CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125418Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Clinical Pharmacology Review	
BLA	125418
Submission Date	February 3, 2012
Submission Type	Original BLA
Brand Name	ZALTRAP™
Generic Name	Aflibercept
Dosage Form / Strength	25 mg/mL solution for IV infusion
Dosing Regimen	4 mg/kg every 2 weeks in combination with a FOLFIRI chemotherapy regimen
Proposed Indication	Metastatic colorectal cancer that is resistant to or has progressed after an oxaliplatin-containing regimen
Applicant	Sanofi-Aventis
Clinical Pharmacology Reviewer	Ruby Leong, Pharm.D.
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1. EXECUTIVE SUMMARY

Aflibercept (Vascular Endothelial Growth Factor [VEGF] Trap), a recombinant human soluble fusion protein that binds endogenous VEGF, is proposed for the treatment of metastatic colorectal cancer (mCRC) that is resistant to or has progressed after an oxaliplatin-containing chemotherapy regimen. The intravitreal dosage form of aflibercept as EYLEA™ was approved on November 18, 2011 for the treatment of patients with neovascular (wet) age-related macular degeneration.

The proposed intravenous dosing regimen is 4 mg/kg every two weeks (Q2W) in combination with a FOLFIRI (5-FU/leucovorin and irinotecan) chemotherapy regimen. In the registrational trial, mCRC patients were randomized to receive either aflibercept 4 mg/kg IV Q2W plus FOLFIRI (n=612) or placebo plus FOLFIRI (n=614). An overall survival benefit of 1.4 months was demonstrated in the aflibercept arm (median: 13.5 months) versus the placebo arm (median: 12.1 months), with a stratified hazard ratio (HR) of 0.816 (95% CI: 0.713, 0.934; p = 0.0032). The addition of aflibercept to FOLFIRI increases the incidence of adverse events (AEs) associated with VEGF inhibition including hypertension, dysphonia, proteinuria, and hemorrhage (epistaxis, gastrointestinal bleeding), and less frequent but potentially severe AEs such as arterial and venous thromboembolic events (ATE and VTE). Aflibercept also increases the frequency and severity of background chemotherapy toxicities including stomatitis, diarrhea, and neutropenia.

A total of 19 clinical studies containing pharmacokinetic (PK) and immunogenicity data were submitted to support the Clinical Pharmacology Section of the BLA, which includes two Phase 1 pharmacodynamic (PD) studies in healthy subjects; four monotherapy and five combination Phase 1 PK trials in patients with advanced solid tumors or lymphomas; four Phase 2 monotherapy trials in patients with advanced ovarian cancer or non-small cell lung cancer (NSCLC); one Phase 3 registrational trial in patients with mCRC; two Phase 3 trials in patients with NSCLC or metastatic pancreatic cancer; and one QT/QTc study. The application also contains reports of population PK and exposure-response (E-R) analyses.

1.1 Recommendations

BLA 125418 is acceptable for approval from a clinical pharmacology perspective, provided that the Applicant and the Agency come to a mutually satisfactory agreement regarding the labeling language.

1.2 Phase IV Commitments and Requirements

The Office of Clinical Pharmacology does not recommend any postmarketing requirement (PMR) or postmarketing commitment (PMC) studies.

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1.3 Summary of Clinical Pharmacology and Biopharmaceutics Findings

Aflibercept (Vascular Endothelial Growth Factor [VEGF] Trap), is a recombinant human soluble fusion protein consisting of sequences derived from the extracellular domains of VEGF receptors 1 and 2 (VEGFR-1 and VEGFR-2) fused to the Fc region of IgG_1.

Mechanism of Action: Aflibercept acts as a soluble receptor that binds to VEGF-A, placenta growth factor (PlGF) and VEGF-B, to form a stable inert complex. By binding to these endogenous ligands, aflibercept can inhibit VEGFR-1 and VEGFR-2 activation, blocking endothelial cell proliferation and new blood vessel formation.

Clinical Dose Selection: Preclinical pharmacological data and *in vivo* determination of the dissociation constant (K_D) suggested that maintaining a free/bound aflibercept ratio above 1 throughout the dosing interval would maximize binding of endogenous VEGF and maintain VEGF levels <20 pg/mL (near the median value of 17 pg/mL in healthy subjects). At doses >2 mg/kg Q2W, the mean ratio of free/bound aflibercept trough concentrations was >1 in all monotherapy and combination Phase 1 studies. The proposed dosing regimen of aflibercept (4 mg/kg IV Q2W) was initially evaluated in Phase 1 monotherapy studies TED6115 and TED6116, and identified as a safe and biologically active dose for further evaluation as monotherapy in Phase 2 studies. In the Phase 1 combination study (TCD6118) with 5-fluorouracil (5-FU), leucovorin, and irinotecan, aflibercept doses from 2 to 6 mg/kg Q2W were investigated and best overall response was evaluated at the selected dosing regimen of 4 mg/kg IV Q2W in an extension cohort (similar to a single arm Phase 2 trial). The applicant justifies that results from the monotherapy studies and the combination study supported the proposed dosing regimen of aflibercept 4 mg/kg IV Q2W in the registrational Phase 3 EFC10262/VELOUR trial.

Pharmacokinetics: When administered intravenously, free aflibercept concentrations appear to exhibit linear pharmacokinetics in the dose range of 2 to 9 mg/kg. Following 4 mg/kg Q2W IV administration, the mean elimination half-life ($t_{1/2}$) of free aflibercept was approximately 6 days (range 4-7 days). Steady state concentrations of free aflibercept were reached by the second dose. Drug accumulation of free aflibercept was approximately 1.3-fold with 4 mg/kg Q2W administration. Based on a population PK analysis with data from 1378 patients who received 2-9 mg/kg of aflibercept Q2W or

every three weeks (Q3W) as monotherapy or in combination with chemotherapy, the estimated $t_{1/2}$ of VEGF-bound aflibercept was approximately 15 days. The estimated time to reach steady state concentrations of VEGF-bound aflibercept was 70 days.

Population Pharmacokinetic Analysis: Population PK analyses (n=1507) showed that age, race, and gender did not have a clinically meaningful effect on the exposure of free aflibercept. Patients weighing ≥100 kg had a 30% increase in drug exposure compared to patients weighing <100 kg. Patients with renal and hepatic impairment had similar exposure as patients with normal organ function.

Exposure-Response Relationship: In the registrational trial, EFC10262/VELOUR, overall survival (OS) was significantly related with free and VEGF-bound aflibercept exposure. For the multivariate Cox proportional regression analysis using model-derived free aflibercept steady state AUC, an increase of 1000 µg·h/mL was associated with a 21% decrease in the survival hazard ratio (HR). The relationship remained significant when endogenous VEGF was included in the model. Consistent results were observed for progression-free survival (PFS) where an increase of 1000 µg·h/mL was associated with a 19% decrease in the survival HR. Incidence of hypertension and hemorrhage during the first two cycles were found to be significantly related to exposure of free aflibercept. The odds of experiencing hypertension increased by 27% for an increase in $AUC_{0\text{-}336h}$ of 1000 µg·h/mL. For an increase of 2000 µg·h/mL in $AUC_{cum\ cycles1,\ 2}$, the odds of hemorrhage increased by 17%.

Immunogenicity: The overall incidence of anti-product antibody (APA) development across fifteen studies enrolling patients with various cancers was 4.2% in patients receiving IV aflibercept (72/1706; of which 19 tested positive at baseline) and 3.5% in patients receiving placebo (41/1156; of which 22 tested positive at baseline). Among patients who tested positive for APA and had sufficient samples for further testing, neutralizing antibodies were detected in 17 of 48 aflibercept-treated patients and in 2 of 40 placebo-treated patients. The presence of neutralizing antibodies appeared to affect exposure of aflibercept. Free aflibercept trough concentrations (C_{trough}) at Cycle 3 were approximately 30-fold lower (near lower limit of quantitation with mean C_{trough} of 192 ng/mL) than those of the overall population (mean C_{trough} of 5.8 µg/mL) after six weeks of aflibercept treatment. The impact of neutralizing antibodies on efficacy and safety could not be assessed due to limited data.

Drug-Drug Interaction: No clinically meaningful drug interactions were observed between aflibercept monotherapy and in combination with chemotherapy including irinotecan/SN-38, 5-FU, oxaliplatin, cisplatin, docetaxel, gemcitabine, erlotinib, and pemetrexed, based on cross-study comparisons and population PK analyses.

QT/QTc Prolongation: The effect of 6 mg/kg IV Q3W on QTc interval was evaluated in 87 patients with solid tumors in a randomized, placebo-controlled study. No large changes in the mean QT interval from baseline (i.e., greater than 20 ms as corrected for placebo) based on Fridericia correction method were detected in the study.

2. QUESTION-BASED REVIEW

2.1 General Attributes

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 as they relate to clinical pharmacology and biopharmaceutics review?

Aflibercept (Vascular Endothelial Growth Factor [VEGF] Trap), is a recombinant human soluble fusion protein, consisting of sequences derived from the extracellular domains of VEGF receptors 1 and 2 (VEGFR-1 and VEGFR-2) fused to the Fc region of IgG_1. The total molecular weight of aflibercept is approximately 115 kDa, consisting of a protein molecular weight of 97 kDA with (b) (4) approximately 15% of the total mass of aflibercept.

Aflibercept drug product is supplied as a sterile, preservative-free 25 mg/mL solution in two presentations, 100 mg/4.0 mL and 200 mg/8.0 mL single-use vials. The solution consists of excipients including sodium phosphate, citrate, and chloride, polysorbate 20, and sucrose, in Water for Injection USP, at a pH of 6.2. The concentrate solution is diluted with 0.9% sodium chloride solution or 5% dextrose prior to IV infusion over one hour.

2.1.2 What are proposed mechanism(s) of action and therapeutic indication(s)?

Aflibercept acts as a soluble receptor that binds to endogenous VEGF-A (equilibrium dissociation constant K_D of 0.5 pM for VEGF-A_{165} and 0.36 pM for VEGF-A_{121}), to human PlGF (K_D of 39 pM for PlGF-2), and to endogenous VEGF-B (K_D of 1.92 pM) to form a stable inert complex. By binding to these endogenous ligands, aflibercept can inhibit VEGFR-1 and VEGFR-2 activation, blocking endothelial cell proliferation and new blood vessel formation. The proposed indication is metastatic colorectal cancer (mCRC) previously treated with an oxaliplatin-containing regimen.

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

The proposed dosing regimen of aflibercept is 4 mg/kg administered intravenously over a 60-minute period every 2 weeks (Q2W) in combination with irinotecan/fluoropyrimidine-based chemotherapy.

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?

In support of the BLA for aflibercept, the applicant submitted 19 clinical studies containing PK and immunogenicity data including two Phase 1 PD studies in healthy subjects; four monotherapy and five combination Phase 1 PK trials in patients with advanced solid tumors or lymphomas; four Phase 2 monotherapy trials in patients with

advanced ovarian cancer or NSCLC; one Phase 3 registrational trial in patients with mCRC; two Phase 3 trials in patients with NSCLC or metastatic pancreatic cancer; and one QT/QTc study. The application also contains reports of population PK and exposure-response (E-R) analyses. Among the 19 clinical studies, the SC dosage form was initially investigated in two monotherapy PK studies. Due to the high injection volume (>10 mL for SC administration), only the IV route was studied in all subsequent clinical trials. Refer to **Table 1** and **Table 2** for details of study design.

Table 1. Summary of Clinical Pharmacology Trials

Trial Number	Design	Objectives	N*	Treatment
Monotherapy; Healthy male subjects				
PDY6655	Phase 1, open label, randomized, single dose, crossover	Pharmacodynamic effects on blood pressure, markers of RAAS, VEGF levels of aflibercept administered SC vs. IV; PK; Absolute bioavailability of SC aflibercept	40	2.0 mg/kg IV and SC; single doses
PDY6656	Phase 1, randomized, double-blind, placebo-controlled, sequential ascending dose	Pharmacodynamic effects on blood pressure, hemodynamics, markers of endothelium dysfunction, renal function, VEGF levels; PK	48	1, 2, or 4 mg/kg IV
Monotherapy; Patients with advanced solid tumors or lymphoma				
TED6113	Phase 1, open label, sequential cohort, dose escalation, safety, tolerability, PK	Safety and tolerability of aflibercept administered SC	38	0.025, 0.05, 0.1, 0.2, 0.4, 0.8 mg/kg SC QW, and 0.8 mg/kg SC twice weekly
TED6114	Phase 1, open label, long-term safety and tolerability (extension of TED6113)	Long-term safety and tolerability of aflibercept administered SC	18	0.025, 0.05, 0.1, 0.2, 0.4, 0.8 mg/kg SC QW, and 0.8 mg/kg SC twice weekly
TED6115	Phase 1, open label, sequential cohort, dose escalation, safety, tolerability, PK	Safety and tolerability of aflibercept administered IV vs. SC	57	0.3, 1.0, 2.0, 3.0, 4.0, 5.0, and 7.0 mg/kg IV Q2W, and 4.0 mg/kg SC Q2W
TED6116	Phase 1, open label, long-term safety and tolerability (extension of TED6115)	Long-term safety and tolerability of aflibercept administered IV vs. SC	44	0.3, 1.0, 2.0, 3.0, 4.0, 5.0, and 7.0 mg/kg IV Q2W, and 4.0 mg/kg SC Q2W
ARD6122	Phase 2, randomized, double-blind, parallel,	ORR, CBR, DR, TMRR, TTP, TTMP, PFS, OS,	215	2 and 4 mg/kg IV Q2W

