

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

BLA APPLICATION NUMBER:

125156

PHARMACOLOGY REVIEW



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER: 125156
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 12/29/2005
DRUG NAME: Lucentis™, ranibizumab
INDICATION: Neovascular age-related macular degeneration
SPONSOR: Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080-4990
Tel: 650-225-1202; Fax: 650-467-3198
DOCUMENTS REVIEWED: Nonclinical Studies
REVIEW DIVISION: Division of Anti-Infective and Ophthalmology Products (HFD-520)
PHARM/TOX REVIEWER: Zhou Chen, MD, PhD
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PROJECT MANAGER: Lori Gorski

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

Approval is recommended.

B. Recommendation for nonclinical studies

No recommendation is necessary.

C. Recommendations on labeling

After a careful review of the draft labeling proposed by the sponsor, the reviewing pharmacologist considers that the pharmacology/toxicology-related parts of the labeling are acceptable. No modifications are recommended.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Ranibizumab is a recombinant, humanized monoclonal IgG1 antibody antigen-binding fragment (Fab) designed to bind and inhibit all active forms of human vascular endothelial growth factor (VEGF). Pharmacology studies demonstrated the high apparent binding affinity of ranibizumab for all active forms of rhVEGF (rhVEGF₁₆₅, rhVEGF₁₂₁, and rhVEGF₁₁₀) and the inhibition of VEGF-mediated endothelial cell proliferation, tissue factor up-regulation and vessel leakage.

In PK studies conducted in rabbits and monkeys, following intravitreal (ITV) administration, ranibizumab was present in vitreous humor, aqueous humor, all layers of the retina, and other ocular tissues (ciliary body, iris, corneal endothelium) as well as in serum. The terminal t_{1/2} was approximately 2–3 days in all ocular matrices in both species. Concentrations in serum following ITV injection were more than one thousand fold lower than concentrations in vitreous humor. Serum levels declined in parallel with the concentrations in the ocular compartments.

ITV toxicology studies of up to 26 weeks in duration were conducted in cynomolgus monkeys and rabbits. Ranibizumab-related effects were limited to ocular tissues. Administration of ranibizumab to monkeys resulted in inflammatory reactions evidenced by transient, dose-dependent anterior chamber flare, cellular responses and the appearance of vitreal cells and floaters. The severity of this inflammation varied from minimal to severe, generally increasing in severity as the dose of ranibizumab increased. In contrast, a low incidence of inflammation was observed in rabbits administered a single ITV injection of ranibizumab. In monkeys, two forms of posterior segment changes, perivenous retinal hemorrhage and perivascular sheathing, were observed. Although predose and post-dose treatment with corticosteroids by oral and ocular topical routes did not alter the inflammatory response, evidence of reversibility of the ocular inflammation was observed during recovery periods.

B. Pharmacologic activity

Ranibizumab is a recombinant, humanized antibody antigen-binding fragment (Fab) that selectively binds with high affinity to human VEGF and neutralizes the biological activities of human VEGF by blocking the binding of VEGF to its receptors. Pharmacology studies demonstrated the effects of ranibizumab on the inhibition of VEGF-mediated endothelial cell proliferation, tissue factor up-regulation and vessel leakage. Studies in the non-human primate eye in a laser-induced CNV (choroidal neovascularization) model demonstrated the ability of ranibizumab to limit the development of CNV lesions and reduce vascular permeability.

C. Nonclinical safety issues relevant to clinical use

Ranibizumab-related effects were limited to ocular tissues. Ranibizumab given by repeated ITV injection caused inflammation in both anterior and post segments of monkey eyes. The inflammation was dose-dependent. Results from four monkey studies demonstrated that 0.5 mg ranibizumab/eye was the maximum tolerated dose. In these studies, the incidence and severity of anterior chamber inflammation and vitreous inflammatory cell findings at 500 µg/eye were much less than those seen at higher doses. In histopathological examinations, the degree of inflammatory cell infiltration was mostly minimal to slight, and the incidence was less than in higher dose groups. Evidence of reversibility of the ocular inflammation was observed during recovery periods. Considering the differences between human and monkey in vitreous volume (human: 4.5 ml vs. monkey: 1.5 ml), dosage (human: 0.3 mg/eye vs. monkey: 0.5 mg/eye) and dosing frequency (human: once a month vs. monkey: every 2 weeks in the 26-week study), the reviewing pharmacologist concludes that nonclinical data are adequate to support the proposed clinical use of the drug. Approval is recommended.

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

BLA number: BLA 125156

Review number: 001

Sequence number/date/type of submission: 000/December 29, 2005/Commercial

Information to sponsor: Yes () No (X)

Sponsor and/or agent: Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080-4990
Tel: 650-225-1202; Fax: 650-467-3198

Manufacturer for drug substance: Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080-4990
U.S. License No. 1048

Reviewer name: Zhou Chen, MD, PhD

Division name: Division of Anti-Infective and Ophthalmology Products

Review completion date: May 15, 2006

Drug:

Trade name: **Lucentis™**

Generic name: **Ranibizumab**

Code name: rhuFab V2

Chemical name: Immunoglobulin G1, anti-(human vascular endothelial growth factor) Fab fragment

CAS registry number: 347396-82-1

Other names: Recombinant humanized anti-VEGF monoclonal IgG1 antibody antigen binding fragment (Fab), RFB002, rhuFab VEGF (V2), anti-VEGF Fab, AMD rhuFab, rhuFab (2nd generation)

MW: approximately 48 kilodaltons.

Structure:

Relevant INDs/NDAs/DMFs: BB-IND — BLA 125085, DMFs

Drug class: Recombinant humanized anti-VEGF monoclonal antibody

Indication: Neovascular (wet) age-related macular degeneration

Clinical formulation (10 mg/ml)

Ingredient	10 mg/ml vial	Function	Reference standard
Ranibizumab		Active ingredient	
α,α-trehalose dihydrate			
Histidine HCl			Ph. Eur.
			USP and Ph. Eur.
Polysorbate 20			NF and Ph. Eur.
Water for Injection			USP and Ph. Eur.

Route of administration: Intravitreal injection

Proposed use: (0.05 ml) administered by intravitreal injection once a month

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

Studies reviewed within this submission: (All studies are reviewed by the current reviewing pharmacologist.)

Pharmacology:

Primary pharmacodynamics

4.FBV.0.RPT.3_0: Binding of Ranibizumab to Various Isoforms of VEGF by Surface Plasmon Resonance

05-0852-1757 (Genentech, Inc.): Analysis of Ranibizumab Binding to VEGF Isoforms

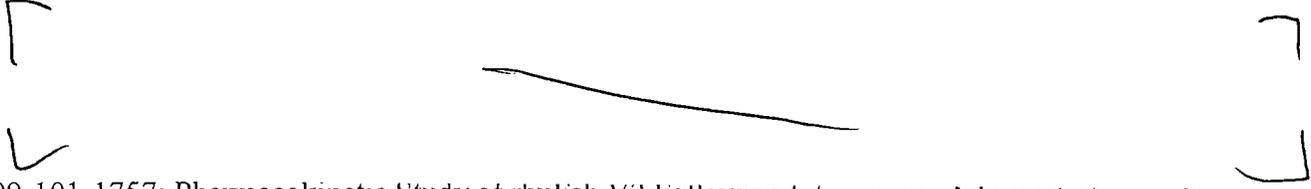
05-1201(Genentech, Inc.): Binding Affinity of anti-VEGF (ranibizumab; rhuFab V2; Y0317-Fab) to Rabbit VEGF

01-401E-1757 (Part II): Inhibition of VEGF-Induced Changes in Permeability Following Intradermal Administration of rhuFab V2 to Guinea Pigs

01-401G-1757: Inhibition of VEGF-Induced Changes in Permeability Following Intradermal Administration of rhuFab V2 to Guinea Pigs

PK:**Distribution:**

98-223-1757: Pharmacokinetics of rhuFab VEGF Following Intravitreal Administration in Normal Rabbits
02-311-1757: Pilot Pharmacokinetic Study of rhuFab V2 Following Subconjunctival, Intracameral and Intravitreal injections in Rabbits
03-0520-1757 (Genentech, Inc.): Pharmacokinetic Study of Ranibizumab (Lucentis™) following Subconjunctival, Intracameral and Intravitreal injections in Rabbits

Other PK studies:

99-101-1757: Pharmacokinetic Study of rhuFab V2 Following Intravenous Administration in Normal Rabbits
00-141-1757: A Single Intravitreal-Dose Pharmacokinetic Study with rhuFab V2 in Male and Female Cynomolgus Monkeys
98-223A-1757: Pharmacokinetics of rhuMAB VEGF Following Intravitreal Administration in Normal Rabbits (Extended Pilot Study)
00-142-1757: A Pilot Single Intravitreal-Dose Pharmacokinetic Study with rhuFab V2 in Male and Female Cynomolgus Monkeys
05-0269-1757: Pharmacokinetic Modeling of Ranibizumab in Rabbits and Monkeys following Intravitreal and Intravenous Administration

Toxicology:**Repeated dose studies**

98-358-1757: 4-Week Intravitreal Toxicity Study with rhuFab VEGF in Cynomolgus Monkeys with a 4-Week Recovery
98-361-1757: 13-Week Intravitreal Toxicity Study with rhuFab VEGF in Cynomolgus Monkeys with a 4-Week Recovery
99-539-1757: 16-Week Intravitreal Toxicity Study with rhuFab VEGF in Cynomolgus Monkeys
01-463-1757: 26-Week Intravitreal injection Toxicity Study with rhuFab VEGF in Cynomolgus Monkeys with an 8-Week Recovery

Local tolerance

98-359-1757: Intravitreal Local Tolerance Study with rhuFab VEGF in Rabbits
22-205-1757: Intravitreal Local Tolerance Bridging Study with rhuFab VEGF in Rabbits
02-406-1757: Intravitreal Local Tolerance Bridging Study with rhuFab VEGF (ranibizumab) in Rabbits

Special toxicology studies

98-279-1754: Cross-Reactivity of Biotinylated Second Generation Humanized Monoclonal Antibody rhuMAb VEGF (GN1754) with Normal Human Tissue

98-360-1757: Hemolytic Potential, Blood Compatibility, and Vitreal fluid Compatibility Testing with rhuFab VEGF

00-580-1757: Assessment of the Safety of Intravitreal Injections of rhuFab VEGF (V2) in Combination with Intravenous Verteporfin Photodynamic Therapy following Laser-Induced Choroidal Neovascularization (CNV) in Cynomolgus Monkeys

02-108-1757: Validation study: Assessment of the Safety and Efficacy of Intravitreal Injections of rhuFab VEGF in a Laser-induced Choroidal Neovascularization (CNV) Model in Cynomolgus Monkeys

Studies not reviewed within this submission:

Pharmacokinetics

Analytical methods and validation reports

4.FBV.1. AVR_0: rhuFab V2 Antigen ELISA

4.FBV.7. AVR_0: rhuFab V2 Antibody ELISA

~~4.FBV.0.RPT.6_0: Pharmacokinetic Assay for rhuFab V1 and rhuFab V2~~

~~4.FBV.0.RPT.5_0: Anti-rhuFab V1 and V2 Antibody Assay~~

~~4.FBV.0.RPT.4_0: Anti-ECP Antibody Assay~~

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Ranibizumab is a recombinant humanized antigen-binding fragment (Fab) of a recombinant humanized monoclonal antibody directed against human vascular endothelial growth factor (VEGF). Pharmacology studies showed little difference in the kinetics of ranibizumab binding to different isoforms of rhVEGF (rhVEGF₁₆₅, rhVEGF₁₂₁, and rhVEGF₁₁₀). The apparent affinity rates were high, and the apparent dissociation rates were very low. In a guinea-pig skin model, ranibizumab inhibited VEGF-induced vascular permeability in a concentration-dependent manner.

No in vivo safety pharmacology studies were conducted with ranibizumab. Several safety endpoints were incorporated into the repeat-dose toxicology studies in cynomolgus monkeys. No treatment-related effects on physical examination parameters, including respiratory rate, heart rate, and body temperature, were observed.

2.6.2.2 Primary pharmacodynamics

~~4.FBV.0.RPT.3_0: Binding of Ranibizumab to Various Isoforms of VEGF by Surface Plasmon Resonance~~

The purpose of this study was to determine the binding affinities of ranibizumab for the rhVEGF₁₆₅, rhVEGF₁₂₁, and rhVEGF₁₁₀ isoforms by surface plasmon resonance (SPR) analysis. Each VEGF isoform

(10 µg/ml) was coupled onto one of the four different flow cells of a _____ using a standard _____. The dissociation equilibrium constant (K_D), association equilibrium constant (K_A), dissociation rate constant (kd), and association rate constant (ka) were calculated with _____ evaluation Software.

The results of this study (see table below) indicated that all three isoforms of VEGF tested (rhVEGF₁₆₅, rhVEGF₁₂₁, and rhVEGF₁₁₀) bound to ranibizumab with high affinities. The apparent k_d was low (below the instrument's limit of detection, 10^{-5} sec^{-1}) and the apparent k_a of ranibizumab was similar for each of the three VEGF isoforms tested.

Apparent affinity of ranibizumab for three recombinant human VEGF isoforms

	VEGF ₁₆₅	VEGF ₁₂₁	VEGF ₁₁₀
ka ($\text{M}^{-1} \text{sec}^{-1}$)	$(5.6 \pm 0.28) \times 10^4$	$(10.1 \pm 2.3) \times 10^4$	$5.2 \pm 0.02) \times 10^4$
kd (sec^{-1})	$\leq 10^{-5}$	$\leq 10^{-5}$	$\leq 10^{-5}$
K_A (M^{-1})	$\geq 5.6 \times 10^9$	$\geq 10.1 \times 10^9$	$\geq 5.2 \times 10^9$
K_D (pM)	≤ 179	≤ 99	≤ 192

M: molar

05-0852-1757: Analysis of Ranibizumab Binding to VEGF Isoforms

The purpose of this study was to determine the kinetics of ranibizumab binding to three biologically relevant forms of VEGF: VEGF₁₁₀, VEGF₁₂₁, and VEGF₁₆₅ (2-5 µg/ml). The apparent association rate (ka) and apparent dissociation rate (kd) were measured using the _____ surface plasmon resonance system. The binding affinity, as described by an apparent equilibrium dissociation constant K_D , was then calculated as $K_D = kd/ka$.

The results (see table below) showed similar apparent association rates for the binding of ranibizumab to VEGF₁₁₀, VEGF₁₂₁, and VEGF₁₆₅. Apparent dissociation rates were below the limit of accurate detection ($1 \times 10^{-5} \text{ sec}^{-1}$). Based upon _____ kinetics measurements, there is little difference in the kinetics of ranibizumab binding to these isoforms. Apparent dissociation equilibrium constants were all in the subnanomolar range.

Apparent affinity of ranibizumab for three recombinant human VEGF isoforms (mean ± SD)

	VEGF ₁₆₅	VEGF ₁₂₁	VEGF ₁₁₀
ka ($\times 10^4 \text{ M}^{-1} \text{sec}^{-1}$)	3.85±0.10	4.04±0.15	7.37±0.61
kd (sec^{-1})	$< 10^{-5}$	$< 10^{-5}$	$< 10^{-5}$
K_D (pM)	<260	<248	<136

M: molar

05-1201(Genentech, Inc.): Binding Affinity of anti-VEGF (ranibizumab; rhuFab V2; Y0317-Fab) to Rabbit VEGF

The purpose of this study was to measure the *in vitro* binding affinity of ranibizumab to recombinant rabbit VEGF. Binding affinities were determined at 37°C in _____ kinetics experiments using a surface plasmon resonance instrument. Association and dissociation curves were analyzed to determine the apparent association rate (k_{on}) and the apparent dissociation rate (k_{off}) of antibody with antigen. The apparent equilibrium dissociation constant, K_D was calculated as the ratio k_{off}/k_{on} .

The data indicated that ranibizumab had an apparent affinity ($K_D \pm SD$) of 8.8 ± 8.1 nM for rabbit VEGF. The results suggested that the affinity of ranibizumab to rabbit VEGF was lower than that for human VEGF.

01-401E-1757 (Part II): Inhibition of VEGF-Induced Changes in Permeability Following Intradermal Administration of rhuFab V2 to Guinea Pigs

The purpose of this study was to investigate the inhibition of VEGF-induced vascular permeability following intradermal administration of VEGF and rhuFab V2 to male hairless guinea pigs. A 32-square grid was drawn onto the dorsum of each animal (4×8). Each animal was given an intracardiac injection of 1 ml of 1% Evans Blue dye. One hr following dye injection, 0.1 ml of numerous test solutions and controls was injected intradermally into each square of the grid. Test solutions composed of VEGF₁₆₅ (100 ng/ml) pre-mixed with rhuFab V2 at concentrations of 1, 10, 30, 60, 100, 300, 600, 1000, and 6000 ng/ml were each injected in triplicate (3 squares). Histamine (300 μ M), PBS/BSA (single location), and VEGF (100 ng/ml; duplicate) were injected as positive and negative controls. rhuFab V2 vehicle was diluted to 1:100,000 using PBS/BSA (equivalent dilution of rhuFab V2 at 1000 ng/ml) and was additionally injected in duplicate. Animals were euthanized one hr following injections of test solutions. The pelt of each animal was collected, cleaned, and photographed to assess dye leakage or vascular permeability. Photographs of each dorsum were scanned into a digital image. As a measurement of VEGF-induced permeability, pixel counts of the Evans Blue dye, which migrated into the injection sites, were quantitated using Adobe Photoshop Version 7.0.

Following an intracardiac administration, the Evans Blue dye leaked into injection sites containing the positive controls [histamine (300 μ M) and VEGF (100 ng/ml)]. Dye leakage was not observed for the negative controls (PBS/BSA or the rhuFab V2 vehicle). When injected in combination with VEGF, rhuFab V2 inhibited VEGF-induced permeability in a concentration-dependent manner with a mean IC_{50} of 56.7 ± 17.0 ng/ml. The mean IC_{90} was 113 ± 29.4 ng/ml. These results suggested that rhuFab V2 was capable of completely inhibiting VEGF vascular permeability effects when present at greater than or equal concentrations of VEGF.

