

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

125104

PHARMACOLOGY REVIEW

Product: Natalizumab

STN# _____

Sponsor: Biogen Idec

Indication: Treatment of relapsing form of multiple sclerosis

Pharmacology & Toxicology Branch, OTRR (HFD-579)

Reviewer: Iftekhar Mahmood, Ph. D.

Martin David Green, Ph. D.

Introduction

Natalizumab is a recombinant humanized IgG4 antibody, is a selective adhesion molecule inhibitor, and binds to the $\alpha 4$ -subunit of human integrin which is highly expressed on the surface of all leukocytes, with the exception of neutrophils. Natalizumab is produced in murine myeloma cells. The molecular weight of natalizumab is 149 kilodaltons.

Natalizumab specifically binds to the $\alpha 4\beta 1$ integrin, blocking the interaction with its cognate receptor, vascular cell adhesion molecule-1 (VCAM-1). Blockade of the molecular interactions of $\alpha 4\beta 1$ with its targets reduces inflammatory activity present in the brain in MS and inhibits further recruitment of immune cells into inflamed tissue, thus reducing the formation or enlargement of MS lesions.

The recommended dose of natalizumab to MS patients is 300 mg IV _____ infusion,

The pharmacokinetics and pharmacodynamics of natalizumab were evaluated in a total of 3 single-infusion studies in healthy volunteers and in 9 single- and repeat-infusion studies in multiple sclerosis (MS) patients. Throughout clinical development, natalizumab was administered as a 30-60 minute intravenous (IV) infusion. In the single-infusion studies, natalizumab was administered at doses ranging from 0.03 mg/kg to 6 mg/kg. In the repeat-infusion Phase 2 studies, natalizumab was administered monthly at doses of 3 mg/kg and 6 mg/kg for up to 6 months. In the Phase 3 studies, natalizumab dosing was no longer weight based and was administered monthly as a 300 mg fixed dose for a minimum of 6 months. Two studies evaluated the pharmacokinetics and pharmacodynamics of natalizumab when concurrently administered with interferon β -1a (AVONEX) and one study evaluated the pharmacokinetics and pharmacodynamics of natalizumab given with glatiramer acetate (Copaxone) but the results of the study are inconclusive.

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Comments

1. Although from study #6 it appears that there is no effect of gender on the pharmacokinetics of antegen, it should be noted that the sample size is unbalanced. The number of female subjects in the study is almost 4 times than the male subjects. Therefore, the study may not reflect the true impact of gender on the pharmacokinetics of antegen.
2. Study #7 is not adequate to conclude that natalizumab has no effect on the PK of $\text{INF}\beta\text{-1a}$. A single dose of natalizumab may not have any impact on the PK of $\text{INF}\beta\text{-1a}$ but under steady state condition of natalizumab this conclusion may not hold true.
3. It appears that AVONEX alters the pharmacokinetics of natalizumab. In study #10 when patients were not given AVONEX, the pharmacokinetics of natalizumab remained unchanged after the first and the sixth dose. Therefore, the change in PK parameters in study #11 is not simply due to the drug accumulation rather may be an effect of AVONEX on natalizumab pharmacokinetics.
4. In study #12 the PK parameters were calculated only after the first dose. In order to assess the impact of glatiramer acetate (GA) on the PK of natalizumab the sponsor should submit the PK data following the administration of natalizumab on week 12. At this time from this study there is no evidence that GA has no impact on the PK of natalizumab.

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Recommendation

From clinical pharmacology point of view, this submission is acceptable. The sponsor should modify the labeling according to the labeling comments.

Iftexhar Mahmood, Ph.D.
Clinical Pharmacology Reviewer

Martin David Green, Ph. D.
Assistant to the Director for Pharmacology and Toxicology ODE VI



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	STN# 125104
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	5/28/04
DRUG NAME:	Natalizumab
INDICATION:	relapsing multiple sclerosis
SPONSOR:	Biogen
DOCUMENTS REVIEWED:	Toxicology
REVIEW DIVISION:	Division of Biological Internal Medicine Drug Products (HFD-576)
PHARM/TOX REVIEWER:	Barbara J. Wilcox, Ph.D.
PHARM/TOX SUPERVISOR:	Martin D. Green, Ph.D.
DIVISION DIRECTOR:	Marc Walton, M.D.
PROJECT MANAGER:	Cathleen Michaloski Beverly Conner, D.Ph.

Date of review submission to Division File System (DFS):

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

Non-clinical toxicology studies indicated that natalizumab is generally well tolerated in the animal models studied. The toxicities observed in animals were primarily extensions of the known pharmacologic activity of the drug. The clinical population can be selected and/or monitored appropriately to avoid unreasonable risk.

B. Recommendation for nonclinical studies

No further non-clinical studies are required at this time.

C. Recommendations on labeling

Non-clinical reproductive toxicology studies demonstrate that treatment of natalizumab has potential for reducing fertility through impairment of embryonic implantation. In monkeys and guinea pigs a small tendency toward postimplantation loss and decreased fetal survival was noted. In monkeys and guinea pigs, natalizumab was found to undergo transport across the placenta and fetal drug levels were roughly 30% of maternal levels. Infants exposed to natalizumab before birth were born with hematologic findings characteristic of natalizumab exposure (increased WBC, nRBC, increased circulating lymphocytes). Therefore, Pregnancy category C is recommended for this drug.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

This document contains only reviews of the non-clinical toxicology data including single-dose toxicology, repeated dose toxicology, genotoxicity, carcinogenicity, and reproductive toxicology. The pharmacology, pharmacodynamics, pharmacokinetics and safety pharmacology are reviewed elsewhere.

Non-clinical single-and repeat dose toxicology studies were performed to evaluate the potential toxic effects of natalizumab in a variety of animal models. The species studied included CD-1 mice, guinea pig, juvenile cynomolgus monkeys, young adult cynomolgus monkeys. Treatment durations were up to 6 months and evaluated doses up to 60 mg/kg in monkeys and 250 mg/kg in mice.

Treatment with single and repeated dosing of natalizumab at doses up to 60 mg/kg in monkeys was generally well tolerated. The high dose of 60 mg/kg administered weekly represents over 50X the anticipated human monthly dose. The major study drug -related effect observed in all species tested included dose dependent increases in circulating WBC (in the presence of serum drug levels above 1-5 ug/ml), attributable primarily to increases in circulating lymphocytes. These findings are expected effects of the pharmacological activity of natalizumab.

Additional, findings noted with less consistency but also related to natalizumab treatment are: dose dependent increases in reticulocytes and/or nucleated red blood cells (nRBCs), increased spleen weight, mild to moderate follicular hypertrophy in spleen and lymph node, and minimal to mild focal leukocyte infiltrates in the liver. Most findings appeared to be reversible after sufficient non-treatment periods. However, in some cases, the findings persisted until the end of the recovery period.

In a study evaluating the potential toxicity of natalizumab administered in combination with Avonex, findings at the end of the treatment period consisted of lymphoid hyperplasia in the spleen and peripheral lymph nodes and leukocytosis and focal leukocyte aggregates in the liver. These findings appeared to be more prominent in animals receiving natalizumab or the combination relative control or Avonex treatment alone. There was no evidence that the combination of Avonex and natalizumab potentiated the incidence or severity of these changes.

Anti-drug antibodies were detectable in treated animal serum in an apparent inverse relationship in incidence with dose of natalizumab. Infusion reaction was observed in two studies using cynomolgus monkeys and these reactions appeared to be immune mediated and correlated with high levels of anti-drug antibodies. Although it appears that the higher doses of natalizumab may have had a tolerizing effect, masking of the antibody may have interfered with detection at the higher dosing levels. A finding of glomerulonephritis in 4 monkeys after 6 months chronic dosing was also correlated with the detection of anti-drug antibodies and elevations in circulating immune complexes. However, in another study where cynomolgus monkeys were dosed every other day with doses up to 30 mg/kg, no such glomerulonephritis was observed and no immune complex deposition was observed.

Studies to assess genetic toxicity were conducted on natalizumab. The testing included: chromosomal aberration study in human whole blood lymphocytes and mutagenicity in L5178Y K+/- mouse lymphoma cells. No genotoxic effects were observed.

Tumor promotion potential of natalizumab was evaluated by first identifying human tumor cells lines that bind natalizumab *in vitro*. In a subsequent study the tumor promotion potential of natalizumab was evaluated by assessing tumor growth in athymic nude mice after subcutaneous implantation followed by treatment with natalizumab, vehicle, or Taxol. Natalizumab did not show any tumor promoting potential on two $\alpha 4$ expressing tumors (leukemia, melanoma) in this nude mouse xenograft model under the conditions of the studies.

Treatment of pregnant female guinea pigs and cynomolgus monkeys with natalizumab revealed potential reproductive toxicities. In a study of the effects of natalizumab on female guinea pig fertility, a dose of 30 mg/kg administered resulted in a significantly reduced pregnancy rate. This was determined to be a result of implantation failure rather than failure of ovulation. Corpora lutea were identified in ovaries of animals from all groups with no group differences in frequency. However, the number of animals pregnant for the high dose group was roughly one half that of other groups. In that study, a reduced pup survival was noted during the interval from birth to PND 14. No cause of death was identified. In one other study where pregnant guinea pigs were dosed with up to 30 mg/kg through the fertilization and implantation period, only a slightly higher

implantation loss was observed. For that study, if early and late resorptions are combined with fetal death, a slightly higher rate of loss is noted for natalizumab treated females (30 mg/kg).

No teratogenic effects of natalizumab treatment were noted for either guinea pigs or monkeys. A slightly higher rate of visceral findings was reported for fetuses (guinea pigs) from treated mothers (26% for control, 32% for treated fetuses). The malformations were present in a single pup and no pattern of organ system defects was observed. The findings consisted of abnormalities commonly observed for this species. In one monkey study of four studies conducted in monkeys and guinea pigs, an increase in rate of abortions was noted. Effects fetuses from natalizumab treated cynomolgus monkeys were noted. In one study, a dose dependent trend toward decreased fetal weight was reported. In addition, decreased thymic weight and thymic atrophy was noted for fetuses from the mid and high dose groups, as well as dose dependent increases in circulating lymphocytes, reticulocytes, monocytes and segmented neutrophils. Also noted are increased fetal spleen weights from the mid and high dose groups (10 and 30 mg/kg, respectively). These findings are not surprising in light of the fact that toxicokinetic analysis confirmed exposure of the fetuses to approximately 30% of the levels in the dams.