Table 1. Summary of Clinical Pharmacology Trials

Trial Number	Design	Objectives	N*	Treatment
	two-stage trial in advanced ovarian cancer patients resistant to platinum and topotecan and/or liposomal doxorubicin	safety (including immunogenicity), HRQL, exploratory biomarkers, PK		
ARD6123	Phase 2, open label, single arm, two-stage trial in locally advanced or metastatic non-small-cell lung adenocarcinoma patients resistant to platinum and erlotinib	ORR, DR, PFS, OS, safety (including immunogenicity), HRQL, exploratory biomarkers, PK	96	4 mg/kg IV Q2W
ARD6772	Phase 2, open label, single arm trial in advanced ovarian cancer patients with recurrent symptomatic malignant ascites	Repeat paracentesis response rate, time to repeat paracentesis, frequency of paracentesis, PFS, OS, safety (including immunogenicity)	16	4 mg/kg IV Q2W
EFC6125	Phase 2, randomized, double-blind, placebo-controlled trial in advanced ovarian cancer patients with recurrent symptomatic malignant ascites	Time to repeat paracentesis, ascites impact measure, frequency of paracentesis, safety (including immunogenicity), PK	58	4 mg/kg IV Q2W
Combination; Patients with advanced solid tumors				
TCD6117	Phase 1, open label, sequential cohort, dose escalation, safety, tolerability, PK	DLT and RP2D of aflibercept in combination with FOLFOX4 (oxaliplatin, LV, 5-FU), safety (including immunogenicity), PK, efficacy (antitumor response, functional status)	32	Aflibercept 2, 4, and 5 mg/kg IV Q2W plus FOLFOX4
TCD6118	Part 1: Phase 1, open label, sequential cohort, dose escalation, safety, tolerability, PK Part 2: Double-blind,	DLT and RP2D of aflibercept in combination with FOLFIRI (irinotecan, LV, 5-FU), safety (including immunogenicity), PK, efficacy (antitumor	65	Aflibercept 2, 4, 5, and 6 mg/kg IV Q2W plus FOLFIRI or placebo

Table 1. Summary of Clinical Pharmacology Trials

Trial Number	Design	Objectives	N*	Treatment
	randomized (1:1), parallel group, placebo-controlled	response)		
TCD6119	Phase 1, open label, sequential cohort, dose escalation, safety, tolerability, PK	DLT and RP2D of aflibercept in combination with TCF (docetaxel, cisplatin, 5-FU)	44	Aflibercept 2, 4, and 6 mg/kg IV Q3W plus TCF
TCD6120	Phase 1, open label, sequential cohort, dose escalation, safety, tolerability, PK	DLT and RP2D of aflibercept in combination with docetaxel 75 mg/m^2 (VT75), docetaxel, cisplatin (VTC), docetaxel 100 mg/m^2 (VT100), or pemetrexed (V-pemetrexed)	134	Aflibercept 2, 4, 5, 6, 7, and 9 mg/kg IV Q3W plus docetaxel 75 mg/m^2 (VT75); Aflibercept 4, 5, and 6 mg/kg IV Q3W plus docetaxel, cisplatin (VTC) or docetaxel 100 mg/m^2 (VT100); Aflibercept 6 mg/kg IV Q3W plus V-pemetrexed
TCD6121	Phase 1, open label, sequential cohort, dose escalation, safety, tolerability, PK	DLT and RP2D of aflibercept in combination with gemcitabine or gemcitabine plus erlotinib	61	Aflibercept 4 and 6 mg/kg IV Q2W plus gemcitabine (GV); Aflibercept 2 and 4 mg/kg IV Q2W plus gemcitabine, erlotinib (GEV)
TES10897 (QUTIE)	Phase 1, randomized, double-blind, placebo-controlled	Effect of aflibercept on QTcF interval	87	Aflibercept 6 mg/kg IV Q3W plus docetaxel

* Number of patients treated

5-FU: 5-fluorouracil

CBR: Clinical benefit response

DR: Duration of response

GV: Aflibercept IV infusion over 1 hour, followed by gemcitabine 1000 mg/m^2 IV infusion over 30 minutes on Days 1 and 15 of each 28-day cycle. Gemcitabine administered alone on Day 22 of Cycle 1 and Day 8 of each subsequent 28-day cycle (Cycle 2 and beyond).

GEV: Aflibercept IV infusion over 1 hour, followed by gemcitabine 1000 mg/m^2 IV infusion over 30 minutes and erlotinib 100 mg QD on Days 1 and 15 of each 28-day cycle. Gemcitabine and erlotinib administered without aflibercept on Day 22 of Cycle 1 and Day 8 of each subsequent 28-day cycle (Cycle 2 and beyond).

FOLFOX4: Day 1 - Oxaliplatin 85 mg/m^2 IV with leucovorin (LV) 200 mg/m^2 IV over 2 hours, followed by 5-FU 400 mg/m^2 IV bolus over 2-4 minutes, then 600 mg/m^2 IV infusion over 22 hours; Day 2 - LV 200 mg/m^2 over 2 hours, followed by 5-FU 400 mg/m^2 IV bolus then 600 mg/m^2 IV infusion over 22 hours.

FOLFIRI: Irinotecan 180 mg/m^2 IV over 60 minutes with LV 200 mg/m^2 (or levo-LV 100 mg/m^2) IV over 2 hours, followed by 5-FU 400 mg/m^2 IV bolus over 2-4 minutes, then 600 mg/m^2 IV infusion over 22 hours on Day 1; LV 200 mg/m^2 (or levo-LV 100 mg/m^2) over 2 hours, followed by 5-FU 400 mg/m^2 IV bolus then 600 mg/m^2 IV infusion over 22 hours on Day 2.

Table 1. Summary of Clinical Pharmacology Trials

Trial Number	Design	Objectives	N*	Treatment

LV: Leucovorin

ORR: Objective response rate

OS: Overall survival

PFS: Progression-free survival

RAAS: Renin angiotensin aldosterone system

TCF: Docetaxel 75 mg/m² and cisplatin 75 mg/m² IV over 1 hour on Day 1, then 5-FU 750 mg/m² IV infusion over 24 hours on Days 1 to 5.

TMRR: Tumor marker response rate

TTMP: Time to tumor marker progression

TTP: Time to tumor progression

VT75: Aflibercept IV infusion over 1 hour, followed by docetaxel 75 mg/m^2 IV infusion over 1 hour Q3W.

VT100: Aflibercept IV infusion over 1 hour, followed by docetaxel 100 mg/m^2 IV infusion over 1 hour Q3W.

VTC: Aflibercept IV infusion over 1 hour, followed by docetaxel 75 mg/m^2 IV infusion over 1 hour, then cisplatin 75 mg/m^2 IV infusion over 1 hour Q3W.

V-pemetrexed: Aflibercept IV infusion over 1 hour, followed by pemetrexed 500 mg/m^2 IV over 10 minutes Q3W.

Table 2. Summary of Clinical Efficacy/Safety Trials

Trial Number	Design	Objectives	N*	Treatment
EFC10262 (VELOUR)	Phase 3, randomized, placebo-controlled, double-blind trial in patients with metastatic colorectal cancer that previously failed an oxaliplatin based regimen	OS, PFS, ORR, safety (including immunogenicity), PK	1216 (612 aflibercept)	Aflibercept 4 mg/kg IV Q2W or placebo plus leucovorin* 400 mg/m^2 IV, irinotecan 180 mg/m^2 IV, 5-FU 400 mg/m^2 IV bolus and 5-FU 2400 mg/m^2 IV over 46-hour infusion *200 mg/m^2 dose for l-isomer
EFC10261 (VITAL)	Phase 3, randomized, placebo-controlled, double-blind trial in patients with locally advanced or metastatic non-small-cell lung cancer (NSCLC) that previously failed a	OS, PFS, ORR, HRQL, safety (including immunogenicity), PK	905 (452 aflibercept)	Aflibercept 6 mg/kg IV Q3W or placebo plus docetaxel 75 mg/m² IV

Table 2. Summary of Clinical Efficacy/Safety Trials

Trial Number	Design	Objectives	N*	Treatment
	platinum based therapy			
EFC10547 (VANILLA)	Phase 3, randomized, placebo-controlled double-blind trial in patients with metastatic pancreatic cancer	OS, PFS, ORR, tumor-related symptoms (pain score, analgesic consumption, ECOG PS, weight change from baseline), safety (including immunogenicity), PK	541 (270 aflibercept)	Aflibercept 4 mg/kg IV Q2W or placebo plus gemcitabine 1000 mg/m^2 IV on Days 1, 8, 15, and 22 of Cycle 1 (28 days), and then Days 1, 8, and 15 of subsequent 28-day cycles
TCD10173[‡]	Phase 1, open label, dose escalation trial in patients with non Hodgkin's B-cell lymphoma	DLT and recommended dose of aflibercept in combination with RCHOP14 or RCHOP21, safety (including immunogenicity), efficacy (PFS), exploratory biomarkers	28	Aflibercept 2 and 4 mg/kg IV Q2W plus RCHOP14; Aflibercept 2, 3, and 6 mg/kg IV Q3W plus RCHOP21

* Number of patients treated
[‡] No PK or immunogenicity data

5-FU: 5-fluorouracil

HRQL: Health Related Quality of Life

GV: Aflibercept IV infusion over 1 hour, followed by gemcitabine 1000 mg/m^2 IV infusion over 30 minutes on Days 1 and 15 of each 28-day cycle. Gemcitabine administered alone on Day 22 of Cycle 1 and Day 8 of each subsequent 28-day cycle (Cycle 2 and beyond).

FOLFIRI: Irinotecan 180 mg/m^2 IV over 60 minutes with LV 200 mg/m^2 (or levo-LV 100 mg/m^2) IV over 2 hours, followed by 5-FU 400 mg/m^2 IV bolus over 2-4 minutes, then 600 mg/m^2 IV infusion over 22 hours on Day 1; LV 200 mg/m^2 (or levo-LV 100 mg/m^2) over 2 hours, followed by 5-FU 400 mg/m^2 IV bolus then 600 mg/m^2 IV infusion over 22 hours on Day 2.

LV: Leucovorin

RCHOP14/RCHOP21: Day 1 - Rituximab 375 mg/m² IV, cyclophosphamide 750 mg/m² IV, doxorubicin 50 mg/m² IV, vincristine 1.4 mg/m² IV. Days 1-5 - Prednisone 40 mg/m² PO.

2.2.2 What is the basis for selecting the response endpoints (i.e., clinical or surrogate endpoints and how are they measured in clinical pharmacology and clinical studies? What is the clinical outcome in terms of efficacy and safety?

The primary efficacy endpoint for the registrational Phase 3 trial EFC10262 was overall survival (OS). Following radiological documented progressive disease as determined by an independent review committee (IRC), patients were followed for survival status every

two months until death, withdrawal of patient consent, or cutoff date (February 7, 2011) for primary analysis, whichever came first.

In Trial EFC10262/VELOUR, mCRC patients were randomized to receive either aflibercept 4 mg/kg IV Q2W plus irinotecan, 5-FU, and leucovorin (FOLFIRI) (n=612) or placebo plus FOLFIRI (n=614). An OS benefit of 1.4 months was demonstrated in the aflibercept arm (median: 13.5 months) versus the placebo arm (median: 12.1 months), with a stratified hazard ratio (HR) of 0.816 [(95% CI: 0.713, 0.934); p = 0.0032]. Median follow up time was 22 months.

The addition of aflibercept to FOLFIRI increases the incidence of adverse events (AEs) associated with VEGF inhibition including hypertension, dysphonia, proteinuria, and hemorrhage (epistaxis, gastrointestinal bleeding), and less frequent but potentially severe AEs such as arterial and venous thromboembolic events (ATE and VTE). Aflibercept also increases the frequency and severity of background chemotherapy toxicities including stomatitis, diarrhea, and neutropenia.

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?

Free aflibercept (active), VEGF-bound aflibercept (stable inert complex), and free endogenous VEGF plasma concentrations were measured using validated enzyme-linked immunosorbent assays (ELISAs). Refer to **2.6 Analytical Section** for bioanalytical methodology.

Total aflibercept concentrations were calculated by adding free and adjusted VEGF-bound concentrations. Adjusted VEGF-bound concentrations were calculated using the following formula:

Adjusted bound aflibercept = 0.717 (measured VEGF:aflibercept complex)

VEGF:aflibercept complex contains 1 molecule VEGF (molecular weight [MW] of approximately 40 kDa) and 1 molecule of aflibercept (MW of approximately 115 kDa).

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.

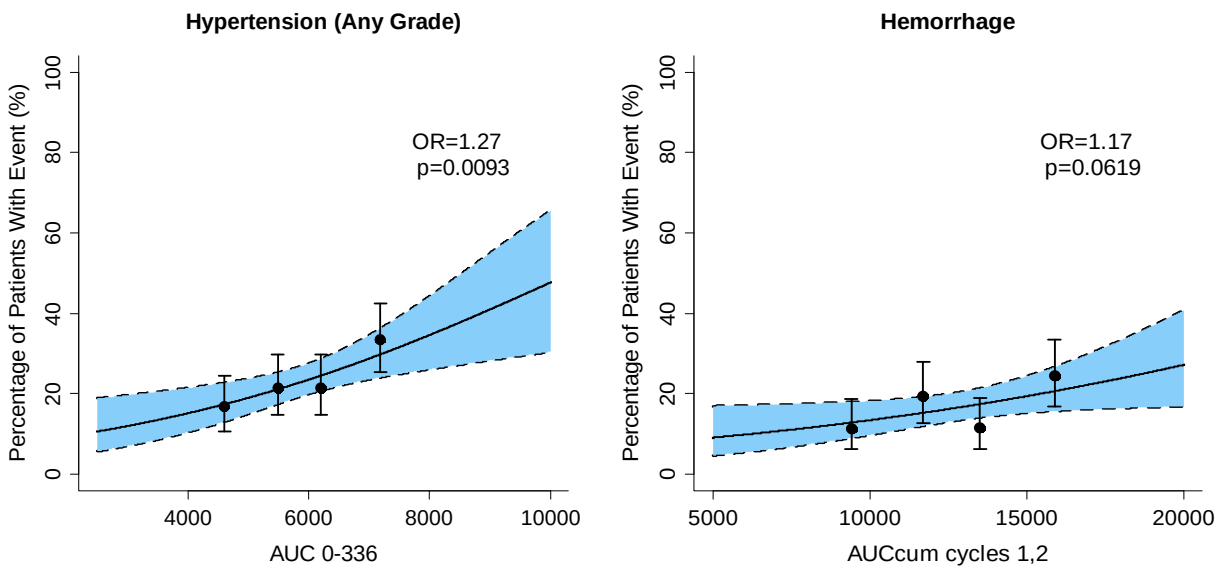
In the registrational trial, EFC10262/VELOUR, OS was significantly related with free and VEGF-bound aflibercept exposure. For the multivariate Cox proportional regression analysis using model-derived free aflibercept steady state AUC, an increase of 1000 µg·h/mL was associated with a 21% decrease in the survival HR. The relationship

remained significant when endogenous VEGF was included in the model. Consistent results were observed for PFS where an increase of 1000 µg·h/mL was associated with a 19% decrease in the survival HR. Refer to the Pharmacometrics review by Dr. Kevin Krudys.

