# CENTER FOR DRUG EVALUATION AND RESEARCH

***APPLICATION NUMBER:***

# 125387Orig1s000

# PHARMACOLOGY REVIEW(S)

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

# PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

| | |
|---|---|
| Application number: | 125387 |
| Supporting document/s: | IND 12462 [redacted] (b) (4) Memo to File dated June 17, 2009 |
| Applicant's letter date: | February 17, 2011 |
| CDER stamp date: | February 18, 2011 |
| Product: | Aflibercept Ophthalmic Solution |
| Indication: | Neovascular "wet" age-related macular degeneration (AMD) |
| Applicant: | Regeneron Pharmaceuticals, Inc. |
| Review Division: | Anti-infective and Ophthalmology Products |
| Reviewer: | María I. Rivera, Ph.D. |
| Supervisor/Team Leader: | William Taylor, Ph.D. |
| Division Director: | Renata Albrecht, M.D. |
| Project Manager: | Michael Puglisi |

## Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 125387 are owned by Regeneron Pharmaceuticals, Inc. or are data for which Regeneron Pharmaceuticals, Inc. has obtained a written right of reference. Any information or data necessary for approval of BLA 125387 that Regeneron Pharmaceuticals, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of BLA 125387. Applicant material has been used in this review.

# TABLE OF CONTENTS

# Table of Tables

## Table of Figures

# 1 Executive Summary

## 1.1 Introduction

VEGF Trap is a recombinant protein that is composed of two domains of the human VEGF cell surface receptors (VEGF R1 and VEGF R2) fused to the Fc region of human IgG. This recombinant molecule binds with high affinity to VEGF-A ($K_D$=0.36-0.76 pM) along with the related Placental Growth Factor (PlGF; $K_D$=29-392 pM). VEGF Trap has demonstrated anti-angiogenic activity in several preclinical animal models. In this BLA, this molecule is intended for the treatment of neovascular (wet) age-related macular degeneration (AMD) by intravitreal (ITV) injection of a 2 mg dose once every 2 months, following 3 initial 2 mg monthly injections.

## 1.2 Brief Discussion of Nonclinical Findings

The monkey was selected as the relevant species. Findings observed in ocular toxicity studies following ITV administration of VEGF Trap included mild and transient increases in anterior segment and vitreous cellularity (interpreted as a mild inflammation) that was not associated with other ocular abnormalities. These findings occurred at doses 0.5 times the intended clinical dose when correcting for vitreous volume (i.e., assuming a vitreous volume of 2 mL in monkeys and 4 mL in humans). However, the mild and transient nature of the finding does not represent a major clinical concern.

Epithelial erosion/ulceration of the nasal turbinates accompanied with chronic-active inflammation was noted in the ocular toxicity studies following ITV administration of VEGF Trap. Partial recovery was observed. Similar, albeit more severe lesions in the nasal cavity were noted in systemic toxicity studies in monkeys following repeated, IV administration. These findings occurred at exposures 42 and 56 times higher those observed after ITV administration in humans based on $C_{max}$ and AUC, respectively. The reviewer is not aware of the observation of similar nasal findings with any other approved VEGF inhibitor following ITV injection. The applicant monitored for this finding in a subset of patients in the clinical trials. The finding is acknowledged in the proposed label.

Systemic toxicity studies in monkeys identified toxicities mostly related to the pharmacology of VEGF Trap. The main target organs included the bone, kidney, adrenals, ovary and, as noted above, nasal cavity. Other microscopic findings included vascular alterations in the brain choroid plexus and digestive tract (duodenum, stomach, gallbladder, pancreas), vascular degeneration and fibrosis in several tissues including the heart, and hepatic portal inflammation and periportal necrosis. Findings in the bone, nasal cavities, digestive system, liver, and brain (choroid plexus) were still present at recovery. A NOAEL was not established but these systemic adverse effects occurred at systemic exposures well in excess of the exposure observed in humans.

VEGF Trap adversely affected the female and male reproductive systems. Absent or irregular menses associated with alterations in female reproductive hormone levels,

decreases in ovarian and uterus weights, ovarian and uterine microscopic alterations, reduction in sperm motility, and sperm morphological abnormalities were observed at all dose levels. All changes were reversible. A NOAEL was not established but these systemic adverse effects occurred at systemic exposures over 1500 times higher than the exposure observed in humans. These findings are well known class effects.

As expected, given the role of VEGF in organogenesis, VEGF Trap was embryotoxic and teratogenic in rabbits. Dose-related increases in fetal resorptions, abortions, and numerous fetal (external, visceral and skeletal) malformations were observed. A developmental NOAEL was not identified but systemic exposures were at least 600 times higher than those in humans. Free VEGF Trap was detected in amniotic fluid samples in the dose range-finding study in rabbits.

VEGF inhibitors, as a class, are known to increase blood pressure. Elevations in blood pressure were primarily observed in rats and mice after systemic administration. No effects were noted after ITV administration in monkeys. The blood pressure remained elevated above pre-treatment baseline values until circulating VEGF Trap levels fell below ~ 1 µg/mL in both rats and mice. The mean $C_{max}$ observed in humans is ~50 times lower than the identified threshold in rodents. The applicant monitored for changes in blood pressure in the clinical trials.

## 1.3 Recommendations

1.3.1 Approvability

Approval is recommended from the nonclinical perspective.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

### 8.1 Pregnancy

Pregnancy Category C

(b) (4)

[redacted]

**Reviewer's recommendations (2nd paragraph):** Aflibercept produced embryo-fetal toxicity (b) (4) *when administered during organogenesis in pregnant rabbits* (b) (4) at intravenous (b) (4) doses of (3 to 60 mg/kg). *A series of external, visceral, and skeletal malformations were observed in the fetuses.* The maternal No Observed Adverse Effect Level (NOAEL) was (b) (4) 3 mg/kg, *whereas the fetal NOAEL was below 3 mg/kg.* At this dose, the systemic exposures based on $C_{max}$ and AUC for free aflibercept were approximately 2900 and 600 (b) (4) *times* higher, respectively, than corresponding values observed in humans after an intravitreal dose of 2 mg.

## 13 NONCLINICAL TOXICOLOGY

### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

**Reviewer's recommendations:** No studies have been conducted on the mutagenic or carcinogenic potential of aflibercept. Effects on male and female fertility were assessed as part of a 6-month study in monkeys with intravenous administration of aflibercept at doses ranging from 3 to 30 mg/kg. Absent or irregular menses associated with alterations in female reproductive hormone levels and changes in sperm morphology and motility were observed at all dose levels. *In addition, females showed decreased ovarian and uterine weight accompanied by compromised luteal development and reduction of maturing follicles. These changes correlated with uterine and vaginal atrophy.* A No Observed Adverse Effect Level (NOAEL) was not identified. Based on $C_{max}$ and AUC for free aflibercept observed at the *lowest dose used of* 3 mg/kg (b) (4) the systemic exposures were approximately 4900 (b) (4) *times* and 1500 (b) (4) *times* higher, respectively, than the exposure observed in humans after an intravitreal dose of 2 mg. All changes were reversible.

### 13.2 Animal Toxicology and/or Pharmacology

(b) (4)

Similar effects were not seen in clinical studies [*see Clinical Studies (14)*].

**Reviewer's recommendations:** Erosions and ulcerations of the respiratory epithelium in nasal turbinates in monkeys treated with aflibercept intravitreally were observed at (b) (4) *intravitreal doses of 2 or 4 mg/eye*. (b) (4) At the No Observed Adverse Effect Level (NOAEL) *of 0.5 mg/eye*, the systemic exposure was 42 and 56 (b) (4) *times* higher based on $C_{max}$ and AUC, respectively, *than the exposure observed in humans after an intravitreal dose of 2 mg.*

Similar effects were not seen in clinical studies [*see Clinical Studies (14)*].

# 2 Drug Information

## 2.1 Drug

CAS Registry Number: 862111-32-8

Generic Name: Aflibercept

Code Name: VEGF Trap-Eye; BAY 86-5321

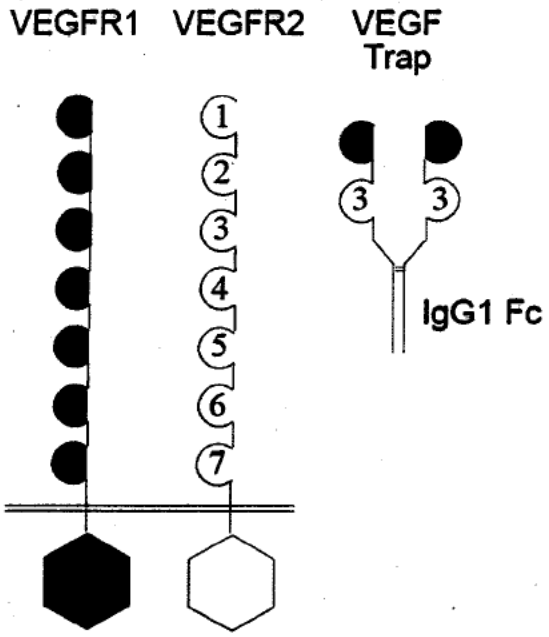
Chemical Name: Des-432-lysine-[human vascular endothelial growth factor receptor 1-(103-204)-peptide (containing Ig-like C2-type 2 domain) fusion protein with human vascular endothelial growth factor receptor 2-(206-308)-peptide (containing Ig-like C2-type 3 domain fragment) fusion protein with human immunoglobulin G1-(227 C-terminal residues)-peptide (Fc fragment)], (211-211':214-214')-bisdisulfide dimer

Molecular Formula/Molecular Weight: Aflibercept is a dimeric glycoprotein with a protein molecular weight of 96.9 kDa. It contains approximately 15 % glycosylation to give a total molecular weight of 115 kDa. Its molecular formula (without glycosylation) is $C_{4318}H_{6788}N_{1164}O_{1304}S_{32}$.

Structure or Biochemical Description: Aflibercept is a recombinant protein consisting of sequences derived from human vascular endothelial growth factor (VEGF) receptor extracellular domains fused to the Fc portion of human immunoglobulin G1 (IgG1). (b) (4) (b) (4) The presumptive Ig domain structure of aflibercept is

provided in Figure 1. The amino acid sequence of the protein was provided in Module 3.2.S.1.2 Structure of the CTD.

**Figure 1 Secondary and Tertiary Structure of Aflibercept**




Pharmacologic Class: Vascular endothelial growth factor (VEGF) inhibitor

## 2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 12462 (b) (4)

## 2.3 Drug Formulation

VEGF Trap-Eye drug product, (b) (4) 40 mg/mL, is a sterile solution for intravitreal (ITV) injection. The drug product is produced by formulating aflibercept drug substance in an aqueous (b) (4) solution at pH 6.2, containing 10 mM sodium phosphate, 40 mM sodium chloride, 5% (w/v) sucrose, and 0.03% (w/v) polysorbate 20 (PS20). The drug product is manufactured in single-dose vials (b) (4)

## 2.4 Comments on Novel Excipients

None

## 2.5 Comments on Impurities/Degradants of Concern

Refer to the product quality and CMC reviews.

## 2.6 Proposed Clinical Population and Dosing Regimen

Aflibercept Ophthalmic Solution is indicated for the treatment of patients with neovascular (wet) age-related macular degeneration (AMD). The recommended dose is 2 mg (50 µL) administered by ITV injection once every 2 months, following 3 initial monthly injections of 2 mg (50 µL). Aflibercept Ophthalmic Solution may be dosed as frequently as 2 mg once per month.

## 2.7 Regulatory Background

(b) (4)

In an applicant meeting held on June 1, 2009 (See Memo to File IND 12462 and (b) (4) dated June 17, 2009), DAIOP concurred that the pharmacology/ toxicology program appeared sufficient for the indications of AMD and (b) (4) as long as there were no findings which warranted further studies. The applicant was asked to justify the use of a single species in the chronic toxicology studies. It was agreed in this meeting that pre- and post-natal development studies were not required for the current indication. Based on ICH S6 recommendations, the Division agreed that conducting genotoxicity and carcinogenicity studies for VEGF Trap were considered inappropriate in this case.

# 3 Studies Submitted

## 3.1 Studies Reviewed

**Primary Pharmacology**

- Determination of Equilibrium Binding Constants for the Interaction of Human VEGF-A165 and Human PlGF-2 with Aflibercept Conformance and Production Lots (Study # VGFT-MX-08021)
- Determination of Equilibrium Binding Constants for the Interaction of Aflibercept with VEGF-Family Related Ligands (Study # VGFT-MX-08022)
- VEGFR2 Bioassays of Aflibercept: Blocking of VEGFR2 Phosphorylation and Calcium Mobilization in HUVE Cells (Study # VGFT-MX-08016)
- VEGFR2 Bioassays of Aflibercept: Blocking of VEGFR2 Phosphorylation and Calcium Mobilization in HUVE Cells (Study # VGFT-MX-08016)

- A Study to Evaluate the Safety and Efficacy of VEGF Trap Administered by Intravitreal Injection and Intravenous Infusion in a Cynomolgus Monkey Choroidal Neovascularization (CNV) Model of Age-Related Macular Degeneration (Study # VGFT-TX-03027)
- Intravitreal Injection of VEGF Trap Reverses Breakdown of Blood-Retinal Barrier in Diabetes (Study # VGT-NC-007)
- VEGF Trap Reverses Breakdown of Blood-Retinal Barrier in Diabetes (Study # VGT-NC-008)
- Intravitreal Administration of VEGF Trap Inhibits Pathological Retinal Neovascularization in a Mouse Model of Oxygen-Induced Retinopathy (Study # VGT-NC-013)

**Secondary Pharmacology**

- Blood Pressure Effects of VEGF Trap (AVE0005) in Telemetered Mice and Rats (Study # VGFT-MX-08018)
- Complement-Dependent Cytotoxicity (CDC) and Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) Activities of Aflibercept (AVE0005, VEGF Trap) (Study # VGFT-MX-07014)

**Safety Pharmacology**

- Effect on the Respiratory Function after a Single 30-Minute Intravenous Infusion in Unrestrained Conscious Rats using Whole Body Plethysmography (Study # VGFT-TX-06009)

**PK/ADME**

- Collection of Samples for a Pharmacokinetic Study of VEGF Trap and Mini-VEGF Trap After Intravitreal Injection in Pigmented Rabbits (Study # VGFT-PK-03028)
- A Single-Dose Intravenous and Subcutaneous Pharmacokinetic Study of VEGF Trap in Cynomolgus Monkeys (Study # VGFT-PK-01012)
- Biodistribution of VEGF Trap in Normal Sprague-Dawley Female Rats (Study # VGFT-PK-01005.2)
- Pharmacokinetics of VEGF Trap Following Intravenous Administration to Sham-Operated and Nephrectomized Sprague Dawley Rats (Study # VGFT-PK-01004.2)
- Pharmacokinetics of VEGF Trap following Intravenous Administration to Sprague Dawley Rat: Correlation between Pharmacokinetic Parameters and Sialic Acid Levels (Study # PK06005-9-SA-01V1)

**Single-Dose Toxicity**

- Summary Report AVE0005: Exploratory Single-Dose Intravenous (30-Minute Infusion) Toxicity Study in Rats with 2-Week Observation Period (Study # VGFT-TX-06007)
- AVE0005: Exploratory Single-Dose Intravenous (30-Minute Infusion) Toxicity Study in Rats with 2-Week Observation Period (Study VGFT-TX-06008)

**Repeat-Dose Toxicity**

- 8-Month Intravitreal Toxicity and Toxicokinetic Study in VEGF Trap in Cynomolgus Monkeys with a 4-Month Recovery (Study # VGFT-TX-05011)
- 13-Week Intravitreal Toxicity and Toxicokinetic Study with VEGF Trap in Cynomolgus Monkeys with a 10-Week Recovery (Study # VGFT-TX-04019)
- 13-Week Exploratory Intravitreal Study of VEGF Trap and Mini-VEGF Trap in Cynomolgus Monkeys (Study # VGFT-TX-03053)
- A 13-Week Exploratory Formulation Intravitreal Toxicity and Toxicokinetic Study of a VEGF Trap in Cynomolgus Monkeys (Study # VGFT-TX-05015)
- An Intravitreal Toxicity and Toxicokinetic Study with VEGF Trap in Cynomolgus Monkeys (Study # VGFT-TX-04025)
- A Non-GLP, Exploratory Study to Determine the Effect of Subcutaneous Administration of VEGF Trap (25 mg/kg) on Nude (T cell deficient) Rats Twice a Week for Four or Eight Weeks (Study # PK01032)
- A non-GLP, Exploratory Study to Determine the Effect of Subcutaneous Administration of VEGF Trap (10 and 15 mg/kg) on Sprague Dawley Rats Three Times per Week for Four Weeks (Study # PK01027)
- A Non-GLP, Exploratory Study to Determine the Effect of Subcutaneous Administration of VEGF Trap (2 and 5 mg/kg) on Sprague Dawley Rats Three Times per week for Four Weeks (Study # PK01034)
- A Non-GLP, Exploratory Study to Determine the Effect of Subcutaneous Administration of VEGF Trap (0.5 and 1 mg/kg) on Sprague Dawley Rats Three Times per Week for Four Weeks (Study # PK01042)
- A 3-Month Toxicity Study of VEGF Trap by Subcutaneous Injection in Rats (Study # VGFT-TX-02006)
- A 6-Month Intravenous Toxicity Study of VEGF Trap in Cynomolgus Monkeys with a 5-Month Recovery Period (Study # VGFT-TX-05009)
- A 4-Week Subcutaneous Toxicity Study of VEGF Trap in Cynomolgus Monkeys Followed by a 4-Week Recovery Period (Module 4.2.3.2, VGFT-TX-03004)
- A 13-Week Subcutaneous Toxicity Study of VEGF Trap in Cynomolgus Monkeys Followed by a 6-Week Recovery Period (Module 4.2.3.2, VGFT-TX-02037)
- A 4-Week Intravenous Toxicity Study of VEGF Trap with a 6-Week Recovery Period in Cynomolgus Monkeys (Module 4.2.3.2, VGFT-TX-02029)
- A 13-Week Repeat-Dose Intravenous Toxicity Study of VEGF Trap in Cynomolgus Monkeys and a 13-Week Recovery Period (Module 4.2.3.2, VGFT-TX-03048)

**Embryonic Fetal Development**

- AVE005: Intravenous (30-Minute Infusion) Embryo-Fetal Toxicity Study in Rabbits (Study # VGFT-TX-06002)
- AVE0005: Intravenous (30-min Infusion) Range-Finding Toxicity Study in Pregnant Rabbits (Study # VGFT-TX-06001; Module 4.2.3.5.2.1)

**Special Toxicology Studies**

- Cross-Reactivity of VEGF Trap with Human Tissue *Ex Vivo* (Study # SPS 01-141)
- Evaluation of VEGF Trap to Induce Hemolysis in Monkey Blood and to Induce Flocculation in Monkey Plasma and Serum (Study # Hem#1)

- Evaluation of VEGF Trap to Induce Hemolysis in Whole Blood from Humans and to Induce Flocculation in Human Plasma and Serum (Study # Hem#3)
- Evaluation of VEGF Trap to Induce Hemolysis in Whole Blood from Humans and to Induce Flocculation in Human Plasma and Serum (Study # Hem#4)
- Evaluation of VEGF Trap to Induce Hemolysis in Whole Blood from Humans and to Induce Flocculation in Human Plasma and Serum (Study # Hem#5)

### 3.2 Studies Not Reviewed

- Pharmacokinetics and Bioavailability of VEGF Trap Following Intravenous and Subcutaneous Administration to Sprague Dawley Rats (Study # VGFT-PK-01001.2)
- Pharmacokinetic Dose Ranging Study of VEGF Trap Administered as a Single Subcutaneous Injection to Sprague-Dawley Rats (VGFT-PK-01002.0)
- Pharmacokinetics and Bioavailability of VEGF Trap Following Intravenous and Subcutaneous Administration to CD-I Mice (VGFT-PK-01007.3)
- Pharmacokinetics of VEGF Trap following Intravenous Administration to Sprague Dawley Rat: Correlation Between Pharmacokinetic Parameters and Sialic Acid Levels (Study # PK06005-9-SA-01V1)
- AVE0005: Exploratory 14-Day Intravenous (30-Min Infusion) Toxicity Study in Nonpregnant Female Rabbits (Study # VGFT-TX-05007)
- AVE0005: Single-Dose Local Intravenous, Intramuscular, and Subcutaneous Tolerance Study in Female Rabbits (Study # VGFT-TX-05008)
- A 3-Month Intravenous Toxicity Study of VEGF Trap in Cynomolgus Monkeys with a 5-Month Recovery Period (Study # VGFT-TX-05010)
- All analytical methods and validation reports

### 3.3 Previous Reviews Referenced

Memo to File: IND 12462 (b) (4) dated 6-17-2009 by James Wild, Ph.D.

## 4 Pharmacology

### 4.1 Primary Pharmacology

**Determination of Equilibrium Binding Constants for the Interaction of Human VEGF-A165 and Human PlGF-2 with Aflibercept Conformance and Production Lots (Study # VGFT-MX-08021)** - The interaction between aflibercept (4 conformance lots and 3 production lots) and human VEGF-$A_{165}$ and PlGF-2 were measured using BiaCore technology. The range for the association rate constant (ka), dissociation rate constant (kd), and equilibrium dissociation constant (KD) are shown in the table below. Similar binding affinities were observed between lots.

**Table 1: Binding Parameters (Range) for the Interaction of Different VEGF Trap Lots with VEGF-$A_{165}$ and PlGF-2**

| Ligand | ka ($M^{-1}s^{-1}$) | Kd ($s^{-1}$) | $K_D$ (pM) |
|---|---|---|---|
| VEGF-$A_{165}$ | 2.28-5.12 x $10^7$ | 1.41-2.54 x$10^{-5}$ | 0.496-0.763 |
| PlGF-2 | 1.75-3.73 x $10^6$ | 1.05-9.62 x$10^{-5}$ | 29.1-42.6 |

**Determination of Equilibrium Binding Constants for the Interaction of Aflibercept with VEGF-Family Related Ligands (Study # VGFT-MX-08022) –** The interaction between aflibercept and eleven VEGF family related ligands was measured using BiaCore technology. As shown in the table below, VEGF Trap exhibited high affinity binding to VEGF-A from human, mouse, rat and rabbit (sub-picomolar $K_D$ values). VEGF Trap also exhibited a high affinity for human and mouse PlGF, although lower than that for VEGF-A. Aflibercept did not demonstrate binding to human VEGF-C and human VEGF-D.

**Table 2: Binding Parameters (Mean) for the Interaction of VEGF Trap to VEGF Family Related Ligands**

| **Ligand** | **ka ($M^{-1}s^{-1}$)** | **kd ($s^{-1}$)** | **$K_D$ (pM)** |
|---|---|---|---|
| Human VEGF-$A_{165}$ | 4.05x$10^7$ | 2.01x$10^{-5}$ | 0.497 |
| Human VEGF-$A_{121}$ | 3.75x$10^7$ | 1.35x$10^{-5}$ | 0.360 |
| Human PlGF-2 | 1.75x$10^6$ | 6.81x$10^{-5}$ | 38.8 |
| Human PlGF-1 | 6.73 x$10^6$ | 2.64x$10^{-3}$ | 392.0 |
| Murine VEGF-$A_{164}$ | 2.80x$10^7$ | 1.64x$10^{-5}$ | 0.585 |
| Murine VEGF-$A_{120}$ | 2.15x$10^7$ | 1.23 x$10^{-5}$ | 0.571 |
| Murine PlGF-2 | 1.64x$10^7$ | 5.45x$10^{-5}$ | 3.33 |
| Rat VEGF-$A_{164}$ | 3.67x$10^7$ | 1.73x$10^{-5}$ | 0.471 |
| Rabbit VEGF -$A_{165}$ | 3.39x$10^7$ | 2.63 x$10^{-5}$ | 0.775 |
| Human VEGF-C | NB[a] | NB | NB |
| Human VEGF-D | NB | NB | NB |
| Abbreviations used: ka = Association rate constant, kd = Dissociation rate constant, $K_D$ = Equilibrium dissociation constant. | | | |

[a] NB= no detectable binding

The applicant stated that the binding affinity of VEGF Trap to VEGF-A from cynomolgus monkey was not tested because the monkey VEGF-A protein sequence is identical on the amino acid level to its human counterpart. Thus, the applicant believes that the binding interaction of human and monkey VEGF-A with VEGF Trap should likely be indistinguishable. The fact that the systemic toxicity studies (Section 6.2) showed

targets of toxicity known for VEGF inhibitors and efficacy (see below) was observed in a monkey model of choroidal neovascularization (CNV) supports the assertion that VEGF Trap binds to monkey VEGF.

**VEGFR2 Bioassays of Aflibercept: Blocking of VEGFR2 Phosphorylation and Calcium Mobilization in HUVE Cells (Study # VGFT-MX-08016) –** Complete inhibition of VEGF-dependent VEGF receptor phosphorylation was observed when VEGF Trap (1 nM) was incubated with 1-3 nM VEGF-$A_{165}$, i.e., at a molar ratio of 1:1 or greater. VEGF Trap effectively inhibited calcium mobilization with an $IC_{50}$ of 1.20-1.73 nM.