01-401G-1757: Inhibition of VEGF-Induced Changes in Permeability Following Intradermal Administration of rhuFab V2 to Guinea Pigs

The purpose of this study was to determine the inhibitory effect of rhuFab V2 on the permeability induced by VEGF₁₂₁ and VEGF₁₁₀ following an intradermal administration of rhuFab V2 to male hairless guinea pigs. A 32-square grid (4×8) was drawn onto the dorsum of each animal, and 1 ml of 1% Evans Blue dye was injected via intracardiac injection. One hr following dye injection, 0.1 ml of numerous test solutions and controls was injected intradermally into each square of the grid in triplicate. All animals received a single intradermal injection of histamine (300 μ M) as a positive control, PBS/BSA as a negative control, and duplicate injections of the respective VEGF alone as a positive control. rhuFab V2 vehicle was diluted 1:100,000 using PBS/BSA and was additionally injected in duplicates as another negative control. Animals were euthanized one hr following injections of test solutions. The pelts of each animal were collected, cleaned, and photographed to assess dye leakage or vascular permeability.

Study design

Group	N	Treatment	VEGF concentration (ng/ml)	rhuFab V2 concentration (ng/ml)	Dosing volume (ml)
1	8	rhuFab V2 + VEGF ₁₂₁	205	0, 1, 6, 10, 30, 60, 100, 300, 600, 1000	0.1
2	8	rhuFab V2 + VEGF ₁₁₀	189	0, 1, 6, 10, 30, 60, 100, 300, 600, 1000	0.1

Following the Evans blue dye injection, dye leakage was seen in the injection sites containing histamine and the respective VEGF (VEGF₁₂₁ or VEGF₁₁₀). Dye leakage was not observed for the negative controls. In both groups, ranibizumab significantly inhibited VEGF-induced permeability in a concentration-dependent manner with a mean IC₅₀ of 35.6 ng/ml for rhVEGF₁₂₁ and 20.6 ng/ml for rhVEGF₁₁₀.

2.6.2.3 Secondary pharmacodynamics

No studies were submitted.

2.6.2.4 Safety pharmacology

Safety pharmacology endpoints were incorporated into repeat-dose toxicity studies. No formal *in vivo* safety pharmacology studies were conducted with ranibizumab. No treatment-related effects on physical examination parameters, including respiratory rate, heart rate, and body temperature, were observed in cynomolgus monkeys administered up to 2.0 mg/eye of ranibizumab ITV every 2 weeks for up to 26 weeks.

2.6.2.5 Pharmacodynamic drug interactions

No drug interaction studies were conducted.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Study	Animal species	Dose/concen.	Findings
4.FBV.0.RPT.3_0: Binding of Ranibizumab to Various Isoforms of VEGF by Surface Plasmon Resonance	In vitro affinity binding study using 10 µg/ml of rhVEGF ₁₆₅ , rhVEGF ₁₂₁ , and rhVEGF ₁₁₀ isoforms	15.6-250 nM	All three isoforms (rhVEGF ₁₆₅ , rhVEGF ₁₂₁ , and rhVEGF ₁₁₀) bind to ranibizumab with high and similar affinities.
05-0852-1757: Analysis of Ranibizumab Binding to VEGF Isoforms	In vitro affinity binding study using 2-5 µg/ml of rhVEGF ₁₆₅ , rhVEGF ₁₂₁ , and rhVEGF ₁₁₀ isoforms	1.95-500 nM	Similar apparent association rates for the binding of ranibizumab to VEGF ₁₁₀ , VEGF ₁₂₁ , and VEGF ₁₆₅ were seen. Apparent dissociation rates were below the limit of accurate detection (1 x 10 ⁻³ sec ⁻¹).
05-1201(Genentech, Inc.): Binding Affinity of anti-VEGF (ranibizumab; rhuFab V2; Y0317-Fab) to Rabbit VEGF	In vitro affinity binding study using 10 nM of recombinant rabbit VEGF	250-2000 nM	Ranibizumab had an apparent affinity (KD) of 8.8 nM for rabbit VEGF. The affinity of ranibizumab to rabbit VEGF was lower than that for human VEGF.
01-401E-1757 (Part II): Inhibition of VEGF-Induced Changes in Permeability Following Intradermal Administration of rhuFab V2 to Guinea Pigs	Male hairless guinea pigs. Each animal was given an intracardiac injection of 1 ml of 1% Evans Blue dye. One hour later, 0.1 ml of test solutions and controls were injected intradermally into each square of the grid.	Ranibizumab at concentrations of 1 to 6000 ng/ml was pre-mixed with rhVEGF ₁₆₅ (100 ng/ml) and injected in triplicate.	Ranibizumab inhibited rhVEGF ₁₆₅ -induced permeability in a concentration-dependent manner with a mean IC ₅₀ of 56.7 ng/ml.
01-401G-1757: Inhibition of VEGF-Induced Changes in Permeability Following Intradermal Administration of rhuFab V2 to Guinea Pigs	Male hairless guinea pigs. Each animal was given an intracardiac injection of 1 ml of 1% Evans Blue dye. One hour later, 0.1 ml of test solutions and controls were injected intradermally into each square of the grid.	Ranibizumab at concentrations of 1 to 1000 ng/ml was pre-mixed with rhVEGF ₁₂₁ (205 ng/ml) or rhVEGF ₁₁₀ (189 ng/ml) and were injected in triplicate.	Ranibizumab inhibited rhVEGF-induced permeability in a concentration-dependent manner with a mean IC ₅₀ of 35.6 ng/ml for rhVEGF ₁₂₁ and 20.6 ng/ml for rhVEGF ₁₁₀ .

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

In both rabbit and monkey PK studies after intravitreal (ITV) administration, ranibizumab was present in vitreous humor, aqueous humor, all layers of the retina, ciliary body, iris, corneal endothelium, and serum. The terminal t_{1/2} was approximately 2–3 days in all ocular tissues in both species. Concentrations in serum following ITV injection were more than one thousand fold lower than concentrations in vitreous humor and declined in parallel with the concentrations in the ocular compartments. In rabbit studies, the PK of ranibizumab from

humor. Antibodies against ranibizumab were detected in the vitreous body and serum after ITV administration. Following ITV, SCJ (subconjunctival) and IC (intracameral) administrations in rabbits, the highest ranibizumab concentrations in the retinal tissue and the lowest serum concentrations were measured after ITV administration.

2.6.4.2 Methods of Analysis

See descriptions under individual study reviews.

2.6.4.3 Absorption

No studies were provided.

2.6.4.4 Distribution

98-223-1757: Pharmacokinetics of rhuFab VEGF Following Intravitreal Administration in Normal Rabbits

Report N^o: 98-223-1757
 Compound: rhuFab V1 (First generation), Lot 23896-62, 12.5 mg/ml
 rhuFab V2 (Second generation), Lot 23896-63, 12.5 mg/ml, 0.5 mg/ml
¹²⁵I labeled rhuFab V2 for Group 11 animals
 Study site: Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080
 Study dates: 12/7/1998–2/5/1999
 Route: Intravitreal injection, both eyes
 Dosing Regimen: Single dose
 Animal: Male NZW rabbits, 2.7-3.3 kg
 GLP compliant: No

The purpose of this study was to evaluate and compare the pharmacokinetics of rhuFab V1 and rhuFab V2 in vitreous humor, aqueous humor, and serum following intravitreal (ITV) administration in rabbits. rhuFab V1 is the first generation of rhuFab VEGF. rhuFab V2, an affinity-matured molecule of rhuFab V1, is the second generation of rhuFab VEGF. Study design is summarized in the table below. Vitreous humor, aqueous humor, and serum samples were collected at termination from 2 rabbits per timepoint/group (Groups 8-10) for the measurement of rhuFab V1 and rhuFab V2 concentrations using an

enzyme-linked immunosorbent assay (ELISA) with an LTR (less than reportable) level < 0.78 ng/ml for vitreous and aqueous humors and < 7.8 ng/ml for serum. The antibodies against rhuFab V1 and rhuFab V2 were measured. For Group 11, three animals were treated with ¹²⁵I labeled rhuFab V2, and one animal was sacrificed on each of Days 1, 2, and 4. The right eye was harvested for microautoradiography, and the left eye was harvested for electron microscopy.

Group	N	Treatment	Dose (µg/eye)	Dosing volume (ml/eye)	Sampling time
8	24	rhuFab V1	625	50	Both eyes: 1, 8 hrs, Days 2, 4, 14, 30 and 50. Right eye: Days 1, 7, 21, 40, 60*
9	24	rhuFab V2	625	50	Both eyes: 1, 8 hrs; Days 2, 4, 14, 30, 50, Right eye only: Days 1, 7, 21, 40, 60*
10**	10	rhuFab V2	25	50	Both eyes: Days 2, 14. Right eye only: Days 1, 7, 21*
11	3	¹²⁵ I-rhuFab V2	625 (50 µCi)	50	Both eyes: Days 1, 2, 4#

*Left eyes were harvested for immunohistochemistry analysis.

#Left and right eyes were harvested for electron microscopy and microautoradiography analyses, respectively.

** Following administration of 25 µg/eye, PK analysis was not conducted because of insufficient or undetectable rhuFab V2 concentration data in aqueous humor and serum.

Results:

PK parameters are shown in the table below. Following ITV administration, rhuFab V2 cleared from the vitreous humor with a similar half-life as the lower affinity antibody rhuFab V1. The concentrations in the aqueous humor and serum were much lower than in the vitreous humor.

PK parameters of rhuFab V1 and rhuFab V2 following a single intravitreal injection

Group	Vitreous humor			Aqueous humor		serum	
	8	9	10	8	9	8	9
Treatment	rhuFab V1	rhuFab V2	rhuFab V2	rhuFab V1	rhuFab V2	rhuFab V1	rhuFab V2
C _{max} (µg/ml)	743±389	1280±308	24.2	17.66±7.72	57.11±23.37	30 ng/ml	55 ng/ml
AUC _{0-∞} (µg-day/ml)	2640	4850	97.1	131.5	286.3	0.114	0.271
T _{1/2} (day)	2.39	2.89	2.40	2.06	2.98		
T _{max} (hr)	1	1	24	2 days	2 days	1 day	1 day

In vitreous humor, antibodies against rhuFab V1 were detected in Group 8 animals (22 of 40 eyes) and against rhuFab V2 in Group 10 animals (8 of 16 eyes) starting on Day 14. Following administration of rhuFab V2 at 625 µg/eye (Group 9), antibodies were found in 19 of 44 eyes starting on Day 7.

In serum, antibodies against rhuFab VEGF in serum were detected in all dose groups as early as Day 14 or 15.

The ocular distribution of human Fab was determined by immunohistochemical method. The sponsor indicated that the presence of ocular inflammation reduced the immunohistochemistry staining intensity. rhuFab V1 at 625 µg/eye was present on Days 1 and 7 in the vitreous, all retinal layers, ciliary body epithelium and stroma of the pars plana, iris stroma posterior epithelium, Schlemm's canal, limbal blood vessels and corneal endothelial cells. The staining intensity decreased on Days 14 and 15. Patchy and/or weak retinal staining of specific components [ganglion cell layer, inner nuclear layer and RPE (retinal pigment epithelium)] persisted up to Day 40. For rhuFab V2, positive staining in similar tissues was only seen on Day 7. The staining was seen last time in the vitreous, ciliary body epithelium and iris on Day 21. The sponsor indicated that the staining on both Days 1 and 7 was reduced relative to rhuFab V1. No staining was seen for Group 10 animals (25 µg/eye).

In microautoradiographic distribution assay in Group 11 animals with ^{125}I labeled rhuFab V2, a microautoradiographic signal was present in all retinal layers, perivascularly in the optic nerve head with extension going a variable distance into the optic nerve, vitreous, ciliary body, lens capsule, zonula fibers, iris, and corneal endothelium at all three time points (Days 1, 2 and 4). No signal was seen in choroidal and scleral tissues.

In electron microscopy examinations, radioactive Fab fragments injected into the vitreous of rabbit eyes penetrated into the retina and diffused intercellularly through the various inner layers. In addition, Fab fragment appeared to be internalized by different cell types, including the bipolar cells and the RPE cells. After crossing the Bruch's membrane, radioactive Fab reached the blood vessel in the choroid.

02-311-1757: Pilot Pharmacokinetic Study of rhuFab V2 Following Subconjunctival, Intracameral and Intravitreal injections in Rabbits

Report N^o: 02-311-1757
 Compound: rhuFab V2, Lot M4-TOX8
 Study site: Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080
 Study dates: 7/23/2002-8/23/2002
 Route: Intravitreal, subconjunctival or intracameral injection, both eyes
 Dosing Regimen: Single dose, 500 $\mu\text{g}/\text{eye}$, 25 $\mu\text{l}/\text{eye}$, both eyes
 Animal: Male NZW rabbits, 2.5-3.5 kg, 4/group
 GLP: No

The purpose of this study was to investigate the pharmacokinetics of ranibizumab (rhuFab V2) in vitreous humor, aqueous humor, and retinal tissue after subconjunctival, intracameral, and intravitreal administration in New Zealand White rabbits. Vitreous humor, aqueous humor, and retinal tissues were collected from both eyes at 6 hrs and 4 days postdose (4 eyes/2 animals per timepoint). Ocular humors and retinal tissues were analyzed for ranibizumab concentrations by ELISA.

Results:

Results are summarized in the table below. Ranibizumab concentrations in the retina, vitreous and aqueous humor were all highest after the ITV administration. Ranibizumab concentrations from all ocular matrices declined more rapidly after subconjunctival and intracameral administrations when compared with ITV administration. In conclusion, the retinal ranibizumab concentrations after intravitreal administration were higher than those by intracameral or subconjunctival administration, and declined less rapidly.

Summary of PK parameters (ng/ml, mean \pm SD)

Treatment	Timepoint (day)	Vitreous humor	Aqueous humor	Retina tissue
Subconjunctival injection	0.25	391 \pm 79.8	98.9 \pm 64.6	12.4 \pm 16.6
	4	2.29 \pm 0.392	3.64 \pm 1.52	Not applicable
Intracameral injection	0.25	83.6 \pm 49.0	12500 \pm 13600	1.08 \pm 1.12
	4	1.98	19.2 \pm 14.2	Not applicable
Intravitreal injection	0.25	424000 \pm 31300	15800 \pm 13000	56.8 \pm 15.6
	4	223000 \pm 36600	23300 \pm 8890	32.7 \pm 7.03

03-0520-1757 (Genentech, Inc.): Pharmacokinetic Study of Ranibizumab (Lucentis™) following Subconjunctival, Intracameral and Intravitreal injections in Rabbits

Report No: 03-0520-1757
 Compound: Ranibizumab, Lot 36917-16
 Study site: Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080
 Study dates: 9/25/2003-10/10/2003
 Route: Intravitreal (IVT), subconjunctival (SCJ) or intracameral (IC) injection, both eyes
 Dosing Regimen: Single dose, both eyes
 Animal: Male NZW rabbits, 2.3-2.9 kg
 GLP: No

The purpose of this study was to investigate the pharmacokinetics of ranibizumab in vitreous humor, aqueous humor, and retinal tissue after subconjunctival, intracameral, or intravitreal administration in New Zealand White rabbits. Vitreous humor, aqueous humor, and retinal tissues and blood samples were collected from both eyes from three animals/group/timepoint as shown in the table below. Blood samples were collected from all animals at 4 hr post-dose. Ranibizumab concentrations were analyzed using a rhuFab V2 ELISA (the LOQ was 1.56 ng/ml in aqueous humor, vitreous humor, and retinal tissue homogenate, and 7.8 ng/ml in serum). Serum samples on Study Day 11 were analyzed for antibodies against ranibizumab using an anti-rhuMab VEGF Fab (HA) Antibody ELISA.

Study design

Group	N	Route	Dose (µg/eye)	Dose volume (µl/eye)	Sampling timepoints
1	18	SCJ	500	50	4 and 12 hr, and Days 1, 2, 4 and 11
2	18	IC	500	25	4 and 12 hr, and Days 1, 2, 4 and 11
3	15	IVT	500	25	4 and 12 hr, and Days 1, 4 and 11
4	21	SCJ	4000	2 x 50	4 and 12 hr, and Days 1, 2, 4, 7 and 11

Results:

Results are summarized in the tables below. The highest ranibizumab concentrations in retinal tissue and the lowest serum concentrations were measured after ITV administration. SCJ administration (Group 4) showed delivery of ranibizumab to the retinal tissue, although not to the concentrations seen for a lower dose by intravitreal injection, suggesting that extraocular administration can provide exposure in the vitreous body and retinal tissue.

Summary of PK data

Group	Route	Retina		Vitreous humor		Aqueous humor		Serum	
		AUC _{0-last} (µg-day/ml)	T _{last} (hr)						
1	SCJ	2.94	48	0.222	48	0.116	96	0.252	48
2	IC	0.329	24	0.059	96	21.3	96	0.118	24
3	ITV	490	264	1360	264	259	264	0.0193	24
4	SCJ	28.7	264	1.34	264	0.660	96	1.64	48

PK parameters following ITV administration

Matrix	C _{max} (µg/ml)	T _{max} (hr)	AUC _{inf} (µg-day/ml)	T _{1/2} (day)
Vitreous	Not applicable	Not applicable	1710	5.36
Aqueous	37.2	96.0	305	3.47
Serum	0.0257	12.0	0.0463	1.02
Retina	112	4.0	589	4.31

Antibodies against ranibizumab in serum were detected at Study Day 11 in 9 of 12 animals [3 of 3 animals in Group 1 (SCJ, 500 µg/eye), 2 of 3 animals in Group 2 (IC, 500 µg/eye), 3 of 3 animals in Group 3 (ITV, 500 µg/eye), and 1 of 3 animals in Group 4 (SCJ, 4000 µg/eye)].

2.6.4.5 Metabolism

No studies were provided.

