between individual animals for the same time point, as well as between time points for the individual animals. Therefore, no statistically significant differences in cell activity in either peripheral blood leukocytes or splenic lymphocytes were observed between saline controls and either vehicle or natalizumab animals at any time point.

Lymphocyte blastogenesis: This parameter was evaluated *in vitro* by culture with [redacted] No statistically significant effects were noted among groups except for the high dose group on day 28. At this time point a significant increase in blastogenesis was noted relative to the saline control. The biological significance is not clear for this finding but as it did occur in the high dose group at the end of dosing, a relationship to test article cannot be ruled out.

FACS analysis of leukocyte phenotype populations:
Blood samples were processed for double color FACS analysis to identify different leukocyte subsets, including T lymphocytes (with helper and suppressor subpopulations), B lymphocytes, monocytes (single stained), and natural killer cells, as well as minor populations within these subsets.

- On SD22, elevations of all subset for the high dose group (both sexes) were noted except for CD2-CD8B and CD14 (monocytes) in females.
- CD14 cell were increased in mid and high dose males.
- The effects noted are most likely due to the pharmacological activity of the study drug.
- Only absolute numbers were effected and not percentages (except CD14+ effect seen in males in the mid and high dose groups).
- No effects were noted in the low dose group.
- The increases of cell counts in the males were not observed in the males at the end of the recovery timepoint but appeared to persist in the females.
- Cell subset ratios within the spleen were not affected at any dosage, except for a small decrease in the percentage of T suppressor lymphocytes in high dose females. This effect was not reversed in the four week recovery period. (Only one female was analyzed for this parameter at recovery so results are difficult to interpret.)

Gross pathology:

- No treatment related gross lesions were noted.

Organ weights (specify organs weighed if not in histopath table):
Adrenals
Brain
Heart
Kidneys
Liver
Lungs
Spleen
Testes/Ovaries
Thymus

- No effects on organ weights attributable to the test article were noted.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no ( )
brain (3 levels), colon, duodenum, epididymis, esophagus, eyes with optic nerve, gall bladder heart (rt.& lt. vent.,intervent. septum), ileum, infusion sites and surrounding tissue cecum, jejunum, kidneys, liver, lung (with main-stem bronchi), lymph nodes (mandibular, mesenteric, regional at infusion site), mammary glands, ovaries, pancreas, pituitary, prostate, rectum, salivary gland (mandibular), sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord (cervical, lumbar), spleen, stomach (fundic,, pyloric), testes, thymus, thyroid/parathyroids, tongue, trachea, urinary bladder, uterus, vagina

- No treatment related microscopic findings are noted.
- No deposition of immune complexes or albumin was noted in the renal glomeruli of any animal.
- Binding of natalizumab was observed on lymphoid cells of all animals in the high dose group.
- Binding was seen to be localized to the periphery of the cells indicating a pattern of binding to the cellular membrane.

Toxicokinetics:

- Detection of natalizumab levels in serum samples was somewhat variable but generally showed a dose relate increase with increasing dose for SD 8 and 15. For each dose group, values appeared to fall for the 22 and 28 samples.
- No detectable anti-drug antibodies were noted for the two control groups.
- Anti-drug antibodies were detected for the mid and low dose groups on SD15 and later for most animals.
- For the high dose group, anti-drug antibodies were detectable for only one animal (male) beginning on SD 15.

Study title: Avonex/Antegren: a four week combination toxicity study of Antegren administered intravenously and Avonex administered intramuscularly in the rhesus monkey, followed by an eight-week recovery

Key study findings:
The objective of this study was to evaluate the potential toxicity of natalizumab and Avonex when administered alone and in combination to rhesus monkeys.
Weekly administration of 30 or 60 mg/kg natalizumab IV for 4 weeks alone, or in combination with 30 ug Avonex by intramuscular injection, followed by an 8-week recovery period was apparently well tolerated. Test-article-related effects were limited to dose-independent, natalizumab-related increases in circulating lymphocytes and lymphocyte subsets (T- lymphocytes, T-helper, T-cytotoxic/suppressor and B-lymphocyte subpopulations), with a smaller effect on monocytes, eosinophils, basophils, and secondary increases in white blood cell counts. Slight increases in reticulocytes and a clearly increased incidence of nucleated red blood cells, and smaller increases in the incidence of anisocytosis and polychromasia, were noted. These effects likely reflect the pharmacologic activity of natalizumab and did not occur in animals administered only Avonex. There was no evidence of an additive effect on circulating lymphocytes or

lymphocyte subsets or hematology parameters when Avonex and natalizumab were co-administered. Effects on circulating lymphocytes and lymphocyte subsets appeared to be reversible, although increased levels persisted in animals receiving the high dose (60 mg/kg) of natalizumab only.
Weekly administration of natalizumab intravenously at 30 mg/kg or 60 mg/kg alone or in combination with Avonex administration (30 ug, IM) had no effect on IFN β 1 a serum levels during the study. Weekly administration of Avonex (30 ug, IM) had no effect on natalizumab serum concentrations when administered in combination. The presence of Avonex also had no impact on the saturation of a4 integrin on peripheral blood mononuclear cells.

Histologic findings considered related to Avonex, natalizumab or the combination of these drugs consisted of lymphoid hyperplasia in the spleen (which correlated with increased spleen weights) and peripheral lymph nodes and leukocytosis and focal leukocyte aggregation in the liver. These findings often appeared to be more prominent in animals that received natalizumab or natalizumab plus Avonex than in animals administered Avonex alone. There was no clear evidence of a potentiated or additive effect on splenic lymphoid hyperplasia when Avonex and natalizumab were in combination. Changes in the intramuscular injection sites, including myofiber degeneration and necrosis in a few Avonex treated animals were possibly related to Avonex administration. The findings from the microscopic and immunohistochemical evaluations are consistent with hyperplasia of both T-lymphocyte and B-lymphocyte populations in the spleen. There were no differences in the mesenteric lymph node between control and treated animals in the microscopic or the immunohistochemical analyses. After an 8-week recovery period, spleen weights were considered fully or partially recovered and histological changes were resolved. No immunohistochemical changes considered treatment-related were observed in tissues from animals in the recovery groups. All the test article- related microscopic changes were of minimal or mild severity and reversible, under the conditions of this study.

Study no.: — # 1164-87/ Biogen study #P00002-01-01
Volume #, and page #:
Conducting laboratory and location: ———————
Date of study initiation: 6/5/2001
GLP compliance: YES
QA report: yes (x) no ( )
Drug, lot #, and % purity:

Receipt Date	Supplier	Description	Lot Number(s)	No. Vials	Contents
12-Jun-01*	Elan	Antegren	N/A	250	20 mg/mL; 5.3 mL
07-Jun-01	Elan	Antegren Vehicle	E85001	1200	5 mL/vial
06-Jul-01	Biogen	Antegren	MFG1330131	250	20 mg/mL; 5.3 mL
07-Jul-01**	Biogen	Avonex® Vehicle	N/A	400	5 mL/vial
07-Jul-01	Biogen	Avonex®	727-00-0158A	160	30 µg/vial
20-Jul-01	Biogen	Antegren	MFG1330131	100	20 mg/mL; 5.3 mL
24-Jul-01	Elan	Antegren Vehicle	E85001	398	5 mL/vial

* All materials received on this date were returned to Elan Pharmaceuticals and were not used on this study.

** Vials of Avonex® Vehicle were received unlabeled. A sample was returned to the Sponsor and was confirmed to be Avonex® Vehicle.

Methods

Doses: 6 groups were designated. See table below doses and combinations.

Species/strain: rhesus monkeys

Number/sex/group or time point (main study): 3/sex/group (See design below)

Route, formulation, volume, and infusion rate:

Satellite groups used for toxicokinetics or recovery: 2/sex/group (See table below)

Age: 2-6 years

Weight: 2.2 – 5.8 for the males, 2.2- 4.6 for the females.

Unique study design or methodology (if any): The study design and dosing scheme is illustrated in the table below, supplied by the sponsor:

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

1 IM = Intramuscular injection administered on Days 1, 8, 15 and 22

2 IV = 30-minute intravenous infusions administered on Days 1, 8, 15 and 22

3 Avonex® was administered first

Observations and Results

Mortality: All animals survived to scheduled necropsy.

Clinical signs: Observation (cageside) at least twice daily.

- No significant effects related to treatment were recorded with the exception of low food consumption. This finding was most apparent in animals receiving

Avonex. Other clinical signs appeared to be equally evident in all groups. The low food consumption was resolved after dosing was stopped.

Body weights: Data collected twice weekly beginning two weeks prior to study initiation

- All groups showed an increased mean weight. A slightly lower weight gain was noted for the groups receiving Avonex plus 60 mg natalizumab. For group 5, during the recovery period, the group mean weight declined. This is possibly due to the fact that the heavier animals were sacrificed after day 22.

Food consumption: Qualitatively assessed daily. A reduction in food consumption was noted in a dose-dependent manner with increasing natalizumab dos and with the addition of Avonex.

Physical exams: Performed twice prior to study initiation and the day prior to necropsy. Exams included heart rate, body temperature, abdominal palpation, assessment on skin condition, respiratory rate. No test article related effects were noted during the study period.

Ophthalmoscopy: Once prior to study initiation and during week 4.
No treatment related effects were noted, so this testing was not repeated for the recovery period.

EKG: Performed once during the pre-study period, once after dosing (approximately 2 hours) on SD1 and 15 for groups 1, 3, 4, 5, and 6 and approximately 2.5 hours after dosing for group 2 on SD1 and 15. Also performed after dosing on SD28 for all groups.

- No treatment related effects were noted.

Hematology: Blood samples were colleted for hematology, clinical chemistry and coagulation parameters twice prior to study initiation, on SD2 and 16, 28, 56, 84.
The following parameters were assessed (table supplied by the sponsor):

Coagulation Parameters	
Activated partial thromboplastin time (APTT)	Prothrombin time (PT)
Fibrinogen	

Hematology Parameters	
Red blood cell (RBC) counts	Mean cell hemoglobin (MCH)
White blood cells (WBCs)*+	Mean corpuscular volume (MCV)
Hemoglobin concentration	Mean corpuscular hemoglobin concentration (MCHC)
Hematocrit	Platelet counts
Reticulocyte counts	Blood cell morphology**

* Total and differential: included polysegmented neutrophils, bands, lymphocytes, monocytes, eosinophils, and basophils

** The blood smear from all animals was examined at each time point (including prestudy).

\+ Absolute lymphocyte counts determined from hematology samples collected on Days –14, –7, 1 (predose), 2, 16, and 28 were used in conjunction with the results of flow cytometric analyses (Section VI.E) to determine the absolute numbers of each lymphocyte subset.

- Only expected effects due to the pharmacological action of natalizumab were noted during this study. These changes consisted primarily of increases in

lymphocytes (groups 3, 4, 5, and 6). Increases in nucleated RBCs were also observed in natalizumab treated groups. This finding is consistent with effects noted in other toxicology studies with natalizumab and were not seen in groups treated with Avonex alone or control groups.

- Monocyte, eosinophil and basophil counts were also slightly elevated in groups receiving natalizumab only (Groups 3 and 4) or natalizumab in combination with Avonex (Group 5 and 6) on Days 2, 16 and 28, but not in Avonex only-treated animals and were not statistically different from controls during the recovery period (Day 84). Differences in monocyte counts (differential) attained statistical significance in Group 4 on Day 28 and Groups 5 on Days 2, 16 and 28, in Group 6 on Days 16 and 28, and in eosinophil counts (absolute and/or differential) in Groups 3 and 5 on Day 16. Statistically significant increases in basophil counts were observed only in groups receiving natalizumab at 60 mg/kg (Group 4 on Days 16 and 28, Group 5 on Days 2, 16, 28 and 56 and in Group 6 at Day 28) and were attributed to high dose natalizumab. No evidence of test-article-related elevations in neutrophil counts occurred.
- Statistically significant decreases in absolute neutrophil counts were observed in Group 2 (Avonex) on Day 2 and on Day 16. Since similar changes were not observed in Group 5, and as the vast majority of individual values were within the normal range, the effects do not appear to be related to Avonex or natalizumab.
- No effects of the test article were noted on coagulation parameters listed above.

Clinical chemistry:

Serum Chemistry Parameters	
Sodium	Phosphorus
Potassium	Glucose
Chloride	Urea nitrogen (BUN)
Total carbon dioxide (bicarbonate)	Creatinine
Total bilirubin	Total protein
Alkaline phosphatase (AP)	Albumin
Lactate dehydrogenase (LDH)	Globulin

Serum Chemistry Parameters	
Aspartate aminotransferase (AST)	Albumin/globulin ratio
Alanine aminotransferase (ALT)	Cholesterol
Gamma-glutamyltransferase (GGT)	Triglycerides
Calcium	

- No significant test article related effects were noted on serum chemistry parameter during the study. Sporadic changes in various test results occurred but were not consistent or apparently dose related.

FACS analysis of peripheral lymphocytes: Data collected 3X prior to study initiation, (SD-14, -7 and SD1 prior to dosing, SD 2 and 16 (23 hours after dosing on days 1 and 15), and on SD28, during the recovery period on SD 56 and 84.
The following markers were assessed (table supplied by the sponsor):

Antigen Markers	Cell Type Labeled
CD2/CD20	Total lymphocytes (B- and T-cells, NK cells)
CD3	Total T-lymphocytes
CD3/CD4	T-helper lymphocytes and NK cells
CD3/CD8	T-cytotoxic/suppressor lymphocytes and NK cells
CD20	B-lymphocytes

- Results showed an elevation of CD20+ B-lymphocytes in all groups that received natalizumab by day 22. The effect was dose related with respect to the dose of natalizumab.
- In groups receiving natalizumab increases in total lymphocytes were noted between SD 2 and 28. (Large variability is also noted).
- The effect on lymphocyte numbers was not seen with Avonex treatment and Avonex treatment did not appear to alter the effect of natalizumab on lymphocyte numbers.
- Lymphocyte numbers were not significantly different among groups during the recovery period.