One study of pre-and post-natal development in which cynomolgus monkeys were exposed to natalizumab for either GD20-70 or GD 20-term showed similar findings. Infants showed dose dependent increases in WBC, lymphocyte counts and nRBC. In this study, significant decreases on platelets were detected in the infants from the high dose group at PND 28 -112. The hematologic effects of natalizumab on the infants appeared to be reversible. Final gross necropsy and histopathology data were not available at the cut-off date for filing this BLA. Those data should be submitted when available.

No effect was noted n male guinea pig fertility at doses up to 30/mg/kg that resulted in serum levels of 1557 ±382 ug/ml.

B. Pharmacologic activity

Natalizumab is a humanized IgG4 monoclonal antibody that binds to the $\alpha 4\beta 1$ integrin and blocks the interaction with its cognate receptor, vascular cell adhesion molecule-1 (VCAM-1). Natalizumab also blocks the interaction of $\alpha 4\beta 7$ integrin with the mucosal addressin cell adhesion molecule-1 (MadCAM-1). Disruption of these molecular interactions prevents transmigration of mononuclear leukocytes across the vascular endothelium into inflamed tissue. Natalizumab may also suppress ongoing inflammatory reactions by inhibiting the interaction of $\alpha 4$ -expressing leukocytes with their ligands in the extracellular matrix and on parenchymal cells. Through these mechanisms, natalizumab is thought to suppress inflammatory activity, and inhibit further recruitment of immune cells into inflamed tissues.

C. Nonclinical safety issues relevant to clinical use

Natalizumab, as used under the conditions of the studies reviewed here, is generally well tolerated. As a product of its mechanism of action (preventing recruitment of immune cells to sites of inflammation), this drug has an immune suppressant effect and may result in vulnerability of the user to infection or neoplasms.

In monkeys and guinea pigs, this molecule was shown to be immunogenic. Occasional occurrences of infusion reactions were observed in monkeys that appeared to be immune mediated. Deposition on immune complexes was also noted in one study that resulted in minimal to moderate glomerulonephritis.

Use during pregnancy may result in fetal loss (low rates of fetal loss were observed in both guinea pigs and monkeys). Although no teratogenic effects were demonstrated in non-clinical studies, exposure to natalizumab did result in effects on the fetuses of both guinea pigs and monkeys. Those effects appeared to be reversible, under the conditions of the studied described.

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: BLA STN# 125104

Review number:

Sequence number/date/type of submission: BLA Original submission

Information to sponsor: Yes () No (X)

Sponsor and/or agent: Biogen IDEC

Manufacturer for drug substance:

Biogen Idec
5000 Davis Drive
Research triangle Park, NC 27709

Reviewer name: Barbara J. Wilcox, Ph.D.

Division name: Division Of Biological Internal Medicine Products

HFD #: HFD-108

Review completion date: 11/3/04

Drug:

Trade name:

Generic name: Natalizumab

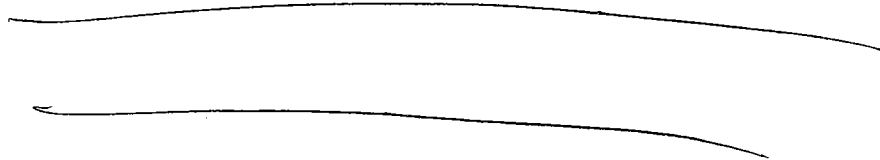
Code name:

Chemical name: recombinant humanized IgG₄ anti- α 4 integrin monoclonal antibody

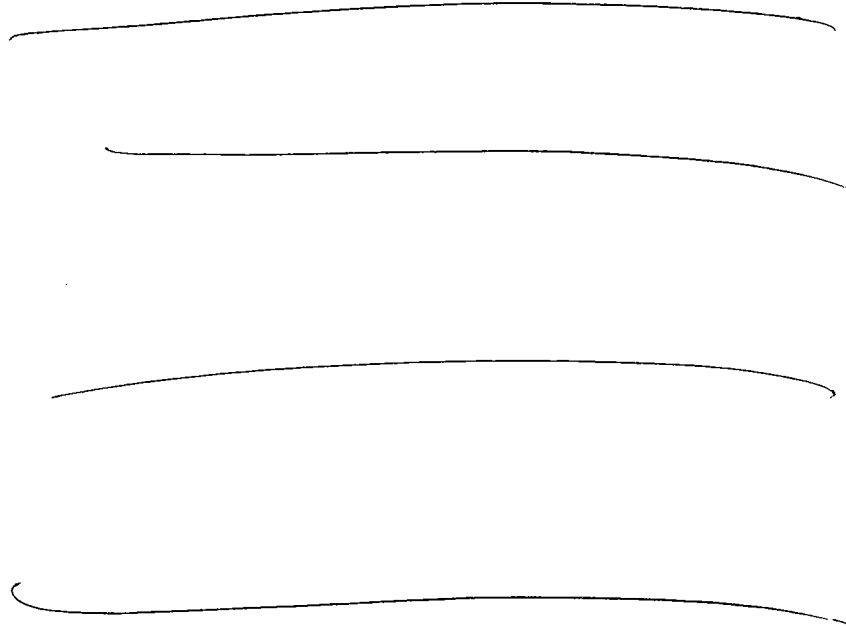
CAS registry number:

Molecular formula/molecular weight: mw 149kD

Structure:



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§ 552(b)(4)



Relevant INDs/NDAs/DMFs:

Drug class: Monoclonal antibody

Indication: Treatment of relapsing forms of multiple sclerosis to reduce the frequency of clinical exacerbations.

Clinical formulation:

The formulation contains natalizumab, monobasic sodium phosphate monohydrate, dibasic sodium phosphate heptahydrate, sodium chloride, polysorbate 80 and water for injections (WFI). Natalizumab is the active ingredient, sodium phosphate is a

Route of administration:

Intravenous

Proposed use:

To be used for treatment of relapsing forms of multiple sclerosis to reduce the frequency of clinical exacerbations

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission: Toxicology section is included in this review.

Studies not reviewed within this submission: Pharmacology, pharmacodynamics, pharmacokinetics are reviewed in a separate document by Dr. Anne Pilaro, Ph.D.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

Drug activity related to proposed indication:

2.6.2.3 Secondary pharmacodynamics

2.6.2.4 Safety pharmacology

Neurological effects:

Cardiovascular effects:

Pulmonary effects:

Renal effects:

Gastrointestinal effects:

Abuse liability:

Other:

2.6.2.5 Pharmacodynamic drug interactions

2.6.3 PHARMACOLOGY TABULATED SUMMARY

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

2.6.4.2 Methods of Analysis

[see under individual study reviews]

2.6.4.3 Absorption**2.6.4.4 Distribution****2.6.4.5 Metabolism****2.6.4.6 Excretion****2.6.4.7 Pharmacokinetic drug interactions****2.6.4.8 Other Pharmacokinetic Studies****2.6.4.9 Discussion and Conclusions****2.6.4.10 Tables and figures to include comparative TK summary****2.6.5 PHARMACOKINETICS TABULATED SUMMARY****2.6.6 TOXICOLOGY****2.6.6.1 Overall toxicology summary**General toxicology:

Non-clinical single- and repeat dose toxicology studies were performed to evaluate the potential toxic effects of natalizumab in a variety of animal models. The species studied included CD-1 mice, guinea pig, juvenile cynomolgus monkeys, young adult cynomolgus monkeys. Treatment durations were up to 6 months and evaluated doses up to 60 mg/kg in monkeys and 250 mg/kg in mice.

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Additional findings noted with less consistency but also related to natalizumab treatment are: dose dependent increases in reticulocytes and/or NRBCs, increased spleen weight, mild to moderate follicular hypertrophy in spleen and lymph node, and minimal to mild focal leukocyte infiltrates in the liver. Most findings appeared to be reversible after sufficient non-treatment periods. However, in some cases, the findings persisted until the end of the recovery period.

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Genetic toxicology:

Studies to assess genetic toxicity were conducted on natalizumab. The testing included: chromosomal aberration study in human whole blood lymphocytes and mutagenicity in L5178Y K+/- mouse lymphoma cells. No genotoxic effects of natalizumab were observed under the conditions of these studies..

Carcinogenicity:

Tumor promotion potential of natalizumab was evaluated by first identifying human tumor cells lines that bind natalizumab *in vitro*. In a subsequent study the tumor promotion potential of natalizumab was evaluated by assessing tumor growth in athymic nude mice after subcutaneous implantation followed by treatment with natalizumab, vehicle, or Taxol. Natalizumab did not show any tumor promoting potential on two $\alpha 4$ expressing tumors (leukemia, melanoma) in this nude mouse xenograft model under the conditions of the studies.

Reproductive toxicology:

Treatment of pregnant female guinea pigs and cynomolgus monkeys with natalizumab revealed potential reproductive toxicities. In a study of the effects of natalizumab on female guinea pig fertility, a dose of 30 mg/kg administered resulted in a significantly reduced pregnancy rate. This was determined to be a result of implantation failure rather than failure of ovulation. Corpora lutea were identified in ovaries of animals from all groups with no group differences in frequency. However, the number of animals pregnant for the high dose group was roughly one half that of other groups. In that study, a reduced pup survival was noted during the interval from birth to PND 14. No cause of death was identified. In one other study where pregnant guinea pigs were dosed with up to 30 mg/kg through the fertilization and implantation period, only a slightly higher implantation loss was observed. For that study, if early and late resorptions are

combined with fetal death, a slightly higher rate of loss is noted for natalizumab treated females (30 mg/kg).

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One study of pre-and post-natal development in which cynomolgus monkeys were exposed to natalizumab for either GD20-70 or GD 20-term showed similar findings. Infants showed dose dependent increases in WBC, lymphocyte counts and NRBC. In this study significant decreases on platelets were detected in the infants from the high dose group at PND 28 -112. The hematologic effects of natalizumab on the infants appeared to be reversible. Final gross necropsy and histopathology data were not available at the cut-off date for filing this BLA. Those data should be submitted when available.

No effect was noted in male guinea pig fertility at doses up to 30/mg/kg. This dose resulted in serum levels of 1557 ± 382 ug/ml.

