CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

STN/BLA 125075/0

Pharmacology Review(s)

Note:

This will be the Standard CDER Coversheet

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EXECUTIVE SUMMARY

1. Recommendations

1.1 Recommendation on approvability

It is recommended that efalizumab be approved.

1.2 Recommendation for nonclinical studies

No additional nonclinical studies are recommended.

1.3 Recommendations on labeling

PRECAUTIONS
Immunizations

[ ]

Carcinogenesis, Mutagenesis, Impairment of Fertility

[ ]

Genotoxicity studies were not conducted.

Pregnancy Category C

Animal reproduction studies have not been conducted with RAPTIVA. It is also not known whether RAPTIVA can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. RAPTIVA should be given to a pregnant woman only if clearly needed.

[

Nursing Mothers

2. Summary of nonclinical findings

2.1 Brief overview of nonclinical findings

Introduction: The nonclinical evaluation of efalizumab encompassed pharmacology (primary pharmacodynamics and pharmacokinetics) and toxicology (general, reproductive and immunotoxicology) studies. The sponsor used the chimpanzee and the mouse as the primary animal models for these studies. The chimpanzee studies were conducted with efalizumab. However, because efalizumab recognizes only human and chimpanzee CD11a, the sponsor developed an antibody specific for mouse CD11a, muM17, to allow for more comprehensive toxicology studies to be conducted.

Pharmacology: The results of the *in vitro* pharmacology (primary pharmacodynamics) studies were consistent with efalizumab's ability to block the binding of LFA-1 to ICAM-1 and inhibit T-cell function. These studies showed that efalizumab inhibited [1] the human one-way mixed lymphocyte reaction (IC_{50} 0.063 and 0.0942 ug/mL), [2] the adhesion of activated T cells to human umbilical vein cells (IC_{50} 0.13 ug/mL), [3] the adhesion of T cells to keratinocytes (IC_{50} 0.1 to 0.4 ug/mL), and [4] chemoattractant-induced transendothelial migration of T cells (IC_{50} 0.01 ug/mL).

Pharmacology studies demonstrated that muM17 is an appropriate surrogate for efalizumab. These studies revealed that [1] the affinity of muM17 for murine CD11a (2.7 nM) was similar to that of efalizumab for human CD11a (3.0 nM), [2] muM17 inhibited the proliferation of murine T cells in a mixed lymphocyte reaction test (IC_{50} 0.1 – 0.3 ug/mL), [3] *in vivo* treatment with muM17decreased CD11a expression and increased saturation, and [4]

treatment of mice *in vivo* with ≥ 3 mg/kg of muM17 administered subcutaneously resulted in a significant decrease in cell-mediated immune function.

Pharmacokinetics: Studies revealed that the kinetics of efalizumab and muM17 are saturable in the chimpanzee and the mouse, respectively, due to their elimination via binding to CD11a. For example, in mice treated with muM17, clearance decreased from 166 mL/kg/day following sc administration of 1 mg/kg to 15 – 20 mL/kg/day following sc administration of ≥ 3 mg/kg. Clinical pharmacokinetics studies conducted in psoriasis patients revealed that kinetics are saturable in humans as well.

Toxicology: The sponsor conducted general, reproductive, and immunotoxicology studies.

General toxicology studies were conducted in the chimpanzee and the mouse, using efalizumab (administered iv) and muM17 (administered sc), respectively, as the test articles. A number of the chimpanzee studies were conducted using iv administration because in the early stages of development, iv was the intended route of clinical administration. The duration of exposure employed in these studies ranged from a single dose to 6-months of weekly dosing, with the 6-month studies being the most relevant to the clinical scenario. The doses used in 6-month chimpanzee study are shown in the table below along with a comparison of exposure achieved in that study to that achieved in psoriasis patients.

Dose	Chimpanzee Peak Plasma Levels (ug/mL)		Human Mean Peak Levels*
	Days 2 – 13*	Days 21 – 203	
0 mg/kg	---	---	12 ug/mL
Day 1: 2 mg/kg Days 2 – 14: 2 mg/kg/day Week 3 – 6 months: 8 mg/kg/week	118 - 190	318 - 585	
Day 1: 40 mg/kg Days 2 – 14: 10 mg/kg/day Week 3 – 6 months: 40 mg/kg/week	494 - 1220	1770 - 3380	

*The human mean peak plasma levels were obtained from psoriasis patients receiving the recommended clinical dosing regimen (an initial 0.7 mg/kg sc dose followed by weekly sc doses of 1 mg/kg).

The data obtained from the 6-month study in chimpanzees showed that CD11a expression was reduced throughout the study, with recovery being observed upon cessation of treatment. The effects observed in this study were consistent with decreased CD11a expression. First, animals in all dose groups exhibited a decreased ability to mount antibody response to tetanus toxoid. Second, lymph node biopsies revealed a decrease in CD3 T cells in paracortical areas of the lymph nodes in all treatment groups due to

efalizumab-induced alterations in T cell trafficking. The chimpanzees in this study did not, however, demonstrate an increase in infections. One animal receiving 8 mg/kg from Week 3 through 6 months died on Day 153 of the study. Prior to death, this animal exhibited hypoactivity, soft stools to bloody diarrhea, dehydration, and decreased body weight. The cause of death was considered to be moderate to severe necrotizing inflammation of the small intestine, which was considered to be of possible viral origin. The relationship of this death to treatment is uncertain. Toxicology studies of shorter duration (single dose to 14 days) did not reveal any unique effects. Efalizumab-treated chimpanzees immunized with tetanus toxoid after the product had cleared and CD11a levels had recovered were able to mount an immune response. The lowest dose tested in the chimpanzee toxicology studies, 2 mg/kg, markedly decreased CD11a expression within 24 hours.

The sponsor conducted a 6-month toxicology study in the TSG-p53 wild type mouse. In this study, muM17 was administered as sc doses of 0, 3, 10, and 30 mg/kg/week (3 to 10 times the clinical dose), which are known to be pharmacologically active in TSG-p53 wild type mice. The plasma levels of muM17 observed in this study were 1.4 to 68 times those observed for efalizumab in psoriasis patients. muM17-induced effects observed in the mice were consistent with decreased CD11a expression. Mice in all treatment groups exhibited reversible increases in circulating lymphocytes, neutrophils, and eosinophils. Psoriasis patients frequently exhibited increased lymphocyte counts. Histopathological evaluation in mice, which was limited to the 30 mg/kg group, revealed [1] hypercellularity of the splenic white pulp and [2] decreased lymphocytic infiltration of various organs (pancreas mandibular salivary gland, and kidney). The latter finding was not found to be reversible at the end of a 3-month recovery period although plasma levels of muM17 were undetectable. The mice in this study did not exhibit an increase in infections. One female mouse in the high dose group was found dead on Day 141 of the study. Histopathology revealed changes consistent with a disseminated infection of unknown origin. The relationship of this death to treatment is not known. TGS-p53® wild type mice receiving 3, 10, or 30 mg/kg/week for 4 weeks exhibited decreased cellularity in lymph nodes, a finding not observed in the 6-month study. The reason for this difference is not clear. The lowest dose tested in mice, 0.1 mg/kg in CD-1 mice, had no effect on CD11a expression or any other endpoints.

The sponsor conducted reproductive toxicology studies in CD-1 mice using 3, 10, and 30 mg/kg of muM17, doses shown to be pharmacologically active in CD-1 mice. Treatment with muM17 had no effect on fertility or early embryonic development. Similarly, muM17 had no effect on embryofetal development in spite of being detected in the amniotic fluid. In a study designed to assess prenatal and postnatal development, muM17 had no effect on reproductive endpoints. In contrast, immunological effects were observed in offspring born to dams treated with $\geq$ 3 mg/kg of muM17 during gestation

and lactation. At 11 weeks of age, offspring born to these dams exhibited a 35% to 80% decrease in their antibody response to sheep red blood cells, a T cell dependent antigen. A trend towards reversal was observed at 25 weeks of age. The offspring did not, however, exhibit an increase in infections.

The sponsor evaluated the immunotoxic potential of decreased CD11a expression in CD-1 mice treated with 0, 3, 10, and 30 mg/kg/week of muM17 for 4 weeks. A subset of the 3 mg/kg group was maintained for a 28-day treatment-free recovery period. The effects observed in this study were not unexpected based on the pharmacology of muM17. All dose groups exhibited increased splenic lymphocytes, a marked decrease ($\geq$ 90%) in IgM response to a T cell dependent antigen, and decreased NK cell activity (48% to 68% and 71% to 88% in males and females, respectively). In males, the effects on spleen cell numbers fully reversed and the effect on IgM partially reversed. In contrast, the effect on NK cell activity was not reversible. All effects were reversed in the female recovery group.

In summary, the sponsor evaluated the pharmacological and toxicological consequences of decreased Cd11a expression in *in vitro* and *in vivo* models. The animals used for the latter were the chimpanzee and the mouse. The chimpanzees were treated with efalizumab, which recognizes only human and chimpanzee CD11a. Mice were treated with muM17, a monoclonal antibody specific for mouse CD11a. The sponsor submitted data supporting that muM17 is an acceptable surrogate for efalizumab. The effects observed in studies conducted in the chimpanzee and the mouse studies were consistent with the pharmacology of efalizumab and muM17.

2.2 Pharmacologic activity: Efalizumab binds with high affinity to lymphocyte function antigen-1 (LFA-1) and blocks its' binding to intercellular adhesion molecule-1 (ICAM-1) and inhibits T-lymphocyte-mediated functions. LFA-1 is expressed on all leukocytes and is the primary B_2 integrin on lymphocytes. It is also expressed on platelets.

2.3 Nonclinical safety issues relevant to clinical use

The nonclinical issues relevant to clinical use are [1] decreased ability of efalizumab-treated chimpanzees to mount an immune response to tetanus toxoid when CD11a expression is decreased, and [2] diminished ability of mouse pups born to dams treated with muM17 during gestation and lactation to mount an immune response to a T cell dependent antigen. These concerns have been discussed with the clinical review staff and are being addressed through labeling and/or post-marketing commitments.

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

Studies reviewed within this submission: The list of studies reviewed within this submission is located in Appendix 2.

Studies not reviewed within this submission: The list of studies not reviewed within this submission is located in Appendix 3.

3.2 PHARMACOLOGY

3.2.1 Brief summary

The results of the *in vitro* pharmacology (primary pharmacodynamics) studies were consistent with efalizumab's ability to block the binding of LFA-1 to ICAM-1 and inhibit T-cell function. These studies showed that efalizumab inhibited [1] the human one-way mixed lymphocyte reaction (IC_{50} 0.063 and 0.0942 ug/mL, [2] the adhesion of activated T cells to human umbilical vein cells (IC_{50} 0.13 ug/mL), [3] the adhesion of T cells to keratinocytes (IC_{50} 0.1 to 0.4 ug/mL), and [4] chemoattractant-induced transendothelial migration of T cells (IC_{50} 0.01 ug/mL).

Pharmacology studies demonstrated that muM17 is an appropriate surrogate for efalizumab. These studies revealed that [1] the affinity of muM17 for murine antibodies (2.7 nM) was similar to that of efalizumab for human CD11a (3.0 nM), [2] muM17 inhibited the proliferation of murine T cells in a mixed lymphocyte reaction test (IC_{50}), [3] *in vivo* treatment with muM17decreased CD11a expression and increased saturation, and [4] treatment of mice *in vivo* with $\geq$ 3 mg/kg of muM17 administered subcutaneously resulted in a significant decrease in cell-mediated immune function.

3.2.2 Primary pharmacodynamics

Mechanism of action:

Drug activity related to proposed indication:

The primary pharmacodynamics studies that the sponsor submitted for this BLA are summarized in the table below.

Study	Summary
Study 00-118-1046 Compare rhuMAB CD11a from Genentech and from XOMA for binding to human and chimpanzee T-lymphocytes	The objective of this study was to compare the binding characteristics of efalizumab manufactured at Genentech to that manufactured at XOMA in human and chimpanzee lymphocytes using flow cytometry. For human lymphocytes, the EC_{50} for the Genentech and the XOMA products

PHARMACOLOGY/TOXICOLOGY REVIEW

3.1 INTRODUCTION AND DRUG HISTORY

BLA number: 125075
Review number: 1
Sequence number/date/type of submission: 125075/0
December 27, 2002
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Genentech, South San Francisco, CA
Manufacturer for drug substance: Genentech, South San Francisco, CA

Reviewer name: Andrea B. Weir, Ph.D.
Division name: Therapeutic Biotechnology Internal Medicine Products
HFM #: HFM-579
Review completion date: October 20, 2003

Drug:

Trade name: Raptiva
Generic name: Efalizumab
Code name: G176CR, hu1124
Chemical name: recombinant humanized monoclonal antibody to CD11a
CAS registry number: 214745-43-4
Molecular formula/molecular weight: ~ 150,000 daltons
Structure: The structure of efalizumab is provided in Appendix 1.