Urinalysis: Samples were collected at necropsy from all animals: The following parameters were tested (table supplied by the sponsor:

Urinalysis Parameters	
Color/Character	Protein
pH	Glucose
Specific gravity	Ketones
Leukocyte esterase	Bilirubin
Nitrite	Occult blood
Urobilinogen	Microscopics

- No effects on urinalysis parameters were noted.

Gross pathology:
Full necropsy was performed on all animals sacrificed during this study. No apparent test article related effects were noted for gross necropsy examinations.

Organ weights (specify organs weighed if not in histopath table):

Organs Weighed	
Adrenals	Thyroid with parathyroids
Epididymides	Brain
Kidneys	Heart
Lungs	Liver (with drained gall bladder)[10]
Pituitary (post fixation)	Ovaries
Testes	Spleen
Prostate gland	Thymus

Organ/body weight ratios were calculated (using the final body weight obtained prior to necropsy), as well as organ/brain weight ratios.

- Increased spleen weights were reported for all groups receiving natalizumab. Weights were closer to control weights after the recovery period but remained somewhat greater in natalizumab treated groups.
- One animal treated with Avonex alone also had slightly increased spleen weight resulting from mild lymphoid hyperplasia. This finding was attributed to Axonex treatment and was resolved by day 85.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no ( )

- Spleen: lymphoid hyperplasia (increase in the number and size of lymphoid follicles) showed a dose related increase in incidence and severity in groups 2-6, rated mild to moderate. The incidence and severity of the findings among groups indicated that the lymphoid hyperplasia was related to both natalizumab and Avonex. There did not appear to be a potentiating effect between the two drugs. On day 85, lymphoid hyperplasia (minimal or mild) was observed in all groups, including controls. Based on the comparisons between groups and the fact that hyperplasia was apparent in control animals, the sponsor concluded that the hyperplasia had resolved. However, without a clearer understanding of why the increase occurred in the control, it is not clear that the finding was fully resolved.
- Lymph nodes: lymph node hyperplasia was reported for all treatment groups in the incidence illustrated in the table below, supplied by the sponsor:

Incidence of Lymphoid Hyperplasia in the Inguinal, Mandibular, Cervical and Axillary Lymph Nodes, Day 29

Group		1	2	3	4	5	6
Treatment	Avonex® (µg)	0	30	0	0	30	30
	Antegren (mg/kg)	0	0	30	60	60	30
Lymph Nodes	Inguinal	0/6*	3/6	4/6	5/6	4/6	6/6
	Mandibular	4/6	2/6	5/6	5/6	5/6	3/6
	Cervical	6/6	6/6	5/6	6/6	6/6	5/6
	Axillary	4/6	3/6	5/6	5/6	6/6	5/6
	Combined	14/24	14/24	19/24	21/24	21/24	19/24

* Number Affected / Number Evaluated

Mean Group Severity Scores for Lymphoid Hyperplasia in the Inguinal, Mandibular, Cervical and Axillary Lymph Nodes, Day 29

Group		1	2	3	4	5	6
Treatment	Avonex® (µg)	0	30	0	0	30	30
	Antegren (mg/kg)	0	0	30	60	60	30
Lymph Nodes	Inguinal	0.00	0.50	0.83	1.33	0.67	1.33
	Mandibular	0.83	0.50	1.17	1.50	1.33	1.00
	Cervical	1.00	1.33	1.00	1.50	1.33	0.83
	Axillary	0.67	0.50	0.83	0.83	1.50	0.83
	Combined Mean	0.63	0.71	0.96	1.29	1.21	1.00

Analysis of incidence and severity data suggests that this finding is related to natalizumab treatment alone or natalizumab with Avonex. The does not appear to be an additive or potentiating effect of the combination of the two drugs. By day 85 the lymphoid hyperplasia appears to have resolved.

- Central lymph node; On day 29, minimal lymphoid hyperplasia was noted in the mesenteric lymph nodes of groups 1-4 with highest incidence in group 4 (60 mg/kg natalizumab). However, group 5, also receiving 60 mg /kg natalizumab did not have this finding. Therefore, the biological significance of this finding is not clear. It appears that neither drugs had a true dose related effect on the hyperplasia in the mesenteric lymph node.
- Liver: leukocytosis, consisting of increased leukocytes (mainly granulocytes), was observed in hepatic sinusoids. This finding was rated minimal to mild in 2 of 6, 4 of 6, 4 of 6, 6 of 6 and 5 of 6 animals in Groups 2, 3, 4, 5 and 6, respectively, but did not occur in controls (Group 1
- Focal leukocyte aggregation, discrete parenchymal aggregates of histiocytes, lymphocytes and/or granulocytosis associated with necrosis or loss of hepatocytes, were observed. This finding was rated minimal to moderate in 2 of 6, 3 of 6, 3 of 6,4 of 6 and 4 of 6 animals in Groups 2, 3, 4, 5 and 6, respectively, but was absent in controls. In Groups 3 - 6, dosed with natalizumab , or natalizumab plus and Avonex, the incidence and severity of leukocytosis and focal leukocyte aggregation were similar These changes were considered related to natalizumab treatment or natalizumab and Avonex in combination but were not related to the dose of natalizumab. The relationship to Avonex treatment is uncertain. These findings were not observed in animals sacrificed on day 85 (recovery).

  Necrosis and single-cell necrosis of hepatocytes, minimal or mild, each occurred in one or two animals in Groups 3, 5 and 6. Due to the low incidence and lack of relationship to the dose of natalizumab or Avonex treatment, these changes were not considered direct effects of the test-article. They may have been secondary to the hepatic leukocytosis and leukocyte aggregation. This finding was not reported for day 85.

The tissues examined are listed in the table below, supplied by the sponsor:

The following tissues and organs (or portions of), when present, from all animals sacrificed were collected and preserved in neutral-buffered 10% formalin (except for the eyes, which were preserved in Davidson's fixative for optimum fixation and selected tissues frozen for immunohistochemical analysis). Special care was given to the inclusion of Peyer's patches within the preserved intestinal segments.

Tissues Collected	
Cardiovascular	Urogenital
Aorta	Kidneys
Heart	Ureter
Digestive	Urethra
Salivary Gland (Mandibular)	Urinary Bladder
Tongue	Testes
Esophagus	Epididymis
Stomach	Prostate Gland
Cardiac	Seminal Vesicles
Fundic	Ovaries
Pyloric	Uterus
Small Intestine	Cervix
Duodenum	Vagina
Jejunum	Endocrine
Ileum (including Peyer's Patches)	Adrenal Gland
Large Intestine	Pituitary Gland
Cecum	Thyroid gland/Parathyroid Glands[a]
Colon	Skin/Musculoskeletal
Rectum	Skin/Mammary Gland
Pancreas	Bone (Distal Femur)[c]
Liver	Bone (7th rib)
Gallbladder	Joint (Femoral-Tibial)[d]
Respiratory	Skeletal Muscle (Rectus Femoris)
Trachea	Nervous/Special Sense
Lung & Bronchi	Eyes with Optic Nerve
Lymphoid/Hematopoietic	Sciatic Nerve
Bone Marrow (Sternum)	Brain
Thymus[b]	Optic Chiasm
Tonsils	Lateral Ventricle/Cerebrum
Spleen[b]	Cerebellum/Medulla
Lymph Nodes	Spinal Cord (Cervical, Thoracic, and Lumbar)
Axillary	Other
Cervical	Animal Number Tattoo
Mandibular	Gross Lesions
Mesenteric[b]	Injection/Infusion sites[e] 11
Inguinal	
Peyer's Patches (Ileum)	

a The occasional absence of the parathyroid gland from the routine tissue section did not require a recut.
b Samples of spleen (at least two portions from different areas of the organ), thymus and mesenteric lymph nodes were
c Including articular surface and growth plate
d The femoral-tibial joint was evaluated macroscopically, collected and retained for possible histologic processing and evaluation.
e On Days 29 and 85, the last IM and/or last IV injection/infusion site was collected.

Toxicokinetics: Samples were collected pre- and post-dose on SD1, 8, 15, 22, 28.

Natalizumab receptor saturation: Samples collected from groups 3, 4, 5, and 6 approximately 2 hours after natalizumab infusion on SD22, 29, 56 and 84.

- Binding of natalizumab was not altered y the presence of Avonex

Other:

Sections of spleen, thymus and mesenteric lymph node were double-stained with the following antibodies to identify T- and B-lymphocyte populations (the appropriate isotype antibody was also used as a negative control). Processing and staining of samples were performed according to —— SOP.

Experimental Antibody	Isotype Control Antibody
CD3	Rabbit IgG
CD20	Mouse IgG

Bone marrow smears from the 7th rib of all animals were retained for possible examination. Because no abnormalities were noted in the sternal bone marrow section, this analysis of the smears was not performed.

Study title: A dose range-finding subacute intravenous toxicity study of AN100226 in female cynomolgus monkeys

Key study findings:

The purpose of this study was to evaluate the potential toxicity of natalizumab when administered by IV infusion every other day for a total of 14 doses and to assess reversibility of any toxic effects. In addition, this study was designed to identify appropriate dose levels for a subsequent definitive study.

Test article was administered at dose levels of 0 (saline), 0 (vehicle), 3, 10, and 30 mg/kg, Groups I to V, respectively. Each dose level was administered to 3 animals, of which 1 animal from each group was designated to undergo a 4-week recovery period. Evaluation of parameters for toxicity included twice daily clinical observations, body weights, physical examinations, serum chemistry, hematology, and urinalysis. Animals in Groups I to V were sacrificed on Study Day 28. Recovery Group (Ir to Vr) animals were sacrificed on Study Day 56.

Based on increased lymphocyte production, correlated with splenic and thymic enlargement and microscopic findings in the lymph node adjacent to the injection site, it appears that repeated intravenous administration of AN100226 induces an immunologic response. Changes appeared to be reversible following a 4-week recovery period.

Study no.: —— #940911
Volume #, and page #:
Conducting laboratory and location: ——————
Date of study initiation:
GLP compliance: YES
QA report: yes (X) no ( )
Drug, lot #, and % purity: The test articles are listed below:

Test or vehicle article	Lot number	TSC number
AN100226	AN-100226-0001 (8502/86)	319
AN226 Buffer	8562/86	0316
Sodium chloride (0.9%)	330283	0320

Methods

Doses: 0, 3, 10, 30 mg/kg
Species/strain: female cynomolgus monkeys
Number/sex/group or time point (main study):
Route, formulation, volume, and infusion rate:
Satellite groups used for toxicokinetics or recovery:
Age: 5-12 years
Weight: 2.15- 3.49 kg
Unique study design or methodology (if any): Study design is illustrated in the table below, supplied by the sponsor:

Group number	Group designation	Dose level (mg/kg)	Concentration (mg/mL)	Dose volume (mL/kg/dose)	Animal numbers
I	Saline	0	0	10	PR0979 PR0583
Ir*					PR1151
II	Vehicle	0	0	10	PR0985 PR1245
IIr*					PR1199
III	AN100226	3	0.3	10	PR1007 PR1249
IIIr*					PR1117
IV	AN100226	10	1.0	10	PR1159 PR0941
IVr*					PR1319
V	AN100226	30	3.0	10	PR0999 PR1255
Vr*					PR1001

* *The letter "r" denotes groups of animals assigned to undergo a 4-week recovery phase after completion of dose administration.*

Observations and Results

Mortality: All animals survived to scheduled necropsy.

Clinical signs: Observation made twice daily. No clinical signs were reported indicative of study drug toxicity.

Body weights: No effects noted.

Food consumption: No effects noted.

Ophthalmoscopy: Not done

EKG: Not Done.

Hematology:
Prestudy erythrocyte sedimentation rates were significantly increased for Group 5 when compared to Group 2. A statistically significant decrease in segmented neutrophils (%) was seen for Group 3 and 5on Study Days 15 and 28, respectively. Group 5 showed a statistically significant increase in lymphocytes (%) on SD28, relative to control. A statistically significant increase in absolute lymphocyte levels was also seen in Groups 4 and 5on SD15. Parameters for recovery animals returned to near prestudy levels. Lymphocyte elevations in treatment groups may reflect effects due to test article administration.

Clinical chemistry: No test article related effects are noted. Any changes observed were not dose related and appeared to be incidental.

Urinalysis: No test article related effects are noted.

Gross pathology:

Organ weights (specify organs weighed if not in histopath table):

- Elevated spleen and thymus weights are noted for both SD28 and SD56 kills. This effect appeared to be dose dependent.

Histopathology: Adequate Battery: yes ( ), no ( )—explain
Peer review: yes ( ), no ( )

- postinflammatory injection site perivascular fibrosis was noted in Group 4 (recovery) and arteriolar fibrinoid change in the regional lymph node was noted in Groups 3(r) and 4(r).

Toxicokinetics: Not Done.

Other:

6.6.6.4 Genetic toxicology

Study title: Mutagenicity test on AN100226 in a chromosome aberrations study in human whole blood lymphocytes with a confirmatory assay with multiple harvests

Key findings: The purpose of this *in vitro* study was to assess the potential for AN100226 (natalizumab) to induce chromosomal aberrations in cultured whole blood lymphocytes with and without metabolic activation. All test results are negative. Under the conditions of this study, natalizumab does not appear to have significant genotoxic potential.

