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APPLICATION NUMBER:

125387Orig1s000

**CLINICAL PHARMACOLOGY AND
BIOPHARMACEUTICS REVIEW(S)**

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

BLA:	125-387
Submission Date(s):	February 17, 2011
Proposed Brand Name	EYLEA™
Generic Name	Aflibercept Injection
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Applicant	Regeneron Pharmaceuticals, Inc.
Relevant IND(s)	12462, (b) (4)
Submission Type; Code	1P (NME)
Formulation; Strength(s)	Aflibercept Ophthalmic Solution for Intravitreal Injection; 40 mg/mL (2 mg/50 µL)
Indication	For the treatment of neovascular (wet) age-related macular degeneration

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1. EXECUTIVE SUMMARY

Aflibercept injection (Vascular Endothelial Growth Factor [VEGF] Trap-Eye) is a new chemical entity drug product developed for the treatment of neovascular (wet) age-related macular degeneration (AMD) administered as an intravitreal (IVT) injection. Aflibercept is a fully human, water-soluble recombinant decoy VEGF receptor. It is biologically engineered to contain key extracellular VEGF-binding domains of VEGF receptor-1 and VEGF receptor-2 fused to the constant Fc region of IgG1.

In support of the BLA, the Applicant submitted 10 clinical studies.

Among the six Phase 1 studies, four were following intravenous (IV) administration and two following IVT administration. Four studies following IV administration were conducted in either healthy volunteers (Studies PDY6655 & PDY6656), or in patients with AMD or choroidal neovascularization (CNV) (Studies VGFT-OD-0305 & VGFT-OD-0306).

The pharmacokinetic (PK) and pharmacodynamic (PD) information generated from IVT-administered aflibercept was derived from two Phase I clinical studies in patients with neovascular AMD (Studies VGFT-OD-0502 and VGFT-OD-0603).

Two Phase II studies were conducted in AMD patients receiving IVT injections of aflibercept (Studies VGFT-OD-0508 and VGFT-OD-0702). PK information was obtained in all patients in VGFT-OD-0508 and in a subset of patients in VGFT-OD-0702 (VGFT-OD-0702.PK sub-study). PD information including central retina/lesion thickness (CR/LT), choroidal neovascularization (CNV) area, and blood pressure (BP) measurements were obtained in both studies.

Two Phase III studies are being conducted in AMD patients receiving IVT-administered aflibercept [Studies VGFT-OD-0605 (VIEW 1) and 311523 (VIEW 2)]. These studies are randomized, double-masked, active-controlled (vs. Lucentis[®]), repeat-dose studies that are evaluating the efficacy, safety, and tolerability of aflibercept. Patients were randomly assigned in a 1:1:1:1 ratio to either of three aflibercept arms (0.5 mg q4w, 2 mg q4w, 2mg q8w following three loading doses at 2 mg q4w) or a ranibizumab arm (0.5 mg q4w). These studies are still ongoing with the total duration of 2 years. The reports submitted in the application contain the first-year data from the studies.

1.1. Recommendation

The Clinical Pharmacology information provided by the Applicant in the BLA submission is acceptable.

The reviewer's proposed label changes in Appendix 4.1 should be forwarded to the sponsor.

1.2. Phase IV Commitments

None.

1.3. Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

In patients with neovascular AMD, following intravitreal administration of aflibercept ophthalmic solution, a fraction of the administered dose is expected to bind with endogenous VEGF in the eye to form an inactive aflibercept:VEGF complex. Once absorbed into the systemic circulation, aflibercept presents in the plasma as free aflibercept (unbound to VEGF) and a more predominant stable inactive form with circulating endogenous VEGF (i.e., aflibercept:VEGF complex).

Absorption/Distribution

Following intravitreal administration of 2 mg per eye of aflibercept ophthalmic solution (Study VGFT-OD-0702.PK) to patients with AMD, the mean plasma C_{max} of free aflibercept was 0.02 mcg/mL (range: 0 to 0.054 mcg/mL) and was attained in 1 to 3 days. The free aflibercept plasma concentrations were undetectable two weeks post-dosing in all patients. Aflibercept did not accumulate in plasma when administered as repeat doses intravitreally every 4 weeks.

The volume of distribution of free aflibercept following intravenous (I.V.) administration of aflibercept has been determined to be approximately 6 L.

The aflibercept: VEGF complex plasma concentrations reach C_{max} in 14 to 28 days following a 2-mg intravitreal administration with a mean plasma C_{max} of approximately 0.186 mcg/mL (range from 0.100 to 0.286 mcg/mL).

Metabolism/Elimination

Aflibercept is a therapeutic protein and no drug metabolism studies have been conducted. Aflibercept is expected to undergo elimination through both target-mediated disposition via binding to free endogenous VEGF and metabolism via proteolysis. The terminal elimination half-life ($t_{1/2}$) of free aflibercept in plasma was approximately 5 to 6 days after I.V. administration of doses of 2 to 4 mg/kg aflibercept.

The exploratory subgroup analyses in Phase 3 study VIEW2 did not reveal any clinically relevant influence of the covariants including age, sex, BMI, renal function (determined as creatinine clearance), or geographic region (Europe vs. Japan) on the plasma concentrations of free aflibercept or aflibercept :VEGF complex.

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Concurrence:

cc:
Division File: BLA 125387
HFD-520 (CSO/Puglisi)
HFD-520 (MO/Boyd)
HFD-520 (Chambers)
HFD-880 (Lazor)

2. QUESTION BASED REVIEW

2.1. General Attributes of the Drug

2.1.1. What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product?

Aflibercept is a recombinant protein consisting of sequences derived from human vascular endothelial growth factor (VEGF) receptor extracellular domains fused to the Fc portion of human immunoglobulin G1 (IgG1). The extracellular domain sequences come from two different VEGF receptors, VEGFR1 and VEGFR2. The amino acid sequence structure of a single aflibercept subunit chain comprises Ig domain 2 from VEGFR1, fused to Ig domain 3 from VEGFR2, which is in turn fused to an Fc domain fragment of IgG1. The presumptive Ig domain structure of aflibercept is provided in **Figure 2.1.1-1**. Aflibercept is a dimeric glycoprotein with a molecular weight (mw) of 96.9 kDa. It contains approximately 15 % glycosylation to give a total mw of 115 kDa. Its molecular formula (without glycosylation) is $C_{4318}H_{6788}N_{1164}O_{1304}S_{32}$. Recombinant aflibercept protein is expressed in CHO K1 cells, and then purified ^{(b) (4)}

^{(b) (4)} This formulation of aflibercept, which is specially purified and specifically formulated for IVT injection, is called aflibercept injection (VEGF Trap-Eye).