Relevant INDs/BLAs/DMFs:

IND 6809 (XOMA, psoriasis), IND 8369 (Genentech, psoriasis), IND —— IND ——

Drug class: Monoclonal antibody

Indication: Treatment of adult patients (18 years or older) with moderate or severe plaque psoriasis

Clinical formulation: 100 mg/mL of efalizumab, 0.2% polysorbate 20, 40 mM histidine, and 240 mM sucrose

Route of administration: subcutaneous (sc)

Proposed use: Treatment of adult patients (18 years or older) with moderate or severe plaque psoriasis

Study	Summary
	were 0.050 ± 0.004 ug/mL and 0.045 ± 0.009 ug/mL, respectively. In the case of the chimpanzee lymphocytes, the EC_{50} for the Genentech and XOMA products were 0.081 ± 0.024 ug/mL and 0.098 ± 0.025 ug/mL, respectively. These results show comparable affinity between the Genentech and XOMA products for human and chimpanzee lymphocytes.
Study 00-174-1046 In vitro modulation of CD11a on human T-cells with XOMA and Genentech anti-CD11a	The objective of this study was to compare the ability of efalizumab manufactured by Genentech to that manufactured by XOMA to down modulate cell surface CD11a expression in vitro using human peripheral T lymphocytes as a test system. CD11a down modulation was measured using a flow cytometry assay. In the presence of goat anti-human IgG, an Ab fragment that induces cross-linking of efalizumab and down modulation of CD11a in vitro, the Genentech and XOMA products behaved similarly.
01-404-1049 Effect of efalizumab in a human one-way mixed lymphocyte reaction (MLR)	The purpose of this study was to determine whether efalizumab inhibits the human one-way mixed lymphocyte reaction (MLR) in vitro. The MLR is a model of T-cell recognition of allogeneic major histocompatability gene products. It is characterized by cellular proliferation that is induced by contact between isolated peripheral blood mononuclear cells from two different individuals. In this study, efalizumab inhibited human one-way MLR in vitro in a dose-dependent manner. The IC_{50} values were 0.063 and 0.0942 ug/mL.
01-436-1049 Effect of efalizumab on T-cell action	The purpose of this study was to determine the effect of efalizumab on anti-CD3-induced T-cell activation by measuring CD69 expression in human lymphocytes using flow cytometry. Efalizumab inhibited anti-CD3-induced expression of CD69 in a dose dependent manner. The IC_{50} was 0.24 ug/mL.
01-263-1046 Effect of Xanelim (efalizumab) on the adhesion of human T-lymphocytes to endothelial cells	The purpose of this study was to determine if efalizumab inhibits the binding of human T lymphocytes to human endothelial cells (human umbilical vein cells, HUVEC). Efalizumab inhibited the adhesion of activated T-cells to HUVECs in a dose-dependent manner. The IC_{50} was 0.13 ug/mL.
01-426-1046 Effect of Xanelim (efalizumab) on the adhesion of human T lymphocytes to keratinocytes	The purpose of this study was to determine if efalizumab inhibits the binding of human T-lymphocytes to human keratinocyte monolayers. Efalizumab inhibited the adhesion of T-cells to keratinocytes in a dose-dependent manner. The sponsor conducted three experiments using isolated keratinocytes. The individual IC_{50} values ranged from 0.1 to 0.4 ug/mL.
01-437-1049 Effect of efalizumab on transendothelial migration of T-cells	The purpose of this study was to investigate the effect of efalizumab on the transendothelial migration of human T-cells induced by rhuSDF-1alpha/PBSF, a T-cell chemoattractant. Efalizumab inhibited rhuSDF-1alpha/PBSF-induced migration of T-cells in a dose dependent manner with an IC_{50} of 0.01 ug/mL.
91-328-1049 Expression of lymphocyte adhesion molecules in psoriatic skin and affects of anti-LFA-1	Biopsies were obtained from plaque-type psoriatic skin lesions from 11 patients and skin from 4 normal individuals. The tissues were processed and evaluated using immunohistochemistry for ICAM-1, HER-2, CD11b, LFA-1b, CD3, CD68, B cell, HLA-DR, and gp20. ICAM-1, HER-2 and HLA appeared to be increased in the epidermis of psoriatic skin compared to the control skin.
01-427-1046 Anti-CD11a ameliorates disease in the human psoriatic skin-SCID mouse transplant model: Comparison of antibody to CD11a with cyclosporine	The purpose of this study was to evaluate the applicability of human skin SCID (severe combined immunodeficiency) mouse chimeras in testing therapies for psoriasis. The mice were treated with cyclosporine (20 mg/kg, ip), efalizumab (6 mg/kg, ip), or clobetasol propionate (a potent topical steroid used in the treatment of psoriasis and other dermatoses; topically applied), for 14 days. The effects of

Study	Summary
and clobetasol propionate	the treatments were assessed by measuring skin thickness and by histopathological evaluation of the lesions. All treatments reduced the thickness of psoriatic skin as assessed by measuring skin thickness and histopathological evaluation. Data from this study indicate that the SCID mouse model is a potentially useful model for the evaluation of antipsoriatic agents.
01-143-1049 Comparative binding affinities of Xanelim (efalizumab), muM17 and M17	muM17 is a chimeric rat/mouse IgG anti-mouse CD11a antibody. It was developed as a surrogate for efalizumab, which is specific for human and chimpanzee CD11a. M17 is the parent rat anti-mouse CD11a used to develop muM17. The purpose of this study was to compare the *in vitro* binding affinity of efalizumab in whole human blood with the affinity of muM17 and M17 in murine blood. The affinity of efalizumab in human blood was 3.0 ±1.5 nM. The affinities of muM17 and M17 in mouse blood were 2.7 ± 1.2 nM and 7.3 ± 1.9 nM, respectively. These data suggest that the binding affinities were comparable.
01-142-1049 Effect of the muM17 antibody on a mixed lymphocyte reaction	The objective of this study was to determine if muM17, the mouse surrogate antibody for efalizumab, inhibits the mixed lymphocyte reaction. Different concentrations of murine T-cells were incubated with increasing concentrations of muM17 and with a constant number of allogeneic stimulator cells (murine splenocytes) muM17 inhibited the proliferation of murine T-cells. The IC_{50} value ranged from 0.1 – 0.3 ug/mL of muM17.
01-245-1049 Assessment of cell-mediated immune function in mice treated with muM17	The objective of this study was to evaluate the potential for muM17 to induce alterations in cell-mediated immune function in female mice using contact hypersensitivity as an indicator of cell-mediated function. Groups of 6 female CD-1 mice received muM17 vehicle or muM17 (3, 10, or 30 mg/kg). An additional group received 30 ug epicutaneous dose of dexamethasone (DEX) as a positive control for immunomodulation. Four of the six groups were treated on Days 1 and 7. One group receiving 30 ug of muM17 and the DEX group were treated on Days 7 and 9, respectively. All animals were sensitized to oxazolone (OXA) on Day 3 (abdominal application) and challenged with OXA on Day 9 (application to the ear). The animals were observed twice daily. At approximately 24, 48, and 72 after challenge, the thickness of the treated ear was measured. The animals were sacrificed after the last measurement. Treatment of mice with ≥3 mg/kg of muM17 on Days 1 and 7 resulted in a significant decrease in cell-mediated immune function. In the 10 and 30 mg/kg groups, treatment resulted in a total abrogation of the hypersensitivity response. In mice receiving DEX or muM17 on Day 7, the hypersensitivity response was decreased by 85% to 95% compared to the control group. Detailed kinetics data are provided in Appendix 4.
01-319-1049 Assessment of cell-mediated immune function in TSG-p53 wild type mice treated with muM17	The purpose of this study was to evaluate the potential for muM17 to alter a cell-mediated allergic response in TSG-p53® Wild Type mice using a contact sensitization model of delayed type hypersensitivity. Groups of 6 female mice received 0, 3, or 10 mg/kg sc injections of muM17 on Days 1 and 7; a 4th group received a 30 ug epicutaneous dose of dexamethasone (DEX) as a positive control for immunoodulation on Day 9. All animals were sensitized to oxazolone (OXA) on Day 3 (abdominal application) and challenged on Day 9 (application to the ear). The animals were observed twice daily. At approximately 24, 48, and 72 hours after challenge, the thickness of the treated ear was measured. The animals were sacrificed after the last measurement. Treatment of mice with 3 and 10 mg/kg of muM17 resulted in a significant decrease in the allergic response. The control mice exhibited a maximum increase in ear thickness of ~ 120%. In contrast, mice receiving 3 or 10 mg/kg of muM17 exhibited an increase of ~ 25%. Treatment with DEX also resulted in a marked decrease in ear thickness. Detailed kinetics data are provided in

Study	Summary
	Appendix 4.
00-520A-1047 Multiple dose percent saturation and toxicokinetics of an anti-mouse CD11a (muM17) antibody in female mice	The purpose of this study was to determine the relationship between plasma concentrations of muM17 and down modulation and saturation of CD11a on T-lymphocytes in female mice using flow cytometry. Female mice CD-1 mice (2 to 4 and 4 per time point for the control and treated animals, respectively) received a sc injection of vehicle or muM17 (3 or 10 mg/kg) on Days 1 and 7. A control group receiving no treatment was also included. Blood samples were obtained at the following time points for determination of antibodies against muM17, muM17 concentration, CD11a expression, and CD11a saturation: muM17 vehicle or 3 mg/kg muM17 (Days 3, 7, 14, 21, 28, 35, and 49), 10 mg/kg muM17 (Days 3, 7, 28, 35, and 49), and no treatment (Days 7, 10, 14, 21, 28, 35, and 49). In both the 3 and 10 mg/kg groups, CD11a expression was down-modulated to approximately 6% of the baseline level on Day 3, the first time point measured. On Day 3, the plasma concentrations were 17 ug/mL and 81 ug/mL for the 3 and 10 mg/kg groups, respectively. CD11a down-modulation was maintained until Day 28 and 49 in mice receiving 3 mg/kg and 10 mg/kg, respectively, on Days 1 and 7. An inverse relationship was observed between CD11a expression and saturation. Detailed kinetics data are provided in Appendix 4.

3.2.3 Secondary pharmacodynamics

The sponsor did not conduct any studies in this category.

3.2.4 Safety pharmacology

The sponsor did not conduct safety pharmacology studies *per se*. However, cardiovascular endpoints were included in selected toxicology studies. According to ICH S7a, Safety Pharmacology Studies for Human Pharmaceuticals, this approach is acceptable for biotechnology-derived products with high target specificity such as efalizumab.

Neurological effects: NA (See above)

Cardiovascular effects: NA (See above)

Pulmonary effects: NA (See above)

Renal effects: NA (See above)

Gastrointestinal effects: NA (See above)

Abuse liability: NA (See above)

Other: NA (See above)

3.2.5 Pharmacodynamic drug interactions

The sponsor did not conduct any nonclinical studies for this category.

3.3 PHARMACOKINETICS/TOXICOKINETICS

3.3.1 Brief summary Studies revealed that the kinetics of efalizumab and muM17 are saturable in the chimpanzee and the mouse, respectively, due to their elimination via binding to CD11a. For example, in mice treated with muM17, clearance decreased from 166 mL/kg/day following sc administration of 1 mg/kg to 15 – 20 mL/kg/day following sc administration of $\geq$ 3 mg/kg. Clinical pharmacokinetics studies conducted in psoriasis patients revealed that kinetics are saturable in humans as well. Additional pharmacokinetics data are available in Appendix for a number of the studies addressed in this section of this review.

3.3.2 Absorption

Study 99-393-1049 & 99-393B-1049
The pharmacokinetics and pharmacodynamics of muM17 (mouse chimera)

Methods: Female CD-1 mice (3/4 females/time point) received the following single dose treatments of muM17(in 40 mM histamine, 240 mM sucrose, and 0.08% polysorbate 20): [1] 1 mg/kg sc or iv [2] 10 mg/kg sc or iv, and [3] 3 or 5 mg/kg sc.

Blood samples were obtained for pharmacokinetics at the following time points: [1] mice receiving 1 or 10 mg/kg sc or iv = pre-dose, 10 and 30 min, 1, 2, 6 and 24 hours, and 2, 4, 7, 10, 14, 21 and 28 days post-dose, and [2] mice receiving 3 or 5 mg/kg sc = pre-dose, and 1, 2, 6 and 24 hours, and 2, 4, 7, 10, 14, 21 and 28 days post-dose.

Blood samples were obtained for assessing CD11a expression at the following time points: [1] mice receiving 1 or 10 mg/kg sc or iv = pre-dose, and 6 and 24 hours, and 2, 4, 7, 10, 14, 21, and 28 days after treatment, and [2] mice receiving 3 or 5 mg/kg sc = pre-dose and 1, 2, 6, and 24 hours, and 2, 4, 7, 10, 14, 21, and 28 days after treatment.