Study no.: [redacted] number: 16568-0-449C0
Biogen Idec number: am001

Volume # and page #:
Conducting laboratory and location: [redacted]

Date of study initiation: 12/6/1994
GLP compliance: YES
QA reports: yes (X) no ( )
Drug, lot #, and % purity: AN100226 lot number: 25644PC, buffer lot number: 8562/86

Methods

Strains/species/cell line: human blood lymphocytes

Doses used in definitive study: 191, 383, 765, 1530 and 2040 ug/ml

Basis of dose selection: the highest test article concentration chosen (2040 ug/ml) is more than 300-fold greater than the highest anticipated therapeutic steady state (6 ug/ml).

Negative controls: In non-activation assays, negative controls were cultures with only cells and culture medium. Solvent controls were cultures containing the buffer at the same concentration as the highest concentration of the test article used in test cultures.

In activation assays the negative controls were the same as those for the non-activation assays but with the addition of S9 activation mix.

Positive controls: mitomycin C for the non-activation series, cyclophosphamide in the metabolic activation series.

Incubation and sampling times:

- Chromosomal aberrations without activation: An incubation time of 22.25 hours was used for the first confirmatory trial. For the second confirmatory trial, an incubation time of 46.17 hours was used.
- Chromosomal aberrations with metabolic activation assay: same as above.

Results
No significant increase in cells with chromosomal aberrations or in polyploidy cells was observed at the concentrations of test article analyzed.

Study validity (comment on replicates, counting method, criteria for positive results, etc.):
Appropriate controls, replicates, counting methods and criteria were used.

Study outcome:
Under the conditions of this study, natalizumab appear to have no genotoxic potential.

Study title: Mutagenicity test on AN100226 in the L5178Y K+/- mouse lymphoma forward mutation assay with a confirmatory assay

Key findings:
The purpose of this *in vitro* study was to assess the potential for AN100226 (natalizumab) to induce forward mutations at the thymidine kinase locus in the mouse lymphoma L5178Y cell line in the presence and absence of Aroclor induced rat liver homogenate. The test article, AN100226 (natalizumab), was nonmutagenic with and without metabolic activation in an initial and confirmatory mouse lymphoma forward mutation assay under the conditions of testing.

Study no.: [redacted] number: 16568-0-431R/AM002

Volume #, and page #:
Conducting laboratory and location: [redacted]

Date of study initiation: 12/6/1994
GLP compliance: YES
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Natalizumab lot #: Lot #25644PC, (50 mM L-histidine buffer (150 mM NaC1, pH 6.0),

Methods

Strains/species/cell line: L5 178Y TK+/- mouse lymphoma cells

Doses used in definitive study:
The following concentrations were used to initiate the mutation assays: 47.8, 95.6, 191,382,765 and 1530 pg/mL.

Basis of dose selection:
The highest concentration that could be evaluated in this study and not interfere with the assay was about 1500 pg/mL, which resulted in an osmolality of [redacted] water in the mutation assays, The mutation assays were initiated with concentrations up to 1530 pg/mL. The anticipated steady state levels of AN100226 in human blood is [redacted]

Negative controls: Negative (media) controls were prepared by carrying cells unexposed to the test article through all of the assay operations. In the activation portion of the assay, the negative control cultures were exposed to the S9 metabolic activation mix. A single culture was used in the cytotoxicity assays and in the mutation assays.

Positive controls: Methyl methanesulfonate (MMS) [redacted] is mutagenic via alkylation of cellular DNA and is highly mutagenic to mouse lymphoma cells without S9 metabolic activation. MMS was used at 10 nl/mL and 15 nl/mL as a positive control for nonactivation mutation studies.
20-Methylcholanthrene (MCA) requires metabolic activation by microsomal enzymes to become mutagenic. MCA [redacted] was used at 2.0 pg/mL and 4.0 pg/mL as a positive control for assays performed with S9 metabolic activation.

Results

Study outcome:
The test article, AN1 00226, was nonmutagenic with and without metabolic activation in an initial and confirmatory mouse lymphoma forward mutation assay under the conditions of testing.

2.6.6.5 Carcinogenicity

Carcinogenicity studies are not usually required for approval of biological therapeutics. However, to comply with regulatory agencies for regions outside the United States, the sponsor has performed 4 studies to demonstrate that natalizumab has no carcinogenic or tumor promotion potential:
These studies are as follows:

Study title: In vitro studies of compound binding and proliferative response of human tumor cells with natalizumab.

Key study findings:
This experiment was designed to evaluate the binding properties of natalizumab against the cell lines [redacted]

[redacted] Flow cytometric analysis of the binding of study drug to these cell lines was conducted using biotinylated natalizumab. The data from the flow analysis

were used to select cell lines for use in the cell adhesion assay and the cell proliferation and cytotoxicity assays, which were then performed as part of this study. Cells showing greater than 20% binding were used for subsequent assays.

Results: Relative binding of the various cell lines is illustrated in the table below: supplied by the sponsor:

Cell Line	Tumor Type	% BG00002 Positive
	Bladder	
	Bladder	
	Bladder	
	Bladder	
	Bladder	
	Bladder	
	Colon	
	Colon	
	Colon	
	Colon	
	Colon	
	Colon	
	Colon	
	Colon	
	Colon	
	Colon	
	Colon	
	Colon	
	Colon	
	Leukemia	
	Leukemia	
	Lymphoma	
	Melanoma	
	Melanoma	
	Melanoma	
	Melanoma	
	Melanoma	
	Melanoma	
	Prostate	

Appears This Way On Original

Cell adhesion assay and cytotoxic/proliferation assays were performed for a select number of the above cell lines.
Interpretation of results is equivocal. Selected cell lines were to be used in subsequent in vivo studies.

Study no.: p00002-02-05
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation: 8/13/2002
GLP compliance: NO
QA report: yes (X) no ( )

Drug, lot #, and % purity:
CAC concurrence: NA

Study title: An evaluationof the tumorigenic potential of natalizumab in the ——— Xenograft SCID mouse tumor model

Key study findings:

A select number of human tumor cell lines were screened for the expression of a4 by immunohistochemical evaluation, FACS analysis, and the ability of natalizumab to block the adhesion of the tumor cells to soluble human rVCAM-1. Based on these in vitro experimental data, the human a4-expressing ——— cell line, ——— was selected as an appropriate tumor cell line for the in vivo study in the SCID mouse xenograft model to evaluate whether natalizumab is capable of inhibiting or potentiating human tumor cell growth and/or metastasis.

Tumors were implanted subcutaneously on Day 0. Approximately 30 - 40 mg fragments of ——— from an in vivo passage were implanted in mice near the right axillary area using a——— needle.

Treatment with natalizumab neither supported nor induced expansion of the ——— xenograft in female SCID mice. Growth of established primary ——— tumor xenografts was not affected by the administration of natalizumab. Re-growth of the solid tumor at the site of the excision of the primary tumor was inhibited by the administration of natalizumab. Also, the pre-administration of natalizumab prior to tumor implantation inhibited the growth of the xenografts, although the natalizumab treatment did not affect tumor latency.

It is concluded that natalizumab treatment at a loading dose of 10 mg/kg followed by twice weekly maintenance doses at 5 mg/kg did not exacerbate primary tumor growth or increase metastastic tumor formation in a mouse xenograft model with the ——— cell line. A suggestion of an inhibitory effect of natalizumab on tumor growth when natalizumab is administered prior to establishment of a tumor is indicated by these data.

Study no.: p00002-03-01
Volume #, and page #:
Conducting laboratory and location: Biogen
Date of study initiation:
GLP compliance: NO
QA report: yes ( ) no ( )
Drug, lot #, and % purity:
CAC concurrence: NA

Methods

Doses: 3 groups received a loading dose of 10 mg/kg followed by subsequent doses of 15 mg/kg. 2 groups of 20 received control IgG4 at a dose of 9.7 mg/kg loading dose followed by 5 mg/kg. 2 groups were treated with Taxol.

Basis of dose selection (MTD, MFD, AUC etc.):

Species/strain: female SCID mice

Number/sex/group (main study): 10 groups of 20/group, one group of 30
Route, formulation, volume:
Frequency of dosing:
Satellite groups used for toxicokinetics or special groups:
Study design. Illustrated in the table below, supplied by the sponsor

6.1 Study Design Table

Group No.	Treatment	N	Loading Dose (mg/kg)	Maintenance Dose (mg/kg)	Dose Vol. (ml/kg)	Study No.[a]	Group No.[b]
1	Saline[c]	20	0	0	10	BGN-2	1
2	Natalizumab[c]	20	10	5	10	BGN-2	2
3	Human IgG_4[c]	20	9.7	5	10	BGN-2	3
4	Taxol[d]	20	15	15	10	BGN-2	4
5[e]	Saline[c]	20	0	0	10	BGN-2	5
6[e]	Natalizumab[c]	20	10	5	10	BGN-2	6
7[f]	Natalizumab	30	10	5	10	BGN-2	7
8	Saline[c]	20	0	0	10	BGN-2A	1
9	Natalizumab[c]	20	10	5	10	BGN-2A	2
10	Human IgG_4[c]	20	9.7	5	10	BGN-2A	3
11	Taxol[g]	20	15	15	10	BGN-2A	4

[a] [redacted] study number. Refer to Appendices A and B.
[b] Animal Group numbers as specified in the respective [redacted] study reports.
[c] Saline, natalizumab and human IgG_4, were administered intraperitoneally every 3 – 4 days (on a Monday/Friday schedule) for five weeks
[d] Taxol was administered IV once daily for five consecutive days starting on Day 14.
[e] Tumors were excised for Groups 5 and 6 at Day 28
[f] Animals assigned to Pharmacokinetic group (5 animals/timepoint). Animals were dosed intraperitoneally every 3 – 4 days (on a Monday/Friday schedule) for four weeks
[g] Taxol was administered IV once daily for five consecutive days starting on Day 1.

Groups 1 - 4: Tumor implantation (subcutaneous) occurred on Day 0. The treatment with saline, Taxol (positive chemotherapeutic control), human IgG4 (negative control) or natalizumab was initiated on Day 14 on a preformed established tumor of approximately 75- 150 mgs in size. Saline, human IgG4, and natalizumab were administered as an intraperitoneal bolus injection twice per week and treatment was terminated on Day 45. Taxol was administered as a daily intravenous injection for five days beginning on Day 14. Animals were sacrificed on Day 47/48.

Groups 5 - 6: Tumor implantation (subcutaneous) occurred on Day 0. The treatment with saline or natalizumab was initiated on Day 14 on a preformed established tumor of approximately 75-1 50 mgs in size. Saline and natalizumab were administered as an intraperitoneal bolus injection twice per week and treatment was terminated on Day 45. The primary tumor was excised from each Group 5 and 6 animal on Day 28. The site of the resection was monitored for tumor re-growth twice a week and when a new solid tumor was formed, its dimensions were recorded. Animals were scheduled for sacrifice on Day 80.

Groups 8 -11: The treatment with saline, human IgG4 (negative control), or natalizumab was initiated on Day -7. Tumor implantation (subcutaneous) occurred on study Day 0. Saline, human IgG4, and natalizumab were administered as an intraperitoneal bolus injection twice per week and treatment was terminated on Day 45. Taxol (positive chemotherapeutic control) was administered as a daily intravenous injection for five days beginning on Day 1. Animals were scheduled for sacrifice on Day 46/47.

Group 7: Pharmacokinetic subset. Tumor implantation (subcutaneous) occurred on study Day 0. The treatment with natalizumab was initiated on Day 14. Animals were sacrificed (5 animals/time point) on Day 14 at 8 hours post-dose, and (pre-dose) on Days 21, 28, 35, 38 and 8 hours post dose on Day 38. Blood was collected via retro-orbital eye bleed under C02 anesthesia. The animals were euthanized following blood collection by C02-anesthesia and exsanguination .

Observation times Results

Clinical signs: the number of partial and complete tumor regressions, number of tumor free survivors, time to evaluation size of tumor were determined.

Body weights: Animals were weighed and tumors measured on treatment days. For those animals that had undergone tumor excision, tumor and body weight data were collected twice per week following the completion of treatment.

Food consumption: Not reported.