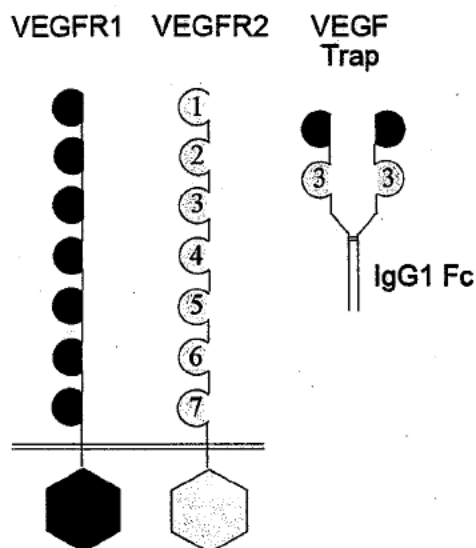


Figure 2.1.1-1: Secondary and tertiary structure of aflibercept, i.e. VEGF Trap

The physicochemical and biochemical properties of aflibercept are provided in **Table 2.1.1-1**. The aqueous solubility of aflibercept in water containing 10 mM sodium phosphate at pH 6.2 is >100 mg/mL at either 5°C or 25°C.

Table 2.1.1-1: Physicochemical and biochemical properties of aflibercept

Characteristic	Data
Description	Aflibercept is a recombinant homodimeric glycoprotein with a molecular weight of approximately 115,000 Daltons.
Quaternary structure	Covalent (disulfide linked) dimer

(b) (4)

Drug Product:

Aflibercept drug product (DP) is a sterile, clear, and colorless to pale yellow aqueous solution with a nominal pH of 6.2. It is approximately iso-osmotic and available in (b) (4) (b) (4) 2 mg, corresponding to the concentration of (b) (4) 40 mg/mL, (b) (4). The product is intended for ophthalmic use and delivered by intravitreal injection (Table 2.1.1-2).

The sponsor submitted (b) (4) aflibercept injection for approval (Table 2.1.1-3). (b) (4)

(b) (4)

- (b) (4)
- (b) (4)
- (b) (4) Vials (10 mg/mL and 40 mg/mL)
- (b) (4) (b) (4)

(b) (4)

During aflibercept clinical development, drug product was supplied primarily in vials filled at (b) (4). In 2010, drug product in vials was also supplied by (b) (4).

The drug product manufactured at (b) (4) is supplied in the same vials as those used during clinical development.

Table 2.1.1-2: Nominal Composition of Aflibercept Injection, (b) (4) 40 mg/mL Formulation

(b) (4)

Ingredient	Function	Reference to Quality Standard	Drug Product Concentration (mg/mL)	Quantity per Unit (0.278 mL/Vial) (mg)
VEGF Trap-Eye	Active Ingredient	Regeneron	40	11.120
Sodium phosphate, (b) (4)	(b) (4)	USP, BP	(b) (4)	(b) (4)
Sodium phosphate, (b) (4)		USP		
Sodium chloride		USP, Ph. Eur., JP		
Sucrose		NF, Ph. Eur., JP		
Polysorbate 20		NF, Ph. Eur., JPE		
(b) (4) (b) (4)		USP, Ph. Eur.		

USP = United States Pharmacopeia, BP = British Pharmacopeia, Ph. Eur. = European Pharmacopeia, JP = Japanese Pharmacopeia, JPE = Japanese Pharmaceutical Excipients, NF = National Formulary.

Table 2.1.1-3: Forms of Aflibercept Drug Product Described in BLA 125387

Primary container	Strength (mg/mL)	Dose (mg)	DP filling contractor	DP secondary packaging contractor	DP package sterilization method
(b) (4)					
vial	(b) (4) 40	(b) (4) 2	(b) (4)	(b) (4)	(b) (4)
vial	40	2	(b) (4) (b) (4)	(b) (4)	(b) (4)

2.1.2. *What is the proposed mechanism of drug action and therapeutic indication?*

VEGF-A and placental growth factor (PlGF) are members of the VEGF family of angiogenic factors that can act as potent mitogenic, chemotactic, and vascular permeability factors for endothelial cells. VEGF acts via two receptor tyrosine kinases, VEGFR-1 and VEGFR-2, present on the surface of endothelial cells. PlGF binds only to VEGFR-1, which is also present on the surface of leucocytes. Excessive activation of these receptors by VEGF-A can result in pathological neovascularization and excessive vascular permeability. PlGF can synergize with VEGF-A in these processes, and is also known to promote leukocyte infiltration and vascular inflammation. A variety of ocular diseases, including wet AMD, are associated with pathologic neovascularization and vascular leakage, and can result in thickening and edema of the retina, which is thought to contribute to vision loss.

Aflibercept acts as a soluble decoy receptor that binds VEGF-A and PlGF with higher affinity than their natural receptors, and thereby can inhibit the binding and activation of these cognate VEGF receptors. The equilibrium dissociation constant (K_D) for aflibercept binding to human VEGF-A₁₆₅ is 0.5 pM and to human VEGF-A₁₂₁ is 0.36 pM. The K_D for binding to human PlGF-2 is 39 pM.

Aflibercept ophthalmic solution is indicated for the treatment of patients with Neovascular (Wet) Age-Related Macular Degeneration (AMD).

2.1.3. *What are the proposed dosage(s) and route(s) of administration?*

The recommended dose regimen for aflibercept injection is 3 initial monthly intravitreal (IVT) injections of 2 mg (50 microliters), followed by 2 mg (50 microliters) administered by IVT injection once every 2 months.

2.2. General Clinical Pharmacology

2.2.1. *What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?*

The PK and/or PD information generated following IV or IVT administration of aflibercept was derived from six Phase I studies.

