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RESEARCH**

APPLICATION NUMBER:

125418Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Zaltrap (Aflibercept)

Date: July 31, 2012

To: File for BLA 125418

From: John K. Leighton, PhD, DABT

Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review of Dr. Putman and secondary review memorandum and labeling provided by Dr. McDougal. Nonclinical pharmacology and toxicology submitted to support the proposed indication are described in the Integrated Summary and Safety Evaluation provided by Dr. Putman and key points are discussed in the memorandum provided by Dr. McDougal.

I agree with Dr McDougal's conclusion that Zaltrap may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
07/31/2012

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

PHARMACOLOGY/TOXICOLOGY BLA MEMORANDUM

To: The file

FROM: Andrew J. McDougal, Ph.D., D.A.B.T.
Acting Supervisory Toxicologist. Team 2, Division of Hematology
Oncology Toxicology (DHOT), Office of Hematology and Oncology
Products (OHOP), Office of New Drugs (OND), CDER. HFD-108.

BLA: STN 125418

APPLICANT: Sanofi-aventis U.S. Inc.

PRODUCT: Intravenous Aflibercept

INDICATION Treatment of patients with metastatic colorectal cancer previously
treated with an oxaliplatin-containing regimen

RECOMMENDATION: As the Acting Supervisory Toxicologist for the team charged with reviewing the nonclinical Pharmacology and Toxicology of biologics license application (BLA) 125418, I concur with Dr. Alexander H. Putman's recommendation that aflibercept be approved for the treatment of patients with metastatic colorectal cancer previously treated with an oxaliplatin-containing regimen.

BASIS OF RECOMMENDATION:

As noted in Dr. Putman's primary review, aflibercept is a recombinant human soluble fusion protein that traps endogenous vascular endothelial growth factors. More specifically, aflibercept is a recombinant fusion protein, with a total molecular weight of 115 kilodaltons, consisting of Vascular Endothelial Growth Factor (VEGF)-binding portions from an extracellular domain of the human VEGF Receptor 1 and an extracellular domain of the human VEGF Receptor 2, fused to the Fc portion of human IgG1.

Aflibercept's apparent mechanism of action is via binding and sequestration of the endogenous ligands for VEGFR. Vascular endothelial growth factor A and B (VEGF-A, VEGF-B), and placental growth factor (PIGF) are members of the VEGF family of angiogenic factors that can act as mitogenic, chemotactic, and vascular permeability factors for endothelial cells. VEGF-A acts via two receptor tyrosine kinases, VEGFR-1 and VEGFR-2, present on the surface of endothelial cells. PIGF and VEGF-B bind only to VEGFR-1, which is also present on the surface of leucocytes. Activation of these

receptors by VEGF-A can result in neovascularization and vascular permeability. PlGF is also linked to neovascularization and recruitment of inflammatory cells into tumors. Aflibercept acts as a soluble receptor that binds to human VEGF-A (equilibrium dissociation constant K_D of 0.5 pM for VEGF A165 and 0.36 pM for VEGF-A121), to human PlGF (K_D of 39 pM for PlGF-2), and to human VEGF-B (K_D of 1.92 pM). By binding to these endogenous ligands, aflibercept can inhibit the binding and activation of their cognate receptors.

For the use of aflibercept in the treatment of patients with metastatic colorectal cancer, the nonclinical development program was consistent with both ICH S6^{1,2} and ICH S9³. Regeneron Pharmaceuticals received approval of Aflibercept (Eylea®; BLA 125387) in 2011 for the treatment of patients with Neovascular (Wet) Age-Related Macular Degeneration (AMD), and the FDA review⁴ of the supporting nonclinical studies is referenced by Dr. Putman and this reviewer. Notably, Sanofi-aventis U.S. Inc. submitted BLA 125418 as a BLA under 21 CFR Part 601 with a full, stand-alone nonclinical package.

For BLA 125418, Sanofi-aventis U.S. LLC submitted nonclinical studies including: *in vitro* pharmacodynamic (PD) studies elucidating the mechanisms of action of aflibercept; *in vivo* PD studies demonstrating anti-tumor activity in tumor-bearing mice under the conditions tested; a tissue cross reactivity study verifying the specificity of aflibercept in human tissues; *in vitro* studies investigating blood compatibility (i.e. potential to induce hemolysis or flocculation); wound healing studies in rabbits; *in vivo* safety pharmacology studies; a distribution study in rats following intravenous administration; evaluations of nonclinical pharmacokinetics (intravenous and subcutaneous dosing); a rabbit local tolerance study (intravenous, intramuscular, subcutaneous); single-dose and repeat-dose toxicology studies; and an embryofetal toxicity study in rabbits.

As noted in Dr. Putman's primary review, the results of the nonclinical studies verified the proposed mechanism of action; the toxicities observed in animals exposed to aflibercept are consistent with exaggerated primary pharmacology. General toxicology studies of 3 or 6 month duration with aflibercept in cynomolgus monkeys observed bone

¹ Guidance for Industry. ICH S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals. 1997. Accessed online via:

<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM074957.pdf>

² Guidance for Industry. ICH S6 Addendum to Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals. 2012. Accessed online via:

<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM194490.pdf>

³ Guidance for Industry. ICH S9 Nonclinical Evaluation for Anticancer Pharmaceuticals. Accessed online via:

<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM085389.pdf>

⁴ By Dr. María I. Rivera. Publically-available version of the review accessed online via:

http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/125387Orig1s000PharmR.pdf

effects (exososes and related effects on growth plate, and the axial and appendicular skeleton), nasal cavity (atrophy/loss of the septum and/or turbinates), kidney (glomerulopathy with inflammation), ovary (decreased numbers of maturing follicles, granulosa cells, and/or thecal cells), and adrenal gland (decreased vacuolation with inflammation). Alterations in sperm morphology and decreased sperm motility were observed. Aflibercept's effects on the ovaries and sperm suggest that aflibercept might impair fertility and reproductive function in both sexes.

As noted in Dr. Putman's primary review, intravenous administration of aflibercept to pregnant rabbits caused postimplantation losses (the study designs not being timed to investigate the potential to cause preimplantation losses). Aflibercept caused fetal anomalies were observed at all doses tested; the lowest dose of 3 mg/kg correlated with a systemic exposure approximately 30% of the systemic exposure observed in patients receiving the recommended label dose of 4 mg/kg. Adverse embryo-fetal effects included increased incidences of postimplantation losses and external (including anasarca, umbilical hernia, diaphragmatic hernia and gastroschisis, cleft palate, ectrodactyly, and atresia), visceral (in the heart, great vessels, and arteries), and skeletal fetal malformations (including fused vertebrae, sternebrae, and ribs; supernumerary arches and ribs, and incomplete ossification).

In accordance with the ICH S6 and ICH S9 guidances, no studies were conducted to evaluate the potential genotoxic potential or the carcinogenic potential of aflibercept. Based on the structure of aflibercept, it is not expected to interact directly with DNA or other chromosomal material; additionally, genotoxicity studies are not considered essential to support clinical trials for therapeutics intended to treat patients with advanced cancer. Carcinogenicity studies are not warranted to support marketing for therapeutics intended to treat patients with advanced cancer.

From a nonclinical Pharmacology/Toxicology perspective, approval is recommended for BLA 125418 for the use of aflibercept in combination with irinotecan-fluoropyrimidine-based chemotherapy for patients with metastatic colorectal cancer (MCRC) previously treated with an oxaliplatin-containing regimen.

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/s/

ANDREW J MCDOUGAL
07/10/2012

**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: STN 125418\0
Supporting document/s: 0000 (Original Application)
Applicant's letter date: February 2, 2012
CDER stamp date: February 3, 2012
Product: Intravenous Aflibercept
Indication: Treatment of patients with metastatic colorectal cancer previously treated with an oxaliplatin-containing regimen
Applicant: Sanofi-aventis U.S. Inc.
Review Division: Division of Hematology Oncology Toxicology
Reviewer: Alexander H. Putman, Ph.D.
Supervisor/Team Leader: Andrew McDougal, Ph.D.
Division Director: John Leighton, Ph.D.
Project Manager: Melanie Pierce

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 125418 are owned by Sanofi-aventis, Inc. or are data for which Sanofi-aventis, Inc. has obtained a written right of reference. Any information or data necessary for approval of BLA 125418 that Sanofi-aventis, 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 125418.

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1 Executive Summary

1.1 Introduction

Sanofi-aventis Inc. submitted a Biologics License Application (BLA) for intravenous aflibercept (Zaltrap®) in patients with metastatic colorectal cancer in combination with irinotecan-fluoropyrimidine-based chemotherapy.

Eylea™ (aflibercept) is FDA approved as an intravitreal injection for the treatment of patients with neovascular (wet) age-related macular degeneration (AMD). To support the use of intravenous aflibercept (Zaltrap®) in patients with metastatic colorectal cancer in combination with irinotecan-fluoropyrimidine-based chemotherapy, the applicant submitted non-clinical studies, many of which were previously reviewed for the approval of Eylea™. The review of these non-clinical studies is documented below.

1.2 Brief Discussion of Nonclinical Findings

See Integrated Summary and Safety Evaluation (11).

1.3 Recommendations

1.3.1 Approvability

From a Pharmacology/Toxicology perspective, approval for intravenous aflibercept is recommended.

1.3.2 Additional Non-Clinical Recommendations

None. From a Pharmacology/Toxicology perspective, no additional post-marketing commitments (PMCs) or post-marketing requirements (PMRs) are recommended for this indication.

1.3.3 Labeling

The recommendations to the sponsor's proposed labeling were discussed internally and communicated to the sponsor. Information in the non-clinical sections of the label reflects findings of studies reviewed within this document.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number

862111-32-8

2.1.2 Generic Name

Aflibercept

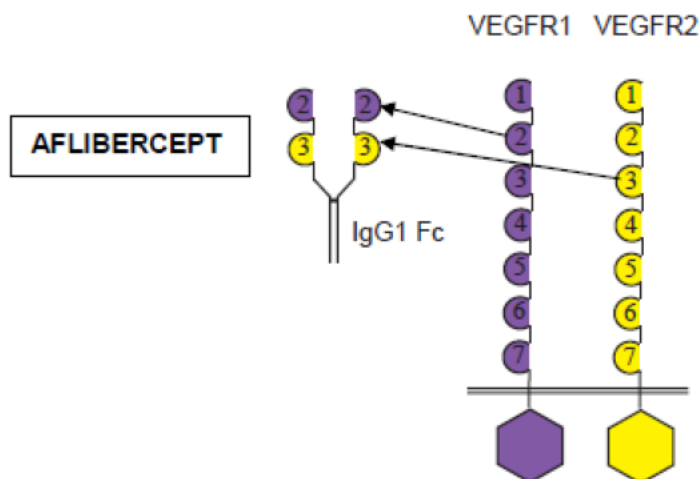
2.1.3 Code NameVEGF Trap
AVE0005**2.1.4 Chemical Name**

Vascular endothelial growth factor receptor type VEGFR-1 ((b) (4) human immunoglobulin domain 2 fragment) fusion protein with vascular endothelial growth factor receptor type VEGFR-2 ((b) (4) human immunoglobulin domain 3 fragment) fusion protein with immunoglobulin G1 ((b) (4) Fc fragment), dimer, OR

(b) (4)

2.1.5 Molecular Formula/Molecular Weight

(b) (4) 115 kDa

2.1.6 Structure

(figure excerpted from applicant's BLA)

2.1.7 Pharmacologic class

Vascular endothelial growth factor (VEGF) inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

The applicant references Eylea™ (aflibercept; BLA 125387), and notes that the holder of the approved application is Regeneron Pharmaceuticals, Inc.

2.3 Drug Formulation

The final drug product for Zaltrap® is different than the final drug product for Eylea™.

Zaltrap® will be available at 25 mg/mL, in 5 mL or 10 mL single use vials, containing a withdrawable amount of 100 mg/4 mL or 200 mg/8 mL aflibercept, respectively. The vials are to be packaged in cartons containing either one 5 mL or one 10 mL vial, and, in cartons containing three 5 mL vials. It is to be diluted before administration. The composition of the 25 mg/mL constitutive solution is provided below.

Composition of ZALTRAP® product at 25 mg/mL

Name of excipient (a)	Concentration (mg/mL) (b)	Concentration (mmol)	Function / characteristic	Reference to standards
Aflibercept (AVE0005 ; VEGF Trap)	25.0	0.22	Drug Substance	In-house monograph
Sodium Phosphate	(b) (4)	(b) (4)	(b) (4)	USP
(b) (4)	(b) (4)	(b) (4)	(b) (4)	USP
(b) (4)	(b) (4)	(b) (4)	(b) (4)	USP, Ph. Eur.
Sodium Citrate	(b) (4)	(b) (4)	(b) (4)	USP, Ph. Eur.
Sodium Chloride	(b) (4)	(b) (4)	(b) (4)	USP, Ph. Eur.
(b) (4)	(b) (4)	(b) (4)	(b) (4)	NF, Ph. Eur.
(b) (4)	(b) (4)	(b) (4)	(b) (4)	NF, Ph. Eur.
Sucrose	200	584	(b) (4)	NF, Ph. Eur.
Polysorbate 20	1.00	0.81	(b) (4)	NF, Ph. Eur.
Water for Injection	(b) (4)	(b) (4)	(b) (4)	USP, Ph. Eur.
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)

(table excerpted from applicant's BLA)

2.3.3 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

Zaltrap® (aflibercept) is indicated in combination with irinotecan-fluoropyrimidine-based chemotherapy for patients with metastatic colorectal cancer, previously treated with an oxaliplatin-containing regimen. Zaltrap® (aflibercept) will be administered as a 4 mg/kg intravenous infusion over 1 hour, every 2 weeks, with combination of irinotecan and infusional 5-fluorouracil/leucovorin.