Gross pathology:
Tumors of animals in Groups 1 - 4 were harvested on Day 47/48. Tumors of animals in Group 8 - 11 were harvested on Day 46/47.
The primary tumor of animals in Groups 5 and 6 was surgically excised on Day 28. The secondary tumor, if any, (which re-grew at the site of the primary tumor) of all surviving animals in Groups 5 and 6 was harvested on Day 80.
Toxicokinetics:

Table 1: Pharmacokinetic Parameter Estimates Following Single Dose IV and IP Administration of 10 mg/Kg BG00002 to Nude Mice

Dose (mg/Kg)	Route	Cmax (µg/mL)	AUC (µg*hr/mL)	Cl (mL/hr/kg)	t½ (h)
10	IV	170	11728	853	77
10	IP	91	10482	954[a]	97

[a] unadjusted for bioavailability

Study title: In vitro studies of the proliferative response of human tumor cells treated with natalizumab

Key study findings:

The purpose of this study was to evaluate the cytotoxic or proliferative effect of natalizumab on a series of tumor cells lines: [redacted]

The assays conducted showed not potential for either cytotoxicity of proliferation by natalizumab under the conditions of the study.
Study no.: p00002-03-03
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: 4/28/03
GLP compliance: No
QA report: yes ( ) no ( ) NA
Drug, lot #, and % purity:
CAC concurrence: NA

Study title: An evaluationof the tumorigenic potential of natalizumab in the [redacted] xenograft athymic NCR-NU nude mouse tumor model

Key study findings:
A select number of human tumor cell lines were screened for the expression of a4 by immunohistochemical evaluation, FACS analysis, and the ability of natalizumab to block the adhesion of the tumor cells to human rVCAM-1. Based on these in vitro experimental data, the human a4-expressing melanoma cell line, [redacted], was selected as an appropriate tumor cell line for the in vivo study in the athymic Ncr-nu mouse xenograft model to evaluate whether natalizumab is capable of inhibiting or potentiating human tumor cell growth and/or metastasis.
Treatment with natalizumab neither increased tumor growth or the formation of metastases of the [redacted] melanoma xenograft in female athymic NCr-nu mice. Growth of established primary [redacted] tumor xenografts was not affected by the administration of natalizumab. Regrowth of the solid tumor at the site of the excision of the primary tumor was not affected by the administration of natalizumab. Also, the pre-administration of natalizumab prior to tumor implantation did not affect the growth of the xenografts.
In summary, natalizumab treatment at a loading dose of 10 mg/kg followed by twice weekly maintenance doses at 5 mg/kg did not exacerbate tumor growth or increase metastastic tumor formation in a mouse xenograft model with the [redacted] melanoma cell line.

Study no.: p00002-03-04
Volume #, and page #:
Conducting laboratory and location: Biogen
Date of study initiation:
GLP compliance: NO

QA report: yes ( ) no ( ) NA
Drug, lot #, and % purity:
CAC concurrence: NA

Methods
The general study design is illustrated in the table below, supplied by the sponsor:

6.1 Study Design Table

Group No.	Treatment	Treatment Initiation[a] (Study Day)	N	Loading/ Maintenance Dose (mg/kg)	Dose Vol. (ml/kg)	— Study No.[b]	— Group No.[c]
1	Saline[d]	14	20	0/0	10	BGN-3	1
2	Natalizumab[d]	14	20	10/0	10	BGN-3	2
3	Human IgG_4[d]	14	20	9.7/0	10	BGN-3	3
4	Dacarbazine[e]	14	20	150/150	10	BGN-3	4
5[f]	Saline[d]	14	20	0/0	10	BGN-3	5
6[f]	Natalizumab[d]	14	20	10/5	10	BGN-3	6
7[g]	Natalizumab	-7	30	10/5	10	BGN-3	7
8	Saline[d]	-7	20	0/0	10	BGN-3A	1
9	Natalizumab[d]	-7	20	10/5	10	BGN-3A	2
10	Human IgG_4[d]	-7	20	9.7/5	10	BGN-3A	3
11	Dacarbazine[h]	14	20	150/150	10	BGN-3A	4

[a] Treatment initiation on Study Day 14 follows establishment of the primary tumor after xenograft implantation. Treatment initiation on Study Day –7 precedes implantation of the tumor xenograft by seven days.
[b] ——— study number. Refer to Appendices A and B.
[c] Animal Group numbers as specified in the respective ——— e study reports.
[d] Saline, natalizumab and human IgG_4, were administered intraperitoneally every 3 – 4 days (on a Monday/Friday schedule) for five weeks
[e] Dacarbazine was administered IV once weekly for three consecutive weeks starting on Day 14.
[f] Tumors were excised for Groups 5 and 6 at Day 26
[g] Animals assigned to Pharmacokinetic group (5 animals/timepoint). Animals were dosed intraperitoneally every 3 – 4 days (on a Monday/Friday schedule) for four weeks
[h] Dacarbazine was administered IV once weekly for three consecutive weeks starting on Day 1.

No effect of natalizumab on tumor promotion was observed.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Study of the effects on male guinea pigs of cohabitation with Antegren (AN100226, natalizumab)-treated female guinea pigs

Key study findings:
The purpose of this study was to investigate of male guinea pigs that were dosed in the course of the male fertility study, 309-007-01 (See below). These male guinea pigs were

previously used for co-habitation with female guinea pigs in ——— study 1147-106. Male guinea pigs were not dosed in that study. Upon initiation of dosing for study 309-007-01, the male guinea pig evidenced severe adverse effects including sudden death. The dose range used was the same as that used in male guinea pigs in previous studies with no adverse effect, 0, 3, 10 or 30 mg/kg. The animals were tested for presence of plasma levels of natalizumab and presence of anti-natalizumab antibodies. The results of these tests were equivocal. The results of the investigations indicate that the sudden death of the males on initial dosing was due to a hypersensitivity reaction and/or anaphylaxis. The conclusion is drawn mainly from the sudden nature of the deaths and the symptoms exhibited by the animals that did not die and were determined to be consistent with anaphylaxis. These findings indicate previous exposure of the male guinea pigs to the drug resulting in sensitization. No attempt to explain how the supposedly naïve male guinea pigs became exposed to the study drug. This study was conducted to assess the surviving guinea pigs for study drug levels and the presence of anti drug antibodies.

Study no.: 309-005-02
Volume #, and page #:
Conducting laboratory and location: ———
Date of study initiation:
GLP compliance: Unknown
QA reports: yes ( ) no ( )
Drug, lot #, and % purity:

Study title: Study of the effects of Antegren (AN100226, natalizumab) on the fertility of male guinea pigs

Key study findings:
The purpose of this study was to evaluate the potential for adverse effects of natalizumab treatment on fertility in male guinea pigs. Male guinea pigs were treated with natalizumab on alternate days for 28 days prior to cohabitation with untreated female guinea pigs. Dosing of the males continued until necropsy, either 3-5 days after littering of the cohabited female or after at least 4 weeks of cohabitation. No test article effects are reported for in-life clinical observations, necropsy findings, or sperm analyses. Natalizumab had no adverse effect on male fertility parameters after doses of up to 30 mg/kg, IV, every other day under the conditions of this study.

Study no.: Site study number 1147-107, Elan study number 309-007-01
Volume #, and page #:
Conducting laboratory and location: ———
Date of study initiation: 1/22/02
GLP compliance: YES
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Study drug lot# F23007, placebo lot # E85001

Methods

Doses: 0, 3, 10 or 30 mg/kg
Species/strain: male Hartley guinea pigs, greater than 16 weeks old, 674-954 g
Number/sex/group: 30 per group
Route, formulation, volume, and infusion rate: IV infusion
Satellite groups used for toxicokinetics:
Study design: Four groups of male guinea pigs received test article or placebo on alternate days for a minimum of 28 days prior to mating and continuing to necropsy (approximately days after the mate littered). Mating was performed with untreated female guinea pigs during the first post-partum estrus. Cohabitation was initiated after 28 days of dosing was completed.

Table 1 Group Assignments and Dose Levels

Group Number	N	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Solution Conc. (mg/mL)
1	30	0 (Vehicle)	1.5	0
2	30	3	1.5	2.0
3	30	10	1.5	6.67
4	30	30	1.5	20.0

Parameters and endpoints evaluated:

- Observation twice daily for mortality and general health, clinical signs
- Male food consumption was monitored weekly until cohabitation
- Physical exams and body weight were performed at randomization and weekly thereafter
- Males were euthanized 3-5 days after mating or after at least 4 weeks of cohabitation
- Blood samples were taken prior to necropsy for hematology, serum drug levels and serum anti-drug antibodies.
- Organ weights were determined fortestes, epididymides, and seminal vesicles. The left testis and epididymis were evaluated histologically, Group 1 and Group 4 only. Sperm analysis (motility and morphology) was done using the right cauda epididymis and the right testis was used for spermatid head analysis.
- Females were euthanized between 29 and 31 days or at the time of the sacrifice of the last scheduled females where evidence of littering/mating was not observed. The uterus was excised, weighed and evaluated for number of implantation sites.

Results

Mortality: All animals survived to terminal sacrifice. (Of the male animals first assigned to this study, several unexplained sudden deaths occurred after the initial dose of test article. The deaths were attributed to anaphylaxis, which remains unexplained as the male guinea pigs were thought to be naïve to the test article. They had been used in a previous study in which they were cohabitated with treated female guinea pigs but were not, themselves, treated. It is unknown how these male animals developed sensitivity to the test article. All the remaining original male animals were removed from the study

and new replaced with new male guinea pigs. See study 309-005 for follow-up information on this incident.)

Clinical signs: No remarkable clinical signs attributable to test article administration are reported.

Body weight: All male animals showed similar weight gain among groups during the course of the study.

Food consumption: Similar food consumption rates and amounts are reported among all groups during the course of the study.

Toxicokinetics:

- Low levels of natalizumab was detected in the blood samples from treated animals. See Table 2, provided by the sponsor below:

Table 2 Mean Natalizumab Levels in Serum from Males at Necropsy

Treatment Group	Antegren (µg/mL)
[redacted]	< LOQ[1]
3 mg/kg	39 ± 40
10 mg/kg	303 ± 157
30 mg/kg	1557 ± 382

[1] LOQ = Limit of quantitation

- Many of the samples from placebo animals tested positive for anti-natalizumab antibodies. The sponsor indicates that this finding may be due to non-specific binding of the guinea pig IgG used in the detection assay and are false positives.

Necropsy:

- No significant effect of test article administration on male organ weights is reported.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

- No significant effects of test article could be detected on sperm numbers, motility, amplitude, path velocity, straightness, linearity, track speed, beat cross frequency.
- No significant effects of test article administration could be detected in number of litters, numbers of corpora lutea, number of implantation sites, number of pre-implantation losses, number of total resorptions, number of live fetuses.
- A small trend toward increased total fetal deaths, early resorptions is noted. These data do not achieve statistical significance. Thus, biological significance is not probable.

Study title: Study of the effects of Antegren (AN100226, natalizumab) on the fertility of female guinea pigs

Key study findings:

The purpose of this study was to evaluate the potential for adverse effects of natalizumab on fertility in female guinea pigs. Female Hartley guinea pigs were given 0, 3, 10, or 30 mg/kg Antegren by IV infusion on alternate days from GD30 of an existing pregnancy through GD30 of a second pregnancy. The second pregnancy was established by mating on the post-partum estrus. Treatment with natalizumab at 3 and 10 mg/kg did not result in any test article-related effects on female fertility or pup survival, growth or development parameters. Treatment at 30 mg/kg resulted in a small reduction (not statistically significant) in the number of live pups born to these dams which, when combined with a slightly higher mortality rate in these pups, resulted in a statistically significant reduction in live pups at PND 14. No reason for this reduction in pup survival could be determined. Fertility was also reduced in females treated with 30 mg/kg of natalizumab but not in animals treated with 3 or 10 mg/kg. Reduction in fertility appears to be result of implantation failure. A 53% reduction in pregnancy rate was observed in the high dose group relative to control. Implantations were seen in only 31% of animals having corpora lutea in the 30 mg/kg group versus 66-69% in the other groups.

Eight deaths occurred during the study in the treated groups. (2 for group 2, 3 for group 3 and 3 for group 4). No deaths occurred in the control group. All but one occurred near the time of parturition. Though these deaths may be due to events related to parturition, the distribution of deaths of pregnant dams indicates that a relationship to study drug administration cannot be ruled out. In other studies in which pregnant female guinea pigs were used to assess reproductive toxicity of natalizumab, maternal deaths occurred at low rates in all groups including controls.

A reduction in pregnancy rate is noted for the high dose group relative to control and lower dose groups. Pregnancy rates were 63.3, 66.7, 66.7, and 29.6% for the control, 3, 10, and 30 mg/kg animals, respectively. The reduced pregnancy rate was not correlated with changes in the ovary. No differences in corpora lutea were noted. Thus, the reduction in pregnancy rate is more likely related to implantation failure. Implantations were seen in only 31% of animals having corpora lutea in the 30 mg/kg group versus 66-69% in the other groups.

Study no.: 309-008-01 (——— study number 1147-106)
Volume #, and page #:
Conducting laboratory and location: ———
Date of study initiation: 11/21/2001
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: natalizumab lot #F23007, placebo lot# E85001

Methods

Doses: 0, 3, 10, 30 mg/kg
Species/strain: female Hartley guinea pigs, 140 presumed pregnant
Number/sex/group:
Route, formulation, volume, and infusion rate: IV infusion in a volume of 1.5 ml/kg
Satellite groups used for toxicokinetics:

Study design: Presumed pregnant females were received from the supplier 3 or 4 days after littering. These females were treated on alternate days with natalizumab beginning on GD 30 to determine the effect on prenatal development in the established pregnancy. These females were dosed every other day for at least 28 days prior to littering and continued throughout the study. The females raised their litters for two weeks, during which time post-natal development and maternal function was assessed. These same females had been mated again after littering and fertility and early embryonic parameters were assessed during the second pregnancy. Necropsies were performed 29-30 days after the second litter.
Group assignments are illustrated in the table provided by the sponsor, below:

Group	Treatment	Dose mg/kg	Concentration mg/mL	Dose mL/kg	Number of Females	Female Animal Numbers
1	Control	0	0	1.5	30	6166-6195
2	Antegren	3	2	1.5	30	6196-6225
3	Antegren	10	6.67	1.5	30	6226-6255
4	Antegren	30	20	1.5	30	6256-6285

Parameters and endpoints evaluated:
Each animal received an intravenous (IV) dose every other day from gestational day (GD) 30 of the existing pregnancy through GD 30 of a second pregnancy established by mating on the post-partum estrus (approximately 12-24 hours post-littering) following littering on the existing pregnancy. All animals (male and female) were observed twice daily for changes in clinical signs.
Physical examinations and body weights for females were done at randomization and weekly thereafter. Males were transferred to Elan Study No. 309-007-01
(_____ : Study No. 1147-107 [male fertility study in which several males dies suddenly after receiving study drug injections]) following mating.
The date of littering of the females was recorded and was assumed to have been the day of mating (GD 1 of the new pregnancy). The date of littering was designated as post-natal day (PND) 1. For purposes of record keeping, all subsequent study days were referred to as PNDs. (PND 1-30 are the same as GD 1-30 of the second pregnancy.) Dams were euthanized at PND 30 or at the last scheduled sacrifice for those females not littering. Blood samples for evaluation of hematology parameters, serum drug concentration, and serum anti-drug antibody concentration were taken at necropsy. Organ weights were determined for the uterus (with implantations) and ovaries. Uteri were examined for the number of implantation sites, live fetuses, dead fetuses, resorptions (early and late), and ovaries were examined for the number of corpora lutea.