Special toxicology: NA

2.6.6.2 Single-dose toxicity

Study title: A single intracardiac dose safety test of AN100226 in the guinea pig

Key study findings:

This study was designed to investigate toxicity of AN100226 after administered via intracardiac injection a single dose in guinea pigs. Deaths occurred in all groups either during or shortly after the injection. These deaths were attributed to the injection procedure. A small weight loss was noted for the male animals in the treated group (3) but was recovered by the terminal sacrifice at day 15. Necropsy revealed apparently enlarged hearts for 5 out of 9 animals from group 3. No histological correlate for the enlargement was identified.

Study no.: ~ Study # 568,

Volume #, and page #:

Conducting laboratory and location: _____

Date of study initiation: 11/24/94**GLP compliance:** YES**QA report:** yes (X) no ()**Drug, lot #, and % purity:** Test article lot # AN-100226-001, control article lot # 3146 (normal saline), histidine vehicle control lot# 8562/86**Methods**

Doses: 0 (saline control), 0 histidine vehicle control), AN10026 126.9 mg/kg

Species/strain: Hartley guinea pigs, 20 each male and female

Number/sex/group or time point (main study): 5/sex/group

Route, formulation, volume, and infusion rate: Intracardiac injection, 1-5 ml/min in a volume of 30 ml/kg.

Satellite groups used for toxicokinetics or recovery: Not done.

Age: approximately 4 weeks

Weight (nonrodents only):

Unique study design or methodology (if any):

Group (treatment)	Dose (mg/kg)	Dose Volume (mL/kg)	Animal Numbers	
			Male	Female
1 (Saline control)	0	30	1001-1005	1501-1505
2 (Histidine vehicle control)	0	30	2001-2005	2501-2505
3 (AN100226)	126.9	30	3001-3005	3501-3505

Observation times and results

Mortality: Monitored twice daily. Deaths were noted in all groups including both formulation control groups. 1 male and 1 female in the saline control group, 2 males in the histidine control group and 2 males in the treated group. Deaths occurred either during dosing or soon thereafter. It was hypothesized that the deaths were due to the procedure (possibly the volume) and not study drug related.

Clinical signs: Animals examined twice daily and 1-2 hours post-dose. All animals showed dyspnea and were prostrate post-dosing. No other clinical signs are reported.

Body weights: At randomization, on day 1 prior to dosing and weekly thereafter. A small drop in weight was recorded for male animals in the treated group compared to saline control animals. The weight was recovered by the scheduled sacrifice on day 15. This drop in weight was not noted for the treated females. When calculated as group mean body weight change, it appears that the weight gain for both males and females in the treated group tended to be less than that of either control group.

Food consumption: Not recorded.

Ophthalmoscopy: Not performed.

EKG: Not performed.

Hematology: Not performed.

Clinical chemistry: Not performed.

Urinalysis: Not performed.

Gross pathology: Animals were sacrificed on day 15 and gross examination was performed. Hearts of all but two animals were removed and preserved in 10% NBF. Control animals # 1003 and 2001 were necropsied prior to the treated animals and showed no treatment related effects. Tissues were discarded and not available for histological examination.

Organ weights (specify organs weighed if not in histopath table):
Visually enlarged hearts were weighed after fixation. Heart weights were not statistically different between groups. However, there appears to be a small trend toward larger hearts in the treated animals (group 3). Evidence for enlargement of the heart was observed for 5 out of 9 treated animals.

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

Peer review: yes (), no (X)

This study was a preliminary toxicity study in which the focus was cardiotoxicity. No other tissues were examined. Twenty-six of 30 hearts were examined microscopically. No histopathological correlate for enlarged hearts was identified.

Toxicokinetics: Not done.

Study title: An acute toxicity study (limit test) of AN100226 in mice via intravenous administration.

Key study findings:

This study was designed to evaluate acute toxicity of trace impurities in the _____ formulations after a single intravenous dose of AN100226. No significant signs of toxicity were observed for this study. The conclusion made by the sponsor is that the two formulations are equivalent in toxicity of impurities. There was a small, transient weight loss after dosing for group 4 that was interpreted as insignificant.

Study no.: — study # 816

Volume #, and page #:

Conducting laboratory and location:

Date of study initiation: 9/27/96

GLP compliance: Yes

QA report: yes (X) no ()

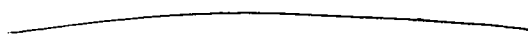
Drug, lot #, and % purity: AN100226-0003 and #AN100226-0004, placebo \ _____
lot # A0003P and placebo \ _____ \ lot # A0006P

Methods

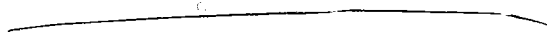
Doses: groups 3 and 4 received 250 mg/kg AN100226
Species/strain: CD-1 mice
Number/sex/group or time point (main study): 4/sex/group
Route, formulation, volume, and infusion rate: IV administration, 50 ml/kg
Satellite groups used for toxicokinetics or recovery:
Age: 6 weeks
Weight (nonrodents only):
Unique study design or methodology (if any):

The four groups are identified as follows: (Taken from the BLA text.)

Group 1

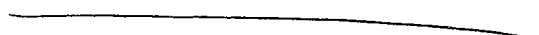


Group 2



Group 3

Group 4



Observation times and results

Mortality: Twice daily.

Clinical signs: Animals were examined 1-3 minutes post-dosing, 1 hour post-dosing, once during the evening of the day of dosing and daily thereafter.

Body weights: Animals were weighed at randomization, on day 1 and weekly thereafter.

- No significant effects on body weight attributable to the test article were noted. However, for the animals in group 4, there was a trend toward less weight gain noted at week 2.

Food consumption: N/A

Ophthalmoscopy: N/A

EKG: N/A

Hematology: Blood samples were collected from all animals at the time of necropsy. For animals that were dosed one day later than the others, blood was collected one day prior to necropsy.

- A significant reduction in WBC was noted for the female animals in groups 3 and 4. This reduction is correlated with a significant reduction in lymphocytes for the same groups. This effect was not observed in the male animals in the same groups.

Clinical chemistry: Blood samples were collected from all animals at the time of necropsy. For animals that were dosed one day later than the others, blood was collected one day prior to necropsy.

- Slightly increased chloride levels are reported for males in groups 3 and 4. The biological significance is not clear.

Urinalysis: N/A

Gross pathology: Animals were sacrificed 14 days after dosing. Gross examination by technicians was performed and tissues showing gross lesions were collected for possible histopathology.

- One female from group3 had a fluid filled ovary.

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

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

Peer review: yes (), no ()

- No histopathology was performed for this study.

Toxicokinetics: N/A

Other: N/A

2.6.6.3 Repeat-dose toxicity

Study title: A 6-month toxicity study with Antegren (AN1000226, natalizumab) via weekly intravenous infusions in juvenile cynomolgus monkeys, with a 17-week recovery period

Key study findings:

The objective of this study was to investigate toxicity of natalizumab when administered weekly for 6-months and to evaluate reversibility of any identified toxicity after a 17-week recovery period.

Weekly intravenous infusions of natalizumab at dose levels of 10, 30 and 60 mg/kg were generally well tolerated by juvenile cynomolgus monkeys. Test-article related increases in total white blood cells (WBC) were observed in all natalizumab treated animals, resulting primarily from increases in lymphocytes (both T and B cells). Test article-related increases in nucleated red blood cells (NRBC) were seen. The morphology of the NRBC was normal, the absolute numbers of circulating NRBC were small (approximately 0.4% of the total RBC count), and other red blood cell parameters

were normal. Increases in NRBC were not seen by the end of the recovery period, indicating reversibility of this finding.

Test article-related gross necropsy findings in animals necropsied 24-hours after the final dose consisted of increased spleen weight. Histologically, the higher spleen weights were correlated with an increased frequency and severity of splenic follicular hypertrophy and hyperplasia (graded as minimal to mild). These findings were not observed after the 17-week recovery period.

In addition to changes in the spleen, there was a dose-related increase in the incidence and severity of leukocytic foci (graded minimal to mild) in the liver of animals administered the 30 mg/kg and 60 mg/kg doses of test article relative to controls. No liver findings were noted for the 17-week recovery animals, indicating reversibility of these findings.

Anti-drug antibodies were found in 10 of 22 animals treated with natalizumab in an inverse relationship to dose. The presence of antibodies was correlated to decreases in total serum complement activity (CH50) and the development of CIC-Raji-reactive immune complexes (IC) in the serum immediately post-infusion. An infusion reaction was seen in one animal in the 60 mg/kg group and appeared to be associated with complement activation and IC formation.

In recovery animals monitored weekly for hematology parameters, serum natalizumab concentration and for $\alpha 4$ saturation levels during Days 177-291, correlations were seen between lymphocyte counts, study drug concentration, and $\alpha 4$ saturation levels. When natalizumab concentrations fell below approximately 1.0 $\mu\text{g/mL}$, $\alpha 4$ saturation levels and lymphocyte counts dropped into the range seen in control animals.

Natalizumab pharmacokinetics following a single infusion on Day 1 showed nonlinear characteristics, with C_{max} and AUC values increasing from 172 $\mu\text{g/mL}$ to 1168 $\mu\text{g/mL}$ for C_{max} and from _____ for AUC with increasing doses from 10 mg/kg to 60 mg/kg groups. Clearance values (CL) showed decreases with increasing dose with associated increases in the serum half-life ($T_{1/2}$). A comparison of juvenile cynomolgus monkey and adult human exposure to natalizumab following a single dose and following 6 months of dosing (26 total doses for monkeys, 6 for humans) was performed. Following a single 60 mg/kg dose, monkey exposure to natalizumab exceeded human exposure by factors of 6.2-fold to 17.1-fold (mean 13.7-fold) for C_{max} and _____ (mean 8.5-fold) for AUC. Following 6 months of dosing, monkey exposure in the 60 mg/kg group exceeded maximum human exposure by factors of 43.7-fold to 94.6-fold (mean 64.8-fold) for AUC.

Study no.: Elan Study # 309-011-00

Volume #, and page #:

Conducting laboratory and location: _____

Date of study initiation: September, 2000

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: natalizumab lot # D23001, control article lot # C85002

Methods

Doses: 10, 30 and 60 mg/kg

Species/strain: cynomolgus monkeys, juvenile

Number/sex/group or time point (main study): 4 groups, 3-5/sex/group

Route, formulation, volume, and infusion rate: IV infusion, 30 minutes for a volume of 10 ml/kg.

Satellite groups used for toxicokinetics or recovery: 2 animals/sex/group, for controls and high dose were designated for recovery evaluation.

Age: 1.5 to 2.5 years. The pre-pubertal status of the animals was determined by radiographs of the epiphyseal plates.