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.

Incidence of hypertension and hemorrhage during the first two cycles were found to be significantly related to exposure of free aflibercept in the registrational trial (**Figure 1**). The odds of experiencing hypertension increased by 27% for an increase in $AUC_{0\text{-}336h}$ of 1000 µg·h/mL. For an increase of 2000 µg·h/mL in $AUC_{cum\ cycles1,2}$, the odds of hemorrhage increased by 17%. Refer to the Pharmacometrics review by Dr. Kevin Krudys.

Figure 1. Exposure-response relationship for hypertension (left) and hemorrhage (right) in Trial EFC10262



Source: Pharmacometrics Review by Dr. Kevin Krudys

2.2.4.3 Does this drug prolong the QT/QTc interval?

A randomized, double-blind, placebo-controlled study (TES10897/QUTIE) was conducted to assess the potential influence of aflibercept on the QT/QTc interval in patients with solid tumors (n=87). Triplicate ECGs were obtained at pre-dose, and 0.5, 1 (end of infusion), 2, 3, 4, and 6 hours post-dose during Cycles 1 and 3. PK samples were collected at pre-dose, 0.5, 1 (end of infusion), 3, and 6 hours post-dose during Cycles 1 and 3, and at Day 60 follow-up.

No large changes (i.e., >20 ms) in QTcF interval were detected following aflibercept dosing. The largest upper bound of the 2-sided 90% CI for the mean QTcF change from baseline (as corrected for placebo) was 15.7 ms at Cycle 3 (2 hours post-dose). In addition, no apparent concentration-QT relationship was identified within the range of concentrations observed in this trial. The 6 mg/kg Q3W dosing regimen used in this study resulted in free aflibercept C_{max} values 2-fold higher than those achieved with the 4-mg/kg Q2W dosing regimen in the registration trial. These exposures also cover the high exposure scenario which is a 30% increase in free aflibercept exposure in patients >100 kg.

Further details can be found in the review performed by the QT-Interdisciplinary Review Team.

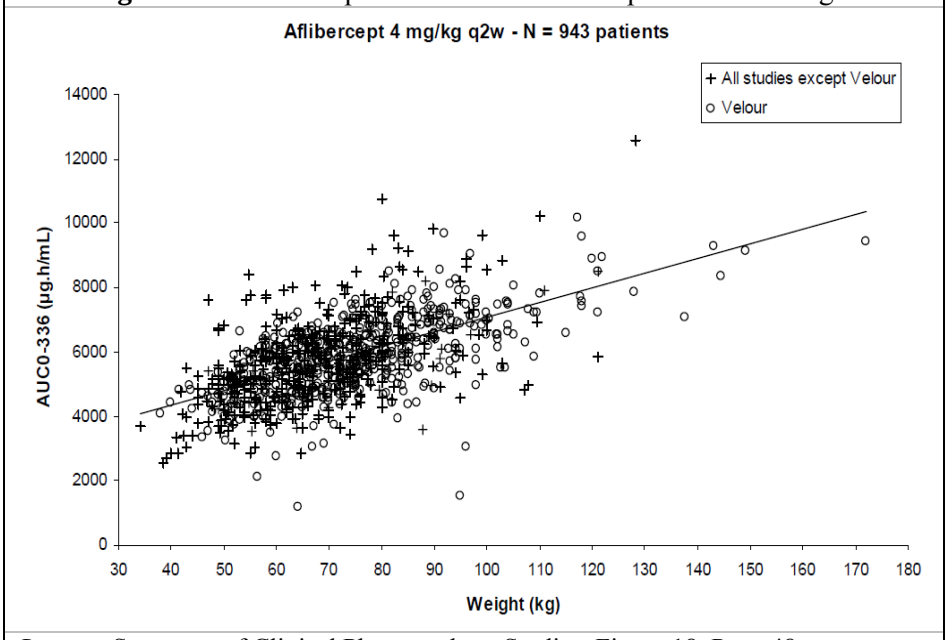
2.2.4.4 Is the dose and dosing regimen selected by the sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

The clinical dose of 4 mg/kg IV Q2W was selected based on maintaining a free/bound aflibercept ratio >1, and acceptable efficacy and safety results from two Phase 1 dose escalation studies. Preclinical pharmacological data and *in vivo* determination of the dissociation constant (K_D) suggested that maintaining a free/bound aflibercept ratio above 1 throughout the dosing interval would maximize binding of endogenous VEGF and maintain VEGF levels <20 pg/mL (near the median value of 17 pg/mL in healthy subjects). At doses >2 mg/kg Q2W, the mean ratio of free/bound aflibercept trough concentrations was >1 in all monotherapy and combination Phase 1 studies.

The proposed dosing regimen of aflibercept 4 mg/kg IV Q2W was initially evaluated in Phase 1 monotherapy studies TED6115 and TED6116 at doses ranging from 0.3, 1, 2, 3, 4, 5, 7 mg/kg, and was identified as a safe and biologically active dose for further evaluation as monotherapy in Phase 2 studies. In the Phase 1 combination study (TCD6118) with 5-fluorouracil (5-FU), leucovorin, and irinotecan, aflibercept doses from 2 to 6 mg/kg Q2W were investigated and best overall response was evaluated at the selected dosing regimen of 4 mg/kg IV Q2W in an extension cohort (similar to a single arm Phase 2 trial). Of note, maximum tolerated dose (MTD) was not reached.

Weight-based dosing of aflibercept (4 mg/kg) in the registrational trial resulted in a strong relationship between body weight and free aflibercept exposure (AUC) (**Figure 2**).

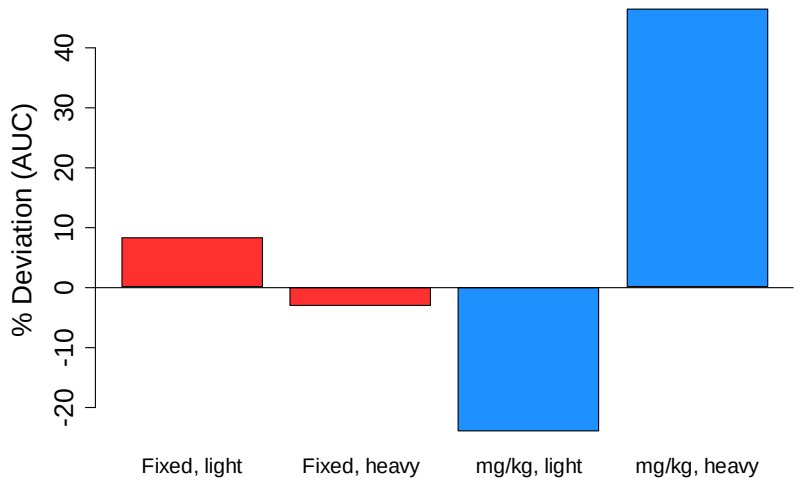
Figure 2. Relationship between free aflibercept AUC and weight



Source: Summary of Clinical Pharmacology Studies, Figure 18, Page 48

Simulations were therefore performed to compare a fixed dose of 300 mg (equivalent to a 4 mg/kg dose in a 75 kg patient) to the weight-based dose of 4 mg/kg. Fixed dosing resulted in tighter distribution of AUC_{ss} values, with less deviation of exposure in heavy (body weight $\geq 90^{th}$ percentile) and light (body weight $\leq 10^{th}$ percentile) patients, as compared to weight-based dosing (**Figure 3**). Given the observed exposure-response relationships for efficacy and safety in the registrational trial, a fixed dose has potential to reduce underexposure in lighter patients possibly improving efficacy outcomes, or reduce overexposure in heavier patients possibly decreasing adverse events. Refer to the Pharmacometrics review by Dr. Kevin Krudys.

Figure 3. Deviation of exposure (AUC_{ss}) from those of patients with median body weight after fixed dose or body weight-based dose



Source: Pharmacometrics Review by Dr. Kevin Krudys

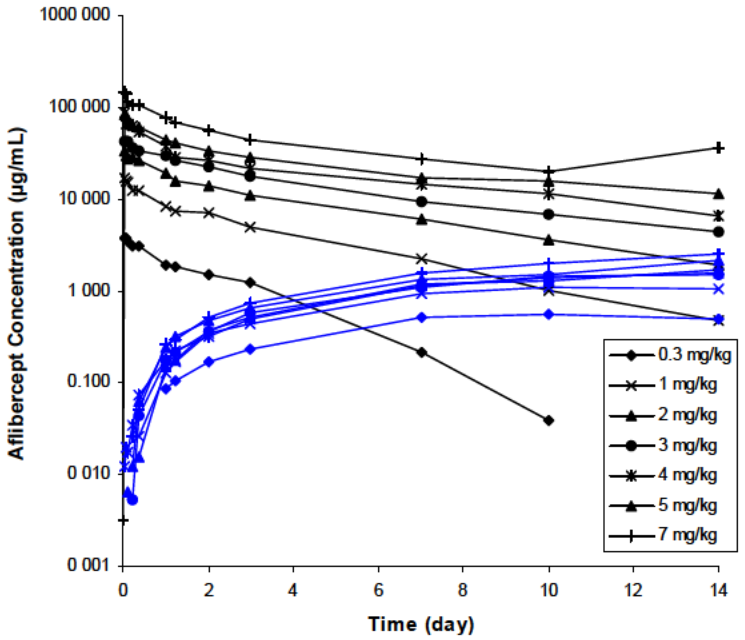
2.2.5 What are the PK characteristics of the drug and its major metabolite?

The PK of IV aflibercept have been evaluated as monotherapy (range of 0.3 to 7 mg/kg IV Q2W) and in combination with chemotherapy (range of 2 to 6 mg/kg Q2W and 2 to 9 mg/kg Q3W). Free aflibercept increased slightly more than dose-proportionally between 1 to 2 mg/kg, and appeared to exhibit linear PK in the dose range of 2 to 9 mg/kg. T_{max} of free aflibercept is reached at the end of infusion (1 hour). Following 4 mg/kg Q2W IV administration, the elimination half-life ($t_{1/2}$) of free aflibercept was approximately 6 days (range 4-7 days). Steady state concentrations of free aflibercept were reached by the second dose. The accumulation ratio for free aflibercept was approximately 1.3 after administration of 4 mg/kg Q2W. Based on a population PK analysis with data from 1378 patients who received 2 to 9 mg/kg of aflibercept Q2W or Q3W as monotherapy or in combination with chemotherapy, the estimated $t_{1/2}$ of VEGF-bound aflibercept was approximately 15 days. The estimated time to reach steady state concentrations of VEGF-bound aflibercept was 70 days, corresponding to the sixth dose.

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

PK characteristics of free and VEGF-bound aflibercept were determined in monotherapy studies (TED6115/TED6116) in the dose range of 0.3 to 7 mg/kg IV Q2W and 5 combination studies (TCD6117, TCD6118, TCD6119, TCD6120, and TCD6121) in the dose range of 2 to 6 mg/kg Q2W and 2 to 9 mg/kg Q3W. Intensive PK sampling was collected in these studies. The concentration-time profile of free and VEGF-bound aflibercept is shown in **Figure 4**. A summary of PK parameters of free and VEGF-bound aflibercept is detailed in **Table 3**, **Table 4**, and **Table 5**.