F_1 generation pups were examined on PND 1 and 14 for the number of live and dead pups, gender, body weight, external abnormalities, eye and pinna opening, incisor eruption and hair growth.

Results

Mortality:

- Seven females were found dead during the study (2 from the 3 mg/kg dose group, 2 from the 10 mg/kg dose group and 3 from the 30 mg/kg dose group). One animal from the 10 mg/kg dose group was euthanized moribund on GD 65.
- All but one of these deaths occurred near the time of parturition. The sponsor suggests that the deaths were related to parturition and not the test article treatment. However, no deaths are reported for the control group.

Clinical signs:

- No test article effects on clinical signs are reported.

Body weight: Treatment with test article did not appear to have a significant effect on female body weight change during this study.

Food consumption: Not reported.

Toxicokinetics: Samples for TK analysis were taken at necropsy, which was performed on GD 30 of the second pregnancy. Study drug levels are illustrated in Table 2, below (provided by the sponsor)

Table 2 Mean Antegren Levels (µg/mL) from Females at Necropsy (GD30)

Treatment Group	Dams
0 mg/kg	0 ± 0
3 mg/kg	40 ± 32
10 mg/kg	246 ± 150
30 mg/kg	802 ± 303

Anti-drug antibodies were detected in 6 of 28 animals in the 3mg group. No anti-drug antibodies were detected in any other dose group.

The data above confirm exposure to the test article for each dose group.

Necropsy:

Dams were euthanized on GD30 of the second pregnancy. Livers were removed for histopathology.

- Gross lesions of the liver are reported. These lesions consisted of discoloration, necrosis, fibrosis and bile duct proliferation, hepatocytes vacuolization and occurred in all liver lobes. These changes are reported across all groups including controls.
- No other gross lesions are reported.
- No test article effects are reported for uterine weights or ovary weights.
- WBC counts were elevated in all treated groups relative to control. These findings correlate with an increase in lymphocytes relative to control in the treated animals. These findings are expected results of the pharmacological activity of the test article.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

- No treatment related effects are reported for gestational length or parturition.

- No effects of the study drug were noted for gender, body weight, external abnormalities, eye and pinna opening, incisor eruption and hair growth of fetuses
- Treated dams had slightly fewer pups relative to control (4.9 and 5.2 pups/litter, respectively). Because this difference was not statistically significant, the sponsor concludes that it is not related to dosing. The small numbers of animals involved and the uncertainly regarding exposure to the test article make this conclusion questionable.
- A small decrease in live pups accompanied by a small increase in dead pups is reported. The sponsor states that these findings did not achieve statistical significance. By PND 14, litters from the 30 mg/kg dose group had significantly fewer live pups relative to control (3.0 and 4.3 pups, respectively.) This is the dose group that may not have received the full dose (males may have been doses in error) so these findings may be an underestimate of the effect of the study drug. Four pups born dead on GD 60 were not counted in this analysis.

Treatment group	PND 1	PND 14
Control	5.2 pups/litter	4.3 pups/litter
Group 4 (30 mg/kg)	4.9 pups/litter	3 pups/litter

- Pregnancy rates were reduced in the group receiving 30 mg/kg (group 4) relative to the lower dose and control groups. Pregnancy rates were 63.3, 66.7, 66.7, and 29.6% for the control, 3, 10, and 30 mg/kg animals, respectively. The data are summarized in the table below, supplied by the sponsor.

Text Table 2: Pregnancy Status

Pregnancy	Antegren Dosage (mg/kg)			
	0	3	10	30
Number of Animals	30	27	27	27
Number with Corpora Lutea	29	27	26	26
Number Pregnant	19	18	18	8
Percent Pregnant	63.3	66.7	66.7	29.6

- Corpora lutea were identified in ovaries of animals from all groups with no group differences in frequency. Mean values for the number of corpora lutea are: 7, 8, 8, and 7 for the control, 3, 10, and 30 mg/kg treatment groups, respectively.
- Implantations were seen in 31% of animals having corpora lutea in the 30 mg/kg group versus 66-69% in the other groups.

Embryofetal development

Study title: Study of the effects of Antegren (AN100226, natalizumab) on embryo/fetal development in guinea pigs

Key study findings:

The purpose of this study was to evaluate the potential for natalizumab to adversely effect embryo/fetal development. Natalizumab was administered IV on alternate days during the period of organogenesis (GD4-30). The results indicate that, under the conditions of this study, natalizumab does not have a significant adverse effect on maternal health or embryofetal development parameters. The abnormalities observed in the pups are among those often observed in this species and were observed in pups from all groups. No treatment related fetal histological changes in the heart, thymus, liver, spleen, or intestinal tract were seen. The maternal NOAEL under the conditions of this study was determined to be 30 mg/kg. The NOAEL for embryofetal development was determined to be 30 mg/kg.

No test article was detectable in the blood from groups 1, 2, or 3 taken at necropsy. Very low levels were detected in blood from the 30 mg/kg group. This lack of detectable study drug may be due to the fact that the last dose was administered on GD30 and blood was not collected until GD60 (necropsy). However, because no useful TK parameters were measured, a question remains regarding the actual exposure of the developing fetuses to the test article.

Study no.: 309-009-01
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation: 11/1/01
GLP compliance: Yes.
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Natalizumab lot # F23007, placebo lot # E85001

Methods

Doses: 0, 3, 10 or 30 mg/kg
Species/strain: female Hartley guinea pigs
Number/sex/group: 30 females per group
Route, formulation, volume, and infusion rate: IV on alternate days from GD4 to GD 30.
Satellite groups used for toxicokinetics:
Study design:

Table 1 Group Assignments and Dose Levels

Group Number	N	Dose Level (mg/kg)	Dose Volume (mL/kg)	(mg/mL)
1	30	0 (Vehicle)	1.5	0
2	30	3	1.5	2.0
3	30	10	1.5	6.67
4	30	30	1.5	20.0

Parameters and endpoints evaluated: All animals were observed twice daily for mortality and clinical signs. Physical exams, body weights and food consumption were done prior to study initiation (at randomization) and on GD 4, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and prior to necropsy.

Dams were euthanized at GD 59-61. Blood samples were collected for hematology, serum natalizumab concentration, and serum anti-natalizumab antibodies at necropsy. Organ weights were recorded for uterus and ovaries. Uteri were evaluated implantation sites, live or dead fetuses, resorptions, placental and embryonic sac development and ovaries evaluated for corpora lutea formation.

Live fetuses were examined externally, weighed and euthanized. Blood samples were collected for serum natalizumab levels. Fetal viscera were examined grossly and spleen, thymus, liver, intestines and heart collected for histology. Fetal skeletal evaluation was performed after staining with Alizarin Red.

Results

Mortality (dams): No group differences in maternal mortality are reported. (3 deaths for control group, 4 deaths each for groups 2 and 3, 2 deaths for group 4). Two animals each from groups 2 and 4 were euthanized moribund.

Clinical signs (dams):

- Animals reported as thin for all treated groups (5 from group 2, 3 from group 3 2 from group 4). This finding was not clearly dose dependent but was not reported for the control animals.

Body weight (dams):

- All group body weight averages showed a slight weight loss after the initial doses. This finding did not achieve statistical significance and occurred in the control animals as well as treated. This apparent slight decrease in group average body weight was not apparent after the first 10-15 days of the study. All groups showed similar weight gains over the course of the study.

Food consumption (dams):

- No significant differences in food consumption were noted across groups during the course of the study.

Hematology: Blood samples were taken at necropsy.

- No significant test article effects on hematology parameters are reported. A dose dependent decrease in RBC, HGB and HCT is noted. The change is small and did not achieve statistical significance.

Toxicokinetics:

- Blood samples were taken at necropsy. The last dose given to the dams was on GD30. There was no measurable natalizumab in groups 1-3 and very low levels detected in group 4 (30 mg/kg).
- Anti-drug antibodies were observed in 100, 81, and 33% of animals in the 3, 10, and 30 mg/kg groups, respectively, on GD60 (day of necropsy). Antibody rates in

the PK study at the GD28 (end of organogenesis) time point demonstrated that 40, 83, and 100% of animals in the 3, 10, and 30 mg/kg dose groups did not have antibody. Therefore the sponsor assumes full exposure to of the animals to natalizumab through organogenesis. However, it is not clear how accurate assessments of antibody levels are in the presence of drug. Additionally, the sensitivity of the anti-drug antibody detection assay may be unacceptably high resulting in false negatives.

Table 2 Mean Serum Antegren Levels (µg/mL) in Females and Fetuses

Treatment Group	Females	Fetuses
0 mg/kg	0 ± 0	0 ± 0
3 mg/kg	0 ± 0	0 ± 0
10 mg/kg	0 ± 0	0 ± 0
30 mg/kg	0.68± 0.24	1.09 ± 0.58

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

- A dose dependent trend toward increased numbers of ovarian cysts over the 4 groups (12 of 30 in the control group, 13 of 30 in group 2, 15 of 30 in group 3, 16 of 30 in group 4). This increase is small in magnitude and biological significance is unclear.
- A small increase in dead fetuses is noted for group 4 compared to control and lower dose groups (0 for control, 1 for 3 mg/kg, 0 for 10 mg/kg, 7 for 30 mg/kg).
- Total fetal deaths were slightly higher for the high dose group compared to control and lower dose groups: control group=4 deaths, group2= 3 deaths, group 3= 3 deaths, group 4= 8 deaths.

Offspring (malformations, variations, etc.):

- No differences across groups are reported for external or visceral malformations.
- A slight increase in total skeletal deformities in fetuses from treated dams is noted. This finding did not achieve statistical significance.

Study title: Study of the effects of Antegren (AN100226, natalizumab) on embryo/fetal development in female guinea pigs

Key study findings:

The purpose of this study was to evaluate the potential for natalizumab to adversely effect embryo/fetal development. Presumed pregnant guinea pigs were dosed on alternate days from GD30 of the first pregnancy through GD30 of the second pregnancy. Under the conditions of this study, natalizumab treatment had no effect on gross pathology lesions or uterine weights in the females. No significant effects were seen on pregnancy rate, number of corpora lutea, number of implantations, number of early or late resorptions, or number of dead and live fetuses.

Treatment with natalizumab did affect maternal hematology parameters. Increased total white blood cell counts determined to be result of increases in absolute lymphocyte counts and absolute monocytes counts were reported. These findings are expected results of the known pharmacological activity of natalizumab. Slightly higher pre-implant loss for the treated group is noted. This difference did not achieve statistical significance. However, pre-implantation loss was noted in another study, therefore, a potential for study drug effect should not be ignored. A slightly higher rate of post-implant loss is noted combining early and late resorptions as well as fetal death. This finding did not achieve statistical significance. Although, the data may be skewed by data collection bias: one animal littered 4 dead pups prior to GD 60 and those dead pups were not included in the statistical analysis. The biological significance of these findings is not clear.

Natalizumab treatment had minimal effect on embryo/fetal development under the conditions of this study. Parameters assessed included number of male and female fetuses, number of implantation sites, total litter weights, fetal weights or fetal examinations. There was no significant effect of the test article on fetal weight, % male fetuses or overall litter weight. Fetal external, skeletal and visceral findings were considered comparable between treatment and control groups. An increased incidence of liver hepatocyte vacuolization was noted in fetuses from the 30 mg/kg treatment group.

Study no.: ——— study #1147-118, Elan study # 309-028-02
Volume #, and page #:
Conducting laboratory and location: ———
Date of study initiation: 12/18/2002
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Natalizumab lot # G23005, placebo lot # E85001 and F85002.

Methods

Doses: two groups, 0 and 30 mg/kg

Species/strain: female Hartley guinea pigs

Number/sex/group: 30 control animals, 70 treated at 30 mg/kg

Route, formulation, volume, and infusion rate: Test article was administered on alternate days by IV infusion in a volume of 1.5 ml/kg

Satellite groups used for toxicokinetics:

Study design:

Animals were dosed from GD30 of the first pregnancy to GD30 of the second. Two groups of presumed pregnant (mated on the post-partum estrus at the breeder) female Hartley guinea pigs were given vehicle or natalizumab (30 mg/kg) by intravenous infusion. Treatment was administered every other day from gestational day (GD) 30 of the existing pregnancy through GD 30 of a second pregnancy established by mating on the post-partum estrus (approximately 12-24 hours post-littering) following littering on the existing pregnancy. Females were euthanized on approximately GD60 of the second pregnancy for evaluation of the fetuses.