2.7 Regulatory Background

Eylea™ (aflibercept) is FDA approved as an intravitreal injection for the treatment of patients with neovascular (wet) age-related macular degeneration (AMD). To support the use of intravenous aflibercept (Zaltrap®) in patients with metastatic colorectal cancer in combination with irinotecan-fluoropyrimidine-based chemotherapy, the applicant submitted non-clinical studies, many of which were previously reviewed for the approval of Eylea™. The review of these non-clinical studies is documented below.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology:

Study #	Study title
VGFT MX 0802 2 (IVT0044)	Determination of equilibrium binding constants for the interaction of aflibercept with VEGF family related ligands
VGFT MX 1101 6 (ONVT0015)	Determination of Equilibrium Binding Constants for the Interaction of VEGF Trap (aflibercept) with VEGF B Ligand
VGFT MX 0801 6 (IVT0043)	VEGFR2 bioassays of aflibercept: blocking of VEGFR2 phosphorylation and calcium mobilization in HUVE cells
IVT0042	AVE0005 (aflibercept): inhibition of VEGF induced proliferation in Human Dermal Microvascular Endothelial Cells
VGFT MX 0701 4 (IVT0037)	Complement dependent cytotoxicity (CDC) and antibody dependent cell mediated cytotoxicity (ADCC) activities of aflibercept (AVE0005, VEGF Trap)
VGFT MX 0801 4 (IVV0066)	Aflibercept effects on tumor blood vessel density
IVV0051	AVE0005: Antitumor activity in mouse preclinical tumor models

Safety Pharmacology:

Study #	Study title
PMA00018 (VGFT TX 06010)	A Study to Determine the Effects of VEGF TRAP on Wound Healing in an Incisional Wound Model in Rabbits
PMA00019 (VGFT TX 06011)	A Study to Determine the Effects of VEGF TRAP on Wound Healing in an Excisional Wound Model in Rabbits

Pharmacokinetics:

Study #	Study title
SNBL.223.3 (VGFT PK 01012)	A Single Dose Intravenous and Subcutaneous Pharmacokinetic Study of VEGF Trap in Cynomolgus Monkeys
VGFT PK 01005.2	Biodistribution of VEGF Trap in Normal Sprague Dawley Female Rats
VGFT PK 01004.2	Pharmacokinetics of VEGF Trap Following Intravenous Administration to Sham Operated and Nephrectomized Sprague Dawley Rats

Repeat-dose Toxicity:

Study #	Study title
670145 (VGFTTX 05009)	A 6 month intravenous toxicity study of VEGF Trap in cynomolgus monkeys with a 5 month recovery period
670144 (VGFTTX 05010)	A 3 month intravenous toxicity study of VEGF Trap in cynomolgus monkeys with a 5 month recovery period

Reproductive and Developmental Toxicology:

Study #	Study title
div0953 (DSE 2005 0569; VGFT TX 05007)	AVE0005: Exploratory 14 day intravenous (30 minute infusion) toxicity study in nonpregnant female rabbits
tep0184 (VGFTTX 06001)	AVE0005: Intravenous (30 minute infusion) range finding toxicity study in pregnant rabbits

Local Tolerance:

Study #	Study title
tol1079 (DSE	AVE0005: Single dose local intravenous, intramuscular and subcutaneous tolerance study in female rabbits

2005 0387; VGFT TX 0500 8)	
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3.2 Studies Not Reviewed

Pharmacology:

Study #	Study title
HVT0136	In vitro effect of AVE0005 (VEGF TRAP) on VEGFR 2 phosphorylation induced by VEGF A and VEGF C
SPRFU 1104	AVE0005 (VEGF Trap): Effect in the rat aortic ring model
VGT_NC_001 (IVV0064)	Aflibercept (VEGF Trap): Dose response study in the rat C6 glioma xenograft model
VGT_NC_002 (IVV0065)	Aflibercept (VEGF Trap): Dose response study in the human A673 rhabdomyosarcoma xenograft model
IVV0091	Activity of aflibercept against orthotopically transplanted renal tumor model RENCA producing ascites
IVV0052	Evaluation of VEGF Trap (aflibercept) in Rip1Tag2 model
SPRFU 1119	AVE0005 (VEGF Trap) : In vivo combination with chemotherapy
IVV0080	Evaluation of the antitumor activity of VEGF Trap (aflibercept) against advanced human colon carcinoma COLO 205 in SCID mice (MIR189)
IVV0081	Evaluation of the antitumor activity of VEGF Trap (aflibercept) in comparison to sunitinib against advanced human carcinoma HCT 116 in SCID mice (MIR243)
IVV0109	Evaluation of the antitumor activity of aflibercept in mice bearing subcutaneous human lung tumors
IVV0082	Evaluation of the antitumor activity of VEGF Trap (aflibercept) against advanced human sarcoma HT 1080 in SCID mice (MIR244)
IVV0103	Activity of aflibercept in combination with oxaliplatin against subcutaneous murine colon adenocarcinoma C51
IVV0043	Efficacy of VEGF Trap +/- CPT 11 combination against HCT 116 Human colon carcinoma xenografts in female NCR Nu/Nu mice

Safety Pharmacology:

Study #	Study title
VGFT MX 0801 9	Effect of VEGF Trap administration on capillary density in normal murine tissues

VGFT MX 08018	Blood Pressure effects of VEGF Trap (AVE0005) in telemetered mice and rats
VGFT MX 08025	Anti hypertensive Screening in VEGF Trap treated Wistar Kyoto Rats
PMA00017 (VGFT TX 06012)	A Study to Determine the Potential Effects of VEGF TRAP (AVE0005) on Venous and Arterial Thrombotic Formation in an Electrolytic Injury Model in Rabbits
RCV0115 (VGFTTX 06009)	Effect on the respiratory function after a single 30 minute intravenous infusion in unrestrained conscious rats using whole body plethysmography
VGFT MX 08020	Effect of VEGF Trap on renal function in C57BL/6 mice

Pharmacokinetics:

Study #	Study title
VGFT PK 01007.3	Pharmacokinetics and Bioavailability of VEGF Trap Following Intravenous and Subcutaneous Administration to CD 1 Mice
VGFT PK 01001.2	Pharmacokinetics and Bioavailability of VEGF Trap Following Intravenous and Subcutaneous Administration to Sprague Dawley Rats
VGFT PK 01002.3	Pharmacokinetic Dose Ranging Study of VEGF Trap Administered as a Single Subcutaneous Injection to Sprague Dawley Rats
VGT_NC_004	Aflibercept (VEGF Trap) Complex Formation Measures Production Rates of VEGF, Providing a New Biomarker for Predicting Efficacious Angiogenic Blockade
PK06005 9 SA 01V1	Pharmacokinetics of VEGF Trap following intravenous administration to Sprague Dawley rat: Correlation between pharmacokinetic parameters and sialic acid levels

Single-dose Toxicity:

Study #	Study title
TXP0166 (VGFTTX 06007)	AVE0005: exploratory single dose intravenous (30 minute infusion) toxicity study in rats with a 2 week observation period
TXA1004 (VGFTTX 06008)	AVE0005: single dose intravenous (30 minute infusion) toxicity study in rats with a 2 week observation period

Repeat-dose Toxicity:

Study #	Study title
pk01017	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
vgt3	A non GLP, exploratory study to determine the effect of subcutaneous administration of VEGF Trap on SCID mice (15 week v. 7 week old mice) for 4 or 8 weeks
pk01027	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
pk01034	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
pk01042	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
pk01032	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
0470rr20 001 (VGFT TX 0200 6)	A 3 month toxicity study of VEGF Trap by subcutaneous injection in rats
snbl 223 11 (VGFT TX 0202 9)	A 4 week I.V. toxicity study of VEGF Trap with a 6 week recovery period in cynomolgus monkey
snbl 223 18 (VGFT TX 0304 8)	A 13 week repeat dose intravenous toxicity study of VEGF Trap in cynomolgus monkeys and a 13 week recovery period
snbl 223 4 (VGFT TX 0300 4)	A 4 week subcutaneous toxicity study of VEGF Trap in cynomolgus monkeys followed by a 4 week recovery period
snbl 223 09 (VGFT TX 0203 7)	A 13 week subcutaneous toxicity study of VEGF Trap in cynomolgus monkeys followed by a 6 week recovery period

Reproductive and Developmental Toxicology:

Study #	Study title
div0953 (DSE 2005 0569; VGFT TX 0500 7)	AVE0005: Exploratory 14 day intravenous (30 minute infusion) toxicity study in nonpregnant female rabbits

Local Tolerance:

Study #	Study title
tol1079 (DSE 2005 0387; VGFT TX 0500 8)	AVE0005: Single dose local intravenous, intramuscular and subcutaneous tolerance study in female rabbits

Special Toxicology:

Study #	Study title
hem no1	Evaluation of VEGF Trap to induce hemolysis in monkey blood and to induce flocculation in monkey plasma and serum
hem no3	Evaluation of VEGF Trap to induce hemolysis in whole blood from humans and to induce flocculation in human plasma and serum
hem no5	Evaluation of VEGF Trap to induce hemolysis in whole blood from humans and to induce flocculation in human plasma and serum
sps 01 141	Cross reactivity of VEGF Trap with Human tissue Ex Vivo

3.3 Previous Reviews Referenced

The Pharmacology/Toxicology and other discipline reviews for:

- BLA 125387 - Eylea™ (intravitreal aflibercept)
- IND 9948 (Regeneron Pharmaceuticals Inc. for treatment of patients with incurable, relapsed, or refractory solid tumors or lymphoma)
- IND 100137 (National Institutes of Health National Cancer Institute for treatment of cancer)

(b) (4)

4 Pharmacology**4.1 Primary Pharmacology**

The primary pharmacology studies submitted by the applicant are adequate, confirming the mechanism of action as described in the label and demonstrating the anti-tumor activity of aflibercept under the non-clinical conditions tested. No basis for new concerns was identified.

VGFT-MX-08022 (IVT0044): Determination of Equilibrium Binding Constants for the Interaction of Aflibercept with VEGF-Family Related Ligands. Reviewed by Maria I. Rivera and slightly modified for this BLA review.

The interaction between aflibercept and eleven VEGF family related ligands was measured (b) (4). 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.

Binding Parameters (Mean) for the Interaction of VEGF Trap to VEGF Family Related Ligands

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

^a NB= no detectable binding

(table excerpted from applicant's BLA)

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 (sequences presented in the study report). Thus, the applicant concludes, and this reviewer concurs, that the binding interaction of human and monkey VEGF-A with VEGF Trap should be indistinguishable. The results of the repeat-dose toxicity study in cynomolgus monkeys (Section 6.2), which showed toxicity consistent with VEGF inhibitors, supports the conclusion that VEGF Trap binds to cynomolgus VEGF.

VGFT-MX-11016 (ONVT0015): Determination of Equilibrium Binding Constants for the Interaction of VEGF Trap (aflibercept) with VEGF-B Ligand.

In this study, the equilibrium binding affinity for the interaction of aflibercept to human VEGF-B was determined by surface plasmon resonance technology (b) (4). The reference standard lot of aflibercept (Lot C07003M500, also designated as RSVEGTO-1) was used in these experiments. The results showed that human VEGF-B exhibits binding to aflibercept in the picomolar range. Specifically, the calculated equilibrium binding constant between human VEGF-B and aflibercept was 1.92pM.

VGFT-MX-08016 (IVT0043): VEGFR2 Bioassays of Aflibercept: Blocking of VEGFR2 Phosphorylation and Calcium Mobilization in HUVE Cells. Reviewed by Maria I. Rivera and slightly modified for this BLA review.

Complete inhibition of VEGF-dependent VEGF receptor phosphorylation was observed when VEGF Trap (1 nM) was incubated with 1-3 nM VEGF-A₁₆₅, i.e., at a molar ratio of 1:1 or greater. VEGF Trap effectively inhibited calcium mobilization with an IC₅₀ of 1.20-1.73 nM.

IVT0042: AVE0005 (aflibercept): Inhibition of VEGF-induced proliferation in Human Dermal Microvascular Endothelial Cells.

The ability of aflibercept to inhibit VEGF-induced endothelial cell proliferation was analyzed using human dermal microvascular endothelial cells (HDMEC) in the presence of 10 ng/ml (238 pM) human recombinant VEGF165 (rh VEGF165). VEGF-induced HDMEC were continuously exposed to aflibercept for 4 days, with doses ranging from 1 pM to 1 nM. The effect of aflibercept was quantified by counting the incorporated 14C-thymidine and determining the concentration able to reduce VEGF-induced HDMEC proliferation by 50% (IC₅₀). The geometric mean IC₅₀ value was 192 pM.

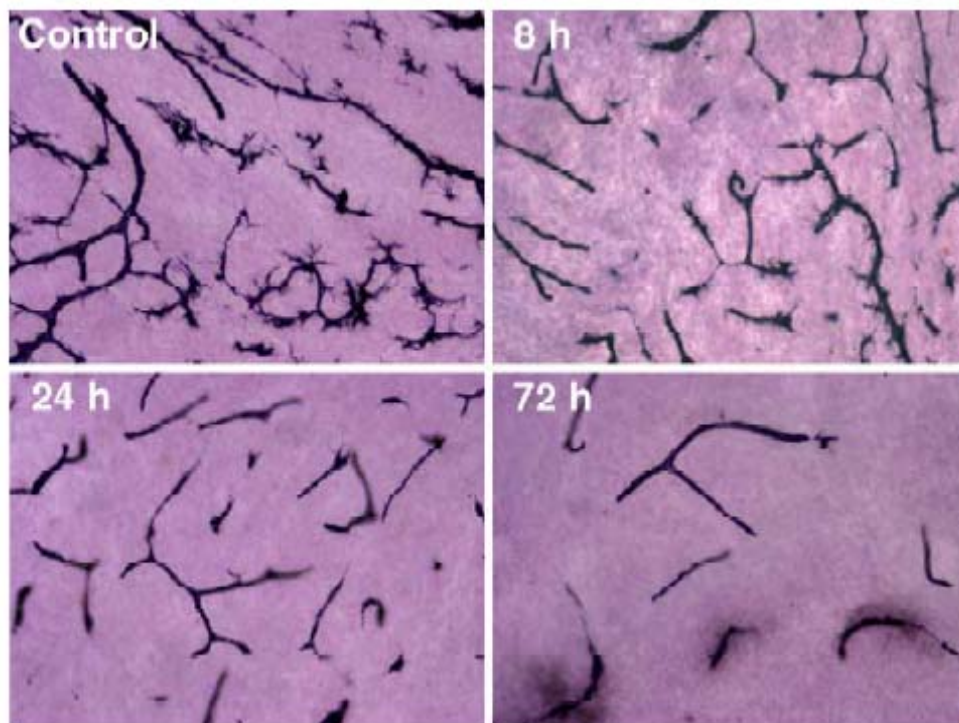
VGFT-MX-07014 (IVT0037): Complement-Dependent Cytotoxicity (CDC) and Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) Activities of Aflibercept (AVE0005, VEGF Trap). Reviewed by Maria I. Rivera and slightly modified for this BLA review.