**A Study to Evaluate the Safety and Efficacy of VEGF Trap Administered by Intravitreal Injection and Intravenous Infusion in a Cynomolgus Monkey Choroidal Neovascularization (CNV) Model of Age-Related Macular Degeneration (Study # VGFT-TX-03027) –** Cynomolgus monkeys (3/sex/goup) were administered placebo or VEGF Trap at concentrations of 50-500 µg/eye biweekly by ITV injection for a total of 3 doses beginning ~ 1 week prior to laser treatment. An additional high-dose group received a single ITV injection 15 days postlaser for a CNV regression group. A separate set of animals received placebo, or 3 or 10 mg/kg VEGF Trap weekly via IV infusion for a total of 6 doses ~ 1 week prior to laser treatment. Evaluations included fluorescein angiography, slit lamp biomicroscopy, indirect ophthalmoscopy, and intraocular pressure. At 35 days postlaser treatment, animals were sacrificed, and the eyes were collected and preserved for microscopic evaluation.

Administration of VEGF Trap prevented the development of clinically significant Grade 4 CNV leak following laser injury at all doses tested and by both routes of administration as assessed by fluorescein angiography. Microscopic evaluation of laser areas showed that when VEGF Trap administration (500 µg biweekly ITV; only dose evaluated) was begun prior to laser injury, choroidal fibroplasia and retinal elevation scores were all lower in VEGF Trap-treated animals relative to placebo controls. A single ITV injection of 500 µg induced rapid and complete regression of established, active CNV leak with microscopic evidence for a trend toward decreased CNV.

Similar to the findings in the repeat-dose ocular toxicology studies (Section 6.2), mild (trace to 1+) inflammatory cells were observed in the anterior chamber or vitreous in all VEGF Trap treated animals. IOP increased immediately after dosing in all groups including controls with reversal to baseline values by the next time it was measured. This increased was attributed to the sudden increase in intraocular volume secondary to the injection. A lower injection volume (25 µL *vs.* 50 µL) resulted in a smaller increase in IOP.

Peak plasma concentrations of free VEGF Trap were measured 24 hrs after ITV injection with mean concentrations of < 0.886 and <1.51 µg/mL at 250 and 500 µg/eye, respectively. At 50 µg/mL, plasma levels were BLQ. After IV administration, mean concentrations were < 77.4 and <278 µg/mL at 3 and 10 mg/kg, respectively.

**Intravitreal Injection of VEGF Trap Reverses Breakdown of Blood-Retinal Barrier in Diabetes (Study # VGT-NC-007) –** Diabetic male Sprague-Dawley rats received a

single, ITV injection of VEGF Trap (3 µg/3 µL) in one eye, and an equal amount of control protein, hFc in the contralateral eye. Forty-eight hours after treatment, animals were injected IV with Evans Blue dye (45 mg/kg), and retinal vessel permeability was assessed by measuring dye concentration in the retina 2 hrs later. Evans Blue concentrations in eyes injected with VEGF Trap were significantly reduced compared to those injected ITV with hFc, and were equivalent to the levels seen in non-diabetic controls, indicating that VEGF Trap suppressed the leaking of fluid from the blood vessels in the diabetic eyes.

**VEGF Trap Reverses Breakdown of Blood-Retinal Barrier in Diabetes (Study # VGT-NC-008)** – Diabetic male Sprague-Dawley rats received a single, IP injection of VEGF Trap (25 mg/kg) and were assessed 24 hrs later for retinal permeability by the extravasation of Evans Blue dye. As noted after ITV injection (Study # VGT-NC-007 above), Evans Blue concentrations in retinas from animals treated with VEGF Trap were significantly reduced compared to those in diabetic eyes, and were equivalent to the levels seen in non-diabetic controls.

**Intravitreal Administration of VEGF Trap Inhibits Pathological Retinal Neovascularization in a Mouse Model of Oxygen-Induced Retinopathy (Study # VGT-NC-013)** – Seven days old C57/B16 pups subjected to hyperoxia for 5 days exhibited marked pathological angiogenesis in the retina, characterized by the presence of vascular tufts penetrating the inner limiting membrane and chaotic sprouting of vessels on the surface of the retina. VEGF Trap effectively blocked the development of these vascular abnormalities when administered as an ITV dose of 0.5 µg at postnatal day 14 (evaluated at postnatal day 17) or 0.24 µg at postnatal day 15 (evaluated at postnatal day 19).

## 4.2 Secondary Pharmacology

**Blood Pressure Effects of VEGF Trap (AVE0005) in Telemetered Mice and Rats (Study # VGFT-MX-08018)** – Single, subcutaneous injections of VEGF Trap (0.5-25 mg/kg) produced relatively rapid increases in blood pressure in Wistar-Kyoto (WKY) rats. No elevations were noted at ≤0.15 mg/kg. Similarly, doses of 2.5 and 25 mg/kg produced rapid increases in blood pressure in C57BL/6 mice. The maximal increases were evident 2-4 days (WKT rats) or 1-2 days postdose (C57BL/6 mice).

The maximal elevation in blood pressure saturated at ≥10 mg/kg (rats). In contrast, the duration of blood pressure elevation was dose proportional throughout the full range of doses tested, in both species. In rats, blood pressure remained above baseline for only 3-4 days in animals receiving 1 mg/kg VEGF Trap, but remained elevated for more than 2 weeks in animals receiving 25 mg/kg VEGF Trap. In mice, the duration of the blood pressure elevation above baseline was ~7 days at 2.5 mg/kg, whereas it was ~ 21 days at 25 mg/kg.

The duration of blood pressure elevation was correlated with the presence of free VEGF Trap in the circulation, such that systolic and diastolic blood pressure remained

elevated above pre-treatment baseline values until circulating VEGF Trap levels fell below ~ 1 µg/mL in both species.

In both rats and mice, heart rate tended to transiently decrease below baseline levels at all VEGF Trap doses tested. This drop in heart rate appears to be a reflexive response to the elevation of blood pressure.

The clinical mean $C_{max}$ after a 2 mg ITV dose every 4 weeks was 0.0193 µg/mL. Thus, the threshold of 1 µg/mL is ~50 times the observed human exposure.

**Complement-Dependent Cytotoxicity (CDC) and Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) Activities of Aflibercept (AVE0005, VEGF Trap) (Study # VGFT-MX-07014)** - VEGF Trap (0.85 pM-50 nM ± 10 nM $VEGF_{165}$) did not demonstrate ADCC activity in either primary human umbilical vein endothelial cells (HUVECs) or tumor cell lines (lung Calu-6, colon DLD1 and epidermoid A431) incubated with human peripheral blood mononuclear cells (PBMCs). Weak activity was observed on HT1080 cells at the highest drug concentrations used (>1 nM). VEGF Trap (0.85 pM-50 nM ± 10 nM $VEGF_{165}$) was unable to mediate CDC activity in either primary HUVECs or tumor cell lines (fibrosarcoma HT1080, lung Calu6, colon DLD1 and epidermoid A431) incubated with normal human serum (with complement components).

### 4.3 Safety Pharmacology

**Effect on the Respiratory Function after a Single 30-Minute Intravenous Infusion in Unrestrained Conscious Rats using Whole Body Plethysmography (Study # VGFT-TX-06009) –** The IV infusion of AVE0005 over 30 minutes at 10, 50 or 250 mg/kg in unrestrained conscious male Sprague-Dawley rats had no significant effects on the respiratory function measured by whole body plethysmography up to 7 days after administration.

## 5 Pharmacokinetics/ADME/Toxicokinetics

### 5.1 PK/ADME

**Collection of Samples for a Pharmacokinetic Study of VEGF Trap and Mini-VEGF Trap After Intravitreal Injection in Pigmented Rabbits (Study # VGFT-PK-03028)** - Male pigmented New Zealand crossbred rabbits received a single ITV injection of VEGF Trap (500 µg/eye) into both eyes. The protein was detected in all ocular tissues examined (vitreous humor, retina and choroid) and plasma. VEGF Trap was eliminated relatively slowly from the vitreous with a half-life ($t_{1/2}$) of ~5 days. The drug penetrated the retina and choroid, though in general never reached more than 10% of the concentrations in the vitreous. The drug was observed in the plasma, though there was a lag in the time of the maximal plasma VEGF Trap concentrations compared to the peak concentration in the eye tissues (see table below). Bound VEGF Trap was measured in both vitreal and plasma samples. In the vitreous humor, free VEGF Trap levels exceeded bound levels for the duration of the study, while in the plasma, bound levels of VEGF

Trap exceeded free. As noted by the applicant, these results suggest that there is sufficient free VEGF Trap in the vitreous to reach the choroid and retina, the sites of action. Given that bound VEGF Trap concentrations exceed the levels of free VEGF Trap in the periphery, the applicant hypothesized that there should be limited ability of the free VEGF Trap to bind a substantial percentage of the available endogenous VEGF. The terminal $t_{1/2}$ of bound complex in the vitreous was calculated to be ~130 days. The applicant noted this estimate is likely an overestimation of the intrinsic bound VEGF Trap $t_{1/2}$, due to the continual conversion of free VEGF Trap to the bound form while in the vitreal compartment. The mean PK parameters are summarized below.

**Table 3: Mean PK Parameters for Free and Bound VEGF Trap in Rabbits after a Single 500 µg/eye Intravitreal Injection of VEGF Trap**

| Tissue | $C_{max}$ (µg/mL or µg/g tissue*) | $AUC_{0\text{-}672\ hrs}$ (µg•hr/mL or µg•hr/g tissue*) | $T_{max}$ (hr) | $t_{1/2}$ (hr) |
|---|---|---|---|---|
| Vitreous | | | | |
| Free | 491 | 64840 | 6 | 115 |
| Bound | 0.610 | 339 | 240 | 3199 |
| Retina | | | | |
| Free | 20.8* | 3206* | 24 | 132 |
| Choroid | | | | |
| Free | 36.2* | 2963 | 1 | 115 |
| Plasma | | | | |
| Free | 0.515 | 199 | 72 | 157 |
| Bound | 1.26 | 518 | 240 | 171 |

**A Single-Dose Intravenous and Subcutaneous Pharmacokinetic Study of VEGF Trap in Cynomolgus Monkeys (Study # VGFT-PK-01012) –** Four groups were dosed once subcutaneously at 0.75, 1.5, 5.0, and 15.0 mg/kg, and one group was dosed once intravenously at 5.0 mg/kg. Three monkeys/sex were assigned to each group. Following SC administration, measures of exposure ($C_{max}$ and AUC) were approximately proportional to the dose at 0.75 and 1.5 mg/kg and at 5 and 15 mg/kg. Group mean terminal $t_{1/2}$ for males and females were ~2 days at 0.75 and 1.5 mg/kg and ~4 - 5 days at 5 and 15 mg/kg. The corresponding free VEGF Trap mean CL/F values were greater and the t1/2 shorter for the lower dose range, as compared to the higher dose range. As noted by the applicant, these observations, in addition to the lack of dose-proportionality, suggest the contribution of a saturable clearance pathway to the total systemic clearance of free VEGF Trap. On the other hand, the nonlinear PK profile could be due to a dose-dependent bioavailability and/or rate of absorption. Absolute bioavailability following SC administration was similar for males and females and averaged 85% at 5 mg/kg (only calculated at this dose).

Following a single 5 mg/kg IV administration, VEGF Trap displayed a multicompartmental PK serum profile. Clearance was slow, the t1/2 was prolonged, and volume of distribution was low compared to total body water (~690 mL/kg for monkeys). In general, no gender differences were distinguished but there was high variability within groups. The mean PK parameters are summarized below.

**Table 4: Mean PK Parameters of Free VEGF Trap in Monkey Serum after a Single IV or SC Dose**

| Route | | IV | SC | SC | SC | SC |
|---|---|---|---|---|---|---|
| No. of animals[a] | | 6 (3/3) | 6 (3/3) | 6 (3/3) | 6 (3/3) | 6 (3/3) |
| Dose | [mg/kg] | 5 | 0.75 | 1.5 | 5 | 15 |
| $C_{max}$ | [µg/mL] | 181.7 ± 46.4 | 3.7 ± 2.0 | 6.5 ± 2.6 | 36.2 ± 13.0 | 101 ± 20.8 |
| $T_{max}$ | [h] | n.c. | 39 ± 25 | 64 ± 29 | 40 ± 28 | 32 ± 24 |
| $AUC_{0-\infty}$ | [µg x h/mL] | 10235 ± 1532 | 511 ± 217 | 1089 ± 389 | 8704 ± 2584 | 24379 ± 5207 |
| $t_{1/2}$ | [h] | 98 ± 31 | 55 ± 18 | 45 ± 10 | 118 ± 19 | 101 ± 39 |
| CL | [mL/h/kg] | 0.50 ± 0.07 | n.c. | n.c. | n.c. | n.c. |
| Vss | [mL/kg] | 62 ± 11 | n.c. | n.c. | n.c. | n.c. |
| CL/F | [mL/h/kg] | n.c. | 1.68 ± 0.64 | 1.61 ± 0.83 | 0.62 ± 0.17 | 0.64 ± 0.13 |
| $MRT_{0-t}$ | [h] | 99 ± 7 | 98 ± 19 | 115 ± 17 | 137 ± 5 | 140 ± 10 |
| F[b] | [%] | n.c. | n.c. | n.c. | 85 | n.c. |

[a] number of all animals (male / female)
[b] bioavailability determined from $AUC_{0-\infty}$ ratio
n.c = not calculated

**Biodistribution of VEGF Trap in Normal Sprague-Dawley Female Rats (Study # VGFT-PK-01005.2) –** Rats received a 1 mg/kg IV dose of $^{125}$I-labeled VEGF Trap. Heart, lungs, liver, kidneys, adrenal glands, spleen, small intestine, large intestine, colon, fat pad, thigh muscle, thyroid gland, and serum were analyzed for radioactive counts. At 5 min postdose, the radioactivity followed a rank order (% dose) of serum (75%) > liver (11.4%) > kidney (1.33%) > spleen (0.42%) > lung (0.34%) > heart (0.19%). The remaining tissues had levels of 0.01-0.04% of the dose. By 24 hrs postdose, the levels in the serum had declined to 12% of the dose and by 168 hrs postdose, the levels were down to 0.76%. By 168 hrs postdose, only 0.16% of the dose was detected in the liver. The results suggest that the distribution of VEGF Trap is limited largely to the circulation and the liver is the main organ for elimination.

**Pharmacokinetics of VEGF Trap Following Intravenous Administration to Sham-Operated and Nephrectomized Sprague Dawley Rats (Study # VGFT-PK-01004.2) –** After administration of a single VEGF Trap IV dose of 1 mg/kg to female rats, there were no substantial differences in the PK parameters between sham-operated (n=7) and functionally nephrectomized (n=10) rats. The mean (± SD) concentration of VEGF Trap at the 1st blood sampling was 27.26 ± 4.82 µg/mL for the sham-operated rats and 26.86 ± 3.52 µg/mL for the nephrectomized rats. $AUC_{0\text{-}10\ hrs}$ was 163.7 ± 14.2 µg•hr/mL and 163.7 ± 17.3 µg•hr/mL for the sham-operated and nephrectomized rats, respectively. These results imply that the kidney does not play a major role in VEGF Trap clearance from the systemic circulation.

## 5.2 Toxicokinetics

The vitreous and plasma concentrations of free VEGF Trap and the plasma concentrations of VEGF Trap complex were determined in 4 separate toxicology studies following repeated ITV dosing to male and female cynomolgus monkeys (VGFT-TX 04019, VGFT-TX-04025, VGFT-TX-05011, and VGFT-TX-05015). Refer to each toxicology study under Section 6.2.

# 6 General Toxicology

## 6.1 Single-Dose Toxicity

**Summary Report AVE0005: Exploratory Single-Dose Intravenous (30-Minute Infusion) Toxicity Study in Rats with 2-Week Observation Period (Study # VGFT-TX-06007) –** According to the summary (full report not submitted), single IV administration of AVE0005 to Sprague Dawley Crl:CD®(SD)IGS BR rats at doses of 150 and 500 mg/kg resulted in transient skin lesions, and at 150 mg/kg only, discoloration of the tail (administration site). A moderate decrease in body weight gain (30-55%) was noted in both male and female rats at both dose levels, associated with a moderate decrease (51-90%) in food consumption in female rats at 500 mg/kg. The applicant concluded that under the conditions of this study, the lethal dose and the MTD were >500 mg/kg. The reviewer concurs.

**AVE0005: Exploratory Single-Dose Intravenous (30-Minute Infusion) Toxicity Study in Rats with 2-Week Observation Period (Study VGFT-TX-06008) –** A single 30-min IV infusion of AVE0005 to Sprague Dawley Crl:CD®(SD)IGS BR rats at doses of 50, 150 or 500 mg/kg resulted in lesions at the injection site in a few rats at 50 and 500 mg/kg and moderate dose-related decrease in mean body weight gain (38-65%) in males at all dose levels associated with slight decreases in mean food consumption (16-27%). The applicant noted that from Days 8-14 mean body weight gain of males was comparable in all groups. However, although the data showed some recovery, lower body weight gains were still observed at all dose levels (26-43%) compared to controls. The applicant concluded that under the conditions of this study, the lethal dose was above 500 mg/kg and the NOAEL was considered to be 150 mg/kg since at this dose level the effect on body weight gain was reversed by the end of the study and the effect on food consumption at the end of the study was minimal (<10% decrease). However, the reviewer considers that given that there was still a significant decrease in body weight gain even at the low dose (26%) at the end of the study, a NOAEL was not identified in males. In females, the NOAEL was > 500 mg/kg.

## 6.2 Repeat-Dose Toxicity

**Ocular Route Specific Studies**

**Study title: 8-Month Intravitreal Toxicity and Toxicokinetic Study in VEGF**

**Trap in Cynomolgus Monkeys with a 4-Month Recovery**

| | |
|---|---|
| Study no.: | VGFT-TX-05011 |
| Study report location: | Module 4.2.3.2 |
| Conducting laboratory and location: | (b) (4) 2595 |
| Date of study initiation: | July 6, 2006 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | VEGF Trap (10 mM Sodium Phosphate, 0.03% PS20, 40mM NaCl, 5% Sucrose, pH 6.2) 10 mg/mL, lot # C06002D640X21A, 99.5% pure<br><br>VEGF Trap (10 mM Sodium Phosphate, 0.03% PS20, 40mM NaCl, 5% Sucrose, pH 6.2) 40 mg/mL, lot # C06002D640N11A, 99.3% pure<br><br>VEGF Trap (10 mM Sodium Phosphate, 0.03% PS20, 40mM NaCl, 5% Sucrose, pH 6.2) 80 mg/mL, lot # C06002D640X11A, 98.6% pure<br><br>VEGF Trap (10 mM Sodium Phosphate, 0.03% PS20, (b) (4) NaCl) 40 mg/mL, lot # C06002D640X31A, 99.2% pure<br><br>***Note:*** *The (b) (4) 40 mg/mL VEGF Trap formulations (10 mM Sodium Phosphate, 0.03% PS20, 40mM NaCl, 5% Sucrose, pH 6.2) are the intended commercial formulations.* |

Key Study Findings

- ITV administration of VEGF Trap to cynomolgus monkeys at 4-week intervals for 9 doses at levels of 500-4000 µg/eye produced a mild and transient anterior segment and vitreous inflammatory response that was not associated with other ocular abnormalities.
- Epithelial erosion/ulceration of the nasal turbinates was noted at ≥ 2000 µg/eye.
- No marked differences in these findings were noted between both formulations evaluated, including the commercial formulation.
- Compared with the free VEGF Trap concentration in the vitreous, the systemic concentration was low (<3%). However, there was substantial systemic exposure of VEGF Trap (free and/or bound) after ITV injection as levels were in the µg/mL

range. The applicant acknowledged these levels may be underestimated because of the long storage period of three samples prior to analysis.

- Ten out of 48 animals were positive for the presence of anti-VEGF Trap antibodies. There was no obvious correlation between the appearance of these putative antibodies and any change in either free and/or bound VEGF Trap levels in these animals.

Methods

Doses:

| Group | No. of Animals Male | No. of Animals Female | Dose Level (µg/eye) | Dose Concentration (mg/mL) |
|---|---|---|---|---|
| Formulation: 0.03% PS20, 40mM NaCl, 5% Sucrose | | | | |
| 1 (Control) | 6 | 6 | 0 | 0 |
| 2 (Low) | 6 | 6 | 500 | 10 |
| 3 (Mid) | 6 | 6 | 2000 | 40 |
| 4 (High) | 6 | 6 | 4000 | 80 |
| Formulation: 0.03% PS20, (b) (4) | | | | |
| 5 (Control) | 6 | 6 | 0 | 0 |
| 6 (Mid) | 6 | 6 | 2000 | 40 |

Frequency of dosing: Once every 4 weeks for 8 months (9 doses/eye)

Route of administration: ITV to both eyes

Dose volume: 50 µL/eye using pre-filled 1-mL glass syringes

Formulation/Vehicle: As shown above, two formulations were evaluated: (1) 10 mM Sodium Phosphate, 0.03% PS20, 40 mM NaCl, and 5% sucrose and (2) 10 mM Sodium Phosphate, 0.03% PS20, and (b) (4) NaCl

*Note: Formulation (1) is the commercial formulation*

Species/Strain: Cynomolgus monkeys (*Macaca fascicularis*)

Number/Sex/Group: 6

Age: 2-4 yrs old

Weight: 2.1-4.0 kg for males and 2.0-2.9 kg for females

Satellite groups: Two animals/sex/group underwent at least 4 months without treatment following the final dose for recovery assessment.

Unique study design: Complement activation was evaluated.

Deviation from study protocol: None that could adversely affect interpretation of the study results.

Observations and Results

Mortality (Twice daily) – One high-dose male was sacrificed on Day 164 due to a sore on the left foot. This was considered unrelated to the test article.

Clinical Signs (Once daily cageside observation; at least once during the predose phase, once weekly during the dosing and recovery phases, and on the day of scheduled sacrifice for detailed observations) – No test article-related findings

Body Weights (At least once prior to treatment, on the first day of treatment, and weekly thereafter during the dosing phase; on Day 1 of the recovery phase an weekly thereafter during the recovery phase) – No test article-related effects

Feed Consumption (Once daily qualitatively) – This data was difficult to evaluate as presented. The applicant claims there was no test article-related effect. The lack of an effect in body weights, supports this claim.

Ophthalmoscopy

Ophthalmic Examinations (OE) (During the predose phase, prior to each dose, 2 days after each dose, approximately weekly during the dosing phase during non-dose weeks, every 2 weeks during recovery, and before the terminal and recovery necropsies by slit lamp biomicroscope and indirect ophthalmoscope) – The two VEGF Trap formulations and, to a lesser extent, the two vehicle controls typically resulted in a mild anterior segment/vitreous inflammatory response. The severity was usually trace to 1+ (mild) with some occasions of 2+, 3+, or 4+ (moderate-severe), particularly in the low dose (Group 2) and also at 2000 µg/eye (Group 6) and 4000 µg/eye (Group 4). This finding was not continuous, *i.e*, it was not observed at all dose intervals. It is unclear why the low-dose VEGF Trap group would exhibit anterior chamber cell scores ≥1+ more frequently than groups treated with greater amounts of the test article using either the same or different vehicles. After usually peaking 2 days after each dose, anterior chamber cell scores spontaneously declined in the interval between doses in all six groups. By 1 week after each dose, anterior chamber cell scores usually had returned to 0 values in all six groups. This finding was not present at recovery.

Vitreal cells (generally trace to 1+) were observed in the majority of eyes through the dosing phase in all groups including controls. Some instances of 2-4+ were observed but this was not persistent (lower severity in subsequent injections). This finding was continuous throughout dosing and gradually disappeared during the recovery period.

Intraocular Pressure (IOP) (In conjunction with OE) – There was a ≤5-fold increase in IOP immediately postdose across all groups including controls. This increase could be expected from the sudden increase in intraocular volume after a bolus injection into the vitreous. IOP returned to normal range by the next time point measured.

Electroretinography (ERG) (During the predose phase, during Weeks 5/6, 13/14, 24, and 32 of the dosing phase (~1 week after the 2$^{nd}$ and 4$^{th}$ doses, ~3 weeks after the 6$^{th}$ dose and within 2 weeks before the terminal sacrifice), and during Week 7/8 of the recovery phase and within 2 weeks before the recovery sacrifice) – No test article-related effects

Ocular Photographs (OP) (Once during the predose phase and during Weeks 6, 14, 25, and 33 of the dosing phase (~2 weeks after the 2nd and 4th doses, ~1 week after the 7th dose, and within 1 week before the terminal sacrifice), and during Week 8/9 of the recovery phase and within 1 week before the recovery sacrifice) – No test article-related effects

Fluorescein Angiography (FA) (In conjunction with ERG evaluations) - No test article-related effects

Hematology and coagulation (Twice during the predose phase, once during Weeks 5, 13, 26, and 34 of the dosing phase, and during Weeks 9 and 17 of the recovery phase) – No test article related effects

Clinical Chemistry (Twice during the predose phase, once during Weeks 5, 13, 26, and 34 of the dosing phase, and during Weeks 9 and 17 of the recovery phase) – The applicant mentioned that the only statistically significant difference considered potentially test article-related was mildly lower serum chloride concentration observed at Day 176 and 232 of the dosing phase for high dose males treated with the first formulation (0.03% PS20, 40mM sodium chloride, 5% sucrose). The individual animal listings showed the values were within predose and control range on Day 176. At Day 232, only 1/5 monkeys had a value below the range observed in the concurrent control (99 nmol/L at the high-dose compared to control range of 100-112 nmol/L). Therefore, the reviewer believes the decrease was unrelated to treatment.