Figure 4. Concentration time-profile of free and VEGF-bound aflibercept



Free aflibercept represented in black; VEGF-bound aflibercept represented in blue

Table 3. Summary of Free and VEGF-Bound Aflibercept PK Parameters After Single Dose Administration in Healthy Subjects (Study PDY6656)

		Pharmacokinetic Parameters [a]					
		AUC[b] (µg·day/mL)	C_{max} (µg/mL)	T_{max} (day)	$t_{1/2}$ (day)	CL (L/day)	V_{ss} (L)
Free	1.0 (n=12)	63.6 (20)	17.9 (18)	0.042 (0.042, 0.333)	4.48 (35)	1.22 (22)	5.77 (25)
	2.0 (n=12)	177 (20)	38.6 (27)	0.042 (0.042, 0.333)	5.26 (6.1)	0.903 (19)	5.91 (15)
	4.0 (n=12)	412 (21)	77.8 (15)	0.042 (0.042, 1.00)	5.75 (18)	0.780 (19)	6.36 (23)
Bound	1.0 (n=12)	35.8 (11)	1.20 (12)	17.5 (14.0, 28.0)	-	-	-
	2.0 (n=12)	72.0 (14)	2.38 (16)	21.0 (21.0, 28.0)	-	-	-
	4.0 (n=12)	76.8 (21)	2.62 (31)	28.0 (7.0, 35.0)	-	-	-

	Pharmacokinetic Parameters [a]					
	AUC[b] (µg·day/mL)	C_{max} (µg/mL)	T_{max} (day)	$t_{1/2}$ (day)	CL (L/day)	V_{ss} (L)

[a] AUC and C_{max} presented as geometric mean (%CV); t_{max} presented as median (min, max)
[b] $AUC_{0-\infty}$ for free aflibercept; AUC_{0-last} for bound aflibercept

Table 4. Summary of First-Dose (Day 1) PK Parameters of Free and VEGF-Bound Aflibercept Administered Q2W as Monotherapy in Patients (Study TED6115)

		Pharmacokinetic Parameters [a]					
		AUC[b] (µg·day/mL)	C_{max} (µg/mL)	t_{max} (day)	$t_{1/2}$ (day)	CL (L/day)	V_{ss} (L)
Free	0.3 (n=3)	9.27 (15)	3.99 (9.0)	0.042 (0.042, 0.125)	1.7 (21)	1.95 (42)	4.51 (29)
	1.0 (n=7)	45.6 (54)	17.2 (31)	0.042 (0.042, 0.124)	2.58 (50)	1.87 (51)	5.88 (22)
	2.0 (n=6)	117 (35)	34.3 (11)	0.042 (0.042, 0.083)	3.76 (42)	1.13 (31)	5.58 (21)
	3.0 (n=7)	210 (34)	46.7 (30)	0.083 (0.042, 0.125)	6.18 (38)	1.14 (48)	7.74 (33)
	4.0 (n=7)	291 (15)	90.6 (43)	0.045 (0.040, 0.212)	5.51 (18)	1.10 (38)	7.88 (38)
	5.0 (n=4)	362 (64)	82.4 (34)	0.045 (0.021, 0.088)	7.43 (38)	1.27 (65)	9.89 (31)
	7.0 (n=12[c])	557 (46)	155 (21)	0.083 (0.042, 0.376)	5.14 (37)	0.915 (39)	6.12 (29)
Bound	0.3 (n=3)	5.53 (5.0)	0.574 (5.0)	9.96 (7.03, 9.98)	-	-	-
	1.0 (n=7)	9.67 (30)	1.18 (29)	9.98 (7.00, 14.0)	-	-	-
	2.0 (n=6)	11.8 (41)	1.51 (32)	13.5 (9.01, 13.9)	-	-	-
	3.0 (n=7)	15.3 (38)	1.68 (23)	13.9 (7.08, 23.0)	-	-	-
	4.0 (n=7)	10.0 (38)	1.16 (45)	9.99 (3.04, 14.0)	-	-	-
	5.0 (n=4)	12.7 (18)	1.90 (25)	10.5 (6.98, 21.8)	-	-	-
	7.0 (n=13[d])	20.3 (26)	2.20 (37)	14.0 (7.02, 27.9)	-	-	-

	Pharmacokinetic Parameters [a]					
	AUC[b] (µg·day/mL)	C_{max} (µg/mL)	t_{max} (day)	$t_{1/2}$ (day)	CL (L/day)	V_{ss} (L)

[a] AUC and C_{max} presented as geometric mean (%CV); t_{max} presented as median (min, max)
[b] $AUC_{0-\infty}$ for free aflibercept; AUC_{0-last} for VEGF-bound aflibercept
[c] n=12 for C_{max} and T_{max}; n=11 for all other PK parameters
[d] n=13 for C_{max} and T_{max}; n=10 for AUC_{0-last}

Table 5. Summary of first-dose (Cycle 1) PK parameters of free and VEGF-bound aflibercept administered in combination with chemotherapy (Study TCD6117, TCD6118, TCD6119, TCD6120, TCD6121)

		Pharmacokinetic Parameters [a]					
		AUC[b] (µg.day/mL)	C_{max} (µg/mL)	t_{max} (day)	$t_{1/2}$ (day)	CL (L/day)	V_{ss} (L)
Free	2.0 (n=28)	127 (39)	36.7 (25)	0.04 (0.04, 0.33)	3.5 (34)	1.2 (42)	4.9 (32)
	4.0 (n=102)	298 (37)	83.1 (35)	0.05 (0.004, 0.35)	4.5 (32)	1.0 (44)	5.1 (38)
	5.0 (n=34)	396 (35)	96.7 (37)	0.06 (0.04, 1.0)	5.5 (36)	1.0 (43)	6.0 (43)
	6.0 (n=127)	428 (38)	113 (35)	0.05 (0.01, 1.0)	5.2 (28)	1.0 (40)	6.3 (40)
	7.0 (n=4)	584 (54)	151(18)	0.05 (0.04, 0.33)	5.8 (28)	0.9 (55)	5.6 (25)
	9.0 (n=3)	823 (33)	195 (18)	0.17 (0.08, 0.17)	5.5 (5.0)	0.9 (30)	5.2 (17)
Bound	2.0 (n=28)	18.1 (39)	1.65 (34)	14.0 (7.0, 29.0)	-	-	-
	4.0 (n=98)	17.6 (48)	1.78 (34)	14.0 (2.0, 33.0)	-	-	-
	5.0 (n=36)	19.2 (70)	1.96 (31)	17.9 (7.0, 28.0)	-	-	-
	6.0 (n=124)	25.0 (46)	2.11 (34)	21.0 (7.0, 35.0)	-	-	-
	7.0 (n=5)	37.5 (11)	2.79 (12)	21.0 (14.0, 23.0)	-	-	-
	9.0 (n=3)	26.9 (20)	2.36 (4.0)	21.0 (19.9, 21.1)	-	-	-

[a] AUC and C_{max} presented as geometric mean (%CV); t_{max} presented as median (min, max)
[b] $AUC_{0-\infty}$ for free aflibercept; AUC_{0-last} for VEGF-bound aflibercept

Table 6. Accumulation Ratios of Aflibercept

Dose[b] (mg/kg)	Pharmacokinetic Parameters (Accumulation Ratio)[a]	
	AUC (µg·day/mL) Cycle 2[c]/Cycle 1	C_{max} (µg/mL) Cycle 2[c]/Cycle 1
2.0 (n=11)	1.1 (29)	1.3 (28)
4.0 (n=61)	1.3 (28)	1.1 (25)
5.0 (n=16)	1.2 (24)	1.0 (22)
6.0 (n=20)	1.3 (32)	1.1 (28)

[a] Data presented as mean ratio (%CV)
[b] Doses administered Q2W
[c] Cycle 2 PK data available only from Study TCD6117, TCD6118, and TCD6121

2.2.5.2 How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

Free aflibercept exposure (AUC and C_{max}) were slightly higher in healthy volunteers (**Table 3**) as compared to those in patients (**Table 4**), possibly due to lower endogenous VEGF levels available to bind to aflibercept.

2.2.5.3 What are the characteristics of drug absorption?

The subcutaneous (SC) dosage form of aflibercept was initially investigated in two monotherapy PK studies. After a single dose of 2 mg/kg aflibercept by SC administration, the absolute bioavailability of free aflibercept was 54.9% (n=38 subjects in Study PDY6655). The observed C_{max} of free aflibercept, reached approximately 2 days (t_{max}) following SC administration, were about 5-fold lower than those observed at the end of the 1-hour IV infusion. Due to the high volume (>10 mL) of 25 mg/mL solution required for SC injection, only the IV route was subsequently studied in all clinical trials.

2.2.5.4 What are the characteristics of drug distribution?

Aflibercept exhibited an estimated steady state volume of distribution (V_{ss}) of 7.8 L, which is slightly greater than the blood volume.

2.2.5.5 Does the mass balance study suggest renal or hepatic as the major route of elimination?

Not applicable. No radiolabeled mass balance study with aflibercept has been conducted in humans to determine the proportion of administered dose cleared through specific mechanisms. Mass balance studies are not generally performed for therapeutic proteins due to target-mediated drug disposition and protein catabolism as the common elimination pathways.

2.2.5.6 What are the characteristics of drug metabolism?

Not applicable. Metabolism studies are generally not performed for therapeutic proteins.

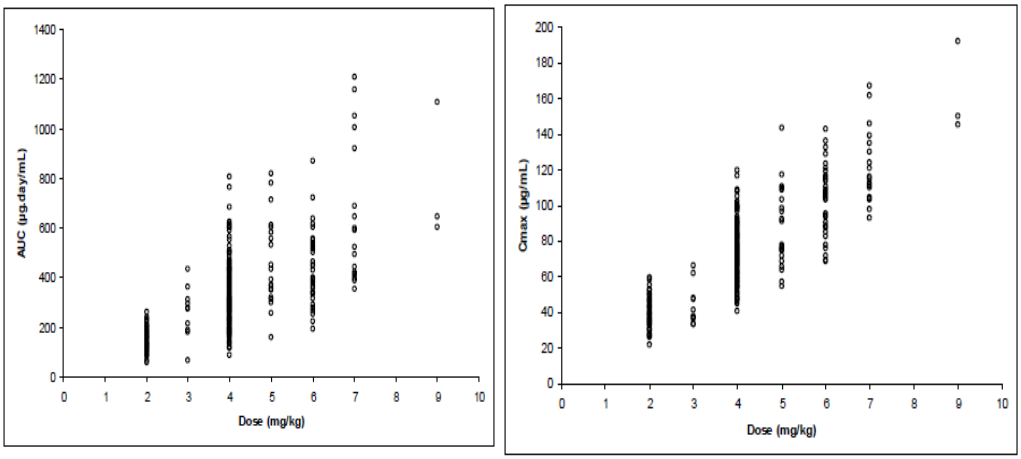
2.2.5.7 What are the characteristics of drug excretion and elimination?

In addition to VEGF target-mediated drug disposition, free aflibercept is most likely eliminated through proteolysis.

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

Free aflibercept increased slightly more than dose-proportionally between 1 to 2 mg/kg, and appear to exhibit linear PK in the dose range of 2 to 9 mg/kg (**Figure 5**).

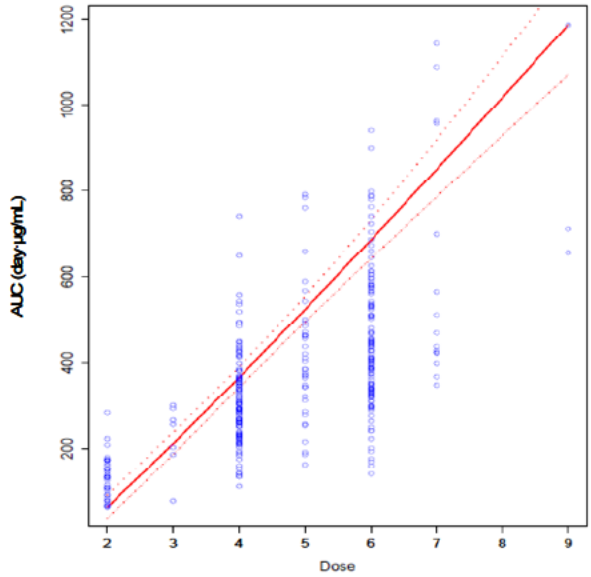
Figure 5. Free aflibercept AUC and C_{max} by dose



Source: Summary of Clinical Pharmacology Studies, Figure 6, Page 37

A power model was applied to test dose proportionality in the range of 2 to 9 mg/kg. The slope of the power model on the logarithmic scale was 1.12 for AUC with a 90% confidence interval of (1.02, 1.22) (**Figure 6**).

Figure 6. Dose proportionality of aflibercept



Source: Analysis by Clinical Pharmacology Reviewer

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

Free and VEGF-bound aflibercept trough concentrations (C_{trough}) were measured throughout the duration of treatment in each patient. Drug accumulation of free aflibercept was minimal, with an accumulation ratio of approximately 1.3 following administration of 4 mg/kg Q2W.

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?

Inter-individual variability (CV%) in CL and V_{ss} ranged from approximately 20% to 40% in Phase 1 studies. In the registrational trial, the variability in CL, V_{ss}, C_{max}, and AUC_{0-336h}, were 33%, 14%, 19%, and 20%, respectively.

The population PK covariate analysis identified several factors as significant factors explaining variability in V_c and CL. V_c increases with an increase in weight, CL_{Cr}, and in males, and decreases with an increase of total protein. CL increases with an increase in ALK, CL_{Cr}, BW, total protein, in males, irinotecan/LV5-FU2, and docetaxel. CL decreases with an increase in albumin. There was no effect of total bilirubin, aspartate amino transferase, or alanine amino transferase on free aflibercept clearance. Sex had the largest effect on free aflibercept PK with men having a 16% higher clearance and 21% higher V_c. The remaining interindividual variability not explained by covariates was 28% for CL and 20% for V_c. Refer to the Pharmacometrics review by Dr. Kevin Krudys.

2.3 Intrinsic Factors

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

Body weight

With weight-based dosing, patients weighing ≥100 kg had a 30% increase in drug exposure compared to patients weighing <100 kg. Given the observed exposure-response relationship for safety in the registrational trial, higher exposures in heavier patients may lead to increased incidence of adverse events. Based on an analysis of incidence of adverse events by BMI status (**Table 7**), aflibercept-related toxicities including hypertension, neutropenia, diarrhea, and pulmonary embolism were increased in obese patients (BMI ≥30). However, increases in these toxicities may not be solely attributed to the higher exposures in heavier patients due to other potential confounding factors.