Four **Phase I** studies following IV administration were conducted in either healthy volunteers, or in patients with AMD or choroidal neovascularization (CNV):

1. Study PDY6655: an open-label (OL), single dose, crossover study with both the IV and SC formulations in healthy male volunteers.
2. Study PDY6656: a randomized, double-blind, placebo-controlled, sequential ascending dose study with the IV formulation in healthy male volunteers.
3. Study VGFT-OD-0305: a double masked, placebo-controlled, sequential-group, dose-escalating study with the IV formulation in patients with neovascular AMD.
4. Study VGFT-OD-0306: an open-label, long-term study with the IV formulation in patients with neovascular AMD.

The PK and PD information generated from IVT administered aflibercept were derived from two Phase I clinical studies in patients with neovascular AMD (VGFT-OD-0502 and VGFT-OD-0603).

1. VGFT-OD-0502: an OL dose-escalation study;
2. VGFT-OD-0603: a double-masked, randomized study that compared aflibercept formulated in either the ITV-1 or ITV-2 formulations.

Two **Phase II** studies were conducted in AMD patients receiving IVT injections of aflibercept (VGFT-OD-0508 and VGFT-OD-0702):

1. Study VGFT-OD-0508: a double-masked, prospective, randomized, multiple-dose study;
2. Study VGFT-OD-0702: a randomized, single-masked, long-term extension study in patients who had previously participated in either VGFT-OD-0502, VGFT-OD-0508, or VGFT-OD-0603.

Pharmacokinetics information was obtained in all patients in VGFT-OD-0508 and in a subset of patients in VGFT-OD-0702 (VGFT-OD-0702.PK). Pharmacodynamic information including central retina/lesion thickness (CR/LT), choroidal neovascularization (CNV) area, and blood pressure (BP) measurements were obtained in both studies.

Two **Phase III** studies are being conducted in AMD patients receiving IVT-administered aflibercept [VGFT-OD-0605 (VIEW 1) and 311523 (VIEW 2)]. These studies are randomized, double-masked, active controlled repeat-dose studies that evaluated the efficacy, safety, and tolerability of aflibercept. Patients were randomly assigned in a 1:1:1:1 ratio to either of three aflibercept arms (0.5 mg q4w, 2 mg q4w, 2mg q8w following three loading doses at 2 mg q4w) or a ranibizumab arm (0.5 mg q4w). The first year (through the primary endpoint) of these studies has been completed and the reports were submitted in this application.

Pharmacokinetic information was obtained only in Study 311523 (VIEW 2). Pharmacodynamics information (central retinal thickness [CRT] and change in CNV area) and blood pressure (BP) measurements were obtained in both studies.

2.2.2. What is the basis for selecting the response endpoints (i.e., clinical or surrogate endpoints) or biomarkers (collectively called pharmacodynamics [PD]) and how are they measured in clinical pharmacology and clinical studies?

Optical coherence tomography (OCT) was used to measure retinal and subretinal fluid accumulation and thickness of AMD lesions. Central retinal/lesion thickness (CR/LT) is known to be a fast reacting variable that shows PD response to anti-VEGF treatments in a reproducible way. This makes CR/LT an accepted response marker even though it is known to not always correlate on an individual patient basis with the main efficacy variable, best corrected visual acuity (BCVA). In addition, measurements of choroidal neovascularization (CNV) area by fluorescein angiography (FA) were carried out providing additional morphological insight into the PD effects at the target site.

2.2.3. Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

Yes, free aflibercept (active) and bound aflibercept (inactive) plasma concentrations were measured using validated enzyme-linked immunosorbent assay (ELISA) methods (*Refer to Section 2.6*).

2.2.4. Exposure-response

- 2.2.4.1. *What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy? If relevant, indicate the time to the onset and offset of the desirable pharmacological response or clinical endpoint.*

The dose-PD response in the eye was studied with respect to dose, in Study VGFT-OD-0502, and to dosing regimen, in Study VGFT-OD-0508. The PD parameters used to assess the dose-response relationship in the eye include CR/LT and CNV area. Following IVT administration of aflibercept, a dose-dependent reduction in CR/LT was observed in AMD patients, with clear effects seen at 0.5 mg/eye, and the maximum improvement being achieved at a dose of 2 mg/eye. There was little further improvement observed at 4 mg/eye. These data suggest that the maximal decrease in CR/LT is achieved between 0.5 mg/eye and 2 mg/eye and that higher doses of aflibercept beyond 2 mg/eye are not warranted. Data obtained from the Phase-2 studies suggest that a dosing interval of Q12 weeks (0.5 mg, 2 mg or 4 mg) did not provide a sustained PD improvement over the entire dosing interval, indicating that the optimal dosing interval would be less than every 12 weeks. However, specific dosing intervals between every 4 weeks and every 12 weeks were not evaluated in these trials.

- 2.2.4.2. *What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for safety? If relevant, indicate the time to the onset and offset of the undesirable pharmacological response or clinical endpoint.*

The increases in both diastolic BP (DBP) and systolic BP (SBP) seen with systemic administration of drugs that inhibit or interfere with the VEGF signaling pathway are attributed to the reduction of free systemic VEGF. There is a dose-dependent correlation between plasma free aflibercept concentrations (systemic administration) and the appearance of systemic PD effects (BP change, refer to Section 4.2.6). When administered via the IVT route, aflibercept was not observed to cause increases in DBP (**Figure 2.2.4.2-1**) or SBP. This is further illustrated by the comparison between the systemic exposure (C_{max}) of free and bound aflibercept following IV and IVT administration of aflibercept (**Figure 2.2.4.2-2**), and the dose-response (BP change) relationship over time following IV and IVT administration of aflibercept (**Figure 2.2.4.2-3**).