Urinalysis (Once during the predose phase, once during Weeks 5, 13, 26, and 34 of the dosing phase, and during Weeks 9 and 17 of the recovery phase) - The applicant mentioned that the only statistically significant difference considered potentially test article-related was decreased urine pH and mildly higher urine sodium, potassium, and chloride excretion observed at Day 232 of the dosing phase for males treated with the first formulation (0.03% PS20, 40mM sodium chloride, 5% sucrose) at 4000 µg/eye. The individual animal listings showed a range of values similar to that seen at predose in all groups and/or within the concurrent control, and therefore, these differences were considered incidental.

Gross Pathology (After 8 months of treatment or after 4-months of recovery phase) – No test article-related findings

Organ Weights (Performed in the following organs from all animals) – No test article-related changes

| | |
|---|---|
| adrenal (2) | prostate |
| brain[a] | seminal vesicles |
| epididymides (2) | spleen |
| heart[a] | thyroid (2) with parathyroid[a] |
| kidney (2) | testis (2) |
| liver with gallbladder (drained) | thymus[a] |
| ovary (2) | uterus |
| pituitary[a] | |

a Due to the upper body perfusion, these weights will be considered postfixation weights and will be collected on the day of necropsy.

Histopathology (From control and high-dose groups, eye, eyelids, lacrimal glands, and nonocular tissues (as appropriate) were examined microscopically. The adrenals, brain, femur with marrow (including knee joint), vertebrae and surrounding muscle, heart, duodenum, colon, rectum, kidneys, lesions, nasal turbinates, urinary bladder, ovaries, and uterus of all animals were examined.

Adequate Battery - Yes

*Note: Bone marrow smears were prepared but not examined.*

Peer Review – Only conducted for ocular tissues.

Histological Findings – At the end of the dosing phase there was a test article-related increased incidence of epithelial erosion/ulceration, often accompanied by chronic-active inflammation of the nasal turbinates in animals given 2000 µg/eye (regardless of formulation) and 4000 µg/eye. At recovery, minimal to slight chronic active inflammation was noted in 1/2 animals in most groups including male controls (except for an incidence of 2 in the low dose females) but the other findings were not present. The incidence (and severity) is given in Table 5.

**Table 5: Incidence and Severity of Microscopic Findings in the Nasal Turbinates following ITV Dosing of VEGF Trap to Cynomolgus Monkey Monthly for 8 Months**

| Controls from group(s): 1 | | -- Animals Affected -- | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Animal sex: | -- Males -- | | | | | | -- Females -- | | | | | |
| | Dosage group: | Ctls | 2 | 3 | 4 | 5 | 6 | Ctls | 2 | 3 | 4 | 5 | 6 |
| Tissues With Diagnoses | No. in group: | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Nasal Turbinates ..... Number examined: | | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Inflammation, Chronic-Active | | | | | | | | | | | | | |
| | -> | 4 | 2 | 2 | 2 | 4 | 0 | 3 | 3 | 1 | 2 | 4 | 0 |
| | 1> | 0 | 2 | 1 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 2> | 0 | 0 | 0 | 1 | 0 | 2 | 1 | 1 | 2 | 2 | 0 | 3 |
| | 3> | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ..... Total Incidence of Finding Observed: | | 0 | 2 | 2 | 1 | 0 | 4 | 1 | 1 | 3 | 2 | 0 | 4 |
| Erosion/Ulceration, Respiratory Epithelium | | | | | | | | | | | | | |
| | -> | 4 | 4 | 3 | 3 | 4 | 2 | 4 | 4 | 3 | 3 | 4 | 0 |
| | 1> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| | 2> | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 1 |
| | 3> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 4> | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ..... Total Incidence of Finding Observed: | | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 4 |
| Hyperplasia, Respiratory Epithelium | | | | | | | | | | | | | |
| | -> | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| ..... Total Incidence of Finding Observed: | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hemorrhage | | | | | | | | | | | | | |
| | -> | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 |
| | 2> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| ..... Total Incidence of Finding Observed: | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Squamous metaplasia (conversion from respiratory to -squamous epithelium) | | | | | | | | | | | | | |
| | -> | 4 | 4 | 4 | 3 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 2> | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| ..... Total Incidence of Finding Observed: | | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |

All Diagnoses; Phases: P2; Death types: Scheduled FS; Date of death range: 06.Mar.07 To 15.Mar.07

One male at 4000 µg/eye (#I08593) had a B-astrocytoma in the brain, one female at 4000 µg/eye µg/eye (#I08629) had a moderate liver granuloma with thick connective tissue capsule. Slight lymphoid hyperplasia in the lung was observed at 4000 µg/eye in one male (#I08593) and one female (#I08632) and in one male at 2000 µg/eye (#I08609). These findings were not observed in the 6-month systemic toxicity study (Study # VGFT-TX-05009) at weekly IV doses up to 30 mg/kg and therefore, they were considered incidental. In addition, the applicant considered the astrocytoma in the brain to be spontaneous and not related to test article treatment for the following reasons:

- Similarly, other marketed drugs of a similar anti-VEGF mechanistic class (Lucentis™, Avastin™, and Macugen™) have not been associated with neoplasia (manufacturer package insert prescribing information).
- While spontaneous central nervous system neoplasia in the nonhuman primate is a rare event, it has been shown to occur.

Special Evaluations

C-reactive protein (Twice once during the predose phase, on Day 5 and once during Weeks 5, 13, 26, and 34 of the dosing phase, and during Weeks 9 and 17 of the recovery phase) - No test article-related effect

Complement Analysis (Once during the predose phase and once before dosing on Days 29, 85, 169, 197, and 225 of the dosing phase and during the last week of the recovery phase for C3a, Cd4, and soluble C5b-9) – No test article-related effect

Serum Anti-VEGF Trap Antibody Analysis [Once during the predose phase, once before each dose on Days 1, 29, 57, 85, 113, 141, 169, 197, and 225 of the dosing phase, during Week 26 (from males in Groups 1 through 4), on the day of terminal necropsy (Week 34 of the dosing phase), and during Weeks 9 and 17 (Groups 1 through 4) or 18 (Groups 5 and 6) of the recovery phase] – Ten out of 48 animals had samples collected from the dosing phase that were positive in the anti-VEGF Trap antibody (914-6070 mIU/mL). Of the animals that received VEGF Trap, 2 animals in Group 2 (I08585 and I08617), 1 animal in Group 3 (I08627), 3 animals in Group 4 (I08631, I08632 and I08634), and 4 animals in Group 6 (I08606, I08607, I08608 and I08641) had samples that were positive in the anti-VEGF Trap antibody assay at various time points of the study. There was no obvious correlation between the appearance of these putative antibodies and any change in either free and/or bound VEGF Trap levels in these animals.

Vitreous Fluid Collection (On days of schedule sacrifice; only free VEGF Trap analyzed) – VEGF Trap levels in vitreous humor samples were approximately dose proportional in the highest two dose groups and less than dose proportional at 500 µg/eye. At the end of the 4-month recovery period, there was no detectable free VEGF Trap in the vitreous of any of the animals. No apparent difference was noted in the 2 cohorts receiving the different formulations of VEGF Trap at 2000 µg/eye (Group 3 and Group 6) with respect to the concentration of free VEGF Trap detected in vitreal samples. See Table 6 for mean vitreous levels (both gender combined).

Toxicokinetics (Both free and bound VEGF Trap were analyzed predose and 1 and 3 days postdose on Days 1, 29, 57, 85, 113, 141, 169, 197, and 225 of the dosing phase; weekly during the non-dosing weeks of the dosing phase; and every 2 weeks during the recovery phase, and on the day of terminal necropsy) – Plasma samples were analyzed ~4-24 month after initial sample collection and some samples underwent more than the number of freeze-thaw cycles that the assay was validated for. Therefore, concentrations in the samples may be underestimated.

Free VEGF Trap in plasma –The $T_{max}$ was observed 24 hrs after ITV injection. Free VEGF Trap concentrations at the 24-hr time point were approximately dose proportional in the highest two dose groups and greater than dose proportional at 500 µg/eye when compared with either the 2000 or 4000 µg/eye cohorts. Free VEGF Trap was not detected in the majority of analyzed plasma samples beyond the 1st week postdose in the 500 µg/eye dosing cohort (Group 2). In the two 2000-µg/eye cohorts (Groups 3 and 6), free VEGF Trap was not detected in the majority of analyzed plasma samples beyond the 2nd week postdose. In the 4000-µg/eye cohort (Group 4), free VEGF Trap was detected in most of the Week 3 postdose samples but in only a few animals just prior to the next monthly ITV injection. This suggests that at least some high-dose animals had drug exposure during the entirety of several of the

month-long dosing intervals. However, in general, there appears to be little to no accumulation of free drug in the plasma.

Mean free VEGF Trap levels (males and females combined) ranged from 0.802-1.37 µg/mL ($AUC_{0\text{-}28d} \leq$ 6.66 µg•day/mL) in Group 2 animals receiving 500 µg/eye, from 3.99-6.49 µg/mL ($AUC_{0\text{-}28d} \leq$ 84.3 µg•day/mL) in Group 3 animals receiving 2000 µg/eye and from 8.36-15.3 µg/mL ($AUC_{0\text{-}28d} \leq$ 140 µg•day/mL) in the 4000 µg/eye cohort (Group 4) at 24 hrs post each dose administration. The mean systemic levels are shown in Table 6.

In the 2 cohorts receiving the different formulations of VEGF Trap at 2000 µg/eye (Group 3 and Group 6), Group 6 females had slightly higher (1.7-fold) mean free VEGF Trap levels (based on $AUC_{0\text{-}28d}$). Similarly, Group 6 females had higher mean $AUC_{0\text{-}28d}$ values (≤1.7 fold) compared to Group 6 males up to dose # 5.

Free VEGF Trap was detected in plasma samples collected through the 2nd week of recovery in the 4 monkeys at 4000 µg/eye (0.199-0.346 µg/mL), at Day 1 in the 2 of 4 recovery animals at 500 µg/eye (0.221-0.193 µg/mL), at Day 1 in all 8 animals in the two 2000 µg/eye dose groups (0.901-2.52 µg/eye), and at Week 2 in only 1 of the 8 animals at 2000 µg/eye (0.157 µg/mL).

VEGF:VEGF Trap complex (bound VEGF Trap) – Apparent $T_{max}$ was achieved ~1 week post-dose in the 500 µg/eye cohort and ~ 2 weeks postdose in the 2000 and 4000 µg/eye cohorts. There was a less than dose proportional increase in bound VEGF Trap levels. The applicant noted that the low levels of free VEGF Trap observed in circulation after each of these ITV injections is not believed to be sufficient to completely bind all of the endogenous systemic VEGF available *in vivo*. However, the presence of systemically circulating free VEGF Trap for at least 1 week postdose suggests that at least some of the free VEGF Trap can reach systemic circulation via the eye and be detected prior to binding to endogenous VEGF.

Unlike free VEGF Trap, the bound complex has a much slower clearance and elimination rate as it was still detected well into the recovery period. VEGF Trap complex was detected during the recovery period for all VEGF Trap treated cohorts. In the 500 µg/eye dose group (Group 2), bound VEGF Trap was detected in all animals through Week 6 of the recovery period; one monkey in this cohort (#I08585) had detectable complex through recovery Week 14. In the two cohorts administered 2000 µg/eye, complex was measured in all recovery animals through recovery Week 14 for the Group 3 animals and through recovery Week 10 for the Group 6 animals, with 2 of the 4 recovery animals from Group 6 demonstrating bound VEGF Trap at recovery Week 14. In the 4000 µg/eye cohort (Group 4), bound VEGF Trap was measured in all animals through recovery week 10, with 3 out of 4 animals demonstrating measurable complex at recovery Week 14 and 1 of the 4 with detectable complex at the end of the study (recovery Week 18).

As noted by the applicant, the results indicate that the majority of the total drug concentration in circulation (Free VEGF Trap + Adjusted Bound VEGF Trap) after each ITV administration of VEGF Trap was the free form directly following administration and the bound form at later time points. Although in general most of the animals may not have been systemically exposed to free VEGF Trap for the entire time period between doses, the monkeys were exposed to VEGF:VEGF Trap complex throughout the study.

The table below shows the mean concentration of free and bound VEGF Trap in the plasma and free VEGF Trap in the vitreous 7 days after the 9th ITV injection.

**Table 6: Mean Plasma and Vitreous Free VEGF Trap and Plasma Bound VEGF Trap Concentrations in Monkeys 7 Days after the Last of 9 ITV Doses Administered Every 4 Weeks**

| Group | Dose (mg/eye) | No. of animals (M/F) | Plasma | | Vitreous |
|---|---|---|---|---|---|
| | | | Free VEGF Trap (µg/mL) | Adjusted VEGF Trap Complex (µg/mL)[a] | Free VEGF Trap (µg/mL) |
| 2 | 0.5[b] | 6/6 | 0.384 | 1.12 | 42.0 |
| 3 | 2.0 | 6/6 | 4.19 | 2.74 | 148 |
| 4 | 4.0 | 6/6 | 6.14 | 3.13 | 265 |
| 6 | 2.0[c] | 6/6 | 2.15 | 2.41 | 129 |

[a] The bound VEGF Trap concentrations are reported as total complex weight per volume (e.g. µg/mL) and must be normalized for the VEGF Trap portion of the weight alone by multiplying by 0.717 to generate adjusted-bound concentrations.

[b] Formulation ITV-2 (Phase 3 commercial enabling) was: 10 mM sodium phosphate, 40 mM sodium chloride, 0.03% PS20, 5% sucrose, pH 6.2

[c] Formulation 3 was: 10 mM sodium phosphate, [redacted] (b) (4) sodium chloride, 0.03% PS20, pH 6.2

**Dosing Solution Analysis –** Stability analysis at Time 0 and at End of Study demonstrated dosing solutions were >97% of nominal concentration.

**Study title: 13-Week Intravitreal Toxicity and Toxicokinetic Study with VEGF Trap in Cynomolgus Monkeys with a 10-Week Recovery**

| | |
|---|---|
| Study no.: | VGFT-TX-04019 |
| Study report location: | Module 4.2.3.2 |
| Conducting laboratory and location: | [redacted] (b) (4) |
| Date of study initiation: | August 10, 2004 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | VEGF Trap (1 mg/mL), lot # C04005D620F11A, 99.8% pure<br><br>VEGF Trap (5 mg/mL), lot # C04005D620E11A, 99.8% pure |

VEGF Trap (10 mg/mL, lot # C04005D620C11A, 98.6% pure

Key Study Findings

- Mild and reversible ocular inflammation (generally 1+ or less) was observed at all doses in the anterior segment and/or vitreous with a trend for an increased incidence with dose.
- No anti-VEGF Trap antibodies were detected.
- Dose-dependent levels of free VEGF Trap were detected in vitreous humor samples collected 7 days after the last ITV injection. At the end of the 10-week recovery period, there was no detectable free VEGF Trap in the vitreous of any of the animals except for small amounts in 2 high-dose monkeys.
- VEGF-Trap (free and/or bound) was detected systemically after ITV administration at the mid and high doses. Compared with the vitreous concentration of free VEGF Trap, the systemic concentration was low (<1%).

Methods

| | |
|---|---|
| Doses: | 0, 50, 250, or 500 µg/eye |
| Frequency of dosing: | Once every 4 weeks (4 doses/eye) |
| Route of administration: | ITV to both eyes |
| Dose volume: | 50 µL/eye |
| Formulation/Vehicle: | 10 mM Sodium phosphate, (b) (4) sodium chloride, and (b) (4) |
| Species/Strain: | Cynomolgus monkeys (*Macaca fasciculari)* |
| Number/Sex/Group: | 6/sex in control, mid, and high-dose groups; 4/sex in the low dose group |
| Age: | 3-5 yrs old |
| Weight: | 3.8-6.2 kg for males and 2.2-3.2 kg for females |
| Satellite groups: | Two animals/sex/group in the control, mid and high-dose underwent a 10 week recovery period. |
| Unique study design: | None |
| Deviation from study protocol: | None that could adversely affect interpretation of the study results |

Observations and Results

Mortality (Twice daily) - None

Clinical Signs (Detailed observations prior to initiation of treatment and weekly during the study; cageside observations daily) – No test article-related effects

Body Weights (Prior to initiation of treatment, on the first day of treatment, and weekly thereafter) - No test article-related effects

Feed Consumption (Daily qualitatively) – Apparently, there was no test article-related effects. However, due to the qualitative evaluation, the data is not considered very informative.

Ophthalmoscopy

OE [Once prior to initiation of treatment and predose on each day of dosing (Days 1, 29, 57, and 85; on Days 3, 31, and 87 (2 days after dosing on Days 1, 29, and 85); on Days 8, 34/35, and 64 (~7 days after dosing on Days 1, 29, and 57); on Days 15, 43, 71, and 98/99 (~2 weeks after dosing); and during recovery on Days 112/113, 126/127, 140/141, and on the day of sacrifice (Days 92 and 157/158)] - Mild ocular inflammation (trace or 1+) was observed in the anterior segment and/or vitreous. In the anterior segment, this inflammatory response was apparent at 2 days postdose and diminished with time prior to the next dose. Although observed in all groups including the control group, there was a trend for an increase in the incidence of inflammation with dose. For example, >1+ anterior chamber cell was seen at one or more time points during the study in 4/24 (16.7%), 8/16 (50%), 11/24 (45.8%), and 21/24 (87.5%) eyes at 0, 50, 250, or 500 µg/eye, respectively. During recovery, this finding had resolved.

Inflammatory cells in the vitreous were slower to appear (peak scores typically occurred 1 week postdose) and were slower to leave the viscous vitreal gel than the water-like aqueous humor. The frequency of vitreal cell also exhibited a weak dose response effect. Over the entire study, vitreal cells were seen in 15/24 (62.5%), 12/16 (75%), 20/24 (83.3%), and 24/24 (100%) eyes at 0, 50, 250, or 500 µg/eye, respectively. Overall, however, vitreal cells did not increase in frequency or score between Day 8 (1 week after the first dose) and Day 92 (1 week after the fourth dose) indicating that the 4-week dosing interval does not result in accumulation of inflammatory cells in the vitreous.

Indirect ophthalmoscopy showed focal chorioretinal depigmentation in the right eye in one high dose female beginning on Day 1 and throughout the study. Given the isolated event, a relationship to the test article is not clear.

IOP (In conjunction with OEs) - There was a ≤5.5-fold increase in IOP immediately postdose across all groups including controls. This increase could be expected from the sudden increase in intraocular volume after a bolus injection into the vitreous. IOP returned to normal by the next time point measured (2 days postdose).

ERG [Once before initiation of treatment and during Weeks 6 and 10 and Week 21 (recovery animals)] – No test article-related effects

OP and FA – [Once before initiation of treatment and during Weeks 7 and 13, and Week 22 (recovery animals)] – The applicant stated that there was no test article-related effect (the data was not submitted).

Hematology and coagulation [Prior to initiation of treatment and on Day 31/32, Weeks 13 (Day 87) and 18, and on the day of recovery sacrifice (Day 157/158)] – No test article-related effects

Clinical Chemistry [Prior to initiation of treatment and on Day 31/32, Weeks 13 (Day 87) and 18, and on the day of recovery sacrifice (Day 157/158)] – No test article-related effects

Urinalysis (Prior to each schedule sacrifice and Week 17 during the recovery period) - No test article-related effects

Gross Pathology [On Days of schedule necropsy, *i.e.,* Day 92 and after 10 weeks of recovery (Day 157/158)] – Red focus were noted in the cecum of one male and one female each in the mid and high dose groups and in the colon of one female each in the mid and high dose groups. There was no microscopic correlate.

Organ Weights (Performed in the following organs from all animals: adrenals, brain, heart, kidney, liver with gallbladder, spleen, ovary, testis, and thymus) – No test article-related changes

Histopathology (For all animals; full battery) –

Adequate Battery: Yes

*Note: Bone marrow smear was collected but not evaluated.*

Peer Review: No

Histological Findings - No test article-related effects

Special Evaluation

Vitreous Fluid Collection (On the day of dosing and recovery necropsy) – Dose-dependent levels of VEGF Trap were detected in vitreous humor samples collected 7 days after the last dose. The mean vitreous levels (both genders combined) are shown in Table 7.

At the end of the 10-week recovery phase, VEGF Trap was not detected in the vitreous humor of any animal in the 50 or 250 µg/eye. In vitreous collected from animals in the 500 µg/eye, only two samples from two animals had detectable, although low, VEGF Trap levels (~86 ng/mL).

Serum Antibody Analysis [Before the initiation of treatment and before dosing on Days 1, 29, 57, and 85; on Day 99, during Week 18, and on the day of recovery necropsy (Day 157/158)] – No antibodies were detected

Toxicokinetics (On Days 1, 29, 57, and 85 predose, ~1 hr and 1 and 3 days postdose, and approximately weekly throughout the remainder of recovery) -

Free VEGF Trap - Free VEGF Trap was not detected in plasma after ITV administration of 50 µg/eye. Plasma concentrations of free VEGF Trap were highest at ~24 hrs postdose and were approximately dose proportional in the two highest dose level groups. The applicant noted that the lack of detectable free VEGF Trap in the 50 µg/eye group may be partially a result of the sensitivity of the assay. After the third dose, free VEGF Trap was not detected in the 250 or 500 µg/eye groups at 2, 3, or 4 weeks after injection. Overall, results of free VEGF Trap plasma analyses indicate that systemic exposure to VEGF Trap at or beyond 14 days postdose was minimal. The plasma levels ranged from 403-615 ng/mL and 699-1175 ng/mL in the 250 µg/eye and 500 µg/eye cohorts, respectively. The mean levels are shown in Table 7.

Free VEGF Trap was not detected during the recovery period from Day 99 onward at 250 µg/eye and Day 99 at 500 µg/eye, except for high-dose female # AI02506. This animal continued to demonstrate detectable levels of free VEGF Trap in most samples collected after the 4$^{th}$ ITV injection out to 73 days, the last sample collected for this recovery animal. The level at Day 73 postdose was 1460 ng/mL (as high as those observed at $T_{max}$ postdose), indicating that there was a collection or analysis error.

AUC values were estimated for free VEGF Trap plasma levels. Mean $AUC_{0\text{-}28\ days}$ at both 250 and 500 µg/eye decreased as the study progressed. The applicant noted this difference is most likely due to the limited number of sampling time points following the first 2 drug administrations as compared to the last two. $AUC_{0\text{-}28\ days}$ ranged from 729-2862 ng•day/mL (males) and 2221-7062 ng•day/mL (females) at 250 µg/eye, and 2465-8352 ng•day/mL (males) and 4663-16395 ng•day/mL (females) at 500 µg/eye. Exposure in females was ~2-fold higher compared to males.

Systemic exposure was also estimated for the first 72 hrs postdose. In this case, the first 3 injections showed comparable free VEGF Trap levels, with a decrease at the 4$^{th}$ injection. The applicant attributed this decrease to the fact that the estimates for the 4$^{th}$ injection were based only on data from the first 7 days following injection.

VEGF:VEGF Trap complex - Peak VEGF:VEGF Trap complex (bound VEGF Trap) levels were observed at 7 days postdose after the 3$^{rd}$ and 4$^{th}$ injections. Bound VEGF Trap levels after the first two sets of injections were still increasing 72 hrs postdose; which was the last sample collected in each of these first two cycles. An approximate dose proportional or less than dose proportional increase in bound VEGF Trap levels was observed. Bound VEGF Trap was detected at the end of the 10-week recovery period. Although in general, the animals may not have been exposed to free VEGF Trap for the entire period between doses, they were exposed to VEGF Trap complex throughout the study.

For the two highest dosing cohorts, the majority of the total VEGF Trap (free plus bound VEGF Trap) levels observed at 24 hrs postdose were due to the presence of free VEGF Trap levels. At 72 hrs, the levels of the bound form had exceeded the free form suggesting that the test article is rapidly converted to the bound form limiting systemic exposure to free VEGF Trap. At 7 days post dose, the majority of the detected total VEGF Trap levels were observed to be mainly due to the presence of the bound VEGF Trap species.

The following table shows the mean free and bound VEGF Trap concentrations observed in the plasma and free VEGF Trap concentrations in the vitreous.

**Table 7: Mean Plasma and Vitreous Free VEGF Trap and Plasma Bound VEGF Trap Concentrations in Monkeys 7 Days after the Last of 4 ITV Doses Administered Every 4 Weeks**

| Dose (mg/eye) | No. of animals (M/F) | Plasma | | Vitreous |
|---|---|---|---|---|
| | | Free VEGF Trap (µg/mL) | Adjusted VEGF Trap Complex[a] (µg/mL) | Free VEGF Trap (µg/mL) |
| 0.050 | 4/4 | 0 | 0.126 | 5.32 |
| 0.250 | 6/6 | 0.0344 | 0.630 | 26.8 |
| 0.500 | 6/6 | 0.268 | 1.06 | 50.2 |

[a] The bound VEGF Trap concentrations are reported as total complex weight per volume (e.g. µg/mL) and must be normalized for the VEGF Trap portion of the weight alone by multiplying by 0.717 to generate adjusted-bound concentrations.

Dosing Solution Analysis – Stability was conducted in samples stored for the duration of the study (~3 months). The preliminary acceptance criteria of 90% main peak purity as determined by SE-HPLC was not met due to aggregation in the 1 mg/mL and 5 mg/mL dosing solutions (50.7-76.9% main peak area). The bioassay and binding assay results met the specifications. Therefore, the aggregation did not affect the bioactivity of the test article.