Weight (nonrodents only): 1.7 to 2.9 kg

Unique study design or methodology (if any):

Three animals/sex/group were sacrificed with full necropsy on 177. The remaining 2 animals in the control and high dose group were sacrificed on day 291.

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

Table 1: Group Assignments and Dose Levels

Group Number	No. Males/ Females	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Solution Conc. (mg/mL)	Duration of Infusion (Minutes)	No. Sacrificed:	
						Day 177	Day 291
1	5/5	0 (Vehicle)	10	0	30	3/3	2/2
2	3/3	10	10	1.0	30	3/3	0/0
3	3/3	30	10	3.0	30	3/3	0/0
4	5/5	60	10	6.0	30	3/3	2/2

Observation times and results

Mortality: Twice daily. All animals survived to scheduled sacrifice.

Clinical signs: Twice daily. The animals were observed 2 hours post injection for clinical signs. The sponsor reports no remarkable observations related to test article were noted. However, one female animal in the high dose group was removed from the study due to an adverse reaction on days 64 and 71. No other remarkable clinical signs are reported.

- One female in the high dose group exhibited adverse reactions to the test article approximately 2.5 to 3 hours following infusion on Days 64 and 71. The reactions were characterized by generalized petechial hemorrhages, severe bruising/ecchymotic hemorrhages around the face, bruising of the arms and femoral area, and on Day 71, swelling of the left side of the face. On both occasions, the animal was treated with dexamethasone and benadryl, and the lesions gradually subsided within 3 to 5 days. The animal was given a dose of vehicle on Day 78, and no adverse findings occurred. The animal was no longer dosed with Antegren after Day 71, but remained on study and underwent scheduled blood collections and evaluations. The data subsequently collected suggest that the reactions on Days 64 and 71 may have been complement-mediated responses, which were likely associated with an antibody response against natalizumab.

Body weights: Prior to the first dose and weekly thereafter. No effect on body weight related to the test article was noted.

Food consumption: Food consumption was qualitatively assessed daily. No effects of the test article on food consumption were noted. All animals showed similar weight gain profiles.

Physical exam: Prior to study initiation and 1 hour after dosing on days 1, 22, 85 and 169. For recovery animals, an additional exam was done on day 288. Exams included ophthalmologic exam, ECG, blood pressure, heart rate. No remarkable findings related to the test article are reported. A slight reduction in body temperature was noted for all groups over time. A small increase in heart rate was noted for the 30 mg/kg group, but this finding was not observed in the 60 mg/kg group. The biological significance is unknown and probably not related to test article.

Ophthalmoscopy: Prior to study initiation and 1 hour after dosing on days 1, 22, 85 and 169. For recovery animals, an additional exam was done on day 288. No abnormalities were noted for any group at any time point.

EKG: See above for schedule.

- No test article related effects were reported.

Hematology: Samples for hematology were collected pre-study, and on days 23, 86 and prior to necropsy (day 177 or 291).

- Increases in circulating lymphocytes related to test article administration were noted
- A dose dependent increase in WBC is reported for all treatment groups. Statistical significance is reported for the 60 mg/kg group at all time points measured (day 23, 86, 176, 177, 179, 183, 190, 197, 204, 211, 218, 239). By day 291, WBC levels for the high dose group appear to approach baseline levels.
- A statistically significant increase in reticulocytes for the high dose group over controls is reported for days 23, 86, 176 and day 183. However, this increase for the high dose group is relatively small and not significantly greater than baseline for that group except for day 183. Therefore, the biological significance of these findings is not clear.
- A reduction in neutrophil levels compared to control and group baselines is noted for the 30 and 60 mg/kg treatment groups beginning day 23 and persisting through day 218 for the high dose group and through pre-necropsy testing for the 30 mg/kg group.
- In three recovery animals from the 60 mg/kg treatment group who were tested weekly following the last dose for study drug serum levels and lymphocyte counts, normal lymphocyte counts were associated with the reduction of serum drug levels to below the level of 1 µg/mL

Table 4: Mean Lymphocyte Counts Day 177^a

Group	Treatment	Lymphocyte Count ($10^3/\mu\text{L}$) ^c	# of Animals with Elevated Counts ^b
1	Vehicle	8.04 ± 1.42	0/6
2	10 mg/kg	13.07 ± 12.05	1/6
3	30 mg/kg	18.85 ± 9.46	4/6
4	60 mg/kg	25.09 ± 3.56	6/6

^a24 hours post last dose

^bElevated counts defined as $>13.02 \times 10^3/\mu\text{L}$ (upper end of normal range)

^cValues are the mean ± SD of 6 animals

Clinical chemistry: Samples for hematology were collected pre-study, and on days 23, 86 and prior to necropsy (day 177 or 291).

- A 50% decrease in total bilirubin is reported for the high dose group at day 177 compared to baseline. A similar, but smaller decline is noted for the control group. Biological significance is not clear, but all values are within the reported normal range for cynomolgus monkeys.
- All groups (mean) showed an increase in alkaline phosphatase pre-necropsy compared to baseline. The elevation did not revert to baseline after the recovery period. Because this finding was present for all groups, it does not appear to be related to the test article. Biological significance is not clear.
- A reduction in lactate dehydrogenase was present for all groups at pre-necropsy compared to baseline and did not recover. The severity for all groups was similar. The biological significance is not clear since the finding was present for the control animals as well as the treated groups.
- Reductions in group mean AST is reported for all groups. The values did not reflect recovery at day 291. This finding is noted for all groups and a large degree of variation within groups is present. Values taken prestudy appear for all groups to be somewhat high. Therefore, biological significance is not clear and it does not appear to be related to test article administration.

Urinalysis: Samples collected from cage pan on day 59 and by bladder puncture at necropsy.

- An increase in incidence of increased urine protein findings for treated animals (groups 2-4) relative to control is noted for urinalysis results from day 177 (necropsy). There dose not appear to be a clear dose relationship for this finding and the severity was rated as 1 or 2+. Therefore, the biological significance of these findings is not clear. No kidney gross or microscopic findings are reported that might correlate.

Radiographic images: The pre-pubertal status of the monkeys was established prestudy by radiographic imaging of the epiphyseal growth plates at the knee joint. Images were also taken at weeks 13 and 25 to monitor the prepubertal status of the animals.

Other analyses:

Antegren and anti-antegren serum analysis

Table 7: Pharmacokinetic Parameters of Antegren in Juvenile Cynomolgus Monkeys Following a Single Dose

Group	Treatment	C _{max} (µg/mL)	AUC (µg-hr/mL)	CL (mL/hr/kg)	T _{1/2} (days)
2	10 mg/kg	172 ± 67	11,340 ± 3353	0.95 ± 0.29	2.94 ± 1.03
3	30 mg/kg	492 ± 133	55,264 ± 17,621	0.65 ± 0.40	4.80 ± 1.47
4	60 mg/kg	1168 ± 311	143,057 ± 76,479	0.51 ± 0.21	5.28 ± 2.08

Table 8: AUCs for Antegren in Juvenile Cynomolgus Monkeys Following 6 Months of Weekly Dosing

Group	Treatment	AUC _{all} (ug-hr/mL)
2	10 mg/kg	172,660 ± 143,870
3	30 mg/kg	1,814,932 ± 1,418,766
4	60 mg/kg	6,527,648 ± 1,697,479

Table 6, supplied by the sponsor, summarizes the incidence of anti-drug antibodies from this study:

Table 6: Frequency of Anti-Antegren Antibody Positive Animals

Group	Treatment	Anti-Antegren Positive
1	Vehicle	0/10
2	10 mg/kg	6/6
3	30 mg/kg	3/6
4	60 mg/kg	1/10

Complement analysis:

Treatment of juvenile cynomolgus monkeys with 10 or 30 mg/kg of natalizumab was associated with the reduction of total serum complement hemolytic capacity as measured by CH50, and the formation of IC which had activated complement as measured by the

CIC-Raji assay. Reductions in CH50 were statistically significant at Day 85 and for CIC-Raji were statistically significant at Days 85 and 176. Most animals (9/10) in the 60 mg/kg group and half of the animals in the 30 mg/kg group (3/6) did not show these effects, resulting in an inverse dose-response, associated with a lack of measurable anti-natalizumab antibodies in the circulation of these animals. This lack of antibodies may be the result of tolerization to natalizumab due to the frequent high dose treatment, or to the masking of antibodies that are produced by immediate one-to-one binding of the antibody to the excess of natalizumab with subsequent clearance and/or inability to be detected in the antibody assay. Only one animal experienced adverse reactions during the course of the study which appeared to be associated with this IC formation and complement activation.

Natalizumab receptor saturation analysis:

Eight animals, 4 from the control group and 4 from the 60 mg/kg group, were evaluated at 7 time points during a 17-week, non-dosing recovery period for the extent of $\alpha 4$ integrin receptor saturation of their peripheral blood mononuclear cells (PBMC). Samples were taken 30 minutes post-dose on Day 176 (last dose) and on Days 177, 183, 190, 238, 266, and 291 (day of necropsy). The animal in the 60 mg/kg group, removed from dosing after Day 71 due to adverse events, had no measurable study drug in circulation after Day 85. Therefore, this animal was treated as a control for the purposes of this evaluation. Thus, 5 control animals and 3 test animals were available for the saturation analysis.

The mean percentage of PBMC saturation in control animals (resulting from non-specific assay factors) was 7.42 ± 6.85 . Serum natalizumab levels exceeding $1.0 \mu\text{g/mL}$ were associated with an increase in PBMC saturation above 14.27%, the mean saturation + 1 SD for controls. These increases in saturation were associated with increases in lymphocyte counts to greater than the normal range ($3.11\text{-}13.02 \times 10^3/\mu\text{L}$). These data are consistent with the direct correlation of natalizumab levels and lymphocyte counts which show increased lymphocyte counts when natalizumab levels are above $1.0 \mu\text{g/mL}$.

Flow cytometry (immune cell phenotyping):

**Appears This Way
On Original**

Table 11: Mean WBC, CD3+ and CD20+ Cell Counts: Day 135^a

Group	Treatment	WBC (10 ³ /μL)	CD3+ Count (10 ³ /μL)	CD20+ Count (10 ³ /μL)
1	Vehicle	15.80 ± 3.32	6.20 ± 1.68	2.38 ± 1.23
2	10 mg/kg	21.57 ± 15.66	8.78 ± 6.18	6.02 ± 6.67
3	30 mg/kg	27.05 ± 9.85	12.51 ± 6.44 ^b	8.14 ± 2.37 ^d
4	60 mg/kg	34.23 ± 7.68 ^c	15.21 ± 5.83 ^c	11.01 ± 3.06 ^e

^a 24 hours post-dose 20

^b p = ≤ 0.01

^c p = ≤ 0.001

^d p = ≤ 0.001

^e p = ≤ 0.0001

Gross pathology: No remarkable gross findings at necropsy.