Free aflibercept plasma concentrations following IVT administration of doses of up to 4 mg/eye (approximately 0.057 mg/kg, based on a 70 kg body weight) were approximately 1/1000th to 1/100th of the free aflibercept plasma concentrations observed following I.V. administration of doses ≥ 1 mg/kg (**Figure 2.2.4.2-2**). Concentrations of bound aflibercept in plasma following IVT administration of doses of up to 4 mg/eye were approximately 1/20th of the plasma bound aflibercept concentrations determined following IV administration of doses of 1 to 4 mg/kg. Considering that IV doses ≥ 1 mg/kg are required to completely bind the endogenous systemic VEGF present at the time of dosing as well as VEGF that is synthesized in the weeks thereafter, the IVT doses investigated in the application were far from saturating the endogenous systemic VEGF. This conclusion is further supported by the observation that the peak concentrations of bound aflibercept continue to increase in a dose-dependent manner with increasing IVT doses (Refer to Section 4.2.2: Study VGFT-OD-0502). Moreover, the absence of systemic PD findings following IVT administration (**Figure 2.2.4.2-1 & 3**) support the observation that these IVT doses and the resulting low systemic exposures are unable to reduce systemic free endogenous

VEGF concentrations to a level sufficient to cause inhibition of systemic VEGF activity throughout the dosing interval.

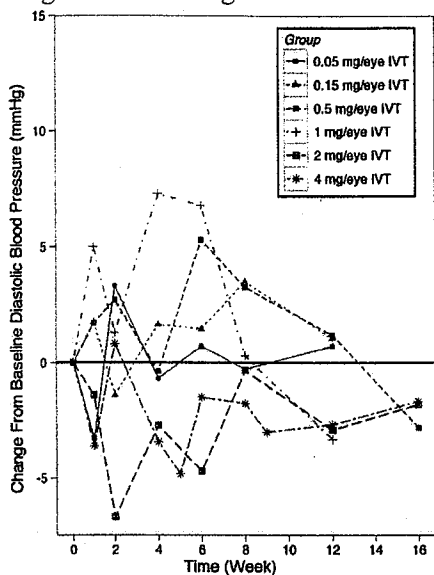
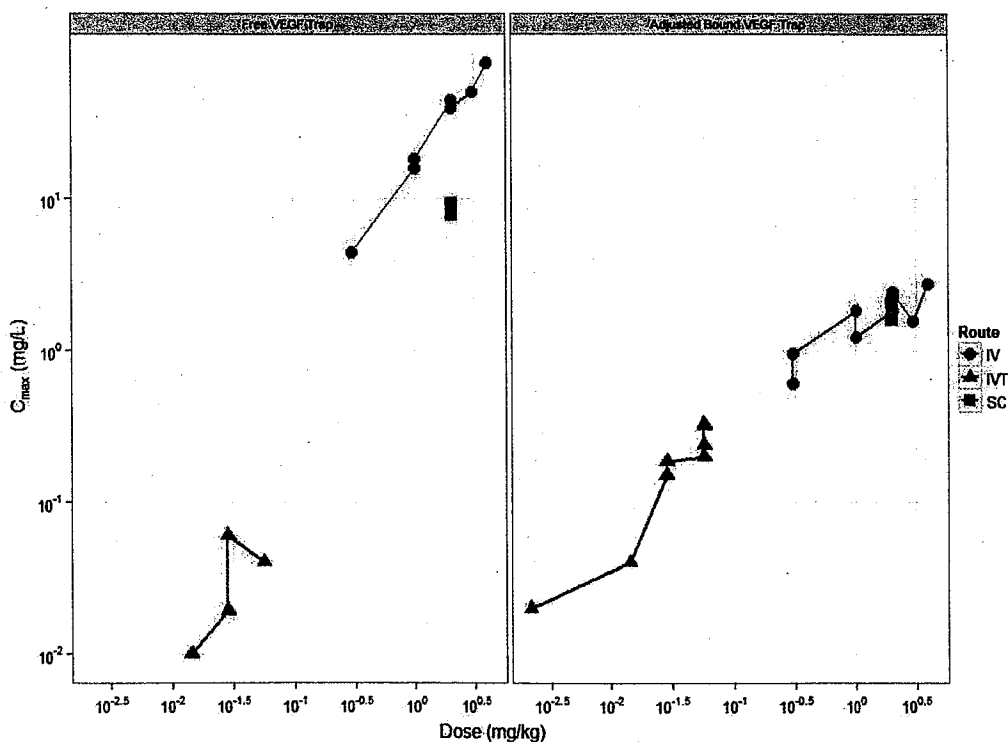
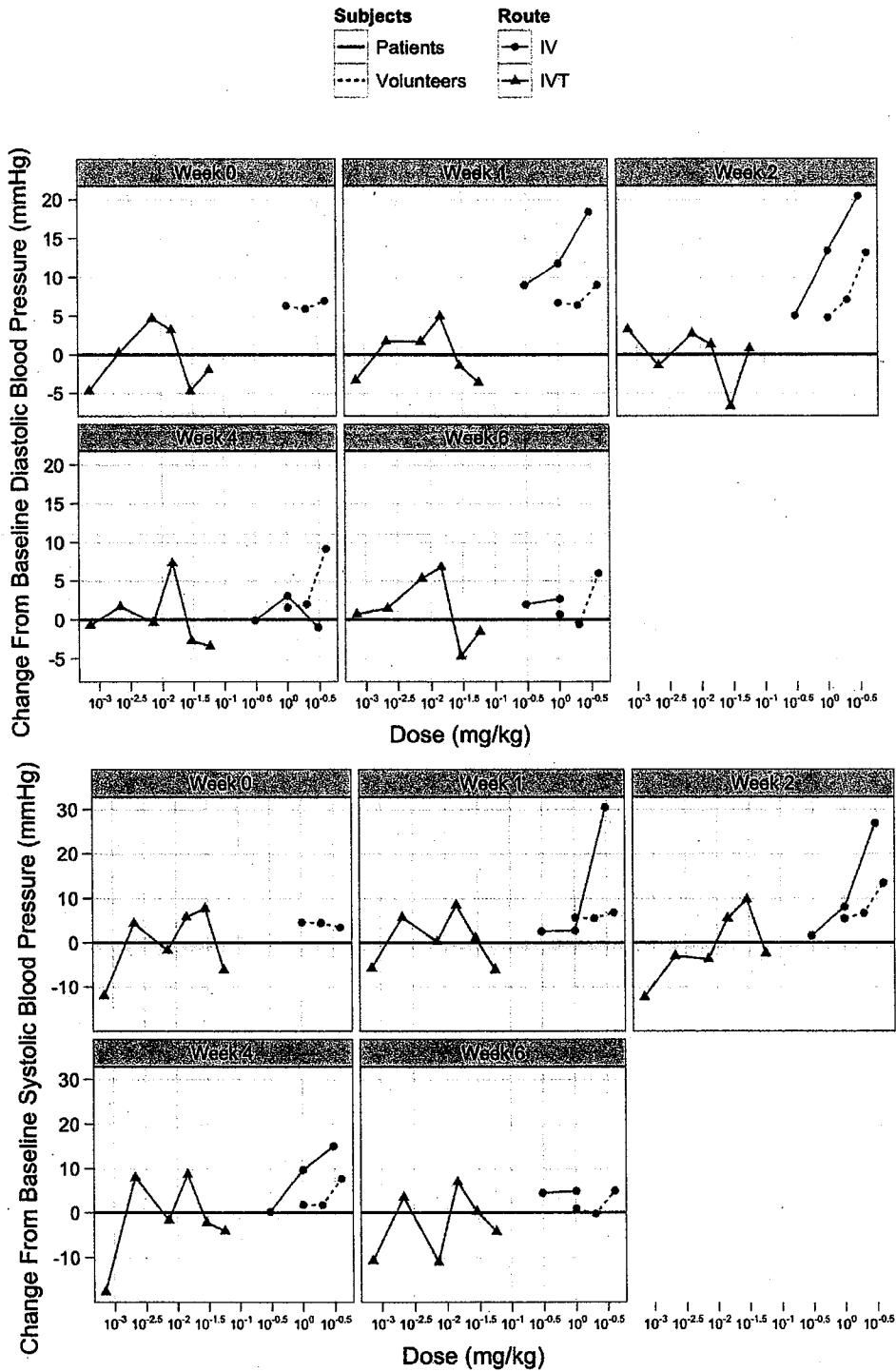