Results: The key findings for this study are as follows: [1] Saturable kinetics were observed following iv and sc administration. For example, clearance decreased from 166 mL/kg/day following sc administration of 1 mg/kg to 15 – 20 mL/kg/day following sc administration of $\geq$ 3 mg/kg. The half-lives increased from 1.2 days to 4.4 days following administration of 1 to 10 mg/kg, sc. [2] The absolute bioavailability of 1 mg/kg, sc (62.8%) was less than that observed with 10 mg/kg, sc (88.7%). [3] Down modulation of CD 11a expression by peripheral blood T cells was observed within 24 hours of sc and iv dosing, with a return to baseline occurring after the clearance of muM17.

8758-9810
A bioequivalence study of intravenous and subcutaneous formulation in chimpanzees

Methods: The purpose of this study was to determine the bioavailability and dermal reactivity of an sc formulation of efalizumab. The study consisted of 2 groups. One group of 3 male chimpanzees received a single 2 mg/kg, sc dose. Blood samples were collected for pharmacokinetics at pre-study; baseline; 1, 4, 8, and 12 hours; and 2, 3, 5, 7, 10, 14, 21, 28, 35, and 42 days. Flow cytometry data were collected to monitor CD11a levels pre-study, at baseline, and on Days 2, 7, 28, and 42. This group of animals received a second 2 mg/kg dose of efalizumab following CD11a re-expression to determine if any adverse reactions occur with re-exposure and to measure chimp-anti-human antibodies.

A second group of 4 chimps (2/sex) was assigned to a crossover study in which they received a 0.5 mg/kg sc dose followed by a 0.5 mg/kg iv dose or vice versa. The two doses were followed by a 3-week washout period. Blood samples were collected at pre-study, baseline, and 1, 4, 8, and 12 hours; and 2, 3, 5, 7, 10, and 14 days after each dose for pharmacokinetics and at baseline and on Days 2, 7, 10, and 14 following each of the two doses for CD11a expression.

In both groups, dermal reactivity was assessed by obtaining skin biopsies approximately 24 hours following subcutaneous dosing. The biopsies were processed and evaluated histopathologically.

Results: The bioavailability of a 2 mg/kg sc dose was estimated to be 58% of a model-estimated area under the curve for a 2 mg/kg dose administered iv. A model-estimated area-under-the-curve was used for the iv dose because empirical data were not available.

Based on plasma levels measured up to 10 days after dosing with 0.5 mg/kg, the bioavailability of an sc dose was calculated to be 16.8 to 42.1%.

A comparison of data obtained for 0.5 mg/kg sc and 2 mg/kg sc revealed that clearance (30.56 ± 1.47 and 149 to 487 mL/kg/day for the 2 and 0.5 mg/kg doses, respectively) and $t_{1/2}$ increased with dose (3.43 ± 0.18 days and 20.6 to 35.7 hours for the 2 mg/kg and 0.5 mg/kg doses, respectively).

In the chimpanzees receiving 2 mg/kg, CD11a expression was reduced to ~ 10% of baseline in 2/3 animals and to 34% of baseline in the third animal one day after dosing. By Day 28, CD11a expression exceeded baseline by 1.6 X to 3.6 X. In the chimpanzees receiving 0.5 mg/kg sc or iv, CD11a expression was reduced to less than 10% to 25% of baseline one day after treatment. Within 7 to 10 days after treatment, CD11a expression returned to or exceeded (up to 3.4 X) baseline.

The animals did not exhibit any signs of dermal reactivity.

None of chimpanzees in the first group exhibited an antibody response. One animal from the second group exhibited a low level response.

973409
A single dose subcutaneous bioavailability pilot study in chimpanzees

Methods: One male chimpanzee (3.5 years old, 12.47 kg) received a single subcutaneous injection of ~8 mg/kg of efalizumab in the following formulation (25 mM histidine, 300 mM trehalose, and 0.05% Tween 20 in sterile distilled water). Plasma drug concentrations were determined immediately prior to dosing, 5 min, 1, 4, and 8 hours, and 2, 6, 8, 11, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78 days after injection. CD11a expression on lymphocytes was determined at the same time points as plasma levels and 85 days after treatment.

Results: Pharmacokinetic parameters were as follows: AUC = 474.06; Tmax = 48 hours; Cmax = 52 ug/mL; Cl = 21.1 mL/day/kg $t_{1/2}$ = 6.07 days. The apparent bioavailability was 36.3%. CD11a expression decreased to the limit of detection within 3 days of treatment. A trend towards recovery was apparent as plasma levels of efalizumab decreased, but a full recovery was not achieved.

3.3.3 Distribution

Study 95-271-1049: Whole body autoradiography, tissue distribution, and cellular localization of a rat anti-CD11a antibody, M17, in male CD-1 mice

Methods: The design of this study is shown in the table below. (Table copied from the submission.)

Study Design

Study No.[a] (Organs Harvested)	Group[a]	Timepoints (No. Mice per Timepoint)	Material	Lot No.	Specific Activity	Dose
95-271 (WBA)	1	1 and 72 hrs (2)	^{125}I-M17	21537-57	25	25 µCi
	2	1 and 72 hrs (2)	^{125}I-M17 M17	21537-57 20705-33	0.05	25 µCi 20 mg/kg
95-271A (L, SP, KD, Br, Bm, Bl)	3	1 hr (2)	Rat ^{125}I-IgG2A	21537-69B	11	25 µCi
	4	1 hr (2)	^{125}I-M17	21537-69A	20	25 µCi
	5	1 hr (2)	^{125}I-M17 M17	21537-69A 20705-33	0.04	25 µCi 20 mg/kg
95-271B (SP, KD, Bl)	6	1 hr (2)	^{125}I-M17	21537-57	21	25 µCi
95-271C (SP, KD, Bl, LN)	7	1 and 24 hrs (3)[a]	^{125}I-M17	24284-1	3.3	240 µCi

WBA=Whole body autoradiography.
L=Liver; KD=Kidney; SP=Spleen; Br=Brain; Bm=Bone marrow; Bl=Blood.
[a] Groups 3–7 were renamed to simplify the report. Groups 3, 4, and 5 in this study were Groups 1, 2, and 3, respectively, in the original protocol for 95-271A. Group 6 in this table was Group 1 in the original protocol for 95-271B. Group 7 in this table was Group 1 in the original protocol for 95-271C.

Results: This study showed that following treatment with ^{125}I-labeled M17, radioactivity was specifically associated with tissues and cells known to express CD11a. In the blood,

radioactivity was specifically associated with leukocytes and platelets. Additionally, radioactivity was specifically associated with leukocytes in the spleen, liver, kidney, and lymph nodes.

3.3.4 Metabolism

Study 02-328-1046: Internalization and clearance of a murine antibody specific for mouse CD11a

Methods: muM17 or ^{125}I-labeled muM17 was incubated with purified murine T cells isolated from the lymph nodes of female C57BL/6 mice to assess *in vitro* internalization of muM17. *In vivo* internalization was assessed using whole blood and single cell suspensions of lymph node and spleen cells from CD-1 female mice treated with ^{125}I-labeled muM17. Samples were analyzed using flow cytometry [redacted] analysis.

Results: Data from the *in* vitro segment of this study showed that muM17 was internalized *in vitro* by purified T cells in a time dependent manner following binding to the CD11a receptor, with peak internalization occurring within 1 hour. Data from the *in vivo* segment showed that muM17 was internalized primarily by cells from the spleen and peripheral blood, and to a lesser extent by cells from the lymph node. Approximately 70% of the internalized antibody was cleared from the spleen and peripheral blood cells within 24 hours. These data support the hypothesis that internalization by CD11a-expressing cells is one mechanism for efalizumab clearance.

3.3.5 Excretion

The sponsor did not conduct any studies. Classic excretion studies are not needed for monoclonal antibodies.

3.3.6 Pharmacokinetic drug interactions

The sponsor did not conduct any studies.

3.3.7 Other Pharmacokinetics Studies

00-092-1046
A single dose subcutaneous study to evaluate the pharmacokinetics and pharmacodynamics of two formulations of humanized anti-CD11a in a chimpanzee model

Methods: One group of 2 female chimpanzees received a single sc dose of Genentech-manufactured efalizumab. A second group of one female chimpanzee received the same dose of XOMA-manufactured product. Blood samples were obtained for pharmacokinetics analysis prior to dosing, on the day of dosing (6 and 12 hours), and 1,

2, 4, 7, 10, 14, 16, 21, 28, and 35 days after dosing. Blood samples were obtained for analysis of CD11a expression and % saturation prior to dosing and 1, 2, 4, 7, 10, 14, 16, 21, 28, and 35 days after dosing. Blood samples were collected for anti-efalizumab antibody analysis prior to dosing (2 times) and 35 days after dosing.

Results: The chimpanzee receiving the XOMA-manufactured inadvertently received a dose 20% lower than the animals receiving the Genentech product. Therefore, Cmax and AUC were dose-adjusted. Data for the pharmacokinetic endpoints are shown in the table below. Clearance for the XOMA-manufactured product was greater than for the Genentech product, an effect that might be due to the dose-mediated clearance of efalizumab.

Parameter	Genentech Product	Genentech Product	XOMA Product
Dose (mg/kg)	2.16	2.16	1.71
Cmax (ug/mL)	6.08	7.15	6.53
AUC (ug.day/mL/dose)	29.9	34.9	23.1
Terminal $t_{1/2}$ (days)	1.14	1.14	0.89
Clearance/F (mL/day/kg)	33.4	28.7	43.3

Efalizumab-induced decreases (~10% to 20% of baseline) in CD11a expression were generally comparable between the 2 groups through Day 7. However, at Day 10, the CD11a expression of the animal receiving the XOMA product was 55% of baseline, while those of the animals receiving the Genentech product were 11.2% and 16.5% of baseline. CD11a expression in the animal receiving the XOMA product reached baseline by Day 14 and exceeded baseline for the remainder of the study. A recovery and rebound was not seen in the animals receiving the Genentech product until Days 16 and 21 of the study. Reversal of CD11a saturation followed a pattern similar to CD11a expression, with the animal receiving the XOMA product making a more rapid recovery.

None of the animals exhibited antibodies to efalizumab.

The remaining pharmacokinetics data that the sponsor submitted are summarized in the table below.

Study	Summary
99-538A-1046 Pharmacokinetics of anti-CD11a (XOMA formulation) in rabbits following intravenous administration	A group of 5 New Zealand White male rabbits received a single 2 mg/kg iv dose of efalizumab. Plasma samples were obtained for analysis of efalizumab pre-dose; 10 and 30 min; 1, 2, 4, 8, and 24 hours; and 2, 3, 4, 7, 10, 15, 21, 28, and 35 days after treatment. Samples were obtained for analysis of antibodies to efalizumab pre-dose, and on Days 4, 7, 10, 15, 21, 28, and 35. The noncompartmental pharmacokinetic parameters were as follows: C_{max} = 32.9 ± 2.66 ug/mL; AUC_{last} 137.02 ± 20.2 ug.day/mL; Clearance = 13.9 ± 3.24 mL/kg/day; and $t_{1/2}$ = 9.74 ± 3.58 days. Anti-efalizumab antibodies were detected in one rabbit.