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

Adrenals	Brain
Epididymides	Heart
Kidneys	Liver
Lungs	Ovaries
Pituitary (post fixation)	Spleen
Testes	Thymus
Thyroid with parathyroids	

- A dose dependent increase in spleen weight for animals sacrificed on SD177 was observed for all treated groups relative to control (5.324 gm for group 4 versus 2.931 for controls). The increase in weight was statistically significant for groups 3 and 4. This difference in spleen weigh was not noted for the animals sacrificed on SD291.
- Thyroid gland weight for group 3 was significantly increased relative to control and other treated groups. Since this increase was not noted for group 4, the biological significance is not clear and may have been incidental. The mean body weight and mean brain weight for that group is also slightly greater than the other groups, though not statistically significant.
- A reduction in mean testicular weight and mean epididymal weight is noted for the treated groups relative to control. These weight differences did not reach statistical significance. The animals used for this study were juvenile and may not have been fully mature. This difference in mean testicular and epididymal weights was also noted for the animals sacrificed in SD 291. Only two per group. For testes: 1.713 grams versus 0.962 grams for group 4; epididymes: 1.051 grams for control versus 0.600 grams for group 4.

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

Peer review: yes (), no ()

The following tissues were collected and fixed:

Tissues Collected	
Cardiovascular	Urogenital
Aorta	Kidneys
Heart	Urinary Bladder
Digestive	Testes
Salivary Gland (mandibular)	Epididymis
Tongue	Prostate
Esophagus	Seminal Vesicles
Stomach	Ovaries
Small Intestine	Uterus
Duodenum	Cervix
Jejunum	Vagina
Ileum	Endocrine
Large Intestine	Adrenals
Cecum	Pituitary
Colon	Thyroid/Parathyroids*
Rectum	Skin/Musculoskeletal
Pancreas	Skin/Mammary Gland
Liver	Bone (femoral head)
Gallbladder	Bone (7th rib)
Respiratory	Skeletal Muscle (thigh)
Trachea	Nervous/Special Sense
Lung	Eyes with optic nerve
Lymphoid/Hematopoietic	Sciatic Nerve
Bone Marrow (sternum)	Brain
Thymus	Spinal Cord (thoracic)
Spleen	Other
Lymph Nodes	Animal Number Tattoo
Mandibular	Gross Lesions
Mesenteric	Injection Site(s)**

* The occasional absence of the parathyroid gland from the routine tissue section did not require a recut of the section.

- “enhanced follicular pattern” (follicular hypertrophy, rated minimal to mild) and nodules in the spleens of animals in treated group sacrificed on SD177. These findings correlate with the increase spleen weight observed for groups 3 and 4.
- A dose related increase in incidence and severity of leukocyte foci observed in the livers (rated minimal to mild) for groups 3 and 4.
- Thyroid findings showed a small increase for the groups receiving natalizumab. These findings included mononuclear cell infiltrates, follicular epithelial cell degeneration generally rated mild. A clear dose relationship was not seen.
- An increase in kidney findings is noted for the treated groups relative to control. Minimal to mild mononuclear cell infiltrate, minimal fibrosis, and minimal

glomerulosclerosis are reported for group 3. These findings were not noted for group 4 so the biological significance is not clear and may have been due to background, pre-existing conditions.

- Immature testes and epididymes are noted. Incomplete spermatogenesis is noted for animals in groups 2-4.

Toxicokinetics:

Samples for pharmacokinetics and other special analyses were collected by the schedule presented in the following table provided by the sponsor:

Table 2: Blood Sampling for Clinical Pathology and Special Assays

Treatment Day	Dose	Clinical Chemistry ^a	Hematology	PK/Ab ^b	Complement Analysis ^c	Receptor Saturation	Immuno-phenotyping ^d
-7		X	X		X		
1	X			X			
2				X			
4				X			
8	X			X			
15	X						
22	X			X			
23		X	X				
29	X						
36	X						
43	X						
50	X			X			
57	X						
64	X						
71	X						
78	X						
85	X			X	X		
86		X	X				
92	X						
99	X						
106	X						
113	X						
120	X						
127	X						
134	X						
135			X ^e				X
141	X						
148	X						
155	X						
162	X						
169	X						
176	X		X	X	X	X	
177		X	X	X		X	
179			X	X			
183			X	X		X	
187				X			
190			X	X		X	
197			X	X			

Treatment Day	Dose	Clinical Chemistry ^a	Hematology	PK/Ab ^b	Complement Analysis ^c	Receptor Saturation	Immuno-phenotyping ^d
204			X	X			
211			X	X			
218			X	X			
225				X			
232				X			
238						X	
239			X	X			
246				X			
253				X			
260			X	X			
266						X	
267				X			
274				X			
281			X	X			
288				X			
291		X	X	X		X	

^aConsisting of serum chemistry and coagulation

^bPK/Ab (pharmacokinetic/antibody) samples on dosing days consist of one sample within 1 hour pre-dose and one sample 0.5 hour post-dose

^cConsisting of CH50, C1q and CIC-Raji assays, on dosing days samples are taken 0.5 hours post-dose

^dConsisting of FACS analysis for CD20, CD4, CD3, CD8 and CD34

^eFollowing overnight shipment of the whole blood samples to , _____

TK results: See above.

Other: NA

Study title: A 6- month weekly intravenous infusion toxicity study with Antegren (natalizumab) in cynomolgus monkeys with a 6-week recovery period.

Key study findings:

The purpose of this study was to evaluate the potential toxicity of natalizumab administered by IV infusion weekly for 26 weeks followed by a 6-week recovery period. An additional group of animals were monitored for an additional 6-week recovery period. Evaluations included standard clinical observations, clinical pathology and necropsies, with full histopathology. Additional blood samples were collected to evaluate natalizumab concentration, anti-drug antibody levels, natalizumab receptor saturation levels, specific complement parameters, and CD34+ stem cells in peripheral blood. Supplementary evaluations were conducted on 3 animals that exhibited transient treatment-related clinical signs, which were suggestive of a hypersensitivity reaction to a foreign protein.

Intravenous administration of natalizumab at dose levels of 3, 10, 30, or 60 mg/kg was well tolerated. Infusion-related incidences were observed in three animals from different dose groups, which appeared to be due to a hypersensitivity response to the study drug. Test-article-related changes in clinical pathology included dose-related increases in circulating lymphocytes, monocytes, and eosinophils. These findings were

attributed to the known pharmacologic activity of natalizumab. Microscopic findings noted included minimal to mild lymphoplasmacytic inflammation of the intestinal mucosa of the large intestine, which occurred across all treated groups. In addition, minimal to moderate glomerulonephritis was observed in the kidneys of 4 animals at Week 26 and glomerulosclerosis (chronic manifestation of glomerulonephritis) was observed in 1 animal at the end of the recovery period. The glomerulonephritis findings are believed to be the result of immune complex deposition or other antibody-dependent phenomena, which is likely related to the immunogenicity of the test article in the monkey.

Study no.: Elan study # 723-013-98

Volume #, and page #:

Conducting laboratory and location: _____

Date of study initiation: 5/23/98

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: natalizumab lot#CO237A
Control lot # DO157

Methods

Doses: 0, 3, 10, 30, 60 mg/kg

Species/strain: cynomolgus monkeys

Number/sex/group or time point (main study): 42 monkeys total were used

Main study: 3/sex/group

Recovery: additional 2/sex/group for groups 1, 4 and 5

Route, formulation, volume, and infusion rate: Test article was administered weekly by IV infusion in a volume of 10 ml/kg (20 ml/kg for group 5) for an infusion duration of 30 minutes (60 minutes for group 5).

Satellite groups used for toxicokinetics or recovery: Recovery (see above) 2/sex/group for groups 1, 4, and 5

Age: Pre-pubertal to young adults

Weight (nonrodents only): 1.6 to 4.1 kg

Unique study design or methodology (if any): The study design is illustrated in the table below, provided by the sponsor.

Animals were sacrificed at week 26 (3/sex/group) and week 32 (recovery, 2/sex/group 1, 4 and 5)

Group No.	Number of Males/Females	Dose Level (mg/kg)	Dose Volume (mL/kg)	Duration of Infusion (Minutes)	Dose Solution Conc. (mg/mL)	Number Sacrificed in:	
						Week 26	Week 32
1	5/5	0 (vehicle)	10	30	0	3/3	2/2
2	3/3	3	10	30	0.3	3/3	
3	3/3	10	10	30	1.0	3/3*	
4	5/5	30	10	30	3.0	3/3	2/2
5	5/5	60	20	60	3.0	3/3	2/2

*Note one female (F6621F) was removed from the study following two episodes of collapse during dosing on Days 43 and 50.

Observation times and results

Mortality: Cageside observations were recorded at least twice daily, am and pm. Additional observations were recorded on dosing days 1-3 hours post-dosing.

- All animals survived to scheduled sacrifice.
- One female group 3 collapsed after dosing on day 43 and day 50. The animal recovered after stopping the dose. This animal was dosed with placebo on day 57 as part of the investigation in to the cause of the collapse. No further dosing was performed on this animal.

Physical exams: Physical exams were performed on all animals prior to study initiation, 1 hour post-dosing on day 1, then during week 4, 13 and 25. Exam included body weight, blood pressure, heart rate, respiration, EKG.

Clinical signs: As above. Twice daily and 1-3 hours after each dose administration.

- Two animals (one from group 5 and one from group 2) developed a red/ purple face after dosing on day 134, 148, 155. The low dose animals also developed drowsiness post -dosing on days 148 and 155. The sponsor suggests that this effect may be indicative of a hypersensitivity reaction. But offers no direct data to support this hypothesis.

Body weights: See above.

- No test article related effects noted.

Food consumption: Evaluated qualitatively daily beginning 7 days prior to study initiation.

- No test article related effects were noted.

Ophthalmoscopy: Once prior to study initiation, then during week 4, 13, 25.

- No abnormalities reported.

EKG: See above.

- No abnormal findings related to the test article reported.