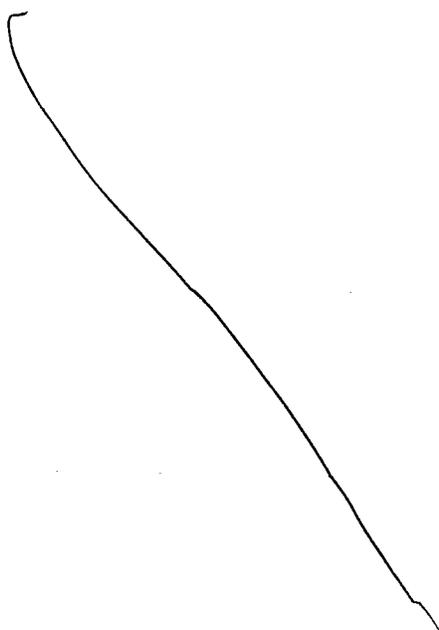
2.6.4.6 Excretion

No studies were provided.

2.6.4.7 Pharmacokinetic drug interactions

No studies were provided.

2.6.4.8 Other pharmacokinetic studies



1 Page(s) Withheld

 ✓ § 552(b)(4) Trade Secret / Confidential

 § 552(b)(4) Draft Labeling

 § 552(b)(5) Deliberative Process

99-101-1757: Pharmacokinetic Study of rhuFab V2 Following Intravenous Administration in Normal Rabbits

Report N^o: 99-101-1757
 Compound: rhuFab V2, Lot K9806AX/G170AJ
 Study site: Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080
 Study dates: 3/25/1999-3/26/1999
 Route: Intravenous injection
 Dosing Regimen: Single dose
 Animal: Male NZW rabbits, 3.0-3.2 kg
 GLP: No

The purpose of this study was to evaluate the pharmacokinetics of ranibizumab following intravenous (IV) administration of doses of either 625 or 2500 µg to male rabbits. Blood samples were collected pre-dose, 5, 15, and 30 min, and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 16, and 24 hr after dosing. ranibizumab concentrations were analyzed by an enzyme-linked immunosorbent assay (ELISA). Additional serum samples were collected at pre-dose and 24 hr post-dose and analyzed for antibody against ranibizumab by ELISA.

Study design

Group	N	Route	Treatment	Dose (µg)
1	5	IV	rhuFab V2	625
2	5	IV	rhuFab V2	2500

Results:

Results are summarized in the table below. The pharmacokinetics of rhuFab V2 appeared to be dose dependent. Antibodies against rhuFab V2 were not detected in serum at pre-dose or 24 hr post-dose.

Serum PK parameters following a single iv administration in rabbits

Dose (µg)	C _{max} (µg/ml)	AUC _{inf} (µg-day/ml)	T _{max} (min)	T _{1/2} (hr)
625	5.16± 0.746	4.86± 0.614	5	3.05± 1.22
2500	31.5± 5.18	29.5± 4.34	5	5.26± 0.666

00-141-1757: A Single Intravitreal-Dose Pharmacokinetic Study with rhuFab V2 in Male and Female Cynomolgus Monkeys

Report N^o: 00-141-1757
 SBi Study N^o: 0940-10
 Compound: rhuFab V2, Lot 23896-63
 Study site: 
 Study dates: 5/16/2000-5/26/2000
 Route: Intravitreal injection (Groups 1 and 2, both eyes) and intravenous injection (Groups 3 and 4)
 Dosing Regimen: Single dose, intravenous injection (1 mg or 4 mg/animal) or intravitreal injection, 500 or 2000 µg/eye
 Animal: Cynomolgus monkeys, 3.2-8.0 years old, 2.2-4.5 kg for males and 2.2-3.2 kg for females
 GLP: No

The purpose of this study was to determine the vitreous and retinal pharmacokinetics of ranibizumab when administered as a single intravitreal (ITV) bolus at doses of 500 and 2000 µg/eye to male and female cynomolgus monkeys. On Days 1 (6 hr postdose), 2, 3, 5, 8, and 11, two animals (one each from Groups 1 and 2, alternating male and female) were sacrificed and ocular tissues were collected and analyzed for ranibizumab concentrations. Blood samples were collected from Groups 1 and 2 animals at predose, 2, 6, 12, 24, 36, 48 hr post-dose and daily from Days 3 to 11, and from Groups 3 and 4 animals at predose, 3, 4, 5, 6, 7, 8, 9, 10, 16, 24, 36 and 48 hr after dosing. Blood samples collected from Groups 1 and 2 animals prior to dosing on Day 1, and on Days 5, 8, and 11 were analyzed for anti-rhuFab V2 antibodies. Samples for antibody analysis were also collected from the animals in Groups 3 and 4 on Days 15 and 30. Ranibizumab concentrations were determined by an enzyme-linked immunosorbent assay (ELISA). Antibodies against ranibizumab in serum were measured using an ELISA. An additional analysis to measure VEGF concentrations in vitreous, aqueous, and retinal tissue samples was conducted using an ELISA.

Group	N/sex	Route	Dose	Dosing volume
1	3	ITV	500 µg/eye	50 µl/eye
2	3	ITV	2000 µg/eye	50 µl/eye
3	2	IV	1000 µg/animal	1 ml
4	2	IV	4000 µg/animal	1 ml

Results:

PK parameters are shown in the tables below. Following ITV administration of 500 and 2000 µg/eye (total dose 1000 and 4000 µg/animal, respectively), ranibizumab PK appeared to be dose-linear in vitreous humor, aqueous humor, retinal tissues, and serum. The terminal $t_{1/2}$ was approximately 2–3 days in all ocular matrices and rapid penetration into retinal tissues was observed. Concentrations in serum following ITV injection were 1000-fold lower than concentrations in vitreous humor and declined in parallel with the concentrations in the ocular compartments. These data suggested that the slower elimination half-life in serum after ITV administration, compared to IV, was caused by the slow disappearance of the drug from the vitreous body into the serum compartment. The bioavailability was 60% and 50% after ITV administration of 500 and 2000 µg/eye, respectively.

PK parameters of ranibizumab following a single intravitreal injection

Group	Vitreous humor		Aqueous humor		Retina		Serum	
	1	2	1	2	1	2	1	2
Treatment	500 µg/eye	2000 µg/eye	500 µg/eye	2000 µg/eye	500 µg/eye	2000 µg/eye	500 µg/eye	2000 µg/eye
C _{max} (µg/ml)	169	612	116	478	78.6 ng/ml	227 ng/ml	0.150	0.616
AUC _{0-∞} (µg-day/ml)	687	3230	221	1550	223*	909*	0.464	1.57
T _{1/2} (day)	2.32	2.37	2.40	2.14	2.52	2.31	4.51	3.89
T _{max} (day)	0.25	1	0.25	1	0.25	1	0.25	0.25

*ng-day/mg

PK parameters of ranibizumab following a single intravenous injection

Group	Serum	
	3	4
Treatment	1000 µg/animal	4000 µg/animal
AUC _{0-2 hr} (µg-day/ml)	0.246± 0.009	1.18± 0.276
T _{1/2} (day)	0.648± 0.481	0.585± 0.233

VEGF concentrations in the ocular matrices did not appear to fluctuate between doses of 500 and 2000 µg/eye. Ranibizumab concentrations in the ocular matrices at both doses were at least 10³-fold higher than VEGF. The assay had some deficiencies. VEGF concentrations were measured from separate animals

at each timepoint, and predose VEGF concentrations were not determined. Thus, it could not be concluded whether concentrations consistently increased or decreased over time following rhuFab V2 administration.

No positive titers for the antibody were detected to rhuFab V2.

98-223A-1757: Pharmacokinetics of rhuMAB VEGF Following Intravitreal Administration in Normal Rabbits (Extended Pilot Study)

Report N^o: 98-223A-1757
 Compound: rhuFab V1 (First generation), Lot FD1868-1
 rhuFab V2 (Second generation), Lot FD1868-2
 _____ rhuFab V1 (First generation), Lot 96-22-195-1
 ECP (endotoxin and *E. coli* Protein) solution, Lot 30135-30
 _____, Lot E9857AX/G15BP
 DNase, Lot FD1215
 Study site: Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080
 Study dates: 9/8/1998-3/2/1999
 Route: Intravitreal injection
 Dosing Regimen: Single dose, 50 µl/eye
 Animal: Male NZW rabbits
 GLP: No

The initial purpose of these non-GLP pilot studies was to investigate the pharmacokinetics of rhuFab V1 and V2 following intravitreal (ITV) administration as an immediate-release formulation or as a modified formulation with _____ in order to evaluate molecule and dose selection. However, after ocular inflammation was observed following administration of the immediate-release formulation, the original objective was changed to the evaluation of factors contributing to ocular inflammation. Several experiments were conducted sequentially to investigate the toxicity of rhuFab V1 and rhuFab V2 at different dose levels and ECP concentrations. Determination of toxicity was based upon ocular examinations performed by either in-house technicians or a veterinarian. No PK data were reported.

Results:

In this series of pilot studies, following ITV administration in rabbits, 625 µg/eye containing lower ECP content (≤0.35 µg/eye) of rhuFab V1 and rhuFab V2 was determined to be highest well-tolerated dose and was selected for additional PK studies. Additionally, an ECP content of ≤0.5 µg/eye in the dose solution appeared to be well tolerated.

00-142-1757: A Pilot Single Intravitreal-Dose Pharmacokinetic Study with rhuFab V2 in Male and Female Cynomolgus Monkeys

Report N^o: 00-142-1757
 Compound: rhuFab V2, Lot M4-TOX8
 Study site: _____
 Study dates: 4/14/2000-4/21/2000
 Route: Intravitreal injection

Dosing Regimen: Single dose, 2000 µg/eye, 50 µl/eye
 Animal: Cynomolgus monkeys, one male and one female, 2-3 years old, 2.0-3.0 kg
 GLP: No

The purpose of this study was to determine the vitreous and retinal pharmacokinetics of ranibizumab when administered as a single intravitreal (ITV) bolus at doses of 2000 µg/eye to one male and one female cynomolgus monkeys. The animals each received a single ITV dose of rhuFab V2 at 2000 µg/eye in the left eye on Day 1 and in the right eye on Day 6. Serum samples for analysis of ranibizumab concentration and anti-rhuFab V2 antibody analysis were obtained prior to dosing and at 2, 6, 12, 24, 36, 48 hr postdose and daily from Days 4 to 8. Both animals were euthanized on Day 8, and ocular samples were collected. The retina was divided into two sections: the neural retina and the retinal pigmented epithelium/Bruch's membrane/choriocapillaris. Ranibizumab concentrations, ocular VEGF concentrations, and antibodies against rhuFab V2 were measured using ELISA methods.

Results:

Results are summarized in the table below. Following ITV administration, ranibizumab concentrations were measurable in the vitreous humor, aqueous humor, neural retina layer, RPE/Bruch's layer, and serum. VEGF concentrations were higher in the vitreous humor compared with the aqueous humor. Antibodies against rhuFab V2 were not detected in vitreous humor, aqueous humor, or serum.

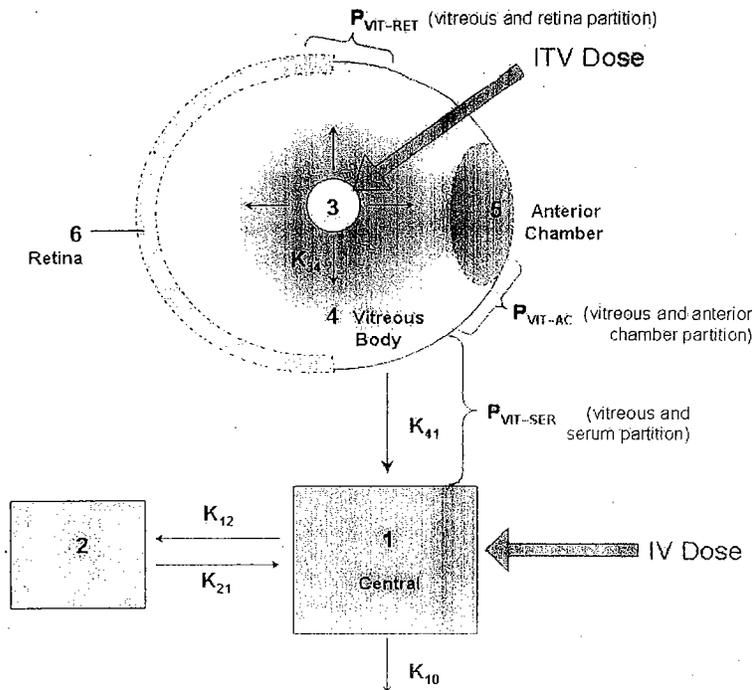
PK parameters

Treatment day	rhuFab V2 concentration (µg/ml)					VEGF concentration (pg/ml)	
	Vitreous humor	Aqueous humor	Retina (NR) (mg/total protein)	Retina (RPE/Bruch's) (mg/total protein)	Serum (Cmax)	Vitreous humor	Aqueous humor
Day 2 (right eye)							
Male	319	145	1.37	0.308	0.248	2310	544
Female	311	120	1.53	1.06	0.225	1512	341
Mean	315	133	1.45	0.684	0.237	1910	443
Day 7 (left eye)					Tmax (day)		
Male	129	48.9	0.256	0.1373	0.08	3487	930
Female	98.6	38.2	0.208	0.0983	0.08	2852	529
Mean	114	43.6	0.232	0.118	0.08	3170	730

05-0269-1757: Pharmacokinetic Modeling of Ranibizumab in Rabbits and Monkeys following Intravitreal and Intravenous Administration

Report N^o: 05-0269-1757
 Study site: Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080
 GLP: No

The purpose of this study was to develop a pharmacokinetic model to describe ocular and systemic disposition of ranibizumab in rabbits and monkeys after intravitreal (ITV) and intravenous (IV) administration. The sponsor summarized and analyzed 6 rabbit PK studies and one monkey PK study. Based on ranibizumab concentrations measured in the eye and serum, ocular drug elimination concepts, and ocular physiology, a six-compartment PK model was developed and fit to the data (see figure below).



After ITV dosing, the ratio between ranibizumab concentrations in the vitreous and the retina, the target tissue, was similar between species. Serum concentrations were at least 1000-fold less than vitreous concentrations in rabbits and monkeys. Following an ITV dose, the model-predicted curves indicated dose proportionality in ocular tissues and serum in both species. Following IV administration, ranibizumab disposition was biphasic and also dose proportional in both species.

Serum ranibizumab concentrations after an IV dose were modeled as two-compartmental because individual study results indicated biphasic elimination. For the ITV route, the eye was divided into four compartments: the vitreous center (Compartment 3), vitreous body (Compartment 4), anterior chamber (Compartment 5), and retina (Compartment 6). Ranibizumab concentrations were measured in the vitreous humor, aqueous humor, and retinal tissue and supported Compartments 4, 5, and 6, respectively. Ranibizumab concentrations in the retina and anterior chamber paralleled concentrations in the vitreous body; thus Compartments 5 and 6 were modeled as being in equilibrium with Compartment 4. The sponsor indicated that the PK model developed herein could be used to predict retina and serum ranibizumab exposure and C_{max} under simulated dosing regimens in these species and provided inferences to expected ocular concentrations in humans. The reviewing pharmacologist considers this PK model acceptable based on the PK study data.

2.6.4.9 Discussion and Conclusions

In both rabbit and monkey PK studies, following intravitreal injection, ranibizumab rapidly penetrated into all layers of retina and other ocular tissues. Serum drug concentrations were very low (more than one thousand fold lower than the drug concentrations in vitreous humor) and declined in parallel with the concentrations in the ocular compartments. The elimination half-life for ocular ranibizumab was two to three days in both species. The half-life for serum ranibizumab after an intravenous injection was much shorter (3 to 5 hr in rabbits and 0.6 day in monkeys) than after ITV dosing (one day in rabbits and 4.5 days in monkeys). The drug elimination from the aqueous humor, retina and serum after ITV dosing was closely

In the 26-week study, a cataractogenic effect was noted in animals receiving the treatment at 1000 and 2000 µg/eye doses. In each case, a new cataract developed only after a relatively long period of intense inflammation following multiple doses, suggesting that the lens changes were secondary to chronic inflammation. Similar findings were not observed among animals at 500 µg/eye.

In the 26-week study, color fundic photography showed some abnormal findings including venous dilatation and tortuosity, venous beading, possible peripapillary retinal thickening, macular thickening, possible papillary swelling, and avascular papillary tufts. These findings were associated with the inflammation observed during the ophthalmic examinations. The incidence of these findings tended to follow a dose-related pattern.

Three local tolerance studies were conducted in rabbits with a single ITV injection of the drug at 2.0 or 2.5 mg/eye followed by a 7-day observation. The ocular inflammation in rabbits was not as severe as in monkeys. Therefore, the monkey is the more sensitive model. Two lots of ranibizumab used in nonclinical studies (Lot M4-TOX8 and Lot M4-TOX14, both were _____ that was reconstituted with water for injection) produced similar ocular responses. In a study to compare the local tolerability of ranibizumab of two different lots (Lot M3-TOX61, the to-be-marketed formulation, and Lot M4-TOX14), more inflammatory responses were seen with Lot M4-TOX14. Histopathological examinations showed a higher frequency and severity of inflammatory cell infiltrates with Lot M4-TOX14.

No cross-reactive binding of humanized monoclonal antibody rhuMab VEGF was observed to any human tissues. Ranibizumab at concentrations of up to 20 mg/ml did not cause hemolysis of human erythrocytes, and was compatible with monkey and human serum and plasma, and human vitreal fluid. In two non-GLP monkey studies with a laser-induced CNV model, ITV injection of ranibizumab prevented formation of CNV lesions and decreased leakage of already formed CNV lesions. ITV injection of ranibizumab in combination with verteporfin PDT did not induce a significant increase in toxicity compared to PDT alone, and the toxicity of ranibizumab in combination with PDT was similar to that of ranibizumab alone.

Ranibizumab given by ITV injection caused inflammation in both anterior and post segments. The inflammation was dose-dependent. Results from four monkey studies demonstrated that 0.5 mg ranibizumab/eye was the maximum tolerated dose. In these studies, the incidence and severity of anterior chamber inflammation and vitreous inflammatory cell findings were much less than those seen at higher doses. In histopathological examinations, the degree of inflammatory cell infiltration was mostly minimal to slight, and the incidence was less than in higher dose groups. No abnormal IOP, cataract or fluorescein leakage changes were noted.

2.6.6.2 Single-dose toxicity

No single dose studies were provided. Three single ITV dose local tolerance studies in rabbits were reviewed under "Local tolerance" section.