Table 7. Grade 3-4 AEs by BMI Status

PT	Placebo/FOLFIRI (n, %) N=602			Aflibercept/FOLFIRI (n, %) N=608		
	Underweight/ Normal[a] (N=260)	Overweight[b] (N=233)	Obese[c] (N=109)	Underweight/ Normal[a] (N=252)	Overweight[b] (N=243)	Obese[c] (N=113)
Neutropenia	68 (26)	47 (20)	17 (16)	63 (25)	57 (24)	31 (27)
Diarrhea	17 (7)	21 (9)	9 (8)	49 (19)	40 (16)	29 (26)
Hypertension	2 (1)	4 (2)	3 (3)	47 (19)	44 (18)	26 (23)
Fatigue	30 (12)	11 (5)	6 (6)	39 (15)	24 (10)	14 (12)
Stomatitis	16 (6)	9 (4)	3 (3)	39 (15)	27 (11)	12 (11)
Asthenia	6 (2)	8 (3)	4 (4)	18 (7)	9 (4)	4 (4)
Febrile neutropenia	6 (2)	2 (1)	2 (2)	14 (6)	8 (3)	4 (4)
Dehydration	4 (2)	4 (2)	0	13 (5)	8 (3)	5 (4)
Abdominal pain	9 (3)	2 (1)	3 (3)	9 (4)	12 (5)	6 (5)
Decreased appetite	9 (3)	1 (<1)	1 (1)	11 (4)	7 (3)	3 (3)
Vomiting	9 (3)	7 (3)	5 (5)	8 (3)	6 (2)	3 (3)
Palmar-plantar erythrodysestesia	1 (<1)	1 (<1)	1 (1)	8 (3)	8 (3)	1 (1)
Pulmonary embolism	5 (2)	9 (4)	7 (6)	5 (2)	12 (5)	11 (10)
Intestinal obstruction	6 (2)	4 (2)	2 (2)	4 (2)	4 (2)	0
Nausea	7 (3)	6 (3)	5 (5)	4 (2)	4 (2)	3 (3)
Pneumonia	1 (<1)	3 (1)	0	5 (2)	4 (2)	2 (2)
Ascites	3 (1)	1 (<1)	0	4 (2)	1 (<1)	0
Headache	0	1 (<1)	1 (1)	5 (2)	3 (1)	2 (2)
Proteinuria	0	0	0	5 (2)	8 (3)	5 (4)
Sepsis	2 (1)	1 (<1)	2 (2)	5 (2)	3 (1)	0
Weight decreased	4 (2)	1 (<1)	0	6 (2)	6 (2)	4 (4)
Deep vein thrombosis	4 (2)	4 (2)	3 (3)	3 (1)	9 (4)	1 (1)
Back pain	4 (2)	3 (1)	4 (4)	3 (1)	2 (1)	2 (2)
Device related	3 (3)	2 (1)	2 (2)	2 (1)	1 (<1)	3 (3)

PT	Placebo/FOLFIRI (n, %) N=602			Aflibercept/FOLFIRI (n, %) N=608		
	Underweight/ Normal[a] (N=260)	Overweight[b] (N=233)	Obese[c] (N=109)	Underweight/ Normal[a] (N=252)	Overweight[b] (N=243)	Obese[c] (N=113)
infection						
Syncope	2 (1)	3 (1)	4 (4)	3 (1)	5 (2)	2 (2)
Hyperbilirubinemia	3 (1)	1 (<1)	2 (2)	3 (1)	0	0
Ileus	4 (2)	1 (<1)	0	2 (1)	0	0
Peripheral neuropathy	0	1 (<1)	2 (2)	2 (1)	2 (1)	2 (2)
Neutropenic infections	4 (2)	1 (<1)	2 (2)	2 (1)	3 (1)	1 (1)
Abdominal upper pain	2 (1)	2 (1)	2 (2)	0	5 (2)	2 (2)

[a] Underweight and normal weight: BMI <25
[b] Overweight: BMI 25-29.9
[c] Obese: BMI ≥30

Source: Clinical review by Dr. Sandra Casak

2.3.2 Based upon what is known about exposure-response relationships and their variability and the groups studied, healthy volunteers vs. patients vs. specific populations, what dosage regimen adjustments, if any, are recommended for each of these groups? If dose regimen adjustments are not based upon exposure-response relationships, describe the alternative basis for the recommendation.

No clinically meaningful PK differences have been identified in specific patient populations; therefore no dosing regimen adjustments are recommended for specific patient populations.

2.3.2.1 Elderly Patients

Age was not identified as a significant covariate influencing aflibercept PK based on a population PK analysis which included patients <65 (n=1038, 69%), 65-75 (n=392, 26%), and >75 (n=77, 5%) years of age.

2.3.2.2 Pediatric Patients

The safety and effectiveness of aflibercept in the pediatric patient population have not been established. The applicant requests a full waiver of pediatric studies because mCRC in the pediatric population is rare. Furthermore, colorectal cancer is an adult-related condition that may qualify the drug for a disease-specific waiver.

2.3.2.3 Sex

Sex was identified as a significant covariate and having the largest effect on free aflibercept PK, with a 16% higher clearance and 21% higher volume of distribution in men than women. In the registrational trial (n=201 women; n=299 men), mean (CV%)

aflibercept exposure was similar in both genders with AUC values of 304 (26%) µg·day/mL and 301 (25%) µg·day/mL, respectively. This observation might be due to weight-based dosing as weight was lower in women than in men with mean (5th-95th percentile) of 66 (48-92) kg and 82 (59-115) kg, respectively.

2.3.2.4 Body weight

Population PK analyses revealed a shallow relationship between body weight (BW) and free aflibercept clearance. Relative to a median weight of 67 kg, free aflibercept clearance decreases by 5.8% for a weight of 49 kg and increases by 7.9% for a weight of 100 kg. Given that fixed dosing has not been explored during development, no dose adjustments are recommended. However, use of fixed dose should be considered for future trials with aflibercept.

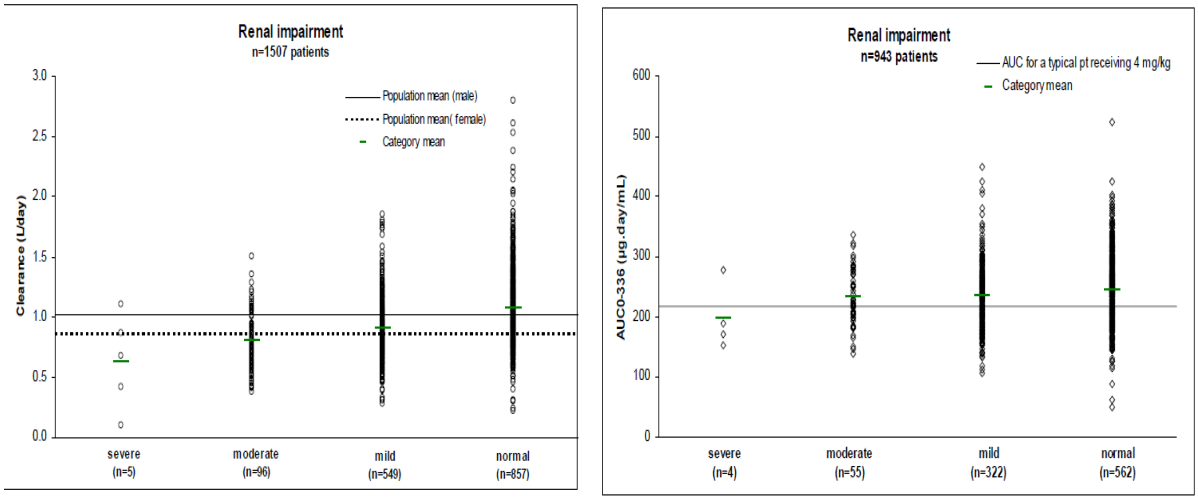
2.3.2.5 Race

Based on a population PK analysis which included 1378 Caucasian (91%), 75 Asian (5%), 27 Black (2%), and 27 Others (i.e., Hispanic, Mixed, 2%) patients, race was not identified as a significant covariate influencing aflibercept PK.

2.3.2.5 Renal Impairment

No dedicated clinical studies have been conducted to evaluate the effect of renal impairment on the PK of aflibercept. Based on a population PK analysis which included patients with mild (CL_{Cr} 50-80 mL/min, n=549), moderate (CL_{Cr} 30-50 mL/min, n=96), and severe (CL_{Cr} <30 mL/min, n=5) renal impairment, the effect of CL_{Cr} on free aflibercept was minor, and not expected to be clinically meaningful (**Figure 7**). Clearance decreases by 6.5% for a CL_{Cr} of 48 mL/min (5th percentile of the population) and increases by 10% for a CL_{Cr} of 148 mL/min (95th percentile of the population). Model-derived $AUC_{0\text{-}336h}$ in patients with mild, moderate, and severe renal impairment were similar to those in patients with normal renal function (**Figure 7**). Given that aflibercept has a molecular weight of 115 kDa which is larger than human serum albumin, renal impairment is not expected to have clinically meaningful effect on aflibercept clearance.

Figure 7. Clearance (left) and AUC (right) by renal function status

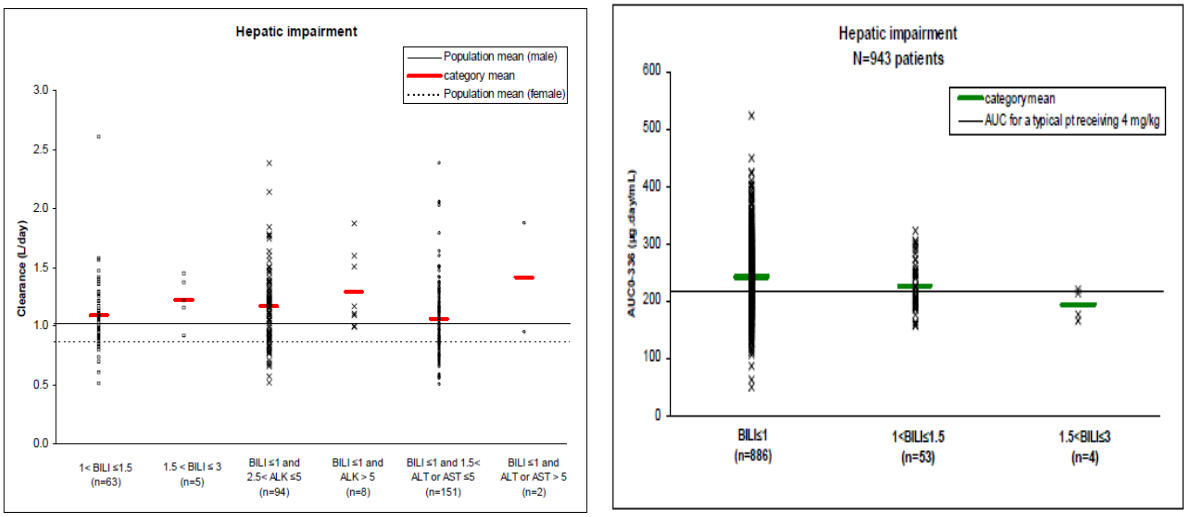


Source: Summary of Clinical Pharmacology Studies, Figure 20 and 21, Pages 50-51

2.3.2.6 Hepatic Impairment

No dedicated clinical studies have been conducted to evaluate the effect of hepatic impairment on the PK of aflibercept. Based on a population PK analysis which included patients with mild (total bilirubin >1.0x-1.5x ULN and any SGOT/AST, n=63) and moderate (total bilirubin >1.5x–3x ULN and any SGOT/AST, n=5) hepatic impairment, there was no effect of total bilirubin, aspartate amino transferase and alanine amino transferase on the clearance of free aflibercept (**Figure 8**). Model-derived $AUC_{0\text{-}336h}$ values in patients with mild and moderate hepatic impairment were similar to those in patients with normal hepatic function (**Figure 8**). There is no data available for patients with severe hepatic impairment (total bilirubin >3x ULN and any SGOT/AST). Given that aflibercept is a therapeutic protein, hepatic impairment is not expected to have clinical meaningful effect on aflibercept clearance.

Figure 8. Clearance (left) and AUC (right) by hepatic function status

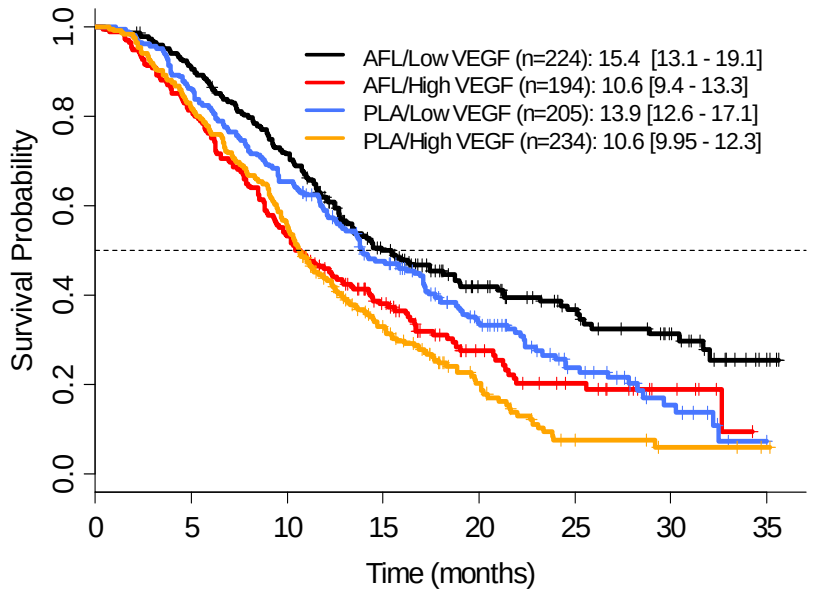


Source: Summary of Clinical Pharmacology Studies, Figure 22 and 23, Pages 52-53

2.3.2.7 What exploratory biomarker information is in the application and is it important or not?

Endogenous free VEGF plasma levels were measured at baseline in patients from the Phase 3 studies. An exploratory analysis showed that aflibercept-treated patients with low baseline VEGF levels had an OS benefit of 4.8 months as compared to those with high baseline VEGF (cutoff between low and high baseline VEGF of 42 pg/mL). A similar trend was observed in the placebo arm with an OS benefit of 3.3 months, suggesting that endogenous free VEGF could be a prognostic factor (**Figure 9**).