Three other 13-week ITV ocular toxicology studies were conducted in monkeys. The main findings are summarized below:

**13-Week Exploratory Intravitreal Study of VEGF Trap and Mini-VEGF Trap in Cynomolgus Monkeys (Study # VGFT-TX-03053) –** The purpose of this study was to evaluate the ocular toxicity of Mini-VEGF Trap (thrombin cleaved VEGF Trap) and VEGF Trap when administered every 2 weeks via ITV injection to cynomolgus monkeys for at least 3 weeks (Mini-VEGF Trap; two doses) or 13 weeks (VEGF Trap; seven doses).

Three different formulations of VEGF Trap were evaluated. The formulation compositions were the following: VEGF Trap lot # L04-064 (10 mM sodium phosphate, (b) (4) NaCl, (b) (4) PS20, (b) (4) VEGF Trap, pH (b) (4)); VEGF Trap lot # L04-065 (10 mM sodium phosphate, (b) (4) M NaCl, (b) (4)% PS20, (b) (4) (b) (4)

(b) (4) VEGF Trap, pH (b) (4)); and VEGF Trap lot # L04-066 (10 mM sodium phosphate, (b) (4) NaCl, 0.03% PS20, (b) (4) VEGF Trap, pH (b) (4)). None of these formulations is identical to the intended clinical formulation.

Only the right eye was treated with the test article; the left eye received the vehicle. The doses used are shown in the table below:

**Table 8: Intravitreal Doses Used in Study # VGFT-TX-03053**

| | Right Eye | Left Eye | No. of | Dose Level[a] | Dose Concentration[a] |
|---|---|---|---|---|---|
| Group | Formulation Lot No. | Placebo Lot No. | Males/Females | (µg/eye) | (mg/mL) |
| 1 (VEGF Trap) | L04-064 | L04-014 | 1 / 2 | 500 | 10 |
| 2 (Mini-VEGF Trap) | RSCH03006 | L04-015 | 1 / 2 | 250 | 5 |
| 3 (VEGF Trap) | L04-065 | L04-016 | 1 / 2 | 500 | 10 |
| 4 (VEGF Trap) | L04-066 | L04-017 | 1 / 2 | 500 | 10 |

a Animals were administered the test article at a volume of 0.05 mL. The concentration for Group 2 was achieved by diluting the Mini-VEGF Trap received at a concentration of 9.79 mg/mL.

Both test articles were intended to be dosed for 13 weeks, but marked ocular inflammation attributed to Mini-VEGF Trap formulation resulted in suspension of dosing on Day 29 after two doses had been administered. Whether this degree of inflammation was attributable to the mini-VEGF Trap or to the high levels of endotoxin (19.2 EU/mg) not observed with other VEGF formulations, is uncertain.

A variable and less marked inflammatory response (anterior chamber and vitreous) was observed in VEGF Trap formulations. The inflammation increased in severity in some animals with repeated dosing and was associated with mild effects on retinal function. No discernable differences were observed between VEGF Trap formulations. Systemic exposure to VEGF trap following ITV administration was minimal (measured on Day 1 and Day 85). Only 3 animals had measurable serum levels of VEGF Trap postdose. Specifically, Group 2 animal # I09283 which had a serum level of 210.9 ng/mL 72 hrs postdose on Day 1, Group 2 animal # I09410, which had a serum level of 206.6 ng/mL 24 hrs postdose on Day 1, and Group 4 animal # I01066, which had measurable serum levels of 234.1 and 404.3 ng/mL 5 hrs and 24 hrs postdose, respectively, on Day 85.

**A 13-Week Exploratory Formulation Intravitreal Toxicity and Toxicokinetic Study of a VEGF Trap in Cynomolgus Monkeys (Study # VGFT-TX-05015)** – This study evaluated the ocular toxicity of 8 formulations of VEGF Trap when administered by ITV injection to cynomolgus monkeys every 4 weeks for at least 13 weeks (four doses) to aid in the selection of the proposed commercial formulation. Groups consisting of males and females for a total of 3 monkeys (2-7 yrs old; 2.7-4.6 kg males and 2.5-4.0 kg females) each received 50 µL/eye ITV doses of one of the 8 formulations, each containing 40 mg/mL VEGF Trap, to provide a VEGF Trap dose of 2 mg/eye. A control group (2 males)

received the ITV-1 formulation vehicle (b) (4) sodium chloride, 10 mM sodium phosphate, (b) (4) pH (b) (4)) on a comparable regimen.

Assessment of ocular toxicity was based on clinical ophthalmic signs, intraocular pressure measurements, electroretinographic and photographic evaluations (color fundus photography and fluorescein angiography), and histopathology. Animal health was assessed by clinical signs, qualitative food consumption, body weights, selected organ weights, and clinical pathology.

The formulations are described in the table below. Formulation ITV-2 is the intended commercial formulation. The purity of the formulations ranged from 98.1-99.5%.

**Table 9: Placebo and VEGF Trap Formulations Evaluated in a 13-Week (4 Doses) Ocular Toxicity Study in Monkeys (Study # VGFT-TX-05015)**

| Group No. | Formulation Designation | Formulation | VEGF Trap Concentration mg/mL | Lot/Batch No. |
|---|---|---|---|---|
| 1 | | (b) (4) (10 mM Sodium phosphate, (b) (4) Sodium chloride, (b) (4) | 0 | C04004V710D02A |
| 2 | 1 (ITV-1) | (b) (4) (10 mM Sodium phosphate, (b) (4) Sodium chloride, (b) (4) | 40 | C04009D640G11B (synonymous with C04009M640G11) |
| 3 | 2 | (b) and PS20 (10 mM Sodium phosphate, (b) (4) Sodium chloride, (b) (4) 0.03% PS20, pH (b) (4) | 40 | C1-5688-39-P20-1A |
| 4 | 3 | PS20 Alone (10 mM Sodium phosphate, (b) (4) Sodium chloride, 0.03% PS20, (b) (4) | 40 | C1-5688-47-SAP-1A |
| 5 | 4 (ITV-2) | Sucrose and PS20 (10 mM Sodium phosphate, 40mM Sodium chloride, 0.03% PS20, 5% Sucrose, pH 6.2) | 40 | C2-5688-103-SPS-1A |
| 6 | 5 | (b) (4) and PS20 (10 mM Sodium phosphate, 40mM Sodium chloride, 0.03% PS20, (b) (4) pH 6.2) | 40 | C3-5688-114-MPS-1A |
| 7 | 6 | (b) (4) PS20 (10 mM Sodium phosphate, (b) (4) Sodium chloride, (b) (4) PS20, pH (b) (4) | 40 | C1-5688-77-PS1-1A |
| 8 | 7 | (b) (4) PS20 (10 mM Sodium phosphate, (b) (4) Sodium chloride, (b) (4) PS20, (b) (4) (b) (4) | 40 | C1-5688-82-PS2-1A |
| 9 | 8 | (b) (4) (b) (4), (b) (4) PS20, 5% Sucrose, pH 6.5) | 40 | L05-288-A |
| (b) (4) PS20 = Polysorbate 20 | | | | |

ITV administration of the VEGF Trap and, to a somewhat lesser extent, the vehicle typically produced a mild anterior segment/vitreal cellular response. In general, the anterior chamber cellular response was greatest 2 days after dosing and usually

achieved trace to 1+ cell scores in all nine groups with some infrequent incidences of 2+ or 3+ in groups receiving formulations 6 and 7 (1/3 animals in each group). Anterior chamber cell scores spontaneously improved in all nine groups. By 2 weeks after each dose, all eyes in all groups were free of anterior chamber cells with one exception; a male that received Formulation 4 had a trace anterior chamber cell score in one eye. Vitreal cells were typically trace to 1+ in all eyes in all groups throughout the study. Vitreal cell was rare in the control article-treated eyes (Group 1), and on only one occasion (Day 59 in one eye) was trace vitreal cell detected in this group.

No clear and consistent differences were observed between any of the VEGF Trap formulations in terms of the anterior chamber or vitreal response except for, as mentioned previously, the incidences of 2 or 3+ anterior chamber cell in one animal each receiving formulations 6 and 7. As noted by the applicant, the low severity as well as limited number of samples, may not provide a good scenario for detecting clear differences between the formulations.

No biologically significant changes from baseline on fundus photography, fluorescein angiography, or electrophysiology were noted in any animal in any group throughout the study. IOP showed values below 10 mm Hg (4-9 m Hg) in one or more animals across VEGF Trap formulations. These decreases, however, were not generally consistent with subsequent measurements, and values as low as 7 mm Hg were observed on Day 1 predose. Therefore, these lower IOP values were considered to be within normal range.

One female dose with ITV-2 formulation had AST values of 129 U/L (compared to 57 and 82 in concurrent controls and 31-43 U/L predose). ALT was also elevated (101 u/L) compared to 51 and 59 n concurrent control and 53-65 predose). No histopathology was conducted; the relevance of the findings is not clear.

Only the samples from animals in the control group and from those groups receiving the ITV-1 formulation, ITV-2 formulation, and Formulation 3 were analyzed for plasma free VEGF Trap, plasma VEGF Trap complex, and serum anti-VEGF Trap antibodies. Vitreous concentrations of free VEGF Trap were detected at 1 week following the last ITV dose of each of these formulations, indicating substantial ocular exposure to free VEGF Trap for at least one week following dosing.

Peak plasma concentrations of free VEGF Trap were reached approximately 24 hrs after an ITV injection, with mean concentrations ranging from ~4660-5526 ng/mL following the 1$^{st}$ injection and 3982-5563 ng/mL following the 4$^{th}$ injection. Free VEGF Trap was detected in the plasma of most animals at 14 days after dosing, but declined to BLQ by 29 days after dosing.

Peak plasma VEGF:VEGF Trap complex levels were achieved within 2 weeks after VEGF Trap ITV injection. The mean concentrations ranged from ~2090-3190 ng/mL 2 weeks following the 1$^{st}$ injection and 2095-3130 ng/mL 72 hrs following the 4$^{th}$ injection. Bound VEGF Trap was cleared more slowly than free VEGF Trap and was still detected

4 weeks after injection and trough concentrations increasing after each successive dose. By 14 days following the last dose, only VEGF Trap complex was detected in the circulation. There was no apparent difference in the TK of the three VEGF Trap formulations evaluated (see Table 10). During the trough period, the majority of total VEGF Trap was in complex form while at peak concentrations free VEGF Trap protein accounted for approximately 2/3 of the total pool.

**Table 10: Mean Plasma Concentrations for Free, Adjusted Bound, Total VEGF Trap Concentrations in Monkeys, 3 Days after the 4th ITV Administration of VEGF Trap 2 mg/eye Every 4 Weeks**

| Formulation | Plasma | | |
|---|---|---|---|
| | Free VEGF Trap (µg/mL) | Adjusted VEGF Trap Complex[a] (µg/mL) | Total VEGF Trap (µg/mL) |
| ITV-1[b] (Formulation 1) | 5.160 | 2.20 | 7.36 |
| ITV-2[c] (Formulation 4) | 3.708 | 1.50 | 5.21 |
| Formulation 3[d] | 4.212 | 2.24 | 6.45 |

[a] The bound VEGF Trap concentrations are reported as total complex weight per volume (e.g.µg/mL) and must be normalized for the VEGF Trap portion of the weight alone by multiplying by 0.717 to generate adjusted-bound concentrations.
[b] 10 mM sodium phosphate, (b) (4) mM sodium chloride, (b) (4), pH (b) (4)
[c] 10 mM sodium phosphate, 40 mM sodium chloride, 0.03% PS 20, 5% sucrose, pH (b) (4)
[d] 10 mM sodium phosphate, (b) (4) mM sodium chloride, 0.03% PS 20, pH (b) (4)

Anti-VEGF Trap antibodies were detected in one animal treated with formulation ITV-1. This positive finding, however, most likely reflected serum interference in the assay because, as noted by the applicant, the levels were low (less than 2-fold above the LLOQ of the assay) and were detected at similar levels prior to administration of the drug (945 mIU/mL at necropsy *vs.* 932-1401 mIU/mL at predose).

**An Intravitreal Toxicity and Toxicokinetic Study with VEGF Trap in Cynomolgus Monkeys (Study # VGFT-TX-04025; GLP)** – This study evaluated VEGF Trap Formulation ITV-1, which is different to the intended commercial formulation. As noted in (b) (4)

Monkeys (6/sex/dose) were administered ITV doses of 1 or 2 mg/eye once every 4 weeks for a total of 4 doses at VEGF Trap concentrations of 20 or 40 mg/mL, respectively, followed by a 10-week recovery period. A placebo control group received the vehicle on a comparable dosing regimen. Two additional groups (3/sex/dose) received a single ITV administration of vehicle or 2 mg/eye VEGF Trap, followed by a 4 week recovery period. A single group (5/sex) received an ITV dose of 4 mg/eye (80 mg/mL VEGF Trap concentration) every 6 weeks for a total of 3 doses followed by a 10-week recovery period.

Assessment of ocular toxicity was based on slit lamp biomicroscopy, funduscopy, IOP measurements, ERG, photographic evaluations with fluorescein angiography, and histopathology. Animal health was assessed by clinical observations including clinical signs, food consumption, body weights, clinical pathology, selected organ weights, and gross necropsy. Limited systemic tissues were evaluated by histopathology. Free VEGF Trap and VEGF Trap complex in plasma, anti-VEGF Trap antibodies in serum, and free VEGF Trap in the vitreous were determined.

The results of the study for both evaluation of toxicity and plasma TK were similar to those observed in previous ITV toxicity studies with this and other VEGF Trap formulations. A mild and transient anterior segment and vitreous cellular response was observed that was not associated with other ocular abnormalities. There were no significant differences between the two different dosing concentrations or dosing regimens. The cellular response gradually reversed once dosing stopped. No systemic toxicity was observed.

A single animal administered 2 mg/eye VEGF Trap developed serum antibodies and exhibited severe ocular inflammation (3+ or 4+) after receiving 2 doses that was interpreted by the applicant to be due to an immune-mediated mechanism. Mild retinal perivenous sheathing was present in both eyes, and some venous dilatation was noted on the fluorescein angiogram. During the recovery phase, the anterior segment inflammatory response spontaneously resolved. Vitreal cell scores also gradually declined to 1+ - 2+ score at the end of the recovery period. The retinal perivascular sheathing gradually improved such that no perivascular sheathing was present on Day 50 of the recovery period. The presence of an anti-VEGF Trap antibody response in this animal appeared to correlate with decreased plasma levels of both free and bound VEGF Trap, suggesting that the presence of anti-drug antibody accelerated the clearance of both the free and bound forms of VEGF Trap.

Dose-dependent levels of free VEGF Trap were detected in vitreous humor samples collected 7 days after the last administration, with mean levels ranging between 94.3 and 284 µg/mL at 1000 and 4000 µg/eye, respectively. At the end of the 10-week recovery period, measurable levels of VEGF Trap were detected in most vitreous samples obtained from recovery animals, with mean levels ranging between 138 and 153 ng/mL at 1000-4000 µg/eye. In Study # VGFT-TX-04019 above, at the end of the 10-week recovery phase after monthly treatment for 13 weeks, VEGF Trap was not detected in the vitreous humor of any animal in the 50 or 250 µg/eye, and only two samples from two animals in the 500 µg/eye had detectable VEGF Trap levels (~86 ng/mL). In Study # VGFT-TX-04019 at monthly doses up to 4000 µg/eye for 8 months, VEGF Trap was not detected in the vitreous following a 4-month recovery period. Therefore, as would be expected, the extent of exposure to VEGF Trap in the vitreous is dose and recovery period-length dependent.

**Systemic Route Specific Studies**

In repeat-dose studies, VEGF Trap was administered SC to cesarean-derived (CD)-1 (Study # VGFT-PK-01017) and severe combined immunodeficiency (SCID) mice (2x/week for up to 8 weeks) and to Sprague-Dawley (Study # PK01027; 3x/week for 4 weeks) and nude (Study # VGFT-PK-01032; 2x/week for up to 8 weeks) rats. In addition, SC toxicity studies were conducted in Sprague-Dawley rats (Study # VGFT-TX-02006) for up to 13 weeks in duration (3x/week dosing). From these studies, the applicant determined that neither rats nor mice were an appropriate species to use for repeat-dose evaluation of VEGF Trap, as these two species developed an anti-VEGF Trap antibody response associated with nephropathy that precluded long-term dosing. Therefore, the applicant selected the cynomolgus monkey as the primary relevant species for safety assessment of the systemic effects resulting from administration of VEGF Trap.

The main findings of the rat and mice studies are listed in the tables below. In addition to nephropathies, other findings in both rodent species included edema, multiorgan vascular dilatation and congestion, and vasculitis. Similar to findings in monkeys, hemorrhage in the nasoturbinates was noted in mice; bone adverse effects were noted in rats. In spite of the identification of adverse effects, the reviewer concurs that the strong antibody response in these species with the associated marked decrease in VEGF Trap levels and concurrent toxicities will preclude the use of these species for chronic systemic toxicity studies.

**Table 11: Main Findings Observed in Rat Systemic Toxicity Studies following Subcutaneous Administration of VEGF Trap**

| |
|---|
| **Study Title:** A Non-GLP, Exploratory Study to Determine the Effect of Subcutaneous Administration of VEGF Trap (25 mg/kg) on Nude (T cell deficient) Rats Twice a Week for Four or Eight Weeks (Study # PK01032; Module 4.2.3.2)<br>**Design:** Animals were randomly assigned to receive SC injections of either 25 mg/kg of VEGF Trap (3/sex) or placebo (2/sex). Animals were treated twice a week for 4 or 8 weeks. Parameters evaluated included body weights, hematology, clinical chemistry, histopathology, and VEGT Trap and anti-VEFG Trap antibodies levels in serum.<br>**Main Findings**: ↓ body weights and ↑ BUN at 8 weeks, ↑ cholesterol, ↑ triglycerides (4 weeks only), ↑ lipase, ↓ albumin, ↓ A/G ratio, anti-VEGF Trap antibodies not detected but VEGT Trap concentrations decreased with repeated dosing starting on Day 15 (after 4 doses), glomerulonephritis and tubular changes more pronounced at 8 weeks compared to 4 weeks |
| **Study Title:** A non-GLP, Exploratory Study to Determine the Effect of Subcutaneous Administration of VEGF Trap (10 and 15 mg/kg) on Sprague Dawley Rats Three Times per Week for Four Weeks (Study # PK01027; Module 4.2.3.2)<br>**Design:** Rats (4/sex/group) were administered SC injections of either 10 mg/kg of VEGF Trap, 15 mg/kg of VEGF Trap, 15 mg/kg of human IgG, or placebo. Animals were treated 3x/week for 4 weeks and then observed for an additional 18 days. An additional 2 females were used as an untreated control. Same parameters as Study # PK01032 were evaluated.<br>**Main Findings:** Mortalities [2/4 females at 15 mg/kg sacrificed due to weight loss and lethargic behavior on Day 19 (after 9 doses), 1 and 2/4 males at 10 mg/kg sacrificed due to edema on Day 21 (after 10 doses) and due to weight loss and lethargic behavior during the post-treatment period on Day 43, respectively, 1/4 males at 15 mg/kg sacrificed on Day 28 due to edema and another found dead during the post-treatment period on Day 32], ↑ body weight due to edema or body weight loss, trend towards ↑ hemoglobin and hematocrit, ↑ cholesterol, ↑ triglycerides, ↑ lipase, ↑ BUN in 4 out 6 moribund animals, glomerulonephritis, renal tubular dilatation, vascular dilatation and congestion in the spleen, liver, lung, |

| |
|---|
| kidneys, uterus, ovaries, testicles, and GI tract, vasculitis (panarteritis, periarteritis) in one male at 15 mg/kg (considered by the pathologist as possibly representing a background lesion), VEGT Trap concentrations markedly decreased starting on Day 14 (after 6 doses) associated with the development of an antibody response in both dose groups that was more evident in the female rats<br><br>*Note: Regarding the renal changes, the pathologist commented that the glomerular changes may or may not be attributable to the test article due to the relatively high incidence of spontaneous progressive nephropathy in these rats.* |
| **Study Title:** A Non-GLP, Exploratory Study to Determine the Effect of Subcutaneous Administration of VEGF Trap (2 and 5 mg/kg) on Sprague Dawley Rats Three Times per week for Four Weeks (Study # PK01034; Module 4.2.3.2)<br>**Design:** Rats (4/sex/group) were administered SC injections of either 2 mg/kg of VEGF Trap, 5 mg/kg of VEGF Trap, or placebo. Animals were treated 3x/week for 4 weeks. Same parameters as Study # PK01032 were evaluated.<br>**Main Findings:** ↓ body weights in 3 males at 5 mg/kg starting 2 weeks after treatment initiation, ascites in 2 of these males at necropsy, ↑ cholesterol, ↑ triglycerides, ↑ lipase, ↑ BUN in 3 males at 5 mg/kg, ↑ serum creatinine in 1 male at 5 mg/kg, ↓ albumin and calcium in 2 males at 5 mg/kg, glomerulonephropathy (swelling of glomerular tufts, mesangial expansion, increased neutrophils, thickening of Bowman's membrane and capillary basement membranes) and renal tubular changes (thickened basement membranes, swollen epithelial cells, disruption, dilation with protein casts) at both doses but higher incidence at 5 mg/kg, vasculitis in a number of tissues in 2/8 rats at 5 mg/kg, inflammation in the lung and intestines, lymphoadenitis at 5 mg/kg, VEGT Trap concentrations decreased starting on Day 14 (after 6 doses) which generally correlated with the development of an antibody response at 2 mg/kg in both genders and in females at 5 mg/kg; in males at 5 mg/kg, no anti-VEGF Trap antibody was detected in spite of the decrease in VEGF Trap concentrations |
| **Study Title:** A Non-GLP, Exploratory Study to Determine the Effect of Subcutaneous Administration of VEGF Trap (0.5 and 1 mg/kg) on Sprague Dawley Rats Three Times per Week for Four Weeks (Study # PK01042; Module 4.2.3.2)<br>**Design:** Rats (4/sex/group) were administered SC injections of either 0.5 mg/kg of VEGF Trap, 1 mg/kg of VEGF Trap, or placebo. Animals were treated 3x/week for 4 weeks and then observed for a further 4 (males) or 5 (females) days. Same parameters as Study # PK01032 above were evaluated.<br>**Main Findings:** All parameters of toxicity were normal except for elevated serum triglycerides in 2 males at 0.5 mg/kg and 1 male at 1 mg/kg without a dose response. In addition, early glomerulopathy was noted in one animal at 0.5 mg/kg, although it was considered a background change by the pathologist. VEGT Trap concentrations decreased starting on Day 14 or 15 (after 6 or 7 doses) which generally correlated with low serum anti-VEGF Trap antibody concentrations. The NOAEL was considered 1 mg/kg. |
| **Study Title:** A 3-Month Toxicity Study of VEGF Trap by Subcutaneous Injection in Rats (Study # VGFT-TX-02006; Module 4.2.3.2).<br>**Design:** Sprague-Dawley rats were administered the vehicle-control or VEGF Trap at 0.1, 0.5, 1.0, or 2.0 mg/kg for 1 (6 rats/sex/group) or 3 months (10 rats/sex/group) SC 3x/week. Parameters evaluated included mortality, clinical observations, body weight and weight gains, food consumption, hematology, coagulation, clinical chemistry, urinalysis, selected organ weights, gross pathology and histopathology.<br>**Main Findings:** Mortalities at ≥ 1 mg/kg at dosing Day 43; ↑ body weight in males at ≥1 mg/kg, ↓ body weight in females at all doses but particularly at 1 mg/kg; at 1-month sacrifice, glomerulopathy at ≥ 1 mg/kg (particularly in males with only one animal affected in females at each dose); at 3-month sacrifice, glomerulopathy at all doses in males with fibrosis at ≥0.5 mg/kg and at ≥1 mg/kg in females (only 1-2 animals at each dose); polyangiitis and osteoporosis with decrease or complete absence of metaphyseal capillaries at 2 mg/kg in rats with more severe renal toxicity; ↓ total protein in males at 2 mg/kg (1-mo), ↓ albumin, ↑ globulin, and ↓albumin/globulin at ≥1 mg/kg , ↑BUN in males at 2 mg/kg (3-mo), ↑ cholesterol in males at ≥1 mg/kg, ↑ triglycerides in males at 2 mg/kg (3-mo); ↑urinary protein at all doses in males and at ≥1 mg/kg in females; ↑kidney and liver weight in males at 2 mg/kg; decreased VEGT Trap concentrations starting ~ 2-4 weeks after dosing which generally correlated with the development of an anti-VEGF antibody response as early as Day 6; NOAEL = 0.5 mg/kg for females, < 0.1 mg/kg in males |

**Table 12: Main Findings Observed in Mouse Systemic Toxicity Studies following Subcutaneous Administration of VEGF Trap**

| |
|---|
| **Study Title:** A Non-GLP, Exploratory Study to Determine the Effect of Subcutaneous Administration of VEGF Trap on SCID Mice (15 Week vs. 7 Week Old Mice) for 4 or 8 Weeks (Study # VGT3; Module 4.2.3.2)<br>**Design:** Mice (1-5 males/group) received SC injections of 2.5 mg/kg or 25 mg/kg of VEGF Trap or placebo 2x/week except for 3x/week during Week 4. Two age groups were compared at each dose: 7 and 15 weeks old. Mice were treated for 4 weeks, 8 weeks, or 8 weeks plus 4 weeks of recovery. Same parameters as Study # PK01032 above were evaluated.<br>**Main Findings:** ↑ weight loss in 2 males at 25 mg/kg (8 weeks), ↑ hematocrit, hemoglobin and RBC at both doses (8 weeks), hemorrhage and/or vascular congestion at 8 weeks of treatment principally in the kidney at both doses but with higher incidence at 25 mg/kg and 7 week old mice, congestion and/or edema in the GI tract including one mouse with typhlitis at 25 mg/kg in 7 week old mice and 8 weeks of treatment, periarteritis and myocarditis in one mouse at 25 mg/kg (15 weeks old, 8 wk treatment), hemorrhage in the nasoturbinates in one 7-week old and one 15-week old mouse at 2.5 mg/kg (8 wk treatment), no anti-VEGF Trap antibodies detected, VEGT Trap concentrations remained elevated; all changes showed reversibility except for mild renal tubular findings (degeneration, dilation, and protein casts) at recovery in 2 mice at 25 mg/kg |
| **Study Title:** A Non-GLP, Exploratory Study to Determine the Effect of Subcutaneous Administration of VEGF Trap (10 and 15 mg/kg) on CD-1 Mice Three Times per Week for Four Weeks (Study # PK01017; Module 4.2.3.2)<br>**Design:** Mice (4/sex/group) received SC injections of either 10 mg/kg of VEGF Trap, 15 mg/kg of VEGF Trap, 15 mg/kg human IgG, or placebo (2/sex/group). Mice were treated 3x/week for 4 weeks and then observed for an additional 2 weeks. In addition, 3 untreated females were included for comparative purposes. Same parameters as Study # PK01032 above were evaluated (clinical pathology data was not interpretable).<br>**Main Findings:** Mortalities (2 females at 10 mg/kg found dead on Day 18, another female at 10 mg/kg sacrificed moribund on Day 20, 1 female at 15 mg/kg found dead on Day 20, and 2 other females at 15 mg/kg sacrificed moribund on Day 20 and Day 31); edema in all unscheduled sacrifice animals; ↑ body weights in all unscheduled sacrifice animals; glomerulopathy (enlargement of the mesangial tufts, deposition of eosinophilic material, increased numbers of mesangial cells, thickening and some displacement of capillary loops) and dilated tubules at both doses; vascular dilation with fluid exudation into the subcutaneous space and GI tract at both doses; VEGF Trap concentrations declined starting on Day 14 which correlated with the development of an antibody response |

As noted above, the monkey was selected as the relevant species. Therefore, the monkey studies were reviewed in greater detail.