2.6.6.3 Repeated-dose toxicity

98-358-1757: 4-Week Intravitreal Toxicity Study with rhuFab VEGF in Cynomolgus Monkeys with a 4-Week Recovery

Key study findings: Administration of ranibizumab at dose levels of 450 and 1800 µg/kg was associated with dose-related inflammation; this effect was reversible.

Study no.: 98-358-1757

Conducting laboratory and location: C

Date of study initiation: 2/22/1999

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: rhuFab VEGF (ranibizumab), Lot Nos. M4-TOX6 and M4-TOX7, purity =

Study Design:

Groups	Nominal dose (µg/eye)*	Dose volume	N/sex
1 (control)	0	50 µl/eye	2
2 (LD)	500	50 µl/eye	2
3 (HD)	2000	50 µl/eye	4 (2 animals/sex were designated as recovery animals)
4 (LD satellite)	500	50 µl/eye	1
5 (HD satellite)	2000	50 µl/eye	1

* The actual doses were 450 and 1800 µg/eye, respectively.

The purpose of this study was to assess the toxicity of ranibizumab when administered to cynomolgus monkeys by intravitreal injection once every other week for at least 4 weeks (three injections to each eye on Days 1, 15 and 29) and to determine the reversibility or persistence of effects after 4 weeks of recovery.

Methods

Doses: 450 and 1800 µg/eye, both eyes, once every other week for 4 weeks (3 injections/each animal)

Species/strain: Cynomolgus monkeys

Number/sex/group or time point (main study): 2

Route, formulation, volume, and infusion rate: Intravitreal, 50 µl/eye

Satellite groups used for toxicokinetics or recovery: Yes

Age: 3-9 years old

Weight (nonrodents only): 2.3-3.6 kg for males and 2.4-3.0 kg for females

Unique study design or methodology: No

Observation times

Mortality: Twice daily

Clinical signs: Twice daily

Body weights: Weekly

Food consumption: Daily

Physical examination (including rectal body temperatures, heart rates, and respiratory rates): Week -2, predose on Days 15 and 29, and before sacrifice of the recovery toxicity animals

Clinical ophthalmology examination: Before initiation of treatment and on Days 2, 8, 15 (predose), 22, 29 (predose), and 30 for all animals and on Days 43 and 57 for the recovery animals

IOP: Before initiation of treatment and on Days 1 (predose and postdose), 2, 8, 15 (predose and postdose), 22, 29 (predose and postdose), and 30

ERG: Before initiation of treatment and on Day 31

Fluorescein angiography and ocular photography: Prior to initiation of treatment and on Day 30

Hematology and clinical chemistry: Twice prior to dosing (Weeks -2 and -1) and on Days 16 and 30 for all animals and on Day 57 for the recovery animals.

Gross pathology: Day 31, main study animals from Groups 1, 2, and 3; after 4 weeks recovery period: recovery and TK animals

Histopathology: See histopathology inventory table

Toxicokinetics: Before initiation of treatment, 8 and 24 hr postdose on Days 1, 15, and 29 from toxicity animals and on Day 57 from the recovery animals, and on Days 3, 8, 15 (predose), 17, 22, 29 (predose), 31, 36, 43, 50, and 57 from TK animals. Vitreal fluid samples were collected from satellite animals only on Days 1 (predose), 8, 15 (predose), 22, 29 (predose), 43, and 57.

Serum antibody analysis: Before initiation of treatment from all animals, on Days 16 and 30 from the toxicity animals, on Day 57 from the recovery animals, and on Days 17, 31, and 57 from the TK animals

Results:

Mortality: No mortality occurred during the study period.

Clinical observations: No drug-related abnormal findings were noted.

Body weights: No treatment-related differences in body weights were noted.

Food consumption: No treatment-related differences in food consumption were noted.

Clinical pathology: No treatment-related, toxicologically significant differences in hematology and clinical chemistry were noted.

Physical examinations: Physical examination indicated no test material effects on heart rate data, rectal body temperature, or respiratory rate.

Clinical ophthalmic examination: Dose-related inflammation of the eye as evidenced by the presence of cells, fibrin, and flare in the anterior chamber and accumulation of cells in the anterior vitreous was observed in the drug-treated animals. The inflammation was transient in nature and only anterior vitreal cells persisted beyond 7 days postdose. The degree of inflammation noted on Day 30 (24 hr after the third dose) was markedly attenuated compared with that following the first dose. Two HD recovery animals developed a transient retinal vasculitis. It was observed in one animal on Day 30 and was resolved by Day 43. For the other animal, it was noted in the right eye on Day 8 and in the left eye on Day 22. The vasculitis in the right eye and left eye was resolved by Day 29 and Day 43, respectively.

Repeated intravitreal dosing and vitreal fluid sampling resulted in temporal vitreal opacities in both eyes of all TK animals. The repeated sampling of vitreal fluid and intravitreal dosing for the satellite animals were performed at identical sites for each sampling/dosing event. These procedures resulted in focal scleral weakening at the sites of vitreal dosing and sampling when viewed with operating loupes. The conducting

laboratory _____ was advised that the injection and sampling sites should be rotated in the future studies.

IOP: There was no drug-related, toxicologically significant effect on intraocular pressure. Similar changes were found in both control and treated animals. Intravitreal injection of either ranibizumab or vehicle caused immediate and marked increases (up to 31-67 mmHg) in intraocular pressure. This increase was a consequence of an acute increase in intraocular volume (the result of the bolus fluid injections of either the drug or vehicle). Intraocular pressure was back to normal at the next measurement (1 to 7 days after dosing) in all groups including the control group.

Photography and fluorescein angiography: Abnormalities seen on Day 30 included perivascular sheathing in one Group 3 male (left eye), vitreal opacity in one Group 3 female (left eye), and a tiny hemorrhage or microaneurysm in one Group 2 female (right eye). The perivascular sheathing noted in the Group 3 male was consistent with the clinical finding of retinal vasculitis. Small foci of depigmentation of the RPE with corresponding hyperfluorescence were noted in several animals, and the similar findings were noted before the treatment in baseline examinations.

Electroretinography: There were no toxicologically significant findings.

Macroscopic examinations: There were no ocular macroscopic findings among the main study animals in any group. No drug-related abnormal findings in other organs were noted.

Histopathology: Inflammatory cell infiltrates (macrophages, lymphocytes, and neutrophils) near the injection site were noted in all groups with similar incidence and were considered related to the intravitreal dosing procedures and not the result of the test material. At the recovery sacrifice, the changes were less severe and were graded as minimal, indicating recovery was in process.

There were no drug-related lesions noted in any other tissues.

Antibody analysis: Four of 16 monkeys that received the test material (one Group 4 animal given 450 µg/eye and three Group 5 animals given 1,800 µg/eye) developed low to moderate antibody titers to ranibizumab in the serum. No antibodies were detected in the vitreous.

TK: Results are summarized in the table below. There was no unexpected drug accumulation in the vitreal fluid or serum, and ranibizumab disposition appeared to be linear from 450 to 1,800 µg/eye following administration every other week for a total of three doses.

TK data (mean ± SD)

Group	Serum				Vitreous humor	
	2	3	4	5	4	5
Dose (µg/eye)	450	1800	450	1800	450	1800
AUC _{0-Day 29/30} (µg-day/ml)	2.57± 0.562	9.81± 2.71	0.889	2.75		
C _{max} (µg/ml)			0.0582	0.188	215	601
T _{1/2} (day)					2.18	2.50
AUC _{0-last} (µg-day/ml)			1.60	4.28	1630	5640

In summary, intravitreal injection of ranibizumab at 450 and 1800 mg/eye in monkeys (both eyes) once every two weeks for three times produced no systemic toxicity. Dose-related inflammation of the eye as

evidenced by the presence of cell and flare in the anterior chamber and accumulation of cells in the anterior vitreous was observed. The inflammation was reversible in nature and only anterior vitreal cells persisted beyond 7 days postdose. The degree of inflammation noted 24 hr after the Day 29 dose was markedly attenuated relative to that observed following the Day 1 dose. Two HD animals developed a transient retinal vasculitis. Ocular photographic examination on Day 30 showed perivascular sheathing in one Group 3 male (left eye), vitreal opacity in one Group 3 female (left eye), and a tiny hemorrhage or microaneurysm in one Group 2 female (right eye). Histopathology examination revealed inflammatory cell infiltrates in all groups with the similar incidence that were considered related to the intravitreal dosing and sampling procedures. No drug-related positive findings in intraocular pressure and ERG examinations were noted. The repeated sampling of vitreal fluid and intravitreal dosing for the TK animals resulted in scleral weakening and inflammatory changes. Serum antibody titers were detected in 4 of the 16 animals administered the test material. In conclusion, administration of ranibizumab at dose levels of 450 and 1800 µg/kg was associated with dose-related inflammation, but this effect was reversible.

98-361-1757: 13-Week Intravitreal Toxicity Study with rhuFab VEGF in Cynomolgus Monkeys with a 4-Week Recovery

Key study findings: Administration of ranibizumab at dose levels of 750 and 2000 µg/kg was associated with dose-related inflammation, but this effect was reversible.

Study no.: 98-361-1757

Conducting laboratory and location: C

Date of study initiation: 5/13/1999

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: rhuFab VEGF, Lot No. M4-TOX8, purity =

Study Design:

Groups	Dose (µg/eye)	Dose volume	N/sex
1 (control)	0 (vehicle)	50 µl/eye	6 (2 animals/sex were designated as recovery animals)
2 (LD)	250	50 µl/eye	4
3 (MD)	500/750*	50 µl/eye	4
4 (HD)	500/2000*	50 µl/eye	6 (2 animals/sex were designated as recovery animals)

* The first dose was 500 µg/eye.

The purpose of this study was to assess the toxicity of ranibizumab when administered to cynomolgus monkeys by intravitreal injection (once every two weeks for 8 injections) and to determine the reversibility of the effects after 4 weeks of recovery.

Methods

Doses: 250, 750 and 2000 µg/eye, both eyes, Days 1, 15, 29, 43, 57, 71, 85, and 99

Species/strain: Cynomolgus monkeys

Number/sex/group or time point (main study): 4

Route, formulation, volume, and infusion rate: Intravitreal, 50 µl/eye

Satellite groups used for toxicokinetics or recovery: No

Age: 2.5-3.5 years old

Weight (nonrodents only): 2.2-3.4 kg for males and 2.1-3.7 kg for females

Unique study design or methodology: No

Observation times

Mortality: Twice daily

Clinical signs: Twice daily

Body weights: Weekly

Food consumption: Daily

Physical examination: Before the initiation of the treatment and on Days 45 and 87; and on Day 127 (recovery males) or 126 (recovery females)

Clinical ophthalmology examination (slit lamp biomicroscopy and indirect ophthalmoscopy): Before initiation of treatment and predose and 48 hr postdose on Days 1, 15, 29, 43, 85, and 99. Exams were also done 7 days postdose (Days 8, 22, and 92). Recovery animals were examined on Days 113 and 127 (males) and Days 112 and 126 (females).

IOP: Days 1, 3, 8, 15, 17, 22, 29, 31, 43, 45, 57, 71, 85, 87, 92, 99, and 101 for all animals, and on Days 113 and 127 for recovery males and on Days 112 and 126 for recovery females

ERG: Before initiation of treatment and during Week 13/14

Fluorescein angiography and ocular photography: Prior to initiation of treatment, during Week 13/14, and on recovery animals on Days 127 (males) and 126 (females)

Vitreous fluid analysis: At the scheduled sacrifices

Hematology and clinical chemistry: Twice prior to dosing (Weeks -2 and -1) and on Days 45 and 101, 129 (recovery males) and 128 (recovery females)

Gross pathology: On Day 101 for main study animals (4 animals/sex/group) and on Day 129 for recovery animals (two/sex in Groups 1 and 4)

Histopathology: See histopathology inventory table

Toxicokinetics: Approximately 8 and 24 hr postdose on Days 1, 15, and 29, 43, 57, 71, 85, and 99

Serum antibody analysis: Before initiation of treatment and predose on Days 15, 29, 57, 85, and 99, and on the day of scheduled sacrifice from selected animals that had positive antibody titers at a previous time point.

Results:

Mortality: One control male died on Day 91 during an ERG examination. The cause of death was unknown.

Clinical observations: No drug-related abnormal findings were noted. Endophthalmitis evidenced by peri-orbital swelling with or without red skin, dilated or constricted pupil, drooping eyelid, opaqueness, or squinting was seen in one Group 3 male (right eye) on Day 17, one Group 4 male (left eye) on Day 22, and one Group 1 female (right eye) on Day 73. Cultures of swabs from the surface of the eye indicated the presence of *Staphylococcus spp.* These animals remained in the study and were treated with antibiotics. The treatment initiated on the days the infection was noted included intravitreal, subconjunctival, and systemic administration of antibiotics (Vancomycin, Ceftazidime, and Maxitrol). However, dosing of the infected eyes was discontinued.

Body weights: No treatment-related differences in body weights were noted.

Food consumption: No treatment-related differences in food consumption were noted

Clinical pathology: No treatment-related, toxicologically significant differences in hematology or clinical chemistry were noted.

Physical examinations: Physical examinations, including heart rate, rectal body temperature and respiratory rate, indicated no test material-related effects.

Clinical ophthalmic examination: Dose-dependant inflammation was seen in the eyes of drug-treated monkeys. This reaction was the most intense (often 4+ in anterior chamber cell plus aqueous flare) after the first treatment and diminished with subsequent injections, even at the same or greater doses. Anterior chamber inflammation was generally transient with either no or mild (score = 1+) anterior chamber cell persisting beyond 7 days after each administration in most animals. Two HD animals (one male and one female) showed severe (4+) anterior chamber cells many times throughout dosing. Vitreal cell scores were also dose-dependent and the vitreal cells tended to persist, or slowly increase in number throughout the study. This persistence was at least partially due to the injection procedure and slow turnover rate of the vitreous humor because vitreal cell scores in vehicle-treated eyes also tended to increase slowly with repeated injections. The cells remained in the vitreous at the end of each dosing interval in all groups were often brown in color. It is possible that the brown color was due to hemosiderin from previous hemorrhage caused by the injection trauma. During the recovery period, the vitreal cells scores notably decreased.

The majority of eyes in the vehicle- and drug-treated groups showed small vitreal "floaters" during the course of the study. Retinal perivascular infiltrates observable by indirect ophthalmoscopy were noted in five HD animals and two MD animals. One LD animal had a single infiltrate that was not observed upon indirect ophthalmoscopy but was noted on color fundic photography. Three of these animals with perivascular retinal infiltrates (one HD male, one HD female, and one MD male) subsequently developed varying degrees of white exudates over the surface of the optic disk. This reaction was drug-related as it was observed mainly in Groups 3 (750 µg/eye) and 4 (2,000 µg/eye). At recovery sacrifice, sheathing was reduced or absent.

IOP: There were no drug-related, toxicologically significant differences in intraocular pressure between control and drug-treated groups. Intravitreal injection with either vehicle or ranibizumab resulted in an immediate increase (to 40s or 50s mmHg in most cases) in intraocular pressure that typically spontaneously resolved within 15 min postdose. The increase in IOP was due to rapid increase in intraocular volume.

Photography and fluorescein angiography: Significant leakage of fluorescein on fluorescein angiography was only seen in the eyes of animals with endophthalmitis, and the eyes of a HD male on Day 87 with a marked posterior segment inflammatory response where fluorescein leakage was seen from all large vessels. By the end of recovery (Day 127), the angiogram of this animal showed recovery, although it remained somewhat hazy.

Electroretinography: There were no toxicologically significant abnormal findings.

Macroscopic examinations: No drug-related systemic gross lesions were noted. Light focal areas in the retina were noted in three terminal sacrifice females, one each from Groups 1, 2, and 4. There were no microscopic changes corresponding to the macroscopic observation in control and LD animals. In the HD animal, perivascular sheaths and granulomas were noted corresponding to the macroscopic observation. In one MD male, light focal areas in the peripheral retina were noted in the left eye, corresponding to the

perivascular sheathing noted on clinical examination. This also corresponded to inflammatory cell infiltrates, including eosinophils, in several ocular locations at microscopic examination.

Histopathology: Inflammatory cell infiltration (most prominently lymphocytes and macrophages) was noted in many ocular tissues with a higher incidence in MD and HD groups. At the terminal sacrifice, there was a dose-dependent increase in plasma cell infiltrates among females at the higher two doses. Perivascular inflammatory infiltrates (composed of lymphocytes, macrophages, plasma cells, neutrophils, and eosinophils) occurred in two MD animals and one HD animal. One of the MD animals showed an elevated surface of the optic disk, and the HD animal showed granuloma-like inflammatory foci that occupied a portion of the optic disk region, displacing normal disk structures and adjacent retinal structures peripherally. The inflammatory cells were also present within the vessel wall in two animals (one MD and one HD) with extensive perivascular infiltrates and enlarged endothelial cells.

In eyes diagnosed clinically as having endophthalmitis, there were inflammatory changes in ocular structures including vitreous fibrosis, indicating maturation/scar tissue formation. Inflammatory cells of many types were abundant in these eyes, but normal ocular cellular morphology was not affected.

At the recovery sacrifice, all animals in the high dose group had a clinical history of perivascular sheathing, and three of the four HD animals had microscopic perivascular infiltrates of plasma cells and lymphocytes. The number of inflammatory cells was much lower than that seen in animals at the terminal sacrifice.

There were no drug-related lesions noted in any systemic tissues.

Antibody analysis: Fifteen out of 28 treated monkeys (4 HD, 4 MD and one LD males and 2 HD, 3 MD and 1 LD females) developed antibodies to ranibizumab in the serum as early as Day 29. Antibodies were also detected in the vitreal fluid in one MD and two HD males.

During the study period, endophthalmitis was seen in three animals (one each in Groups 1, 3, and 4). For the Group 1 animal, no antibody was found in serum or vitreous humor. For the MD animal, endophthalmitis was seen on Day 17 while antibody was detected on Day 57 in serum and Day 101 in vitreous humor. For the HD animal, endophthalmitis was seen on Day 22. Vitreal fluid collected on the same day did not show antibody. The antibody was detected on Day 57 in serum and Day 129 in vitreous humor.