Figure 2.2.4.2-1: Mean Change from Baseline in Diastolic Blood Pressure by Dose (Phase I and II Studies including VGFT-OD-0502, -0603, and -0508)



Note: The IVT doses were converted to an approximate mg/kg value by assuming a patient weight of 70 kg.
 Data source: VGFT-OD-0603, VGFT-OD-0502, VGFT-OD-0702.PK, PDY6656, and VGFT-OD-0305/306,, PDY6655 (not reviewed).

Figure 2.2.4.2-1: Mean or Median Cmax of Free and Adjusted Bound aflibercept After IVT, IV, or SC (not reviewed) Administration versus Weight-Normalized Dose of Aflibercept



Note: The IVT doses were converted to an approximate mg/kg value by assuming a patient weight of 70 kg.

Data source: VGFT-OD-0603, VGFT-OD-0502, VGFT-OD-0508, PDY6656, and VGFT-OD-0305, VGFT-OD-0307, PDY6655 (not reviewed).

Figure 2.2.4.2-3: Comparison of Mean Change from Baseline Diastolic and Systolic Blood Pressures Following IV and IVT Administration of Aflibercept (Phase I and Phase II Studies)

2.2.4.3. Does this drug prolong the QT or QTc interval? (You must answer this question, unless this is addressed in the question above.)

No, aflibercept did not prolong the QT or QTc interval following IVT administration in the clinical trials population. A thorough QT study was not conducted.

2.2.4.4. Is the dose and dosing regimen selected by the sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

The dosing rationale for both Phase 3 pivotal trials was based on the dose-response relationship derived from Phase 1 and 2 studies (Refer to *Section 2.2.4.1*). Both Phase 3 studies compared three aflibercept regimens (0.5 mg and 2 mg dosed monthly, and 2 mg dosed every two months) to ranibizumab 0.5 mg dosed monthly.

The 0.5-mg dose was chosen as the lowest dose for which efficacy could be expected. In particular, during the prn part of the Phase-2 study VGFT-OD-0508, the 0.5-mg dose did not perform as well as the 2 mg dose. No dose higher than 2 mg was selected for the Phase-3 program because the highest dose administered during Phase-2, i.e. 4 mg, was not associated with improvements in the efficacy parameters beyond those achieved with a dose of 2 mg. The rationale for including a prolonged dosing interval of two months for 2 mg aflibercept was based on the observation from VGFT-OD-0508 that, at Week 8, improvements in visual acuity after a single 2-mg dose were similar to those obtained with 2 mg dosed monthly, suggesting that a longer and less burdensome dosing interval (i.e. every 8 weeks) may be possible without compromising efficacy.

There is no unresolved dosing or administration issues.

2.2.5. What are the PK characteristics of the drug?

2.2.5.1. What are the single dose and multiple dose PK parameters?

For the single dose Study VGFT-OD-0502, systemic exposure (C_{max} and AUC) to free aflibercept following IVT administrations of aflibercept is summarized in **Table 2.2.5.1-1**. PK parameters including C_{max} and AUC for free aflibercept were highly variable (CV>69%).

Systemic PK parameters obtained from Study VGFT-OD-702.PK (repeated IVT doses) were not considered significantly different from that reported in the 2 mg single IVT dose group of Study VGFT-OD-0502, given the wide inter-subject variability and range of AUC and C_{max} :

- Mean C_{max} of free aflibercept: 0.0193 mg/L (Study # -0702, range from 0 to 0.054 mg/L) vs. 0.0636 ± 0.0404 mg/L (Study # -0502).
- Mean AUC_{0-last} of free aflibercept: 0.153 day*mg/L (Study # -0702, range from 0 to 0.586 day*mg/L) vs. 0.302 ± 0.215 day*mg/L (Study # -0502).

Table 2.2.5.1-1: Summary of PK Parameters of Free Aflibercept by Dose (Single IVT dose)

Source: VGFT-OD-0502 PK report

Dose (mg) / Parameter	N	Mean	SE	CV%	Min	Median	Max
C_{max} (mg/L)	0.05	3	0	0	N/A	0	0
	0.15	17	0	0	N/A	0	0
	0.5	3	0	0	N/A	0	0
	1	6	0.00796	0.00507	156.	0	0
	2	4	0.0636	0.0404	127.	0	0.0430
	4	16	0.0411	0.00715	69.5	0	0.0341
AUC_{all} (day*mg/L)	0.05	3	0	0	N/A	0	0
	0.15	17	0	0	N/A	0	0
	0.5	3	0	0	N/A	0	0
	1	6	0.0302	0.0196	158.	0	0
	2	4	0.302	0.215	143.	0	0.147
	4	16	0.407	0.0739	72.6	0	0.362

Notes: Winnonlin NCA Model 200. In the noncompartmental model, dose was expressed in mg.