Study	Summary
99-214-1046 Pharmacokinetics of XOMA hu1124 (anti-CD11a) in rabbits	Three groups of 5 male rabbits received a single sc 0.2, 2, or 20 mg/kg of efalizumab. Blood samples were collected for analysis of efalizumab at pre-dose; 10 and 30 min; 1, 2, 4, 8, and 24 hours; and 2, 3, 4, 7, 10, 14, and 21 days after treatment. Linear pharmacokinetics were observed because efalizumab does not bind to rabbit CD11a. In contrast, humans and chimpanzees exhibit nonlinear pharmacokinetics. Pharmacokinetic parameters for the 0.2, 2, and 20 mg/kg groups, respectively, are as follows: Cl/F = 16.2, 14.9, and 20.1 mL/day/kg; $t_{1/2}$ = 5.62, 6.23, and 4.72 days; AUC = 10, 118, and 860 day.ug/mL; Cmax = 1.04, 11, and 96.3 ug/mL.
00-119-1046 and 00119A-1046 Comparability pharmacokinetics of anti-CD11a (Genentech vs XOMA formulation in rabbits)	Four groups of male New Zealand white rabbits were treated with efalizumab as follows: Group 1 = 2 mg/kg iv Genentech-manufactured material; Group 2 = 2 mg/kg iv XOMA-manufactured material; Group 3 = 2 mg/kg sc Genentech-manufactured material; Group 4 = 2 mg/kg sc XOMA-manufactured material. Groups 1 and 2 had 6 animals per group. Groups 3 and 4 had 8 animals per group. Blood samples were obtained for analysis of efalizumab at pre-dose; 10 and 30 min; 1, 2, 4, 8, and 24 hours; and 2, 3, 4, 7, 10, 14, 21, 28, 35, 42, 49, and 56 days after treatment. Additionally, samples were analyzed for antibodies to efalizumab. Pharmacokinetics were comparable for the Genentech- and XOMA-manufactured materials. Antibodies to efalizumab were detected in 1 animal.
01-260-1046 Comparability pharmacokinetics of humanized anti-CD11a (Genentech vs XOMA formulation) in rabbits	Groups of 6 male rabbits received the following sc treatments: Group 1 = 0.5 mg/kg Genentech-manufactured material; Group 2 = 0.5 mg/kg XOMA-manufactured material; Group 3 = 1 mg/kg Genentech-manufactured material; Group 4 = 1 mg/kg XOMA-manufactured material; Group 5 = 2 mg/kg Genentech-manufactured material; Group 6 = 2 mg/kg XOMA-manufactured material. Samples were collected for efalizumab analysis at pre-dose, 10 and 30 min; 1, 2, 4, 8, and 24 hours; and 2, 3, 4, 7, 10, 14, 21, 28, 35, 42, 49, and 56 days. Additionally, samples were analyzed for antibodies to efalizumab. Pharmacokinetics were comparable for the Genentech- and XOMA-manufactured materials. Anti-efalizumab antibodies were detected in 5 animals.
02-223-1046 Effects of glycosylation on the pharmacokinetics of mum17 following a single dose in mice	This study was performed to investigate whether the extent of glycosylation on the Fc region of muM17 could affect the pharmacokinetics of muM17. Groups of 12 male mice were treated with muM17

Study	Summary
	produced to express mostly G0 (69%) or mostly G2 (84%) galactose populations. All mice in the study received a total dose of 3 mg/kg. The treatment groups are as follows: Group 1 = ^{125}I- muM17G0 and ^{131}I-muM17 G2, sc; Group 2 = ^{131}I-muM17 G0 and ^{125}I- muM17G2, sc; Group 3 = -^{125}I-muM17G0 and ^{131}I-muM17 G2, iv; Group 2 = ^{131}I-muM17 G0 and ^{125}I- muM17G2, iv. Blood samples were collected for analysis of TCA precipitable radioactivity at 3, 10, 30, and 60 minutes; 6, 24, and 48 hours; and 7, 10, 14, 17, and 21 days after treatment. The data obtained from this study suggest that the extent of glycosylation does not affect the pharmacokinetics of muM17 in mice.
02-224-1046 Effects of glycosylation and formulation on the pharmacokinetics of muM17 following a single dose in mice	The objective of this study was to determine if formulation differences, changes in carbohydrate configuration, or increased protein content at the injection site affect the clearance or absorption of muM17. Groups of 12 male mice were treated iv/sc with radiolabeled muM17, muM17 (G0), and muM17 (G2). Blood samples were collected for analysis of TCA precipitable radioactivity in plasma at selected time points up to 35 days after treatment. Data obtained in this study indicated neither formulation differences nor differing galactose ratios affected muM17 clearance or bioavailability.

3.3.10 Tables and figures to include comparative TK summary

The summary tables that the sponsor provided as part of the submission, to include toxicokinetics, are located in Appendix 4.

3.4 TOXICOLOGY

3.4.1 Overall toxicology summary

General toxicology: General toxicology studies were conducted in the chimpanzee and the mouse, using efalizumab (administered iv) and muM17 (administered sc), respectively, as the test articles. The duration of exposure employed in these studies ranged from a single dose to 6-months of weekly dosing, with the 6-months being the most relevant to the clinical scenario. The doses used in 6-month chimpanzee study are shown in the table below along with a comparison of exposure achieved in that study to that achieved in psoriasis patients.

Dose	Chimpanzee Peak Plasma Levels (ug/mL)		Human Mean Peak Levels*
	Days 2 – 13	Days 21 – 203	
0 mg/kg	---	---	12 ug/mL
Day 1: 2 mg/kg Days 2 – 14: 2 mg/kg/day Week 3 – 6 months: 8 mg/kg/week	118 - 190	318 - 585	
Day 1: 40 mg/kg Days 2 – 14: 10 mg/kg/day Week 3 – 6 months: 40 mg/kg/week	494 - 1220	1770 - 3380	

*The human mean peak plasma levels were obtained from psoriasis patients receiving an initial 0.7 mg/kg sc dose followed by 11 weekly sc doses of 1 mg/kg.

The data obtained from this study showed that CD11a expression was reduced throughout the study, with recovery being observed upon cessation of treatment. The effects observed in this study were consistent with decreased CD11a expression. First, animals in all dose groups exhibited a decreased ability to mount antibody response to tetanus toxoid. Second, lymph node biopsies revealed a decrease in CD3 T cells in paracortical areas of the lymph nodes in all treatment groups due to efalizumab-induced alterations in T cell trafficking. The chimpanzees in this study did not, however, demonstrate an increase in infections. Toxicology studies of shorter duration (single dose to 14 days) did not reveal any unique effects. Efalizumab-treated chimpanzees immunized with tetanus toxoid after the product had cleared and CD11a levels had recovered were able to mount an immune response. The lowest dose tested in chimpanzees, 2 mg/kg, markedly decreased CD11a expression within 24 hours.

The sponsor conducted a 6-month toxicology study in the TSG-p53 wild type mouse. In this study, muM17 was administered as sc doses of 0, 3, 10, and 30 mg/kg/week (3 to 10 times the clinical dose), which are known to be pharmacologically active in TSG-p53 wild type mice. The plasma levels of muM17 observed in this study were 1.4 to 68 times those observed for efalizumab in psoriasis patients. muM17-induced effects observed in the mice were consistent with decreased CD11a expression. Mice in all treatment groups exhibited reversible increases in lymphocytes, neutrophils, and eosinophils. Histopathological evaluation, which was limited to the 30 mg/kg group, revealed [1] hypercellularity of the splenic white pulp and [2] decreased lymphocytic infiltration of various organs (pancreas mandibular salivary gland, and kidney). The latter finding was not found to be reversible at the end of 3-month recovery although plasma levels of muM17 were undetectable. The mice in this study did not exhibit an increase in infections. TGS-p53® wild type mice receiving 3, 10, or 30 mg/kg/week for 4 weeks exhibited decreased cellularity in lymph, a finding not observed in the 6-month study. The reason for this difference is not clear. The lowest dose tested in mice, 0.1 mg/kg in CD-1 mice, had no effect on CD11a expression or any other endpoints.

Genetic toxicology: Based on ICH S6, "Guideline for the Safety Evaluation of Biotechnology-Derived Pharmaceuticals, no studies were conducted.

Carcinogenicity: Based on ICH S6, "Guideline for the Safety Evaluation of Biotechnology-Derived Pharmaceuticals, no studies were conducted.

Reproductive toxicology: The sponsor conducted reproductive toxicology studies in CD-1 mice using the mouse anti-CD11a antibody, muM17. In all of the reproductive toxicology studies, mice were treated sc with 3, 10, and 30 mg/kg/week, doses shown to be pharmacologically active in CD-1 mice. Treatment with muM17 had no effect on fertility or early embryonic development. Similarly, muM17 had no effect on embryofetal development in spite of muM17 being detected in the amniotic fluid. In a study designed to assess prenatal and postnatal development, muM17 had no effect of reproductive endpoints. In contrast, immunological effects were observed in offspring born to dams treated with $\geq$ 3 mg/kg of muM17 during gestation and lactation. At 11 weeks of age, offspring born to these dams exhibited a 35% to 80% decrease in their antibody response to sheep red blood cells, a T cell dependent antigen. A trend towards reversal was observed at 25 weeks of age. The offspring did not, however, exhibit an increase in infections.

Special toxicology: The sponsor evaluated the immunotoxicity of decreased CD11a expression in CD-1 mice treated with 0, 3, 10, and 30 mg/kg/week of muM17 for 4 weeks. A subset of the 3 mg/kg group was maintained for a 28 day treatment-free recovery period. The effects observed in this study were not unexpected based on the pharmacology of muM17. All dose groups exhibited increased splenic lymphocytes, a marked decreases ($\geq$ 90%) in IgM response to a T cell dependent antigen, and decreased NK cell activity (48% to 68% and 71% to 88% in males and females, respectively). In males, the effects on spleen cell numbers fully reversed and the effect on IgM partially reversed. In contrast, reversal of the effect on NK cell activity was not observed. All effects were reversed in the female recovery group.

3.4.2 Single-dose toxicity

Study title: Effect of an anti-mouse CD11a (muM17) antibody on white blood cell counts and differentials in female CD1 mice

Key study findings: The animals in the 5 and 50 mg/kg groups exhibited a dose dependent increase in WBCs. CD11a expression was reduced on T cells in the thymus (70% to 80%) and lymph nodes (95% to 97%).

Study no.: 00-299-1047
Volume #, and page #: Not applicable (electronic submission)
Conducting laboratory and location: Genentech, South San Francisco, CA
Date of study initiation: July 11, 2002
GLP compliance: No
QA report: yes ( ) no (X)
Drug, lot #, and % purity: muM17, Lot# 33775-29

Methods

Doses: 0 (no treatment), 0 (muM17 vehicle), 5 and 50 mg/kg; single dose
Species/strain: CD-1
Number/sex/group or time point (main study): 0 (no treatment) = 6F; vehicle, 5 mg/kg and 50 mg/kg = 24F
Route, formulation, volume, and infusion rate: sc; 0.5 and 5 mg/mL for 5 and 50 mg/kg, respectively; 10 mL/kg; bolus
Satellite groups used for toxicokinetics or recovery: No
Age: 65 – 75 days old
Weight (nonrodents only):
Unique study design or methodology (if any):

The endpoints evaluated in this study are shown in the table below:

Endpoint	Timing
General antemortem observations	Weekly
Body weight	Weekly
Hematology	24, 48, 72, and 144 hours post-dose
Flow cytometry analysis of harvested lymph nodes and thymuses (5 mg/kg only)	24, 48, 72, and 144 hours post-dose

Results: The animals in the 5 and 50 mg/kg groups exhibited a dose dependent increase in WBCs. The maximum increase (~ 3-fold) was observed in the 50 mg/kg group 24 hours after treatment and was due to approximately equivalent increases in neutrophils and lymphocytes. The WBC count remained elevated through the 144 hour time point. The increases observed at the later time points were due primarily to increases in lymphocytes.

CD11a expression was reduced on T cells in the thymus (70% to 80%) and lymph nodes (95% to 97%). CD2 and CD28, markers of T cell activation, were also slightly reduced. muM17 did not appear to activate T cells.

Study title: hu1124: A 5-day intravenous dose-range finding study in chimpanzees
***[Comment:** In addition to data from animals receiving 5 doses, this study contained data from animals treated with a single dose of hu1124.]*

Key study findings: Effects were limited to decreased expression of CD11a. : CD11a expression decreased to ~10% of the pretreatment level following treatment and remained suppressed at this level through Day 28/49. Recovery began at Day 49/56, with values reaching pretreatment levels by Day 56/63. The reduced level of CD11a present was saturated with efalizumab.

Study no.: 960809
Volume #, and page #: Not applicable (electronic submission)
Conducting laboratory and location: [redacted]

Date of study initiation: June 1996
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: hu1124; GC606001-1

Methods

Doses: 10 mg/kg, single dose
Species/strain: Chimpanzee
Number/sex/group or time point (main study): 1/sex/group
Route, formulation, volume, and infusion rate: iv; 4 mg/mL; 2.5 mL/kg; 7 to 9 mL/min
Satellite groups used for toxicokinetics or recovery: None
Age: 10 to 26 years
Weight (nonrodents only): 18 to 25 kg
Unique study design or methodology (if any): There was no vehicle control group in this study. Because this study was conducted in chimpanzees, it was not a terminal study.

Observation times and results

Endpoint	Timing
Mortality	Daily
Clinical signs	Daily
Body weight	Days 1, 2 7, 14, 21, 28, and weekly from Day 49 – 147
Food consumption	Daily
Ophthalmoscopy	Days 2, 7, 14, and 28
EKG	Days 2, 7, 14, and 28
Hematology	Days 1 (pre-dose and 4 hours), 2, 7, 28, and every 2 weeks from Day 70 - 140
Clinical chemistry	Days 1 (pre-dose and 4 hours), 2, 6, and 28, and every 2 weeks from Day 70 to 140
Coagulation	Days 1 (pre-dose and 4 hours), 2, 6, and 28
Urinalysis	Pre-dose, Days 2, 6, and 14
Gross pathology	Not monitored
Organ weights	Not monitored
Histopathology	Not monitored
Toxicokinetics	Days 1 (0, 5, and 15 min, and 1, 4, and 8 hours), 2 – 4, 6, 7, 10, 14, 21, 28, 49, 56, and 63
Immunogenicity	Days 1 (pre-dose), 7, 14, and 28 Weekly for Days 70 - 147
Tetanus anti-toxoid levels	Day 70: 1 animal immunized with tetanus toxoid Day 119: Second animal immunized with tetanus toxoid Days 77, 84, 91, 98, 105, 112, 119, 126, 133, 140, 147: Anti-toxoid antibodies measured
Mixed lymphocyte reactions	Days 1 (0, 1, 4, and 8), 2 – 4, 6, 7, 10, 14, 21, 28, and 49, 56, 63, and 70
Lymphocyte blastogenesis transformation	Days 49, 56, 63, and 70

Endpoint	Timing
Flow cytometry	Days 1 (0, 1, 4, and 8), 2 – 4, 6, 7, 14, 21, and 28, and weekly from Days 49 to 70

Mortality: There were no mortalities in this study.