Clinical pathology: Blood samples were collected for testing of hematology, serum chemistry and coagulation parameters twice pre-study, 24 hours after dosing on day one, 4, 13, and prior to necropsy on week 26 and 32. On day 177 (26 week) samples were taken only for animals scheduled for necropsy that day.

Hematology: Testing for hematology parameters listed in the table below, supplied by the sponsor:

Red blood cell (RBC) counts	Mean cell hemoglobin (MCH)
White blood cells (WBCs) (total and differential*)	Mean corpuscular volume (MCV)
Hemoglobin concentration	Mean corpuscular hemoglobin concentration (MCHC)
Hematocrit	Platelet counts
Reticulocyte counts	Abnormal blood cell morphology

*Includes relative percentage and absolute counts for neutrophils, band cells, lymphocytes, monocytes, eosinophils, basophils, and immature cells

Additional samples for hematology were collected 1 hour pre-dose, 0.5 hours post dose on days 176, 179, 183, 190, 196, 203, 211 (recovery animals).

- Dose related elevation in groups 4 and 5 for WBC counts were noted for all time points from SD23 to SD177. These elevations were primarily due to elevations in circulating lymphocytes. Similar elevations in lymphocytes were recorded for group 3 but achieved statistical significance only on SD 23.
- An apparent recovery effect was noted for the WBC and lymphocyte parameters with a general decrease over time after SD177. (The elevated lymphocyte counts persisted for one animal in each of groups 4 and 5 through the recovery necropsy.
- Increases in monocytes and eosinophil counts were also elevated in treatment groups relative to control. (Statistical significance was noted for groups 3, 4 and 5 at various time points during the study. For the recovery animals, these values were persistently high the recovery period but were not statistically significant except for monocytes counts on SD179 for group 4.
- A 2 to 3 fold increase in absolute neutrophils counts relative to baseline was reported on SD176. However, the increase was apparent for all groups. Therefore, it was not considered related to the test article.
- NRBCs not specifically evaluated for this study.
- One group 4 animal showed a reduction in platelets on post-dose sampling on 3 occasions (SD23, 51 and 86). The platelet levels recovered within one week. No other animals showed this finding.
- No effects on coagulation parameters were noted during the study.

Clinical chemistry: Samples were tested for serum chemistry parameters listed in the table below, supplied by the sponsor:

Sodium	Calcium
Potassium	Phosphorus
Chloride	Glucose
Carbon dioxide (CO ₂)	Urea nitrogen (BUN)
Total bilirubin	Creatinine
Direct bilirubin	Uric acid
Indirect bilirubin	Total protein
Alkaline phosphatase (AP)	Albumin
Lactate dehydrogenase (LDH)	Globulin
Aspartate aminotransferase (AST)	Albumin/globulin ratio
Alanine aminotransferase (ALT)	Cholesterol
Gamma-glutamyltransferase (GGT)	Triglycerides

- No test article related effects were reported on serum chemistry. Sporadic group differences in various parameters were noted. However, these findings did not show a relationship to dose or duration of dosing.

Coagulation parameters:

Activated partial thromboplastin time (APTT)
Prothrombin time (PT)
Fibrinogen*

*Determined only on samples taken prior to necropsy in Weeks 28 and 32.

Urinalysis: Urine samples were collected at necropsy and the following analyses were conducted (Table supplied by the sponsor):

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

* Microscopic examination was not performed when the specimen was clear and negative for protein, blood, nitrite and leukocyte esterase

- Two animals with mild to moderate glomerulonephritis had 3+ occult blood and 3+ protein in the urine samples collected at necropsy in Week 26. Although glomerulonephritis can cause proteinuria, other animals, including animals in the control group, that did not have lesions of glomerulonephritis, had similar levels of blood and protein in the urine sample at Week 26. The urine samples were collected by bladder puncture and may have been contaminated with blood. Therefore, the relationship of the alterations in the urinalysis was of uncertain relationship to the presence of glomerulonephritis.

Gross pathology: Animals were sacrificed 24 hours after the last dose during week 26 (main study) and 32 (recovery)

A complete gross necropsy was conducted by qualified personnel on all animals sacrificed during the study. The necropsy included examination of:

- Carcass and muscular/skeletal system
- All external surfaces and orifices
- Cranial cavity and external surface of the brain
- Neck with associated organs and tissues
- Thoracic, abdominal and pelvic cavities with their associated organs and tissues

Gross findings 26 week necropsy:

- In one Group 4 animal there was red foci and paleness of the kidneys, diffuse reddening of the left and right diaphragmatic lung lobes, and red foci in the thymus. These lesions correlated histologically to membranous glomerulonephritis and intra-tubular hemorrhage in the kidneys, intra-alveolar hemorrhage in the lung, and interstitial hemorrhage in the thymus.

Glomerulonephritis is almost always an immune-mediated condition, and there is the possibility that it was indirectly related to test article administration.

- In four animals (1 per group, Groups 2-5) the spleen was minimally enlarged. This finding correlated histologically in two of the animals with minimal to mild follicular hyperplasia. In the third animal it correlated with congestion of the red pulp, and in the fourth animal there was no histologic correlate.

Gross findings 32 week necropsy:

- No findings related to test article administration were reported. Any lesions noted, were ascribed to background pre-existing conditions since they were sporadic and did not show any dose relationship to the study drug.

Organ weights (specify organs weighed if not in histopath table): Selected organs were removed at necropsy and organ weights recorded as illustrated in the table below, supplied by the sponsor.

Adrenals	Brain
Epididymides	Heart
Kidneys	Liver
Lungs	Ovaries
Pituitary (post fixation)	Spleen
Testes	Thymus
Thyroid with parathyroids	

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

- The mean spleen weights for group 5 were relative to control and other groups (not statistically significant). This finding is consistent with results from other studies. One animal per group 2-5 showed enlargement of the spleen that was correlated with mild follicular enlargement. These findings were not considered related to the test article by the sponsor but since they were observed in another toxicology study using natalizumab, and could be related to the known biological activity of the drug, a relationship should not be ruled out.

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

Peer review: yes (), no ()

Cardiovascular	Urogenital
Aorta	Kidneys
Heart	Urinary Bladder
Digestive	Testes
Salivary Gland (mandibular)	Epididymis
Tongue	Prostate
Esophagus	Seminal Vesicles
Stomach	Ovaries
Small Intestine	Uterus
Duodenum	Cervix
Jejunum	Vagina
Ileum	Endocrine
Large Intestine	Adrenals
Cecum	Pituitary
Colon	Thyroid/Parathyroids
Rectum	Skin/Musculoskeletal
Pancreas	Skin/Mammary Gland
Liver	Bone (femoral head)
Gallbladder	Bone (7th rib)
Respiratory	Skeletal Muscle (thigh)
Trachea	Nervous/Special Sense
Lung	Eyes with optic nerve
Lymphoid/Hematopoietic	Sciatic Nerve
Bone Marrow (sternum)	Brain
Thymus	Spinal Cord (thoracic)
Spleen	Other
Lymph Nodes	Animal Number Tattoo
Mandibular	Gross Lesions
Mesenteric	Injection Site(s)*

*Cephalic and saphenous veins

- A finding of membranous glomerulonephritis was noted for a total of 4 animals from groups 3 and 4. This condition was not seen in any group 5 animals so a relationship to the study drug is doubtful. However, all 4 animals tested positive for anti-drug antibodies and evidence of complement activation. Therefore the kidney findings may have been a result of immune-mediated processes and related indirectly to the study drug. Glomerulonephritis was not noted in any animal after the recovery period. However, one animal in group 5 showed evidence of glomerulosclerosis, a condition that may be representative of chronic glomerulonephritis.
- A finding of “lymphoplasmacytic inflammation” in the mucosa of the large bowel (cecum, colon, rectum) was noted for all treated groups relative to control. The severity/incidence was given as slightly increased for groups 4 and 5. The lesion is characterized as “increased numbers of lymphocytes and plasma cells within the lamina propria, with occasional crypt abscesses (crypts that were dilated and filled with mucus and neutrophils)”. Although it is possible that these animals had low level infections, the pattern of incidence and severity indicates that there may be a study drug effect. These findings were noted only in groups 4 and 5 for the 32 week sacrifice and rated as minimal. Although there was not an apparent dose relationship in severity and incidence between groups 4 and 5, the fact that there was no finding in the control animals and the consistency with the findings

at the 26 week sacrifice suggests that this effect is related to the study drug. The effect appears to show reversibility.

- One animal from group 4 showed hypertrophy of the smooth muscle of the duodenum, jejunum and colon at the recovery sacrifice. The etiology was not determined. Relationship to the study drug is doubtful since only one animal showed the finding.
- Test article effects on the spleen were reported for all treated groups relative to control: Six animals treated with natalizumab had findings of minimal to mild follicular hyperplasia in the spleen (2, 1, 1, and 2 animals in Groups 2, 3, 4, and 5, respectively). One animal in Group 4 had red pulp leukocytosis. No animals in the control group had these findings. This can be a normal finding in cynomolgus monkeys, but there appears to be a drug-related increase in incidence, which does not appear to be dose-related. For this study, the sponsor considers the finding to be not related to the test article. However, because splenic enlargement correlated histologically with follicular hypertrophy is observed consistently with natalizumab exposure, this finding is most likely study drug related.

Complement analysis:

Blood samples were collected from all animals, on Day 176 (0.5 hr post-dose), for analysis of complement parameters [CH50, complement split products (C3a and C4a), and immune complexes]. For a sub-population of monkeys, there was an apparent correlation between high anti-drug antibody responses and complement activation, as measured by CH50, complement split products (C3a and C4a), and immune complexes. These results suggest that the monkeys responded to natalizumab, however, the response was not consistent from animal to animal or dose to dose. Similar findings were seen in the monkey that was removed from study in Week 8 due to two shock-like episodes.

The data indicate that treatment with natalizumab may be related to increases in circulating immune complexes and complement activation. These findings were apparently not dose related. There were increased incidences in the lower dose groups relative to the high dose groups. A rough correlation of these findings is observed with the incidence of anti-drug antibodies. The sponsor suggests that the circulating immune complexes and complement activation are due to the antibody response to natalizumab rather than a direct effect of the drug.

Flow cytometry:

Blood samples were collected at necropsy and analyzed for percent CD34+ cells by flow cytometry. The samples from SD177 showed no increase in CD34+ levels. Samples were not analyzed from the recovery animals. Analysis of CD34+ levels from bone marrow samples was not considered interpretable since the quality of the samples was poor.