Figure 9. Relationship between endogenous baseline VEGF and overall survival



Source: Pharmacometrics Review by Dr. Kevin Krudys

2.3.2.8 What pregnancy and lactation use information is in the application?

Aflibercept is categorized as Pregnancy Category C. There are no adequate and well-controlled studies with aflibercept in pregnant women. Aflibercept was embryotoxic and teratogenic in rabbits at exposure levels lower than human exposures at the recommended dose, with increased incidences of external, visceral, and skeletal fetal malformations. Aflibercept should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. It is not known whether aflibercept is excreted in human milk.

2.3.3 Immunogenicity

2.3.3.1 What is the incidence (rate) of the formation of the anti-product antibodies (APA), including the rate of pre-existing antibodies, the rate of APA formation during and after the treatment, time profiles and adequacy of the sampling schedule?

The overall incidence of anti-product antibody (APA) development across 15 studies enrolling patients with various cancers was 4.2% in aflibercept-treated patients (72/1706; of which 19 tested positive at baseline) compared to 3.5% in placebo-treated patients (41/1156; of which 22 tested positive at baseline) (**Table 8**,**Table 9**). Among patients who tested positive for APA and had sufficient samples for further testing, neutralizing antibodies were detected in 17 of 48 aflibercept-treated patients and in 2 of 40 placebo-treated patients (**Table 9**).

Table 8. Immunogenicity Across Phase 1 and Phase 2 Studies

Trial Number	No. patients evaluable[a]	Positive at any time point	Positive APA post-baseline with negative (or missing) at baseline
TED6115/6116	42	0	0
TCD6117	30	1	1
TCD6118	60	4	0
TCD6119	37	2	1
TCD6120	125	6	6
TCD6121	37	3	1
ARD6122[b]	186	5	5
ARD6123[b]	64	0	0
ARD6772[b]	7	0	0

[a] Patients with at least one post-baseline measurement were considered evaluable.
[b] APA determined by PCL2375 assay method. PCL059 assay method used for the other studies.

Table 9. Immunogenicity Across Studies with Placebo Arm

Trial Number	Positive at any time point/evaluable patients[a]		Positive APA post-baseline with negative or missing at baseline/evaluable patients		Neutralizing antibodies/evaluable patients		Neutralizing antibodies/positive APA at any time point	
	Placebo	Aflibercept	Placebo	Aflibercept	Placebo	Aflibercept	Placebo	Aflibercept
EFC10262	18/526 (3.4%)	9/521 (1.7%)	8/526 (1.5%)	5[b]/521 (1.0%)	2/526 (0.4%)	1/521 (0.2%)	2/18 (11%)	1/9 (11%)
EFC10547	4/202 (2.0%)	5/201 (2.5%)	3/202 (1.5%)	4/201 (2.0%)	0/202 (0%)	2/201 (1.0%)	0/4 (0%)	2/5 (40%)
EFC10261	16/377 (4.2%)	32/344 (9.3%)	7/377 (1.9%)	29[b]/344 (8.4%)	0/377 (0%)	14/344 (4.1%)	0/16 (0%)	14/32 (44%)
EFC6125	1/12 (8.3%)	3/17 (18%)	0/12 (0%)	2/17 (12%)	-	-	-	-
TES10897	2/39 (5.1%)	2/35 (5.7%)	1/39 (2.6%)	1/35 (2.9%)	0/39 (0%)	0/35 (0%)	0/2 (0%)	0/2 (0%)

[a] Patients with at least one post-baseline measurement were considered evaluable.

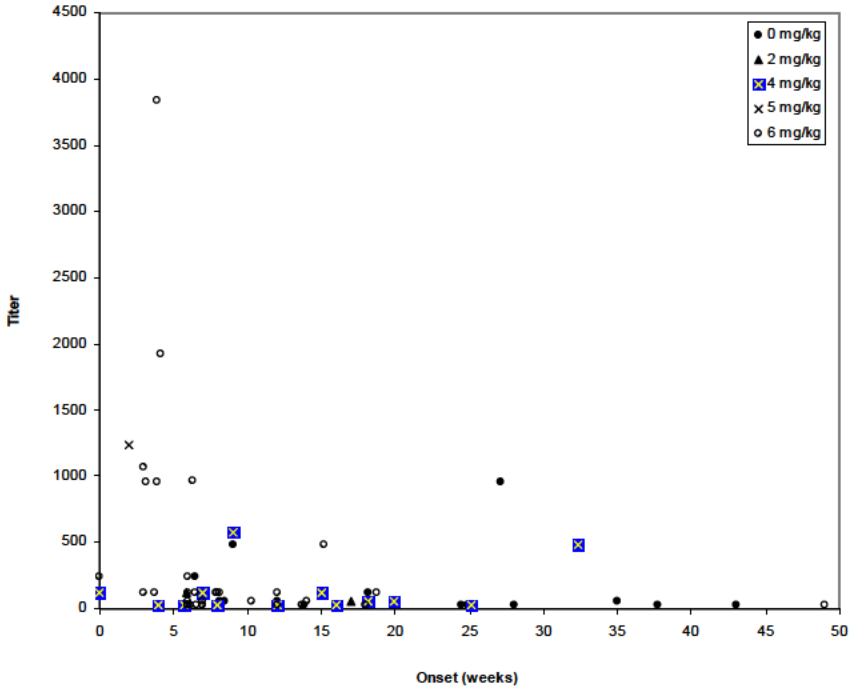
[b] Includes one patient with "treatment-boosted" APA positive response (e.g., pre-existing APA that were boosted to at least 2- to 3-fold higher following aflibercept administration).

Applicant's analysis:
Overall, of the patients evaluable for immunogenicity in clinical studies with aflibercept, 35/1105 (3.2%) placebo-treated patients and 63/1671 (3.8%) aflibercept-treated patients exhibited a positive low titer assay response at any time point, baseline or post-baseline, with positive response for neutralizing anti-aflibercept antibodies in 2 (0.2%) placebo-treated patients and 17 (1.3%) aflibercept-treated patients, respectively. In the pivotal study of metastatic CRC patients, 18/526 (3.4%) placebo-treated evaluable patients and 9/521 (1.7%) aflibercept-treated evaluable patients exhibited a positive assay response including one positive for neutralizing antibody in the aflibercept arm. Of note, most of the samples positive in the APA assay exhibited only the minimum assay titer (30), and none of the patients with a positive assay response exhibited a high titer result (>500) or a greater than 4-fold increase in the titer in subsequent samples. Since the level of low titer APA assay responses in the aflibercept-treated patients is similar to that observed in the placebo-treated patients, it is likely that most if not all the positive assay responses observed in the aflibercept-treated patients are due to high assay background levels and not due to treatment-emergent immune response to aflibercept.

Reviewer's comment: *The applicant's analysis did not include immunogenicity data from TES10897 and placebo-treated patients from Study EFC6125.*

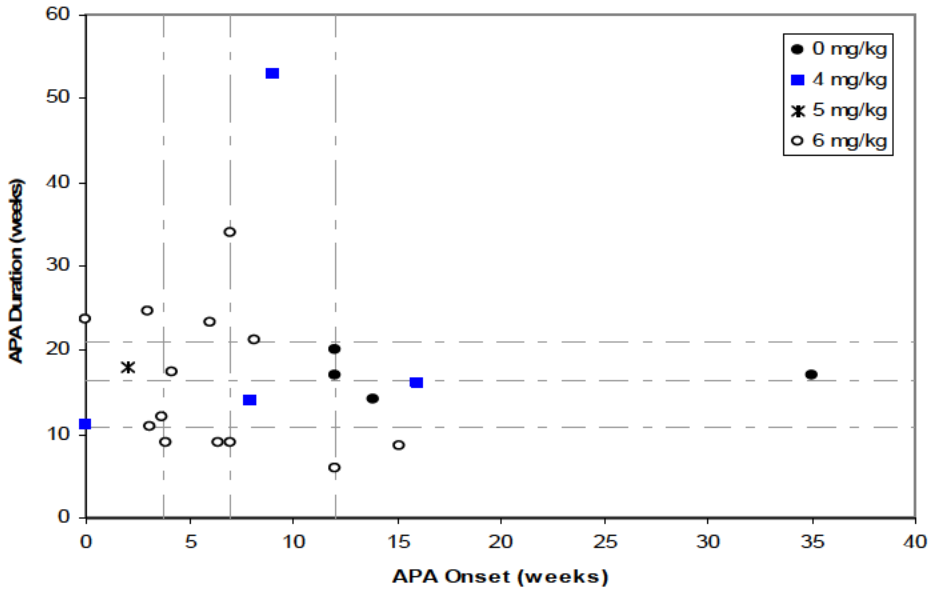
Titers were mostly ≤240 at onset of APA for the majority of patients (56/68) with a minimum assay titer of 30 (**Figure 10**). There appeared to be a trend towards higher titers with higher doses of 5 mg/kg and 6 mg/kg.

Figure 10. Titer vs. onset of APA



A descriptive illustration of APA kinetics for aflibercept is shown in **Figure 11** from 22 evaluable patients. Of the 22 patients with persistent APA, defined as treatment-induced APA detected at 2 or more sequential sampling time points during treatment (including follow-up period, if any) where the first and last APA positive samples were separated by a period exceeding 16 weeks or longer, the median duration of positivity was 16.5 weeks.

Figure 11. APA kinetic profile

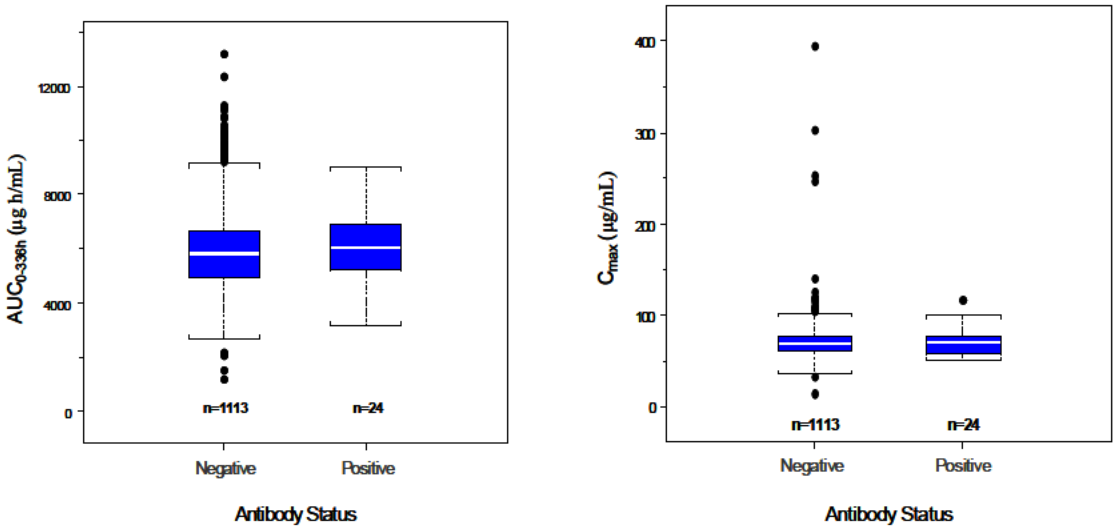


Dotted lines represent quartiles

2.3.3.2 Does the immunogenicity affect the PK and/or PD of the therapeutic protein?

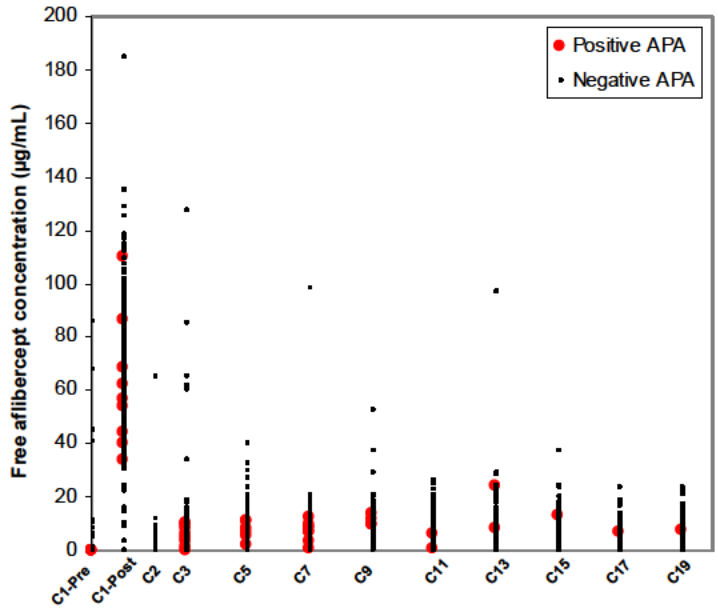
Overall, the presence of positive antibodies with the 4 mg/kg Q2W dosing regimen did not appear to impact the PK of free aflibercept in the registrational trial (**Figure 12**). Free aflibercept trough concentrations were also not affected (**Figure 13**).

Figure 12. Box plots of dose-normalized (4 mg/kg) $AUC_{0\text{-}336h}$ (left) and C_{max} (right) of free aflibercept after Q2W administration of aflibercept on Cycle 1 Day 1 by antibody status



The whiskers represent the 95% CI. The solid line is the median. Refer to **Table 1** for trial design.