AUC_{all} = AUC_{0-last}

2.2.5.2. How does the PK of the drug in healthy volunteers compare to that in patients?

The systemic exposure to free aflibercept following IVT administration was only evaluated in patients.

2.2.5.3. What are the characteristics of drug absorption?

The free aflibercept plasma concentration reached the maximal concentration (C_{max}) in 1 to 3 days following a 2-mg intravitreal administration (Study VGFT-OD-0702.PK) with a mean C_{max} of approximately 0.02 µg/mL (range from 0 to 0.054 µg/mL), and were undetectable two weeks post-dosing in all patients. Free aflibercept did not accumulate in the plasma when administered intravitreally every 4 weeks.

2.2.5.4. What are the characteristics of drug distribution?

In patients with neovascular AMD, following IVT administration of aflibercept, a fraction of administered dose is expected to bind with endogenous VEGF in the eye to form an inactive aflibercept: VEGF complex. Once absorbed into systemic circulation, aflibercept presents as free aflibercept and a more predominant inactive form with circulating endogenous VEGF, i.e., the aflibercept: VEGF complex. The terminal elimination half-life (t_{1/2}) and volume of distribution of free aflibercept following IV administration has been determined to be approximately 5 to 6 days and 6 L, respectively (Study PDY6656).

2.2.5.5. Does the mass balance study suggest renal or hepatic as the major route of elimination? (This may include table with results of mass balance study.)

A mass balance study was not performed.

2.2.5.6. What are the characteristics of drug metabolism?

No drug metabolism studies were conducted.

2.2.5.7. What are the characteristics of drug excretion?

Free aflibercept is eliminated both through binding to VEGF, as well as through a slower non-saturable clearance (e.g. protein catabolism by proteolysis) mechanism. Free aflibercept is not expected to be eliminated by the kidney due to its relatively large molecular size (115 KDa).

2.2.5.8. Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

As shown in **Table 2.2.5.1-1**(Study VGFT-OD-0502), C_{max} and AUC values of free aflibercept were highly variable (CV% >69%) and generally increase as the dose increases.

2.2.5.9. How do the PK parameters change with time following chronic dosing?

Refer to *Section 2.2.5.1*.

2.2.5.10. What is the inter- and intra-subject variability of PK parameters in volunteers and patients, and what are the major causes of variability?

The systemic exposure to free aflibercept following IVT administration was only evaluated in patients. As shown in **Table 2.2.5.1-1**(Study VGFT-OD-0502), C_{max} and AUC values of free aflibercept were highly variable (CV% >69%). This level of inter-subject variability in systemic exposure is not considered unexpected following IVT administration, particularly when considering that drug absorption to systemic circulation may vary due to the potential permeability differences in the retina-blood barrier among AMD patients.

2.3. Intrinsic Factors

2.3.1. What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) influence exposure (PK usually) and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

The exploratory subgroup analyses in Phase 3 study VIEW2 did not reveal any clinically relevant influence of the covariants including age, sex, BMI, renal function determined as creatinine clearance, or geographic region (Europe vs. Japan) on the plasma concentrations of free aflibercept or bound aflibercept. For example, pharmacokinetic analysis of a subgroup of patients (n=496) in one Phase 3 study, of which 41% had renal impairment (mild N=118, moderate N=72, and severe N=15), revealed no differences with respect to plasma concentrations of free aflibercept after intravitreal administration every 4 or 8 weeks. As a therapeutic protein of 115 KDa, aflibercept is not expected to be eliminated by the kidney. It is expected to undergo elimination through both target-mediated disposition via binding to endogenous VEGF and metabolism via proteolysis. Therefore from a PK perspective, there is no mechanistic basis for dose adjustment based on renal or hepatic impairment status following IVT administration of aflibercept.

2.4. Extrinsic Factors

2.4.1. *What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence dose-exposure and/or -response and what is the impact of any differences in exposure on response?*

Based upon what is known about exposure-response relationships and their variability, what dosage regimen adjustments, if any, do you recommend for each of these factors? If dosage regimen adjustments across factors are not based on the exposure-response relationships, describe the basis for the recommendation.

No extrinsic factors have been shown to influence aflibercept exposure-response. Therefore, no dosage adjustments for extrinsic factors are recommended.

2.4.2. *Drug-drug interactions*

2.4.2.1. *Is there an in vitro basis to suspect in vivo drug-drug interactions?*

There is no in vitro basis to suspect in vivo drug-drug interactions with aflibercept.

2.4.2.2. *Is the drug a substrate of CYP enzymes? Is metabolism influenced by genetics?*

As a fusion protein, aflibercept is expected to be degraded into small peptides and individual amino acids. Therefore, it is not expected to be a substrate of CYP enzymes nor have metabolism influenced by genetics.

2.4.2.3. *Is the drug an inhibitor and/or an inducer of CYP enzymes?*

As a fusion protein, aflibercept is not expected to be an inhibitor and/or an inducer of CYP enzymes.

2.4.2.4. *Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes?*

As a fusion protein, aflibercept is not expected to be an inhibitor and/or substrate of P-glycoprotein transport process.

2.4.2.5. *Are there other metabolic/transporter pathways that may be important?*

Since aflibercept is a fusion protein, other metabolic/transporter pathways are not expected to be of importance.

2.4.2.6. *Does the label specify co-administration of another drug and, if so, has the interaction potential between these drugs been evaluated?*

No, the label does not specify co-administration of another drug.

2.4.2.7. *What other co-medications are likely to be administered to the target patient population?*

No co-administered drugs can be specified.

2.4.2.8. Are there any *in vivo* drug-drug interaction studies that indicate the exposure alone and/or exposure-response relationships are different when drugs are co-administered?

In vivo drug-drug interactions studies have not been conducted and are not needed.

2.4.2.9. Is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any?

There is no known mechanistic basis for PD drug-drug interaction.

2.4.2.10. Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions, or protein binding?

There are no unresolved questions related to metabolism, active metabolites, metabolic drug interaction, or protein binding.

2.4.3. What issues related to dose, dosing regimens, or administration are unresolved and represent significant omissions?