VEGF Trap (0.85 pM-50 nM ± 10 nM VEGF₁₆₅) 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 VEGF₁₆₅) 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).

VGFT-MX-08014 (IVV0066): Aflibercept effects on tumor blood vessel density.

The objective of this study was to determine whether treatment of tumor bearing mice with aflibercept results in a decrease of tumor vessels, as measured histologically by vessel area density. Male immunodeficient mice (SCID) were injected subcutaneously with C6 (rat glioma), U87 (human glioblastoma/astrocytoma), or 786-0 (renal cell carcinoma) tumor cells. Once tumors grew to approximately 100 mm³ in size, the mice were treated systemically with a single subcutaneous injection of 25 mg/kg aflibercept or a control protein (human Fc, also 25 mg/kg). Four to 72 hours later, the tumors were processed histologically to stain for blood vessels (using antibodies to CD31/PECAM). The stained sections were analyzed morphometrically to assess tumor vessel density.

As shown by the representative figure below of immunohistochemical staining, treatment of mice bearing subcutaneous C6 tumors with a single dose with aflibercept resulted in a reduced density of tumor vessels, most notable after 24 hours.

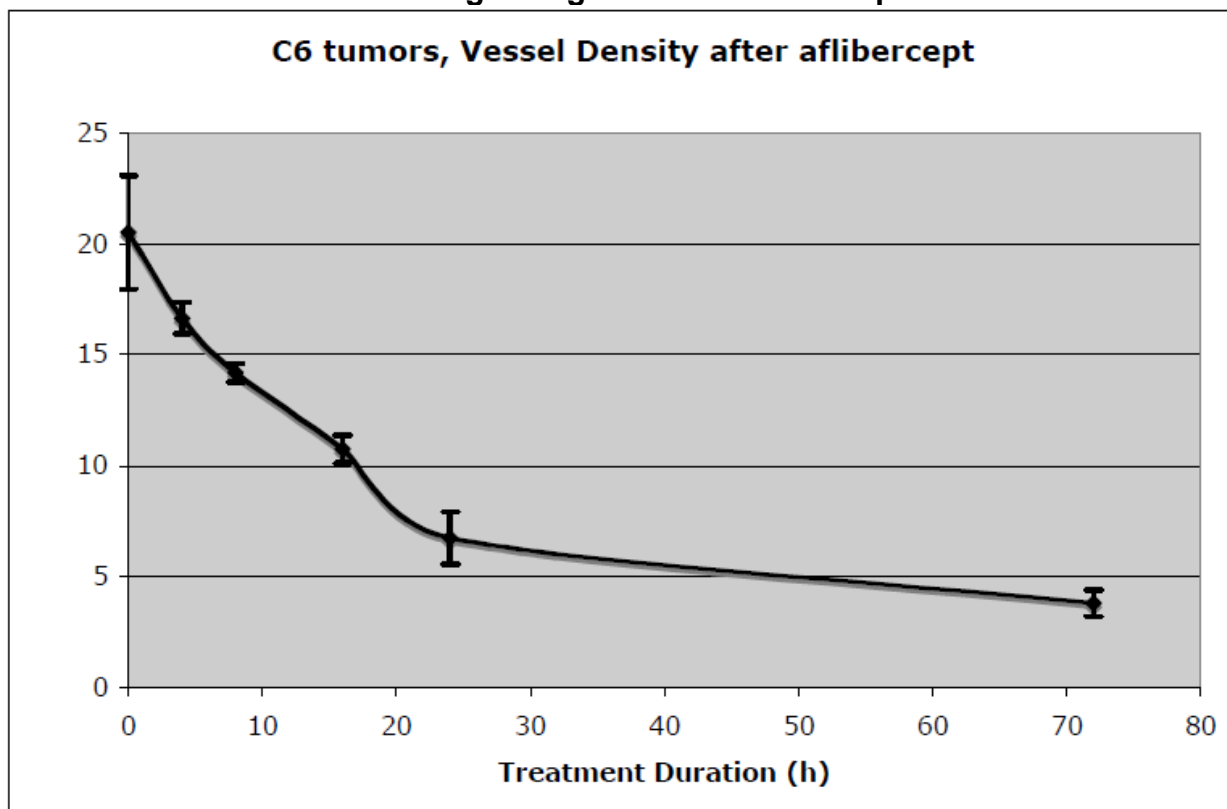
Immunohistochemical staining for blood vessel appearance and density in C6 tumors treated with control or single dose of aflibercept

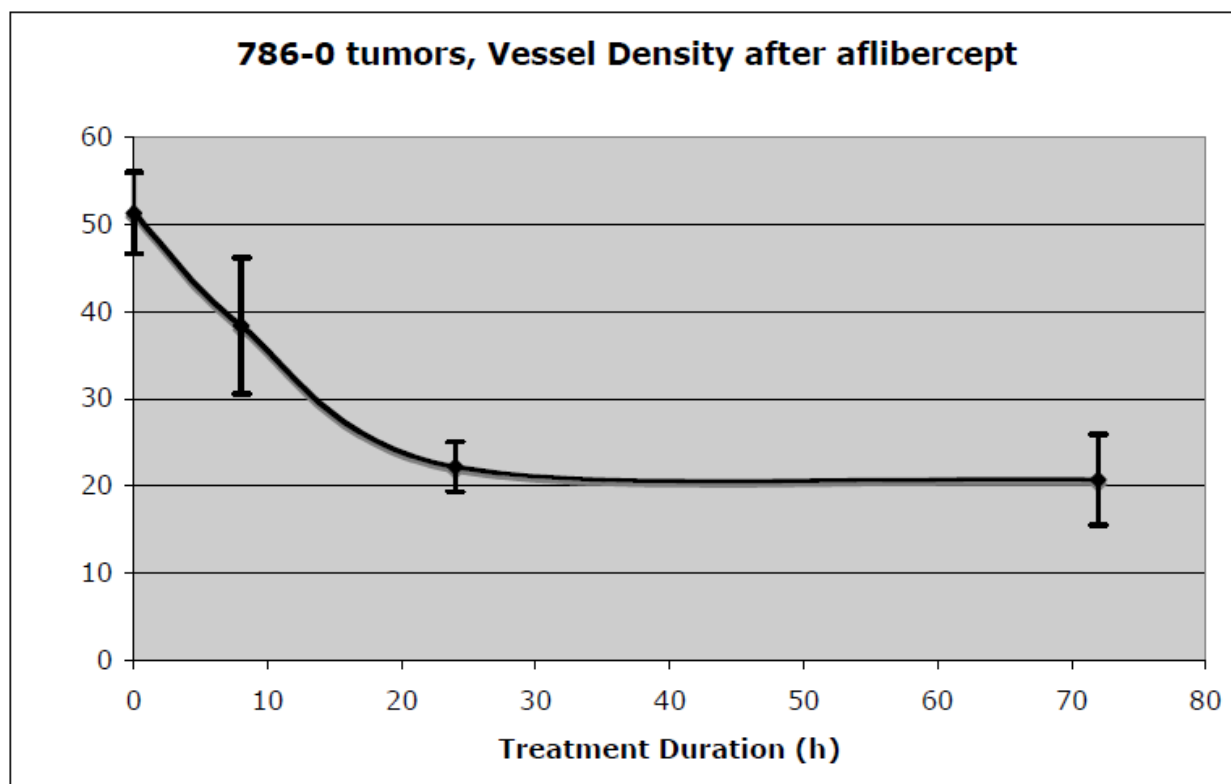
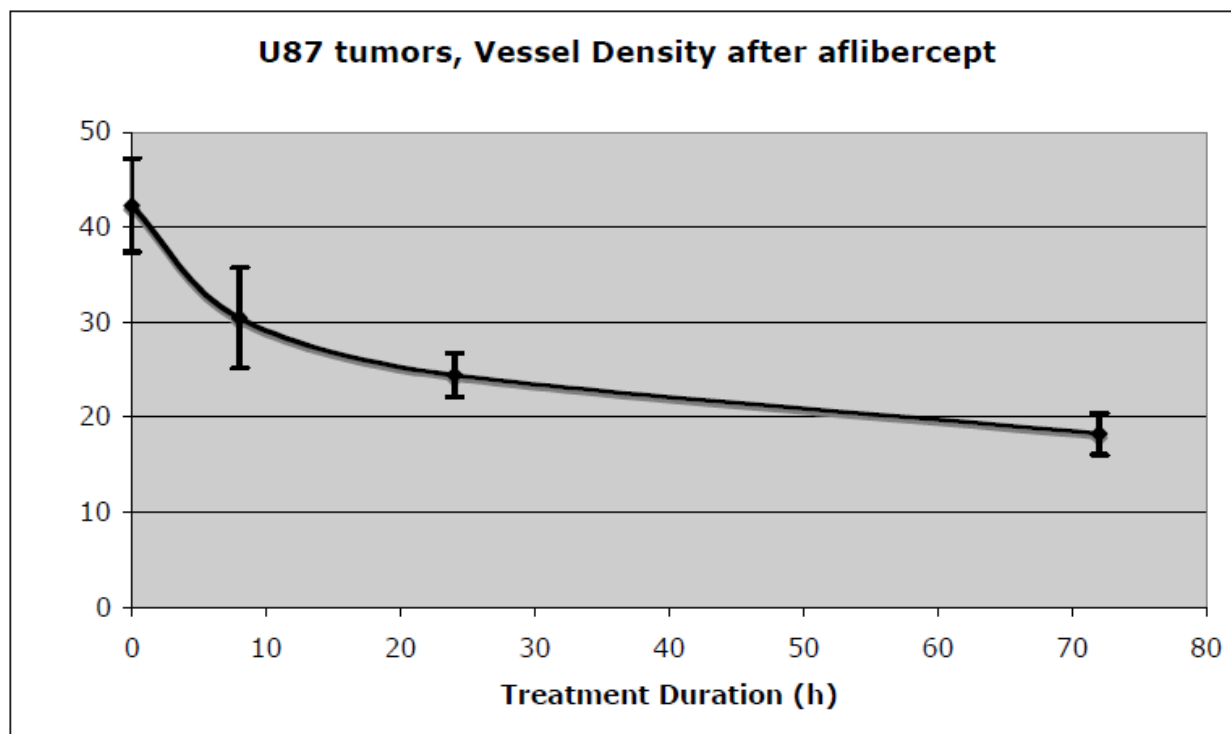
(figure excerpted from applicant's BLA)

A morphometric assessment of vessel density was conducted in each tumor type (C6, U87, and 786-0) following aflibercept. As shown in the figures below, following a single treatment with 25 mg/kg aflibercept, the vessel density of C6 tumors (rat glioma) was only 20% of control tumors at 72 hours after treatment; U87 tumors (human glioblastoma / astrocytoma) was 43% of controls; 786-0 tumors (human renal cell

carcinoma) was 40% of controls. Based on these results, aflibercept appears to reduce the density of tumor blood vessels in several types of tumors grown subcutaneously in mice.

Average blood vessel density of C6, U87, and 786-0 tumors over 72 hours following a single dose of aflibercept





(figures excerpted from applicant's BLA)

IVV0051: AVE0005: Antitumor activity in mouse preclinical tumor models

The following studies were performed to assess the spectrum of anti-tumor efficacy of aflibercept in tumor models representing various tissues, including melanoma, prostate, ovarian, mammary, pancreatic, gastric, colon tumors, neuroblastoma, rhabdomyosarcoma, Ewing's sarcoma, and lymphoma.

The results showed that aflibercept was active in 20/24 tumors of murine and human origin; ovary SK-OV-3, A2780, NIH: OVCAR-3, B16 and A-375 melanoma, prostate DU 145, mammary UISO-BCA-1, pancreas BxPC-3, gastric MKN-45, Hs746T and SNU-5, colon C51, HT-29 and HCT 116, SKN-MC neuroblastoma, RH-30 rhabdomyosarcoma, TC-71 and SK-ES-1 Ewing's sarcoma, NAMALWA, and WSU-DLCL2 lymphoma.

Only a few tumors, LOX melanoma, prostate PC-3, pancreatic PANC-1 and SK-N-AS neuroblastoma were not sensitive to aflibercept.

In three tumor models (SK-N-MC, TC-71 and SK-ES-1), aflibercept was directly compared to sorafenib, and exhibited greater anti-tumor activity (more than 1 log cell kill gross/net at their respective highest dose tested).

In several models, dose-response studies were conducted to evaluate the pharmacological index (ratio of the highest active dose and the lowest active dose, antitumor activity being declared for log cell kill gross ≥ 0.7) of aflibercept. Aflibercept showed anti-tumor activity with a pharmacological index ≥ 16 in 11 tumor models (SK-OV-3, A2780, NIH: OVCAR-3, DU 145, B16, BxPC-3, MKN-45, SNU-5, C51 at early stage, HT-29 and HCT 116).

The detailed anti-tumor efficacy results in each tumor model are shown in the following tables. The study endpoints used within these tables are described below.

End points for assessing tumor activity:

Toxicity:

Anti-tumor activity evaluation was completed at the highest non-toxic dose (HNTD) or the highest dose tested (HDT) when toxicity could not be reached. A dose producing a 20% weight loss at nadir (mean of group) or 10% or more drug deaths, was considered an excessively toxic dose.

Tumor growth inhibition:

To evaluate the tumor growth inhibition (T/C), the tumors from the treatment (T) and control (C) groups were measured when the median of the control group reached approximately 650 to 1000 mg. The tumor weight used for this calculation is reported in each individual table. The median tumor weight of each group was determined.