TK: Results are summarized in the table below. The serum and vitreous drug concentrations increased in proportion to dose. The maximum serum concentrations were approximately 300 to 600-fold higher than in the vitreal fluid.

TK data on Day 101 (mean ± SD)

Group	Serum			Vitreous humor		
	2	3	4	2	3	4
Dose (µg/eye)	250	750	2000	250	750	2000
AUC ₀₋₁₀₁ (µg·day/ml)	4.34±1.77	16.6±8.81	30.1±21.5	Mean concentration (µg/ml)		
C _{max} (ng/ml)	132±102	624±266	899±774	53.5±7.16	186±24.1	545±87.6
T _{max} (day)	75±34	93±11	75±26			

In summary, monkeys were treated by intravitreal injection with ranibizumab at 250, 750 and 2000 µg/eye once every two weeks for a total of 8 times. There were no systemic drug-related effects as assessed by clinical observations, body weights, food consumption, clinical pathology, or anatomical pathology of

Unique study design or methodology: No

Observation times

Mortality: Twice daily

Clinical signs: Twice daily

Body weights: Weekly

Clinical ophthalmology examination (slit lamp biomicroscopy, IOP and indirect ophthalmoscopy): Before initiation of treatment, predose, and approximately 48 hr and 7 days postdose on Days 1, 15, 29, and 57 (Days 1, 3, 8, 15, 17, 22, 29, 31, 36, 57, 59, and 64), and on Days 50, 71, 85, 95, and 113.

Ocular photography: Prior to initiation of treatment and on Days 53 and 109

Vitreous fluid analysis: At the scheduled sacrifices on Day 113

Hematology and clinical chemistry: Before initiation of treatment and on Day 113

Gross pathology: On Day 113, all animals

Histopathology: Ocular tissues only from all animals [Reviewer's comments: It is acceptable since histopathological examinations on systemic tissues were performed in other studies and no lesions attributed to drug were found.]

Toxicokinetics: Before initiation of treatment (Day -12) and at approximately 24 hr postdose on Days 1, 15, 29, and 57

Serum antibody analysis: Before initiation of treatment (Day -12), predose on Days 15, 29, and 57, and on Days 71, 85, 98, and 113

Results:

Mortality: One Group 1 male was euthanized on Day 77 due to poor health associated with persistent diarrhea. On Day 50 dehydration was noted in this animal. A fecal sample for culture sent for analysis on Day 58 was positive for *Campylobacter spp.* A decrease in body weight and food consumption was also noted. Macroscopic observations showed light reddish-brown fluid in the rectum and distal colon. The sponsor indicated that this condition was unrelated to treatment. The reviewer agrees.

Clinical observations: No drug-related abnormal findings were noted.

Body weights: No treatment-related differences in body weights were noted.

Food consumption: No treatment-related differences in food consumption were noted.

Clinical pathology: No treatment-related, toxicologically significant differences in hematology and clinical chemistry were noted.

Clinical ophthalmic examination: ITV injection of ranibizumab produced an inflammatory response in the eyes of monkeys. Less inflammation was seen on subsequent injections when the same dose (250 µg/eye), or a two-fold increase in the dose (500 µg/eye) was administered. When the dose was increased four-fold (2000 µg/eye) from the previous dosing or dosing was 4 weeks from the previous dose (Group 2, Days 1 to 29, or all groups, Days 29 to 57), inflammation was not diminished. [Reviewer's comments: There was no explanation why the inflammatory reactions after the 4-week interval were more serious than that after the two-week interval. It was possibly due to the high dose (2000 mg/eye) of the drug given after the 4-week

interval.] Inflammatory cells in the anterior chamber were generally transient in nature with peak scores at 48 hr (4+) after dosing and spontaneously diminishing to no or a mild anterior chamber cell (trace or 1+) beyond 7 days after dosing in most animals. Vitreal cell scores also tended to demonstrate a dose-response, but this was less obvious than in the anterior chamber. As expected, inflammatory cells in the vitreous were slower to appear (peak scores typically occurred 1 week after dosing) and were slower to clear than in the more fluid aqueous humor. Systemic and topical corticosteroids administered prior to and after dosing did not alter the inflammatory response. The inflammatory response notably diminished over time during the recovery period. Anterior chamber cells were not present at examinations more than 2 weeks post-dosing, and, by the end of this period, vitreal cells were either absent, or at trace to the level of 1+ in all eyes in all groups.

Two forms of posterior segment inflammation of the eye evidenced by changes around the peripheral retinal venules were noted. The first form, seen in 22 of 24 animals, was an acute response characterized by focal or multifocal, perivenous retinal hemorrhages with white centers in the far peripheral retina. These typically appeared at 48 hr after doses of 500 µg/eye or greater were administered for the first time, resolved one week after dosing, and did not reoccur or were notably diminished on subsequent injections. The second form of posterior segment inflammation, seen in 14 of 24 animals, was characterized as focal or multifocal, white, perivascular sheathing around peripheral retinal venules. Perivascular sheathing was more prominent at the dose of 2000 µg/eye, and was completely resolved in every animal by the conclusion of the study. The majority of eyes in all groups developed small vitreal “floaters” over the course of the study.

IOP: An increase in intraocular pressure (30-50 mm Hg) above baseline values was seen immediate after ITV injection. IOP was within normal limits at 48 hr after dosing by the next time IOP measurement. Four-week and 13-week studies demonstrated that this increase typically resolved within 15 min, and it was not directly drug-related.

Photography: Eleven animals (two males each in Groups 1, 2, and 3, respectively, and one Group 1, two Group 2 and two Group 3 females) showed small foci and/or streaks of depigmentation of the RPE that showed no differences between baseline and Day 53 or 109. Seven animals had small retinal hemorrhages including 4 animals at baseline, one Group 2 male at Day 53, and 4 animals at Day 109 (one Group 1 male, two Group 2 males, and one Group 1 female). The sponsor indicated that these hemorrhages may be associated with the capture or restraint of the animals and not the test material

Macroscopic examinations: No drug-related abnormal systemic and ocular findings were noted.

Histopathology: Positive microscopic observations within the left eyes are summarized in the table below. The data from the right eyes were similar. There were numerous microscopic observations within the eyes. Group 1 males had fewer observations than animals in other groups but all groups had multiple observations. Inflammatory cell infiltrates (neutrophils, plasma cells, lymphocytes, or eosinophils) were noted in various ocular structures among all groups. There were no steroid application-related effects to distinguish one group from another.

Positive ocular histopathology findings

	Sex	Males			Female		
	Group	1	2	3	1	2	3
	N	4	4	4	4	4	4
Left conjunctiva/eyelid, infiltrate, lymphocytes, plasma cells, neutrophils	Minimal	2	0	1	1	0	0
	Slight	0	0	0	0	1	0
--infiltrate, neutrophils	Minimal	0	1	1	2	0	1
	Slight	0	0	0	0	1	0
--infiltrate, lymphocytes	Minimal	0	0	0	0	1	0
--infiltrate, plasma cell	Minimal	0	0	0	0	1	1
Left iris, infiltrate, lymphocytes	Minimal	0	0	0	1	0	0
	Slight	0	1	0	0	0	0
--infiltrate, plasma cell	Slight	0	1	0	0	0	0
--infiltrate, neutrophils	Minimal	0	0	0	2	0	1
Left ciliary body, infiltrate; lymphocytes, pars plicata	Minimal	0	0	0	0	1	2
	Slight	0	1	0	1	1	0
--infiltrate, plasma cell, pars plicata	Minimal	0	0	0	0	1	1
	Slight	0	1	0	1	1	1
--infiltrate, eosinophils	Minimal	0	0	0	0	1	0
Left vitreous, infiltrate, neutrophils	Minimal	0	0	1	1	0	0
	Minimal	0	0	0	1	0	0
--hemorrhage	Minimal	0	0	0	1	0	0
Left neuroretina, detachment	Minimal	0	0	0	0	1	0
Left choroids, infiltrate, lymphocytes	Minimal	0	0	0	0	1	0
	Minimal	0	0	0	0	1	0
--infiltrate, plasma cell	Minimal	0	0	0	0	1	0
Left sclera, infiltrate, eosinophils, perivascular	Minimal	0	0	1	0	0	0
	Minimal	0	0	1	0	0	0
--infiltrate, plasma cell and lymphocytes	Minimal	0	0	1	0	0	0

Antibody analysis: Eleven of 28 treated monkeys (3 Group 1, 5 Group 2, and 3 Group 3 animals) developed low to moderate antibody titers to ranibizumab in the serum. Antibodies were detected in 6 animals after 2 injections, 4 animals after 3 injections, and 1 animal after 4 injections. Antibodies were not detected in the vitreal fluid.

TK: Serum drug concentrations are summarized in the table below. Vitreous humor drug concentrations on Day 113 were below the reportable level () in most eyes (39/46) and ranged from in the remaining 7 eyes.

Serum concentrations of ranibizumab (ng/ml, mean ± SD)

Group	Serum		
	1	2	3
Day 1	LTR (lower limit of detection, <15.6 ng/ml)	LTR	19.6
Day 2	43.4±6.33	39.5±4.12	71.3±8.21
Day 16	78.7±8.49		252±47.4
Day 30	331±54.4	246±28.5	364±148
Day 58	566±354	460±180	465±232

In summary, monkeys were treated by intravitreal injection with ranibizumab at 250, 500 and 2000 µg/eye every two to four weeks (on Days 1, 15, 29, and 57). There were no systemic drug-related effects as assessed by clinical observations, body weights, food consumption, clinical pathology, or post-mortem gross examinations of non-ocular tissues. Administration of ranibizumab was associated with a transient inflammatory response. The inflammatory reaction in the anterior chamber appeared most intense following the first injection. Changes in the posterior segment of the eye included acute focal or multifocal, perivenous retinal hemorrhages in the venules of the far peripheral retina. The hemorrhages typically occurred following initial doses of 500 or 2000 µg/eye, and generally resolved within 1 week. An additional change in the posterior segment of the eye was focal or multifocal perivascular sheathing around peripheral retinal venules, which resolved by the end of the study. Histopathologic examination revealed infiltrates (including neutrophils, plasma cells, lymphocytes, or eosinophils) in different ocular tissues in all groups. In

conclusion, ITV treatment of cynomolgus monkeys with ranibizumab at dose levels from 250 to 2000 µg/eye caused reversible ocular inflammation. Administration of a steroid did not appear to alter the inflammatory response of the drug.

01-463-1757: 26-Week Intravitreal injection Toxicity Study with rhuFab VEGF in Cynomolgus Monkeys with a 8-Week Recovery

Key study findings: Intravitreal administration of rhuFab VEGF produced a dose-dependent anterior and posterior segment inflammation in monkeys. The inflammatory response was reversible.

Study no.: 01-463-1757

Conducting laboratory and location: 

Date of study initiation: 1/16/2002

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: rhuFab VEGF, Lot Nos. TS01-036, TS01-037, and TS01-038, purity =
The formulation was similar to the proposed clinical formulation.

Study Design:

Group	Dose	N/sex
1	Vehicle	6*
2	500 µg/eye	4
3	500 µg/eye on Day 1 and 1000 µg/eye thereafter	4
4	500 µg/eye on Day 1, 1000 µg/eye on Day 15, and 2000 µg/eye thereafter	6*

* Two animals/sex in Groups 1 and 4 were designated as recovery animals.

The purpose of this study was to assess the toxicity of ranibizumab when administered to cynomolgus monkeys 14 times over a 26-week period followed by 8 weeks of recovery.

Methods

Doses: 500, 1000 and 2000 µg/eye, both eyes, on Days 1, 15, 29, 43, 57, 71, 85, 99, 113, 127, 141, 155, 169 and 183

Species/strain: Cynomolgus monkeys

Number/sex/group or time point: 4 to 6

Route, formulation, volume, and infusion rate. Intravitreal, 50 µl/eye, once every 2 weeks for 14 times followed by a 8-week recovery period

Satellite groups used for toxicokinetics or recovery: No

Age: 2-3.5 years old

Weight (nonrodents only): 2.1-2.7 kg

Unique study design or methodology: No

Observation times

Mortality: Twice daily

Clinical signs: Twice daily

Body weights: Weekly

Clinical ophthalmology examination (including IOP): Before initiation of treatment and on Days 1, 3, 8, 15, 17, 22, 29, 31, 36, 43, 45, 50, 57, 71, 85, 87, 99, 113, 127, 129, 134, 141, 155, 169, 171, 183, and 185, and during recovery on Days 196/197, 210/211, and 240/241 (pre-dose if it was a dosing day)

Ocular photography, fluorescein angiography and ERG: Prior to initiation of treatment and during Weeks 13/14, 25/26, and 33/34

Hematology and clinical chemistry: Before initiation of treatment and on Days 45, 101, 185 and 240/241

Gross pathology: On Day 185 for terminal sacrifice and Day 240/241 for recovery sacrifice

Histopathology: All animals. See Histopathological Inventory Table

Toxicokinetics: Samples were collected pre-dose on each dosing day, approximately 24 and 48 hr postdose, and on the days of necropsy [Day 185 (48 hr after last dose) and Day 240/241].

Serum antibody analysis: Before initiation of treatment (Day -12/-13), predose on Days 15, 29, 57, 85, 113, 141, 169, and 183, and prior to the recovery necropsy on Day 240 (females) and Day 241 (males)

Vitreous fluid collection: From each animal at scheduled necropsies

Results:

Mortality: No mortality occurred during the study period.

Clinical observations: No drug-related abnormal findings were noted. Eye squinting was noted at low incidence in all groups and was considered secondary to ocular inflammation or the dosing and examination procedures.

Physical examinations: There were no test article-related effects on physical examination parameters including heart rate, body temperature, and respiratory rate.

Body weights: No treatment-related differences in body weights were noted.

Clinical pathology: No treatment-related, toxicologically significant differences in hematology and clinical chemistry were noted.

Clinical ophthalmic examination: Mild and reversible inflammation (trace to 1+ anterior chamber and vitreal cell scores) was seen in vehicle-treated animals, and was considered not toxicologically significant. Five HD animals (three females and two males) were discontinued from treatment starting from Day 57 to Day 169 due to severe ocular inflammation in both eyes with a degraded view of the fundus. The right eye of one LD animal was discontinued from treatment due to a suspected ocular infection.

Dose-related inflammation in the anterior segment of the eye [cells and flare, levels ranging from trace to severe levels (4+)] was noted in drug-treated animals. In this 26-week study, the first treatment in all treatment groups resulted in an anterior chamber inflammatory reactions that were most prominent at 48 hr after dosing and spontaneously diminished to 0 or trace levels by 7 days postdose. Anterior chamber inflammation after doses 2 and 3 in Groups 2 through 4 was generally transient with either no or mild (trace to 1+) anterior chamber cell persisting beyond 7 days after dosing. However, for the following doses (doses 4 through 14), the duration and intensity of the anterior chamber inflammatory responses in Groups 2 through 4 were increased. The number of eyes with no aqueous cells at 14 days post-dose declined from 14/16 and 14/24 on Day 43 to 2/15 and 0/16 for Groups 2 and 4 animals on Day 183, respectively. On Day 43, no eye in any group had anterior chamber cell scores $\geq 2+$ (25-50 cells seen in a single field of slit-lamp

beam) and aqueous flare. On Day 183, the number of eyes with $\geq 2+$ anterior chamber cells was 4/16 for Group 3 and 6/16 for Group 4, and aqueous flare was seen in 1/15 eyes of Group 2, 4/16 eyes of Group 3, and 6/16 eyes of Group 4. Beginning Day 31, there was a clear dose-related increase in aqueous cells that persisted through the end of the treatment period. Other positive findings including fibrin clots, hypopyon, and/or conjunctival follicles were commonly seen after the fourth dose in HD animals. The increased duration and intensity of the inflammatory effect seen after doses 4 through 14 suggested that the 2-week dosing interval was not long enough to allow the eye to fully recover from the inflammatory reactions before the next dosing.

Vitreous cell scores also showed a dose- and time-effect. Inflammatory cells in the vitreous body were slower to appear and to disappear from the vitreous body than the aqueous humor. With the 2 week dosing interval, the cells tended to accumulate in the vitreous with subsequent dosing. A portion of the cells that persisted for more than 2 weeks post-dosing was brown in color (noted in all groups, including the vehicle control). It is possible that the brown color was due to hemosiderin from previous hemorrhage caused by the injection trauma. The majority of eyes in Group 1 had only mild (trace to 1+) vitreous cell scores at all time points.

Two forms of posterior segment inflammation of the eye evidenced by changes around the peripheral retinal venules were noted in drug-treated groups. The first form was an acute response characterized by focal or multifocal, perivenous retinal hemorrhages with white centers in the far peripheral retina. These typically appeared at 48 hr after the first dose of 500 $\mu\text{g}/\text{eye}$ were administered, resolved one week after dosing, and did not recur or were notably diminished after subsequent injections. During the study period, this response was seen in 26 eyes of 15/28 drug-treated animals. The second form of posterior segment inflammation, first seen on Day 36 for males and Day 22 for females, was characterized as focal or multifocal, white, perivascular sheathing around peripheral retinal venules. These changes tended to become more prominent with repeated administration of the drug. This response was not seen in the Group 1 animals but observed during one or more examinations in most LD animals (13/16 eyes in 7/8 animals) and in all MD and HD animal eyes. In general, the greater the doses, the earlier in the course of the study the lesions appeared. The sponsor indicated that the rapidity with which perivascular sheathing appeared also predicted to some extent which animals would be ultimately withdrawn from the study. Varying amounts of white inflammatory material were also seen over the surface of the optic disc at one or more time points over the course of the study in one eye of one LD animal, 4 eyes of two MD animals, and 10 eyes of 6 HD animals. These posterior segment inflammatory changes diminished when the ITV treatment was discontinued or following scheduled recovery.