No issues related to dose, dosing regimens, or administration remain unresolved.

2.5. General Biopharmaceutics

Not applicable. Aflibercept is formulated as an ophthalmic solution for intravitreal administration.

2.6. Analytical Section

This section summarizes the bioanalytical methods (Table 2.6-1) utilized to assess therapeutic protein concentrations and the formation of the anti-product antibodies. Details for the bioanalytical methodology used to determine free and bound aflibercept plasma concentrations and anti-drug antibody are presented in the individual study review in Section 4.2.

Table 2.6-1: Summary of Bioanalytical Methods Used in Clinical Studies

Methods Report (Report Location)	Matrix (Anticoagulant)	Analytes	Calibration Range (ng/mL)	LLOQ ^a (Sensitivity)	Intra-Assay Precision (%CV) ^b	Intra-Assay Accuracy (AR%) ^c	Inter-Assay Precision (%CV)	Inter-Assay Accuracy (AR%)
VGFT-AS-01026-SA-01V2	Plasma (CTAD)	Free VEGF Trap	3.13 – 100	31.3 ng/mL	3.3 to 3.8	103 to 112	2.4 to 10.5	91 to 106
VGFT-AV-01026-PV01-01V2			1.56 – 100	15.6 ng/mL	9.6 to 13.7	106 to 110	1.1 to 16.2	92 to 103
VGFT-AS-02016-SA-01V2		Bound VEGF Trap	8.78 - 100	43.9 ng/mL ^d	2.0 to 4.4	79 to 84	1.6 to 10.0	80 to 110
VGFT-AV-02016-PV01-01V2					1.1 to 1.6	116 to 123	0.4 – 16.2	94 to 110
VGFT-AS-03019/03036-SA-01V6	Serum	ADA ^e	11.92-200	238.4 ng/mL	2.3 to 6.5	96 to 107	1.0 to 10.0	93 to 106
VGFT-AV-08035-SA-01V1								
VGFT-AV-07023-SA-01V1		NAb ^g	(940 ng/mL)					

^a LLOQ: Lower limit of quantitation; LLOQ differs from calibration range due to dilution of samples for analysis

^b %CV: Percent coefficient of variance

^c AR%: Analyte recovery expressed as a percentage

^d LLOQ of 43.9 ng/mL is for the bound VEGF Trap; the LLOQ for the adjusted bound VEGF Trap is 31.5 ng/mL, see Section 1.5.3

^e Anti-drug antibodies

^f Accuracy and precision not applicable for non-quantitative assays

^g Neutralizing antibodies

2.6.1. *How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?*

Free aflibercept (active, aflibercept) plasma concentrations were measured using a validated enzyme-linked immunosorbent assay (ELISA) method.

2.6.2. *Which metabolites have been selected for analysis and why?*

The bound aflibercept (inactive) concentrations in the plasma were analyzed by a validated ELISA method.

In addition, as a fusion protein administered into the body, aflibercept has the potential to induce an immune response resulting in the development of anti-drug antibodies (ADA). The potential immunogenicity of aflibercept in subjects participating in clinical studies was evaluated using one of two different ADA assays.

2.6.3. *For all moieties measured, is free, bound, or total measured? What is the basis for that decision, if any, and is it appropriate?*

Not applicable as aflibercept is a fusion protein.

2.6.4. *What bioanalytical methods are used to assess concentrations?*

Assay for free aflibercept:

Plasma concentrations of free aflibercept were determined using a validated ELISA performed in 10% human CTAD (buffered citrate, theophylline, adenosine and dipyridamole) plasma in a microplate coated with human VEGF₁₆₅ to capture free aflibercept in the sample matrix (*Validation report VGFT-AV-01026-PV01-SA-01V3*). A mouse monoclonal antibody, specific to an epitope on aflibercept, was used as the primary detection reagent in the assay. An enzyme-linked antibody (peroxidase-conjugated Affinipure goat anti-mouse IgG Fc- γ) was then used as a secondary antibody to detect the captured aflibercept. To determine free aflibercept, a luminol-based substrate specific for peroxidase was used to achieve a signal intensity that is directly proportional to the concentration of free aflibercept.

Assay for bound aflibercept:

Plasma concentrations of bound aflibercept were determined using a validated ELISA (*Validation report VGFT-AS-02016-PV01-SA-01V2*). The assay did not detect the free form of aflibercept in human plasma samples. The bound aflibercept assay used a microplate coated with goat anti-VEGF polyclonal antibody to capture bound aflibercept. This antibody only captured aflibercept that is bound to VEGF. A mouse monoclonal antibody specific to an epitope on aflibercept was used to detect bound aflibercept that has been captured by the goat anti-VEGF polyclonal antibody. An enzyme-linked antibody (peroxidase-conjugated Affinipure goat anti-mouse IgG Fc- γ) is used as a secondary detection reagent to detect the captured aflibercept. To determine bound aflibercept, a luminol-based substrate specific for peroxidase was used to achieve a signal intensity that is directly proportional to the concentration of bound aflibercept.

Assay for anti-aflibercept antibody:

Two assays for anti-drug antibody response (ADA) were developed: an original, quasi-quantitative, ELISA-based assay used in the Phase I/II studies with a sensitivity of ~ 240 ng/mL, and a more sensitive, titer-based, non-quantitative, bridging immunoassay used in the Phase III

studies (VIEW 1 and VIEW 2) with a sensitivity of ~ 5.4 ng/mL. Samples that were positive in the anti-drug antibody (ADA) assays (VIEW 1 and VIEW 2 trials) were further characterized by a neutralizing antibody (NAb) assay (*Not reviewed*).

2.6.4.1. What is the range of the standard curve? How does it relate to the requirements for clinical studies? What curve fitting techniques are used?

Assay for free aflibercept:

This assay was calibrated using a standard curve generated from seven free aflibercept standards (100, 50, 25, 12.5, 6.25, 3.13 and 1.56 ng/mL).