The percent T/C value is an indication of tumor effectiveness: $T/C (\%) = (\text{Median tumor weight of the Treated}) / (\text{Median tumor weight of the Control}) \times 100$. According to NCI standards, a $T/C \leq 42\%$ is the minimal level to declare activity. A $T/C < 10\%$ is

considered to indicate high anti-tumor activity and is the level used by NCI to justify further development (Decision Network-2 level, DN-2).

Tumor growth delay:

To evaluate the tumor growth delay (T-C), T and C are the median times (in days) required for the treatment group and the control group tumors, respectively, to reach a predetermined size (650 to 1000 mg). Tumor free survivors are excluded from these calculations and tabulated separately.

Tumor doubling time:

The tumor doubling time (Td) is the time in days for the tumor burden to double in size. The doubling time (Td) is estimated from a semi-log plot of tumor burden versus time, over the period of exponential growth. The Td could be calculated by taking the Td values of the median growth curve, estimated from the control group when this median growth curve is representative of the entire control group.

Tumor cell kill:

For subcutaneously growing tumors, the total log cell kill is calculated from the following formula:

$$\text{Log cell kill (gross or total)} = (\text{T-C value in days}) / (3.32 \times \text{Td})$$

where T-C is the tumor growth delay and Td is the tumor volume doubling time in days, as described above. The log cell kill value can be converted to an arbitrary activity rating according to the SRI criteria:

SRI activity		Duration of treatment 5 to 20 days log cell kill gross
Highly active	++++	>2.8
	+++	2.0 to 2.8
	++	1.3 to 1.9
	+	0.7 to 1.2
Inactive	-	<0.7

(figure excerpted from applicant's BLA)

The log cell kill net is used for duration of treatment over 10 days. The log cell kill net is calculated from the following formula:

$$\text{Log cell kill (net)} = ((\text{T-C value in days}) - \text{duration of treatment in days}) / (3.32 \times \text{Td})$$

where T-C is the tumor growth delay and Td is the tumor volume doubling time in days, as described above. The log cell kill net is negative when the tumors grow under treatment. The log cell kill net is positive when the compound has a cytotoxic effect. When the value of the log cell kill is 0, the compound has a cytostatic effect. A

complete regression (CR) corresponds to a regression below the limit of palpation (< 63 mg). A partial regression (PR) corresponds to a regression greater than 50% reduction in tumor mass.

Pharmacological index:

The ratio of the highest active dose and the lowest active dose, anti-tumor activity being declared for log cell kill gross ≥ 0.7 .

Detailed anti-tumor efficacy results (from report # (VV0066)):

Evaluation of aflibercept against advanced human ovarian adenocarcinoma SK-OV-3 in SCID female mice

Agent (batch)	Route	Dosage in mg/kg/ adm	Schedule in days	Total dose in mg/kg	Drug death (Day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 700 mg in days	T-C in day	log cell kill gross/ net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C04003M600B11)	S.C.	40.0	20, 24, 27,	240.0	0/5	-4.4 (29)	73.7	46.3	4.2 / 2.5	HDT, highly active	0.0017
		10.0	31, 34, 38	60.0	0/5	-3.3 (29)	68.9	41.5	3.8 / 2.1	Highly active	0.0246
		2.5		15.0	0/5	-5.5 (36)	71.6	44.2	4.0 / 2.3	Highly active	0.0306
Control						-7.1 (36)	27.4*				

Tumor doubling time = 3.3 days.

Tumor size at star of therapy was 75 – 263 mg with a median tumor burden per group of 122 - 147 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 20.06 - 23.28 g), dosages were adjusted to the individual body weights.

Treatment duration: 19 days.

Abbreviations used: (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

* Kruskal-Wallis multiple comparisons test versus control (Statistical report n*: RP08048-EN-E01). A probability less than 5% ($p < 0.05$) was considered as significant; NS = Non-significant.

Evaluation of aflibercept against human ovarian adenocarcinoma A2780 in SCID female mice

Agent (batch)	Route	Dosage in mg/kg/ adm	Schedule in days	Total dose in mg/kg	Drug death (Day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 1000 mg in days	T-C in day	log cell kill gross/ net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C04003M600B11)	S.C.	40.0	11,14,18	160.0	0/5	-1.5 (13)	29.9	13.2	2.3 / 0.4	HDT, active	0.0265
		10.0	21	40.0	0/5	-0.8 (13)	30.8	14.1	2.5 / 0.5	Active	0.0187
		2.5		10.0	0/5	-5.5 (36)	24.6	7.9	1.4 / -0.5	Active	0.0273
Control						-1.4 (13)	16.7				

Tumor doubling time = 1.7 days.

Tumor size at star of therapy was 75 – 262 mg with a median tumor burden per group of 128 - 174 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 17.91 – 22.37 g), dosages were adjusted to the individual body weights.

Treatment duration: 11 days.

Abbreviations used: (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

* Log-rank multiple comparisons test versus control (Statistical report n*: RP08064-EN-E01). A probability less than 5% ($p < 0.05$) was considered as significant.

Evaluation of aflibercept against advanced human ovarian adenocarcinoma NIH:OVCAR-3 in SCID female mice

Agent (batch)	Route	Dosage in mg/kg/ adm	Schedule in days	Total dose in mg/kg	Drug death (Day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 750 mg in days	T-C in days	log cell kill gross/ net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C04003M600B11)	S.C.	40.0	21, 24, 28,	240.0	0/5	-2.8 (22)	80.0	44.5	2.0 / 1.3	HDT, active	<0.0001
		10.0	31, 35, 37	60.0	0/5	-2.4 (25)	69.5	34.0	1.6 / 0.8	Active	0.0035
		2.5		15.0	0/5	-4.3 (27)	54.5	19.0	0.9 / 0.1	Active	0.0741
Control						-1.8 (23)	35.5				

Tumor doubling time = 6.6 days.

Tumor size at start of therapy was 86 – 163 mg with a median tumor burden per group of 126 - 131 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 19.90 – 26.3 g), dosages were adjusted to the individual body weights.

Treatment duration: 17 days.

Abbreviations used: (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

* Kruskal-Wallis multiple comparisons test versus control (Statistical report n°: RP08063-EN-E01). A probability less than 5% (p< 0.05) was considered as significant; NS = Non-significant.

Evaluation of aflibercept against early B16 melanoma in Swiss nude female mice

Agent (batch)	Route	Dosage in mg/kg/ adm	Schedule in days	Total dose in mg/kg	Drug death (day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Median tumor weight in mg on day 17 (range)	T/C in % day 17	Time for median tumor to reach 1000 mg in days	T-C in days	log cell kill gross/ net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C03002D600B21A)	S.C.	40.0	3,6,10,13,17,20	240.0	0/6	-0.8 (5)	0 (0-136)	0	30.7	19.8	5.4/ 0.5	HDT, highly active	0.0008
		25.0		150.0	0/6	-1.6 (5)	0 (0-1565)	0	29.2	18.3	5.0/ 0.1	Highly active	0.0004
		10.0		60.0	0/6	+9.0 (21)	20 (0-116)	0	25.1	14.2	3.9/ -1.0	Highly active	0.0008
		2.5		15.0	0/6	-0.5 (5)	605 (254-2088)	15	19.7	8.8	2.4/ -2.5	Active	0.0008
Vehicle (C03001V700A21A)	S.C.		3,6,10,13,17		0/8		4059 (3244-7963)		10.9				
Control							5172 (1056-6527)		10.1				

Tumor doubling time = 1.1 days.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 22.64 g; Vehicle = 21.32 g), dosages were adjusted to the individual body weights.

Treatment duration: 18 days.

Abbreviations used: T/C = tumor growth inhibition on day 17, (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

* Log-rank multiple comparisons test versus control (Statistical report n°: RP08021-EN-E01). A probability less than 5% (p<0.05) was considered as significant.

Evaluation of aflibercept against advanced B16 melanoma in BALB/c nude female mice

Agent (batch)	Route	Dosage in mg/kg/ adm	Schedule in days	Total dose in mg/kg	Drug death (day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 1000 mg in days	T-C in days	log cell kill gross	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C03002D600B21A)	S.C.	40.0	7,10,13	120.0	0/5	+4.6 (14)	18.3	8.6	2.6	HDT, active	0.0001
		25.0		75.0	0/5	+6.0 (14)	16.7	7.0	2.1	Active	0.0049
		10.0		30.0	0/5	+4.7 (14)	15.9	6.2	1.9	Active	0.0081
		2.5		7.5	0/5	+1.0 (14)	12.6	2.9	0.9	Active	NS
Control							9.7				

Tumor doubling time = 1 day.

Tumor size at start of therapy was 63 - 262 mg with a median tumor burden per group of 113 - 125 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 17.81 - 19.58 g), dosages were adjusted to the individual body weights.

Treatment duration: 7 days.

Abbreviations used: (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

* Kruskal-Wallis multiple comparisons test versus control (Statistical report n°: RP08015-EN-E01). A probability less than 5% (p< 0.05) was considered as significant; NS = Non-significant.

Evaluation of aflibercept against advanced human melanoma A-375 in BALB/c nude female mice

Agent (batch)	Route	Dosage in mg/kg/adm	Schedule in days	Total dose in mg/kg	Drug death (day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 750 mg in days	T-C in days	log cell kill gross/net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept	S.C.	120	10, 13, 17, 20	480	0/5	-4.8 (24)	23.0	9.5	1.9/-0.3	HDT, active	0.0012
(C03002D600B22A)	0.1 ml	40		160	0/5	-4.7 (32)	19.7	6.2	1.2/-1.0	Active	0.0058
and		10		40	0/5	-8.1 (27)	15.4	1.9	0.4/-1.8	Inactive	0.0099
C04003M600B11)		4		16	0/5	-21.6 (27)	14.5	1.0	0.2/-2.0	Inactive	NS
Control						-26.3 (24)	13.5				

Tumor doubling time = 1.5 days.

Tumor size at start of therapy was 95 – 244 mg with a median tumor burden per group of 166 - 189 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 19.84 - 23.10 g), dosages were adjusted to the individual body weights

Treatment duration: 11 days.

Abbreviations used: (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

* Kruskal-Wallis multiple comparisons test versus control (Statistical report n°: RP08019-EN-E01). A probability less than 5% (p<0.05) was considered as significant; NS = Non-significant.

Evaluation of aflibercept against advanced human melanoma LOX in BALB/c nude female mice

Agent (batch)	Route	Dosage in mg/kg/adm	Schedule in days	Total dose in mg/kg	Drug death (day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 750 mg in days	T-C in days	log cell kill gross	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept	S.C.	40	7, 10, 14	120	0/10	-1.5 (21)	17.7	5.2	0.6	HDT, inactive	0.0007
(C04003M600B11)	0.1 ml										
Control							12.5				

Tumor doubling time = 1.5 days.

Tumor size at start of therapy was 63- 169 mg with a median tumor burden per group of 96 - 112 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 18.64 - 23.59 g), dosages were adjusted to the individual body weights.

Treatment duration: 8 days.

Abbreviations used: (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

* Wilcoxon test (Statistical report n°: RP08020-EN-E01). A probability less than 5% (p<0.05) was considered as significant.

Evaluation of aflibercept against advanced human prostate carcinoma DU 145 in SCID female mice

Agent (batch)	Route	Dosage in mg/kg/adm	Schedule in days	Total dose in mg/kg	Drug death (Day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 800 mg in days	T-C in days	log cell kill gross /net	Regressions	Tumor free survivors day 127	Comments	Statistical difference on time-to-reach versus control (p-value)*	
Partial Complete														
Aflibercept	S.C.	40.0	18, 22, 25,	240.0	0/5	-4.7 (27)	86.7 *	54.6	4.7/3.1	3/5	2/5	1/5	HDT, highly active	0.0030
(C04003M600B11)	0.1 ml	25.0	29 32, 35	150.0	0/5	-4.1 (33)	72.8	40.7	3.5/2.0	1/5	1/5	0/5	Highly Active	0.0030
		10.0		60.0	0/5	-3.3 (20)	60.5	28.4	2.4/0.9	0/5	0/5	0/5	Active	0.0030
		2.5		15.0	0/5	-4.5 (20)	45.4	13.3	1.1/-0.4	0/5	0/5	0/5	Active	0.0030
Control						-2.4 (19)	32.1			0/10	0/10	0/10		

Tumor doubling time = 3.5 days.

Tumor size at start of therapy was 103 - 201 mg, with a median tumor burden per group of 138 - 145 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 18.23 – 23.65 g), dosages were adjusted to the individual body weights.

Treatment duration: 18 days.

Abbreviations used: (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

* Log-rank multiple comparisons test versus control (Statistical report n°: RP08022-EN-E01). A probability less than 5% (p<0.05) was considered as significant; NS = Non-significant.

Evaluation of aflibercept against advanced human prostate adenocarcinoma PC-3 in SCID female mice

Agent (batch)	Route	Dosage in mg/kg/ adm	Schedule in days	Total dose in mg/kg	Drug death (Day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 1000 mg in days	T-C in days	log cell kill gross/net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C04003M600B11)	S.C. 0.1 ml	40	13, 16, 20, 23	160	0/5	-9.0 (33)	25.7	3.6	0.3/-0.6	HDT, inactive	0.0303
Control						-10.0 (30)	22.1				

Tumor doubling time = 4.0 days.

Tumor size at start of therapy was 100 - 262 mg, with a median tumor burden per group of 144 - 163 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 20.12- 21.85 g), dosages were adjusted to the individual body weights.

Treatment duration: 11 days.

Abbreviations used: (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

* Wilcoxon test (Statistical report n°: RP08023-EN-E01). A probability less than 5% (p<0.05) was considered as significant.