Text Table 4: Study Design

Group	Treatment	Test Article Dosage (mg/kg)	Test Article Concentration (mg/mL)	Females	
				N	Animal Numbers
1	Antegren Placebo	0	0	30	9296 – 9325
2	Antegren	30	20	70	9326 – 9395

N = number of animals per group

Parameters and endpoints evaluated:
Cageside observations were made twice daily for mortality and general health. Clinical observations were made on GD30 of the first pregnancy and every 5 days thereafter until littering. Clinical observations were made during the second pregnancy on GD1, GD5 and every 5 days thereafter and prior to necropsy. Food consumption was measured at each body weight measurements from GD 5 of the second pregnancy to necropsy. Animals were euthanized on GD59-62 of the second pregnancy.

Blood collection for hematology and TK:

Text Table 7: Blood Collection

Parameter	Hematology	Toxicokinetics
Collection Day	Prior to necropsy	Prior to necropsy
Collection Method	Abdominal aorta or vena cava, after CO_2 inhalation	
Volume Collected	~1 mL	~1 mL
Tubes Used		

Study termination:
Dams that survived to scheduled termination underwent gross examination, the uterus was removed and examined for the number and placement of implantation sites, live and dead fetuses, early and late resorptions and any abnormalities of placenta and embryonic sac. Ovaries were examined for number of corpora lutea. Uteri with no evidence of implantations were treated with a solution of 10% ammonium sulfide for detection of early embryonic death.

Live fetuses were examined externally and weighed. After euthanization, blood samples were collected drug levels and anti-drug antibodies. Visceral examinations were performed by fresh dissection and sex determined. The fetuses were finally preserved and stained with Alizarin Red S for examination of the skeleton.

Histopathology: Tissues retained for histological exam from each fetus included spleen, liver, intestines and heart.

Results

Mortality: Five animals were found dead from group 1 during the study, 1 animal from group 2 was found dead. One control animal was killed moribund and three treated

animals were killed moribund. Four treated animals were removed from the study (failure to litter for first pregnancy). The cause of death was determined to not be test article related.

Clinical signs: No significant clinical signs attributable to test article administration were reported for the dams.

Hematology:
A statistically significant increase in WBC levels was noted for the treated dams compared to control. WBC counts were 127% those of control animals and were primarily the result of statistically significant increases in circulating lymphocytes and monocytes. These findings are an expected result of natalizumab biological activity.

Body weight: No test article effect on maternal body weight was detected.

Food consumption: No test article effect on food consumption was noted.

Toxicokinetics:

Table 4 Mean Serum Antegren Levels (µg/mL) in Females and Fetuses

Treatment Group	Females	Fetuses
0 mg/kg	0	0
30 mg/kg	31.2	9.1

The final dose of test article was administered on GD 30, 30 days prior to blood sampling at necropsy. These data confirm exposure of the fetuses to the test article throughout the pregnancy. Although none of the dams or fetuses that were tested from group 1 had detectable levels of natalizumab, thirteen of 24 dams and 40 of 72 fetuses did have detectable anti-natalizumab antibodies (0.5-90.8 ug/ml and 0.51-29.1 ug/ml). For group 2, no detectable anti-drug antibodies were detected in either dams or fetuses. Detectable levels of natalizumab were detected in 51 of 62 dams and 125 of 173 fetal serum samples tested from group two.

Necropsy: No apparent test article effects are reported for gross examination of the dams and no differences in uterine weights between groups are reported.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

- Slightly higher pre-implant loss for the treated group is noted. This difference did not achieve statistical significance. The result is most likely not biologically significant.
- A slightly higher rate of post-implant loss is noted combining early and late resorptions as well as fetal death. This finding did not achieve statistical significance. One animal littered 4 dead pups prior to GD 60 and those dead pups were not included in the statistical analysis. The biological significance of these findings is not clear.
- No effect of test article on number of corpora lutea is reported.
- There was no significant effect of the test article on fetal weight, % male fetuses or overall litter weight.

- A slightly higher rate of external and visceral abnormalities as well as skeletal deformities is reported for the treated group. Incidence of fetuses showing defects in the control group is 26% (19 of 73). Incidence of treated fetuses showing defects is 32% (55 of 171). Most individual visceral findings were present in a single pup and no pattern was observed in affected organ systems. Skeletal malformations and variations consisted primarily of short rib, discontinuous rib, supernumerary rib, and extra sites of ossification and were seen in pups from both groups. These findings are not unusual for this species.

A summary table showing combined malformations for study 309-028-02 and 309-009-01 is provided by the sponsor, below:

Table 2 Litter Incidence of Malformations and Variations Observed in Guinea Pigs

Finding	Study 309-009-01		Study 309-028-02		All[e]	Rabbits[f]
	Control[a]	Antegren[b,c]	Control	Antegren[d]	Control + Antegren	Control
Limb Hyperflexion	1/20 (5.0%)	0/53 (0.0%)	0/12 (0.0%)	1/34 (2.9%)	2/119 (1.68%)	2/1638 (0.12%)
Umbilical Hernia	1/20 (5.0%)	0/53 (0.0%)	0/12 (0.0%)	1/34 (2.9%)	2/119 (1.68%)	5/1638 (0.31%)
Asplenia	0/20 (0.0%)	1/53 (1.9%)	0/12 (0.0%)	0/34 (0.0%)	1/119 (0.84%)	0/1716 (0.00%)
Small Spleen	0/20 (0.0%)	0/53 (0.0%)	0/12 (0.0%)	1/34 (2.9%)	1/119 (0.84%)	7/1716 (0.41%)
Small Lung	0/20 (0.0%)	1/53 (1.9%)	0/12 (0.0%)	0/34 (0.0%)	1/119 (0.84%)	5/1716 (0.29%)
Enlarged Intestine	1/20 (5.0%)	0/53 (0.0%)	0/12 (0.0%)	0/34 (0.0%)	1/119 (0.84%)	1/1716 (0.06%)
Dilated Renal Pelvis	0/20 (0.0%)	0/53 (0.0%)	0/12 (0.0%)	1/34 (2.9%)	1/119 (0.84%)	2/1716 (0.12%)
Misshapen Liver	0/20 (0.0%)	1/53 (1.9%)	0/12 (0.0%)	0/34 (0.0%)	1/119 (0.84%)	0/1716 (0.00%)
Atresia of Uterus	1/20 (5.0%)	0/53 (0.0%)	0/12 (0.0%)	0/34 (0.0%)	1/119 (0.84%)	0/1716 (0.00%)
Dilated Pulmonary Trunk	0/20 (0.0%)	0/53 (0.0%)	0/12 (0.0%)	1/34 (2.9%)	1/119 (0.84%)	1/1716 (0.06%)
Cardiomegaly	0/20 (0.0%)	1/53 (1.9%)	0/12 (0.0%)	0/34 (0.0%)	1/119 (0.84%)	1/1716 (0.06%)
VSD	0/20 (0.0%)	1/53 (1.9%)	0/12 (0.0%)	0/34 (0.0%)	1/119 (0.84%)	9/1716 (0.52%)

[a] Represents 4 different pups from 3 separate litters, one pup presented with both umbilical hernia and atresia of the uterus; limb hyperflexion was observed in two pups in the same litter

[b] Represents 1 pup from a single litter (low dose group, 3 mg/kg) that presented with all findings

[c] Pups from all Antegren treated animals, dose levels of 3, 10 and 30 mg/kg

[d] Represents 4 different pups from four separate litters, one pup presented with both dilated pulmonary trunk and small spleen

[e] All litters (control + Antegren) examined on the program to date

[f] Rabbit data from the MARTA/MTA historical control database project (http://www.hcd.org/search/abnormality.asp) as of March 18, 2004

Histopathology: Performed on heart, thymus, liver, spleen and intestinal tract of the fetuses.

- Liver cytoplasmic vacuolization was seen at a higher rate in treated fetuses when analyzed on a per fetus basis (sexes combined). However, liver vacuolization was not significantly different when analyzed on a litter basis. Heart vacuolization was observed but in equal frequency between groups.

A tabulated summary of the liver microscopic findings is presented in table 3 below, provided by the sponsor:

Table 3 Incidence of Fetal Liver Findings

Parameter	Control		30 mg/kg Antegren	
	Male	Female	Male	Female
Number examined	35	38	88	85
Number livers within normal limits (%)	24 (69%)	25 (66%)	66 (75%)	70 (82%)
Number livers with vacuolation (%)	8 (23%)	12 (32%)	39 (44%)	36 (42%)
Number of livers with other findings[1]	5	7	11	8

[1] Findings included telangectasia, congestion, and mineralization.

- A slightly higher rate of cardiac myocytes and focal mineralization is reported for fetuses from the 30 mg/kg group relative to control.

Study title: Intravenous preliminary embryo-fetal development study in the cynomolgus monkey

Key study findings:
This study was intended as a preliminary study, the objective of which, was to provide information about the maternally toxic and embryotoxic effects of the test article in embryo-fetal development when administered to pregnant cynomolgus monkeys during the period of organogenesis. No placebo group was run with this study. All comparisons are made to historical control data. Under the conditions described, no adverse effects of natalizumab treatment (up to 30 mg/kg, approximately 7 X the recommended human dose) during the period of organogenesis are reported. Without TK information, it is not clear what exposure to the test article the fetuses received.

Study no.: — study #1438-001 (BLA study al302)
Volume #, and page #:
Conducting laboratory and location: ————————
Date of study initiation:
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 1) ——— natalizumab bulk lot #26093PC, —— finished lot# 0003.
2) ——— natalizumab bulk lot # 27785RC, —— finished lot# 0005.
Formulation: 5 mg/mL AN100226 in 50 mM histidine, 150 mM NaCI;
pH 6.0

Methods

Doses: 0.06, 0.30 and 30.0 mg/kg
Species/strain: cynomolgus monkeys
Number/sex/group: 5 pregnant females per group, sexually mature

Route, formulation, volume, and infusion rate: Test article administered by IV infusion via infusion pump on alternate days beginning on GD 20 through GD 50. Two animals from the 30 mg/kg group were also dosed on day 99 of the study.

Satellite groups used for toxicokinetics:

Study design: Animals were dosed as above between GD20 and 50 and pregnancies terminated on GD100 by Cesarean section. Day of mating was designated as GD0.

The study design is illustrated by the table below, provided by the sponsor:

Group No.:	Group Designation	Color Code	Number of Pregnant Females	Animal Number	Dose Level mg/kg/day	Application Volume (mL/kg/day)	Duration of Infusion
1	Low	blue	5	6645 6823 6574 6396 6370	0.06	10	30 min.
3	Mid	green	5	6277 5843 6579 6517 7509	0.30	10	30 min
3	High	red	5	6280 9528 6267 6608 6248	30.00	10	30 min.

Parameters and endpoints evaluated:

Pregnancy was verified on day 20 after mating by ultrasound examination. Ultrasound was used to monitor each pregnancy with exams on days 30, 44, 58, 72 and 86 of gestation.

Blood samples were collected from all animals on days 20, 21, 28, 35, 42 and 50, 64, 78 and 100. On dosing days, samples were collected prior to dosing.

Animals were observed 2X daily for morbidity and mortality.

Observation for clinical signs were performed twice daily on weekdays an at least once daily on weekends and holidays for health signs, behavioral changes and feces. Vaginal smear was performed daily from day 20 until the day prior to cesarean section.

Body weight was recorded on days 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 55, 62, 69, 76, 83, 90, 97 and on the day of Cesarean section.

Food consumption was determined semi-quantitatively daily from day 20 of gestation until cesarean section and evaluated once per week.

Pregnancies terminated by Cesarean section on GD100.

The fetuses were weighed, sexed, examined for external abnormalities, and measured as

follows: distance from coccyx to cranium, distance from tip of nose to os occipitale, distance from os frontale to os occipitale, width of head, distance between the eyes. Macroscopic and/or stereomicroscopic visual inspection for external defects as follows: body form, symmetry of head, facial form, formation of the lower jaw, eyebrows, eyes and eyelids, hair on head, nipple formation, anus, fingers, toes, and nails of fingers and toes, ears, tail, upper and lower extremities, external genitals, palpation of the vertebral column, umbilical cord, palate.

Each fetus was photographed. The placentae were also weighed and examined macroscopically, where possible.

Full necropsy was performed on any fetus with macroscopic, stereomacroscopic or visual defects of the following organs: stomach, cecum, and small and large intestine, testes, epididymides, and vas deferens, ovaries and uterus, ureters, kidneys and adrenals, urinary bladder, liver, spleen, and gall bladder, diaphragm, thymus and thyroids, heart with valves, aortic arch, ventricular septum, and cardiac auricles, lungs, esophagus and trachea, eyes, brain.

The following fetal organs were weighed:
Adrenals, brain, eyes, heart, kidneys, liver, lungs, ovaries, spleen, testes, thymus, uterus, Paired organs were weighed separately. Each organ was preserved and retained for possible histopathological examination.
Each carcass including the skull of each fetus was processed to stain the ossified skeleton and was examined for skeletal defects.

Results

Mortality: No animal deaths are reported.

Clinical signs: No treatment-related clinical findings are reported.

Body weight: No treatment related effect on body weight is reported. Body weight change was similar among groups and consistent with historical control data.

Food consumption: No treatment related effects on food consumption are reported.

Toxicokinetics: Not done.

Necropsy:

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

- The incidence of abortions in the low (0.06 mg/kg/day), mid (0.30 mg/kg/day) and high dose groups (30.00 mg/kg/day) was 0/5 (0%), 0/5 (0%), and 1/5 (20%). The sponsor reports that one abortion in the high dose group is not above the historical norm, and concluded that this fetal loss is not of biological significance. With so few animals in the study, this conclusion seems reasonable but is not confirmable.