Small, injection-associated vitreous "floaters" were noted in most eyes in all groups over the course of the study. Many times in drug-treated animals, mainly in HD animals, white infiltrates obscured the optic disc. In some drug-treated animals in each group, the intraocular inflammatory response following dosing was severe enough to obscure the view of the fundus at one or more time points (first seen on Day 31). These changes were dose-related, were most serious at 48 hr after dosing and gradually improved with time until the next ITV injection. The obscured view was believed to be the entry of protein and/or cells into the vitreous cavity. The incidence for Groups 2 and 3 was similar, whereas that for group 4 was increased (8/16 eyes in 4 LD animals, 10/16 eyes in 5 MD animals, and 24/24 eyes in all HD animals). In several animals, protein and inflammatory debris persisted within the vitreous cavity for more than 2 weeks after dosing and continued to severely impair the view of the fundus. Five HD animals affected to this degree were withdrawn from dosing after 5 to 11 injections because it was no longer possible to evaluate the fundus and their vision was believed to be severely impaired. Four of the five animals had perivascular sheathing early

in the study (before or soon after the third dose). The sponsor suggested that early-onset of the perivascular sheathing might be an indicator for the development of more severe inflammatory responses later. Treatment was stopped on the right eye of one Group 2 animal due to a suspected ocular infection.

A cataractogenic effect in Groups 3 (two males) and 4 (5 males) was noted that was correlated with the intensity and duration of the inflammatory response. Cataracts tended to occur in the second half of the study period after multiple doses. In each case, a new cataract developed only after a relatively long period of intense inflammation suggesting that the lens changes were secondary to chronic inflammation. Similar findings were not observed among Group 2 animals.

IOP: An immediate, usually 2-5 fold increase in intraocular pressure (IOP) was seen after ITV injection in all groups. This IOP spike completely resolved by the next time IOP was measured 48 hr after dosing. The increase in IOP was related to the rapid increase in intraocular volume induced by the injection of a fluid bolus. No significant alterations in IOP between groups were detected for females, but Group 4 males had lower IOP than Group 1 vehicle control males at most time points after Day 85 (9.9-11.4 mmHg vs. control's 12.6-15.0 mmHg). The cause of this phenomenon is unclear. The sponsor indicated that the decreased intraocular pressure was commonly associated with inflammation of the anterior segment of the eye. The reviewer considers that the decreased IOP might be related to the long-term inflammation that damaged the normal ocular structure. In clinical practice, the dosing frequency (once a month) and dosage (0.3 mg/eye) are much lower than the dose (2 mg/eye) in this study. The reviewer considers the lower IOP in Group 4 males is not clinically significant.

Photography: Color fundic photographs were consistent with the clinical ophthalmologic examination findings. Additional findings including venous dilatation and tortuosity (two eyes of one LD animal and 5 eyes of three HD animals), venous beading (one eye of one MD animal and 4 eyes of two HD animals), possible peripapillary retinal thickening (6 eyes of three LD animals, 6 eyes of 3 MD animals, and 7 eyes of 4 HD animals), macular thickening (one eye of one LD animal), possible papillary swelling (two eyes of one LD animal, 6 eyes of 3 MD animals, and 8 eyes of 5 HD animals), avascular papillary tuft (5 eyes of 3 LD animals, 4 eyes of two MD animals, and 16 eyes of 8 HD animals) were associated with the inflammation observed during the clinical ophthalmic examinations. The incidence of these findings tended to follow a dose-related pattern. On fluorescein angiography, questionable fluorescein leakage was noted in the papillary region of three HD animals (6 eyes) and in the peripapillary region of two MD animals (four eyes).

ERG: ITV injections of the vehicle control or test article showed no toxicologically significant electrophysiological retinal effects on the monkeys.

Macroscopic examinations: No drug-related abnormal systemic or ocular findings were noted.

Histopathology: No drug-related abnormal findings in systemic tissues were noted. Drug-related inflammation in ocular tissues is summarized in the table below. Because of differences between left and right eyes in tissue processing and staining [paraffin embedded complete circumferential sections and H&E staining (left eyes) versus epoxy embedding of segments and toluidine blue staining (right eyes)], the microscopic assessments of left and right eyes differed somewhat. However, they showed similar inflammatory responses. There were no degenerative changes in any ocular structure. The inflammatory finding was characterized by a general progression from lymphohistiocytic infiltrates to mixtures of

lymphocytes, macrophages, and neutrophils to granulomatous inflammation with increasing dose of the test article. Fewer inflammatory reactions were noted following the recovery period.

Positive ocular histopathology findings

	Group	Males						Females					
		Terminal				Recover		Terminal				recover	
		1	2	3	4	1	4	1	2	3	4	1	4
Left eye	N	4	4	4	4	2	2	4	4	4	4	2	2
Inflammation, acute, vitreous	Slight	0	0	0	1								
Inflammation, lymphohistiocytic, ciliary body	Minimal	0	3	1	0			1	1	1	1	1	0
	Slight	0	0	2	0			0	1	0	1		
	Moderate					0	1	0	1	1	2	0	2
Inflammation, chronic-active, ciliary body	Moderate	0	0	0	2								
	Moderate-severe							0	0	1	0		
Inflammation, granulomatous, ciliary body	Slight	0	0	0	1			1	0	0	0		
	Moderate	0	0	0	2			0	0	1	0		
Inflammation, chronic-active, limbus	Minimal	0	1	2	0								
	Slight	0	0	0	1								
Inflammation, chronic-active, sclera	Minimal							0	1	0	0	0	1
	Slight	0	0	0	2			0	0	0	2		
Inflammation, chronic-active, optic disc	Minimal	0	1	1	0			0	2	2	2	0	2
	Slight	0	0	1	1								
Inflammation, chronic, perivascular, retina	Moderate							0	1	2	0		
	Minimal	0	0	0	1			0	1	0	0		
Inflammation, granulomatous, perivascular, retina	Moderate							0	1	0	0		
	Minimal	0	4	3	1			0	1	2	1	0	2
Inflammation, neutrophils, macrophages, lymphocytes, vitreous	Slight	0	0	1	2			0	1	1	0		
	Moderate							0	0	1	1		
Inflammation, granulomatous, optic disc	Minimal	0	1	1	1	0	2						
	Slight	0	2	0	1								
Inflammation, chronic-active, optic disc	Moderate	0	0	0	1								
	Minimal					0	1						
Inflammation, neutrophils, lymphocytes, choroid, anterior	Minimal					0	1						
Inflammation, lymphohistiocytic, iris	Minimal					0	1					0	1
	Slight	0	0	1	1			0	0	2	1		
Inflammation, lymphohistiocytic, sclera	Slight							0	1	0	0		
	Minimal	0	0	2	1			0	0	1	0	0	1
Inflammation, chronic-active, perivascular, retina	Slight							0	1	1	0		
	Moderate							0	0	1	0		
Infiltrate, lymphocytes, ciliary body	Minimal					0	1						
	Moderate	0	0	0	1								
Hemorrhage, ciliary body	Slight							0	0	0	1		
	Slight							0	1	0	1		
Hemorrhage, vitreous	Slight							0	1	0	1		
	Minimal	0	0	2	1			0	0	1	0	0	1
Right eye	N	4	4	4	4	2	2	4	4	4	4	2	2
	Minimal	0	2	2	2	0	1	0	2	4	3	0	2
Inflammation, lymphocytes, neutrophils, macrophages, angle	Slight	0	2	2	0			0	1	0	1		
	Moderate	0	0	0	1								
Inflammation, lymphocytes, neutrophils, macrophages, perivascular retina	Minimal	0	0	0	2								
	Slight	0	0	1	1								
Inflammation, granulomatous, ciliary body	Slight											0	1
	Moderate	0	0	0	3	0	1						
Inflammation, chronic, perivascular, retina, anterior	Minimal					0	1						
	Minimal	0	2	2	2			0	2	2	2		
Inflammation, neutrophils, macrophages, lymphocytes, vitreous	Slight	0	0	0	1								
	Minimal	0	0	1	1			0	1	1	0	0	1
Inflammation, granulomatous, sclera (likely injection site)	Slight	0	1	1	0			0	1	0	0		
	Minimal							0	0	1	0		
Inflammation, neutrophils, lymphocytes, macrophages, choroids, anterior	Minimal							0	0	1	0		
	Minimal	0	3	3	2			0	1	2	1		
Inflammation, lymphocytes, neutrophils, macrophage, iris	Moderate	0	0	0	1			0	0	0	2		
	Minimal							0	1	2	1		
Inflammation, chronic-active, perivascular, retina	Minimal							0	1	2	1		
	Slight							0	1	1	0		
Inflammation, lymphocytes, neutrophils, macrophages, ciliary body	Minimal	0	0	2	0			0	1	1	0	0	1
	Slight	0	0	1	0			0	2	2	2		

	Group	Males						Females					
		Terminal				Recover		Terminal				recover	
		1	2	3	4	1	4	1	2	3	4	1	4
Right eye	N	4	4	4	4	2	2	4	4	4	4	2	2
Infiltrate, lymphohistocytic, choroids	Minimal	1	0	0	1								
Thrombus, vascular, retina	Slight	0	0	0	1								
Hemorrhage, sclera	Moderate							0	0	1	0		
Hemorrhage, ciliary body	Minimal							0	0	1	0		
Hemorrhage, vitreous	Slight							0	0	1	0		

TK: TK data are summarized in the table below. The mean vitreous ranibizumab concentrations on Day 185 (the day of terminal necropsy) increased in proportion to dose. Mean serum concentrations were approximately 460- to 1000-fold lower than the concentrations in the vitreous. Higher AUC levels were noted in animals with positive antibodies (3-5 folds greater than in antibody-negative animals), suggesting that the antibodies may interfere with the PK of the drug possibly by decreasing drug clearance or overestimation of the drug concentration in the ELISA method.

TK data (mean ± SD)

Group	Serum			Vitreous humor		
	2	3	4	2	3	4
Dose (µg/eye)	500	500/1000	500/1000/2000	500	500/1000	500/1000/2000
AUC _{0-Day 184} (µg-day/ml)	20.5±14.1	23.1±6.47	88.7±54.3	Vitreous concentration		
AUC in Ab positive animals	29.8±14.9					
AUC in Ab negative animals	11.1±2.35					
Mean concentration 48 hr after the last dose (Day 184, µg/ml)	0.166±0.125	0.265±0.198	1.22±1.06	127±38.9	276±57.5	569±53.5
C _{max} (µg/ml)	0.362±0.303	0.354±0.222	2.05±1.24			
T _{max} (day)	83.5±51.2	113±54.4	93.3±43.2			

Antibody analysis: No antibody was detected in control or MD animals. Serum antibodies to ranibizumab were detected in 4/8 LD animals and 11/12 HD animals. To explain why antibodies were not detected in MD animals, the sponsor indicated that antibodies were detected sporadically and not necessarily in a dose-dependent manner in a teleconference on 5/10/06. Antibodies first appeared on Day 29, two weeks after the second dose. There was no apparent correlation between the degree of ocular inflammation and the appearance of antibodies.

In summary, monkeys were treated by ITV injection with ranibizumab at 500, 1000 and 2000 µg/eye every two weeks for 14 doses followed by an 8-week recovery period. There were no systemic drug-related effects as assessed by clinical observations, body weights, clinical pathology, or anatomical pathology of non-ocular tissues. ITV injection of ranibizumab at all dose levels produced a dose-dependent anterior and posterior segment inflammation (single to multifocal perivenous retinal hemorrhages and focal to multifocal perivascular sheathing around peripheral retinal venules) which tended to increase in severity and duration with subsequent doses. Cataracts secondary to chronic inflammation were noted in MD and HD animals in the second half of the study period. The increased duration and intensity of the inflammatory effect seen after doses 4 through 14 suggested that the 2-week dosing interval did not allow time for the eye to fully recover. Inflammation observed at the ophthalmic examinations was concordant with the results of the ocular photographs. Retinal function as assessed by ERG was not affected. Microscopic examination of the ocular tissues revealed inflammatory cell infiltrates without indication of a degenerative process. Ocular inflammation was reduced when dosing was discontinued for animals with severe inflammatory reactions (five HD animals) or during the recovery period, suggesting that the inflammatory process was reversible.

At the low dose, 500 µg/eye, the incidence and severity of anterior chamber inflammation and vitreal cell findings were much less than those seen in MD and HD animals. On histopathological examinations, the degree of inflammatory cell infiltration was mostly minimal to slight, and the incidence was less than in MD and HD groups. No abnormal IOP, cataract and fluorescein leakage changes were noted. The sponsor considered 500 µg/eye the maximum tolerated dose. The reviewer believes that it is reasonable.

Appears This Way
On Original

Appears This Way
On Original

Histopathology inventory

Study	98-358-1757	01-463-1757	98-361-1757
Species	Monkey	Monkey	Monkey
Adrenals	X	X	X
Aorta	X	X	X
Bone Marrow smear	X	X	X
Bone (femur)	X	X	X
Brain	X	X	X
Cecum	X	X	X
Cervix	X	X	X
Colon	X	X	X
Duodenum	X	X	X
Epididymis	X	X	X
Esophagus	X	X	X
Eye	X	X	X
Gall bladder	X	X	X
Gross lesions	X	X	X
Harderian gland			
Heart	X	X	X
Ileum	X	X	X
Injection site			X
Jejunum	X	X	X
Kidneys	X	X	X
Lachrymal gland	X	X	X
Larynx			
Liver	X	X	X
Lungs	X	X	X
Lymph nodes, axillary	X	X	X
Lymph nodes inguinal	X	X	X
Lymph nodes, mesenteric	X	X	X
Mammary Gland	X	X	X
Nasal cavity			
Optic nerves	X	X	X
Ovaries	X	X	X
Pancreas	X	X	X
Parathyroid	X	X	X
Pituitary	X	X	X
Prostate	X	X	X
Rectum	X	X	X
Salivary gland	X	X	X
Sciatic nerve	X	X	X
Seminal vesicles	X	X	X
Skeletal muscle	X	X	X
Skin	X	X	X
Spinal cord	X	X	X
Spleen	X	X	X
Sternum	X	X	X
Stomach	X	X	X
Testes	X	X	X
Thymus	X	X	X
Thyroid	X	X	X
Tongue	X	X	X
Trachea	X	X	X
Ureter	X	X	X
Urethra	X	X	
Urinary bladder	X	X	X
Uterus	X	X	X
Vagina	X	X	X

X, histopathology performed

6.6.6.4 Genetic toxicology

No genotoxicity studies were conducted with ranibizumab.

2.6.6.5 Carcinogenicity

No carcinogenicity studies were conducted with ranibizumab.

2.6.6.6 Reproductive and developmental toxicology

No reproductive toxicity studies with ranibizumab were conducted.

2.6.6.7 Local tolerance**98-359-1757: Intravitreal Local Tolerance Study with rhuFab VEGF in Rabbits**

Key study findings: Administration of ranibizumab as a single intravitreal injection to male NZW rabbits produced a slight inflammatory response by 7 days postdose. Lower intraocular pressure in some drug-treated eyes might be associated with a mild, transient cyclitis.

Study no.: 98-359-1757

Conducting laboratory and location: _____

Date of study initiation: 4/21/1999

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: rhuFab VEGF, Lot No. M4-TOX8, purity: _____

The purpose of this study was to assess the local tolerability of ranibizumab when administered to rabbits as a single ITV injection. The day of dosing was designated as Day 1.

Methods

Doses: 2 mg/eye, left eyes. The right eye was treated with vehicle.

Species/strain: Male Hra:(NZW)SPF rabbits

Number/sex/group or time point: 9

Route, formulation, volume, and infusion rate: Intravitreal, 50 µl/eye, single dose

Satellite groups used for toxicokinetics or recovery: No

Age: 13 weeks old

Weight (nonrodents only): 2.1-2.6 kg

Unique study design or methodology: No

Observation times

Mortality: Twice daily

Clinical signs: Twice daily

Body weights: Days 1, 2, 4, and 8

Ophthalmology (IOP, indirect ophthalmoscopy and slit lamp biomicroscopy): Days -1, 2, 4, and 8

Gross pathology: Days 2, 4, and 8, three animals per time

Histopathology: All animals; the anterior and posterior ocular segments, iris, ciliary body, central and peripheral retina, and retina/optic nerve

Results:

Mortality: No mortality occurred during the study period.

Clinical observations: Low food consumption was seen in two animals during Days 1 through 3 and on Day 4, respectively.

Body weights: All animals had similar body weights at initiation of treatment and on Days 2, 4, and 8.

Ophthalmology: No active inflammation characterized by flare or the presence of inflammatory cells was seen. Vitreal "floaters" and iris inflammation were each observed in one of nine test material-treated eyes on Day 2 only (the animal with vitreal "floaters" was sacrificed on Day 2). Four of nine drug-treated eyes exhibited decreased intraocular pressure on Day 2 (6-9 mmHg vs. 12 mmHg at pretest).

Necropsy: No toxicologically significant abnormal findings in systemic or ocular tissues were noted.

Histopathology: In the treated eyes, drug-related changes were limited to minimal to slight subacute inflammation in the vitreous of animals sacrificed on Days 4 and 8. The inflammation was evidenced by neutrophilic and mononuclear cell infiltration in the vitreal areas adjacent to but not including the retina, ciliary body, or iris. The severity of the inflammation was increased from minimal in all animals sacrificed at Day 4 to minimal or slight in each of the three animals sacrificed at Day 8. Inflammatory cells were not seen in the vitreous of the three animals sacrificed at Day 2.

In summary, NZW rabbits were treated with a single ITV injection of ranibizumab (2 mg/eye, left eye only) and were observed for 7 days. Ophthalmology examination of the anterior and posterior ocular segments showed transient iris inflammation in one drug treated eye and vitreal "floaters" in another drug-treated eye on Day 2 only. Lower intraocular pressure was seen in 4 drug-treated eyes. Histopathological examinations showed a minimal to slight subacute inflammatory response in vitreous by 4 and 8 days postdose.

00-205-1757: Intravitreal Local Tolerance Bridging Study with rhuFab VEGF in Rabbits

Key study findings: Single ITV injection of the two lots of ranibizumab (Lot Nos. M4-TOX8 and M4-TOX14) in rabbits produced similar effects.

Study no.: 00-205-1757

Conducting laboratory and location:

Date of study initiation: 6/13/2000

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: rhuFab VEGF, Lot Nos. M4-TOX14 and M4-TOX8, purity

The purpose of this study was to assess the local tolerability of ranibizumab of two different lots when administered to rabbits as a single ITV injection. The day of dosing was designated as Day 1.