Evaluation of aflibercept against advanced human mammary adenocarcinoma UISO-BCA-1 in BALB/c nude female mice

Agent (batch)	Route	Dosage in mg/kg/ adm	Schedule in days	Total dose in mg/kg	Drug death (day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 750mg in days	T-C in days	log cell kill gross/net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C03002D600B22A)	S.C. 0.1 ml	40.0 25.0 10.0 2.5	19,24,28 32,35,39	240.0 150.0 60.0 15.0	0/5 0/5 0/5 0/5	-2.9 (34) - 3.0 (34) - 3.2 (23) - 2.9 (38)	63.1 50.2 49.4 33.5	29.9 17.0 16.2 0.3	2.1/ 0.6 1.2/-0.3 1.1/ -0.3 0.1/ -1.4	HDT, active Active Active Inactive	0.0017 0.0653 0.0144 NS
Control						- 5.5 (47)	33.2				

Tumor doubling time = 4.3 days.

Tumor size at start of therapy was 70 - 219 mg with a median tumor burden per group of 93 - 112 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 20.65 - 23.89 g), dosages were adjusted to the individual body weights.

Treatment duration: 21 days.

Abbreviations used: (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

* Kruskal-Wallis multiple comparisons test versus control (Statistical report n°: RP08024-EN-E01). A probability less than 5% (p<0.05) was considered significant; NS = Non-significant.

Evaluation of aflibercept against early and advanced human pancreatic tumor BxPC-3 in BALB/c nude female mice

Agent (batch)	Route	Dosage in mg/kg/ adm	Schedule in days	Total dose in mg/kg	Drug death (day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Median tumor weight in mg on day 39 (range)	T/C in % day 39	Time for median tumor to reach 750 mg in days	T-C in days	Log cell kill gross/net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Early stage BxPC-3													
Aflibercept (C03002D600B22A)	S.C. 0.1 ml	40.0 25.0 10.0 2.5	3, 6, 10, 13, 17, 20	240.0 150.0 60.0 15.0	0/5 0/5 0/5 0/5	+ 7.7 (21) + 5.9 (21) - 8.4 (12) + 6.9 (21)	112 (54-132) 103 (50-218) 175 (50-383) 269 (63-486)	11 10 17 26	70.8 66.8 74.4 54.8	38.1 34.1 41.7 22.1	1.9/1.0 1.7/0.8 2.1/1.2 1.1/0.2	HDT, active Active Active Active	0.0102 0.0102 0.0065 0.0991
Control							1024 (383-1697)		32.7				
Advanced stage BxPC-3													
Aflibercept (C03002D600B22A)	S.C. 0.1 ml	40	10, 13, 17, 20, 24, 27	240	0/5	- 2.9 (12)			56.2	25.8	1.3/0.4	Active	0.0007
Control									30.4				

Tumor doubling time = 6.1 days.

For advanced stage, tumor size at start of therapy was 84 - 172 mg, with a median tumor burden per group of 115 mg.

Mice weight (average): aflibercept at early stage = 18.57 g. At advanced stage, due to body weight heterogeneity (range for aflibercept = 19.21 - 21.73 g), dosages were adjusted to the individual body weights.

Treatment duration: 18 days.

Abbreviations used: T/C = tumor growth inhibition on day 39, (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

* For early stage: Log-Rank multiple comparisons test versus control; For advanced stage: Log-Rank test (Statistical report n°: RP08016-EN-E01). A probability less than 5% (p<0.05) was considered significant; NS = Non-significant.

Evaluation of aflibercept against advanced human pancreatic carcinoma PANC-1 in SCID female mice

Agent (batch)	Route	Dosage in mg/kg/ adm	Schedule in days	Total dose in mg/kg	Drug death (Day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 1000 mg in days	T-C in days	log cell kill gross/net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C04003M600B11)	S.C.	40.0	10,13,17,20	160.0	0/5	-7.2 (22)	19.5	2.9	0.4/-1.2	HDT, inactive	0.0063
		10.0		40.0	0/5	-8.3 (28)	17.7	1.1	0.2/-1.5	Inactive	NS
		2.5		10.0	0/5	-6.3 (28)	17.6	1.0	0.2/-1.5	inactive	NS
Control						-3.4 (21)	16.6				

Tumor doubling time = 2 days.

Tumor size at start of therapy was 93 - 221 mg with a median tumor burden per group of 152 - 168 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 18.16 - 22.70 g), dosages were adjusted to the individual body weights.

Treatment duration: 11 days.

Abbreviations used: (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

* Kruskal-Wallis multiple comparisons test versus control (Statistical report n°: RP08027-EN-E01). A probability less than 5% (p<0.05) was considered significant; NS = Non-significant.

Evaluation of aflibercept against advanced human gastric adenocarcinoma MKN-45 in SCID female mice

Agent (batch)	Route	Dosage in mg/kg/ adm	Schedule in days	Total dose in mg/kg	Drug death (Day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 650 mg in days	T-C in days	log cell kill gross/net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C04003M600B11)	S.C.	40.0	8, 12, 15, 19	160.0	0/5	-6.4 (63)*	56.4	38.2	2.8/1.9	HDT, highly active	<0.0001
		10.0		40.0	0/5	-5.1 (47)*	39.7	21.5	1.6/0.7	Active	0.0118
		2.5		10.0	0/5	-4.9 (39)*	33.6	15.4	1.1/0.2	Active	0.0273
Control							18.2				

Tumor doubling time = 4.1 days.

Tumor size at start of therapy was 72 - 201 mg, with a median tumor burden per group of 112 - 115 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 18.64 - 21.91 g), dosages were adjusted to the individual body weights.

Treatment duration: 12 days.

Abbreviations used: (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

Comment: * Drug body weight losses were calculated by subtracting the tumor induced- body weight loss from the total body weight loss (a maximal body weight loss of 11.8% was recorded on day 26 in the control group)

* Kruskal-Wallis multiple comparisons test versus control (Statistical report n°: RP08028-EN-E01). A probability less than 5% (p<0.05) was considered significant

Evaluation of aflibercept against advanced human gastric carcinoma Hs746T in SCID female mice

Agent (batch)	Route	Dosage in mg/kg/ adm	Schedule in days	Total dose in mg/kg	Drug death (Day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 1000 mg in days	T-C in days	log cell kill gross/net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C04003M600B11)	S.C.	40.0	10, 13, 17, 20	160.0	0/5	-3.1 (11)	17.6	4.1	1.1/-1.9	HDT, active	0.0063
		10.0		40.0	0/5	-1.1 (11)	18.4	4.9	1.3/-1.7	Active	0.0024
		2.5		10.0	0/5	-0.0 (11)	15.3	1.8	0.5/-2.5	Inactive	0.0445
Control							13.5				

Tumor doubling time = 1.1 day.

Tumor size at start of therapy was 107-227 mg, with a median tumor burden per group of 163 - 174 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 18.90 - 24.41 g), dosages were adjusted to the individual body weights.

Treatment duration: 11 days

Abbreviations used: (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous

* Kruskal-Wallis multiple comparisons test versus control (Statistical report n°: RP08029-EN-E01). A probability less than 5% (p<0.05) was considered significant

Evaluation of aflibercept against advanced human gastric carcinoma SNU-5 in SCID female mice

Agent (batch)	Route	Dosage in mg/kg/ adm	Schedule in days	Total dose in mg/kg	Drug death (Day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 750 mg in days	T-C in days	log cell kill gross/net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C04003M600B11)	S.C. 0.1 ml	40.0	10, 14, 17,	240.0	0/5	-4.6 (11)	46.0	26.8	2.4/ 0.7	HDT, active	0.0005
		10.0	21, 24, 28	60.0	0/5	-3.2 (11)	46.5	27.3	2.5/ 0.8	Active	0.0004
		2.5		15.0	0/5	-3.8 (11)	34.0	14.8	1.4/-0.4	Active	0.0628
Control						-2.3 (11)	19.2				

Tumor doubling time = 3.3 days.

Tumor size at start of therapy was 102 - 188 mg, with a median tumor burden per group of 119 - 126 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 19.86 - 23.05 g) dosages were adjusted to the individual body weights.

Treatment duration: 19 days

Abbreviations used: (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

* Kruskal-Wallis multiple comparisons test versus control (Statistical report n*: RP08034-EN-E01). A probability less than 5% (p<0.05) was considered significant.

Evaluation of aflibercept against early murine adenocarcinoma C51 in SCID female mice

Agent (batch)	Route	Dosage in mg/kg/ adm	Schedule in (days)	Total dose mg/ kg	Drug death (day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Median tumor weight in mg on day 11 (range)	T/C in % day 11	Time for median tumor to reach 1000 mg in days	T-C in days	log cell kill gross/ net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C04003M600B11)	S.C. 0.1 ml	40.0	3, 6, 10, 13	240.0	0/5	- 1.6 (8)	36 (0-58)	4	32.2	20.7	4.2/ 0.5	HDT, highly active	<0.0001
		10.0	17, 20	60.0	0/5	- 2.3 (6)	72 (14-80)	8	26.8	15.3	3.1/ - 0.5	Highly active	0.0039
		2.5		15.0	0/5	- 3.6 (7)	203 (75-378)	24	16.9	5.4	1.1/ - 2.5	Active	0.0628
Control							848 (651-1564)		11.5				

Tumor doubling time = 1.5 days.

Mice average weight for aflibercept = 20.96 g.

Treatment duration: 18 days.

Abbreviations used: T/C = tumor growth inhibition on day 11, (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

* Kruskal-Wallis multiple comparisons test versus control (Statistical report n*: RP08035-EN-E01). A probability less than 5% (p<0.05) was considered significant.

Evaluation of aflibercept against advanced murine adenocarcinoma C51 in SCID female mice

Agent (batch)	Route	Dosage in mg/kg /adm	Schedule in days	Total dose in mg/kg	Drug death (day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 750 mg in days	T-C in days	log cell kill gross	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C04003M600B11)	S.C. 0.1ml	40.0	9,14	80.0	0/5	+ 2.9 (15)	19.1	7.9	2.0	HDT, active	0.0016
		25.0		50.0	0/5	- 2.3 (11)	15.9	4.7	1.2	Active	0.0025
		10.0		20.0	0/5	- 1.1 (11)	11.7	0.5	0.1	Inactive	NS
		2.5		5.0	0/5	- 7.1 (18)	12.1	0.9	0.2	Inactive	NS
Control						- 10.7 (16)	11.2				

Tumor doubling time = 1.2 days.

Tumor size at start of therapy was 200 - 397 mg with a median tumor burden per group of 261 - 271 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 19.40 - 22.55 g), dosages were adjusted to the individual body weights.

Treatment duration: 6 days.

Abbreviations used: (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

* Kruskal-Wallis multiple comparisons test versus control (Statistical report n*: RP08037-EN-E01). A probability less than 5% (p<0.05) was considered significant; NS = Non-significant.

Evaluation of aflibercept against advanced human colon adenocarcinoma HT-29 in SCID female mice

Agent (batch)	Route	Dosage in mg/kg/adm	Schedule in days	Total dose in mg/kg	Drug death (day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 750 mg in days	T-C in days	log cell kill gross/net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C04003M600B11)	S.C.	40.0	10, 13, 17,	240.0	0/5	+ 2.6 (28)	47.3	27.2	2.2/0.7	HDT, active	0.0017
	0.1 ml	10.0	20, 24, 27	60.0	0/5	+ 2.0 (28)	43.1	23.0	1.8/0.4	Active	0.0017
		2.5		15.0	0/5	- 10.6 (31)*	28.9	8.8	0.7/-0.7	active	0.0017
Control						- 11.2 (30)*	20.1				

Tumor doubling time = 3.8 days.

Tumor size at start of therapy was 103-193 mg with a median tumor burden per group of 132 – 136 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 19.20 – 22.38 g), dosages were adjusted to the individual body weights.

Treatment duration: 18 days.

Abbreviations used: (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

Comment: * Delayed body weight loss which could be attributed to tumor ulceration.

* Log-rank multiple comparisons test versus control (Statistical report n*: RP08038-EN-E01). A probability less than 5% (p<0.05) was considered significant.

Evaluation of aflibercept against early human colon carcinoma HCT 116 in Swiss nude female mice

Agent (batch)	Route	Dosage in mg/kg /adm	Schedule in days	Total dose in mg/kg	Drug death (day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Median tumor weight in mg on day 20 (range)	T/C in % day 20	Time for median tumor to reach 1000 mg in days	T-C in days	log cell kill gross/net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C03002D600B22A)	S.C.	40.0	4,7,11,14	160.0	0/5	- 4.6 (10)	32 (14-37)	4	47.9	25.2	2.5/1.4	HDT, active	0.0121
	0.1 ml	25.0		100.0	0/5	- 1.4 (6)	14 (0-40)	2	44.8	22.1	2.2/1.1	Active	0.0159
		10.0		40.0	0/5	- 13.2 (14)	18 (0-75)	2	44.6	21.9	2.2/1.1	Active	0.0174
		2.5		10.0	0/5	- 1.1 (6)	171 (72-284)	22	35.9	13.2	1.3/0.2	Active	NS
Control							776 (14-1469)		22.7				

Tumor doubling time = 3 days.

Mice average weight (range for aflibercept = 24.66 - 25.58 g).

Treatment duration: 11 days.

Abbreviations used: T/C = tumor growth inhibition on day 20, (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

* Kruskal-Wallis multiple comparisons test versus control (Statistical report n*: RP08039-EN-E01). A probability less than 5% (p<0.05) was considered significant; NS = Non-significant

Evaluation of aflibercept against advanced murine carcinoma HCT 116 in BALB/c nude female mice

Agent (batch)	Route	Dosage in mg/kg/ adm	Schedule in days	Total dose in mg/kg	Drug death (day of death)	Average body weight change in % per mouse (day) ^a	Time for median tumor to reach 1000 mg in days	T-C in days	Log cell kill gross/net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C03002D600B22A)	S.C.	40.0	13,17,20	240.0	0/5	-1.1 (32)	56.9	34.1	2.6/1.2	HDT, active	0.0027
	0.1ml	25.0	24,27,31	150.0	0/5	- 1.8 (32)	61.8	39.0	3.0/1.5	Active	0.0027
		10.0		60.0	0/5	- 2.8 (32)	48.4	25.6	2.0/0.5	Active	0.0064
		2.5		15.0	0/5	- 9.3 (32)	43.3	20.5	1.6/0.1	Active	0.0232
Control						- 20.6 (33)	22.8				

Tumor doubling time = 3.9 days.