The Relative Light Unit (RLU) readings of the standards are calibrated against their respective nominal concentrations using a 4-parameter logistic curve fit (see equation below), from which all other measurements (samples and QCs) are subsequently computed.

$$y = ((A-D) / (1 + (x / C)^B)) + D$$

A is the y-value corresponding to the bottom asymptote, D is the y-value corresponding to the top asymptote, C is x-value at the inflection point of the sigmoidal curve and B is the slope. The curve parameters are automatically computed for each equation using the Levenberg-Marquardt curve-fitting algorithm. The y-axis represents the RLU values and the x-axis represents the nominal aflibercept concentrations. The RLU response from each sample is then back-calculated from the respective calibration equation. The triplicate back-calculated dose-response (ng/mL) of each sample is then averaged and the relative standard deviation is described in term of the percent coefficient of variance (%CV). The mean back-calculated concentration from each sample is finally corrected for dilutions to estimate aflibercept levels.

The range of the standard curve is adequate for purposes of determining plasma concentrations of free aflibercept in the clinical studies.

Assay for bound aflibercept:

This assay was calibrated using a standard curve generated from seven bound aflibercept standards (100, 66.67, 44.44, 29.63, 19.75, 13.17 and 8.78 ng/mL).

The Relative Light Unit (RLU) readings of the Standards are calibrated against their respective nominal concentrations using a Log-Log curve fit (see equation below) from which all other measurements (samples and QCs) are subsequently computed.

$$\text{Log}_{10}(y) = A + B(\text{Log}_{10}(x))$$

Where A is the log₁₀ y-intercept (when log₁₀ x = 0), B is the slope of the line, x = dose or back calculated concentration of bound aflibercept and y is the corresponding RLU. Based on this equation, the RLU readings of the QCs and plasma samples were used to calculate their bound aflibercept concentration.

The range of the standard curve is adequate for purposes of determining plasma concentrations of bound aflibercept in the clinical studies.

Assay for anti-aflibercept antibody:

ELISA assay - This assay was calibrated using a standard curve generated from seven standards prepared using the anti-R1 monoclonal antibody at concentrations of 200, 125, 78.13, 48.83,

30.52, 19.07 and 11.92 ng/mL. The absorbance of the calibration standards was plotted against the nominal concentrations of anti-aflibercept antibody and fitted with a four-parameter logistic (4PL) curve fit and the results reported in terms of mass equivalent antibody unit per volume (ng/mL). The ranges of standard curve are adequate for purposes of determining serum concentrations of ADA in the clinical studies.

2.6.4.2. What are the lower and upper limits of quantification (LLOQ/ULOQ)?

Assay for free aflibercept:

The assay was performed in a 10% human (CTAD) plasma matrix. The LLOQ and ULOQ are 15.6 ng/mL and 1000 ng/mL in the undiluted plasma sample, respectively.

Assay for bound aflibercept:

The assay was performed in a 20% human (CTAD) plasma matrix. The LLOQ and ULOQ are 43.9 ng/mL and 500 ng/mL in the undiluted plasma sample, respectively.

Assay for anti-aflibercept antibody:

ELISA assay - The assay was performed in a 5% human serum matrix. The LLOQ and ULOQ are 238.4 ng/mL and 4 µg/mL in the undiluted serum sample, respectively.

Non-quantitative bridging immunoassay- The sensitivity of the bridging immunoassay is approximately 5.4 ng/mL in the absence of aflibercept and about 25.2 ng/mL in the presence of 20 µg/mL of aflibercept.

2.6.4.3. What are the accuracy, precision, and selectivity at these limits?

Assay for free aflibercept:

The assay accuracy and precision were determined from the assay standards and QCs. The accuracy values ranged from 92.2% to 109.9%. The precision values ranged from 1.05% to 16.18%. Assay selectivity was confirmed by analyzing 10 naïve human plasma samples and none yielded above BLQ results.

Assay for bound aflibercept:

The assay accuracy and precision were determined from the assay standards and QCs. The accuracy values ranged from 93.38% to 123.34%. The precision values ranged from 0.38% to 16.17%. Assay selectivity was not evaluated.

Assay for anti-aflibercept antibody:

ELISA assay- The assay accuracy and precision were determined from the assay standards and positive QCs. The accuracy values ranged from 94.53% to 103.76%. The precision values ranged from 0.14% to 6.44%. Assay selectivity was confirmed by analyzing 10 naïve human plasma samples and none yielded above BLQ results.

2.6.4.4. What is the sample stability under the conditions used in the study (long-term, freeze-thaw, sample-handling, sample transport, autosampler)?

Assay for free aflibercept:

Free aflibercept was stable when stored at ~ -80°C up to 15 months, in human plasma after 9 freeze-thaw cycles, stored at room temperature for 4 hours, and stored overnight at 2°C to 8°C.

Assay for bound aflibercept:

Samples were stable when stored at ~ -80°C up to 24 months and after 10 freeze-thaw cycles.

Assay for anti-aflibercept antibody:

ELISA assay- The positive QCs for the assay (the mouse monoclonal) in human serum was shown to be stable after 3 freeze-thaw cycles, storage at room temperature for 4 hours and storage overnight at 2°C to 8°C. Long-term stability of the mouse monoclonal in human serum was demonstrated for up to 12 months at -80°C.

Non-quantitative bridging immunoassay- The positive QCs were shown to be stable after 10 freeze-thaw cycles, storage at room temperature for 5 hours, storage overnight at 2°C to 8°C and after long-term storage at -80°C for up to 24 months.

2.6.4.5. *What is the QC sample plan?*

Assay for free aflibercept:

Three QCs prepared in plasma at a concentration of 700, 350 and 40 ng/mL of free aflibercept were diluted to 10% plasma (final concentrations of 70, 35 and 4 ng/mL) and included in each analysis.

Assay for bound aflibercept:

Three QCs prepared in plasma at a concentration of 400, 200 and 125 ng/mL of VEGF: aflibercept were diluted to 20% plasma (final concentrations of 80, 40 and 25 ng/mL) and included in each analysis.

Assay for anti-aflibercept antibody:

ELISA assay- The positive QCs were prepared in serum at a concentration of 3200, 1280 and 512 ng/mL of anti-R1 monoclonal antibody and then diluted to 5% serum (final concentrations of 160, 64 and 25.6 ng/mL) and included in each analysis.

Non-quantitative bridging immunoassay- Three positive QC samples, prepared with the mouse anti-aflibercept monoclonal antibody were included in each assay run: a high QC (6000 ng/mL), a mid QC (600 ng/mL), and a low QC (30 ng/mL) sample.

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