- No treatment-related effects are reported for fetal body weight, organ weights, fetal body measurements or placental weights.
- With the exception of the one abortion noted above for group 3, all fetuses were removed alive by cesarean section.
- No treatment related abnormalities of external appearance, visceral or skeletal development are reported. Minor skeletal findings were observed in all fetuses. These findings included mainly variations in the ossification of sternebra(e), ribs, vertebra(e), tarsal bones and pelvic girdle. These findings were judged to be unrelated to test article exposure because they occurred with equal frequency in all groups and are consistent with historical controls. No placebo group was run with this study.

Study title: An Embryotoxicty and Teratogenicity study of natalizumab (AN100226) by intravenous infusion in cynomolgus monkeys

Key study findings:
The purpose of this study was to evaluate the embryotoxic and teratogenic potential of natalizumab in cynomolgus monkeys when administered IV between days 20 and 80 of gestation. No maternal deaths occurred during the study nor were there any observed effects on fetal viability. Hematology results in the maternal blood confirmed that the test article was active during the study with expected dose-dependent changes in WBC populations the treated animals.
Treatment of pregnant cynomolgus monkeys with natalizumab on alternate days during the period of organogenesis (GD20 to GD70) at doses up to 30 mg/kg was well tolerated. Treatment-related findings in dams were restricted to hematology changes (primarily increases in lymphocyte counts) which are the expected pharmacologic response to natalizumab exposure. No group differences in fetal loss across dose groups were observed. Fetal losses were distributed across all groups and were within the normal range for pregnancy losses historically observed at the testing facility. These data indicate that, under the conditions of this study, natalizumab does not have abortifacient activity under the experimental conditions in this study.
Total test article maternal exposure for the 3 mg/kg group was lower than expected compared to the exposures determined for mid- and high-dose groups. This finding could be accounted for by the presence of serum antibodies at the lower natalizumab dose. All dams in the 3 mg/kg dose group developed measurable serum antibodies to natalizumab by GD52 and with more than half of the dams in that group testing positive (5/9) by GD36. Analysis of toxicokinetic data demonstrated that development of antibodies in these animals was associated with rapid natalizumab clearance. The antibody response declined over the mid dose group and no dams in the high dose group tested positive for anti-natalizumab antibodies. Toxicokinetics confirmed that the fetuses were exposed to the test article during the dosing period.

All fetuses removed by Cesarean section on GD100 were alive. There were no treatment related external abnormalities. No differences were found in fetal weight, placental

morphology or weight, amniotic fluid volume, or fetal external measurements. No visceral abnormalities or variations, or skeletal abnormalities were seen. Apparent treatment-related effects seen in most, but not all, fetuses in the 10 and 30 mg/kg groups were mainly hematological (as in the mothers). Decreases in RBC, hematocrit, hemoglobin concentration and increases in mean corpuscular volume and mean corpuscular hemoglobin.

- Increases in lymphocyte counts by automated hematology analysis of 6.8-7.5-fold that of control fetuses
- Decreases in lymphocyte percentages by FACS of ~72% compared to control fetuses
- Increases in NRBC in the fetal circulation as determined by differential counts
- Increases in background (non-PHA stimulated) proliferation of peripheral WBC
- Significantly low or trends towards low liver and thymus weights (absolute and relative) and significantly high spleen weights (absolute and relative)
- Histopathologic changes consisting of very slight thymic atrophy, increases in splenic and decreases in hepatic extramedullary hematopoiesis, and very slight to slight increase in immature NRBC in the bone marrow
- Decreased numbers of CD20+ cells in the thymus, spleen, liver and lymph node by immunohistochemistry While increases in lymphocyte counts are expected with natalizumab treatment, the level of increases seen in the fetuses was above the range normally associated with such treatment (1.5-3-fold that of controls). Absolute counts by FACS (with a limited N) did not indicate significant changes in lymphocyte counts seen by hematology analysis. The dramatic changes in lymphocyte percentages by FACS were also surprising as treatment in juvenile and adult animals have demonstrated little or no effect on lymphocyte subpopulation percentages in spite of overall increased lymphocyte counts.

Study no.: 309-012-00
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation: 6/21/2000
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Test article lot #D23001
Placebo lot #C85002

Methods

Doses: 0, 3, 10 or 30 mg/kg
Species/strain: cynomolgus monkeys
Number/sex/group: See table below. Total of 50 monkeys used.
Route, formulation, volume, and infusion rate: intravenous infusion, 30 minutes, 10 ml/kg
Satellite groups used for toxicokinetics:
Study design: Test article was administered every other day from GD20 to GD 70 (During organogenesis plus and additional 20 days for a total of 26 doses). Pregnancies were terminated by cesarean section on DG 100.

Study design is illustrated in the Table below, provided by the sponsor:

Table 1 Group Assignments and Dose Levels

Group Number	No. Pregnant Females	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Solution Conc. (mg/mL)	Duration of Infusion (Minutes)
1	15	0 (Vehicle)	10	0	30
2	10	3	10	0.3	30
3	10	10	10	1.0	30
4	15	30	10	3.0	30

Parameters and endpoints evaluated:

- Clinical signs: Once per day on non-dosing days, 2X per day on dosing days
- Body weight: Every 5th day from GD 20 -80, GD90 and GD100
- Ultrasound: Same as body weight recording.
- Clinical chemistry and hematology: Samples collected pre-dose and 30 minutes post-dose on GD 20, 45, 71 and 100.
- Samples from some dams tested for mitogen (PHA) induced proliferation and immune cell subset profiles.
- Hormone measurements: progesterone, 17 beta estradiol, prolactin
- Samples tested for test article and anti-test article antibodies pre-dosing and 30 minutes post-dosing on GD20, 28, 36, 44, 52, 60 and 70, once on GD 80 and 90, and from dams and fetuses on GD100.

Results

Mortality (dams): No maternal death is reported.

Clinical signs (dams): No test article related effects on clinical signs are reported.
Clinical pathology:

- Increased WBCs are reported for the treated mothers from the 10 and 30 mg/kg groups, including significant increases in lymphocytes and monocytes accompanied by a decrease in percent neutrophils beginning at day 45 and continuing through day 100. These findings are dose dependent and expected pharmacological effects of the test article. Smaller changes are reported for the 3 mg/kg group at day 45.
- Analysis of lymphocyte sub-populations showed a percent decrease in CD8+ cells and percent increase in CD20+ cells in the 10 and 30 mg/kg group.

(The discrepancies between hematology results, FACS percentage results, and FACS absolute count results were investigated in Elan Study 309-028-01. The results of this investigation indicate that the data from the automated hematology analysis and percentage FACS assays are unreliable as normally conducted due to the presence of, and increased levels of, nRBC in the fetal circulation. nRBC are picked up as lymphocytes in the hematology analysis and artificially increase the counts. They are counted within the

lymphocyte gate in FACS but do not carry a lymphocyte marker therefore artificially depressing the percentages).

- Serum chemistry: No changes related to test article administration are reported for the dams.

Body weight (dams):
No effect of test article administration on body weight of the dams is reported.

Food consumption (dams):
No effect of the test article administration on food consumption is reported.

Toxicokinetics:
Blood was drawn from the femoral vein (dams) at pre-dosing and approximately 30 minutes after the end of infusion on Days 20, 28, 36, 44, 52, 60, 70 of gestation, and once on Days 80 and 90 of gestation and from the umbilical vein (all fetuses) on the day of the scheduled cesarean section (on Days 100, 101 or 102 of gestation). Mean PK parameters for dams are illustrated below in Table 10, provided by the sponsor:

Table 10 Mean Antegren Pharmacokinetic Parameters

Dose	C_{max} (µg/mL)	$AUC_{(0\text{-}244)}$[1] (µg-hr/mL)	AUC_z[2] (µg-hr/mL)	AUC_{inf} (µg-hr/mL)	$T_{1/2}$ (days)
3 mg/kg	76 ± 16	22,265 ± 4,585	23,134 ± 6,422	ND	ND
10 mg/kg	3,366 ± 2,361	469,657 ± 308,855	1,660,532 ± 1,100,144	1,878,447 ± 994,064	3.96 ± 1.19
30 mg/kg	13,487 ± 7,993	2,926,875 ± 2,191,711	8,690,898 ± 5,251,820	8,738,099 ± 5,273,531	6.75 ± 2.09

Mean ± SD
ND= Not Determined; insufficient data available
[1] AUC from GD20 through GD 44
[2] AUC from GD 20 through the final sample

Total natalizumab exposure in the 3 mg/kg group is lower than expected compared to the mid- and high-dose AUC. This result is hypothesized to be due to increased clearance in the presence of serum anti-natalizumab antibodies at the lower exposure. All animals in the 3 mg/kg dose group developed measurable antibodies to natalizumab by GD52 and more than half (5/9) by GD36. Development of antibodies in these animals was associated with rapid natalizumab clearance as evidenced by little or no measurable natalizumab within 30 minutes post-dose. Only two animals in the 10 mg/kg dose group developed measurable antibodies, one at GD44 and one at GD80, while none in the 30 mg/kg group developed antibodies. The sponsor points out that the single 10 mg/kg animal with antibodies at GD44 had limited total natalizumab exposure similar to the 3 mg/kg animals.

Table 11 provided by the sponsor, below, summarizes the anti-test article anibody results for fetuses.

Table 11 Mean Antegren and anti-Antegren Antibody Exposure in Dams and Fetuses at GD100 Cesarean

	Antegren			Anti-Antegren Antibody		
Dose	Dam (µg/mL)	Fetus (µg/mL)	Ratio (F/D)	Dam (µg/mL)	Fetus (µg/mL)	Ratio (F/D)
3 mg/kg	ND	ND	ND	67.2 ± 64.7	19.4 ± 13.7	0.40 ± 0.15
10 mg/kg[1]	27.4 ± 17.0	6.7 ± 10.4	0.18 ± 0.15	55.0 ± 43.5	17.3 ± 8.4	0.37 ± 0.14
30 mg/kg[2]	170.2 ± 130.6	54.1 ± 46.7	0.35 ± 0.19	ND	ND	ND

Mean ± SD
ND = Not Determined; all individual samples BLOQ
[1]Antegren detected in 6/8 dams and fetuses; anti-Antegren antibody in 2/8 dams and fetuses
[2]Antegren detected in all dams and fetuses

Levels of natalizumab and anti-natalizumab antibodies were detected in fetal blood at approximately half to one third the maternal values. Test article was detected in all fetuses tested.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): Fetuses were removed by C-section on days 100, 101 or 102. Hysterectomies were performed on four dams (one control, one low dose and two mid-dose) after embryonic death was confirmed (day 30, day 32 and day 45, respectively). Three additional embryonic deaths were confirmed but hysterectomies were not performed (one control, and two high-dose animals).

Offspring (malformations, variations, etc.):
Standard teratology evaluation was made on all fetuses including viability, sex, body weight, placental weight, full external, visceral, and skeletal examinations. In addition, histopathological and immunohistochemical examinations of the lymph tissues (spleen, thymus, mesenteric lymph node, and/or bone marrow) and mitogen induced proliferation and immune cell subset profiles of the fetal cord blood were performed. Cord blood of some fetuses (last 19 on study) was also evaluated for hematology parameters to help understand apparent changes in lymphocyte populations (CD8+, CD20+, CD34+) seen in fetuses necropsied earlier in the study.

- Fetal death was not different between groups: (2 of 15 for group1, 1 of 10 for group 2, 2 of 10 for group 3, 2 of 15 for group 4.
- A trend toward reduction in fetal weight is apparent in a dose dependent relationship for treatment groups. However, this trend did not achieve statistical significance due to variability in results within groups. No significant effects on placental weight or fetal head width or other fetal measurements are reported across groups.
- Reduced thymic organ weight is noted for fetuses from 10 and 30 mg/kg groups. The reduction appears to be dose dependent with a 28% decrease in the high dose group compared to control.
- Fetal hematology results show decreases in RBC, HCT, HGB and PLC in treated groups that appear to be dose dependent. MCV, MCH, RET, lymphocytes, monocytes, and segmented neutrophils appear to be elevated in a dose dependent

manner. Percent erythroblasts is elevated (approximately 2X) in fetuses from the 10 and 30 mg/kg group.

- Immunostaining showed a dose dependent decrease in % CD20+ lymphocytes accompanied by dose dependent decreases in % CD3+, CD4+ and CD8+ lymphocytes. These changes are expected from the known pharmacologic activity of the test article and confirm fetal exposure to the drug.
- Dose dependent increases in BrdU-labeled cells is noted with and without PHA stimulation.
- Increases in spleen and liver organ weights are reported for fetuses from the 10 and 30 mg/kg groups.
- Histological examination revealed thymic atrophy in fetuses from the 10 and 30 mg/kg groups, as well as decreased extramedullary hematopoiesis in the liver and increased extramedullary hematopoiesis in the spleen.
  Immunostaining analysis showed a dose-dependent decrease in CD20+ cells in thymus, spleen, mesenteric lymph nodes and liver in the fetuses from the 10 and 30 mg/kg groups. Decreased numbers of cells were observed in the medulla/cortico-medullary junction (10 and 30 mg/kg groups) and in the cortex (30 mg/kg group only)
- No fetal skeletal deformities related to test article administration are reported.