Clinical signs: The animals did not exhibit any treatment-related effects.

Body weights: Treatment had no effect on body weight.

Food consumption: Treatment had no effect on food consumption.

Ophthalmoscopy: The animals did not exhibit any treatment-related effects.

EKG: The animals did not exhibit any treatment-related effects.

Hematology: The animals did not exhibit any apparent treatment-related effects.

Clinical chemistry: The animals did not exhibit any treatment-related effects.

Urinalysis: The animals did not exhibit any treatment-related effects.

Gross pathology: Not monitored

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

Histopathology: Not monitored

Toxicokinetics: The toxicokinetics parameters are shown in the tables below. The animals receiving the single dose were CA0183 and CA0213. (The tables were copied from the submission.)

Animal	AUC mg/mL-day	Vc mL/kg	Vss mL/kg	Clearance mL/kg/day	plasma MRT days	MRT days
CA0183	1.94 ± 0.16[1]	36.0 ± 3.9	98.8 ± 7.9	5.13 ± 0.43	7.02 ± 0.97	19.25 ± 2.45
CA0213	1.11 ± 0.05	48.9 ± 3.2	116.9 ± 8.4	9.03 ± 0.42	5.41 ± 0.43	12.95 ± 1.13
CA0154	1.19 ± 0.05	46.5 ± 2.4	123.6 ± 4.5	8.38 ± 0.35	5.55 ± 0.37	14.74 ± 0.87
CA0208	1.23 ± 0.07	46.9 ± 4.5	106.4 ± 5.1	8.09 ± 0.44	5.79 ± 0.65	13.15 ± 0.94
mean	1.37 ± 0.19[2]	44.6 ± 2.9	111.4 ± 5.5	7.66 ± 0.86	5.94 ± 0.37	15.02 ± 1.46

Table 2 Continued: Pharmacokinetic parameters values of hu1124 in Chimpanzees

Animal	$t_{1/2}\alpha$ hours	$t_{1/2}\beta$ days	FAUCα	FAUCβ
CA0183	2.91 ± 1.02[1]	13.6 ± 1.7	0.016 ± 0.005	0.984 ± 0.005
CA0213	16.29 ± 3.91	10.1 ± 1.0	0.122 ± 0.029	0.878 ± 0.029
CA0154	3.94 ± 0.80	10.5 ± 0.6	0.027 ± 0.005	0.973 ± 0.005
CA0208	1.35 ± 0.62	9.2 ± 0.7	0.008 ± 0.003	0.992 ± 0.003
mean	6.12 ± 3.43[2]	10.8 ± 0.9	0.043 ± 0.027	0.957 ± 0.027

[1]Standard error from individual curve fit.
[2]Standard error from mean value of parameters of individual curve fits.

AUC=area under the curve
Vc=volume of distribution of the central compartment
Vss=steady state volume of distribution
CL=clearance
plasma MRT=plasma mean residence time
MRT=total body mean residence time
$t_{1/2}\alpha$=alpha half-life
$t_{1/2}\beta$=beta half-life
FAUCα=fraction of AUC contributed by the α-phase
FAUCβ=fraction of AUC contributed by the β-phase

Immunogenicity: The male animal, CA0183, exhibited an antibody response on Days 70, 126, and 140.

Tetanus anti-toxoid levels: The animals exhibited anti-tetanus toxoid antibodies.

Mixed lymphocyte reaction: No effects were apparent.

Lymphocyte blastogenesis transformation: No effects were apparent.

Flow cytometry: CD11a expression decreased to ~10% of the pretreatment level following treatment and remained suppressed at this level through Day 28/49. Recovery began at Day 49/56, with values reaching pretreatment levels by Day 56/63. The reduced level of CD11a present was saturated with efalizumab.

3.4.3 Repeat-dose toxicity

Chimpanzee

Study title: hu1124: An intravenous repeated dose safety/toxicity study with reversibility phase in chimpanzees

Key study findings: The following findings were noted in all treatment groups: [1] decreased the antibody response to tetanus toxoid following immunization on Day 2 of the study, [2] paracortical atrophy (CD3+ cell decrease), infiltration of neutrophils into lymph nodes, and reticulum cell hyperplasia of the paracortex in lymph node biopsies (reversal was apparent following the recovery period), and [3] decreased expression of CD11a on the surface of circulating lymphocytes.

Study no.: 961109
Volume #, and page #: Not applicable (electronic submission)
Conducting laboratory and location: [redacted]

Date of study initiation: July 1996
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: hu1124; GH606003-1 and GH608005-1

Methods

Doses: The doses used are shown in the table below.

Group	N	Dose
I	2M + 2F	0 mg/kg
II	2M + 2F	Day 1: 2 mg/kg Days 2 – 14: 2 mg/kg/day Week 3 – 6 months: 8 mg/kg/week
III-A	2M + 2F	Day 1: 40 mg/kg Days 2 – 14: 10 mg/kg/day Week 3 – 6 months: 40 mg/kg/week
III-B	1M + 1F	Day 1: 40 mg/kg Days 2 – 14: 10 mg/kg/day

Species/strain: Chimpanzee
Number/sex/group or time point (main study): See above under "Doses"
Route, formulation, volume, and infusion rate: iv; 4 mg/mL (0.01M sodium acetate and 4% mannitol); 10 mL/kg; 5 – 8 mL/min
Satellite groups used for toxicokinetics or recovery: Yes – Group III-B did not receive test article following Day 14. It was designated as a recovery group. The other groups were observed for an additional 6 months after the last treatment.
Age: 10 – 26 years
Weight (nonrodents only): 38 to 56 kg
Unique study design or methodology (if any): None

Observation times and results

The endpoints monitored in this study are shown in the table below.

Endpoint	Timing
Mortality	Twice daily
Clinical signs	Twice daily
Body weights	Daily for the first 14 days, then weekly thereafter
Food consumption	Daily qualitative assessment
Ophthalmoscopy	Prestudy, Days 13, 98, 182, and 351
EKG	Pretreatment Days 14, 28, 84, 182, 210, and 272
Hematology	Pretreatment Day 1 (4 hours after treatment), 2, 4, 7, 14, 21, 28, 35, 42, 49, 56, 84, 91, 98, 105, 112, 119, 126, 133, 140, 147, 154, 161, 168, 175, and 182
Clinical chemistry	Pretreatment Day 1 (4 hours after treatment), 2, 4, 7, 14, 21, 28, 35, 42, 49, 56, 84, 91, 98, 105, 112, 119, 126, 133, 140, 147, 154, 161, 168, 175, and 182
Coagulation	Pretreatment Day 1 (4 hours after treatment), 2, 4, 7, 14, 21, 28, 35, 42, 49, 56, 84, 91, 98, 105, 112, 119, 126, 133, 140, 147, 154, 161, 168, 175, and 182
Urinalysis	Pretreatment Days 2, 7, 14, 28, 84, and 182
Gross pathology	Not monitored
Organ weights	Not monitored
Histopathology	Not monitored
Toxicokinetics	Pretreatment Days 1 (0, 5 min, 1, 4, and 8 hr) 2 ,3, 5, 7, 10, 14, 15, weekly for Days 21 – 182
Immunogencity	Pretreatment Days 1, 7, 14, 28, 28, 56, 84, 112, 140, 168, and 182
Antibody titers to tetanus toxoid	Day 2: Immunization with tetanus toxoid Prestudy and Days 28, 56, and 343: Analysis for tetanus anti-toxoid
Flow cytometry	Pretreatment Days 1, 2, 3, 4, 7, 9, weekly for Days 14 to 182
Immunopathology	Days 183 and 272

Mortality: One animal from Group II died on Day 153 of the study. Prior to death, this animal exhibited hypoactivity, soft stools to bloody diarrhea, and dehydration. The animal's body weight declined from 34 kg on Day 77 to 27 kg on Day 153. A complete necropsy was conducted within 1 hour of death. The cause of death was considered to be moderate to severe necrotizing inflammation of the small intestine. This finding was considered to be of possible viral origin.

Clinical signs: The animals did not exhibit any treatment-related effects. It was noted in the report that animal CA0102 was not dosed on day 42 do to "animal health concerns". This animal's "overall body condition" was rated as "poor" on Day 42, "fair" on Day 49, then "good" for essentially all of the remaining days in the study. The relationship of the

condition noted on Day 42 to treatment is not clear.

Body weights: The animals did not exhibit any treatment-related effects.

Food consumption: The animals did not exhibit any treatment-related effects.

Ophthalmoscopy: The animals did not exhibit any treatment-related effects.

EKG: The animals did not exhibit any treatment-related effects.

Hematology: The animals did not exhibit any treatment-related effects.

Clinical chemistry: The animals did not exhibit any treatment-related effects.

Urinalysis: The animals did not exhibit any treatment-related effects.

Gross pathology: Not monitored

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

Histopathology: Not monitored

Toxicokinetics: Results are provided in the table below. Presence of detectable levels of efalizumab in the control animals indicates inadvertent exposure to the product occurred during the study. (The table was copied from the submission.)

Table 2: Pharmacokinetic Parameters of hu1124 in Chimpanzees

Group	Animal	Sex	Total Dose mg/kg	Total AUC mg/mL-day	CL mL/day/kg	$T_{1/2}$ Days
1	CA0120	M	1			3.47 ± 0.02
1	CA0155	F	1			3.43 ± 0.01
1	CA0162	M	8			10.21 ± 0.65
1	CA0185	F	8			8.52 ± 0.36
1	Mean		5 ± 2	0.50 ± 0.23	9.88 ± 1.28	6.41 ± 1.74
2	CA0003	F	242			8.07 ± 0.05
2	CA0096	M	242			9.74 ± 0.67
2	CA0145	M	241			8.62 ± 0.34
2	CA0204	F	186			not determined
2	Mean[1]		242 ± 0	28.19 ± 1.30	8.61 ± 0.41	8.81 ± 0.49
3A	CA0017	F	1209			6.16 ± 0.55
3A	CA0102[2]	M	1168			9.84 ± 0.31
3A	CA0126	F	1209			5.74 ± 0.40
3A	CA0158[3]	M	1172			3.66 ± 0.30
3A	Mean		1189 ± 11	141.44 ± 20.33	9.02 ± 1.44	11.35 ± 2.12
3B	CA0050[4]	F	180			7.82 ± 0.21
3B	CA0089	M	169			9.84 ± 0.22
3B	Mean		174 ± 5	18.85 ± 2.52	9.39 ± 0.98	8.83 ± 1.01

[1]Because animal CA0204 died during the study and dosing was not completed, its pharmacokinetic parameters were not included in the mean values.
[2]Not dosed on day 42 due to animal health concerns.
[3]Incompletely dosed on day 49.
[4]Received an excess of dose on the first day.

Immunogenicity: No anti-efalizumab antibodies were detected.

Antibody titers to tetanus toxoid: As shown in the table below, treatment with efalizumab decreased the antibody response to tetanus toxoid following immunization on Day 2 of the study. According to the study report, the nonresponsive animal in the control group, CA010 received a partial dose of efalizumab on Day 1 of the study.

Group	Animal #	Sex	Day Pre	28/29	56	343 - 347
0 mg/kg	CA0120	M	<0.10	1.76	1.63	<0.10
	CA0162		<0.10	<0.10	<0.10	<0.10
	CA0155	F	<0.10	0.29	0.41	<0.10
	CA0185		<0.10	0.20	0.52	<0.10
Day 1: 2 mg/kg	CA0096	M	<0.10	0.07	0.24	<0.10
Days 2 – 14: 2 mg/kg/day	CA0145		<0.10	<0.10	<0.10	<0.10
Week 3 – 6 months: 8 mg/kg/week	CA0003	F	<0.10	<0.10	<0.10	<0.10
	CA0204		0.15	2.50	1.14	----
Day 1: 40 mg/kg	CA0102	M	<0.10	<0.10	0.07	<0.10
Days 2 – 14: 10 mg/kg/day	CA0158		<0.10	<0.10	0.18	<0.10
Week 3 – 6 months: 40 mg/kg/week	CA0017	F	<0.10	<0.10	0.07	<0.10
	CA0126		<0.10	<0.10	0.18	<0.10
Day 1: 40 mg/kg	CA00089	M	<0.10	<0.10	<0.10	---
Days 2 – 14: 10 mg/kg/day	CA00050		<0.10	<0.10	<0.10	---

Flow cytometry: According to the study report, expression of CD11a on the surface of circulating lymphocytes "was reduced, but not completely eliminated, within 24 hours

following dosing" with efalizumab. This reduction persisted as long as efalizumab was present in the plasma. CD11a expression reportedly recovered following plasma clearance of efalizumab. These results could not be confirmed because data were not available for independent review.

