the 3.0 and 30 mg/kg dose groups showed a third phase of accelerated elimination beginning on Study Day 11 to Study Day 14, and corresponding to the development of anti-natalizumab antibody, which resulted in increased clearance of AN100226 (data not shown; please see Immunogenicity results section, below). In cynomolgus monkeys, the volume of distribution of AN100226 at steady state was approximately equal to the plasma space. The calculated values for C_{max}, T_{max}, half-life, $AUC_{0\text{-}last}$, area under the moment curve (AUMC), initial and steady state volumes of distribution (V_z and V_{dss}, respectively), and clearance (Cl) for AN100226 are presented in Table 32 below. The means and standard deviations for each pharmacokinetic parameter were calculated from the individual animal values derived by the sponsor from the serum AN100226 concentrations in each dose group, as included in the sponsor's final study report.

Table 32. Pharmacokinetic profiles of natalizumab (AN100226) after a single, intravenous infusion in cynomolgus monkeys

Pharmacokinetic Parameter	Mean Value, ± S.D. (n = 4/sex/group)		
	0.3 mg/kg	3.0 mg/kg	30 mg/kg
C_{max} (µg/ml)	8.1 ± 1.6	124 ± 34.7	1630 ± 551
T_{max} (h)	1.4 ± 2.1	0.9 ± 0.6	1.0 ± 0.7
$t½_{elim}$ (h)	8.2 ± 3.8	58.0 ± 24.2	73.8 ± 23.4
$AUC_{0\text{-}last}$ (µg*h/ml)	66 ± 29	2537 ± 628	93490 ± 62120
AUMC (µg*h^2/ml)	649 ± 356	140900 ± 54230	13170000 ± 14410000
MRT (h)	10 ± 5	56 ± 22	113 ± 53
V_z (ml/kg)	62 ± 35	109 ± 57	42 ± 15
V_{dss} (ml/kg)	52 ± 29	70 ± 31	40 ± 10
Cl (ml/h/kg)	5.1 ± 1.4	1.3 ± 0.4	0.4 ± 0.2

Immunogenicity: Anti-natalizumab antibody responses varied in both time to onset and magnitude of response, with relationship to dose of AN100226. In most animals, detectable anti-AN100226 titers began to appear on Study Day 11; however, time to reach peak anti-natalizumab antibody levels varied between the dose groups, and between individual animals in each AN100226 dose group. In the low-dose group, 2/6 monkeys that developed anti-AN100226 antibody reached peak titer on Study Day 14, 3/6 reaching peak titer on Study Day 17, and the remaining monkey reaching peak titer on Study Day 21. Determination of peak antibody titers and time to peak titer for monkeys in the 3.0 mg/kg group was confounded by missing samples at Study Days 14 and 17; however at Study Day 11 anti-natalizumab titers were detectable in all 6 monkeys who had sample analyzed, and in all 8 animals at Study Day 21. In the high-dose group, only 1/8 monkeys had a detectable anti-AN100226 titer at Study Day 11. The incidence of detectable antibody titer increased to 4/8, 5/8, and 7/8 animals at Study Days 14, 17, and 21, respectively. In this dose group, the majority of animals did not reach peak anti-natalizumab antibody titers until Study Day 28, with the exception of monkey #1327F, whose peak titer of 142 µg/ml anti-AN100226 was detected on Study Day 24. Two female monkeys in the 0.3 mg/kg dose group (animals #1361F and #1323F), and female monkey #1363F in the 30 mg/kg group never developed detectable anti-natalizumab antibodies while on study. The development of relatively high, anti-AN100226 antibody titers in animals #1528M, #1500M, #1347F, and #1329F in the mid-dose group, and in monkeys #1522M, #1536M, #1327F, and #1357F correlated with decreased serum natalizumab concentrations, and was associated in these animals with an apparent, accelerated elimination phase beginning on Study Days 11-14 (data not shown). The mean values for each of the different antibody responses for the monkeys treated with 0.3, 3.0, or 30 mg/kg natalizumab are shown in Table 33, below. There were no significant

differences in anti-AN100226 antibody development noted between the dose groups, or between the male and female monkeys on this study ($p > 0.05$, ANOVA). Neutralizing antibody directed against natalizumab was not measured in this study.