Methods

Doses: 2.5 mg/eye, Lot M4-TOX14 for left eyes and Lot M4-TOX8 for right eyes
Species/strain: Male Hra:(NZW)SPF rabbits
Number/sex/group or time point: 9
Route, formulation, volume, and infusion rate: Intravitreal, 50 µl/eye, single dose
Satellite groups used for toxicokinetics or recovery: No
Age: 86-93 days old
Weight (nonrodents only): 2.06-2.44 kg
Unique study design or methodology: No

Observation times

Mortality: Twice daily

Clinical signs: Twice daily

Body weights: Days 1, 2, 4, and 8

Ophthalmology (IOP, indirect ophthalmoscopy and slit lamp biomicroscopy): Days -5, 2, 4, and 8.

Gross pathology: The animals were necropsied on Days 2, 4, and 8 (3 animals/day)

Histopathology: All animals; the anterior and posterior ocular segments, iris, ciliary body, central and peripheral retina, and retina/optic nerve

Results:

Mortality: No mortality occurred during the study period.

Clinical observations: Low food consumption was seen in 6 animals during Day 2, and two of these animals on Day 3, and for one animal on Day 6. No detailed information was provided. The sponsor indicated that low food consumption was considered to be related to dose administration procedures.

Body weights: Body weight loss was seen in three animals on Day 2. Animals gained weight in all other examination times.

Ophthalmology: No significant anterior segment changes were noted in either eye. Vitreal "floaters" were noted in three Lot M4-TOX14-treated (left) eyes and one Lot M4-TOX8-treated (right) eye on Day 4. Vitreal flare was observed in one Lot M4-TOX8-treated (right) eyes on Day 2. The sponsor stated that these findings were indicative of a low grade cyclitis which might be related to the intravitreal injection. A similar decrease in average intraocular pressure (mean IOP = 9-13 mmHg vs. 17 mmHg pretest) was noted in both groups on Days 2, 4 and 8. Overall, the ocular findings revealed no meaningful difference in the ocular tolerance of the two lots of test material.

Necropsy: There were no macroscopic observations in the eyes

Histopathology: Mild inflammatory cell (neutrophils, lymphocytes, macrophages, and/or plasma cells) infiltration in various ocular tissues (vitreous, conjunctiva/eyelid, anterior chamber, ciliary body, optic disk) was seen in eyes treated with both lots. On Day 8, there were more observations from Lot M4-TOX14 (inflammatory cell infiltration in the choroids and optic disk). Despite more locations containing inflammatory cells following administration of Lot M4-TOX14, the cellular composition of the infiltrates was comparable between the two lots.

In summary, NZW rabbits were treated with a single ITV injection of ranibizumab (2.5 mg/eye, Lot M4-TOX8 for right eyes and Lot M4-TOX14 for left eyes) and were observed for 7 days. Ophthalmologic examination showed vitreal flare in one Lot M4-TOX8 eye on Day 2, and vitreal “floaters” in three M4-TOX14-treated eyes and one Lot M4-TOX8-treated eye on Day 4. Histopathological examinations showed inflammatory cell infiltration in various locations in the globe treated with both lots. Overall, the two lots of ranibizumab produced similar ocular responses.

02-406-1757: Intravitreal Local Tolerance Bridging Study with rhuFab VEGF (ranibizumab) in Rabbits

Key study findings: More inflammatory responses were seen with Lot M4-TOX14. Microscopic findings indicated higher frequency and severity of inflammatory cell infiltrates in the left eye (Lot No. M4-TOX14) than in the right eye (M3-TOX61).

Study No.: 02-406-1757

Conducting laboratory and location:

Date of study initiation: 10/22/2002

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: rhuFab VEGF, Lot Nos. M4-TOX14 and M3-TOX61, purity = Lot M3-TOX61 was the to-be-marketed formulation.

Ingredient	Lot M3-TOX61	Lot M4-TOX14
Ranibizumab		
α,α-trehalose dehydrate		
histidine HCl		
Polysorbate 20		
Water for Injection	qs	qs

The purpose of this study was to assess the local tolerability of ranibizumab of two different lots when administered to rabbits as a single ITV injection. The day of dosing was designated as Day 1.

Methods

- Doses: 2.0 mg/eye, Lot M4-TOX14 for left eyes and Lot M3-TOX61 for right eyes
- Species/strain: Male Hra:(NZW)SPF rabbits
- Number/sex/group or time point: 9
- Route, formulation, volume, and infusion rate: Intravitreal, 50 µl/eye, single dose
- Satellite groups used for toxicokinetics or recovery: No
- Age: 86-93 days old
- Weight (nonrodents only): 2.06-2.44 kg
- Unique study design or methodology: No

Observation times

Mortality: Twice daily

Clinical signs: Twice daily

Body weights: Days 1, 2, 4, and 8

Food consumption: Once daily

Ophthalmology (IOP, indirect ophthalmoscopy and slit lamp biomicroscopy): Days -5, 2, 4, and 8.

Gross pathology: Days 2, 4, and 8 (3 animals/day)

Histopathology: All animals; the anterior and posterior ocular segments, iris, ciliary body, central and peripheral retina, and retina/optic nerve

Results:

Mortality: No mortality occurred during the study period.

Clinical observations: Periorbital swelling was noted on Day 2 for two of the nine animals. This finding might be related to the method of administration and not a direct result of the test articles.

Body weights and food consumption: There were no apparent effects on body weight or food consumption.

Ophthalmology: Positive findings on Day 2 included inflammation of the iris in two left eyes, conjunctival chemosis in two right eyes and three left eyes, vitreal flare in two left eyes, vitreal “floaters” in one left eye, and aqueous humor flare in one right eye. On Day 4, vitreal “floaters” were seen in two left eyes and vitreal flare in one left eye. On Day 8, there were one instance of vitreal “floaters” and one instance of vitreal flare in Lot M4-TOX14-treated (left) eye. No toxicologically significant intraocular pressure changes were noted. Overall, the ocular findings revealed no toxicologically significant observations in local tolerance and no meaningful difference between the two test article lots.

Necropsy: There were no macroscopic observations in the eyes.

Histopathology: Positive findings are summarized in the table below. There were inflammatory cell infiltrates in several intraocular locations and in the conjunctiva/eyelids of both eyes. On Days 4 and 8, there were more significant microscopic observations from Lot M4-TOX14 (left eyes) than from Lot M3-TOX61 (right eyes). Despite more locations containing inflammatory cells following administration of Lot M4-TOX14, the cellular composition of the infiltrates was not different between the two lots.

Individual histopathology findings

Animal number	1	2	3	4	5	6	7	8	9
Sacrifice day	2	2	2	4	4	4	8	8	8
Left conjunctiva/eyelid: --infiltrates, neutrophils	2	2	3						
-- infiltrates, neutrophils, macrophages, lymphocytes					1		1		1
Left anterior chamber: --infiltrates, neutrophils				1	1	1			
Left ciliary body: --infiltrates, neutrophils					1				
--infiltrates, macrophages, between basal lamina and pigmented epithelial cells, pars plana		2							
Left angle, --infiltrates, neutrophils				1					
-- infiltrates, neutrophils, macrophages, lymphocytes		1			3	2			
Left retina: -- infiltrates, neutrophils, macrophages, lymphocytes, perivascular						1	1	1	4
-- infiltrates, neutrophils, macrophages, lymphocytes				2	1				
Left optic disc: -- infiltrates, neutrophils, macrophages, lymphocytes, perivascular					3		1	2	
Left vitreous: --infiltrates, neutrophils	1								
-- infiltrates, neutrophils, macrophages, lymphocytes			2	2	3	3	2	2	3
Left sclera: -- infiltrates, neutrophils, macrophages, lymphocytes		1							
Right conjunctiva/eyelid: --infiltrates, neutrophils		1	1		1				
-- infiltrates, neutrophils, macrophages, lymphocytes	1							1	
Right retina: hypertrophy, retinal pigmented epithelium								1	
Right vitreous: --infiltrates, neutrophils			1						
-- infiltrates, neutrophils, macrophages, lymphocytes				1	1		1		1

1=minimal, 2=slight, 3=moderate, 4=moderately severe

In summary, NZW rabbits were treated with a single ITV injection of ranibizumab (2 mg/eye, Lot M3-TOX61 for right eyes and Lot M4-TOX14 for left eyes) and were observed for 7 days. Ophthalmologic examination showed vitreal flare and iris inflammation in Lot M4-TOX14-treated eyes, and aqueous flare in one Lot M3-TOX61 treated eye on Day 2, and vitreal “floaters” and flare in Lot M4-TOX14-treated eyes on Days 4 and 8. It appeared that more inflammation responses were seen with Lot M4-TOX14. Histopathological examinations showed inflammatory cell infiltration in various locations in the globe treated with both lots. Higher frequency and severity of inflammatory cell infiltrates were seen in the left eye (Lot No. M4-TOX14) than in the right eye (M3-TOX61).

2.6.6.8 Special toxicology studies

98-279-1754: Cross-Reactivity of Biotinylated Second Generation Humanized Monoclonal Antibody rhuMAb VEGF (GN1754) with Normal Human Tissue

Key study findings: No cross-reactive binding of rhuMAb VEGF was observed to any human tissues.

Study no.: 98-279-1754

PAI study #: IM483

Conducting laboratory and location: C

GLP compliance: Yes

Drug, lot #, and % purity: Biotinylated rhuMAb VEGF (GN1754), Lot: 31217-12, purity =

Study initiation: 11/30/1998

The purpose of this immunohistochemistry study was to evaluate the potential cross-reactivity of rhuMAb VEGF with cryosections of normal human tissues. To detect binding, the biotinylated test article, rhuMAb VEGF, was applied to cryosections of tissues from three adult human donors at three concentrations (400, 25, and 10 µg/ml). Human tissues sectioned included adrenal, blood vessels, bone marrow, brain, cervix, esophagus, retina, granulocytes, heart, kidney, liver, lung, lymph node,

lymphocytes/monocytes, mammary gland, ovary, pancreas, pituitary, placenta, platelets, prostate, salivary gland, skin, large and small intestine, spinal cord, spleen, stomach, skeletal muscle, testis, thymus, thyroid, ureter, urinary bladder, and uterus. Cryosections of _____ with _____ were used as the positive control material; cryosections of normal human cerebellum were used as the negative control tissue. Other controls were produced by omission of the test article or substitution of the test article with a biotinylated negative control humanized monoclonal antibody of the same immunoglobulin subclass as the test article but with antigenic specificity to IgE (rhuMAb E25). Immunoperoxidase staining was used in this study.

Results:

rhuMAb VEGF stained the positive control material, _____ at all antibody concentrations. Staining by the negative control rhuMAb E25 antibody ranged from negative to equivocal at the 400 µg/ml concentration level, while no reactivity was observed at the 25 or 10 µg/ml concentration levels. Neither the test article nor the negative control antibody demonstrated specific reactivity with the human cerebellar negative control tissue. In human test tissues, neither antigen-specific nor cross-reactive binding was demonstrated regardless of concentration examined. In conclusion, no cross-reactive binding of second generation humanized monoclonal antibody rhuMAb VEGF was observed to any human tissues.

98-360-1757: Hemolytic Potential, Blood Compatibility, and Vitreal fluid Compatibility Testing with rhuFab VEGF

Key study findings: Ranibizumab at concentrations of 20, 7.5, or 2.5 mg/ml did not cause hemolysis of human erythrocytes, and were compatible with cynomolgus monkey, human serum and plasma, and with human vitreal fluid.

Study no.: 98-360-1757

Conducting laboratory and location: _____

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: rhuFab VEGF, Lot No. M4-TOX8

The purpose of this study was to assess the hemolytic potential of ranibizumab for cynomolgus monkey and human whole blood, the blood compatibility of ranibizumab with cynomolgus monkey and human serum and plasma, and the compatibility of ranibizumab with fresh human vitreal fluid. The tests were performed with ranibizumab at concentrations of 20, 7.5, and 2.5 mg/ml.

The hemolytic potential was evaluated by measuring the concentration of soluble hemoglobin in the supernatant after mixing equal volumes of ranibizumab or ranibizumab vehicle with cynomolgus monkey or human whole blood. During Phase 1, blood samples were collected from one cynomolgus monkey and one human. Test mixtures were incubated for 45 min at 37°C. Following a weakly positive initial result in cynomolgus monkey blood, the test was repeated with monkey blood following dilution of ranibizumab vehicle with isotonic sodium chloride. Test mixtures were incubated for 41 min at 39°C. During Phase 2, blood samples were tested from two cynomolgus monkeys and two humans. One of the cynomolgus monkeys and one of the humans were the same donors as were used in Phase 1. Test mixtures were incubated for 43 minutes at 37°C. Saponin, a hemolytic agent, was used as a positive control.

Compatibility with serum and plasma was determined by the absence of precipitation or coagulation in mixtures of equal volumes of ranibizumab or ranibizumab vehicle and cynomolgus monkey or human serum or plasma that had been incubated for 30 min at room temperature (23.6°C). [Reviewer's comments: Three incubation durations and temperatures were used in this study. No explanations were provided.]

Compatibility with human vitreal fluid was determined by the absence of precipitation in mixtures of equal volumes of ranibizumab or ranibizumab vehicle and human vitreal fluid that had been incubated for 31 min at 39°C.

Results:

Ranibizumab at final matrix concentrations of 20, 7.5, or 2.5 mg/ml and/or the vehicle did not cause hemolysis of human erythrocytes, and were compatible with cynomolgus monkey and human serum and plasma, and with human vitreal fluid.

Weakly positive results were noted for the hemolytic potential test for ranibizumab vehicle and all ranibizumab concentrations with cynomolgus monkey blood in Phase 1. Since the hemoglobin concentrations among these samples were comparable (586-879 mg/dl), the positive result was considered due to ranibizumab vehicle. The test was negative for ranibizumab vehicle following dilution [1:1 and 1:3 (v:v)] with isotonic sodium chloride. In Phase 2, positive hemolytic results were again obtained for all ranibizumab concentrations with the same monkey blood (530-651 mg/dl). The result of the Phase 2 hemolytic potential test for vehicle with the same monkey donor as in Phase 1 was negative (341 mg/dl), although the test mixture hemoglobin concentration was only slightly below the criterion for a positive test (500 mg/dl). Phase 2 hemolytic potential test results were negative with samples from the second monkey donor and both humans. It was not clear if the weakly positive effect in one monkey blood was due to the drug.

In conclusion, ranibizumab, at final matrix concentrations of 20, 7.5, or 2.5 mg/ml did not cause hemolysis of human erythrocytes and were compatible with cynomolgus monkey and human serum and plasma and with human vitreal fluid.

00-580-1757: Assessment of the Safety of Intravitreal Injections of rhuFab VEGF (V2) in Combination with Intravenous Verteporfin Photodynamic Therapy following Laser-induced Choroidal Neovascularization (CNV) in Cynomolgus Monkeys

Key study findings: Ranibizumab ITV injection in combination with verteporfin PDT induced no significant increase in toxicity over that observed following administration of PDT alone. The toxicity of ranibizumab in combination with PDT was similar to that of ranibizumab alone.

Study no.: 00-580-1757

Conducting laboratory and location: C

Date of study initiation: 12/18/1999

GLP compliance: No

QA report: yes () no (X)

Drug, lot #, and % purity: rhuFab VEGF, Lot No M4-TOX8 and Verteporfin for injection (Visudyne®)

Study Design:

Group	N	Day of ranibizumab dosing	Ranibizumab ($\mu\text{g}/\text{eye}$)	Day of PDT dosing	Laser-induced CNV	Treatment order
1	7	14, 28, 42, 56	500, 2000 x 3	21, 35, 49	Yes	rhuFab V2 followed by PDT
2	5	21, 35, 49	500, 2000 x 2	14, 28, 42	Yes	PDT followed by rhuFab V2
3	4*	0, 14, 28, 42	500, 2000 x 3	7, 21, 35	No	rhuFab V2 followed by PDT
4	4**	7, 21, 35	500, 2000 x 2	0, 14, 28	No	PDT followed by rhuFab V2
5	2	14, 28, 42	500, 2000 x 2	14, 28, 42	Yes	Concomitant rhuFab V2 and PDT

* One animal received an additional treatment on Day 50

** Two animals received two additional treatments on Days 49 and 63.

The purpose of this non-GLP study was to assess the safety and pharmacological activity of ITV injections of ranibizumab in combination with intravenous verteporfin photodynamic therapy (PDT) following laser-induced choroidal neovascularization (CNV) in cynomolgus monkeys. Additionally, safety of the combination treatment was assessed in normal animals with no laser-induced CNV lesions. In all groups, the right eye received combination treatment of ranibizumab and PDT while the left eye received treatment of ranibizumab vehicle with PDT. Two regimens were included based on whether ranibizumab was administered first followed by PDT one week later for up to three cycles or PDT followed by ranibizumab one week later. These two dosing regimens were carried out both in animals with eyes with CNV as well as in animals with normal eyes. Additionally, one group of monkeys with laser-induced CNV lesions received ranibizumab and PDT on the same day for three cycles of treatment over a 28-day period. For laser irradiation for PDT, 15 min after the start of IV infusion of verteporfin, the retina was irradiated with 689 nm light at $600 \text{ mW}/\text{cm}^2$. The light dose was $100 \text{ J}/\text{cm}^2$ and the spot size was 3 mm. The second eye was treated within 5 min of the first eye so that treatments were completed within 20 min of the start of verteporfin infusion.

Methods

Doses: Ranibizumab: 500 and 2000 $\mu\text{g}/\text{eye}$, right eyes only. The left eye was treated with vehicle. Verteporfin for injection, 6 mg/m^2 , 10 ml/animal, intravenous injection

Species/strain: Cynomolgus monkeys, 1-7 years old, 2-5 kg

Route, formulation, volume, and infusion rate: Ranibizumab: intravitreal, 50 $\mu\text{l}/\text{eye}$. Verteporfin: intravenous injection

Observation times

Clinical signs: Daily

Body weights: Biweekly

Clinical ophthalmology examination: Approximately weekly

Gross pathology: All animals, at termination (Days 63, 56, 49, 42, and 56 for Groups 1, 2, 3, 4, and 5)

Histopathology: Ocular tissues, all animals

Toxicokinetics: On each ranibizumab dosing day, predose and 24 hr post-dose

Serum antibody analysis: Days 14, 18, and 42

Vitreous fluid collection: At termination

Results:

Clinical observations: No drug-related abnormal findings were noted.