**Study title: A 6-Month Intravenous Toxicity Study of VEGF Trap in Cynomolgus Monkeys with a 5-Month Recovery Period**

| | |
|---|---|
| Study no.: | VGFT-TX-05009 |
| Study report location: | Module 4.2.3.2 |
| Conducting laboratory and location: | (b) (4) (b) (4) |
| Date of study initiation: | March 17, 2006 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | VEGF Trap, lot # C06001J600X1, ≥97% pure |

Key Study Findings

- One 3 mg/kg/dose male was euthanized prior to the end of the study primarily due to anemia and nasal bleeding secondary to extensive nasal lesions.
- The remaining animals exhibited hunching, nose bleeding, sneezing, reduced activity, appetite, and body weights.
- Absent or irregular menses associated with alterations in female reproductive hormone levels, and changes in sperm morphology and motility were considered test article-related.
- Test article-related changes in hematology, serum chemistry, urinalysis parameters and bone resorption markers were observed.
- Radiological diagnoses included kyphosis, degenerative joint disease (DJD), and periosteal reactions at all VEGF Trap dose levels.
- Macro- and microscopic test article-related changes were present in bones, nasal cavities, adrenals, brain (choroid plexus), liver, kidneys, ovaries, and digestive system. Vasculitis was noted in several tissues.
- At the end of a 5-month recovery period, principal findings included kyphosis with osteocartilaginous exostoses, nasal cavities deformation, DJD, and changes in the digestive system and the brain (choroid plexus).
- Based upon the above observations, the NOAEL was <3 mg/kg, the lowest dose evaluated.
- Based on $C_{max}$ and $AUC_{0\text{-}168hrs}$, the systemic exposure was 4900-fold and 1550-fold higher, respectively, than the exposure observed in humans after an ITV dose of 2 mg/eye once every 4 weeks ($C_{max}$ and $AUC_{0\text{-}last}$ of 0.0193 µg/mL and 2.856 µg•hr/mL, respectively).

Methods

| | |
|---|---|
| Doses: | 0, 3, 10, or 30 mg/kg |
| Frequency of dosing: | Once weekly for 15 weeks<br>For the final 12 weeks, the dosing frequency was reduced to every other week due to toxicity (decreased body weights). |
| Route of administration: | IV (30-min infusion); peripheral vein (brachial and/or saphenous) |
| Dose volume: | 3.75 mL/kg |
| Formulation/Vehicle: | (b) (4) sodium phosphate, (b) (4) sodium citrate, 33 mM sodium chloride, 0.033% (w/v) PS20 and (b) (4) sucrose, pH (b) (4) |
| Species/Strain: | Cynomolgus monkeys (*Macaca fascicularis*) |
| Number/Sex/Group: | 4 |
| Age: | 5-12 yrs old males; 3-5 yrs old females |
| Weight: | 4.1-12.3 kg for males; 2.3-4.1 kg for females |
| Satellite groups: | An additional 2 monkeys/sex/dose were used for a 5-month recovery period. |
| Unique study design: | Based on target organs identified in earlier studies, a series of special evaluations were performed. |
| Deviation from study protocol: | None that could adversely affect interpretation of |

the study results

Observations and Results

Mortality (Twice daily) – A male at 3 mg/kg (# 202), was euthanized on Day 182 (Week 26) primarily due to a rapid clinical deterioration secondary to a marked anemia due to nasal bleeding. Extensive macroscopic lesions were observed in the nasal cavities, including blood clots (or hemorrhages) in the right caudal nasal cavity and maxillary sinus, a bent nasal septum and an absence of the right middle concha. These observations, as well as corresponding microscopic lesions (atrophy/loss of the nasal septum and/or turbinates associated with necrotizing inflammation), were similar to those noted in several other VEGF Trap-treated animals. Therefore, they were regarded as compound-related. Macroscopic findings also included pale discoloration of the kidneys, liver, and stomach and a darkly discolored digestive content. Histopathologically, erythroid hyperplasia in the bone marrow and extramedullary hematopoiesis in several tissues was consistent with the hematological evidences of a marked regenerative anemia detected prior to euthanasia.

Clinical Signs (Daily; detailed examination once prior to the start of treatment and weekly throughout the treatment and recovery periods) – Main treatment-related clinical signs included sneezing with or without discharge, red fur staining, dry skin, swelling, scabbing and/or redness of the muzzle/lower jaw, hunched or abnormal posture, reduced appetite, thin aspect and/or decreased activity at all dose levels.

During recovery, one male at 30 mg/kg (# 406) was noted with discharge from the muzzle during the first week of the recovery period (Week 28); skin scab or redness of the muzzle/lower jaw was seen in one male in each VEGF Trap treated group (# 210, 308, and 406); and one male at 30 mg/kg (# 406) started to show a hunched posture during the recovery period (Week 32), which persisted until the end of the recovery period. Similarly, signs of hunched posture noted during the treatment period in animals assigned to the recovery phase persisted until the end of the recovery period.

Body Weights (Weekly) - Decreases in mean body weight were noted compared to control values at all VEGF Trap dose levels starting around Week 13 in males (5-14%) and Week 9 in females (8-13%). As a result of this weight loss, the treatment interval was changed to every other week after Week 15 for the remaining 12 weeks. Body weights were still lower than controls for the remainder of the study. The decrease was dose-dependent only in females.

By the end of treatment (Week 27), mean body weight gain in females was dose-dependently decreased (0.4, 7.8, and 20.4 % respectively at 3, 10, and 30 mg/kg). Males did not show a dose response. The percent decreases for males were 7.6, 15.1, and 8.4% respectively at 3, 10, and 30 mg/kg.

The decrease in body weight was reversed during the recovery period.

Feed Consumption (Once daily; qualitatively) – Reduced appetite (associated with thin aspect) was noted in all males treated with VEGF Trap as well as with the vehicle control. In females, there was an increased incidence of reduced appetite, particularly at the 2 highest doses (3, 4, 6, and 6 at 0, 3, 10, and 30 mg/kg, respectively). Reduced appetite was noted in all control and test article-treated animals during the recovery period. Animals were provided with food supplementation as required.

Ophthalmoscopy [Once prior to the start of treatment (all animals) and during Weeks 13 and 26 of treatment and at the end of the recovery period (Week 47) using funduscopic (direct and indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations] - One male at 3 mg/kg (# 210) showed focal areas of retinal edema and subretinal depigmentation in the peripapillary and macular regions at both Weeks 13 and 26 in both eyes. One male at 30 mg/kg (# 408) showed fine granular opacities scattered in the macular area of each eye at Week 26. Another male at 30 mg/kg (# 406) had focal area of edema-like retinal opacity in the inferior papillary area at Week 26 on the left eye only. No histopahtological correlates were noted.

The lesions in animal # 210 were still present during recovery and had become larger and degenerative with retinal edema no longer present. One recovery male at 10 mg/kg (#308) appeared to be functionally blind and had a vertical nystagmus. Histopathological findings included bilateral severe chronic perineural inflammation and moderate nerve fiber degeneration with gliosis of cranial nerves II, III, IV and VI.

Because these lesions were of low incidence and not observed in the 13-week or 8-month ITV toxicity studies, the relationship to treatment is uncertain.

ECG and Blood Pressure [Twice prior to commencement of treatment (three weeks apart) and again during Weeks 4, 12, and 26 of the treatment period and during Weeks 37 and 48 of the recovery period; QTc calculated using Fredericia.s equation] – No test article-related effects

Hematology and coagulation (Once prior to the start of treatment and during Weeks 4, 12, and 25 of the treatment period and during Weeks 37 and 48 of the recovery period) – A series of parameters showed differences when compared with the vehicle control and predose values. The following % change is based on vehicle control values.

In females at all doses, mean hematocrit was increased (12-14%; $p \leq 0.05$) at Week 4. In males at Week 4, mean hemoglobin was increased (11%; $p \leq 0.01$) at the mid and high dose, although the mean values were within physiological range.

At Week 12, mean # reticulocytes and fibrinogen were increased at the high dose (15%) and at all doses in males [72% ($p \leq 0.01$), 69% ($p \leq 0.01$), and 33%, at 3, 10, and 30 mg/kg, respectively]. At Week 12 in females, the high dose showed increased mean % neutrophils (58%; $p \leq 0.05$) and decreased % lymphocytes (22%; $p \leq 0.05$); mean hematocrit was increased (10%; $p \leq 0.05$) and mean APTT was decreased (10-30%; $p \leq 0.05$ at the high dose only) at all doses.

At Week 25, increased mean # reticulocytes (83%, 29%, and 112% at 3, 10, and 30 mg/kg, respectively) and fibrinogen (39%, 26%, and 18% at 3, 10, and 30 mg/kg, respectively) were observed at all doses in males. At the high dose in males, increases were observed in mean red cell distribution width (19%) and platelet volume (24%). At Week 25 in females, mean hemoglobin (13%) and # reticulocytes (70%) were increased and APTT was decreased (22%; $p \leq 0.01$) at the high dose. Except where indicated, the difference was not statistically significant.

Most of these changes were reversible.

Clinical Chemistry (Once prior to the start of treatment and during Weeks 4, 12, and 25 of the treatment period and during Weeks 37 and 48 of the recovery period) -The applicant noted that cholesterol was increased in animals of both sexes treated at all dose levels during Weeks 4, 12, and 25. However, values were within normal biological range, except for one male at 10 mg/kg (# 310) with values of 223 mg/dL (Week 4) and 251 mg/dL (Week 12) but normal at Week 25 (186 mg/dL). Treatment-related and marked increases in GGT, AST, ALP and/or ALT were noted at all doses in individual males and females. The elevation in mean values compared to controls was 2-3x, although individual values showed increases up to 5x, 8x, 10x, and 10x, respectively. These elevations were observed primarily at Week 25 with some animals showing changes at Week 12. Females were more affected than males. At the end of the recovery, some parameters were still elevated.

These changes are described in more detail below:

Treatment-related marked increase in AST was noted at Week 25 in two females at 10 mg/kg (# 355 and 357) and two females at 30 mg/kg (# 454 and 455) relative to controls or acclimation values. At the end of the recovery period, these values largely returned to their acclimation values for animal # 355 and 454.

Females # 355, 357, 454, and 455 with increases in AST, also had increases in GGT relative to controls or acclimation values at Week 25. By the end of the recovery period, female # 355 continued to have elevated GGT. Treatment-related marked increase in GGT was also noted at Weeks 12 and/or 25 in one male at 3 mg/kg (# 207), one male at 10 mg/kg (# 307) and one male at 30 mg/kg (# 405). Except as noted above, elevated GGT values were not noted by the end of the recovery.

Females # 355, 357, 454 and 455 also showed increases in ALP relative to controls or acclimation values at Week 25. An additional female at 10 mg/kg (#361) also had elevated ALP. A slight increase in ALP was noted at Weeks 12 and/or 25 in two males at 3 mg/kg (# 202 and 207) and one male at 30 mg/kg (#408). Elevated ALP values were not noted by the end of the recovery.

Females # 355, 357, 454 and 455 also had increases in ALT relative to controls or acclimation values at Week 25. By the end of the recovery period, female # 355 continued to have elevated ALT levels.

Urinalysis (Once prior to the start of treatment and during Weeks 4, 12, and 25 of the treatment period and during Weeks 37 and 48 of the recovery period) - Mean microalbumin (5-100x) and protein (2-8x) levels were increased in males. Relative to controls and baseline values at Weeks 4, 12 and/or 25, microalbumin and protein were increased in 3 males (# 201, 208 and/or 210) at 3 mg/kg, in 4 males (# 306, 307, 309 and/or 310) at 10 mg/kg, and in 4 males (# 406, 407, 409 and 410) at 30 mg/kg. Increased levels of microalbumin were noted in 2 females (# 453 and 455) at 30 mg/kg at Week 25, but these levels were within the range of values seen in control animals. In general, microscopic kidney changes identified in VEGF Trap treated animals explain the proteinuria and albuminuria. These changes reverse during recovery.

Gross Pathology (All animals euthanized at the end of the treatment and recovery periods) - Macroscopic findings were noted for several tissues, affecting primarily the nasal cavities and skeletal system as well as the adrenal glands, duodenum, and gallbladder. The findings in the bones and nasal cavities are summarized in the table below:

**Table 13: Incidence of VEGF Trap-related Macroscopic Findings in Bones and Nasal Cavities in the 6-Month Intravenous Toxicity Study in Cynomolgus Monkeys**

| Tissue/Finding | Sex | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Dose (mg/kg/dose) | 0 | 3 | 10 | 30 | 0 | 3 | 10 | 30 |
| | Number of animals examined | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Bone-Vertebra[#] | | | | | | | | | |
| | Area raised | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| | Bent: kyphosis | 0 | 2 | 2 | 3 | 0 | 1 | 4 | 3 |
| | Fusion | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| | Thickening | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| Bone-Other sites | | | | | | | | | |
| | Femur-Mass | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 |
| | Ilium-Mass | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| | Radius[&]-Mass | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | Sternum-Mass | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Cavity nasal/Sinuses | | | | | | | | | |
| | Area dark | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 |
| | Area raised | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Bent: septum | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| | Clot | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Discoloration dark | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| | Material pale | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| | Not present*: septum | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 |
| | Not present*: turbinate/concha | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| | Perforation: septum | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 |
| | Small: turbinate/concha | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | Thickening: septum | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |

# The number of animals that presented the finding in at least one segment of the cervical, thoracic and/or lumbar vertebral column.

& Recorded under Bone miscellaneous.

* Finding subdivided according to anatomical modifier.

In the skeletal system, the most common macroscopic finding was noted in the thoracic and/or lumbar vertebrae being described as bent: kyphosis. This change found in males and females at ≥3 mg/kg was centered on the caudal thoracic vertebrae and cranial lumbar vertebrae and extended maximally from T5 to L6.

In other skeletal sites of animals given ≥3 mg/kg, pale and firm mass(es) were occasionally noted with a polyostotic and/or bilaterally symmetric distribution, such as in the left proximal femur and both ilia of one high-dose animal (# 408) and in the right proximal femur, right ilium and both radii in a second high-dose animal (# 455). The most frequently affected long bone was the proximal femur and, in one low dose animal (# 208), the mass was associated with ankylosis of the coxofemoral joint. In addition to these above listed sites, a single occurrence of sternal mass was found in one female treated with 3 mg/kg.

Thickening of the gallbladder wall was seen in 2 females at 30 mg/kg. Dark areas on the duodenal mucosa were present in 1 male at 10 mg/kg and in 1 male and 2 females at 30 mg/kg. Dark discoloration of the adrenal glands was noted in 2 and 1 males at 3 and 30 mg/kg, respectively, and in 1 and 2 females at 10 and 30 mg/kg, respectively. These gross changes were regarded as VEGF Trap-related in light of their histological correlates.

These macroscopic findings persisted in several tissues after the 5-month recovery period, affecting primarily the nasal cavities, skeletal system, and the gallbladder. No findings were noted in the adrenal glands or duodenal mucosa. A dark depressed area on the stomach mucosa was noted in one female at 10 mg/kg.

Organ Weights (adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries/testes, pituitary, prostate, seminal vesicles, spleen, thymus, thyroid/parathyroids, and uterus) – A dose-related decrease in mean absolute uterine weights was observed in females; 26%, 37% ($p \leq 0.05$), and 42% ($p \leq 0.05$) at 3, 10, and 30 mg/kg, respectively. Relative to body weight, a 5, 12, and 10% decrease was observed, respectively. This variation correlated with endometrial and myometrial atrophy noted histologically in most females of these groups.

Marked but non-significant decreases in mean absolute ovary weights (77, 83, and 83% at 3, 10, and 30 mg/kg, respectively) were noted in all VEGF Trap treated groups when compared to the controls, which correlated with the scant luteal activity noted during histological examination. Relative to body weight, decreases of ~70% (no statistical significance) were observed at all VEGF Trap doses.

At recovery, only the high-dose females still showed decreased weight of the ovaries (23% absolute weight and 9% relative to body weight) compared to controls. The reduced magnitude of the change suggests partial recovery.

In males, decreases in absolute seminal vesicle weight were observed without a dose response (16, 52, and 6% at 3, 10, and 30 mg/kg, respectively; no statistical significance). Relative to body weight, decreases of 7, 39, and 14% were observed, respectively. These decreases were still observed at recovery (10, 50, and 25% absolute weight; 38, 51, and 26%, relative to body weight, respectively), although the data is not reliable as the control group consisted of one animal. There was no histopathological correlate for this change but sperm motility and morphology was affected at ≥3 mg/kg (see below).

Thymus weight was decreased at the high dose compared to controls; 41% in males and 74% in females (absolute values); 34% in males and 62% in females relative to body weights. No statistical significance was observed. Recovery was observed in females. In males, thymus weight was still decreased at the end of recovery, but the data was no reliable as only 1 control animal was left. This decrease correlated with a higher incidence of thymic lymphoid atrophy.

Histopathology

Adequate Battery - Yes

*Note: Bone marrow smears were collected but not evaluated. Testicular histopathological evaluation included assessment of the spermatogenic cycle.*

Peer Review - Yes

Histological Findings - Microscopic findings were observed in the nasal cavities, various bones, kidneys, female reproductive system, digestive system (liver, gallbladder, duodenum and stomach), adrenal glands, brain, thymus, and trachea. Microvascular effects were noted in most of these tissues and, sporadically, in the heart and a few other tissues.

Nasal cavities: Histopathological findings that correlated with macroscopic observations noted during trimming were seen in the nasal cavities of some males and females dosed at ≥3 mg/kg. These alterations included an atrophy/loss of the septum and/or turbinates associated with necrotizing inflammation and various other epithelial, microvascular, cartilaginous, and osseous findings (Table 14). In animals allowed a drug-free 5-month recovery period after treatment completion, there was reversibility of most compound-related changes, either inflammatory, vascular and targeting the epithelia, but no reversibility of some of the changes affecting the osseous and cartilaginous support in the nasal cavities.

**Table 14: Incidence and Severity of VEGF Trap-related Histopathological Findings in Nasal Cavities in the 6-Month Intravenous Toxicity Study in Monkeys**

| Tissue/Finding | Sex | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dose (mg/kg/dose) | | 0 | 3 | 10 | 30 | 0 | 3 | 10 | 30 |
| **Nasal cavity/sinus** | Number examined | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Atrophy/loss: septum | | | | | | | | | |
| | Total number affected | 0 | 2 | 3 | 1 | 0 | 2 | 1 | 3 |
| | Minimal | - | - | - | - | - | 1 | 1 | 2 |
| | Slight | - | - | 1 | - | - | 1 | - | 1 |
| | Moderate | - | 2 | 1 | - | - | - | - | - |
| | Marked | - | - | 1 | 1 | - | - | - | - |
| Atrophy/loss: turbinate | | | | | | | | | |
| | Total number affected | 0 | 1 | 3 | 1 | 0 | 1 | 0 | 1 |
| | Slight | - | 1 | 1 | - | - | 1 | - | - |
| | Moderate | - | - | - | - | - | - | - | 1 |
| | Marked | - | - | 2 | 1 | - | - | - | - |
| Cartilaginous metaplasia: ethmoturbinate | | | | | | | | | |
| | Total number affected | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 2 |
| | Minimal | - | 1 | 1 | - | - | - | 1 | 2 |
| | Slight | - | - | - | 1 | - | - | - | - |
| Degeneration/regeneration: respiratory epithelium | Total number affected | 0 | 4 | 4 | 3 | 0 | 4 | 4 | 4 |
| | Minimal | - | 1 | - | - | - | 1 | 2 | - |
| | Slight | - | 1 | 1 | - | - | 2 | - | 3 |
| | Moderate | - | 2 | 1 | 2 | - | 1 | 2 | 1 |
| | Marked | - | - | 2 | 1 | - | - | - | - |
| Degeneration/regeneration: olfactory epithelium | Total number affected | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 |
| | Minimal | - | - | - | - | - | 1 | - | - |
| | Slight | - | - | 1 | - | - | - | - | 1 |
| | Moderate | - | - | - | 1 | - | - | - | - |
| Eosinophilic cartilage/activated chondrocytes: septum | Total number affected | 0 | 4 | 4 | 3 | 0 | 4 | 3 | 4 |
| | Minimal | - | 2 | 1 | 1 | - | 2 | 1 | 1 |
| | Slight | - | 1 | 3 | - | - | 1 | - | 2 |
| | Moderate | - | 1 | - | 1 | - | 1 | 2 | 1 |
| | Marked | - | - | - | 1 | - | - | - | - |
| Exudate | | | | | | | | | |
| | Total number affected | 1 | 4 | 4 | 3 | 2 | 3 | 3 | 4 |
| | Minimal | 1 | 1 | - | - | 2 | 1 | 2 | 1 |
| | Slight | - | 1 | 2 | 2 | - | 2 | 1 | 2 |
| | Moderate | - | 2 | 2 | - | - | - | - | 1 |
| | Marked | - | - | - | 1 | - | - | - | - |

Cont'd Table 14

| Tissue/Finding | Sex | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dose (mg/kg/dose) | | 0 | 3 | 10 | 30 | 0 | 3 | 10 | 30 |
| **Nasal cavity/sinus** | Number examined | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Hemorrhage | | | | | | | | | |
| | Total number affected | 0 | 4 | 4 | 3 | 0 | 1 | 3 | 2 |
| | Minimal | - | 2 | 1 | 1 | - | 1 | 3 | 2 |
| | Slight | - | 1 | 3 | 1 | - | - | - | - |
| | Moderate | - | 1 | - | 1 | - | - | - | - |
| Inflammation: necrotizing | | | | | | | | | |
| | Total number affected | 0 | 2 | 3 | 1 | 0 | 1 | 1 | 1 |
| | Slight | - | - | - | - | - | 1 | 1 | - |
| | Moderate | - | 1 | 3 | - | - | - | - | 1 |
| | Marked | - | 1 | - | 1 | - | - | - | - |
| Proliferation/degeneration: vascular | | | | | | | | | |
| | Total number affected | 0 | 2 | 3 | 3 | 0 | 1 | 2 | 3 |
| | Minimal | - | - | 3 | 1 | - | 1 | 2 | 2 |
| | Slight | - | 2 | - | 2 | - | - | - | 1 |
| Thrombosis | | | | | | | | | |
| | Total number affected | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 |
| | Minimal | - | 1 | - | 1 | - | - | - | 1 |
| Ulceration: respiratory epithelium | | | | | | | | | |
| | Total number affected | 0 | 2 | 2 | 3 | 0 | 2 | 2 | 3 |
| | Minimal | - | - | - | 1 | - | 2 | 1 | 1 |
| | Slight | - | - | 2 | 1 | - | - | 1 | 2 |
| | Moderate | - | 2 | - | 1 | - | - | - | - |

Bones/Muscles: The most important finding was the development of osteocartilaginous exostoses in males and females dosed with ≥3 mg/kg. In the spine, the exostoses were most frequently observed on the arches of the thoracic and lumbar vertebrae and often correlated macroscopically with a bent in kyphosis deformation of the vertebral column. Exostoses, correlating with mass(es) at necropsy, were less common in other bones and predominantly involved the proximal femur. Muscular myofiber atrophy and less commonly vascular proliferation/degeneration were often seen as concurrent findings with the exostoses. There was no reversal of these osseous and muscular findings after recovery.