Table 33. Total anti-natalizumab binding antibody titers in cynomolgus monkeys following a single intravenous infusion of AN100226

AN100226 Dose Group	Mean Anti-Natalizumab Binding Antibody Titer, ± S.D. (n = 8/group)					
	Day 11	Day 14	Day 17	Day 21	Day 24	Day 28
0.3 mg/kg	3.6 ± 8.4	16.5 ± 33.9	15.1 ± 23.1	10.0 ± 9.7	8.7 ± 8.8	5.0 ± 6.3
3.0 mg/kg	30.0 ± 55.4[a]	28.8 ± 23.5[b]	67.7 ± 49.2[c]	99.5 ± 78.5	97.1 ± 66.0	67.7 ± 41.1
30 mg/kg	0.64 ± 1.8	3.3 ± 3.1	6.4 ± 6.5	14.3 ± 10.4	43.4 ± 43.8	58.4 ± 38.9

[a] n = 6 (sample not received for 2 monkeys at this time point)
[b] n = 3 (sample not received for 4 monkeys, insufficient sample received for 1 monkey at this time point)
[c] n = 4 (sample not received for 4 monkeys at this time point)

Comment: Only the raw data for the immunogenicity analyses were provided in the final study report. Calculation of the mean values and standard deviations for each group, as well as statistical analyses were performed as part of the review.

Study Conclusion: Intravenous infusion of a single dose of 0.3, 3, or 30 mg/kg AN100226 was well-tolerated in cynomolgus macaques, with no clinical, hematologic, or serum biochemical evidence of toxicity. Elevations in both total lymphocyte and neutrophils counts as compared to pre-study values were observed in all dose groups at Study Day 28 without an increase in the total leukocyte count, and with a decrease in the differential (percentage) of lymphocytes present. The pharmacokinetics of natalizumab after a single, intravenous infusion in monkeys demonstrated non-linear increases in C_{max}, $AUC_{0\text{-last}}$, AUMC, and half-life, and decreases in clearance. Anti-AN100226 antibody titers were present in the majority of animals in the 3 and 30 mg/kg dose groups at Study Day 28.

Study title: A single dose pharmacokinetic study with intravenous infusion of AN100226 in female cynomolgus monkeys.

Key study findings: Treatment of female cynomolgus monkeys by a single intravenous infusion with 3 mg/kg natalizumab formulated in 50 mM L-histidine buffered saline, with or without 0.02% polysorbate 80 was well-tolerated, with no clinical, hematologic, or serum biochemistry toxicities noted. Pharmacokinetic parameters derived from monkeys treated with either formulation of AN100226 were not statistically different, with the exception of the elimination half-life which was decreased in the animals receiving the polysorbate 80 formulation. Addition of 0.02% polysorbate 80 had no effect on the immunogenicity of natalizumab; anti-AN100226 antibodies developed in 5/6 monkeys by Study Day 15, and no differences in titers were noted between the two groups.

Study no.: #AL-300 (also [redacted] Study #960711, dosing and administration; Athena Study #AQS-232, analysis of serum samples)
Volume #, and page #: EDR files: BLA 125104\000\module4\pharmacokinetics\al-300.pdf
Conducting laboratory and location: [redacted], and Athena Neurosciences, Inc., 800 Gateway Drive, South San Francisco, CA 94080

Date of study initiation: July 26, 1996 (— Study #960711, final report dated June 11, 1997); Athena Study #AL-300, final study report dated January 30, 1998; Athena Study #AQS-232, final study report dated February 24, 1998
GLP compliance: Yes
QAU statement: yes (X) no ( )
Drug, lot #, and % purity: AN100226 (natalizumab), lot #AN10026-0003; 4.9 mg/ml, purity — IgG light and heavy chains, SDS-PAGE; and — monomeric IgG at 164 kD (— — HPLC); (copy of sponsor's Certificate of Analysis was included as an Appendix to the final study protocol)

Methods

Doses: AN100226, 3 mg/kg
Species/strain: *Macaca fasicularis* (cynomolgus monkey); experimentally naïve; source not specified
Number/sex/group or time point (main study): 3 females/dose group
Route, formulation, volume, and infusion rate: intravenous infusion; natalizumab formulated in 50 mM L-histidine in 150 mM sodium chloride, with or without 0.02% polysorbate 80, pH 6.1; 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 5, 15, 30, and 90 min, and 3, 6, and 24 hours after the end of the infusion. Additional samples were collected once daily until Study Day 7, then twice weekly for 3 additional weeks. Blood samples were allowed to clot for at least 30 min, —
and methanol, then stored at -70°C until shipped to the sponsor for analysis.
Age: estimated 2.5 – 11 years
Weight (nonrodents only): 2.72 – 3.94 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: All monkeys were observed twice daily for general health and behavioral or other clinical signs of overt toxicity, beginning 5 days prior to dosing and continuing until Study Day 29. Additionally, each animal was observed for signs of toxicity immediately after infusion and at hourly intervals until 4 h after completion of the infusion. Body weights were determined once prior to dose administration, then weekly thereafter until study termination on d 29. Food consumption was qualitatively assessed daily for each animal, as either normal or decreased from expected. At the completion of the study, all animals were returned to the colony without necropsy.