Immunopathology: Findings from lymph node biopsies are shown in the table below. Treatment related findings were paracortical atrophy (CD3+ cell decrease), infiltration of neutrophils into lymph nodes, and reticulum cell hyperplasia of the paracortex. Reversal was apparent following the recovery period. (The table was copied from the submission.)

Table 1. Summary Incidence of Lymph Node Changes (sexes combined)

Change	Group 1 Control		Group 2 (8/2/8 mg/kg)		Group 3 (40/10/40 mg/kg)		Group 3 Recovery (40/10/0 mg/kg)	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
Follicular hyperplasia/ CD20 increase	1/4	2/4	0/3	0/3	0/4	0/4	0/2	0/2
Follicular atrophy/ CD20 decrease	0/4	0/4	1/3	1/3	0/4	0/4	0/2	0/2
Paracortical atrophy/CD3 decrease	0/4	0/4	2/3	1/3	3/4	1/3*	1/2	0/2
Reticulum cell hyperplasia, paracortex	0/4	0/4	1/3	1/3	1/4	1/4	0/2	0/2
Sinus histiocytosis	4/4	3/4	3/3	3/3	4/4	4/4	2/2	2/2
Paracortex, Neutrophil infiltration	0/4	0/4	0/3	0/3	3/4	1/4	0/2	0/2
Eosinophil infiltration	0/4	0/4	0/3	0/3	1/4	1/4	0/2	1/2

*paracortex from one sample was too small to evaluate

Study title: hu1124: A single dose re-exposure safety study in chimpanzees **[Comment**: This study is included in the repeat-dose section of this review because it an extension of study 961109.]

Key study findings: Cd11a expression was decreased in all treatment groups.

Study no.: 980309
Volume #, and page #: Not applicable (electronic submission)
Conducting laboratory and location: [redacted]
Date of study initiation: February 1998
GLP compliance: Y
QA report: yes (X) no ( )
Drug, lot #, and % purity: hu1124 (efalizumab); Gc609003-11

Methods

Doses: 2 and 10 mg/kg
Species/strain: Chimpanzee

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

Group	Animal # and sex	Previous treatment	Dose (mg/kg)	N
I	CB0627 (M) CB0556 (F)	Protein naïve	0	2
II	CA0162 (M) CA0185 (F)	Received efalizumab formulation buffer in study 961109; possibly received a low but detectable dose of efalizumab during the first week of study 961109 (July 1996)	10	2
III	CA0145 (M) CA0003 (F)	Treated with efalizumab in study 961109; received 8 mg/kg loading dose on Day, 2 mg/kg on Days 2 to 14, and 8 mg/kg/week thereafter for ~ 6 months (dosing completed February 1997)	2	2
IV	CA0102 (M) CA0183 (M) CA0017 (F)	CA0102 and CA0017: Treated with efalizumab in study 961109; received 40 mg/kg loading dose on Day, 10 mg/kg on Days 2 to 14, and 40 mg/kg/week thereafter for ~ 6 months CA0183: Received a single 10 mg/kg of efalizumab in study 960809 (June 1996)	10	3

Route, formulation, volume, and infusion rate: iv; 4 mg/mL in 0.01M sodium acetate and 4% mannitol; 2.5 mL/kg; 8 mL/min

Satellite groups used for toxicokinetics or recovery: No

Age: 10 to 28 years

Weight (nonrodents only): 27 to 59 kg

Unique study design or methodology (if any): This study was conducted in animals that were previously exposed to efalizumab. The primary objectives were to [1] evaluate the potential for allergic or other adverse reactions upon re-exposure following an extended treatment-free period and [2] to monitor for the production of anti-efalizumab antibodies.

Observation times and results

Endpoint	Timing
Mortality	Twice daily
Clinical signs	Twice daily
Body weights	Days 1, 2, 29, 71, and 99
Food consumption	Daily qualitative assessment
Physical exams (body temperature, vital signs, pulse rate, respiration rate, and systolic and diastolic blood pressure)	Days 1, 2, 29, 71, an d99
Body temperatures	Pretest Study Day 1 (day of dosing): ` 5 min intervals

Endpoint	Timing
	during the infusion, after the completion of the infusion, and at 4, 15, and 30 min and 2, 4, and 6 hours after dosing.
Hematology	Prestudy Days 1, 2, 29, 57, and 85
Clinical chemistry	Prestudy Days 1, 2, 29, 57, and 85
Urinalysis	Not monitored
Gross pathology	Not monitored
Organ weights	Not monitored
Histopathology	Not monitored
Toxicokinetics	Day 1 (prior to dosing, 5 min and 6 hr after dosing), 2, 8, 29, 43, 57, 71, 85, and 99
Immunogencity	Days 1, 8, 29, 57, 71, 85, and 99
Antibody titers to tetanus toxoid	Day 1 (prior to treatment) = Measurement of anti-tetanus antibodies (According to the sponsor's summary on page 11/39 in section 2.6.6.3 of the electronic submission, animals were immunized with tetanus toxoid approximately 1 year the last dose of efalizumab they received in the study to which they were previously assigned. However, the specific time of immunization was not clear.)
Flow cytometry	Pretest, Days 1 (prior to dosing, 6 hr), 2, 8, 29, 43, 57, 71, 85, and 99

Mortality: No mortality occurred during the study.

Clinical signs: The animals did not exhibit any treatment-related effects.

Body weights: The animals did not exhibit any treatment-related effects.

Food consumption: The animals did not exhibit any treatment-related effects.

Physical Exams: The animals did not exhibit any treatment-related effects.

Ophthalmoscopy: Not monitored

EKG: Not monitored

Hematology: The animals did not exhibit any treatment-related effects

Clinical chemistry: The animals did not exhibit any treatment-related effects

Urinalysis: Not monitored

Gross pathology: Not monitored

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

Histopathology: Not monitored

Toxicokinetics: The clearance and $t_{1/2}$ of 1/3 chimpanzees definitely exposed to efalizumab in previous studies, CA0183, was significantly decreased relative to those obtained following the its initial exposure to efalizumab in the previous study. Consistent with this observation, this animal had anti-efalizumab antibodies.

Immunogenicity: Only one animal, CA0183, exhibited an antibody response to efalizumab.

Antibody titers to tetanus toxoid: [Comment: According to the sponsor's summary on page 11/39 in section 2.6.6.3 of the electronic submission, animals were immunized with tetanus toxoid approximately 1 year the last dose of efalizumab they received in the study to which they were previously assigned.] As shown in the table below, the effects observed in study 961109 were reversible.

Group	Animal #	Sex	Ab
1	CB0627	M	0.13
	CB0556	F	< 0.1
			> 3.0
2	CA0162	M	> 3.0
	CA0185	F	> 3.0
3	CA0145	M	> 3.0
	CA0003	F	> 3.0
4	CA0102	M	> 3.0
	CA0017	F	> 3.0
	CA0183	M	< 0.1

Flow cytometry: Within 24 hours of infusion, CD11a expression was reduced in animals receiving 2 mg/kg and 10 mg/kg. In the group receiving 2 mg/kg re-expression was apparent by Day 29. In the animals receiving 10 mg/kg, with the exception of animal # CA0183, re-expression occurred by Day 57 or 71. Re-expression occurred by Day 43 in animal CA0183, presumably due to the presence of anti-efalizumab antibodies.

Study title: hu1124: A 5-day intravenous dose-range finding study in chimpanzees

Key study findings: CD11a expression decreased to ~10% of the pretreatment level following the first dose and remained suppressed at this level through Day 28/49. Recovery began at Day 49/56, with values reaching pretreatment levels by Day 56/63. The reduced level of CD11a present was saturated with efalizumab.

Study no.: 960809
Volume #, and page #: Not applicable (electronic submission)
Conducting laboratory and location:
Date of study initiation: June 1996

GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: hu1124; GC606001-1

Methods

Doses: 2 mg/kg daily for 5 days
Species/strain: Chimpanzee
Number/sex/group or time point (main study): 1/sex/group
Route, formulation, volume, and infusion rate: iv; 1 mg/mL; 2 mL/kg; 7 to 9 mL/min
Satellite groups used for toxicokinetics or recovery: None
Age: 10 to 26 years
Weight (nonrodents only): 25 to 34 kg
Unique study design or methodology (if any): There was no vehicle control group in this study.

Observation times and results

Endpoint	Timing
Mortality	Daily
Clinical signs	Daily
Body weight	Days 1 – 5, 6, 7, 10, 14, 21, 28, and weekly from Day 49 - 147
Food consumption	Daily
Ophthalmoscopy	Days 2, 7, 14, and 28
EKG	Days 2, 7, 14, and 28
Hematology	Days 1 (pre-dose and 4 hours), 2, 6, 28, and every 2 weeks from Day 70 - 140
Clinical chemistry	Days 1 (pre-dose and 4 hours), 2, 6, and 28, and every 2 weeks from Day 70 to 140
Coagulation	Days 1 (pre-dose and 4 hours), 2, 6, and 28
Urinalysis	Pre-dose, Days 2, 6, and 14
Gross pathology	Not monitored
Organ weights	Not monitored
Histopathology	Not monitored
Toxicokinetics	Days 1 (0, 5, and 15 min, and 1, 4, and 8 hours), 2 – 4(0 and 15 min), 5 (0 and 15 min, and 1, 4, 8, and 24 hours), 6, 7, 10, 14, 21, 28, 49, 56, and 63
Immunogenicity	Days 1 (pre-dose), 6, 14, and 28 Weekly for Days 70 - 147
Tetanus anti-toxoid levels	Day 70: 1 animal/group immunized with toxoid Day 119: 1 animal/group immunized with toxoid Days 77, 84, 91, 98, 105, 119, 126, 133, 140, and 147: anti-toxoid levels measured
Mixed lymphocyte reactions	Days 1 (0, 1, 4, and 8), 2 – 4(0 and 15 min), 5 (0 and 15 min, and 1, 4, 8, and 24 hours), 6, 7, 10, 14, 21, 28, and 49, 56, 63, and 70
Lymphocyte blastogenesis transformation	Days 49, 56, 63, and 70
Flow cytometry	Days 1 (0, 1, 4, and 8 hours), 2 – 4(0 and 15 min), 5

Endpoint	Timing
	(0 and 15 min, and 1, 4, 8, and 24 hours), 6, 7, 10, 14, 21, and 28, and weekly from Days 49 to 70

Mortality: There were no mortalities in this study.

Clinical signs: The animals did not exhibit any treatment-related effects.

Body weights: Treatment had no effect on body weight.

Food consumption: Treatment had no effect on food consumption.

Ophthalmoscopy: The animals did not exhibit any treatment-related effects.

EKG: The animals did not exhibit any treatment-related effects.

Hematology: The animals did not exhibit any apparent treatment-related effects.

Clinical chemistry: The animals did not exhibit any treatment-related effects.

Urinalysis: The animals did not exhibit any treatment-related effects.

Gross pathology: Not monitored

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

Histopathology: Not monitored

Toxicokinetics: Toxicokinetics are shown in the tables below. The animals that received 5 doses were CA0154 and CA0208. (The tables were copied from the submission.)

Animal	AUC mg/mL-day	Vc mL/kg	Vss mL/kg	Clearance mL/kg/day	plasma MRT days	MRT days
CA0183	1.94 ± 0.16[1]	36.0 ± 3.9	98.8 ± 7.9	5.13 ± 0.43	7.02 ± 0.97	19.25 ± 2.45
CA0213	1.11 ± 0.05	48.9 ± 3.2	116.9 ± 8.4	9.03 ± 0.42	5.41 ± 0.43	12.95 ± 1.13
CA0154	1.19 ± 0.05	46.5 ± 2.4	123.6 ± 4.5	8.38 ± 0.35	5.55 ± 0.37	14.74 ± 0.87
CA0208	1.23 ± 0.07	46.9 ± 4.5	106.4 ± 5.1	8.09 ± 0.44	5.79 ± 0.65	13.15 ± 0.94
mean	1.37 ± 0.19[2]	44.6 ± 2.9	111.4 ± 5.5	7.66 ± 0.86	5.94 ± 0.37	15.02 ± 1.48

Table 2 Continued: Pharmacokinetic parameters values of hu1124 in Chimpanzees

Animal	$t_{1/2}\alpha$ hours	$t_{1/2}\beta$ days	FAUCα	FAUCβ
CA0183	2.91 ± 1.02[1]	13.6 ± 1.7	0.016 ± 0.005	0.984 ± 0.005
CA0213	16.29 ± 3.91	10.1 ± 1.0	0.122 ± 0.029	0.878 ± 0.029
CA0154	3.94 ± 0.80	10.5 ± 0.6	0.027 ± 0.005	0.973 ± 0.005
CA0208	1.35 ± 0.62	9.2 ± 0.7	0.008 ± 0.003	0.992 ± 0.003
mean	6.12 ± 3.43[2]	10.8 ± 0.9	0.043 ± 0.027	0.957 ± 0.027

[1]Standard error from individual curve fit.
[2]Standard error from mean value of parameters of individual curve fits.