Tumor size at start of therapy was 77 - 255 mg with a median tumor burden per group of 100 – 114 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 18.04 – 21.75 g), dosages were adjusted to the individual body weights.

Treatment duration: 19 days.

Abbreviations used: (T-C) = tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

Comment: * An excessive body weight loss was induced by the tumor. The body weight change in the treated groups was indicated 24 hours post last treatment.

* Log-rank multiple comparisons test versus control (Statistical report n*: RP08040-EN-E01). A probability less than 5% (p<0.05) was considered significant

Evaluation of aflibercept and sorafenib against advanced human neuroblastoma SK-N-MC in SCID female mice

Agent (batch)	Route	Dosage in mg/kg/adm	Schedule in days	Total dose in mg/kg	Drug death (Day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 750 mg in days	T-C in days	log cell kill gross/net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C04003M600B11)	S.C. 0.1 ml	40	14,17,21,24,28	200	0/5	-3.2 (22)	62.3	36.6	2.5 / 1.5	HDT, active	0.0007
Sorafenib (P-33789-130-1)	P.O. 0.2 ml	100	14-18, 21-25, 28	1100	0/5	-9.4 (26)	37.3	11.6	0.8 / -0.2	HDT, marginally active	0.0007
Control						-1.2 (15)	25.7				

Tumor doubling time = 4.4 days.

Tumor size at start of therapy was 63 - 132 mg, with a median tumor burden per group of 88 - 96 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 19.40 - 21.30 g, sorafenib = 20.02 - 23.69 g) dosages were adjusted to the individual body weights.

Treatment duration for aflibercept and sorafenib = 15 days.

Abbreviations used: (T-C) = Tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous, P.O. = Per os.

* Wilcoxon test (Statistical report n°: RP08030-EN-E01). A probability less than 5% (p<0.05) was considered significant.

Evaluation of aflibercept against advanced human embryonic neuroblastoma SK-N-AS in SCID female mice

Agent (batch)	Route	Dosage in mg/kg/adm	Schedule in days	Total dose in mg/kg	Drug death (Day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 1000 mg in days	T-C in days	log cell kill gross/net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C04003M600B11)	S.C. 0.1 ml	40	17, 21, 24, 28	160	0/10	-2.0 (19)	26.7	3.9	0.6/-1.3	HDT, inactive	0.0036
Control							22.8				

Tumor doubling time = 1.9 days.

Tumor size at start of therapy was 107 - 198 mg, with a median tumor burden per group of 143 - 147 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 19.63 - 23.34 g) dosages were adjusted to the individual body weights.

Treatment duration: 12 days.

Abbreviations used: (T-C) = Tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

* Log-rank test (Statistical report n°: RP08041-EN-E01). A probability less than 5% (p<0.05) was considered significant.

Evaluation of aflibercept against advanced human rhabdomyosarcoma RH-30 in SCID female mice

Agent (batch)	Route	Dosage in mg/kg/adm	Schedule in days	Total dose in mg/kg	Drug death (Day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 650 mg in days	T-C in days	log cell kill gross/net	Regressions	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C04003M600B11)	S.C. 0.1 ml	40	14,17,21,24	160	0/6	-5.6 (28)	44.3	23.7	2.0 / 1.1	2/6	0/6	0.0002
Control						-10.6 (37)	20.6			0/10	0/10	

Tumor doubling time = 3.6 days.

Tumor size at start of therapy was 97 - 202 mg with a median tumor burden per group of 157 - 158 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 20.73 - 23.70 g), dosages were adjusted to the individual body weights.

Treatment duration in days: 11 days.

Abbreviations used: (T-C) = Tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous.

* Wilcoxon test (Statistical report n°: RP08031-EN-E01). A probability less than 5% (p<0.05) was considered significant.

Evaluation of aflibercept and sorafenib against advanced TC-71 Ewing's sarcoma in SCID female mice

Agent (batch)	Route	Dosage in mg/kg/ adm	Schedule in days	Total dose in mg/kg	Drug death (day of death)	Average body weightchange in % per mouseat nadir (day of nadir)	Time for median tumor to reach 1000 mg in days	T-C in days	log cell kill gross	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C04003M600B11)	S.C. 0.1 ml	40	10, 13, 17	120	0/6	-4.2 (15)	35.1	19.6	3.7	HDT, highly active	0.0002
Sorafenib (P-33789-130-1)	P.O. 0.2 ml	100	10-14, 17	600	0/6	-5.1 (16)	21.6	6.1	1.1	HDT, active	0.0002
Control							15.5				

Tumor doubling time = 1.6 days.

Tumor size at start of therapy was 71 - 201 mg, with a median tumor burden per group of 131 - 138 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 20.42 - 22.64g, sorafenib = 20.44 - 22.68 g) dosages were adjusted to the individual body weight.

Treatment duration: aflibercept and sorafenib = 8 days.

Abbreviations used: (T-C) = Tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous, P.O. = Per os.

* Wilcoxon test (Statistical report n°: RP08032-EN-E01). A probability less than 5% (p<0.05) was considered significant.

Evaluation of aflibercept and sorafenib against advanced SK-ES-1 Ewing's sarcoma in SCID female mice

Agent (batch)	Route	Dosage in mg/kg/ adm	Schedule in days	Total dose in mg/kg	Drug death (Day of death)	Average body weightchange in % per mouseat nadir (day of nadir)	Time for median tumor to reach 1000 mg in days	T-C in days	log cell kill gross/net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C04003M600B11)	S.C. 0.1 ml	40	11, 14, 18, 21	160	0/6	-1.2(12)	45.3	25.5	3.2/1.8	HDT, highly active	0.0002
Sorafenib (P-33789-130-1)	P.O. 0.2 ml	100	11-21	1100	0/6	-11.1(23)	30.8	11	1.4/0.0	HDT, active	0.0002
Control							19.8				

Tumor doubling time = 2.4 days.

Tumor size at start of therapy was 64 - 120 mg, with a median tumor burden per group of 96 - 100 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 18.33 - 21.51 g, sorafenib = 18.05 - 20.46 g), dosages were adjusted to the individual body weights.

Treatment duration: aflibercept and sorafenib = 11 days.

Abbreviations used: (T-C) = Tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous, P.O. = Per os.

Wilcoxon test (Statistical report n°: RP08033-EN-E01). A probability less than 5% (p<0.05) was considered significant.

Evaluation aflibercept against advanced human Burkitt lymphoma NAMALWA in SCID female mice

Agent (batch)	Route	Dosage in mg/kg/ adm	Schedule in days	Total dose in mg/kg	Drug death (Day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 1000 mg in days	T-C in days	log cell kill gross/net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C04003M600B11)	S.C. 0.1 ml	40	10, 14, 17, 21	160	0/5	-1.0 (11)	28.3	12.7	2.3 / 0.1	HDT, active	0.0406
		25		100	0/5	-1.5 (12)	27.7	12.1	2.1 / 0.0	Active	0.0392
		10		40	0/5	-3.0 (11)	28.5	12.9	2.3 / 0.2	Active	0.0041
Cyclophosphamide (084K1328)	I.V. 0.2 ml	286.1	10, 14	572.2	3/5 (2d20, 46)	-28.4 (21)	-	-	-	Toxic	
		177.4		354.8	0/5	-14.5 (19)	46.2	30.6	5.4 / 4.5	HNTD, highly active	<0.0001
		110.0		220.0	0/5	-6.8 (15)	35.3	19.7	3.5 / 2.6	Highly, active	0.0065
		68.2		136.4	0/5	-3.6 (17)	25.5	9.9	1.8 / 0.9	Active	NS
Vincristine (084K1738)	I.V. 0.2 ml	2.4	10, 14	4.8	5/5 (18,20, 3d21)	-31.7 (17)	-	-	-	Toxic	
		1.5		3	0/5	-16.7 (17)	25.1	9.5	1.7 / 0.8	HNTD, active	0.0023
		0.9		1.8	0/5	-3.6 (14)	19.5	3.9	0.7 / -0.2	Marginally active	NS
Control							15.6				

Tumor doubling time = 1.7 days.

Tumor size at start of therapy was 66-166 mg, with a median tumor burden per group of 101-107 mg, except for the highest dose of vincristine and cyclophosphamide.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 17.66 - 22.24 g, cyclophosphamide = 18.22 - 22.64 g,

vincristine = 18.66 - 23.01 g) dosages were adjusted to the individual body weights.

Treatment duration: aflibercept = 12 days, cyclophosphamide and vincristine = 5 days.

Abbreviations used: HNTD = highest nontoxic dose, HDT = highest dose tested, (T-C) = tumor growth delay, S.C. = subcutaneous, I.V. = intravenous.

* Kruskal-Wallis multiple comparisons test versus control (Statistical report n°: RP08036-EN-E01). A probability less than 5% (p<0.05) was considered significant; NS = Non-significant.

Evaluation of aflibercept and vincristine against advanced human lymphoma WSU-DLCL2 in SCID female mice

Agent (batch)	Route	Dosage in mg/kg/ adm	Schedule in days	Total dose in mg/kg	Drug death (Day of death)	Average body weight change in % per mouse at nadir (day of nadir)	Time for median tumor to reach 1000 mg in days	T-C in days	log cell kill gross/net	Comments	Statistical difference on time-to-reach versus control (p-value)*
Aflibercept (C04003M600B11)	S.C. 0.1ml	40	15,19,23	120	0/5	-3.0 (22)	34.9	13.3	2.0/ 0.6	HDT, active	0.0003
Vincristine (084K1738)	I.V. 0.2ml	1.3	15,19	2.6	0/5	-8.1 (18)	29.7	8.1	1.2/ 0.5	HDT, active	0.0003
Control						-1.6 (17)	21.6				

Tumor doubling time = 2 days.

Tumor size at start of therapy was 80 – 132 mg, with a median tumor burden per group of 106 – 109 mg.

Mice average weight: Due to body weight heterogeneity (range for aflibercept = 20.10 - 22.93 g, vincristine = 21.90 - 23.67g), dosages were adjusted to the individual body weights.

Treatment duration: aflibercept = 9 days, vincristine = 5 days.


Abbreviations used: (T-C) = Tumor growth delay, HDT = highest dose tested, S.C. = subcutaneous, I.V. = intravenous.

* Wilcoxon test (Statistical report n°: RP08042-EN-E01). A probability less than 5% (p<0.05) was considered significant.

(tables excerpted from applicant's BLA)

4.3 Safety Pharmacology

Study title: A Study to Determine the Effects of VEGF TRAP on Wound Healing in an Incisional Wound Model in Rabbits

Study no.: PMA00018 (VGFT TX 06010)
 Study report location: Module 4.2.1.3
 Conducting laboratory and location:  (b) (4)

Date of study initiation: February 14, 2007
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #: VEGF Trap, lot # C04008D630B11C

Summary:

The objective of this study was to determine the potential effects of VEGF-Trap on wound repair and healing in the rabbit incisional wound healing model. All animals (6 treatment groups of 12 male New Zealand white rabbits) underwent model induction on Day 1, during which 4 full-thickness incisional wounds, two on each side of the dorsal midline, were created. Animals assigned to Groups 1 and 2 were dosed once daily beginning on Day -1 until necropsy via subcutaneous (SC) injection. Group 1 animals received the positive control, dexamethasone, at a dose level of 2 mg/kg. Group 2 animals received the negative control, 0.9% sodium chloride for injection. Animals assigned to Groups 3–6 were dosed via a 30-minute intravenous (IV) infusion on Days -2, 3, 7, and 11. Group 3 animals were dosed with vehicle control, and animals assigned to Groups 4–6 received test article, VEGF-Trap, at dose levels of 0.3, 3, and 30 mg/kg/administration, respectively.

Recorded assessments included clinical observations (daily), body weights (weekly) and food consumption (daily). Blood samples were collected for antibody (pre-dose and

before necropsy) and toxicokinetic (pre-dose, 5 minutes post infusion on Day -2, 24 hours post-dose on Day -1, Day 1 before surgery, Day 3 before dosing, and before necropsy) analysis. Four animals from each of the six treatment groups were euthanized on Days 4, 8, and 12. All incisional wounds were scored for erythema and swelling at each respective time point and then prepared for either biomechanical or histological evaluation. Three incisional wounds from each of the four animals at each time point underwent tensile strength evaluation. One incisional wound from each of the four animals at each time point underwent histopathology evaluation.


No adverse clinical observations, effects on body weight or food consumption, or macroscopic or microscopic effects associated with treatment with VEGF-Trap in any group were seen. Toxicokinetic analyses indicated that all animals in Groups 4–6 were exposed to VEGF-Trap for the entire duration of the study.

Treatment-related changes in blood vessel density were observed in the VEGF-Trap groups (0.3, 3, or 30 mg/kg/administration) at Day 4. There was also a dose-dependent decrease in the overall density of blood vessels at the wound site with increasing VEGF-Trap dose at Days 8 and 12, though blood vessel densities at Days 8 and 12 at the 0.3 mg/kg/administration were comparable to the controls (Groups 1, 2, and 3). Rabbits at 30 mg/kg/administration had the lowest overall blood vessel density at Days 8 and 12. In addition, the positive control group had less inflammation than the other groups, though overall wound healing in this group was comparable to the other groups.

All the VEGF-Trap treated groups demonstrated reduced tensile strength as compared to Group 2 (negative control) at all time points, attaining statistical significance on Day 12 for the 0.3 and 30 mg/kg/administration groups. The reduced tensile strength in the VEGF-Trap treated groups correlated with a histopathological reduction in vascularization.

In conclusion, wound repair and healing in the rabbit incisional model was inhibited after repeat administration with VEGF-Trap (AVE0005) at dose levels of 0.3, 3, and 30 mg/kg/administration as demonstrated by a reduction in blood vessel density and tensile strength evaluation. Time-to-recovery appeared dose-dependent.