All monkeys survived for the entire study duration, and AN100226 formulated with or without 0.02% polysorbate 80 was well-tolerated after a single, i/v infusion. There were no treatment-related toxicities noted after infusion of natalizumab; several animals had occasional episodes of inappetence, scant or no stool, or changes in stool consistency which were not considered treatment-related. No adverse effects of AN100226 on body weights or food consumption over the duration of the study were observed.

At Study Day 29, there were no remarkable effects of AN100226 treatment of cynomolgus monkeys on levels of serum glucose, hepatic transaminases, γ-GT, ALP, total and direct bilirubin, BUN, creatinine, and electrolyte levels, cholesterol, serum triglycerides, or total protein, albumin, globulin, or A:G ratios, either when compared between dose groups, or when values for individual animals were compared to baseline, pre-study values. One female monkey in the AN100226 + polysorbate 80 formulation dose group had an elevated value for CPK at Study Day 29 that was both outside of the normal range for cynomolgus macaques, and increased by approximately 2.5-fold over baseline. However, similar CPK values were observed in one female monkey in each dose group prior to study initiation, and these findings were considered incidental to natalizumab treatment and likely related to the handling of the animals during the blood collection procedures.

Blood samples for evaluation of hematology and serum biochemistry profiles were obtained from all animals prior to dose initiation, then on Study Day 29 and evaluated for 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. There were no remarkable effects of either natalizumab formulation on erythrocyte parameters at Study Day 29 as compared to the pre-study values. The platelet and total leukocyte counts were slightly elevated (mean values < 10% change) from pre-study values in both dose groups, with no apparent difference in effect related to the different formulations of AN100226 ($p > 0.05$, two-

tailed Student's *t* test). Absolute lymphocyte counts were increased at Study Day 29 by 34% to greater than 2-fold as compared to baseline, but no statistical differences were identified, either between dose groups of natalizumab, or when compared to the baseline values. There was a concomitant, although not statistically significant decrease in both the absolute and differential neutrophil counts at Study Day 29 as compared to baseline in both groups of monkeys treated with AN100226, with or without polysorbate 80. There were no other remarkable effects of natalizumab treatment with either formulation on absolute or differential monocyte, band neutrophil, eosinophil, or basophil counts, and no treatment-related effects on reticulocyte numbers or erythrocyte morphology.

Pharmacokinetics: Pharmacokinetic parameters were calculated by a non-compartmental model, and the area under the concentration vs. time curve was determined using the linear trapezoidal rule. Peak serum concentrations (C_{max}) were observed within 15 min after completion of infusion in both treatment groups, and in both groups of animals, natalizumab concentrations in serum showed a biphasic decline with an initial distribution phase, followed by a longer elimination phase. There were no statistically significant differences in the derived pharmacokinetic parameters C_{max}, T_{max}, $AUC_{0\text{-last}}$, initial and steady state volumes of distribution (V_z and V_{dss}, respectively), MRT, and clearance (Cl) for either formulation of AN100226. The one exception was the elimination half-life, which was significantly less for the group treated with AN100226 in the polysorbate 80 formulation ($p = 0.0477$, two-tailed Student's *t* test, performed by the sponsor). The calculated values for C_{max}, T_{max}, half-life, $AUC_{0\text{-last}}$, MRT, initial and steady state volumes of distribution (V_z and V_{dss}, respectively), and clearance (Cl) for AN100226 are presented in Table 34 below, and were calculated from the reported, individual animal values for each parameter as presented in the sponsor's final study report.