AUC=area under the curve
Vc=volume of distribution of the central compartment
Vss=steady state volume of distribution
CL=clearance
plasma MRT=plasma mean residence time
MRT=total body mean residence time
$t_{1/2}\alpha$=alpha half-life
$t_{1/2}\beta$=beta half-life
FAUCα=fraction of AUC contributed by the α-phase
FAUCβ=fraction of AUC contributed by the β-phase

Immunogenicity: Only 1 animal exhibited antibodies to efalizumab

Tetanus anti-toxoid levels: The animals exhibited anti-tetanus toxoid antibodies.

Mixed lymphocyte reaction: No effects were apparent.

Lymphocyte blastogenesis transformation: No effects were apparent.

Flow cytometry: CD11a expression decreased to ~10% of the pretreatment level following the first dose and remained suppressed at this level through Day 28/49. Recovery began at Day 49/56, with values reaching pretreatment levels by Day 56/63. The reduced level of CD11a present was saturated with efalizumab.

MOUSE

Study title: A six-month subcutaneous toxicology study of muM17 in TSG p53® wild type mice

Key study findings: Males and females in all dose groups exhibited increased WBCs, lymphocytes, neutrophils, eosinophils, and platelet volume and decreased platelets.

Females in all treatment groups exhibited a decrease in globulin (10% to 20% with no dose response) and an increase in albumin/globulin (A/G) ratio (10% to 20% with no dose response). Male and female mice in all treatment groups in the main study exhibited an increase (10% to 20%) in absolute and relative spleen weight. The treatment-related histopathologic changes observed in the high dose group were as follows: [1] hypercellularity of the splenic white pulp and [2] decreased lymphocytic infiltration of various organs (pancreas, mandibular salivary gland, and kidney).

Study no.: 01-292-1049
Volume #, and page #: Not applicable (electronic submission)
Conducting laboratory and location:
Date of study initiation: March 4, 2002
GLP compliance: Yes
QA report: yes (Y) no ( )
Drug, lot #, and % purity: muM17; M3-TOX51 and M3-TOX55

Methods

Doses: 0, 3, 10, and 30 mg/kg/week for 6 months
Species/strain: TSG-p53®Wild type
Number/sex/group or time point (main study): 0 and 30 mg/kg = 36/sex
3 and 10 mg/kg = 24/sex
Route, formulation, volume, and infusion rate: sc; 0, 0.3, 1 and 3 mL/kg; 10 mL/kg; bolus
Satellite groups used for toxicokinetics or recovery: Y
Age: 37 to 51 days
Weight (nonrodents only): NA
Unique study design or methodology (if any):

Observation times and results

Endpoint	Timing
Mortality	2X daily
Clinical signs	Daily during treatment Weekly during recovery
Body weights	Weekly
Food consumption	Not conducted
Ophthalmoscopy	Not conducted
EKG	Not conducted
Hematology	Predosing, Days 29, 85, 141, and 183 (Main study animals) Predosing, Days 29, 85, 141, 190, and 260 (recovery animals)
Clinical chemistry	Day 183 (main study animals) Day 260 (recovery animals)
Urinalysis	Not conducted
Termination	Day 183 (7 days after the last dose)(main study animals) Day 260(recovery animals)
Gross pathology	At termination
Organ weights	At termination

Endpoint	Timing
Histopathology	At termination
Toxicokinetics	Predose, Days 36, 92, 148, and 162 (main study animals) Predose, Days 36, 92, 148, 183, and 246 (recovery animals)
Immunogenicity	Predose, Days 36, 92, 148, and 162 (main study animals) Predose, Days 36, 92, 148, 183, and 246 (recovery animals)

Mortality: The deaths that occurred during the study were not considered to be treatment-related. One female mouse in the high dose group was found dead on Day 141 of the study. Histopathology revealed changes consistent with a disseminated infection of unknown origin. The relationship of this death to treatment is not known.

Clinical signs: The animals did not exhibit any treatment-related effects.

Body weights: The animals did not exhibit any treatment-related effects.

Food consumption: Not monitored

Ophthalmoscopy: Not monitored

EKG: Not monitored

Hematology: Males and females exhibited increased WBCs, lymphocytes, neutrophils, eosinophils, and platelet volume and decreased platelets. The effects in the two genders were qualitatively similar but generally more pronounced in the females than in the males. The effects observed in females are summarized in the table below. No effects were observed at the end of the 3-month recovery period.

		Dose		
Time	Endpoint	3	10	30
1 month	WBC	↑38% (S)	↑47% (S)	↑38% (S)
	Lymphocytes	↑35% (S)	↑45% (S)	↑36% (S)
	Neutrophils	↑45% (S)	↑36% (S)	↑27% (S)
	Eosinophil	↑100% (S)	↑150% (S)	↑100% (S)
	Platelets	↓9% (S)	↓7% (S)	↓14% (S)
	Platelet volume	↑7% (S)	↑6% (S)	↑11% (S)
3 months	WBC	↑35% (NS)	↑73% (S)	↑63% (S)
	Lymphocytes	↑28% (NS)	↑48% (S)	↑46% (S)
	Neutrophils	↑45% (NS)	↑266% (S)	↑183% (S)
	Eosinophil	↑100% (S)	↑100% (S)	↑100% (S)
	Platelets	↓11% (S)	↓7% (NS)	↓9% (NS)
	Platelet volume	↑6% (S)	↑9% (S)	↑6% (S)
5 months	WBC	↑21% (NS)	↑57% (S)	↑55% (S)
	Lymphocytes	↑19% (NS)	↑43% (S)	↑41% (S)
	Neutrophils	↑35% (NS)	↑157% (S)	↑157% (S)
	Platelets	↓19% (S)	↓20% (S)	↓26% (S)
	Platelet volume	↑11% (S)	↑10% (S)	↑13% (S)
Main study	WBC	↑2% (NS)	↑12% (NS)	↑14% (NS)

Time	Endpoint	Dose		
		3	10	30
termination	Lymphocytes	↑13%(NS)	↓5%(NS)	↓6% (NS)
	Neutrophils	No change	↑22% (NS)	↑55% (NS)
	Eosinophils			
	Platelets	↓20% (S)	↓ 12% (S)	↓19% (S)
	Platelet volume	↑10% (S)	↑12% (S)	↑12% (S)
6.5 months	WBC	---	---	↑78% (S)
	Lymphocytes	---	---	↑71% (S)
	Neutrophils	---	---	↑116% (S)
	Eosinophils	---	---	↑100% (NS)
	Platelets	---	---	↓18% (S)
	Platelet volume	---	---	↑8% (S)

S = statistically significant
NS = not statistically significant

Clinical chemistry: The males did not exhibit any effects. Females in all treatment groups exhibited a decrease in globulin (10% to 20% with no dose response) and an increase in albumin/globulin (A/G) ratio (10% to 20% with no dose response). Statistical significance was reached for all differences except for A/G in the high dose group. The difference (15%↓ in globulin and 11%↑ in A/G) persisted through the recovery period.

Urinalysis: Not monitored

Gross pathology: The animals did not exhibit any treatment-related effects.

Organ weights (specify organs weighed if not in histopath table): Male and female mice in the main study group exhibited an increase (10% to 20%) in absolute and relative spleen weight. The differences were statistically significant with the exception of spleen/body weight for the high dose males. A dose-response relationship was not apparent in either gender. No effect was observed in the recovery animals.

Histopathology: Gross lesions were evaluated from animals in all treatment groups. All tissues were examined for the control and high dose group.

The treatment-related histopathologic changes observed in the high dose group were as follows: [1] hypercellularity of the splenic white pulp and [2] decreased lymphocytic infiltration of various organs (pancreas, mandibular salivary gland, and kidney). Data are shown in the table below. In some cases, the decrease in mixed cell/lymphocytic infiltration of persisted through the recovery period (3 months following the final dose).

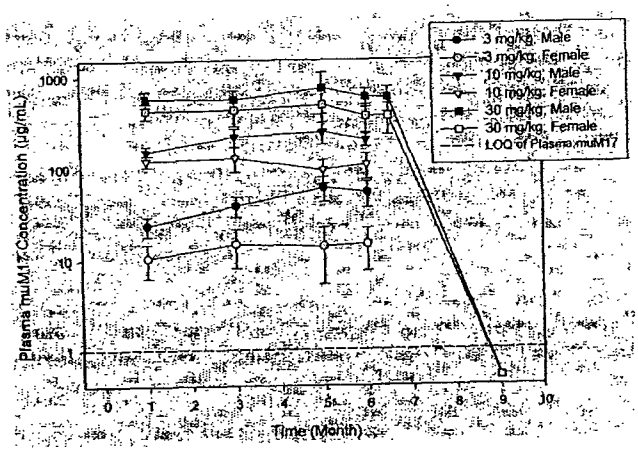
Finding	Male				Female			
	0	3	10	30	0	3	10	30
Liver								
Infiltrate, mixed cell	18/24			3/24	21/24			8/23
Infiltrate, lymphocytic	2/24			0/24	4/24			0/23
Spleen								
Lymphocytic, hyperplasia	8/24			13/24	11/24			12/23
Salivary gland (mandibular)								
Infiltrate, mixed cell	18/24			1/24				

Finding	Male				Female			
	0	3	10	30	0	3	10	30
Infiltrate, lymphocytic	1/24			0/24	12/23			2/23
Pancreas								
Infiltrate, lymphocytic	7/24			0/24	8/24			0/23
Infiltrate, mixed cell					2/24			0/23
Lungs								
Infiltrate, mixed cell	3/24			0/24	5/24			4/23
Kidneys								
Infiltrate, lymphocytic	14/24			0/24	12/24			1/23
Prostate								
Infiltrate, lymphocytic, interstitial	7/24			0/24				
Infiltrate, lymphocytic, mesentery	1/24			0/24				

Toxicokinetics: Trough levels are shown in the chart below. With the exception of one animal, plasma levels of muM17 were below the limit of quantitation at the 9 month time point. It should be noted that detailed pharmacokinetics have not been conducted in this strain of mouse.

Plasma trough concentrations were higher in males than in females and increased in a greater-than-dose-proportional manner in both sexes. The mean trough concentration for patients receiving the receiving the clinical dosing regimen (0.7 mg/kg sc followed by weekly injections of 1 mg/kg sc) was 9 ug/mL. (The chart was copied from the submission.)

Mean (±SD) Trough Plasma muM17 Concentrations Following Weekly Subcutaneous Dose of 3, 10, or 30 mg/kg muM17 for 6 Months in TSG-P53® Mice (n=24–36)



Note: Half of LOQ value was used as the first observed group LTR value.

Immunogenicity: There were no anti-muM17 antibodies detected in any of the animals.

Study title: One-month subcutaneous toxicology study of muM17 in TSG-p53® wild type mice

Key study findings: TSG-p53® wild type mice receiving 3, 10, or 30 mg/kg/week sc injections of muM17 exhibited the following one week after the last injection: [1]

increased absolute and relative spleen weight and [2] increased cellularity in the splenic white pulp, and [3] decreased cellularity in paracortex, lymphoid follicles, and interfollicular area of the mandibular and mesenteric lymph nodes.

Study no.: 01-229-1049
Volume #, and page #: Not applicable (electronic submission)
Conducting laboratory and location: [redacted]
Date of study initiation: October 22, 2001
GLP compliance: Yes
QA report: yes (Y) no ( )
Drug, lot #, and % purity: muM17; M3-TOX41

Methods

Doses: 0 (vehicle), 3, 10, or 30 mg/kg/week for 4 weeks
Species/strain: Mouse/ TSG-p53® wild type
Number/sex/group or time point (main study): 10
Route, formulation, volume, and infusion rate: sc; 0.3, 1.0, and 3 mg/mL; 10 mL/kg; bolus
Satellite groups used for toxicokinetics or recovery: No
Age: 37 to 44 days
Weight (nonrodents only): NA
Unique study design or methodology (if any): None

Observation times and results

Endpoint	Timing
Mortality	2X daily
Clinical signs	2X daily
Body weights	Weekly
Food consumption	Not monitored
Ophthalmoscopy	Not monitored
EKG	Not monitored
Hematology	Day 29 (5 mice/group)
Clinical chemistry	Day 29 (5 mice/group ---- different from those used for hematology)
Urinalysis	Not monitored
Termination	1 week after the last dose
Gross pathology	At termination
Organ weights	At termination
Histopathology	At termination
Toxicokinetics	Day 1: 5/sex/group Day 29: all mice
Immunogenicity	Day 1: 5/sex/group Day 29: all mice

Mortality: The animals did not exhibit any treatment-related effects.