Prenatal and postnatal development

Study title: Developmental reproductive toxicity study of Antegren (AN100226, natalizumab) administered intravenously in cynomolgus monkeys

Key study findings:

The objective of this study was to evaluate the potential for developmental effects in infants born to females treated with natalizumab. The study was designed to evaluate potential effects in offspring following exposure during organogenesis and during full gestation in cynomolgus monkeys. The decision to include treatment through full gestation was based on the fact that certain potentially sensitive organ systems, notably immune system organs, undergo significant developmental events at times later than the initial period of organogenesis.

Four groups of pregnant female cynomolgus monkeys (n = 26) were treated by intravenous (IV) infusion on alternate days with natalizumab or vehicle from gestational day (GD) 20-70 or from GD20-term. Animals were treated intravenously (IV) on alternate days with a dose of 30 mg/ kg. Confirmation of pregnancy at GD 18 by ultrasound was performed prior to being placed on study. Two rounds of animals were placed on study. The first round (1-8/2002) consisted of 14 animals per group. Due to unexpected magnitude of fetal loss for those animals, a second round of 12 animals per group was placed on study (11/02-1/03).

Significant numbers of pregnancies were lost to abortions and stillbirths during the study. The total incidence of abortions in the treated groups was 35% and 27%, in Group 3 and Group 4, respectively, versus 12% and 19% in Group 1 and Group 2, respectively. The higher abortion rate in natalizumab treated animals was attributed to

diffferences in incidence rates in the first enrollment animals in which abortion rates were 43% and 29% in Groups 3 and 4, respectively, versus 7% in both groups of control animals. The incidence of abortion in the second enrollment animals was the same with 25% in both groups of natalizumab-treated animals versus 16% and 33% in control animals (Groups 1 and 2, respectively). The reason for the higher rates of pregnancy losses in natalizumab-treated versus control dams in the first round of pregnancy enrollment is not clear, and was not reproduced in the second round of pregnancy enrollment. The same lot of study drug was used for both rounds of treatment. The abortion rate for all animals on study (23%), as well as the rate for all natalizumab-treated animals (31%) and all control animals (15%), were within the historical normal control range for the test facility. No differences in still births were noted between the control and natalizumab-treated dams and ranged from 11-23% of pregnancies across the groups. These values are consistent with the historical control range of 0-33% for the testing facility. Normal deliveries occurred between GD 134- 183 (mean 162 days) for both control and natalizumab-treated groups, which is consistent with the normal gestational period for this species.

Three infants died during the time period for the interim data cut-off for this study. This resulted in a 5% post-partum loss rate. This rate of loss is within the historical norm reported for this test facility. All infants that died on study were from the first round of pregnancies. Of the three infants, two infants were in Group 2 (controls) and one from group 4. The deaths of these infants were not considered to be treatment-related. Necropsy showed an enlarged spleen and dark red thymus for the group 4 infant. No definitive cause of death was identified. Natalizumab treatment of dams had no observable effect on infant body weights, body weight gains, or clinical observations. No external malformations were observed in any infants.

Treatment of dams with natalizumab resulted in increases in WBC counts, 1.4-1.9-fold versus controls, consisting primarily of increases in lymphocytes, 1.7-2.6-fold versus controls, in dams treated from GD20-70 at GD70, 100, and 150 and in dams treated from GD20-term at GD70, 100, 150 and PND28. FACS analysis demonstrated an overall increase in the percentage of lymphocytes in circulation that was consistent with the hematology findings. In natalizumab-treated dams, shifts in the lymphocyte populations were seen by FACS with small decreases in the relative percentage of CD3+ cells, small increases in CD20+ cells, and moderate decreases in CD16+ cells.

Infants from dams treated from GD20-term demonstrated hematologic changes (increased WBC counts, lymphocyte counts and NRBC counts) that were consistent with the expected pharmacologic effect of natalizumab. Infants from dams treated from GD20-term had increased WBC counts, 1.2-1.6 fold relative to controls. The increases consisted primarily of increases in lymphocytes, (1.2-2.1-fold relative to controls), at PND28, 56 and 84.

For both dams and infants in natalizumab treatment groups, increases in the incidence and degree of nucleated red blood cells (nRBC) present in blood smears was seen at the same time points at which WBC changes were noted. In dams, the cumulative incidence of this finding in control animals was (6%) versus (83%) in natalizumab-treated animals. For the infants, the cumulative incidence of this finding in control infants was (16%) versus (71%) in Group 4 infants. Elevations in nRBC were not

associated with any other changes in RBC parameters and are consistent with effects of natalizumab seen in other animal studies.

Statistically significant decreases in platelet counts were observed in Group 4 infants at PND28, 56, 84 and 112 before returning to normal at PND140. Increases in WBC, lymphocyte and NRBC counts are expected effects of natalizumab treatment and were associated with the presence of drug in the serum. Clearance of natalizumab from circulation resulted in a return to normal control levels for these parameters in both dams and infants. Decreases in platelet counts in the Group 4 infants also appeared to be associated with the presence of serum natalizumab and were reversible following clearance of the drug from circulation.

As of the date of the study, data included here (December 22,2003), IV natalizumab treatment with 30 mg/kg on alternate days of pregnant cynomolgus monkeys either through organogenesis (GD20-70) or through the full pregnancy (GD20-term) does not appear to have had adverse effects on the general health, survival and development of infants born to these dams. Hematology parameters in the affected groups of infants returned to normal following the clearance of natalizumab from circulation. Treatment of dams from GD20-70 or GD20-term had no observable effect on infant IgG, IgM, or IgA levels as tested at 6 months of age. Results of gross necropsy observations, organ weights, and immunohstochemical analysis of immune organs are not yet available.

Study no.: 309-033-01
Volume #, and page #:
Conducting laboratory and location: ———
Date of study initiation: 1/2002
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Natalizumab lot # F23007, vehicle lot # E850001 and F85002

Methods

Doses: 0 or 30 mg/kg
Species/strain: pregnant cynomolgus monkeys,
Age: 4-10 years, 2.5-4.5 kg
Number/sex/group: 14 in first phase, 12 per group for second phase
Route, formulation, volume, and infusion rate: see below, Table 1
Satellite groups used for toxicokinetics:
Study design: Four groups (see below), Animals were dosed from GD 20-70 or GD20-term

Table 1 Group Assignment and Dose Levels

Group Number	Treatment	N of Pregnant Females		Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Solution Conc. (mg/mL)	Duration of Infusion (Minutes)
		1st Cohort[1]	2nd Cohort[2]				
1	Vehicle GD20-70	14	12	0	10	0	30
2	Vehicle GD20-Term	14	12	0	10	0	30
3	Antegren GD20-70	14	12	30	10	3.0	30
4	Antegren GD20-Term	14	12	30	10	3.0	30

[1] Enrolled between January and August, 2002

[2] Enrolled between November, 2002 and April, 2003

Phase 1 consisted of 14 animals per group, placed on study between January and August, 2002. Twelve addtional animals per group were added to the study between November, 2002 and April, 2003 because pregnancy losses in natalizumab-treated groups in the first phase were significantly higher than expected resulting in some groups being too small for reliable evaluation of the data. The same study drug product lot was used for both rounds of enrollment. Infant deliveries were by natural birth at the end of the normal gestational term (-GD 165).

Parameters and endpoints evaluated:

- Animals were observed twice daily for mortality and morbidity
- Body weights recorded weekly on the females during pregnancy, then for both females and infants weekly through PND 28, then monthly.
- Food consumption monitored daily
- Blood samples from females collected pre-dose, and 30 minutes post-dose on GD20, 28, 52, 70, 100, 126 and 150. Then samples collected PMD 12, 28, and 56 for groups 3 and 4 only.
- Blood samples from the infants were collected monthly from PND28 to PND 364 and at PND 546.
- Breast milk was collected on PND 28.
- Infants were tested for the following immune functions:

a. Humoral Immune Function: Tested by the induction of a humoral response following immunization with KLH on PND182 (primary response) and PND340 (secondary response). Blood samples for determination of anti-KLH titers were taken prior to immunization and at PND189, 196,203,210,217,347,354, and 361.

b. Serum Immunoglobulin Levels: Overall levels of IgA, IgG, and IgM were measured at 6, 12, and 18 months post-partum.

- Hematology, clinical chemistry, coagulation, and FACS analyses were performed on maternal samples taken at GD 19,70, 100, and 150, and PND28 and on infant samples on PND28,56,84,112,140,168,196,224,252,280,308,336,364, and 546. The FACS panel consisted of the determination of the percentage of CD3 (lymphocytes), CD4 (T helper cells), CDS (T suppressor cells), CD16 (natural killer [NK] cells), CD20 (B cells), CD34 (stem cells), and CD71 ([infants only], erythroid precursors, replicating cells, macrophages) cells.

Necropsies and full hstopathology were conducted on approximately half of the infants in each group at PND372 ± 7 and at PND546 ± 7. Infants were scheduled for necropsy at at 12 and 18 months of age. Infants were subjected to complete necropsies and complete hstopathologic examinations. Samples from the spleen, thymus, axillary lymph node, mesenteric lymph node, i n p n a l lymph node, Peyer's patches, and tonsils were collected and stained for CD2,CD3, CD4, CDS, and CD20.

Results

Toxicokinetics:

Study drug levels in maternal and infant serum: Results of serum measurements of natalizumab in maternal and infant serum are summarized in the table below, supplied by the sponsor:

Table 8 Antegren and Anti-Antegren Antibody Levels at PND28-84

Study Day, Dosing Time Point	Treatment Group[a]							
	Antegren		Anti-Antegren		Antegren		Anti-Antegren	
	Mean[b]	N[c]	Mean[b]	N[c]	Mean[b]	N[c]	Mean[b]	N[c]
Maternal	30 mg/kg GD20-70				30 mg/kg GD20-term			
PND28	0	0/5	33 ± 42	4/6	412 ± 399	7/7	0	0/6
PND56	0	0/5	10 ± 9	4/6	101 ± 79	7/7	0	0/6
Infants	30 mg/kg GD20-70				30 mg/kg GD20-term			
PND28	0.46	1/5	0	0/5	348 ± 286	7/7	0	0/6
PND56	0	0/5	0	0/5	96 ± 71	6/6	0	0/5
PND84	0	0/5	0	0/5	21 ± 23	6/6	0	0/6

[a] Alternate day dosing, Days GD20-GD70 or Days GD20-Term

[b] Mean value ± SD (µg/mL) for all animals positive for analyte

[c] Number of animals positive for analyte/Number of animals total

- All infants from group 4 had detectable drug levels until PND 84.
- Only one infant from group 3 had detectable serum levels and only on PND 28.
- No infants from the natalizumab treated mothers had detectable anti-drug antibodies.
- 4 of 6 mothers from group 3 (treated GD20-70) had detectable anti-drug antibodies at PND 28 and 56. None from group 4 had detectable antibodies.

Detection of study drug and anti-drug antibodies in breast milk:

- One mother from group 3 had detectable study drug in breast milk on PND28 (measured value of 1.2 ug/ml). No other dams had detectable study drug in breast milk.
- Anti-drug antibodies were detected in breast milk from 4 of 6 mothers from group 3 but no in any of their infants.

F_0 in-life:

- High rates of abortions and still births occurred during the first round of the study: 12 abortions, 11 stillbirths, and 33 normal deliveries out of 56 established pregnancies, for a total loss rate of 41%. Abortions occurred more often in the natalizumab treated animals (groups 3 and 4, 43 and 29%, respectively) than

control (7%). The number of still births were slightly higher for the control groups (21%) relative to the natalizumab treated groups (14%).

Fetal loss for the second round (including abortions, still births) was 38%. In this group the abortion rate for natalizumab and control were comparable.

The following table supplied by the sponsor summarizes pregnancy out comes for the two phases of this study.

Table 2 Pregnancy Outcomes

Group Number	Treatment	Abortion[a]		Stillbirth[b]		Normal Delivery	
		1st	2nd	1st	2nd	1st	2nd
		N=14	N=12	N=14	N=12	N=14	N=12
1	Vehicle GD20-70	1	2	2	2[c]	11	8
2	Vehicle GD20-Term	1	4	4	2[d]	9	6
3	Antegren GD20-70	6	3	3	1[e]	5	8
4	Antegren GD20-Term	4	3	2	1[f]	8	8[g]

[a] Abortion or c-section to remove dead fetus prior to GD150.

[b] Stillbirth or c-section to remove dead fetus after GD150.

[c] Including a GD155 c-section delivery due to incomplete partial delivery, infant dead.

[d] Including a GD159 c-section delivery due to incomplete partial delivery, infant dead.

[e] GD168 c-section delivery due to no heart beat and maternal distress, infant dead.

[f] GD163 c-section delivery due to breech position, infant dead.

[g] Including a GD151 c-section delivery due to breech position, infant alive.

Maternal clinical pathology:

- Significant increases in WBC counts were noted for all dams treated with natalizumab. At GD 70, 100 and 150. For group 4, the increase persisted until at least 28 days post-partum. WBC counts were increased to 1.4-1.9-fold that of controls and appeared to be primarily due to increases in lymphocyte counts, 1.7-2.6-fold that of controls (statistically significant, percentage and absolute counts) at the same time points. Corresponding decreases in the percentage, but not absolute counts, of neutrophils was seen at these time points, an expected result of the increase in lymphocytes in circulation.
- Blood smears demonstrated increases in the presence of NRBC in Groups 3 and 4 at GD70, 100 and 150 (statistically significant). Increases were in both incidence and degree.
- Seven of 15 Group 4 animals had observable NRBC at PND28, (no longer statistically significant). NRBC were not seen in Group 3 dams at PND28. Elevations in NRBC were not associated with any other changes in RBC parameters.

These effects are consistent with the known pharmacological effect activity of natalizumab.