Whenever the physis was not closed in various bone sites, a thickened hypertrophic chondrocyte layer was a common and minor VEGF Trap-induced effect in animals treated with ≥3 mg/kg. In these groups, another minor histological finding was a cartilaginous metaplasia localized in the ventral cortex of vertebral bodies. There was almost complete reversibility of these changes following recovery.

Kidneys: An increased eosinophilic matrix (minimal to moderate) in the glomerular tuft that stained positively with the periodic acid Schiff reaction was noted in males

and females at ≥3 mg/kg. In addition, glomerulopathy (minimal-slight), often with tubulointerstitial inflammation (minimal-slight) and/or cast formation (minimal-moderate), were commonly seen in males at ≥3 mg/kg and in a single female at 30 mg/kg. These changes showed decreased incidence and severity at the end of the recovery phase.

Female Reproductive System: Ovarian luteal development was markedly compromised in females at ≥3 mg/kg (Table 15). This change correlated with a decreased weight of the ovaries at treatment completion. In some of these sexually mature females, follicular maturation was also decreased in number and quality. Uterine endometrial and myometrial atrophy and vaginal epithelial atrophy were noted in most females at ≥3 mg/kg and in a few females at ≥10 mg/kg, respectively. The uterine atrophy correlated with a decreased weight of this organ at completion of treatment. Proliferation/degeneration of the uterus and vagina was noted in 1 female at 30 mg/kg. Reversibility of the effects in the female reproductive tract was observed after the 5-month recovery period, with the exception of the ovarian weights that were still slightly decreased at 30 mg/kg.

**Table 15: Incidence and Severity of VEGF Trap-related Histolopathological Findings in the Female Reproductive System in the 6-Month Intravenous Toxicity Study in Monkeys**

| Tissue/Finding | Sex | Female | | | |
|---|---|---|---|---|---|
| Dose (mg/kg/dose) | | 0 | 3 | 10 | 30 |
| **Ovary** | Number examined | 4 | 4 | 4 | 4 |
| Decreased granulosa cells | | | | | |
| | Total number affected | 0 | 2 | 0 | 3 |
| | Minimal | - | - | - | 1 |
| | Slight | - | 2 | - | 1 |
| | Moderate | - | - | - | 1[#] |
| Decreased maturing follicles | | | | | |
| | Total number affected | 1 | 0 | 1 | 3 |
| | Minimal | - | - | 1 | - |
| | Slight | 1 | - | - | 1 |
| | Moderate | - | - | - | 1 |
| | Marked | - | - | - | 1 |
| Presence of corpus luteum | | | | | |
| | Total number affected | 4 | 3 | 2 | 3 |
| | Very small | - | 1 | 1 | 1 |
| | Small | - | 1 | - | 2 |
| | Large | 2 | 1 | 1 | - |
| | Extensive size | 2 | - | - | - |
| **Uterus** | Number examined | 4 | 4 | 4 | 4 |
| Atrophy: endometrial and myometrial | | | | | |
| | Total number affected | 0 | 3 | 3 | 4 |
| | Minimal | - | 2 | 1 | - |
| | Slight | - | 1 | 2 | 3[&] |
| | Moderate | - | - | - | 1 |
| **Vagina** | Number examined | 4 | 4 | 4 | 4 |
| Atrophy: epithelium | | | | | |
| | Total number affected | 0 | 0 | 1 | 2 |
| | Slight | - | - | 1 | 1 |
| | Moderate | - | - | - | 1[&] |

# Associated with slight decreased theca cells.

& Associated with moderate vascular proliferation/degeneration in one female.

Digestive System: Vascular degeneration/proliferation was noted in the duodenum, stomach, rectum, gallbladder, and pancreas at ≥10 mg/kg. The duodenum and gallbladder were the main target organs, which often presented slight-moderate secondary mucosal damage (atrophy/ulceration) and inflammation, respectively. Following recovery, findings were still observed in the gallbladder, duodenum, and stomach

Liver: Hepatic portal inflammation (minimal to moderate) and periportal necrosis (minimal to slight), usually with diffuse pigment deposits in Kupffer cells, were seen in 1 male and 2 females at 30 mg/kg. Following recovery, one female each at 10 and 30 mg/kg showed slight multifocal chronic active portal inflammation and bile duct hyperplasia. Minimal multifocal chronic portal inflammation was seen in one at 10 mg/kg.

Adrenal Glands: In the cortex, decreased cytoplasmic vacuolation (minimal to moderate) with increased cytoplasmic eosinophilia, which correlated with macroscopic dark discoloration, was observed in 2 and 1 males at 3 mg/kg and 30 mg/kg, respectively, and in 2 and 1 females at 10 mg/kg and 30 mg/kg, respectively. This finding was not observed in recovery animals.

Brain: Minimal infiltration of macrophages was observed in the choroid plexus in 1 and 2 females at 3 and 30 mg/kg, respectively. In addition, minimal vascular degeneration/fibrosis, which affected mainly the small arterioles of the choroid plexus, was noted in 1 male and 3 females at 30 mg/kg. These findings were present in one male at 10 mg/kg and/or 30 mg/kg at the end of the recovery period.

Vasculopathies: In addition to the organs mentioned above, minimal vascular proliferation/degeneration was noted in the heart in 2 males and 1 female at 10 mg/kg. The cardiac vascular findings were not limited to the arterioles; they involved the major coronary artery next to the right atrium in 2 monkeys. Other tissues in which vascular proliferation/degeneration was found included the epididymis in 1 male at 30 mg/kg (slight) and in the femorotibial joint, jejunum, sciatic nerve, and skin in 1 female at 30 mg/kg (minimal).

At recovery, vascular degeneration/fibrosis was noted in the renal papilla of 1 male at 10 mg/kg and vascular proliferation/degeneration was seen in the femorotibial joint of 1 male at 30 mg/kg. The heart was spared in animals of the recovery phase. Overall, there was some evidence of persistence of the compound-related vasculopathy after completion of the treatment-free period (mainly in the digestive system). The applicant noted these changes were generally undergoing repair by fibrosis, as diagnosed by vascular proliferation/fibrosis.

Thymus: Increased incidence of thymic lymphoid atrophy was observed in both males and females compared to controls (0, 2, 4, and 3 in males and 1, 2, 1, and 4 in females at 0, 3, 10, and 30 mg/kg, respectively). The severity ranged from slight-severe in males, slight in the female control, and slight-marked in VEGF Trap-treated females. At recovery, the incidence (1-2 animals/group) and severity was similar to that seen in control groups (marked in the male control and slight-marked in VEGF Trap-treated males and minimal-slight in control and VEGF Trap-treated females).

In 3 males out of 4 in the control and 2 males out of 4 in the low dose groups, there was no thymus or remnant found in the sections of mediastinal fat provided for evaluation, which complicated the interpretation of this finding. The applicant considered that no thymic remnant was indicative of severe thymic atrophy in most control males and consequently, that no compound related effect was present in this organ in males.

In females, the incidence and severity of thymic lymphoid atrophy was more severe at 30 mg/kg (4 animals affected among which 2 were graded moderate or

marked in severity) when compared to controls (1 animal affected and graded slight in severity). However, the applicant regarded the effect upon the thymus as indirect, secondary to the multi-systemic effects induced by the test article and/or stress-related. The reviewer concurs that the existent data do not allow for a clear assessment of a test article-related effect.

Trachea: Atrophy (minimal-moderate) of the epithelium was observed with slightly higher incidence compared to controls in both males and females (0, 1, 1, and 2 at 0, 3, 10, and 30 mg/kg, respectively). At recovery, the finding was noted in 1 male each in control and high-dose groups. As noted by the applicant, the significance of this observation is uncertain because it is possible that it may be a consequence of the endotracheal intubation required when the animals were anesthetized for bone densitometry measurements. However, it may also be an indirect consequence of the observed compound-related effects in the nasal cavities and/or the adjacent cervical spine.

Special Evaluations:

Body Temperature (Twice prior to commencement of treatment (three weeks apart) and during Weeks 2, 4, 12, and 26 of the treatment period and during Weeks 37 and 48 of the recovery period] – No test article-related effects

Vaginal Bleeding (Daily by performing vaginal swabbing using a cotton swab commencing during the acclimation period and extending throughout the treatment and recovery periods) – Arrest of regular menstrual bleeding was observed in 1, 4, 5, and 5 females at 0, 3, 10, and 30 mg/kg which lasted for prolonged durations (7-22 weeks). One or more episodes of abnormally protracted or frequent bleeding that ranged from 10-17 days (and for 23 days extending into the recovery period for one female at 3 mg/kg) was observed in 3 and 1 females at 3 mg/kg and 30 mg/kg, respectively. During the recovery period, the animals started to show more frequent/regular signs of menses or menses duration.

Male Reproductive System (Twice during the acclimation period, Weeks 5, 8, 12, and 25 of the treatment period and at the mid (Week 38) and end of the recovery period; volume, sperm concentration, motility and morphology] – A pronounced reduction in sperm motility and increased morphological abnormalities in spermatozoa were observed at ≥3 mg/kg. These effects, noted at Week 5, persisted throughout the treatment period. Complete reversal of these findings was noted as early as 12 weeks after cessation of compound administration in recovery animals. The administration of VEGF Trap did not induce changes in mean testicular volume and sperm counts.

Hormone Analysis [Males - twice during the acclimation period (two weeks apart), and once every two weeks throughout the treatment and recovery periods and prior to terminal necropsies (blood FSH, LH, and testosterone); Females – once every week or every two weeks during the acclimation period, once a week during the first month of dosing and once every two weeks thereafter, except once a week for females

during the last month of the recovery period) and prior to terminal and recovery necropsies (blood estradiol, progesterone, FSH, and inhibin B] –

Females: Mean progesterone levels were decreased within 1 week of dosing throughout the treatment period, although statistical significance was only reached at the mid- and/or high dose on Weeks 19, 23, and 27. At Week 27, mean levels were decreased by 78, 82, and 86% at 3, 10, or 30 mg/kg, respectively, compared to controls. The individual animal listings showed a wide range of values in the control group (1.76-26.2 ng/mL) which included the range of values observed in VEGF Trap-treated groups (2.12-3.89, 1.26-3.22, and 1.26-3.22 ng/mL at 3, 10, and 30 mg/kg, respectively). The reviewer, therefore, finds it difficult to conclude there was a clear treatment related effect. The applicant counted the number of ovulatory cycles by counting the number of discrete progesterone peaks that were ≥5 ng/mL. A definitive negative effect on progesterone was noted in this parameter. The mean number of progesterone peaks observed per month during the treatment period was 0.69, 0.05, 0.05, and 0.02 in groups receiving 0, 3, 10, or 30 mg/kg, respectively. During recovery, the mean number of progesterone peaks observed per month was 0.91, 0.45, 0.64, and 0.36, respectively, indicating recovery of normal menses was occurring.

Group mean estradiol levels were generally non-significantly decreased in females treated with VEGF Trap, particularly at doses of 10 and 30 mg/kg when compared to controls beginning at Week 15. At Week 27, mean estradiol levels were decreased by 14% (10 mg/kg) and 40% (30 mg/kg). However, individual animal listings showed values within the range observed in control animals at diverse time points throughout the study. The applicant noted that the failure to detect significant decreases in estradiol levels during VEGF Trap treatment was likely attributable to the relatively brief duration of the preovulatory estradiol surge (usually 4-5 days), relatively infrequent blood sampling (usually once every two weeks), and because cycle phases were not temporally aligned. However, that normal follicular development was compromised was clearly evident from the effects of VEGF Trap treatment on inhibin B and FSH levels (see below).

Group mean inhibin B values were statistically significantly reduced compared to controls for all VEGF Trap female groups (≥3 mg/kg/dose) starting from the first week of treatment and throughout the treatment period. At Week 27, mean levels were decreased by 74, 73, and 81% at 3, 10, and 30 mg/kg, respectively. During the recovery period, inhibin B levels were increased within 4-10 weeks of cessation of treatment.

Mean FSH levels exhibited a marked increase (≤5.5x) in all females receiving ≥ 3 mg/kg. The increase in FSH level, was evident within the first 1-2 weeks of the treatment period, and in most cases was sustained for the duration of treatment. Recovery was evident beginning 4-8 weeks following cessation of treatment.

Males: In contrast to the effects noted in sperm motility and morphology, no biologically meaningful effects were detected in FSH and LH levels. During pretreatment Week -2 and the treatment period, group mean testosterone levels were generally lower in groups that received VEGF Trap compared to controls. At Week 27, testosterone levels were decreased by 41, 71 ($p \leq 0.01$), and 62% ($p \leq 0.05$) at 3, 10, and 30 mg/kg, respectively. However, a clear relationship to treatment is not clear because of the following observations: there was wide within group variability, a dose response was not apparent, and for all groups, mean testosterone levels remained at or above pretreatment values throughout the treatment period. As noted previously, there were no histopathological findings in male sex organs, although the weight of the seminal vesicles was decreased.

Blood C-Reactive Protein (Once during the acclimation period, Weeks 4, 13 and 26 of the treatment period, and during Weeks 37 and 48 of the recovery period) – In males, mean levels were higher at 3 mg/kg (6x) and 10 mg/kg (10x) at Week 4 and at all doses (6x, 3x, and 3.5x at 3, 10, and 30 mg/kg, respectively) at Week 26. In females, mean levels were increased at 10 mg/kg (5x; $p \leq 0.05$) and 30 mg/kg (6x) on Week 13 and at all doses (2x, 5x, and 4x at 3, 10, and 30 mg/kg, respectively) on Week 26. The lack of statistical significance reflects the fact that only 1-2 out of 6 animals were affected at the low and mid dose and 3 out of 6 animals were affected at the high dose and showing a wide range of values. These changes were not present at recovery.

Biochemical Markers of Bone Resorption [Once prior to the start of treatment and during Weeks 4, 12, and 25 of the treatment period and during Weeks 37 and 48 of the recovery period; urine C-Telopeptide (CTx) N-Telopeptide (NTx), and Deoxypyridinoline (DPD); normalized by urine creatinine levels] – The data were difficult to interpret due to the wide range of values observed in each group. The range of values observed on each group during acclimation period and in controls, overlapped with the range of values observed in VEGF Trap treated groups. In addition, the changes for most part did not show a dose response or persisted throughout the dosing period. Therefore, in spite of the changes in mean values noted below, it is difficult to definitely attribute these changes to the test article.

CTx: Compared to controls (and acclimation), lower mean CTx levels were noted at Week 4 in both males (30-50%; non-dose dependent) and females (40-45%; dose dependent) at all doses (statistical significance only in high dose females). Females also had statistically significant lower levels at Week 12 (30-45%; non-dose dependent) with movement toward recovery at Week 25. Males showed movement toward recovery at Weeks 12 and 25.

NTx: Lower (statistically non-significant) NTx mean concentrations were observed in males treated with VEGF Trap at all dose levels on Week 4 (8-45%; non-dose dependent) and Week 12 (24-45%; non-dose dependent) and in females treated at all dose levels throughout the study when compared to controls (22-49%; non-dose dependent). At Weeks 48 of the recovery period, the levels of N-Telopeptide

were increased (2-10x) in 2 females relative to their values at Week 25 (end of the treatment period).

DPD: No test article-related effects were noted during the treatment period. At recovery, the levels of DPD were increased in 1 female each at all doses at Week 37 (14.99-18.41 *vs.* 4.94-12.39 nM/mM creatinine in pooled controls and acclimation periods).

Bone Densitometry Measurements [Once during the acclimation period, during Weeks 4, 13, and 26 of the treatment period, and during Weeks 37 and 48 of the recovery period by Dual energy X-ray absorptiometry (DXA) to measure bone mineral density (BMD), bone mineral content (BMC), and total body area] – The applicant noted that the positioning of some animals during the treatment period was not consistent with the initial scan due to the skeletal abnormalities induced by VEGF Trap. As a result, the results obtained for BMD were not considered accurate and BMC values were used to interpret the effects on bone densitometry data. Significant changes in BMC were not observed. The applicant attributed the slight decreases in BMC (whole body and femur) observed at all doses during treatment to body weight losses. Again, given the wide variability within groups with overlapping range of values between controls (and acclimation) and VEGF Trap treated groups, it was difficult to clearly identify a treatment related effect.

Digital Radiographs (Once during the acclimation period and once during Weeks 4, 13, and 26 of the treatment period and during Weeks 37 and 48 of the recovery period) – An increased incidence of kyphosis (generally at the thoraco-lumbar junction), degenerative joint disease (DJD) of articular facets (generally from T10 to L7), periosteal reaction of the femur (at the proximal diaphysis, distally to the lesser trochanter) and periosteal reaction at the lateral aspect of the ilium body (cranial acetabulum) were noted in animals treated with VEGF Trap independent of dose. DJD was more severe in males. The bone changes were still observed at recovery. These findings are summarized in the table below.

**Table 16: Bone Abnormalities Detected by Digital Radiographs in the 6-Month Intravenous Toxicity Study in Monkeys**

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Group | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Kyphosis | | | | | | | | |
| Total number affected | - | 5 | 4 | 5 | - | 1 | 5 | 4 |
| Minimal | - | 1 | - | 1 | - | 1 | 1 | 2 |
| Slight | - | 1 | 3 | - | - | 0 | 2 | 1 |
| Moderate | - | 0 | 1 | 1 | - | - | - | 1 |
| Marked | - | 3 | - | 1 | - | - | 1 | - |
| Severe | - | - | - | 2 | - | - | 1 | - |
| DJD of Articular Facets for Thoracic and Lumbar Spine | | | | | | | | |
| Total number affected | - | 6 | 4 | 5 | - | 1 | 5 | 4 |
| Minimal | - | - | - | - | - | - | - | 1 |
| Slight | - | - | - | 1 | - | - | 1 | - |
| Moderate | - | 4 | 2 | - | - | - | 2 | 1 |
| Marked | - | 2 | - | 2 | - | 1 | 2 | 1 |
| Severe | - | - | 2 | 2 | - | - | - | 1 |
| Periosteal Reaction Ilium | | | | | | | | |
| Total number affected | - | - | 2 | 1 | - | - | - | - |
| Minimal | - | - | - | - | - | - | - | - |
| Slight | - | - | 1 | - | - | - | - | - |
| Moderate | - | - | 1 | - | - | - | - | - |
| Marked | - | - | - | 1 | - | - | - | - |
| Severe | - | - | - | - | - | - | - | - |
| Periosteal Reaction Femur | | | | | | | | |
| Total number affected | - | 3 | 2 | 1 | - | - | 1 | 2 |
| Minimal | - | - | - | - | - | - | - | - |
| Slight | - | 2 | 2 | 1 | - | - | - | 1 |
| Moderate | - | 1 | - | - | - | - | - | 1 |
| Marked | - | - | - | - | - | - | - | - |
| Severe | - | - | - | - | - | - | 1 | - |

Group 1 = Control; Group 2 = 3 mg/kg; Group 3 = 10 mg/kg; Group 4 = 30 mg/kg

Anti-VEGF Trap Antibodies (Prior to the first dose and once a month thereafter (prior to dosing); prior to the terminal necropsy of the dosing period, and during Weeks 37 (mid-recovery) and 48 (prior to recovery necropsy)] – Fourteen animals distributed among all VEGF Trap dose groups exhibited anti-VEGF Trap antibodies during the dosing phase of the study (levels ranging from 908-245000 mIU/mL). Of these animals, one monkey each at 3 and 30 mg/kg (# 255 and # 410, respectively) had a marked reduction in circulating free, bound, and adjusted bound VEGF Trap levels, which correlated to the presence of anti-drug antibodies. It is possible that the accelerated clearance of VEGF Trap and Trap complex was due to anti-VEGF Trap antibodies.

During recovery, one male at 10 mg/kg (# 308) exhibited anti-VEGF Trap antibodies (2140 mIU/mL) at Week 37 but not at Week 48. Unfortunately, except for animal # 308, most animals with a high positive anti-drug antibody response during the dosing period, were not part of the recovery group (i.e., they were sacrificed at the end of dosing). Therefore, limited data was collected to determine the reversibility of the anti-drug antibody response.

Toxicokinetics [Predose, ~5 min, 24 hrs and 168 hrs after the end of the infusions on dosing Days 1, 29 (1st month), 57 (2nd month), 85 (3rd month), 113 (4th month), 141 (5th month) and 183 (7th month - prior to necropsy); Weeks 37 (mid-recovery) and 48 (prior to recovery necropsy); bound and free VEGF Trap) -

Free VEGF Trap - The mean peak and trough plasma concentrations of free and total VEGF Trap increased in a dose-dependent manner. The change in dosing schedule slightly decreased peak free drug levels but did substantially reduce trough levels of free VEGF Trap. The greater decrease in trough levels with increased time between doses was observed at the lower dose, which was considered due to the conversion of free drug to bound drug in plasma over time. At the lower concentrations of drug, proportionally more of the free VEGF Trap is converted to the bound form. During recovery, free VEGF Trap was not detected in all but one male at 10 mg/kg (# 308) during Week 37. No main gender differences were noted.

The average mean free VEGF Trap concentrations at peak (5 min postdose) on Days 29, 57 and 85, when drug was administered weekly, were about 105, 307, and 847 µg/mL in the 3, 10 and 30 mg/kg dose cohorts, respectively. During this same period, average mean trough levels (predose) were about 12, 52, and 130 µg/mL in the 3, 10 and 30 mg/kg cohorts, respectively.

The average mean free VEGF Trap concentration at peak (5 min postdose) on Days 141 and 183, when drug was administered bi-weekly, were about 76.9, 287 and 731 µg/mL in the 3, 10 and 30 mg/kg dose groups, respectively. During this same period, average mean trough levels (predose) were about 0.898, 8.29 and 28.8 µg/mL in the 3, 10 and 30 mg/kg cohorts, respectively.

$VEGF_{165}$:VEGF Trap Complex – The plasma levels increased throughout the first few IV infusions of VEGF Trap reaching steady state levels by approximately Day 29 for the three VEGF Trap-treated cohorts. Unlike free VEGF Trap, the $VEGF_{165}$:VEGF Trap complex was still detected at the end of the recovery period in several of the recovery animals. Generally, females showed a slightly higher (≤1.5x) level than males particularly at ≥10 mg/kg.

The average mean adjusted bound VEGF Trap concentration in the predose samples on Days 29, 57 and 85, when drug was administered weekly, were about 3.8, 5.8, and 4.8 µg/mL in the 3, 10 and 30 mg/kg dose cohorts, respectively. The mean average adjusted bound VEGF Trap concentrations in the predose samples on

Days 141 and 183, when drug was administered bi-weekly, were about 3.24, 4.80 and 4.43 µg/mL in the 3, 10 and 30 mg/kg dose cohorts, respectively.

The majority of the total amount of VEGF Trap (Free + Adjusted Bound) measured in the circulation up to 24 hrs post IV administration at doses of 3, 10 and 30 mg/kg was present in the form of free, active drug. At all of the time points analyzed for these three dosing cohorts, the mean free concentration of VEGF Trap exceeded the amount of adjusted bound form. However just prior to each administration of VEGF-Trap, free VEGF-Trap levels from only the 10 and 30 mg/kg cohorts remained in excess relative to bound VEGF-Trap levels suggesting that complete VEGF ligand binding was maintained in these cohort for the entire dosing period. These data also suggest that doses of VEGF Trap above 3 mg/kg are likely to be saturating for sequestration of endogenous VEGF165.

Dosing Solution Analysis: Dosing solutions were collected over the 6-month dosing period. Overall, the concentrations ranged from 101-113% of the target concentration.

**Four- and 13-Week Systemic Toxicity Studies in Monkeys**: Other repeated-dose GLP compliant toxicity studies of 4- or 13-week duration were conducted in monkeys via the subcutaneous and IV routes. Overall, the doses ranged from 1.5-30 mg/kg 1-3x/week. These studies identified similar findings to those observed in the 6-month studies. Noteworthy is the fact that the effects in RBC parameters, bone, kidney, ovary, and adrenals were observed as early as 4 weeks after treatment at VEGF Trap doses of 2-30 mg/kg 1x/week IV or 1.5-15 mg/kg 3x/week SC. With longer duration of dosing, hunched posture and kyphosis, vasculitis, and nasal bleeding were observed. A NOAEL was not identified in any of these studies. The main findings from each study are summarized below.

**A 4-Week Subcutaneous Toxicity Study of VEGF Trap in Cynomolgus Monkeys Followed by a 4-Week Recovery Period (Module 4.2.3.2, VGFT-TX-03004) -** Doses of 0, 1.5, 5, or 15 mg/kg SC were administered 3x/ week for 4 weeks. A 4-week recovery period was evaluated in control and high-dose groups. The main findings include increased hemoglobin, RBC, and hematocrit at all doses and still present at 15 mg/kg during recovery; effects on the growth plate of the femur (degeneration and disorganization) and effects on the ovary (decreased numbers of maturing follicles, granulosa cells, and/or thecal cells) at doses ≥5 mg/kg; effects on the kidney (increased glomerular mesangial matrix, associated with decreased serum total protein and albumin and increased serum BUN and urine protein levels) at all doses; and decreased vacuolation of adrenal zona fasciculata cells with cytoplasmic eosinophilia at all dose levels. The femoral changes were not observed at the end of the recovery period. Kidney, ovary, and adrenal changes were still observed at 15 mg/kg after the 4-week recovery period.