Table 34. Comparative pharmacokinetic profiles of natalizumab (AN100226) in two different formulation buffers after a single, 3 mg/kg i/v infusion in cynomolgus monkeys

Pharmacokinetic Parameter	Mean Value, ± S.D. (n = 3/group)	
	50 mM L-histidine	50 mM L-his + 0.02% polysorbate 80
C_{max} (µg/ml)	79.2 ± 29.6	57.5 ± 13.9
T_{max} (h)	0.78 ± 0.21	0.56 ± 0.05
t½ (h)	80.1 ± 5.5	65.0 ± 7.4[a]
$AUC_{0\text{-last}}$ (µg*h/ml)	1962 ± 648	1729 ± 601
MRT (h)	61.1 ± 5.9	64.4 ± 4.5
V_z (ml/kg)	186 ± 43	179 ± 73
V_{dss} (ml/kg)	98 ± 22	122 ± 45
Cl (ml/h/kg)	1.6 ± 0.5	1.9 ± 0.6

[a] $p = 0.047$, two-tailed Student's *t* test

Immunogenicity: Anti-natalizumab antibody responses varied in both time to onset and magnitude of response, with relationship to dose of AN100226. In most animals, detectable anti-AN100226 titers began to appear on Study Day 15, and continued to increase until study termination on d 29. However, one monkey in the group treated with AN100226 formulated with polysorbate 80 never developed a detectable anti-natalizumab antibody response. The mean serum anti-AN100226 antibody titers appeared to be higher in the animals that received the formulation without polysorbate 80; however, the difference between the groups was not statistically significant ($p > 0.05$, ANOVA). In both dose groups, the majority of animals did not reach peak anti-natalizumab antibody titers until Study Day 29, with the exception of monkey

#PR1175F, who failed to generate an anti-natalizumab antibody response at any time point on study. The results of this study are shown in Table 35, below.

Table 35. Total anti-natalizumab binding antibody titers in cynomolgus monkeys following a single intravenous infusion of two different formulations of AN100226

AN100226 formulation	Mean Anti-Natalizumab Total Antibody Titer, ± S.D. (n = 3/group)					
	Day 11	Day 15	Day 18	Day 21	Day 24	Day 28
L-histidine	2.0 ± 3.4	49.9 ± 15.1	40.4 ± 8.9	39.3 ± 15.7	44.2 ± 17.8	58.3 ± 18.9
Polysorbate 80	0 ± 0	4.5 ± 7.9	6.9 ± 7.7	8.6 ± 10.5	9.6 ± 12.1	14.0 ± 18.6

Comment: Only the raw data for the immunogenicity analyses were provided in the final study report. Calculation of the mean values and standard deviations for each group, as well as statistical analyses were performed as part of the review.

Study Conclusion: There were no statistically significant differences in the toxicity, pharmacokinetic, or immunogenicity profiles in female cynomolgus monkeys following a single, intravenous infusion of 3 mg/kg natalizumab formulated in 50 mM L-histidine in 150 mM saline, with or without added (0.02%) polysorbate 80. The one exception was the elimination half-life, which was statistically shorter in animals receiving the polysorbate 80 formulation.

Study title: BG00002: A single-dose intravenous pharmacokinetic study of natalizumab — commercial process material versus natalizumab Biogen resupply material in female cynomolgus monkeys.

Key study findings: No differences in the pharmacokinetic, pharmacodynamic, or immunogenicity profiles of AN100226 were noted in samples obtained from female cynomolgus monkeys treated with a single, intravenous infusion of 3 mg/kg of natalizumab produced either by the — commercial process (BG00002B), or by Biogen's "resupply" process (BG00002A). Although no formal analysis of bioequivalence of the two products was performed, there were no statistical differences in any of the parameters evaluated, and the two products were deemed comparable.

Study no.: #P00002-02-01 (— Study #1347-87)

Volume #, and page #: EDR files: BLA 125104\000\module4\pharmacokinetics\P00002-02-01.pdf

Conducting laboratory and location: —

Date of study initiation: February 5, 2003 (final report dated April 16, 2004)

GLP compliance: Yes

QAU statement: yes (X) no ( )

Drug, lot #, and % purity: natalizumab (AN100226; BG00002B-01) manufactured by Biogen at commercial scale and process, lot #MFG-55-02-79; 20.1 mg/ml, pH 6.2; purity — by SDS-PAGE (reduced), — IgG by reducing gel chip, and — monomeric IgG (— aggregate, — low molecular weight impurities by — HPLC); and natalizumab (termed Biogen "resupply" material, or BG00002A), lot #F23014, 18.6 mg/ml, pH 6.2; purity — by SDS-PAGE (reduced), and — monomeric IgG — with no detectable low molecular weight impurities by — n HPLC) (copy of sponsor's Certificate of Analysis for each product was included as an Appendix to the final study protocol)