Body weights: A slight, similar decrease in body weights was seen across all groups.

Clinical ophthalmic examination: Ocular inflammation was observed, characterized by anterior chamber flare along with increased vitreal inflammatory cells. The greatest amount of anterior chamber inflammation was seen following the initial dose of ranibizumab. Following subsequent doses of ranibizumab, the inflammation was either the same or attenuated despite a 4-fold increase in dose (500–2000 $\mu\text{g}/\text{eye}$). Ranibizumab vehicle-treated eyes exhibited minimal or no anterior chamber or vitreal inflammation. Treatment with PDT did not alter the anterior chamber inflammatory response induced by ranibizumab. The combined treatment of ranibizumab and PDT by any regimen, either in normal eyes or eyes with CNV lesions induced by laser, did not alter the inflammatory response.

The posterior pole of the eye, as assessed by indirect ophthalmoscopy, fundic photography, and fluorescein angiography, showed no differences in normal eyes (eyes with no laser-induced CNV lesions, Groups 3 and 4) treated with the combination of ranibizumab and PDT, compared to PDT alone. Typical effects of PDT with or without ranibizumab were seen at the posterior pole of the eyes in Groups 3 and 4 animals (early hypofluorescence in the area of the laser spot with late leakage in the same area). In eyes with CNV lesions (Groups 1, 2, and 5), no differences were seen among ranibizumab treated groups. Fluorescein angiography showed a lower incidence of grades III-IV leakage in the ranibizumab/PDT group than in the PDT group alone. Less leakage was observed on Days 35 and 56 (21 and 42 days, respectively, after first treatment on Day 14) when ranibizumab was injected in combination with PDT than when PDT was used alone. No grade IV leakage was observed in animals treated with ranibizumab/PDT.

Ocular pathology: The only noted difference in eyes that had laser-induced CNV lesions and were subsequently treated with PDT was the presence of blood vessels in most of the lesions in the left eyes (vehicle-treated eyes). Rare or no vessels were seen in lesion sections from the right eyes (ranibizumab-treated eyes). The sizes and cellular makeup of the CNV were similar in both right and left eyes. No changes were seen between the treated groups with regard to order of ranibizumab/PDT treatment.

The normal choroid histology was compared to a previous study performed in the same laboratory investigating the effects of multiple PDT on normal retina and choroid of monkey eyes using verteporfin. Retinas from previous and current studies were similar. Histological changes in the retina in eyes treated with PDT alone and PDT/ ranibizumab were similar.

TK: Twenty-four hr after ITV administration at 500 $\mu\text{g}/\text{eye}$ and 2000 $\mu\text{g}/\text{eye}$, mean serum concentrations ranged from 0.045–0.084 $\mu\text{g}/\text{ml}$ and 0.162–0.207 $\mu\text{g}/\text{ml}$, respectively. Serum concentrations were similar across all groups. Seven days after the last of multiple administrations of 2000 $\mu\text{g}/\text{eye}$ (Groups 1–4), mean vitreal concentrations in the ranibizumab-treated eye ranged from 82.4–222 $\mu\text{g}/\text{ml}$. In Group 5, 14 days after administration of multiple doses of 2000 $\mu\text{g}/\text{eye}$, mean vitreal concentration was 23.7 $\mu\text{g}/\text{ml}$.

Antibody analysis: Serum antibodies to ranibizumab were detected in two of 21 animals (one Group 3 animal and one Group 4 animal). Antibodies first appeared on Day 42 in Group 3, and on Day 35 in Group 4. No antibodies toward ranibizumab were detected in the vitreous.

In summary, monkeys were treated with ITV ranibizumab administration in combination with verteporfin PDT. Ocular inflammation after ranibizumab injection was noted and was consistent with that seen in previous toxicology studies with ranibizumab. Less inflammation was observed after the subsequent injections of ranibizumab. No to minor inflammation was observed in the ranibizumab vehicle-treated eyes.

Other observation times

Laser treatment: On Day 21, 9 areas of laser treatment were placed symmetrically in the macula of each eye.

Clinical signs: Twice daily

Toxicokinetics: On each ranibizumab dosing day, predose and 24 and 48 hr post-dose, and on Day 65

Serum antibody analysis: Days 15, 29, 43, 57, and 65

Vitreous fluid collection: Day 65

Histopathology: Laser treated areas

Results:

General observations: No clinically adverse effects of either laser treatment or ranibizumab administration were noted.

Ocular examinations: Laser treatment-induced CNV with lesions exhibiting extensive vascular leakage was noted in all areas with most of leakage rated I (minimal). Grade IV leakage was present in 11 of 54 laser-induced lesions in three vehicle-treated eyes. Grade IV leakage was observed only in males. The significance of the sex difference was not clear. Grade IV leakage was not observed in the ranibizumab-treated eyes. The number of lesions with moderate to severe leakage was lower in the ranibizumab-treated eyes (see table below). Mild perivenous sheathing and peripapillary swelling were observed in only 2/6 of the ranibizumab-treated eyes. No sustained changes in intraocular pressure were noted.

Individual laser results (Day 20 post laser treatment)

Sex	Grade I		Grade II		Grade III		Grade IV	
	Right eye	Left eye	Right eye	Left eye	Right eye	Left eye	Right eye	Left eye
Male	9	6	0	0	0	0	0	3
Male	9	4	0	1	0	0	0	4
Male	9	5	0	0	0	0	0	4
Female	9	9	0	0	0	0	0	0
female	3	7	0	0	6	2	0	0
Female	7	6	2	3	0	0	0	0
Total	46	37	2	4	6	2	0	11

Scores of laser-induced lesions: Grade I lesions showed no hyperfluorescence on fluorescein angiography. Grade II lesions showed hyperfluorescence without leakage. Grade III lesions had hyperfluorescence early or mid-transit and late leakage. Grade IV lesions had bright hyperfluorescence early or mid-transit and late leakage beyond the borders of the treated area.

Post-mortem examinations: There were no macroscopic observations. Microscopically, the laser sites were similar and characterized by a central area with loss of neuroretinal tissue, retinal pigmented epithelium (RPE), Bruch's membrane, and the choriocapillaris. The destruction of the neural retina followed a pattern beginning with loss of the outer segments and nuclei of rods and cones, and then the inner nuclear layer. The choroid was also altered at laser sites. Its contour was no longer smooth and there were occasional depressions or bulges (relative to the interior of the globe).

The pharmacologic activity of the test-article was determined by evaluating the post-laser ocular response. Histologically, the laser injury sites of both eyes of the males often contained blood vessels in abnormal locations, but the pattern was different between left and right eyes. The changes of "duplication, basal lamina" and "capillaries, subretinal" were not observed in the ranibizumab-treated eyes (right) of the males. The apparent amount of cellular proliferation within the "retinal presence, choroidal cells" was usually greater in left eyes. In "retinal presence, choroidal cells" there was a focus of tissue resembling choroids extending through the breach in Bruch's membrane into the neural retina. In most instances large capillaries

or small arterioles/venules surrounded by a few adventitial cells were present within these choroid-like foci. Anastomoses, especially in left eye sites, often accompanied "retinal presence, choroidal cells", and were recorded when these blood vessels appeared to be contiguous with pre-existing retinal vessels of similar diameter.

The changes induced by the laser injury in left and right eyes of females were not as prominent as in males. The changes, "duplication, basal lamina" and "capillaries, subretinal" were not observed as commonly in ranibizumab vehicle eyes (left) of females as in males. Minimal "capillaries, subretinal" was observed at ranibizumab-treated right eye lesions in one female. The same animal also had chronic inflammation in the choroid at several right eye laser lesions. Chronic inflammation was not observed in any ranibizumab vehicle-treated (left) eye lesions.

TK: Median serum ranibizumab concentrations one day after each dose ranged from 43.1 to 80.4 ng/ml, with an overall range of individual values from . Median serum ranibizumab concentrations two days after each dose ranged from 31.6 to 87.4 ng/ml with an overall range of individual values from 24.3 to 547 ng/ml. At necropsy, the median serum ranibizumab concentration was 17.4 ng/ml. The median concentration of ranibizumab in the vitreous humor 8 days after the final ITV dose was 25650 ng/ml.

Antibody analysis: Antibodies to ranibizumab were detected in 3/6 animals. Antibodies appeared as early as Day 29 in 1 female monkey and on Day 43 in two male monkeys.

In summary, results of this study demonstrated CNV lesions with Grade IV leakage. The mitigative effect of ranibizumab on Grade IV CNV vascular leakage was noted. In addition, the reduced-severity of vascular leakage in ranibizumab-treated eyes compared with that of vehicle-treated eyes generally correlated with the absence of subretinal capillaries and was consistent with the anti-angiogenic effect of ranibizumab. In conclusion, the laser treatment induces neovascularization and ranibizumab effectively reduces the neovascularity.

2.6.6.9 Discussion and Conclusions

Four repeated dose ocular toxicity studies were conducted in monkeys with the duration of up to 6 months. The doses used in these studies ranged from 250 to 2000 µg/eye, and the results were generally considered consistent. Although no drug-induced systemic toxicity was seen, drug-induced, dose-related inflammatory responses evidenced by the presence of cells and flare in the anterior chamber and appearance of cells and "floaters" in the vitreous were noted at all doses in all studies. At the same doses, the inflammatory reaction in the anterior chamber was the most intense after the first treatment and diminished with subsequent injections. The sponsor indicated that this might represent an intrinsic attenuation of the inflammatory response. Anterior chamber inflammation was generally transient with peak scores at 48 hr after dosing and spontaneously diminishing to no or a mild level of anterior chamber cell accumulation within 7 days after dosing.

Posterior segment inflammation of the eye included acute focal or multifocal, perivenous retinal hemorrhages and focal or multifocal perivascular sheathing around peripheral retinal venules. Retinal hemorrhage typically appeared at 48 hr after doses of 500 µg/eye or greater were administered for the first time, resolved one week after dosing, and did not reoccur or were notably diminished on subsequent injections. Perivascular sheathing appeared earlier with higher doses, and the rapidity with which

perivascular sheathing appeared might predict to some extent which animals would be ultimately withdrawn from the study.

Intravitreal injection of either ranibizumab or vehicle caused immediate and marked increases in intraocular pressure (usually 2-5 fold) due to an acute increase in intraocular volume. Intraocular pressure was back to normal at the next measurement as early as 15 min after dosing in all groups. In the 26-week study, males at 2000 mg/eye had lower IOP than vehicle control males at most time points after Day 85 (post-seventh dose). The sponsor indicated that the decreased intraocular pressure was commonly associated with inflammation of the anterior segment of the eye. The reviewing pharmacologist has discussed this issue with a medical officer. The decreased IOP might be related to the long-term inflammation that damaged the normal ocular structure, e.g., ciliary body that was responsible for the production of the aqueous humor. In clinical practice, the dosing frequency (once a month) and dosage (0.3 mg/eye) were much lower than in this study. The reviewer does not consider the lower IOP at 2000 µg/eye to be clinically significant.

There were no toxicologically significant findings on ERG evaluation. Histopathological examinations showed inflammatory cell infiltrates (neutrophils, macrophages, plasma cells, lymphocytes, or eosinophils) in different ocular tissues. Following a recovery period, all inflammatory responses were reversed or reduced.

Serum antibodies to ranibizumab were detected in some animals in many studies. Antibodies were detected in the vitreous in one study at doses ≥ 750 µg/eye.

In the 13-week ocular toxicity study, endophthalmitis was seen in three animals (one each in control, MD and HD groups). For the vehicle control animal, no antibody was found in serum or vitreous humor. For the MD animal, endophthalmitis was seen on Day 17 while antibody was detected on Day 57 in serum and Day 101 in vitreous humor. For the HD animal, endophthalmitis was seen on Day 22. Vitreal fluid collected on the same day did not show antibody. The antibody was detected on Day 57 in serum and Day 129 in vitreous humor. Based on these data, there was no strong evidence that endophthalmitis was due to the antibodies against ranibizumab.

In the pivotal 26-week study, following doses 4 through 14, the duration and intensity of the anterior chamber inflammatory responses were increased, which was different from the inflammatory responses seen after the first 3 doses and from other studies in which inflammation after the first dosing was the most severe. The increased duration and intensity of the inflammatory effect seen after doses 4 through 14 suggested that the 2-week dosing interval was not long enough to allow the eye to fully recover from the inflammatory reactions before the next dosing. The proposed clinical dosing interval is one month. Therefore, this finding does not appear to be a serious safety concern.

In the 26-week study, 5 animals (three females and two males) treated with the drug at 2000 µg/eye were discontinued from treatment starting from Day 57 to Day 169 because of an obscured view of the fundus due to the protein and/or cells in the vitreal cavity. Four of the five animals had perivascular sheathing early in the study (before or soon after the third dose). The sponsor suggested that early-onset of the perivascular sheathing might be an indicator for the development of more severe inflammatory responses later. The reviewer has conveyed this information to the medical team.

Also in the 26-week study, a cataractogenic effect was noted in animals receiving the treatment at 1000 and 2000 µg/eye doses. In each case a new cataract developed only after a relatively long period of intense inflammation following multiple doses, suggesting that the lens changes may be secondary to chronic inflammation. Similar findings were not observed among animals at 500 µg/eye.

Three local tolerance studies were conducted in rabbits with a single ITV injection of the drug at 2.0 or 2.5 mg/eye followed by a 7-day observation. The ocular inflammation in rabbits was less severe than in monkeys. Therefore, monkeys seem to be a more appropriate species used in this application. Two lots of ranibizumab used in nonclinical studies (Lot M4-TOX8 and Lot M4-TOX14, both were produced similar ocular responses. In a study to compare the local tolerability of ranibizumab of two different lots (Lot M3-TOX61, the to-be-marketed formulation and Lot M4-TOX14), more inflammatory responses were seen with Lot M4-TOX14. Histopathological examinations showed a higher frequency and severity of inflammatory cell infiltrates with Lot M4-TOX14.

No cross-reactive binding of humanized monoclonal antibody rhuMAb VEGF was observed to any human tissues. Ranibizumab at concentrations of up to 20 mg/ml did not cause hemolysis of human erythrocytes, and was compatible with monkey and human serum and plasma, and human vitreal fluid. In two non-GLP monkey studies with a laser-induced CNV model, ITV injection of ranibizumab prevented formation of CNV lesions and decreased leakage of already formed CNV lesions. ITV injection of ranibizumab in combination with verteporfin PDT did not induce a significant increase in toxicity compared to PDT alone, and the toxicity of ranibizumab in combination with PDT was similar to that of ranibizumab alone.

Ranibizumab given by ITV injection caused inflammation in both anterior and posterior segments. The inflammation was dose-dependent. Results from four monkey studies demonstrated that 0.5 mg ranibizumab/eye was the maximum tolerated dose. In these studies, the incidence and severity of anterior chamber inflammation and vitreal cell findings were much less than those seen at higher doses. In histopathological examinations, the degree of inflammatory cell infiltration was mostly minimal to slight, and the incidence was less than in that seen at higher doses. No abnormal IOP, cataract or fluorescein leakage changes were noted. Considering the differences between human and monkey in vitreal volume (human: 4.5 ml vs. monkey: 1.5 ml), dosage (human: 0.3 mg/eye vs. monkey: 0.5 mg/eye), and dosing frequency (human: once a month vs. monkey: every 2 weeks in the 26-week study), the reviewing pharmacologist concludes that nonclinical data are adequate to support the proposed clinical use of the drug. The NDA is approvable.

2.6.6 TOXICOLOGY TABULATED SUMMARY

Not applicable

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Ranibizumab is a recombinant, humanized monoclonal IgG1 antibody antigen-binding fragment (Fab) designed to bind and inhibit all active forms of VEGF. In vitro studies demonstrated the high apparent binding affinity of ranibizumab for active forms of VEGF and its inhibition of VEGF-mediated endothelial cell proliferation and vessel leakage. Studies in the non-human primate eye in a laser-induced CNV model

demonstrated the ability of ranibizumab to limit the development of CNV lesions and reduce vascular permeability.

Disposition of ranibizumab was characterized in monkeys and rabbits. Following ITV dosing, ranibizumab was present in vitreous humor, aqueous humor, all layers of the retina, ciliary body, iris, corneal endothelium, and serum. The elimination half-life in the vitreous humor was 3 days. Systemic drug concentrations after bilateral ITV administration were 1000-fold lower than in the vitreous and declined in parallel with the ocular compartments. Pharmacokinetic parameters estimated following repeated dosing in the cynomolgus monkey were consistent with parameters estimated following single-dose administration.

ITV toxicology studies of up to 26 weeks in duration were performed in cynomolgus monkeys and rabbits. Ranibizumab-related effects were limited to ocular tissues. Administration of ranibizumab to monkeys resulted in inflammatory reactions evidenced by transient, dose-dependent anterior chamber flare and cell responses and the appearance of vitreal cells and floaters. The severity of this inflammation varied from minimal to severe, generally increasing in severity as the dose of ranibizumab increased but decreasing overtime. A low incidence of inflammation was observed in rabbits administered a single ITV injection of ranibizumab. In monkeys, posterior segment changes of two general forms, perivenous retinal hemorrhage and perivascular sheathing, were also observed in some animals. Although predose and post-dose treatment by oral and topical ocular routes with corticosteroids did not alter the inflammatory response, evidence of reversibility of the ocular inflammation was observed during recovery periods.

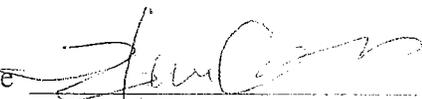
Based on the nonclinical study findings and the proposed clinical dosing regimen, the reviewing pharmacologist concludes that nonclinical data are adequate to support the proposed clinical use of the drug, and approval is recommended. After a careful review of the draft labeling proposed by the sponsor, the reviewing pharmacologist considers that the pharmacology/toxicology-related parts of the labeling are acceptable. No modifications are recommended.

Unresolved toxicology issues: No

Recommendations:

Approval is recommended.

Signatures:

Reviewer Signature  5/15/06

Supervisor Signature  Concurrence Yes No 5/15/06

APPENDIX/ATTACHMENTS