All animals had high plasma levels of free VEGF Trap (from 69-499 µg/ml on Day 26) throughout the treatment period. Free VEGF Trap was detected systemically in plasma during the recovery period at the only dose evaluated (15 mg/kg). Only one monkey (5 mg/kg) developed low levels of antibodies to VEGF Trap, which did not

adversely impact the exposure of this animal to free VEGF Trap. A NOAEL was not determined in this study.

**A 13-Week Subcutaneous Toxicity Study of VEGF Trap in Cynomolgus Monkeys Followed by a 6-Week Recovery Period (Module 4.2.3.2, VGFT-TX-02037) -** Doses of 0, 1.5, 5, 15, or 30 mg/kg IV were administered 2x/week for 13 weeks. A 6-week recovery period was evaluated in control, 15, and 30 mg/kg groups. The main findings include increase in blood pressure on Week 13 in 2 females each at 15 and 30 mg/kg; slight increases in mean hemoglobin, hematocrit and RBC at all doses which were generally not reversible; increased urine protein and/or serum BUN levels; histopathologic findings in the femoral growth plates, kidneys and ovaries at all dose levels at all doses similar to those observed in the 4-week SC study. The histopathologic findings were still present after the 6-week recovery period at 30 mg/kg, with the exception of the ovaries (decreased numbers of maturing follicles, granulosa cells, and/or thecal cells) which were also present at recovery necropsy at 15 mg/kg.

Plasma levels of free VEGF Trap increased in proportion with dose, with a mean concentration ≥ 510 µg/ml measured from the high-dose animals 24 hrs after dosing during the course of the dosing period. At the end of the 6-week recovery period, the plasma levels of free VEGF Trap were 2.62 µg/mL and 17.7 µg/mL at 15 and 30 mg/kg, respectively. The plasma levels of free VEGF Trap were not determined in the other dose groups. Only one monkey developed an antibody response against VEGF Trap, resulting in a marked drop in free VEGF Trap levels in that animal. A NOAEL was not determined.

**A 4-Week Intravenous Toxicity Study of VEGF Trap with a 6-Week Recovery Period in Cynomolgus Monkeys (Module 4.2.3.2, VGFT-TX-02029) -** Doses of 0, 2, 10, or 30 mg/kg were administered once weekly for 4 weeks. A 6-week recovery period was evaluated in control and high-dose groups. Similar targets to those observed after SC administration were identified including the bone growth plates and renal glomeruli (associated with increased urine protein and BUN levels and decreased serum albumin and/or serum total protein) at all doses, the ovaries at ≥10 mg/kg, and adrenal cortex at all doses.

Growth plate changes were characterized by a decrease in metaphyseal capillary invasion, a decrease in primary bony trabeculae, degeneration of the cartilage matrix, disorganization of the chondrocyte columns, increased thickening of the physeal cartilage, and transverse subchondral bony plate.

Kidney and bone growth plate changes were partially, but not completely reversible at the end of the 6-week recovery period at 30 mg/kg.

Mean plasma peak free VEGF Trap levels increased proportionally with dose, with peak levels reaching 7868 µg/ml at the high dose on Day 15. Concentrations of VEGF Trap were detected throughout the dosing interval at all dose levels. At the end of the recovery period, low concentrations (≤2 µg/mL) of VEGF Trap were detected at 30 mg/kg, the only dose group evaluated. A NOAEL was not determined.

**A 13-Week Repeat-Dose Intravenous Toxicity Study of VEGF Trap in Cynomolgus Monkeys and a 13-Week Recovery Period (Module 4.2.3.2, VGFT-TX-03048)** - Doses of 0, 3, 10, or 30 mg/kg IV were administered once weekly for 13 weeks. A 13-week recovery period was evaluated at doses of 0, 10, and 30 mg/kg. Clinical observations included hunched posture and kyphosis (associated with atrophy/degeneration of the muscle fibers of the paravertebral skeletal muscle sections from the thoracic or lumbar regions) at all dose levels, which increased at Weeks 11, 12 and 13 of dosing. Red nasal discharge was very occasionally observed in a few animals at all doses. Slight increases in mean RBC, hemoglobin, and hematocrit were noted at all doses in females on Weeks 4 and/or 13 (statistically significant at ≥10 mg/kg) and in RBC and hemoglobin males at 10 and/or 30 mg/kg at Week 13. One high-dose male (# 54) among all animals with nose bleeding showed decreases in all these parameters. Clinical pathology findings noted during the dosing period consisted of high urinary total protein in 3 monkeys at 10 mg/kg and 1 female at 30 mg/kg and high urinary microalbumin levels in 6 monkeys at 10 mg/kg and 3 monkeys at 30 mg/kg that was associated in some monkeys with a mildly decreased serum albumin and A/G ratio. Increases in blood pressure were not observed in this study.

Ovaries weight was decreased at all doses; uterine weight was decreased at 30 mg/kg. These changes were not observed at the end of the 13-week recovery period.

Histopathologic changes were similar to those described under the 4-week, 13-week, and 6-month IV and/or SC toxicology studies previously reviewed. These findings included effects in the kidneys (increased glomerular mesangial matrix, tubular nephropathy), bones (disorganization of the chondrocyte columns and increased thickening of the growth plate cartilage and transverse subchondral bony plate), adrenals (decreased vacuolation with eosinophilia in the zona fasciculata), gallbladder (inflammation), and ovaries (decrease in the number of maturing follicles, granulosa and/or theca cells, absence of corpora lutea) at all doses. Focal vasculitis was observed in the submucosa of the duodenum (2 males each at 10 and 30 mg/kg), urinary bladder (1 high-dose male), and/or heart (1 male at 10 mg/kg) of individual monkeys.

In recovery animals, kyphosis was observed at ≥10 mg/kg (only doses evaluated) accompanied with atrophy/degeneration/necrosis of muscle fibers. Microscopic findings were noted in the kidneys (increased glomerular mesangial cells in males), ovaries (absence of corpora lutea and decreased maturing follicles) and femur/sternum bones (disorganization of the chondrocyte columns and increased thickening of transverse subchondral bony plate) at ≥10 mg/kg but incidences/severity was lower, suggesting reversibility. Moderate inflammation of the gallbladder was present in 1 high-dose male.

Immunohistochemical staining for IgG, IgM, and C3 was observed in the glomeruli at all VEGF Trap doses, compatible with immune-complex deposition. However, since no antibodies were detected in most of these animals (see below), IgG, IgM, and C3 staining may have been related to increase filtration of these molecules subsequent to damage to the filtration barrier resulting from VEGF antagonism, along with increase

recruitment of IgG, IgM, and C3 to damaged glomerular components. In fact, transmission electron microscopy revealed ultrastructural changes mainly characterized by reduction or loss of filtration slit diaphragms between the terminal foot processes of podocytes (epithelial cells) and hypertrophy, swelling of endothelial cells with irregularity of their cytoplasmic fenestrations in the glomerulus at ≥10 mg/kg (the low dose was not examined). When the severity of changes in podocytes was higher (fused foot processes), variably sized lysosomes and lipid vacuoles were present in endothelial cells, podocytes from glomeruli and cells from the S1 segment of proximal tubules.

At each dose level, both free VEGF Trap and VEGF Trap complex concentrations were detectable over all dose intervals. Mean plasma maximal concentrations ($C_{max}$) and trough concentrations of free VEGF Trap increased proportionally with dose (1300 and 200 µg/mL, respectively). Free drug levels were undetectable at the end of the recovery period but were likely present for a substantial portion of that phase, based on an estimated free VEGF Trap elimination $t_{1/2}$ of 4 to 5 days in the monkey. Bound VEGF Trap was detected throughout the duration of the recovery period. Plasma exposure of free VEGF Trap increased by a factor of approximately 2 between Week 1 and Week 13, at all doses, also consistent with the elimination $t_{1/2}$.

One monkey each at 10 and 30 mg/kg exhibited detectable anti-VEGF Trap antibodies. Both animals had notable reductions in plasma drug concentrations, indicating that the immune response was contributing to increased clearance of the drug. Three additional animals (one predose) showed antibody titers less than 2-fold above the limit of quantitation only at one time point, which were regarded as nonspecific serum interference. One mid-dose animal had reactive antibodies just prior to the last infusion yet none at the end of the recovery period. A NOAEL was not determined.

# 7 Genetic Toxicology

In accordance with ICH Guidance for Industry S6, genotoxicity studies were not conducted because these are not applicable to biotechnology-derived pharmaceuticals.

# 8 Carcinogenicity

No studies were conducted. The Division agreed that these studies were not required in this case (See Memo to File IND 12462 and (b) (4) dated June 17, 2009).

# 9 Reproductive and Developmental Toxicology

## 9.1 Fertility and Early Embryonic Development

The potential effects of VGF Trap in male and female fertility were evaluated as part of the 6-month IV toxicity study in monkeys (Study # VGFT-TX-05009).

Absent or irregular menses associated with reductions in ovarian hormones (progesterone, inhibin B, and likely, estradiol) and increases in FSH levels were observed at ≥ 3 mg/kg during the dosing phase. Ovary weight changes at doses ≥ 3 mg/kg were accompanied by compromised luteal development and reduction of maturing follicles. Following recovery, all VEGF Trap-treated females presented normal ovarian folliculogenesis and presence of medium to large size corpora lutea. In addition, uterine and vaginal atrophy were not seen, indicating complete reversibility. The high-dose females still showed decreased weight of the ovaries (23% absolute weight and 9% relative to body weight) compared to controls. However, the reduced magnitude of the change suggests recovery was ongoing.

There were no clear test article-related effects on male reproductive hormone levels (FSH, LH, and testosterone). Decreased sperm motility and increased sperm abnormalities were evident at all doses in the treatment phase but were fully reversible after the treatment-free phase. Decreases were also observed in the weight of the seminal vesicles but without a histopathological correlate.

Therefore, a NOAEL for fertility was not determined. Based on $C_{max}$ and $AUC_{0\text{-}168hrs}$ for free VEGF Trap observed at the 3 mg/kg IV dose, the lowest dose at which the findings were observed, the exposure was 4902-fold and 1546-fold higher, respectively, than the exposure observed in humans ($C_{max}$ and $AUC_{0\text{-}last}$ of 0.0193 µg/mL and 0.119 µg•day/mL, respectively) after an ITV dose of 2 mg/eye every 4 weeks.

## 9.2 Embryonic Fetal Development

**Study title: AVE005: Intravenous (30-Minute Infusion) Embryo-Fetal Toxicity Study in Rabbits**

| | |
|---|---|
| Study no.: | VGFT-TX-06002 |
| Study report location: | Module 4.2.3.5.2 |
| Conducting laboratory and location: | (b) (4) [redacted] |
| Date of study initiation: | Sept 4, 2006 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | AVE0005 (VEGF Trap); lot # C04008D630B12A |

Key Study Findings

- At all doses, there were slight effects in the dams body weight gain and food consumption primarily during dosing with a movement towards recovery once dosing was stopped.
- When corrected for uterine weight, terminal mean body weight was similar to controls.
- Decreased uterine weight was observed at 60 mg/kg.

- Abortions and increased mean number of postimplantation loss and early resorptions were noted in females at 60 mg/kg. Consequently, the mean number of viable fetuses was slightly lower at 60 mg/kg, corresponding to the decrease in uterine weight. Mean fetal weight was slightly decreased at the high dose.
- A series of external, visceral, and skeletal malformations were observed primarily at 60 mg/kg. A few findings were noted at 3 and/or 15 mg/kg.
- The maternal NOAEL was considered to be 3 mg/kg and the developmental NOAEL was considered to be <3 mg/kg.
- Based on $C_{max}$ and $AUC_{0\text{-}168hrs}$ for free VEGF Trap observed at the 3 mg/kg IV dose, the exposure was 2900-fold and 600-fold higher, respectively, than the exposure observed in humans after an ITV dose of 2 mg/eye every 4 weeks ($C_{max}$ and $AUC_{0\text{-}last}$ of 0.0193 µg/mL and 2.856 µg•hr/mL, respectively)

Methods

| | |
|---|---|
| Doses: | 0, 3, 15 or 60 mg/kg |
| Frequency of dosing: | Gestation Days (GD) 6, 9, 12, 15, and 18 |
| Dose volume: | 4 mL/kg |
| Route of administration: | 30-min IV infusion |
| Formulation/Vehicle: | (b) (4) sodium phosphate, (b) (4) sodium chloride. (b) (4)% (w/v) sucrose and ( (b) (4) (w/v) PS20, pH (b) (4) |
| Species/Strain: | New Zealand White rabbits |
| Number/Sex/Group: | 22-26 mated females (20 pregnant rabbits in the control and 3 and 15 mg/kg groups, 22 pregnant rabbits in the high-dose group) |
| Satellite groups: | 3 mated females in controls (2 pregnant); 6 mated females (5-6 pregnant) in VEGF Trap dose groups |
| Study design: | Standard ICH recommendations; terminal necropsy on GD 29; doses selected based on dose-ranging study # VGFT-TX-06001 (See below) |
| Deviation from study protocol: | None that could adversely affect interpretation of the study results |

Observations and Results

Mortality (Daily) - Three out of 22 pregnant females at 60 mg/kg were euthanized between GD 21 and 26 following compound-related abortion. Clinical signs noted prior to euthanasia consisted of fetal/placental remnant under the cage and/or red material under the cage, and body weight loss associated with reduced food consumption.

Clinical Signs (Daily; main animals only) – No test article-related clinical signs were noted in the females surviving to scheduled euthanasia.

Body Weight (GD 1, 6, 9, 12, 15, 18, 21, 25 and 29 for the main animals and on GD 1, 6, 9,12, 15, 18 and 21 for toxicokinetic animals) – There were no significant effects in mean

body weights compared to controls. On GD 18 (last day of dosing), mean body weights were ~6% lower than controls at all doses. On GD 29, mean body weights were 4.6%, 2.8%, and 2.3% lower at 3, 15, and 60 mg/kg, respectively.

Lower mean body weight gain (non-statistically significant) was observed at all intervals during the dosing period with a trend towards recovery once dosing was stopped. At the interval GD 6-18 (dosing period), this decreases were 30%, 60%, and 64% at 3, 15, and 60 mg/kg, respectively. At the interval GD 6-29, these decreases were 3.6%, 4%, and 10% at 3, 15, and 60 mg/kg, respectively.

When corrected for uterus weight, there was no difference in mean body weight compared to control.

Feed Consumption (Daily; main animals only) – Starting on the GD 7-8 interval, a minimal decrease in mean food consumption was noted at all doses throughout the study. For example, a 9%, 18%, and 12% decrease was observed at the GD 17-18 interval at 3, 15, and 60 mg/kg, respectively. After dosing was stopped on GD 18, food consumption moved toward control values with comparable or higher values after the GD 28-29 interval.

Toxicokinetics (At 0.5, 6, 24, 48 and 72 hrs after the last dosing on Day 18) - Mean plasma concentrations of free and total VEGF Trap on GD18 (after the 5$^{th}$ dose) increased with dose in proportion or less than dose proportional to the dose between 15 and 30 mg/kg but greater than dose proportional between 3 and 15 mg/kg (Table 17). Estimates of $AUC_{0.5\text{-}72hrs}$ were greater than dose proportional between 3 and 15 mg/kg and lower than dose proportional between15 and 30 mg/kg. The amount of VEGF:VEGF Trap complex was similar at all doses. As noted by the applicant, this suggests that sufficient levels of drug were present even at 3 mg/kg to bind essentially all the circulating VEGF present in these animals. The concentration of free VEGF Trap exceeded the concentration of bound VEGF Trap at all time points.

**Table 17: Mean Plasma Concentrations of Free and Bound VEGF Trap in Pregnant Rabbits in the IV Embryofetal Toxicity Study**

| | Time post dose | Free AVE0005 | | | Bound AVE0005 | | | Total AVE0005 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Dose | (mg/kg/administration) | 3 | 15 | 60 | 3 | 15 | 60 | 3 | 15 | 60 |
| Gestation Day 18 | 0.5h | 56.1 | 707 | 2460 | 3.07 | 4.79 | 5.05 | 59.2 | 711 | 2460 |
| | 6h | 53.8 | 624 | 1640 | 6.85 | 6.83 | 5.53 | 60.6 | 630 | 1650 |
| Gestation Day 19 | 24h | 19.5 | 332 | 839 | 4.14 | 5.10 | 4.57 | 23.6 | 337 | 843 |
| Gestation Day 20 | 48h | 23.8 | 275 | 807 | 8.13 | 6.78 | 5.15 | 31.9 | 282 | 812 |
| Gestation Day 21 | 72h | 14.0 | 121 | 333 | 2.84 | 4.10 | 5.05 | 16.8 | 125 | 338 |
| $AUC_{0.5-72}$ | - | 1935 | 24300 | 67018 | NC | NC | NC | NC | NC | NC |

$AUC_{0.5-72}$: Area under the curve from 0.5 h to 72 h; AUC are expressed in µg*h/mL
NC: Not calculated
Plasma exposures are expressed in µg/mL

Anti-Product Antibodies (Before dosing on GD 6 and before euthanasia on GD 29; main animals only) – Detectable anti-product antibodies were noted on GD 29 in 2 out of 22 animals at 3 mg/kg (1750 and 2070 ng/mL), 5 out of 22 rabbits at 15 mg/kg (2370-28800 ng/mL), and 14 out of 26 rabbits at 60 mg/kg (776-25400 ng/mL). Given that the toxicokinetic and antibody determinations were conducted on different animal subgroups, it is not known whether the presence of anti-product antibodies affected the clearance of VEGF Trap from the circulation of pregnant rabbits.

Dosing Solution Analysis – All solutions were 92.5-98.7% of theoretical content.

Necropsy (GD 29; main animals only) – The mean uterus weight was lower at 60 mg/kg (25%; $p \leq 0.05$), compared to control. This effect was considered secondary to the lower mean number of fetuses/litter. No test article-related macroscopic findings were observed.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.) - Increased mean number of postimplantation loss (3.7x; $p \leq 0.05$) and early resorptions (4x) was noted in females at 60 mg/kg, compared to controls. Consequently, the mean number of viable fetuses was slightly lower (26%; $p \leq 0.05$) at 60 mg/kg. A slight, non-statistically significant decrease (5%) in mean fetal weight was observed at the high dose.

Offspring (Malformations, Variations, etc.) – A series of external, visceral, and skeletal malformations were observed at all doses.

External malformations: Findings included anasarca in 2 fetuses (2 litters) at 3 mg/kg, ectrodactyly and umbilical hernia in 1 fetus at 15 mg/kg, and anasarca, gastroschisis, umbilical hernia, anal atresia and/or short tail in 1-10 fetuses (1-7 litters) at 60 mg/kg. Most of these findings were noted in fetuses with multiple external malformations

associated with visceral and/or skeletal anomalies. One polymalformed fetus at 3 mg/kg presented anasarca, paw hyperflexion, umbilical and diaphragmatic hernia associated with skeletal (fused sternebrae) and visceral (dilation of pulmonary trunk and aortic arch and malpositioned kidney) anomalies. At 60 mg/kg, 6 fetuses presented multiple external malformations including anasarca, cleft palate, hyperflexion of hindlimb or paw, umbilical hernia, gastroschisis, anal atresia and/or tail malformation in association with visceral and/or skeletal anomalies. None of these findings were observed in controls.

Visceral malformations: At 3 mg/kg, visceral malformations were limited to dilation of the great vessels of the pulmonary trunk and aortic arch in one fetus with multiple malformations (See External malformations above).

At 60 mg/kg, malformations were mainly noted in the heart, great vessels, and arteries. Malformations in the heart consisted of ventricular septum defect (10 fetuses/7 litters), small (10 fetuses/7 litters) or enlarged (9 fetuses/7 litters) ventricular chamber, and absence of the atrioventricular valve (2 fetuses/2 litters). Malformations in the great vessels and arteries included: narrowed pulmonary trunk (4 fetuses/4 litters), dilated aorta (5 fetuses/2 litters), reduced pulmonary artery branch (1 fetus), absent (2 fetuses/2 litters) or narrowed (9 fetuses/5 litters) ductus arteriosus, retroesophageal (2 fetuses/2 litters) or dilated aortic arch (16 fetuses/9 litters), dilated aorta (5 fetuses/2 litters). None of these findings were observed in controls. Compared to controls, an increase incidence was noted for retroesophageal subclavian artery (10 fetuses/7 litters vs 1 fetus in control) at 60 mg/kg and malpositioned branch subclavian artery at ≥15 mg/kg (5 fetuses/3 litters at 15 mg/kg, 7 fetuses/4 liters at 30 mg/kg vs 2 fetuses/2 litters in controls)

Intestinal atresia (2 fetuses/2 litters), convolution and dilation of the ureter (4 fetuses/3 litters), dilated renal pelvis (4 fetuses/3 litters), and distension of the urinary bladder (2 fetuses/2 litters) were noted at 60 mg/kg. None of these findings were observed in controls.

Skeletal malformations: At 60 mg/kg, there was an increased incidence of skeletal malformations or variations consisting of fused caudal vertebrae (2 fetuses/2 litters vs none in control), fused (4 fetuses/2 litters vs 1 fetus in control) or supernumerary ribs (4 fetuses/2 litters vs none in control), fused sternebrae (17 fetuses/9 litters vs 4 fetuses/4 litters in control), supernumerary arch (2 fetuses/1 litter vs none in control) and/or centrum (5 fetuses/4 litters vs 1 fetus in control) of lumbar vertebrae and absence of arch and/or centrum of sacral vertebrae (3 fetuses/2 litters vs none in control). A compound-related increase in the incidence of absent or small interparietal skull bone (30 fetuses/13 litters vs 15 fetuses/9 litters in control) was also noted at 60 mg/kg.

In addition, incomplete ossification was noted in the hyoid (5 fetuses/3 litters), thoracic (9 fetuses/9 litters), lumbar (4 fetuses/3 litters) and sacral vertebrae (2 fetuses/1 litter), and sternebrae (9 fetuses/5 litters) at 60 mg/kg as compared to none in controls. Compared to controls, a higher incidence was noted at 60 mg/kg for incomplete ossification in the hindpaw phalanx (6 fetuses/4 litters vs 1 fetus in control) and forepaw phalanx (42 fetuses/14 litters vs 17 fetuses/9 litters in control).

Compared to controls, a higher incidence of incomplete ossification was also noted in the ribs (2 fetuses/2 litters vs 1 fetus in control) and talus (2 fetuses/2 litters vs none in control) at 3 mg/kg. Incomplete ossification of the hyoid and thoracic vertebra were observed in 1 fetus and malformation in the thoracic vertebra in 2 fetuses/2 litters (vs none in controls) at 15 mg/kg.

**AVE0005: Intravenous (30-min Infusion) Range-Finding Toxicity Study in Pregnant Rabbits (Study # VGFT-TX-06001; Module 4.2.3.5.2.1)** – VEGF Trap doses of 0 (controls), 3, 15 and 45 mg/kg were given as a 30-min IV infusion to pregnant rabbits (5-6/group) on GD 6, 9, 12, 15 and 18. Main results include abortion in 1 female at 45 mg/kg on GD 21, premature delivery in 1 female at 15 mg/kg on GD 29, decreased mean body weight gain (e.g., 61-210% during GD6-18; non-dose dependent; non statistically significant) associated with decreased food consumption mainly from GD 12-22 (10-30%; non-dose dependent; non-statistically significant), and increased incidence of reduced feces at all dose levels. After dosing was stopped, animals showed a tendency towards recovery of the effects on body weight gain and food consumption. At 45 mg/kg, a slightly higher mean number of postimplantation loss (2.7x) associated with a slightly lower mean number of viable fetuses (15%) was noted as compared to controls. A decrease in mean fetal weight was noted at all dose levels (13, 14, and 9% at 3, 15, and 45 mg/kg, respectively; non-statistically significant). No external abnormalities were noted at any dose level. The maternal NOAEL was considered to be 3 mg/kg (Day 21 Free VEGF Trap $C_{max}$ = 14 µg/mL).

Additional TK information was obtained from this study compared to final study # VGFT-TX-06002. One animal at 15 mg/kg and 3 animals at 45 mg/kg on GD 29 had antibody levels in excess of 14 µg/mL. The presence of anti-VEGF Trap antibodies was associated with lower levels of free and bound (and total) VEGF Trap concentrations on GD 21 and levels below the lower limit of quantitation on Day 29, indicating the antibodies accelerated the clearance of VEGF Trap. Free VEGF Trap was measured in amniotic fluid samples collected on gestation Day 29 (11 days after the last infusion). Mean concentrations of free VEGF Trap in amniotic fluid increased with dose (6.73 ± 6.48, 33.8 ± 31.5, and 731 ± 1435 ng/mL at 3, 15, and 45 mg/kg, respectively). There was substantial variation among the levels of VEGF Trap within each dose cohort.

## 9.3 Prenatal and Postnatal Development

No pre-and post-natal toxicity studies were conducted because they were not considered by the applicant necessary for this indication (treatment of AMD), which affects an older population (≥50 yrs old). At a sponsor meeting held on June 1, 2009 (See Memo to File: IND 12467 (b) (4) dated June 17, 2009), the Division agreed that these studies were not required for the proposed indication. It was stated on this meeting that based on documented adverse reproductive and developmental effects of VEGF Trap and other pharmacological blockers of VEGF signaling, any eventual label will include qualifying statements regarding compound effects on fertility and pregnancy and/or categorization restrictions.