Toxicokinetics:

This TK study was performed to compare the estimated natalizumab exposure of the monkeys in the 6-month toxicology study (study number 723-013-98) with that anticipated in humans treated with 6 mg/kg/month over a 6-month period. The intention

was to assure that the monkeys in the 6-month toxicity study were exposed to adequate (at least 5-10 fold higher) levels of study drug compared to the predicted exposure in humans receiving 6 mg/kg/month, as measured by C_{max}, and total AUC. Exposure data was not available at the time of this report for patients treated at the 6 mg/kg dose level. Therefore, pharmacokinetic data from humans exposed to a single 3 mg/kg dose were used to estimate exposure to a 6 mg/kg/month dose.

The modeling and calculations were performed under the assumption of the worst case scenario, i.e. considering the lowest natalizumab concentrations in the monkey and the highest natalizumab concentrations in humans. This was to assure that this 6-month toxicity study in monkeys represents more than adequate (about 10-fold higher) exposure to natalizumab, as measured by comparing human and monkey maximum serum concentrations and total area under the curve (AUC). The analysis was complicated by the fact that most of the monkeys developed an anti-drug antibody response during the study, which resulted in non-standard pharmacokinetics, and considerable individual-to-individual variation.

All monkeys in Groups 2 and 3 responded with a measurable anti-drug antibody response in Weeks 4, 13 and 26. This response increased throughout the study with the magnitude of the antibody response increasing with dose from 3 mg/kg to 10 mg/kg. Six of 10 monkeys in Group 4 and 4 out of 10 monkeys in Group 5 had measurable anti-drug antibodies at both Weeks 13 and 26. The remaining animals were below the limit of quantitation at both time points. It can not be determined from these data whether the monkeys with no measurable response were non-responsive, or whether the serum antibodies could not be detected due to the presence of natalizumab in the sample (or insufficient sensitivity of the assay). The estimated exposures in most of the animals in the 30 and 60 mg/kg dose groups were at least 10-fold higher than the maximum predicted human exposure at 6 mg/kg/month for 6 months (total dose of 36 mg/kg/6 months), as measured by AUC ratios and ratios. For the 60 mg/kg dose group, individual monkey exposures exceeded the predicted human exposure by factors of 4.4-fold to 23.2-fold in terms of C_{max}, and by factors of 9.5-fold to 87.6-fold for AUC.

Blood samples for detection of drug levels and presence of anti-drug antibodies (ADA) were collected according to the schedule illustrated in the table below, supplied by the sponsor.

TK data indicates that the exposure achieved in the monkeys provided a suitable excess exposure over estimated projected human exposure at the projected recommended dose of 6 mg/kg/month. The fixed recommended dose actually being used for the ongoing Phase 3 clinical study is 300 mg/week or approximately 4.2 mg/kg/week. So the estimate of human exposure made at the time this study was performed was a significant under estimate.

The schedule of sampling for study drug levels and anti-drug antibodies is illustrated below in the table supplied by the sponsor:

Day	During Week	# of Samples Collected	Time point	PK	# of Samples Analyzed	Ab	# of Samples Analyzed
1	1	42	pre-dose, 0.5 hr post-infusion (\pm 0.1 hr)	X	32	X	32
		42		X	32	-	-
2	1	42	1 day post-infusion (\pm 1 hr)	X	32	-	-
4	1	42	3 days post-infusion (\pm 1 hr)	X	32	-	-
8	2	42	Within 1 hour pre-dose (trough) 0.5 hr post-infusion (\pm 0.1 hr)	X	32	X	32
		42		X	32	-	-
22	4	42	Within 1 hour pre-dose (trough) 0.5 hr post-infusion (\pm 0.1 hr)	X	32	X	32
		42		X	32	-	-
85 ^a	13	42	Within 1 hour pre-dose (trough) 0.5 hr post-infusion (\pm 0.1 hr)	X	42	X	42
		42		X	42	X	42
176	26	42	Within 1 hour pre-dose (trough) 0.5 hr post-infusion (\pm 0.1 hr))	X	32	X	32
		42		X	32	-	-
177	26	42	1-day post-infusion (\pm 1 hr) (Necropsy day – terminal sacrifice)	X	8	X	8
Recovery Animals							
179	26	12	3 days post-infusion (\pm 1 hr)	X	8	X	8
183	27	12	7 days post-infusion (\pm 1 hr)	X	8	X	8
190	28	12	14 days post-infusion (\pm 1 hr)	X	8	X	8
196	29	12	20 days post-infusion (\pm 1 hr)	X	8	X	8
203	30	12	27 days post-infusion (\pm 1 hr)	X	2 ^b	X	8
211	31	12	35 days post-infusion (\pm 1 hr)	X	2	X	8
Pre-necropsy	32	12	43, 42, 41, or 40 days post-infusion (\pm 1 hr)	X	2	X	8

PK = Antegren concentration

Ab = anti-Antegren antibody levels

X = Sample analyzed

- = Sample not analyzed

a = Includes analysis of Vehicle (Group 1) samples

b = Samples were not analyzed, at or after this analysis, if results were below the limit of quantitation twice, consecutively.

The Sponsor analyzed Antegren concentration and determined anti-Antegren antibodies in the number of samples specified in the previous table. For the animals in the recovery group, Antegren concentration and antibody levels were evaluated on all animals at the timepoints beginning on Day 177. Analysis of each subsequent timepoint was performed until two consecutive tests showed no Antegren in the samples, at which time testing for Antegren concentration was discontinued. Antibody levels were determined for all Antegren-treated animals in the recovery group.

Saturation of receptors on monkey peripheral blood monocytes:

Whole blood was collected from recovery animals on Days 176 (0.5 hr post-dose), 177, 183, 190, and on the day of necropsy in Week 32. These collection times corresponded to 0.5 hr, and 1, 7, 14, and 43 days after the last injection on Day 176, respectively. Binding of natalizumab was measured by flow cytometry using _____ methods. Groups 4 and 5 showed 72-107% saturation of

CD49d receptors at 0.5 hours after the last infusion of natalizumab. Saturation decreased to less than 5% by day 183 for most animals in groups 4 and 5 (approximately one week after the final dose on SD 176). Two animals (one each from groups 4 and 5) showed continued high saturation (60%+) at the time of sacrifice of the recovery animals in week 32.

Study title: A 14-day repeat dose intravenous safety test of AN100226 in the albino mouse

Key study findings:

The purpose of this study was to evaluate the potential toxicity of natalizumab when administered IV to CD-1 mice daily for 14 days. The doses used were estimated to be up to 30 times the expected human dose. No deaths occurred during the study. Repeated intravenous administration of natalizumab for 14 days did not affect body weight or food consumption of the mice. Furthermore, no adverse clinical signs were evident for the animals throughout the 14-day treatment. At the end of the study (day 14) measurable concentrations of natalizumab were detected in all groups. Concentration increased with dose and no difference was apparent between males and females at each dose. After 14 days on study there was no measurable antigenic response to natalizumab. No effect on organ weights was observed. No findings were observed for clinical pathology or urinalysis parameters. No difference was noted between the saline control or histidine vehicle control groups. Gross lesions occurred sporadically and were not attributed to the test article. No significant microscopic findings attributable to study drug administration were noted with the exception of subacute inflammation at the injection site in two of six high dose females. This finding was not seen in the control mice or the males from the same treatment group.

Repeat intravenous dosing with natalizumab at doses up to 30.1 mg/kg/day was not associated with observable systemic toxicity. The localized inflammation which was seen microscopically was attributed to leakage of test article or histidine vehicle at the injection site. Based on the small group incidence (two of six) and gender specificity of this lesion, the biologic significance of this finding is questionable.

Study no.: — study # 567/ Biogen study #al105

Volume #, and page #:

Conducting laboratory and location: _____

Date of study initiation: 11/23/1994

GLP compliance: YES

QA report: yes (X) no ()

Drug, lot #, and % purity: natalizumab lot# AN-100226-0001

Placebo lot # 8562/86 (50mM L-histidine, 150 mM NaCl,

pH 6.0

Methods

Doses: 0, 3.0, 9.9 or 30.1 mg/kg

Species/strain: CD-1 mice

Number/sex/group or time point (main study): 6/sex/group

Route, formulation, volume, and infusion rate: IV, 10 ml/kg

Satellite groups used for toxicokinetics or recovery:

Age: approximately 6 weeks at initiation of the study.

Weight (nonrodents only):

Unique study design or methodology (if any):

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

Group (treatment)	Dose (mg/kg/d)	Dose Volume (mL/kg/d)	Animal Numbers	
			Male	Female
1 (Saline control)	0	10	1001-1006	1501-1506
2 (Histidine vehicle control)	0	10	2001-2006	2501-2506
3 (AN100226)	3.0	10	3001-3006	3501-3506
4 (AN100226)	9.9	10	4001-4006	4501-4506
5 (AN100226)	30.1	10	5001-5006	5501-5506

Observation times and results

Mortality: Observations made twice daily.

All animals survived to scheduled necropsy with the exception one control animal.

Clinical signs: No clinical signs attributable to test article administration were noted during this study.

Body weights: Prior to study initiation and daily thereafter. On SD1, three animals were replaced (two in the histidine control group and one in the high dose group) due to markedly low body weight. All animals gained small amounts of weight over the course of the study. A slightly lower weight gain (not statistically significant) may have been present for the high dose females.

Food consumption: Measured at initiation of dosing and weekly thereafter.

- There was no clear study drug effect on food consumption. All groups showed a slight decrease in food consumption over the course of the study.

Ophthalmoscopy: Not done.

EKG:

Hematology: Blood samples collected at necropsy. Samples were taken from three animals per group. Due to loss of samples, an n of only two is available for many parameters. Therefore, statistical analysis was not possible for most test results.

- No significant differences were observed in hematology results between the histidine vehicle and saline vehicle controls. With only 2 or three samples per groups statistical analysis is of limited value.
- Total WBC counts were slightly lower for groups 4 and 5 relative to the saline control group but were comparable to values for the histidine control group.

(WBC values for the saline control were higher than those for the histidine control group) lymphocyte values were highly variable among groups.

- Segmented neutrophils levels were slightly decreased in males in groups 4 and 5 relative to control but this trend was not apparent for the females of the same groups.
- A slight drop in hemoglobin measures was noted for groups 4 and 5 relative to control.

Clinical chemistry: Blood sample collected at necropsy.