Clinical signs: The animals did not exhibit any treatment-related effects.

Body weights: The animals did not exhibit any treatment-related effects.

Food consumption: Not monitored

Ophthalmoscopy: Not monitored

EKG: Not monitored

Hematology: Male mice in all treatment groups exhibited a decrease in platelet count (~15%), with no dose response. Platelet volume was slightly increased. All differences were statistically significant with the exception of platelet volume in the high dose group.

In the female mice, platelet counts were significantly decreased (~19%) in the low dose group only. In the mid and high dose females, the platelets were slightly decreased (~6%) relative to the control group. Platelet volume was significantly decreased in all female treatment groups (13%, 10%, and 6% for the low, mid and high dose groups, respectively).

Clinical chemistry: The animals did not exhibit any treatment-related effects.

Urinalysis: Not monitored

Gross pathology: The animals did not exhibit any treatment-related effects.

Organ weights (specify organs weighed if not in histopath table): In males, absolute and relative spleen weights were increased by ~20% in all treatment groups, with no dose response. In female mice, absolute and relative spleen weights were increased by ~28%, ~22%, and ~18% in the low, mid and high dose groups, respectively. All differences for both genders were statistically significant.

Histopathology: Males and females in all treatment groups exhibited treatment-related effects in the spleen and mesenteric and mandibular lymph nodes. The effects observed in the spleen were minimal to mild increased cellularity of the white pulp and minimal to mild red pulp congestion. There was no dose response. The effects observed in the lymph nodes were minimal to marked decreased cellularity in the paracortex, lymphoid follicles, and interfollicular area in all animals. It should be noted that decreased lymph node cellularity was not noted in the 6-month study (01-292-1049).

Toxicokinetics: Summary toxicokinetics data (mean ± SD) are shown in the table below. A greater than dose proportional increase in plasma levels was observed in both males and females. In the 3 and 10 mg/kg groups, plasma levels in males were greater than those in females.

Dose and Collection Time	Plasma muM17 concentration (ug/mL)	
	Male	Female
7 days after 4 weekly 3 mg/kg doses	34.6 ± 8.58	8.27 ± 5.36
7 days after 4 weekly 10 mg/kg doses	214 ± 52.9	141 ± 12.7
7 days after 4 weekly 30 mg/kg doses	854 ± 218	789 ± 287

Immunogenicity: No anti-muM17 antibodies were detected. However, the possibility of muM17 interfering with the detection of antibodies cannot be excluded.

Study title: Multiple dose safety and toxicokinetics of an anti-mouse CD11a (muM17) antibody in CD1 mice

Key study findings: Treatment of female CD-1 mice with 0.1 mg/kg/week of muM17 for 4 weeks did not result in any effects. Treatment with 1 mg/kg resulted in a 50% decrease in CD11a expression but no change in WBC parameters. Treatment with 10 mg/kg resulted in a 90% decrease in CD11a expression and a transient increase in circulating neutrophils and lymphocytes.

Study no.: 99-362-1047
Volume #, and page #: No applicable (electronic submission)
Conducting laboratory and location: Genentech Inc., South San Francisco, CA
Date of study initiation: December 8, 1999
GLP compliance: No
QA report: yes ( ) no (X)
Drug, lot #, and % purity: muM17; Lot# 33775-29

Methods

Doses: 0 (untreated), 0 (muM17 vehicle), 0.1, 1 and 10 mg/kg on Days 1, 7, 14, and 21

Species/strain: Mouse, CD-1

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

Treatment	N	Scheduled sacrifice days
Untreated	4 F	Pre-dose
muM17 vehicle	19F	7, 14, 21, 28, 56
muM17 0.1 mg/kg	16F	7, 14, 21, 28
muM17 1 mg/kg	16F	7, 14, 21, 28
Mum17 10 mg/kg	16F	7, 14, 21, 28, 56
Untreated	4F	56

Route, formulation, volume, and infusion rate: sc; 7.6 mg/mL; 5 mL/kg; bolus

Satellite groups used for toxicokinetics or recovery: No

Age: 106 days

Weight (nonrodents only):

Unique study design or methodology (if any): Toxicokinetics data were collected for the 10 mg/kg group only.

Observation times and results

The endpoints monitored are shown in the table below:

Endpoint	Timing
Antemortem observations	Weekly at the time of dosing
Body weights	On the days of dosing and at termination
Hematology	Days 7, 14, 21, 28, and 56
Clinical chemistry	Days 7, 14, 21, 28, and 56
Gross pathology	At termination
Organ weights	At termination
Histopathology	Days 7, 14, 21, and 28(thymus and spleen only)
Flow cytometry	Days 7, 14, 21, and 28
Toxicokinetics	Days 7, 14, 21, 28, and 56 (trough levels)(10 mg/kg only)
Immunogenicity	Days 7, 14, 21, 28, and 56

Antemortem observations: The animals did not exhibit any treatment-related effects.

Body weights: The animals did not exhibit any treatment-related effects.

Hematology: The mice in the 0.1 and 1 mg/kg groups did not exhibit any treatment-related effects. In the 10 mg/kg group, WBC count was increased approximately 3-fold relative to the vehicle control group. The increase in WBC was due to an increase in lymphocytes of approximately 4-fold and an increase in neutrophils of approximately 2-fold. From Day 14, the WBC counts in the 10 mg/kg group did not differ from the vehicle treated group.

Clinical chemistry: The animals did not exhibit any treatment-related effects.

Gross pathology: The animals did not exhibit any treatment-related effects.

Histopathology: The animals did not exhibit any treatment-related effects.

Toxicokinetics: Plasma concentrations (mean ± SD) of muM17 in the 10 mg/kg group are shown in the table below.

Study Day	Plasma concentration (ug/mL)
7	63.47 ± 10.53
14	124.37 ± 25.17
21	119.30 ± 39.84
28	169.03 ± 11.32
56	20.57 ± 12.33

CD11a expression on T lymphocytes: Treatment with 0.1 mg/kg had no effect on the expression of CD11a. In the mice receiving 1 mg/kg, CD11a was down-modulated 50% by Day 14 and remained decreased until Day 21, the day of termination. In the mice receiving 10 mg/kg, CD11a levels were down-modulated 90% by Day 7 and remained at

that level through Day 56, 35 days after the last injection, when that group was sacrificed.

Immunogenicity: Antibodies to muM17 were detected in 1 animal only.

Study title: Multiple dose safety and toxicokinetics of anti-mouse CD11a (muM17) antibody in female CD1 mice

Key study findings: CD1 mice received a weekly 3 or 30 mg/kg sc injection of muM17 for 4 weeks. CD11a expression was down modulated (~90%) by both doses. The animals exhibited a dose-dependent recovery.

Study no.: 00-297-1047
Volume #, and page #: Not applicable (electronic submission)
Conducting laboratory and location: Genentech, Inc., South San Francisco, CA
Date of study initiation: July 25, 2000
GLP compliance: No
QA report: yes ( ) no (X)
Drug, lot #, and % purity: muM17; Lot# 33775-29, purity not provided

Methods

Doses: 0, 3, and 30 mg/kg, sc, on Days 1, 7, 14, and 21
Species/strain: Mouse CD-1
Number/sex/group or time point (main study):

Group	Treatment	N	Scheduled sacrifice days
1	Untreated	10F	Pre-dose
2	muM17 vehicle	30F	7, 14, 21, 28, 45, 85, 87, 90, 92, 97
3	muM17 3 mg/kg	48F	7, 14, 21, 28, 35, 42, 43, 45, 48, 49, 50, 51, 52, 56
4	muM17 3 mg/kg	4F	Pre-dose, 28, 45
5	muM17 30 mg/kg	46F	28, 35, 42, 56, 63, 70, 77, 78, 84, 85, 87, 90, 92, 95, 97
6	muM17 30 mg/kg	4F	Predose, 28 and 92

Route, formulation, volume, and infusion rate: sc; 0.3 and 30 mg/mL; 10 mL/kg; bolus
Satellite groups used for toxicokinetics or recovery:
Age: 65 – 75 days
Weight (nonrodents only):
Unique study design or methodology (if any):

Observation times and results

Endpoint	Timing
Mortality	Weekly
Clinical signs	Weekly
Body weights	Weekly

Endpoint	Timing
Hematology	Pre-dose Week 7 (Day 45): 3 mg/kg group Week 15 (Day 92) 30 mg/kg group
Toxicokinetics	3 mg/kg: Days 7, 14, 21, 28, 35, 42, 43, 45, 48, 49, 50, 51, 52, 56 30 mg/kg: Days 28, 35, 42, 56, 63, 70, 77, 78, 84, 85, 87, 90, 92, 95, 97
Immunogenicity	Day 7
CD11a expression in peripheral blood	No treatment: Days -5, 35, 36, 42 – 44, 49 – 52, 63, 70, 77, 78, 84, 87, 90, 92, 97 Vehicle: Days 7, 14, 21, 28, 45, 85, 90, 92, 97 3 mg/kg: Days 7, 14, 21, 28, 35, 42, 43, 45, 48 – 52, 56 30 mg/kg: Days 28, 35, 56, 63, 70, 77, 78, 84, 85, 87, 90, 92, 97
Peripheral lymph node (CD3, CD4, CD8, CD11a, CD18, CD28, CD69) and thymic analysis (CD4, CD8, CD4/8, CD11a, CD5)	3 mg/kg only: Days 42, 49, 51, and 52

Mortality: The animals did not exhibit any effects.

Clinical signs: The animals did not exhibit any effects.

Body weights: The animals did not exhibit any effects.

Hematology: The animals did not exhibit any treatment-related effects.

Toxicokinetics: In the mice receiving 3 mg/kg, plasma levels were below the limit of quantitation (~1g/mL) within 3 weeks after the last dose. In the mice receiving 30 mg/kg plasma levels were detected in some of the mice approximately 6 weeks after the last injection.

Immunogenicity: No anti-muM17 antibodies were detected.

CD11a expression in peripheral blood: In the mice receiving 3 mg/kg, CD11a expression was decreased to 7.5% of baseline following the first treatment and remained down-modulated by ~90% for approximately 2 weeks after the last injection. Within 3 to 5 weeks after the last injection, CD11a expression was within the normal range for most mice. Three mice expressed CD11a at levels exceeding the normal range.

In mice receiving 30 mg/kg, CD11a expression was down-modulated ~ 80% one week after the last injection and remained at that level for ~ 8 weeks after the last injection in most of the animals. By Weeks 12 to 14, approximately one-half of the mice expressed CD11a at levels exceeding the normal range of the control animals. The remaining animals were either in the normal range (3/20) or were maximally down-modulated.

Peripheral lymph node and thymic analysis: The animals did not exhibit any effects.

3.4.4. Genetic toxicology

The sponsor did not conduct any studies for this category. Based on ICH S6, Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals, genetic toxicology studies were not needed for efalizumab.

3.4.5. Carcinogenicity

The sponsor did not conduct any studies for this category. Based on ICH S6, Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals, carcinogenicity bioassays are generally inappropriate for biotechnology-derived products.

3.4.6. Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Subcutaneous fertility and general reproductive toxicity study of muM17 in mice, including immunological evaluations

Key study findings: Treatment with muM17 had no effect on reproductive endpoints. In addition to assessing the effect of muM17 on reproductive function, the sponsor assessed the effect of treatment on the immune system. This assessment was limited to the male animals. Treatment with all doses levels (3, 10 and 30 mg/kg/week) resulted in a significant reduction (95% to 99%) in the primary antibody response to sheep red blood cells. This reduction did not appear to be due a decreased number of T-cells or B-cells.

Study no.: 01-006-1049
Volume #, and page #: Not applicable (electronic submission)
Conducting laboratory and location: —

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Date of study initiation: February 17, 2001
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: muM17, M3-TOX35

Methods

Doses: 0, 3, 10, and 30 mg/kg/week
Species/strain: Mouse, Crl:CD1 —
Number/sex/group: 0 = 50M + 25F; 3, 10, and 30 mg/kg = 25/sex
Route, formulation, volume, and infusion rate: sc; 0.3, 1, and 3 mg/mL; 10 mL/kg; bolus
Satellite groups used for toxicokinetics: None