## 10 Special Toxicology Studies

**Cross-Reactivity of VEGF Trap with Human Tissue Ex Vivo (Study # SPS 01-141; Module 4.2.3.7.7; GLP)** – Biotin-VEGF Trap binding was evaluated in a panel of 33 human tissues obtained from 3 separate individuals. No specific staining was observed in any tissues at Biotin-VEGF Trap concentrations of 5 or 25 µg/mL. Five µg/mL was determined to be the lowest concentration of test article that produced the maximum (plateau) binding of the target antigen.

**Evaluation of VEGF Trap to Induce Hemolysis in Monkey Blood and to Induce Flocculation in Monkey Plasma and Serum (Study # Hem#1; non-GLP; Module 4.2.3.7.7)** - VEGF Trap formulated in 5 mM sodium phosphate, 5 mM sodium citrate, 100 mM sodium chloride, 0.1% (w/v) PS20, and 20% (w/v) sucrose at a concentration of 25 mg/mL and pH 6.0 was evaluated at concentrations of 0.69, 2.09, and 4.17 mg/mL. VEGF Trap did not induce hemolysis when incubated with monkey blood and did not cause the formation of flocculants or precipitates when incubated with either monkey serum or plasma.

**Evaluation of VEGF Trap to Induce Hemolysis in Whole Blood from Humans and to Induce Flocculation in Human Plasma and Serum (Study # Hem#3; non-GLP; Module 4.2.3.7.7)** – VEGF Trap formulated in (b) (4) l sodium phosphate, (b) (4) sodium chloride, (b) (4) ) PS20 and (b) (4) (w/v) sucrose sucrose at a concentration of (b) (4) and pH (b) (4) was evaluated at concentrations of 0.5, 2, and 4 mg/mL. VEGF Trap did not induce hemolysis when incubated with human blood and did not cause the formation of flocculants or precipitates when incubated with either human serum or plasma.

**Evaluation of VEGF Trap to Induce Hemolysis in Whole Blood from Humans and to Induce Flocculation in Human Plasma and Serum (Study # Hem#4; non-GLP; Module 4.2.3.7.7)** – VEGF Trap formulated in 0.45% or 0.9% NaCl was evaluated at a concentration of 4 mg/mL. VEGF Trap did not induce hemolysis when incubated with human blood and did not cause the formation of flocculants or precipitates when incubated with either human serum or plasma.

**Evaluation of VEGF Trap to Induce Hemolysis in Whole Blood from Humans and to Induce Flocculation in Human Plasma and Serum (Study # Hem#5; non-GLP; Module 4.2.3.7.7)** - VEGF Trap formulated in 0.9% NaCl was evaluated at a concentration of 8 mg/mL. VEGF Trap did not induce hemolysis when incubated with human blood and did not cause the formation of flocculants or precipitates when incubated with either human serum or plasma.

## 11 Integrated Summary and Safety Evaluation

VEGF Trap is intended for the treatment of wet age-related macular degeneration. The proposed dosing regimen is 2 mg monthly for the first 3 months and every 2 months

afterwards administered by ITV injection. The expected patient population for this indication is male and females ≥50 yrs of age.

The monkey was selected as the relevant species because of identical VEGF-A protein sequence on the amino acid level to its human counterpart. The binding affinity of VEGF Trap to VEGF-A from cynomolgus monkey was not tested but the applicant assumed the binding interaction of human and monkey VEGF-A with VEGF Trap should likely be indistinguishable due to the identical amino acid sequence. The binding of VEGF Trap to monkey VEGF-A was supported by the following observations: (1) the systemic toxicity studies (see below) showed targets of toxicity known for VEGF inhibitors, and (2) efficacy was observed in a monkey model of choroidal neovascularization (CNV).

Binding affinity was demonstrated for mouse, rat, and rabbit VEGF-A. However, mice and rats developed a strong anti-VEGF Trap antibody response after systemic administration of VEGF Trap at doses of 0.1-15 mg/kg (rats) and 10 and 25 mg/kg (rats and mice), 3x/week for 1 month (3 months in one rat study). The anti-drug antibody response was associated with enhanced clearance of VEGF Trap, which precluded the use of these species for long-term toxicity studies. In addition to nephropathies, findings identified in these short term studies in both mice and rats included mortalities, edema, multiorgan vascular dilatation and congestion, and vasculitis. Similar to findings in monkeys (see below), hemorrhage in the nasoturbinates was noted in mice; bone adverse effects were noted in rats. An antibody response associated with enhanced clearance was also detected in pregnant rabbits, which suggested these species may also have limited use for chronic studies.

ITV ocular toxicity studies were conducted in monkeys with duration of 4 and 8 months. The doses ranged from 0.05-0.5 mg/eye or 1 or 2 mg/eye in the 4-month studies and 0.5-4 mg/eye in the 8-month study with a dosing frequency of once every 4 weeks in all studies. An additional 4-month study used a dose of 0.5 µg/mL administered every 2 weeks.

Ocular findings in the ocular toxicity studies were consistent in all studies and included mild and transient increases in anterior segment and vitreous cellularity (interpreted as a mild inflammation) that was not associated with other ocular abnormalities. The anterior segment cell scores declined during the interval between doses and generally, the finding was not present one week postdose. In contrast, vitreal cells were observed throughout the dosing phase and gradually disappeared during the recovery period. These findings were observed at all doses. The lowest dose of 0.5 mg/eye once every 4 weeks evaluated in the longer-term ocular toxicity study (8 months) is 0.5 times the intended clinical dose when correcting for vitreous volume (i.e., assuming a vitreous volume of 2 mL in monkeys and 4 mL in humans). However, the mild and transient nature of the finding does not represent a major clinical concern.

Other VEGF inhibitors (e.g., Lucentis™, Macugen®) have been shown to increase intraocular pressure (IOP) in humans when administered by ITV injection. In all monkey

ocular toxicity studies of VEGF Trap, there was an increase in IOP immediately postdose across all groups including the controls. This increase was considered related to the sudden increase in intraocular volume after a bolus injection into the vitreous. IOP returned to normal range by the next time point measured. In the clinical trials conducted for VEGF Trap, an increase in IOP was observed and warnings are included in the proposed label.

In the monkey 8-month ITV ocular toxicity study, epithelial erosion/ulceration of the nasal turbinates accompanied with chronic-active inflammation was noted at doses of 2 and 4 mg/eye once every 4 weeks. After a 4-month recovery period, the chronic-active inflammation was still present but the other findings were not observed, suggesting partial recovery. Similar, albeit more severe lesions in the nasal cavity were noted in systemic toxicity studies in monkeys following repeated, IV administration of VEGF Trap. Hemorrhage in the nasoturbinates was also noted in mice after SC administration, suggesting the potential for the adverse nasal effects to occur across species. The reviewer is not aware of the observation of similar nasal findings with any other approved VEGF inhibitor following ITV injection. The applicant monitored for nasal adverse effects in a subset of patients in the clinical trials with VEGF Trap and found no evidence for an increase incidence with VEGF Trap (see medical officer review for further details). At the NOAEL of 0.5 mg/eye, the systemic exposure was 42 and 56 times higher based on $C_{max}$ and AUC, respectively, than the exposure observed in humans after an ITV dose of 2 mg every 4 weeks (Table 17). The proposed label includes these nonclinical findings under Section 13.2 Animal Toxicology and/or Pharmacology. The reviewer recommended some changes to the proposed label regarding the safety margins (See Section 1.3.3).

Dose-dependent levels of free VEGF Trap were detected in vitreous humor samples collected 7 days after the last monthly ITV dose in monkeys. The extent that free VEGF Trap was detected in the vitreous was dependent on the dose and length of the recovery period. No VEGF Trap was detected at the end of 10-week recovery period at monthly doses of 0.05-0.250 mg/eye or at monthly doses up to 4 mg/eye following a 4-month recovery period. However, at the end of the 10-week recovery period, measurable levels of VEGF Trap were detected in most vitreous samples obtained from recovery animals after monthly doses of 1 and 4 mg/eye.

Compared with the free VEGF Trap concentration in the vitreous after ITV injection in monkeys, the systemic concentration was low (e.g., <3% of the ITV concentration during administration of 4 mg/eye once every four weeks for 8 months). However, there was substantial systemic exposure of VEGF Trap (free and/or bound) in monkeys after ITV injection as concentrations were in the µg/mL range at doses ≥0.5 mg/eye. However, besides the findings in the nasal turbinates, no other local or systemic toxicity was evident after administration of VEGF Trap by ITV injection at doses up to 4 mg/eye once every 4 weeks for up to 8 months.

Depending on the dose, free VEGF Trap generally cleared from the circulation before the next monthly ITV injection. The systemic $T_{max}$ was observed 24 hrs after ITV injection. Unlike free VEGF Trap, the bound complex has a much slower clearance and

elimination rate, as it was still detected well into the recovery period. As noted by the applicant, the results indicate that the majority of the total drug concentration in the circulation (Free VEGF Trap + Adjusted Bound VEGF Trap) after each ITV administration of VEGF Trap was the free form (active species) directly following administration, and the bound form (inactive species) at later time points. Therefore, although most of the monkeys may not have been systemically exposed to free VEGF Trap for the entire time period between doses, the monkeys were exposed to VEGF:VEGF Trap complex throughout the study.

Anti-VEGF Trap antibodies were detected in the serum of monkeys after ITV administration of VEGT Trap, generally at monthly doses ≥0.5 mg/eye. However, there was no obvious correlation between the appearance of these putative antibodies and any change in either free and/or bound VEGF Trap levels except for one animal administered 2 mg/eye once every 4 weeks in a formulation different to the commercial formulation. This animal showed enhanced VEGF Trap clearance.

The potential systemic toxicity was addressed in monkeys after the SC and IV routes. In these studies, free VEGF Trap produced systemic toxicities mostly related to the its pharmacology. The main target organs identified in these studies include bone (interference with growth plate maturation of long bones, osteocartilaginous exostoses of vertebrae which correlated marcroscopically with kyphosis, degenerative joint disease, degeneration of the cartilage matrix, etc.), kidney (frequently increased glomerular mesangial matrix, occasionally hyperplasia of parietal epithelium, and periglomerular fibrosis), adrenals (decreased vacuolation with eosinophilia in the zona fasciculata), ovary (decreased number of maturing follicles, granulosa cells, and/or theca cells), and nasal cavity (atrophy/loss of the septum, erosion/ulceration of the respiratory and olfactory epithelium of nasal turbinates). Other microscopic findings observed in the longer-term chronic study (6 months) included vascular alterations in the choroid plexus and digestive tract (duodenum, stomach, gallbladder, pancreas), vascular degeneration and fibrosis in several tissues including the heart, and hepatic portal inflammation and periportal necrosis. Alterations on red blood cell parameters and changes in blood markers indicating damage to the kidney and liver/biliary tract were noted. At the end of a 5-month recovery period in the 6-month systemic toxicity study, findings observed included kyphosis with osteocartilaginous exostoses, nasal cavities deformation, degenerative joint disease, and changes in the digestive system, liver, and brain (choroid plexus).

A NOAEL was not established following systemic (IV and SC) administration to monkeys. However, exposure multiples, calculated by comparing free VEGF Trap concentrations ($C_{max}$) and systemic exposure (AUC) at the lowest doses associated with findings in animals to exposures observed in humans administered a VEGF Trap dose of 2 mg ITV in clinical studies, provide adequate support for the proposed dose regimen of VEGF Trap in humans (Table 17).

Effects on male and female fertility were assessed as part of the 6-month systemic toxicity study in monkeys at doses of 3.0-30 mg/kg. Absent or irregular menses associated with alterations in female reproductive hormone levels, ovarian and uterine

changes, reduction in sperm motility, and sperm morphological abnormalities were observed at all dose levels. Ovarian and uterine changes included decreased ovary and uterine weights accompanied with reduction of maturing follicles, granulosa cells, and absence of corpora lutea. These changes correlated with uterine and vaginal atrophy. All changes were reversible after a 5-month recovery period although ovarian weights were still slightly decreased at the high dose. As noted by the applicant, the potential of VEGF inhibition to impair fertility is a known class effect and related to the relevance of VEGF for development and function of male and female reproductive organs. Based on $C_{max}$ and AUC for free VEGF Trap observed at the 3 mg/kg IV dose, the systemic exposures were approximately 4900-fold and 1500-fold higher, respectively, than the exposure observed in humans after an ITV dose of 2 mg (Table 17). The reviewer recommended some changes to the proposed label to more adequately represent these findings (See Section 1.3.3).

In an embryo-fetal toxicity study in the rabbit, IV doses of VEGF Trap ≥ 3 mg/kg produced dose-related increases in fetal resorptions, abortions, and numerous fetal (external, visceral and skeletal) malformations. A compound-related increase in mean number of postimplantation loss (early resorptions) was noted at 60 mg/kg as compared to controls. Consequently, the mean number of viable fetuses was slightly lower at 60 mg/kg as compared to controls. The mean fetal weight was also slightly decreased at 60 mg/kg. Free VEGF Trap was detected in amniotic fluid samples in the dose range-finding study. The maternal NOAEL was considered to be 3 mg/kg, whereas the developmental NOAEL was not identified. At the dose of 3 mg/kg, the systemic exposures based on $C_{max}$ and AUC for free VEGF Trap were approximately 2900 and 600-fold, respectively, the corresponding values observed in humans after an ITV dose of 2 mg (Table 17). The safety margins suggest there is low probability that embryo-fetal toxicity will be observed in humans receiving VEGF Trap at the intended dosing regimen. The reviewer recommended some changes to the proposed label to more adequately represent these findings (See Section 1.3.3).

In humans, VEGF/VEGFR signaling plays a role in modulating blood pressure homeostasis and VEGF inhibitors, as a class, are known to increase blood pressure after systemic administration of pharmacologically active doses. In the SC and IV toxicity studies conducted in monkeys, VEGF Trap generally had no significant effect on arterial blood pressure or EKG parameters. The exception was one study in which an increase in blood pressure was observed in 2 monkeys each administered VEGF Trap at 15 and 30 mg/kg SC 2x/week for 13 weeks. In contrast, single SC injections to Wistar-Kyoto (WKY) rats (>0.15 mg/kg) or C57BL/6 mice (≥2.5 mg/kg) produced relatively rapid increases in blood pressure. In both rats and mice, heart rate tended to transiently decrease below baseline levels at all VEGF Trap doses tested. This drop in heart rate appears to be a reflex reaction to the elevation in blood pressure. The duration of blood pressure elevation was correlated with the presence of free VEGF Trap in the circulation, such that systolic and diastolic blood pressure remained elevated above pre-treatment baseline values until circulating VEGF Trap levels fell below ~ 1 µg/mL in both species. The mean $C_{max}$ (0.0193 µg/mL) observed after ITV injection of 2 mg/eye in humans is ~50x lower the identified threshold in rodents. Thus, these data support that there is a low probability that ITV administration of VEGF Trap-Eye at a monthly dose of 2 mg/eye will result in

systemic exposure to free VEGF Trap in amounts sufficient to affect cardiovascular parameters in patients. Blood pressure changes were monitored in the clinical trials.

Genotoxicity studies were not conducted because these are not applicable to biotechnology-derived pharmaceuticals. Carcinogenicity studies were not considered required in this case per a meeting conducted on June 17, 2009.

The table below (as presented by the applicant), shows the estimates of safety margins for systemic toxicities based on comparisons between the systemic exposure to free VEGF Trap observed at the NOAEL or LOAEL in the nonclinical toxicity studies with that observed in human clinical trials (mean $C_{max}$ of 0.019 µg/mL; range of 0-0.054 µg/mL; mean $AUC_{0\text{-last}}$ of 2.856 µg•hr/mL). Systemic adverse effects identified in the nonclinical studies occurred at systemic exposures well in excess of the exposure observed in humans. In conclusion, the nonclinical studies conducted support the safety of ITV administration of VEGF Trap at the intended dose regimen for the treatment of wet AMD, and the reviewer recommends approval of the BLA.

## Table 18: Safety Margins for Systemic Toxicities Based on the Animal and Human Free VEGF Exposures

| Study Type / Study No. | Free VEGF Trap in Plasma | | | Exposure Ratios[a] | |
|---|---|---|---|---|---|
| | Dose | $C_{max}$[b] (µg/mL) | $AUC_{0-t}$ (µg•h/mL) | Based on $C_{max}$ | Based on AUC |
| **Phase I VEGF Trap-Eye Pharmacokinetic Sub-study in Humans** Module 5.3.3.2, VGFT-OD-0702.PK | 2 mg/eye | 0.0193 | 2.856 | NA | NA |
| ***Cynomolgus Monkeys*** | | | | | |
| **13-Week IVT Toxicity** Module 4.2.3.2, VFGT-TX-04025 | NOAEL: ND | | | | |
| | LOAEL: 1 mg/eye[c] | 2.45 | 384 | 127 | 135 |
| **8-Month IVT Toxicity** Module 4.2.3.2, VFGT-TX-05011 | NOAEL: 0.5 mg/eye[d] | 0.802 | 160 | 42 | 56 |
| | LOAEL: 2 mg/eye[e] | 4.46 | 2023 | 231 | 708 |
| **6-month IV Toxicity** Module 4.2.3.2, VFGT-TX-05009 | NOAEL: ND | | | | |
| | LOAEL 3 mg/kg[f] | 94.6 | 4416 | 4902 | 1546 |
| **3-month IV Toxicity in Juveniles** Module 4.2.3.2, VFGT-TX-05010 | NOAEL: ND | | | | |
| | LOAEL: 0.5 mg/kg[g] | 9.71 | 384 | 503 | 134 |
| ***Rabbits*** | | | | | |
| **Reproductive IV Toxicity** Module 4.2.3.5.2, VFGT-TX-06002 | | | | | |
| Maternal toxicity | NOAEL: 3 mg/kg[h] | 56.1 | 1935 | 2907 | 678 |
| Embryo-Fetal toxicity | NOAEL: ND | | | | |
| | LOAEL: 3 mg/kg | 56.1 | 1935 | 2907 | 678 |

AUC = Area under the concentration time curve; $C_{max}$ = Maximal concentration; GD = Gestation day; IV = Intravenous; IVT = Intravitreal; NA = Not applicable; ND = Not determined; NOAEL = No observed adverse effect level; LOAEL = Lowest observable adverse effect level; $T_{max}$ = Time of maximal concentration, VEGF = Vascular endothelial growth factor.

[a] Exposure ratio = = $C_{max}$ or AUC values in the toxicity studies divided by the $C_{max}$ (from 0.0193 mg/L reported value) or AUC (from 0.0119 mg•day/L reported value) estimated in humans after a 2 mg intravitreal dose every 4 weeks (Module 5.3.3.2, VGFT-OD-0702.PK, Table PK2.1; 21 Sep 2010).

[b] For IVT toxicity studies, $C_{max}$ was generally observed at $T_{max}$ of 1 day. For IV toxicity studies, $C_{max}$ was observed at at $T_{max}$ of 5 minutes after completion of IV infusion. For reproductive IV toxicity studies, $C_{max}$ was observed at at $T_{max}$ of 0.5 hours after dosing.

[c] The $C_{max}$ value after the third 0.5 mg/eye dose was calculated by averaging the observed $C_{max}$ values for individual animals (Module 4.2.3.2, VGFT-TX-04025, Appendix 19 Toxicokinetics (plasma) and antibody determination, Appendix A, Table 3). $C_{max}$ occurred at a calculated average $T_{max}$ of 1.67 days. The mean $AUC_{0-28d}$ (from 16.0 µg•day/mL reported value) after the third 0.5 mg/eye dose in Module 4.2.3..2, VGFT-TX-04025, Appendix 19, Appendix A, Table 43.

[d] The mean $C_{max}$ and $AUC_{0-28days}$ (AUC from 6.66 µg•day/mL reported value) at the NOAEL (0.5 mg/eye) in Module 4.2.3.2, VFGT-TX-05011, Appendix 6 Toxicokinetics (plasma) and antibody determination, Appendix A, Tables 4 and 43; after the 7th dose.

[e] The mean $C_{max}$ and $AUC_{0-28days}$ (AUC from 84.3 µg•day/mL reported value) at 2 mg/eye in Module 4.2.3..2, VFGT-TX-05011, Appendix 6 Toxicokinetics (plasma) and antibody determination, Appendix A, Tables 12 and 46; after the 7th dose.

[f] The mean $C_{max}$ after the first dose and $AUC_{0-168h}$ (AUC from 184 µg•day/mL reported value) at week 21 at 3 mg/kg in Module 4.2.3.2 VFGT-TX-05009, Appendix 12 Analysis of free and bound VEGF Trap and anti-VEGF Trap antibody, Appendix A, Tables 4 and 30.

[g] The mean $C_{max}$ and $AUC_{0-168h}$ (AUC from 19 µg•day/mL reported value) at 0.5 mg/kg in Module 4.2.3.2, VFGT-TX-05010, Appendix 13 Amended toxicokinetics and antibody report, Appendix A Tables 4 and 30; after the fifth dose.

[h] The mean $C_{max}$ and $AUC_{0.5-72h}$ at 3mg/kg in Module 4.2.3.5.2, VFGT-TX-06002, Appendix V Toxicokinetic evaluation phase report, Appendix A Table 18; GD18 data.

***Note:*** *The correct AUC value reported for humans under superscript "a" is 0.119 mg•day/L. The 3-month toxicity study in juvenile monkeys was not reviewed as the intended patient population is at least of 50 years of age.*

**Signatures:**

Reviewer Signature María I. Rivera 6-30-2011
María I Rivera, Ph.D.

Supervisor Signature William H Taylor Concurrence Yes ✓ No ___
William Taylor, PhD 6/30/2011

Comments on BLA 125387 Aflibercept ophthalmic solution
From: Abigail Jacobs, Assoc. Dir
Date: June 30, 2011

There are no outstanding pharm/tox issues for this BLA and the pregnancy category C is appropriate.

I have discussed some comments with the Team Leader and they have been addressed as appropriate.

# PHARMACOLOGY/TOXICOLOGY NDA FILEABILITY CHECKLIST

**BLA Number: 125387** **Applicant: Regeneron Pharmaceuticals** **Stamp Date: 2-18-2011**
**Drug Name: Aflibercept Ophthalmic Solution**

**IS THE PHARM/TOX SECTION OF THE APPLICATION FILEABLE? Yes [ x ] No [ ]**

**The following parameters are necessary in order to initiate a full review, i.e., complete enough to review but may have deficiencies.**

| | Parameters | Yes | No | Comment |
|---|---|---|---|---|
| 1 | On its face, is the Pharmacology/Toxicology section of the NDA organized in a manner to allow substantive review to begin? | X | | |
| 2 | Is the Pharmacology/Toxicology section of the NDA indexed and paginated in a manner to allow substantive review to begin? | X | | |
| 3 | On its face, is the Pharmacology/Toxicology section of the NDA legible so that substantive review can begin? | X | | |
| 4 | Are ALL required* and requested IND studies completed and submitted in this NDA (carcinogenicity, mutagenicity*, teratogenicity*, effects on fertility*, juvenile studies, ocular toxicity studies*, acute adult studies*, chronic adult studies*, maximum tolerated dosage determination, dermal irritancy, ocular irritancy, photocarcinogenicity, animal pharmacokinetic studies, etc)? | X | | In accordance with ICH guideline S61 and CPMP/ICH/302/95 (1998), genotoxicity studies were not conducted. Since VEGF Trap is a large molecule, it is not expected to interact directly with DNA or other chromosomal material.<br><br>Carcinogenicity studies were not conducted. The Division agreed in a sponsor meeting held on 6-1-09 that these studies were not required. |
| 5 | If the formulation to be marketed is different from that used in the toxicology studies, has the sponsor made a appropriate effort to either repeat the studies with the to be marketed product or to explain why such repetition should not be required? | X | | The formulation was optimized as development progressed. A 13-week formulation study and an 8-month study in monkeys were conducted to support long-term intravitreal dosing with the intended clinical formulation. |
| 6 | Are the proposed labeling sections relative to pharmacology appropriate (including human dose multiples expressed in $mg/m^2$ or comparative serum/plasma levels) and in accordance with 201.57? | X | | |
| 7 | Has the sponsor submitted all special studies/data requested by the Division during pre-submission discussions? | X | | |
| 8 | On its face, does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted a rationale to justify the alternative route? | X | | Intravitreal (intended human rotute) as well as systemic route (SC and IV) studies were conducted. |
| 9 | Has the sponsor submitted a statement(s) that all of the pivotal pharm/tox studies been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations? | X | | |
| 10 | Has the Sponsor submitted the data from the nonclinical carcinogenicity studies, in the STUDIES electronic format, for the review by Biometrics? | | X | Not applicable |
| 11 | Has the sponsor submitted a statement(s) that the pharm/tox | | X | The Sponsor did not submit a statement but |

| | | | | |
|---|---|---|---|---|
| | studies have been performed using acceptable, state-of-the-art protocols which also reflect agency animal welfare concerns? | | | the protocols reflect that animals were treated humanely. |
| 12 | From a pharmacology perspective, is this NDA fileable? | X | | |
| 13 | If the NDA is fileable, are there any issues that need to be conveyed to the Sponsor? | | X | |

Reviewing Pharmacologist: María I. Rivera, Ph.D. Date: 3-23-11

Team Leader: Wendelyn Schmidt, Ph.D. Date: 3/23/11

**cc:**

S. Wadhwa/MO
M. Puglisi/PM