Study title: A Study to Determine the Effects of VEGF TRAP on Wound Healing in an Excisional Wound Model in Rabbits

Study no.: PMA00019 (VGFT TX 06011)
Study report location: Module 4.2.1.3
Conducting laboratory and location:  (b) (4)
Date of study initiation: April 27, 2007
GLP compliance: Yes

QA statement: Yes

Drug, lot #: VEGF Trap, lot # C04008D630B11C

Summary:

The objective of this study was to determine the potential effects of VEGF-Trap on wound repair and healing in the rabbit excisional wound healing model. All animals (6 treatment groups of 9 male New Zealand white rabbits) underwent model induction on Day 1, during which 12 full-thickness excisional wounds, six on each dorsal side of the animal, were created. Animals assigned to Groups 1 and 2 were dosed once daily beginning on Day -1 until necropsy via subcutaneous (SC) injection. Group 1 animals received the positive control, dexamethasone, at a dose level of 2 mg/kg. Group 2 animals received the negative control, 0.9% sodium chloride for injection. Animals assigned to Groups 3–6 were dosed via a 30-minute intravenous (IV) infusion on Days -2, 5, 11, and 17. Group 3 animals were dosed with vehicle control, and animals assigned to Groups 4–6 received test article, VEGF-Trap, at dose levels of 0.3, 3, and 30 mg/kg/administration, respectively.

Recorded assessments included clinical observations (daily), body weights (weekly) and food consumption (daily). Blood samples were collected for antibody (pre-dose and before necropsy) and toxicokinetic (pre-dose, 5 minutes post infusion on Day -2, 24 hours post-dose on Day -1, Day 1 before surgery, Day 3 before dosing, and before necropsy). Three animals from each of the six treatment groups were euthanized on Days 8, 14, and 20. All excisional wounds were scored for erythema and swelling at each respective time point and then prepared for either biomechanical or histological evaluation.

No adverse clinical observations or effects on body weight or food consumption were noted. Toxicokinetic analyses indicated that all animals in Groups 4–6 were exposed to VEGF-Trap for the entire duration of the study. Histopathologic and morphometric findings included inflammation and necrosis in Groups 2 and 3 (negative and vehicle control groups, respectively) and were associated with normal wound healing. Groups 1, 4, 5, and 6 (positive control, 0.3, 3, and 30 mg/kg, respectively) had minimal or less inflammation and necrosis at all time points. A robust fibrous response was observed in Groups 2 and 3 (negative and vehicle control groups, respectively). Group 4 (0.3 mg/kg) had a minimal fibrous response by Day 8 that increased considerably by Day 14 so that it resembled Groups 2 and 3 (negative and vehicle control groups, respectively) at the last 2 time points. A minimal fibrous response was observed in Groups 1, 5, and 6 (positive control, 3, and 30 mg/kg, respectively) at Day 8 that increased to mild at Day 14 and moderate by Day 20. Generally, these wounds remained partially cavitated due to incomplete filling by fibrous tissue.

Neovascularization reached high levels in Groups 2 and 3 (negative and vehicle control groups, respectively) at Days 8 and 14, correlating with high levels of fibrosis/granulation tissue in these groups. Group 4 (0.3 mg/kg) had minimal neovascularization at Day 8, that increased by Day 14 so that it resembled Groups 2 and 3 (negative and vehicle control groups, respectively). Neovascularization was

minimally detectable by Day 20 in Groups 2, 3, and 4 (negative control, vehicle control, and 0.3 mg/kg, respectively). In Groups 1, 5, and 6 (positive control, 0.3, and 30 mg/kg, respectively), neovascularization was nearly undetectable at all time points, correlating with the poor fibrous response observed in these groups.

The highest levels of hemorrhage/fibrin were observed at Day 8 in Groups 2 and 3 (negative and vehicle control groups, respectively). These diminished notably by Day 14 and were nearly absent by Day 20. Group 4 (0.3 mg/kg) had initially less hemorrhage/fibrin at Day 8 than Groups 2 and 3 (negative and vehicle control groups, respectively), but trended similarly at Days 14 and 20. Hemorrhage/fibrin was low in Groups 1, 5, and 6 (positive control, 3, and 30 mg/kg, respectively) at Day 8 but persisted or increased at Day 14 and then diminished slightly by Day 20. Groups 2, 3, and 4 (negative control, vehicle control, and 0.3 mg/kg, respectively) had the highest initial epidermal hyperplastic response at Day 8, slight to moderate hyperplasia at Day 14, and a dramatic drop by Day 20. There was wound closure in these groups, and the epidermis began to resemble mature tissue by Day 20. Group 1 (positive control) had minimal epidermal observed at Day 8, followed by a striking increase at Day 14, and then a moderate decrease by Day 20. The epidermis in this group also began to resemble mature tissue by Day 20. Groups 5 and 6 (3 and 30 mg/kg, respectively) had minimal and relatively unchanged levels of epidermal hyperplasia across all time points, indicating a poor hyperplastic response.

Open wounds, defined as incomplete epidermal coverage of the wound surface, were observed in less than 6% of the total wounds in Groups 1, 2, 3, and 4 (positive control, negative control, vehicle control, and 0.3 mg/kg, respectively) at Day 20, while over 19% of the wounds remained open in Groups 5 and 6 (3 and 30 mg/kg, respectively). The relative rates of re-epithelialization for Groups 5 and 6 (3 and 30 mg/kg, respectively) were 12% and 16% slower than the positive control, respectively, and 26% and 30% slower than the negative control, respectively. The time to reach 50% re-epithelialization of Group 4 (0.3 mg/kg) was midway between Group 1 (positive control) and Groups 2 and 3 (negative and vehicle controls, respectively). There was no notable difference in the relative rate of re-epithelialization between the negative and vehicle controls. Statistically significant differences in wound length and percent re-epithelialization between Group 2 (negative control) and the other groups were most prominent at Day 8. By Day 20, significant differences in wound length were only observed with Group 1 (positive control), while significant differences in percent re-epithelialization were only noted in Groups 5 and 6 (3 and 30 mg/kg, respectively). These differences were consistent with the delayed wound healing observed histologically, especially in Groups 5 and 6 (3 and 30 mg/kg, respectively).

In conclusion, repeated intravenous administration of VEGF-Trap at dose levels of 0.3, 3, and 30 mg/kg/administration resulted in an impairment of normal wound repair and healing in the rabbit excisional model on Days 8 and 14 as demonstrated by morphometry and/or histopathological examination. By Day 20, only the dose levels of VEGF-Trap at 3 and 30 mg/kg demonstrated delayed wound repair and healing.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The applicant submitted nonclinical studies assessing aflibercept's absorption in monkeys, distribution in rats, and pharmacokinetics in renally-impaired rats.

Absorption:

SNBL.223.3 (VGFT-PK-01012): A Single-Dose Intravenous and Subcutaneous Pharmacokinetic Study of VEGF Trap in Cynomolgus Monkeys. Reviewed by Maria I. Rivera and slightly modified for this BLA review.

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 subcutaneous (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 $t_{1/2}$ 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 non-linear 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 intravenous (IV) administration, VEGF Trap displayed a multi-compartmental PK serum profile. Clearance was slow, the $t_{1/2}$ 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 1: 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-∞}	[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.
V _{ss}	[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-∞} ratio

n.c = not calculated

*(table excerpted from applicant's BLA)***Distribution:****VGFT-PK-01005.2:** Biodistribution of VEGF Trap in Normal Sprague-Dawley Female Rats. Reviewed by Maria I. Rivera and slightly modified for this BLA review.

Rats received a 1 mg/kg IV dose of ¹²⁵I-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 post-dose, 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 and 168 hrs post-dose, the levels in serum had declined to 12% and 0.76% of the dose, respectively. By 168 hrs post-dose, 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.

Metabolism:

No studies were conducted.

Excretion:**VGFT-PK-01004.2:** Pharmacokinetics of VEGF Trap Following Intravenous Administration to Sham-Operated and Nephrectomized Sprague Dawley Rats. Reviewed by Maria I. Rivera and slightly modified for this BLA review.

After administration of a single 1 mg/kg intravenous dose of VEGF Trap 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 (\pm SD) concentration of VEGF Trap at the 1st blood sampling was 27.26 ± 4.82 $\mu\text{g/mL}$ for the sham-operated rats and 26.86 ± 3.52 $\mu\text{g/mL}$ for the nephrectomized rats. $\text{AUC}_{0-10 \text{ hrs}}$ was 163.7 ± 14.2 $\mu\text{g}\cdot\text{hr/mL}$ and 163.7 ± 17.3 $\mu\text{g}\cdot\text{hr/mL}$ for the sham-operated and nephrectomized rats, respectively. These results indicate that renal clearance is of minor importance for VEGF Trap clearance from the systemic circulation.

6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: A 6-month intravenous toxicity study of VEGF Trap in cynomolgus monkeys with a 5-month recovery period. Reviewed by Maria I. Rivera and slightly modified for this BLA review.

Study no.:	670145 (VGFT-TX-05009)
Study report location:	Module 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 17, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	VEGF Trap, lot # C06001J600X1, $\geq 97\%$ pure

Key Study Findings:

- Of the VEGF Trap doses administered (3, 10, or 30 mg/kg) to cynomolgus monkeys, a NOAEL was not identified. The LOAEL was the lowest dose tested, 3 mg/kg.
- The schedule of administration was changed from weekly to bi-weekly on week 15 due to decreases in body weight following all doses of VEGF Trap.
- 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 VEGF Trap induced nasal lesions.
- Clinical signs included exhibited hunching, nose bleeding, sneezing, and reduced activity and appetite.
- 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.
- Absent or irregular menses associated with alterations in female reproductive hormone levels, and changes in sperm morphology and motility were considered test article-related.
- 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).
- The average cynomolgus monkey exposure level (AUC_{0-168h}) to free VEGF Trap following 3, 10, and 30 mg/kg, on a bi-weekly schedule (dosing week 15-27) as proposed for this BLA, was 179, 686, and 1442 ug.day/mL, respectively.

Methods:

Doses:	0, 3, 10, or 30 mg/kg
Frequency of dosing:	Once weekly for 15 weeks For the final 12 weeks, the dosing frequency was reduced to every other week for the remaining 12 weeks of treatment 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:	1.67 mM sodium phosphate, 1.67 mM sodium citrate, 33 mM sodium chloride, 0.033% (w/v) PS20 and 6.67% (w/v) sucrose, pH 6.0
Species/Strain:	Cynomolgus monkeys (<i>Macaca fascicularis</i>)
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 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]

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 compared to control values were noted 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% at 3, 10, and 30 mg/kg, respectively). Males did not show a dose response. The percent decreases for males were 7.6, 15.1, and 8.4% at 3, 10, and 30 mg/kg, respectively.

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

Feed Consumption [daily]

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 animals 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 [pre-dose; week 13, 16, and 47]

One male at 3 mg/kg (# 210) showed focal areas of retinal edema and sub-retinal 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 areas of edema-like retinal opacity in the inferior papillary area at Week 26 on the left eye only. No histopathological correlates were noted.

The lesions in animal # 210 were still present during recovery and became 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 intravitreal toxicity studies, the relationship to treatment is uncertain.

ECG and Blood Pressure [pre-dose; week 4, 12, 26, 37, and 48]

No test article-related effects.

Hematology and Coagulation [pre-dose; week 4, 12, 25, 37, and 48]

A series of parameters showed differences when compared to the vehicle control and pre-dose 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 [pre-dose; week 4, 12, 25, 37, and 48]

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:

A 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 [pre-dose; week 4, 12, 25, 37, and 48]

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 reversed during recovery.

Gross Pathology [at necropsy: Day 190 for main group animals, D336 for recovery animals]

Macroscopic findings were noted for several tissues, affecting primarily the nasal cavities and skeletal system as well as the adrenal glands, duodenum, and gallbladder. Findings in the bones and nasal cavities are summarized in the table below:

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			
		0	3	10	30	0	3	10	30
	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.

(table excerpted from applicant's BLA)

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 two females at 30 mg/kg. Dark areas on the duodenal mucosa were present in one male at 10 mg/kg, and in one male and two females at 30 mg/kg. Dark discoloration of the adrenal glands was noted in two and one males at 3 and 30 mg/kg, respectively, and in one and two 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 [at necropsy]

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 not statistically 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 these data are 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 these data were not reliable as only one control animal was left. This decrease correlated with a higher incidence of thymic lymphoid atrophy.

Histopathology [at necropsy]

Adequate Battery: Yes

Peer Review: Yes

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 (see table below). 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.

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

Tissue/Finding Dose (mg/kg/dose)	Sex	Male				Female			
		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	-	-	-	-

Incidence and Severity of VEGF Trap-related Histopathological Findings in Nasal Cavities in the 6-Month Intravenous Toxicity Study in Monkeys (cont.)

Tissue/Finding Dose (mg/kg/dose)	Sex	Male				Female			
		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	-	-	-	-

(tables excerpted from applicant's BLA)

Bones/Muscles: The most important finding was the development of osteochondilaginous exostoses in males and females dosed with ≥ 3 mg/kg. In the spine, the exostoses was 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 (see table below). This change correlated with 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 one 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 ovarian weights that were still slightly decreased at 30 mg/kg.

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

Tissue/Finding Dose (mg/kg/dose)	Sex	Female			
		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.

(figure excerpted from applicant's BLA)

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 one male and two 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 animal at 10 mg/kg.

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

Brain: Minimal infiltration of macrophages was observed in the choroid plexus in one and two 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 one male and three 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 two males and one 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 two monkeys. Other tissues in which vascular proliferation/degeneration was found included the epididymis in one male at 30 mg/kg (slight) and in the femorotibial joint, jejunum, sciatic nerve, and skin in one female at 30 mg/kg (minimal).

At recovery, vascular degeneration/fibrosis was noted in the renal papilla of one male at 10 mg/kg and vascular proliferation/degeneration was seen in the femorotibial joint of one 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 males and 1, 2, 1, and 4 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 three of four males in the control and two of four males in the low dose group, 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 no thymic remnant as indicative of severe thymic atrophy in most control males and consequently, that no treatment-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 (four animals affected, two of which were graded moderate or marked in severity) when compared to controls (one animal affected and graded slight in severity). However, the applicant regarded the effect upon the thymus as indirect and 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 one 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 [pre-dose; week 2, 4, 12, 26, 37, and 48]

No test article-related effects.

Vaginal Bleeding [daily]

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 three and one 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 [pre-dose; week 5, 8, 12, 25, and 38]

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 or sperm counts.