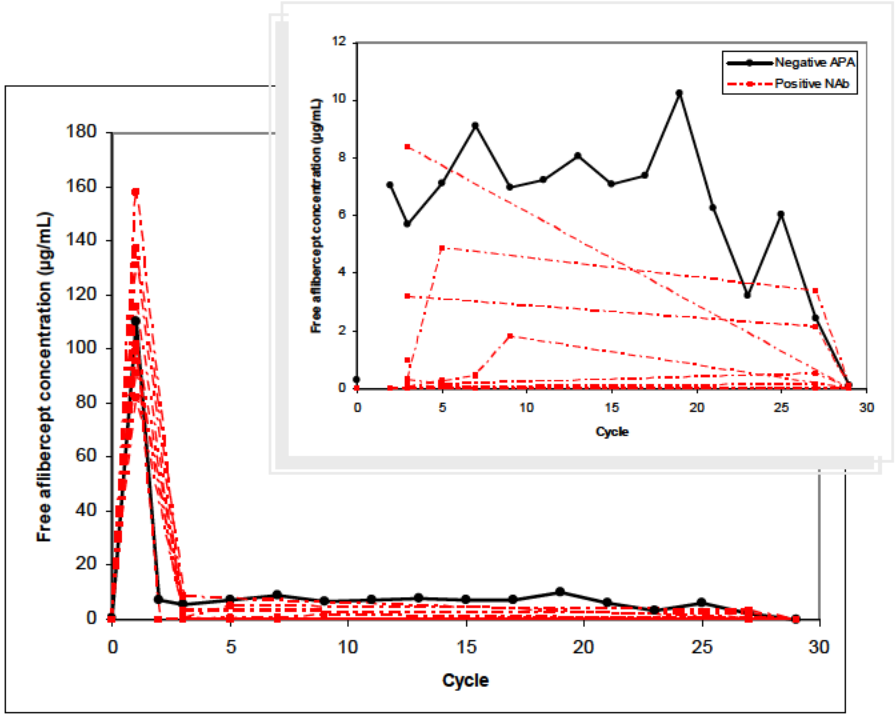
Figure 13. Trough concentrations by antibody status in Study EFC10262



2.3.3.3 Do the anti-product antibodies have neutralizing activity?

Among patients who tested positive for APA and had sufficient samples for further testing, neutralizing antibodies were detected in 35.4% aflibercept-treated patients (17/48) and 5.0% placebo-treated patients (2/40). The presence of neutralizing antibodies appeared to affect exposure of aflibercept. Free aflibercept trough concentrations (C_{trough}) at Cycle 3 (n=9 patients) were approximately 30-fold lower (near LLOQ with mean trough concentration of 192 ng/mL) than those of the overall population (mean trough concentration of 5.8 µg/mL) (**Figure 14**).

Figure 14. Concentration-time profile by immunogenicity status (Study EFC10261)



Applicant's analysis:
Positive response for neutralizing anti-aflibercept antibodies in 2 (0.2%) placebo-treated patients and 17 (1.3%) aflibercept-treated patients, respectively.

Reviewer's comment: *The applicant's analysis of the incidence of neutralizing antibodies was based on the overall population of APA-evaluable patients. It would be more appropriate to present the number of patients who tested positive for neutralizing antibodies over the number of patients who tested positive for APA and were further tested for neutralizing antibodies.*

2.3.3.4 What is the impact of anti-product antibodies on clinical efficacy?

Given the limited data, the presence of APA did not appear to affect clinical efficacy (**Table 10**). Patients with APA positive status were defined as having a negative baseline sample and positive post-baseline measurement. Only evaluable patients defined as having baseline and post-baseline immunogenicity samples were included in the analysis. The impact of neutralizing antibodies on efficacy could not be assessed based on limited available data.

Table 10. Summary of Efficacy by APA Status (Study EFC10262)

	Aflibercept		**Placebo**	
	APA positive[a]	APA negative	APA positive[a]	APA negative
n	14	510	21	506
OS (median in months)	15.0	14.3	8.0	12.7
[95% CI]	[14.4, NA[b]]	[12.9, 16.2]	[6.7, 11.8]	[11.8, 13.8]
PFS (median in months)	9.6	6.9	4.1	5.3
[95% CI]	[7.0, NA[b]]	[6.6, 7.4]	[2.9, 7.2]	[4.5, 5.6]

[a] Patients with APA positive status were defined as a negative baseline sample and positive post-baseline.
[b] Upper bound of 95% CI could not be obtained due to small sample size.

2.3.3.5 What is the impact of anti-product antibodies on clinical safety? (e.g., infusion-related reactions, hypersensitivity reactions, cross-reactivity to endogenous counterparts, etc.)?

No specific association between development of anaphylactic/hypersensitivity reactions and positive APA and neutralizing antibodies could be determined from limited available data. Among 68 patients who experienced anaphylactic/hypersensitivity reactions, three patients tested positive for APA following 6 mg/kg IV aflibercept administration.

2.4 Extrinsic Factors

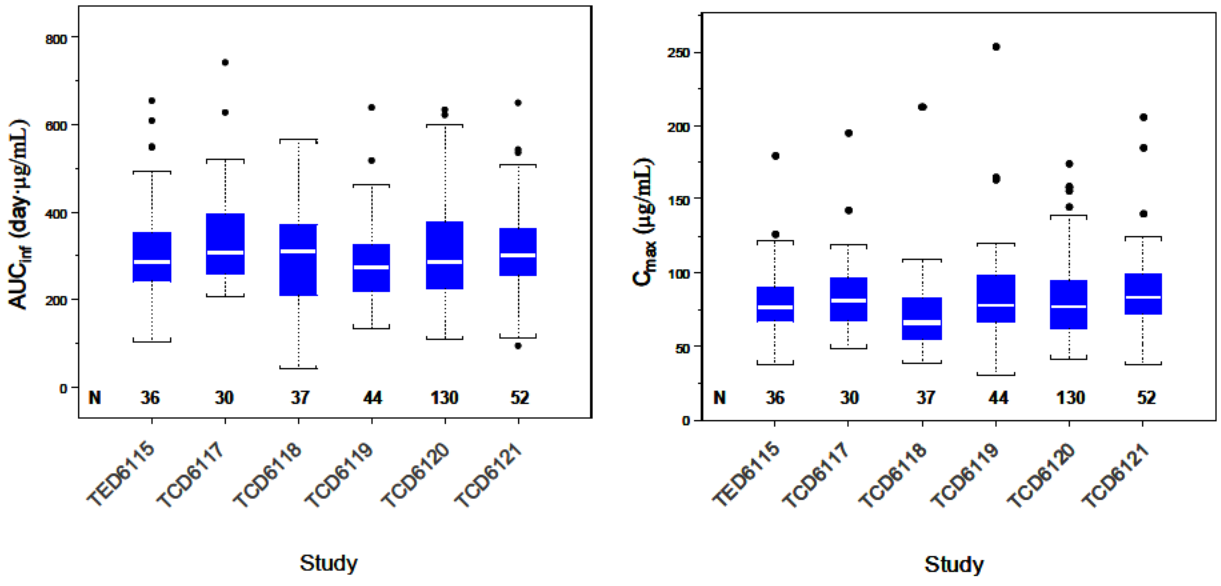
2.4.1 What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence dose-exposure and/or –response and what is the impact of any differences in exposure on response?

No clinically meaningful drug interactions were found between aflibercept and 5-FU or irinotecan/SN-38. Furthermore, no significant drug interactions were observed when aflibercept was administered in combination with other chemotherapies including oxaliplatin, cisplatin, docetaxel, gemcitabine, erlotinib, and pemetrexed.

Effect of combination chemotherapy on aflibercept

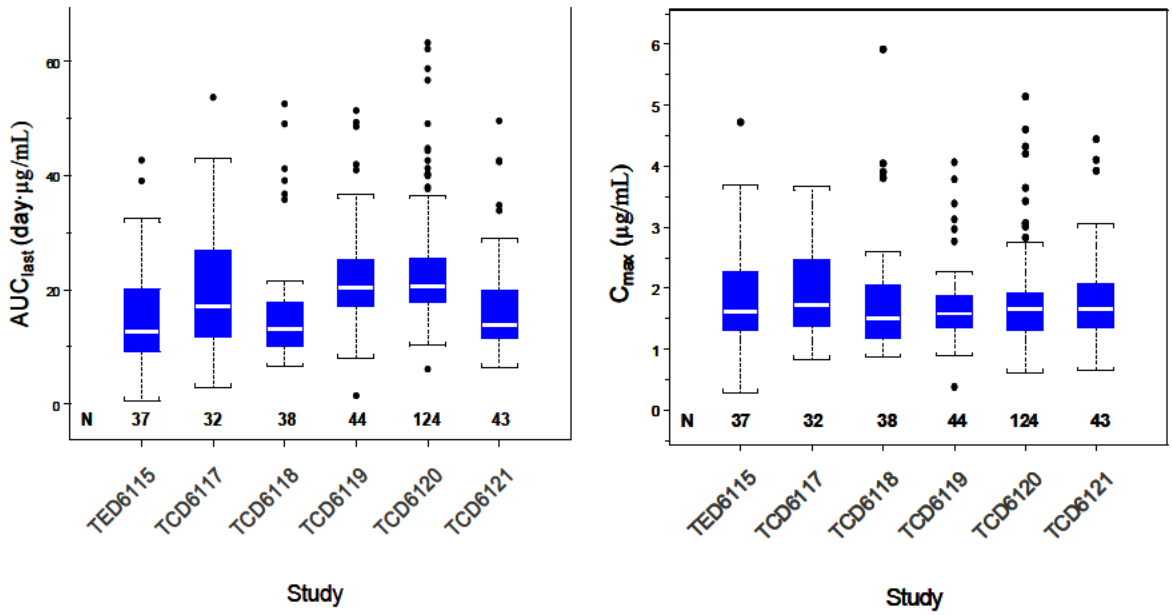
The effect of combination chemotherapy on aflibercept PK was evaluated based on cross-study comparisons and population PK analyses. Free and VEGF-bound aflibercept exposures in the combination studies were comparable to those in the monotherapy study (**Figure 15**, **Figure 16**). Of note, VEGF-bound aflibercept AUC_{last} appeared to be slightly higher in Study TCD6119 and TCD6120 in which the dose schedule was Q3W instead of Q2W. Population PK analyses showed a minor decrease in clearance of free aflibercept in combination with docetaxel (4.7%) and irinotecan/LV5-FU2 (11.3%), which is not expected to be clinically meaningful. No impact of FOLFIRI on free aflibercept clearance was observed.

Figure 15. Box plots of dose-normalized (4 mg/kg) AUC_{inf} (left) and C_{max} (right) of free aflibercept after administration of aflibercept on Cycle 1 Day 1 as monotherapy (Study TED6115) and in combination with chemotherapy (Study TCD6117-TCD6121)



N is the sample size. The whiskers represent the 95% CI. The solid line is the median. Q2W dose schedule in Study TED6115, TCD6117, TCD6118, and TCD6121. Q3W dose schedule in Study TCD6119 and TCD6120. Refer to **Table 1** for details of study design.

Figure 16. Box plots of dose-normalized (4 mg/kg) AUC_{last} (left) and C_{max} (right) of VEGF-bound aflibercept after administration of IV aflibercept on Cycle 1 Day 1 as monotherapy (Study TED6115) and in combination with chemotherapy (Study TCD6117-TCD6121)



N is the sample size. The whiskers represent the 95% CI. The solid line is the median. Q2W dose schedule in Study TED6115, TCD6117, TCD6118, and TCD6121. Q3W dose schedule in Study TCD6119 and TCD6120. Refer to **Table 1** for details of study design.

Effect of aflibercept on combination chemotherapy

The effect of aflibercept on the PK of combination chemotherapies was evaluated based on Phase 1 combination studies and comparison to historical data or published literature.

Irinotecan/SN-38

Irinotecan 180 mg/m^2 was administered IV over 60 minutes in Study TCD6118 (aflibercept in combination with irinotecan, 5-FU, and leucovorin). Irinotecan and SN-38 PK samples were obtained at 5 minutes before dosing and at 1, 1.5, 4, and 23 hours after dosing on Cycle 1 Day 1. Mean exposures (AUC and C_{max}) and CL of irinotecan in the presence of aflibercept were comparable to those of irinotecan monotherapy reported in published literature (**Table 11**).

Table 11. Mean ± SD (%CV) PK Parameters of Irinotecan and SN-38 Metabolite

	AUC[a] (µg·h/mL)	C_{max} (µg/mL)	CL (L/h/m^2)
Irinotecan			
TCD6118 (n=38)[b]	15.4 ± 4.9 (32)	1.97 ± 0.56 (28)	12.6 ± 3.58 (28)
Literature (1) (n=40)	14.8 ± 6.6[c] (45)	2.30 ± 0.73[c] (32)	14.6 ± 6.40 (44)

	AUC[a] (µg·h/mL)	C_{max} (µg/mL)	CL (L/h/m^2)
SN-38			
TCD6118 (n=37)[d]	0.28 ± 0.12 (43)	0.024 ± 0.010 (42)	-
Literature (1) (n=40)	0.46 ± 0.46[c] (100)	0.037 ± 0.015[c] (41)	-

[a] AUC_{inf} for irinotecan from Study TCD6118 and literature; AUC_{0-24h} for SN-38
[b] n=38 for C_{max}; n =36 for AUC_{inf} and CL
[c] AUC and C_{max} dose-normalized to 180 mg/m^2
[d] n=28 for AUC_{0-24h}

5-fluorouracil (5-FU)

5-FU 400 mg/m^2 was administered as an IV bolus over 2-4 minutes, then 600 mg/m^2 IV infusion over 22 hours on Days 1 and 2 of each 2-week cycle in Study TCD6117 and TCD6118. In Study TCD6117, PK samples were collected pre-dose, and 2, 21, 24 (pre-dose of second 5-FU bolus), 25, and 45 hours after the start of 5-FU IV bolus on Cycle 1 Day 1. In Study TCD6118, PK samples were obtained at pre-dose, and 3, 21, 24, 25, and 45 hours after dosing on Cycle 1 Day 1. In Study TCD6119, 5-FU 750 mg/m^2/day was administered as a continuous infusion from Days 1 to 5. PK samples were taken at pre-dose, and 2, 24, and 48 hours after the start of 5-FU infusion on Cycle 1 Day 1.

Published literature indicates that clearance of 5-FU during continuous infusion (at rates 300-1000 mg/m^2/day) ranged from 100 to 350 L/h (2). After IV bolus injection of doses varying between 300 and 600 mg/m^2, clearance ranged from 30 to 120 L/h. Mean 5-FU clearance observed during a 5-day continuous infusion of 1 gram/m^2/day was 235 L/h. Although CL values were consistent with previously published values, there was large variability in AUC consistent with the fact that PK of 5-FU have large inter-patient variability.