- Reduction (slight) in BUN appears to follow a dose-related pattern. But due to variability in the values the differences were not significant. (60 for group 2 versus 29 for groups 4 and 5, males) This finding was not seen for the females.

Toxicokinetics:

Detectable levels of study drug were found in samples taken at necropsy. Concentrations increase with dose. In this study, no measurable anti-drug antibodies were noted for any group.

Urinalysis: results of urinalysis did not reflect any effects of the study drug.

Gross pathology: Animals were euthanized on SD15.

- No gross lesions related to study drug were noted.

Organ weights (specify organs weighed if not in histopath table): adrenals kidneys spleen brain liver thymus testes (with epididymides) ovaries

- No statistically significant effects of study drug are apparent.

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

Peer review: yes (), no ()

Adrenals, bone/marrow (sternum), brain (three levels), colon, epididymides, Esophagus, eyes, gallbladder, heart, injection site, kidneys, liver, lymph nodes, (mandibular, mammary gland, optic nerves, lungs, and mesenteric), ovaries, pancreas, Pituitary, prostate, salivary glands, significant adverse lesions, small intestine, spinal cord, spleen, stomach, testes, thymus, thyroid lobes (and parathyroids), trachea, urinary bladder, uterus

Histopathology was performed on tissues listed above for only the control and high dose groups.

No study drug related effects were noted. Inflammation (characterized as “subacute”) at the injection site was noted in two of six animals in the high dose group. The sponsor suggests that this effect may have been a result of leakage of the drug during the infusion. This is the most reasonable explanation since the finding is limited to only 2 of 6 animals of one sex.

Other findings appear to be incidental and not dose related.

Study title: A 28-day intravenous toxicity study of AN100226 in cynomolgus monkeys

Key study findings:

The purpose of this study was to evaluate the toxicity of natalizumab when administered intravenously every other day for 28 days to cynomolgus monkeys. The doses evaluated were 0.3, 3, or 30 mg/kg. Two control groups received either saline control or histidine-containing vehicle control. The day after the final dose was administered, 4 animals/sex/group were sacrificed and the remaining monkeys, 1/sex/dose group, were maintained for observation for an additional 4 weeks to assess recovery potential for any identified toxicities. The doses and treatment interval were based on previous pharmacokinetic data. Physical examinations, clinical observations, body weight, food consumption, rectal temperatures, ophthalmoscopy and laboratory investigations were carried out at intervals prior to and during the experimental period. Blood samples were taken before and during treatment for serum antibody determinations and immune function assays (lymphocyte blastogenesis, natural killer (NK) cell activity and cell surface marker profile).

At termination, a full macroscopic necropsy including organ weights was performed on all animals. Representative sections of tissues preserved at necropsy were processed and evaluated microscopically. In addition, immunohistochemistry was performed on the kidney, small intestine, spleen, uterus and mandibular lymph node from all animals and examined for the presence of immune-complex disease (kidney) or antibody cross-reactivity.

There were no deaths and no clinical observations indicative of treatment related toxicity reported. Physical examinations, body temperature, body weights, food consumption and ophthalmic examinations were within normal biological variability. Urinalysis and clinical chemistry results showed no evidence of toxicity. Total white blood cell counts were increased (a statistically significant doubling), primarily in the 3.0 and 30 mg/kg dose groups in one or both sexes at one or more weeks during the dosing period. The increase in total white blood cell counts was accompanied by an increase in the number of lymphocytes in these animals. These effects are results of the known pharmacological action of the study drug (inhibition of lymphocyte migration from blood vessels into surrounding tissues and organs). Relative to baseline values, reticulocyte counts were increased for females in the 30 mg/kg group on day 15 and 22 and for females in the 3.0 mg/kg group on day 28. No other findings indicative of anemia were noted except for a small decrease in hematocrit after SD8 and continuing through SD 28 (statistically significant for the high and mid dose only on SD8 for male monkeys). It is thought that the increase in reticulocytes in circulation is a result of the pharmacological activity of the study drug, blocking of the $\alpha 4$ integrin allowing migration of the immature RBS to leave the bone marrow.

Absolute and relative spleen weights were increased in a dose-dependent manner in the 3.0 and 30.0 mg/kg dose groups. This effect was considered to have been related to the normal pharmacodynamic effect of natalizumab, rather than a toxic effect and is consistent with findings in other studies for this product. There were no treatment-related gross lesions or microscopic lesions indicative of adverse toxic effects in any of the monkeys at the end of the dosing or recovery periods.

Study no.: al106

Volume #, and page #:

Conducting laboratory and location: _____

Date of study initiation: 12/14/1994

GLP compliance: YES

QA report: yes (X) no ()

Drug, lot #, and % purity: natalizumab lot # AN-100226-0002

Vehicle lot #4T36QT

Saline lot #s C263418 and C275628 _____

Methods

Doses: 0, 0.3, 3, or 30 mg/kg two control group received either saline control or vehicle (containing histidine).

Species/strain: Cynomolgus monkeys

Number/sex/group or time point (main study): 5/sex/group

Route, formulation, volume, and infusion rate: IV, 30 minute infusion in a volume of 10 ml/kg, every other day for 28 days for a total of 14 doses.

Satellite groups used for toxicokinetics or recovery:

Age: approximately 3 yo

Weight: 2.5-6.5 kg

Unique study design or methodology (if any):

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

<u>Group</u>	<u>Test or Control Article</u>	<u>Dose</u>	<u>Number of Monkeys</u>	
		<u>AN100226 (mg/kg)</u>	<u>Males</u>	<u>Females</u>
1	Saline Control	0	5	5
2	Vehicle Control	0	5	5
3	AN100226	0.3	5	5
4	AN100226	3	5	5
5	AN100226	30	5	5

Observations and Results

Mortality: All animals survived to scheduled necropsy.

Clinical signs: Detailed observations made twice daily (prior to and after dosing, am and pm on non-dosing days). Physical exams performed once pre-study and weekly during the dosing and recovery periods.

- No clinical signs attributable to study drug administration were noted. Sporadic episodes of soft stool and diarrhea were reported but no clear relationship to study drug was observed.
- Physical exams did not reveal any apparent toxic effects.

Body weights: Data collected twice weekly during the pre-study period, on each dosing day (immediately after dosing), weekly during the recovery period and prior to necropsy.

- No test article effects on body weight were observed. It is interesting to note that, during the treatment period, all groups gained small amounts of weight. But during the recovery period, a trend toward a small loss of weight is noted for all groups including controls (n=1 per group).

Body temperature: Data collected once pre-study, once on each dosing day and weekly during the recovery period.

- Rectal temperatures did not reveal any test article effect or indication of infection during the study. Some variability was noted but variation remained within normal limits for this species.

Food consumption: Data collected weekly.

- No test article related effects on food consumption were noted.

Ophthalmoscopy: Performed once prior to study initiation, once during the final week of dosing. Exams will be performed on recovery animals only if toxicity is noted during the dosing period.

- No toxic effects were noted.

EKG: Testing or results are not mentioned.

Clinical pathology: Blood and urine samples for clinical pathology collected once prior to study initiation, on SD 8, 15, 22, 42 and prior to necropsy on SD 28 or 56.

Clinical chemistry:

Testing parameters include the following: Glucose, Urea nitrogen (BUN), Creatinine, BUN/creatinine, Cholesterol, Total bilirubin, Direct bilirubin ratio, Total protein, Albumin, Globulin (by calculation), Albumin/globulin ratio, Inorganic phosphate, Alkaline phosphatase, Creatine kinase, Alanine aminotransferase, Amylase, Lactate dehydrogenase, Gamma glutamyl trans-peptidase, Aspartate amino-transferase, Triglycerides, Sodium, Calcium, Potassium, Chloride, Iron

- BUN values were significantly increased in the high dose group females on days 15 and 22. This effect is not noted for the male animals in the same group.
- BUN/creatinine was elevated for females in all groups on day 22. The sponsor states that these findings are possible due to collection occurring immediately after dosing on day 15, thus increasing the nitrogen levels. These values were analyzed relative to saline control and that value was unusually low on that day. No effects were noted for males in the same groups.
- No effect on creatinine is noted for male or female animals.
- Elevated creatine kinase levels for group 3, 4, and 5 males on day 8. No effects are noted for females in the same groups.

Hematology:

Erythrocyte count and morphology, White blood cell count (total and differential; % and absolute), Hematocrit, Hemoglobin, Mean corpuscular volume, Mean corpuscular hemoglobin, Mean corpuscular hemoglobin concentration, Platelet count, Reticulocyte

count, Prothrombin time, Activated partial thromboplastin time, Erythrocyte sedimentation rate

- Elevated WBC counts were noted for 8 females in the 3 mg group (SD8) and a total of 15 animals (males and females) from the 30 mg group (SD 8, 22 and 28). Elevation relative to the controls was also noted for the low and mid dose males on day 8 but this trend did not reach statistical significance.
- The increased WBC levels were accompanied by increased absolute levels of lymphocytes in the mid dose group on days 8 and 15, and in the high dose group on days 8-28. These findings are dose dependent and are expected effect of the study drug pharmacological activity.
- Relative lymphocyte counts are also elevated for these groups relative to control. These findings are accompanied by decreases in relative levels of other WBC classes.
- Other sporadic statistically significant increases seen in male monkeys: erythrocyte count at the low and mid doses on day 8, hematocrit level at the mid and high doses on day 8, hemoglobin level at the high dose on day 8, and number of eosinophils (absolute) at the high dose on day 22. These findings did not occur in a dose dependent pattern. Therefore, the biological significance is unclear.
- No effects on hemoglobin parameters were observed for the females in the high dose group at the end of the dosing period or recovery.

Urinalysis: No effects of the study drug were noted. A large degree of variability was observed for test values.

The following parameters were evaluated:

Appearance, Color, Specific gravity, Volume, Sodium, PH, Protein, Glucose, Bilirubin, Urobilinogen, Potassium N- Acetyl-glucosaminidase, Nitrite, Ketones, Leukocytes, Occult blood, Microscopy of sediment, Urinary /3-2 microglobulin

Anti-drug antibody detection:

Blood samples for determination of serum antibody titers will be obtained from all study animals on days 0 (pretest), 8, 15, 22, 28, 42, and 56.

Immunotoxicity: Potential immunotoxicity was evaluated by conducting a number of immune function assays

NK cell activity: a large amount of heterogeneity in NK cell response was noted between both male and female individual animals observed for NK cell activity in both peripheral blood leukocytes, as well as splenic lymphocytes. Some variability in response was noted