Hormone Analysis [pre-dose; weekly (females) or bi-weekly (males)]

Females: Mean progesterone levels were decreased within one 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, normal follicular development was clearly compromised as evidenced by 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 ($\leq 5.5x$) 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 pre-treatment 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 pre-treatment 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 [pre-dose; week 4, 13, 26, 37, and 48]

In males, mean levels of c-reactive protein 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 two out of six animals were affected at the low and mid dose and three out of six animals were affected at the high dose, showing a wide range of values. These changes were not present at recovery.

Biochemical Markers of Bone Resorption [pre-dose; week 4, 12, 25, 37, and 48]

These 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 the 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 two males 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 one female per dose group at Week 37 (14.99-18.41 vs. 4.94-12.39 nM/mM creatinine in pooled controls and acclimation periods).

Bone Densitometry Measurements [pre-dose; week 4, 13, 26, 37, and 48]

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 an 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 [pre-dose; week 4, 13, 26, 37, and 48]

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.

**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
(table excerpted from applicant's BLA)

Anti-VEGF Trap Antibodies [pre-dose; week 26, 37, 48]

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 [pre-dose; ~5 min, 24 and 168 hrs post-dose on day 1, 29, 57, 85, 113, 141, 183; recovery week 37 and 48]

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 and 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 post-dose) 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 (pre-dose) 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 post-dose) 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 (pre-dose) were about 0.898, 8.29 and 28.8 µg/mL in the 3, 10 and 30 mg/kg cohorts, respectively.

The average cynomolgus monkey exposure level (AUC_{0-168h}) to free VEGF Trap following 3, 10, and 30 mg/kg, on a bi-weekly schedule (dosing week 15-27) as proposed for this BLA, was 179, 686, and 1442 ug.day/mL, respectively.

VEGF₁₆₅:VEGF Trap Complex – The plasma levels increased throughout the first few intravenous infusions of VEGF Trap, reaching steady state levels by approximately Day 29 for the three VEGF Trap-treated cohorts. Unlike free VEGF Trap, the VEGF₁₆₅: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 ($\leq 1.5x$) level than males particularly at ≥ 10 mg/kg.

The average mean adjusted bound VEGF Trap concentration in the pre-dose 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 pre-dose 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 intravenous 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 cohorts 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.

Study title: A 3-month intravenous toxicity study of VEGF Trap in cynomolgus monkeys with a 5-month recovery period.

Study no.:	670144 (VGFT-TX-05010)
Study report location:	Module 4.2.3.5
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 23, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	VEGF Trap, lot # C05001J600X1, \geq 98.9% pure

Summary:

Young (2-2.5 years of age) skeletally immature cynomolgus monkeys were administered VEGF Trap by intravenous infusion (30 min), once weekly for 3 months at dose levels of 0, 0.5, 3 and 30 mg/kg/dose, followed by a 5-month drug free recovery period.

VEGF Trap-induced adverse effects following the lowest dose of 0.5 mg/kg included histopathological degeneration/regeneration of the respiratory and/or olfactory epithelium. Decreased maturing follicles and granulosa cells were also noted in one female. Hematology (increase RBC, Hb, Ht and FIB), biochemistry (increase CHOL) and marker of bone resorption (decrease CTx and NTx) changes were also evident at this dose level.

Most VEGF Trap-induced adverse effects were seen following \geq 3 mg/kg and consisted of histopathological changes in the bones (osteocartilaginous exostosis of the proximal

femur and vertebral arch associated with muscular and microvascular changes, thickening of the physal cartilage of the femur and tibia as well as sternum and vertebrae and cartilaginous metaplasia in the femoral and sternal periosteum), in the nasal cavities (degeneration/regeneration or necrosis of the olfactory epithelium and respiratory epithelium associated with hemorrhage and suppurative exudate and hypertrophy of the septal chondrocytes and increased osteoclastic resorption in ethmoturbinates), in the kidneys (increased eosinophilic matrix in the glomerular tuft that stained positively with the Periodic Acid Schiff reaction and glomerulopathy with tubular dilatation and cast formation), in the ovaries (decrease in maturing follicles often associated with decreased granulosa cells and theca cells) and in the adrenal glands (decreased cytoplasmic vacuolation with increased cytoplasmic eosinophilia), slight widening of the epiphyseal growth plate evaluated radiographically, clinical signs (hunched posture, sneezing with discharge, reduced activity), decrease in body weight/body weight gains, changes in hematology (increase in RBC, Hb, Ht, FIB), biochemistry (increase in T PROT, ALB, CREAT and GLOB, CHOL, GGT, ALT, AST and decrease in A/G), increase in C-reactive protein and urinalysis (increase microalbumin) parameters, bone resorption markers (decrease CTx and NTx) and decrease in mineral bone density.

At the highest-dose of 30 mg/kg, the following adverse effects persisted throughout the recovery period: vertebral osteocartilaginous exostoses and associated muscular and microvascular changes, and histopathological degeneration/regeneration of the respiratory epithelium and/or olfactory epithelium. All other adverse effects showed complete reversibility by the end of the recovery period.

Based on these findings, the NOAEL could not be determined.

7 Genetic Toxicology

No studies conducted

8 Carcinogenicity

No studies conducted

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

The potential effects of VEGF Trap on male and female fertility were evaluated as part of the 6-month IV toxicity study in monkeys (Study# VGFT-TX-05009, reviewed by Maria I. Rivera and slightly modified for this BLA review).

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 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.

Since adverse findings were observed at all doses, a NOAEL for fertility was not determined. Based on monkey exposure levels to free VEGF Trap following the lowest dose of 3 mg/kg ($AUC_{0-168\text{hrs}} = 179 \text{ ug.day/mL}$), the exposure level in monkeys was equivalent to 61% of the exposure observed in humans ($AUC_{0-\infty} = 293 \text{ ug.day/mL}$) after the clinically recommended 4 mg/kg intravenous dose.

9.2 Embryonic Fetal Development

Study title: AVE0005: Intravenous (30-minute infusion) embryo-fetal toxicity study in rabbits. Reviewed by Maria I. Rivera and slightly modified for this BLA review.

Study no.:	Ter0506; VGFT-TX-06002
Study report location:	4.2.3.5
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 4, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AVE0005 (VEGF Trap); lot # C04008D630B12A

Key Study Findings:

- The maternal NOAEL was 3 mg/kg and a developmental NOAEL was not identified. The lowest dose tested, 3 mg/kg, is the developmental NOAEL for this study.
- At all doses, there were slight effects in the does body weight gain and food consumption primarily during dosing with a movement towards recovery once dosing was stopped.
- Decreased uterine weight was observed at 60 mg/kg. When corrected for uterine weight, terminal mean body weight was similar to controls. Therefore, the decrease at 60 mg/kg appears secondary to the fetotoxic effects.

- Abortions and increased mean number of post-implantation 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.
- At the lowest VEGF Trap dose at which adverse findings were observed, 3 mg/kg, the maternal rabbit exposure level ($AUC_{0.5-72hrs}$) was 81 ug.day/mL. This dose is approximately equivalent to 30% of the exposure observed in humans ($AUC_{Co-\infty} = 293$ ug.day/mL) after the clinically recommended 4 mg/kg intravenous dose.

Methods:

Doses:	0, 3, 15, 60 mg/kg
Frequency of dosing:	Gestation Days (GD) 6, 9, 12, 15, 18
Route of administration:	30-min IV infusion
Dose volume:	4 mL/kg
Formulation/Vehicle:	5 mM sodium phosphate, 5 mM sodium citrate, 100 mM sodium chloride, 20% (w/v) sucrose and 0.1% (w/v) PS20, pH 6.0
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 [twice daily]

Per protocol, three out of 22 pregnant females at 60 mg/kg were euthanized between GD 21 and 26 because abortion was detected. 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]

No test article-related clinical signs were noted in 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 following cessation of dosing. At the interval GD 6-18 (dosing period), body weight gain decreases were 30%, 60%, and 64% at 3, 15, and 60 mg/kg, respectively. At the interval GD 6-29, body weight gain 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]

Starting at the GD 7-8 interval, a minimal decrease in mean food consumption was noted at all doses throughout the study. A 9, 18, and 12% decrease in mean food consumption was observed at the GD 17-18 interval at 3, 15, and 60 mg/kg, respectively. Following cessation of dosing on GD 18, food consumption moved toward control values with comparable or higher values after the GD 28-29 interval.

Toxicokinetics [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 5th dose) were dose proportional between 15 and 30 mg/kg and greater than dose proportional between 3 and 15 mg/kg (see table below). Estimates of AUC_{0.5-72hrs} were greater than dose proportional between 3 and 15 mg/kg and lower than dose proportional between 15 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.

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

(table excerpted from applicant's BLA)

Dosing Solution Analysis

All solutions were 92.5-98.7% of theoretical content.

Necropsy [GD 29]

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.)

An 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 two fetuses (2 litters) at 3 mg/kg, ectrodactyly and umbilical hernia in one 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, six 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 litters 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 a zero incidence in controls. A higher incidence of 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) was noted at 60 mg/kg, 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, compared to controls. Incomplete ossification of the hyoid and thoracic vertebra were observed in one fetus and malformation in the thoracic vertebra in two fetuses / two litters (vs zero in controls) at 15 mg/kg.

Study title: AVE0005: Intravenous (30-min Infusion) Range-Finding Toxicity Study in Pregnant Rabbits (Study # Ter0184; VGFT-TX-06001). Reviewed by Maria I. Rivera and slightly modified for this BLA review.

Study no.: Tep0184 (VGFT-TX-06001)
Study report location: Module 4.2.3.5
Conducting laboratory and location: Sanofi-aventis Research & Development
Drug Safety Evaluation
3 Digue d'Alfortville
94140 Alfortville
France
Date of study initiation: March 23, 2006
GLP compliance: No. The report states, "The study was conducted in line with Good Laboratory Practice Principles but was not audited."
QA statement: No
Drug, lot #: VEGF Trap, lot # C04008D630B11A and C04008D630B11B

Summary:

VEGF Trap doses of 0 (controls), 3, 15 and 45 mg/kg were given as a 30-min intravenous infusion to pregnant rabbits (5-6/group) on GD 6, 9, 12, 15 and 18. Main results included abortion in one female at 45 mg/kg on GD 21, premature delivery in one 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 post-implantation 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 toxicokinetic information was obtained from this study compared to final study # VGFT-TX-06002. One animal at 15 mg/kg and three 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.

11 Integrated Summary and Safety Evaluation

Aflibercept (VEGF Trap) is a recombinant protein that acts as a soluble receptor capable of binding to human vascular endothelial growth factor-A (VEGF; equilibrium dissociation constant K_D of 0.5 pM for VEGF-A₁₆₅ and 0.36 pM for VEGF-A₁₂₁), human placental growth factor (PlGF; K_D of 39 pM for PlGF-2), and human VEGF-B (K_D of 1.92 pM). By binding to these endogenous ligands, aflibercept can inhibit the binding and activation of each ligand's corresponding receptor.

In vitro, aflibercept inhibited VEGF-induced proliferation of endothelial cells, thereby inhibiting the growth of new blood vessels that supply tumors with oxygen and nutrients. In vivo pharmacology studies demonstrated that aflibercept reduces tumor blood vessel density and inhibits tumor growth of a wide variety of murine, rat, and human tumor cell lines implanted in mice.

Safety pharmacology studies were conducted to determine the effects of aflibercept on wound healing. Using full-thickness excisional and incisional skin wound models in rabbits, repeated administration of aflibercept at dose levels of 0.3, 3, and 30 mg/kg/administration was shown to delay wound healing through a reduction in fibrous response, neovascularization, epidermal hyperplasia/re-epithelialization, and tensile strength.

The monkey was selected as the most relevant species for toxicology studies due to identical VEGF-A protein sequence on the amino acid level to its human counterpart. Although the binding affinity of aflibercept to VEGF-A from cynomolgus monkey was not directly tested by the Applicant, toxicities consistent with other VEGF inhibitors were seen in the 6-month toxicology study in monkeys.

Based on results from the 6-month toxicology study in monkeys in which aflibercept was administered weekly/bi-weekly (weekly for first 15 weeks) intravenous doses of 3, 10, or 30 mg/kg, the main target organs included: bone (interference with growth plate maturation of long bones, osteochondrogenous exostoses of vertebrae which correlated macroscopically 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 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, findings observed included kyphosis with osteocartilaginous exostoses, nasal cavities deformation, degenerative joint disease, and changes in the digestive system, liver, and brain (choroid plexus). Since toxicities occurred at all doses, a NOAEL was not established.

Effects on male and female fertility were incorporated into the 6-month toxicology study in monkeys. 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. Since adverse findings were observed at all doses, a NOAEL for fertility was not determined. Based on monkey exposure levels to free aflibercept following the lowest dose of 3 mg/kg ($AUC_{0-168\text{hrs}} = 179 \text{ ug.day/mL}$), the exposure level in monkeys was equivalent to 61% of the exposure observed in humans ($AUC_{0-\infty} = 293 \text{ ug.day/mL}$) after the clinically recommended 4 mg/kg intravenous dose.

In an embryo-fetal toxicity study in the rabbit, intravenous doses of aflibercept $\geq 3 \text{ mg/kg}$ 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 aflibercept 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 lowest aflibercept dose at which adverse findings were observed, 3 mg/kg, the maternal rabbit exposure level ($AUC_{0.5-72\text{hrs}} = 81 \text{ ug.day/mL}$) was 81 ug.day/mL. This dose is approximately equivalent to 30% of the exposure observed in humans ($AUC_{0-\infty} = 293 \text{ ug.day/mL}$) after the clinically recommended 4 mg/kg intravenous dose.

In conclusion, the non-clinical studies support the use of intravenous aflibercept (Zaltrap®) in patients with metastatic colorectal cancer in combination with irinotecan-fluoropyrimidine-based chemotherapy. From a Pharmacology/Toxicology perspective, approval for intravenous aflibercept is recommended.

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/s/

ALEXANDER H PUTMAN
07/05/2012

ANDREW J MCDOUGAL
07/05/2012