Table 12. Mean ± SD (%CV) PK Parameters of 5-FU

	AUC[a] (µg·h/mL)	CL (L/h/m^2)	C_{ss} (ng/mL)
TCD6117 (FOLFOX) (n=32)	52.3 ± 65.0 (124)	80.1 ± 125[b] (156)	2368 ± 2922 (123)
TCD6118 (irinotecan/5-FU/leucovorin) (n=38)	9.08 ± 12.9 (142)	169 ± 323[b] (191)	413 ± 586 (142)
TCD6119 (docetaxel/cisplatin/5-FU) (n=38)	32.6 ± 14.3 (44)	154[d] ± 124 (81)	279 ± 125 (45)
Literature (2) (5-FU alone) (n=21)	-	147[c]	-

	AUC[a] (µg·h/mL)	CL (L/h/m^2)	C_{ss} (ng/mL)
Literature (3) (5-FU alone) (n=18)	8.33 ± 2.74 (33)	70.2 ± 24 (34)	244 ± 88 (36)

[a] AUC = C_{ss} (steady state concentration or median concentration) × T_{CI} (time of continuous infusion: 22 hours)
[b] CL = Dose /AUC
[c] Median BSA of 1.71 m^2
[d] CL = R_{inf}/C_{ss} where C_{ss} was the concentration of 5-FU at 48 hours after the end of the 5-day continuous infusion; R_{inf} was the actual infusion rate

Oxaliplatin

Oxaliplatin 85 mg/m² was administered over 2 hours in Study TCD6117 (aflibercept in combination with FOLFOX). PK samples for oxaliplatin were obtained pre-dose, 1, 3, 7, and 23 hours after the start of oxaliplatin infusion during Cycle 1 only. Oxaliplatin exposures in the presence of aflibercept were comparable to historical data (**Table 13**). Of note, $AUC_{0\text{-}24h}$ represents partial AUC since the PK sampling plan did not cover the full PK profile of oxaliplatin, which has a $t_{1/2\beta}$ of 16.8 hours.

Table 13. Mean ± SD (%CV) PK Parameters of Oxaliplatin

Study	Number of patients	C_{max} (µg/mL)	$AUC_{0\text{-}24}$ (µg.h/mL)
TCD6117	31	1.92 (21)	29.8 (18)
Historical data-INT3010 [a]	17	2.07 (14)	26.6 (10)

a : Clinical pharmacokinetics of oxaliplatin, internal report CSR-BDY-INT3010-EN-E01

Source: Summary of Clinical Pharmacology Studies, Table 12, Page 57

Cisplatin

Cisplatin 75 mg/m^2 was administered over one hour Q3W in combination with aflibercept, docetaxel, and 5-FU in Study TCD6119 and with aflibercept and docetaxel in Study TCD6120 (VTC cohort only). PK samples were obtained on Day 1 Cycle 1 at pre-dose, 5 minutes before the end of cisplatin infusion, then 15 and 30 minutes, and 1, 4, and 24 hours after the end of cisplatin infusion in both studies. Cisplatin exposures in the presence of aflibercept were comparable to those reported in published literature (**Table 14**).

Table 14. Mean ± SD (%CV) PK Parameters of Cisplatin

	AUC_{last}[a] (µg·h/mL)	CL[b] (L/h)	C_{max}[a] (ng/mL)
TCD6119 (TCF) (n=43)	40.3 ± 7.51 (19)	3.66 ± 0.803 (22)	3.43 ± 0.512 (15)
TCD6120 (VTC) (n=29)	42.1 ± 8.78 (21)	3.46 ± 2.76 (80)	3.4 ± 0.544 (16)

	AUC_{last}[a] (µg·h/mL)	CL[b] (L/h)	C_{max}[a] (ng/mL)
Literature (4) (n=10)	37.9 ± 4.39 (12)	3.38 ± 0.463 (14)	-

[a] AUC and C_{max} were determined by noncompartmental analysis
[b] CL=Dose/AUC (µg·h/mL)

Docetaxel

Docetaxel 75 mg/m^2 was administered over one hour in combination with aflibercept, cisplatin, and 5-FU in Study TCD6119. PK samples were obtained at pre-dose, and then 5 minutes before the end of docetaxel infusion, 10 minutes, 2 and 5 hours after the end of docetaxel infusion on Cycle 1 Day 1. The PK of docetaxel were assessed at 75 mg/m^2 (VTC and VT75 cohorts) and 100 mg/m^2 (VT100 cohort) Q3W in combination with aflibercept in Study TCD6120. PK samples were obtained at pre-dose, just before the end of infusion (0.92 hours after the start of infusion), then at 1.17, 3, and 6 hours after the start of docetaxel infusion on Cycle 1 Day 1. PK parameters were estimated by Bayesian analysis and historical population PK data of docetaxel. AUC and CL of docetaxel in the presence of aflibercept were comparable to those reported in published literature (**Table 15**). Slightly higher docetaxel clearance was observed when docetaxel was given in combination with aflibercept and cisplatin/5-fluorouracil (TCD6119), but the difference is most likely not clinically meaningful.

Table 15. Mean ± SD (%CV) PK Parameters of Docetaxel

	AUC_{last}[a] (µg·h/mL)	CL[b] (L/h)
Docetaxel 75 mg/m^2		
TCD6119 (TCF) (n=44)	2.69 ± 0.97 (36)	58.0 ± 16.2 (28)
TCD6120 (VTC) (n=29)	3.41 ± 1.20 (35)	40.6 ± 12.5 (31)
TCD6120 (VT75) (n=54)	3.50 ± 1.49 (43)	43.4 ± 16.0 (37)
Literature (5) (total n=69 at 60, 75, and 100 mg /m^2 doses)	3.41 ± 1.49 (44)	43.7 ± 14.1 (32)
Docetaxel 100 mg/m^2		
TCD6120 (VT100) (n=30)	5.17 ± 1.83 (35)	37.8 ± 13.5 (36)
Literature (5) (total n=69 at 60, 75, and 100 mg /m^2 doses)	5.00 ± 2.80 (56)	43.9 ± 19.6 (45)

[a] AUC calculated as Dose/CL
[b] CL determined by Bayesian analysis

Gemcitabine

Gemcitabine 1000 mg/m^2 IV infusion was administered over 30 minutes on Days 1 and 15 in combination with aflibercept (GV cohort) and aflibercept and erlotinib (GEV cohort) in Study TCD6121. PK samples for gemcitabine and inactive metabolite (dFdU) were collected on Cycle 1 Day 1 and Day 8 at pre-dose, just before the end of infusion (0.5 hours after the start of gemcitabine infusion) then at 0.75, 1, 2, 4, and 23 hours after the start of gemcitabine infusion on Cycle 1 Day 1 and Day 8. PK parameters of gemcitabine and dFdU in the presence of aflibercept were comparable to those reported in published literature (**Table 16**).

Table 16. Mean ± SD (%CV) PK Parameters of Gemcitabine on Day 1

	AUC_{inf}[a] (µg·h/mL)	CL (L/h/m^2)	C_{max} (µg/mL)
Gemcitabine (dFdC)			
TCD6121 (GV) (n=25)	9.1 ± 4.1 (44)	256 ± 204 (90)	15.7 ± 9.11 (58)
TCD6121 (GEV) (n=23)	10.1 ± 4.6 (46)	219 ± 98.3 (45)	16.9 ± 9.32 (55)
Literature (6) (n=21)[c]	10.1 ± 6.4 (63)	196 ± 275 (140)	16.5 ± 10.1 (61)
Literature (7) (n=9)	8.77[b]	114 ± 22.0 (19.3)	11.0 ± 7.04 (64)
Literature (8) (n=12)	12.5 ± 1.62 (13)	104 ± 14.6 (14)	23.5 ± 3.29 (14)
dFdU			
TCD6121 (GV) (n=30)	313 ± 68.3 (22)	-	37.3 ± 6.68 (18)
TCD6121 (GEV) (n=25)	331 ± 87.3 (26)	-	36.0 ± 9.17 (25)
Literature (6) (n=5)	293 ± 389 (32)[d]	-	35.4 ± 18.8 (53)[d]
Literature (7) (n=8)	-	-	37 ± (16)
Literature (9) (n=6)	252 ± 82 (33%)	-	30.2 ± 1.6 (5.0)

[a] AUC and C_{max} were determined by noncompartmental analysis
[b] Calculated as CL=Dose/AUC (µg·h/mL)
[c] Patients were treated with 120-1000 mg/m^2 so AUC and C_{max} were dose-normalized to 1000 mg/m^2.
[d] dFdU AUC and C_{max} were not dose-normalized because dFdU exhibits nonlinear PK.

Erlotinib
Erlotinib 100 mg QD was administered on Days 1 and 15 in combination with aflibercept and gemcitabine (GEV cohort) in Study TCD6121. PK samples were obtained at pre-dose and 1, 2, 4 and 8 hours after dosing on Day 1 and pre-dose on Days 2, 8, 15, and 22 of Cycle 1. Erlotinib exposures in the presence of aflibercept were comparable to those reported in published literature (**Table 17**).

Table 17. Mean ± SD (%CV) PK Parameters of Erlotinib

	AUC_{0-24h}[a] (µg·h/mL)	CL (L/h)	C_{max} (µg/mL)
TCD6121 (n=28)	12.5 ± 6.19 (50)	8[b]	0.802 ± 0.442 (55)
Literature (10) (n=12)	9.95 ± 3.24 (36)[a]	11.2 ± 3.81 (34)	0.560 ± 0.288 (51)
Literature (11) (n=5)	13.2 ± 11.8 (89)	8.83 ± 7.70 (87)	0.943 ± 0.660 (70)

[a] AUC_{inf} used
[b] Calculated as CL=Dose/AUC

Pemetrexed
Pemetrexed 500 mg/m^2 IV was administered over 10 minutes Q3W in combination with aflibercept (TCD6120). PK samples were obtained at pre-dose, just before the end of infusion, then at 0.25, 0.5, 1, 2, 4, 8, 24 and 48 hours after the start of pemetrexed infusion. Pemetrexed exposures in the presence of aflibercept were comparable to those reported in published literature (**Table 18**).

Table 18. Mean ± SD (%CV) PK Parameters of Pemetrexed

	AUC_{inf} (µg·h/mL)	CL (L/h/m^2)	C_{max} (µg/mL)
TCD6120 (n=19)	205 ± 92.5 (45)	2.81 ± 0.964 (34)	122 ± 21.8 (18)
Literature (12)[a] (n=20)	221 ± 58.7 (27)	2.40 ± 0.576 (24)	114 ± 37.9 (33)
Literature (8) (n=12)	188 ± 47.0 (25)	2.89 ± 0.723 (25)	124 ± 14.9 (12)

[a] AUC and C_{max} dose-normalized to 500 mg/m^2

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

No. Aflibercept is a therapeutic protein which is not metabolized by liver cytochrome P450 enzymes or other drug metabolizing enzymes. Given that aflibercept is not

considered a cytokine modulator, it is unlikely to have an effect on CYPs or other drug metabolizing enzymes in terms of inhibition or induction.

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

No. See response in Section 2.4.2.1.

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

No. See response in Section 2.4.2.1.

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

No. See response in Section 2.4.2.1.

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

No. See response in Section 2.4.2.1.

2.4.2.6 Does the label specify co-administration of another drug (e.g. combination therapy in oncology) and, if so, has the interaction potential between these drugs been evaluated?

Yes. The proposed indication is for aflibercept in combination with FOLFIRI. The interaction potential was evaluated by cross-study comparisons and population PK analyses. The results of population PK analyses with sparse PK sampling in Study EFC10262 showed that FOLFIRI did not have an effect on aflibercept PK and cross-study comparisons suggested that there was no clinically meaningful effect of aflibercept on the PK of chemotherapies administered in combination with aflibercept.

2.5 General Biopharmaceutics

2.5.1 What are the manufacturing differences between the to-be-marketed formulation and the formulation used in the pivotal clinical trial?

Not applicable. The formulation used in the registrational clinical trial is the to-be marketed formulation.

2.5.2 What is the in vivo relationship of the proposed to-be-marketed formulation to the pivotal clinical trial formulation in terms of comparative exposure?

Not applicable. The formulation used in the registrational clinical trial is the to-be marketed formulation.

2.6 Analytical Section

This section summarizes the bioanalytical methods utilized to determine therapeutic protein concentrations, endogenous VEGF levels, and anti-product antibodies.

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

Free and VEGF-bound aflibercept plasma concentrations were measured using validated enzyme-linked immunosorbent assays (ELISAs).

2.6.2 Which metabolites have been selected for analysis and why?

Aflibercept is a therapeutic protein that is degraded into amino acids. There are no metabolites of aflibercept; thus only free and VEGF-bound parent compound have been selected for analysis.

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?

Free (active) and VEGF-bound (stable inert complex) aflibercept plasma concentrations were measured. Both were measured to maintain target free/bound ratio >1.

2.6.4 What bioanalytical methods are used to assess therapeutic protein concentrations?

Assay for free aflibercept
Plasma concentrations of free aflibercept were determined using a validated ELISA performed in 10% human plasma CTAD (buffered citrate, theophylline, adenosine, and dipyridamole) in a microplate coated with human $VEGF_{165}$ to capture free aflibercept in the sample matrix. 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. 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 VEGF-bound aflibercept were determined using a validated ELISA performed in 20% human CTAD in a microplate coated with goat anti-VEGF polyclonal antibody to capture VEGF-bound aflibercept. This antibody only captured VEGF-bound aflibercept; the assay did not detect the free form of aflibercept in human plasma samples. A mouse monoclonal antibody specific to an epitope on aflibercept was used to detect VEGF-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. A luminol-based substrate specific for peroxidase was used to achieve a signal intensity that is directly proportional to the concentration of VEGF-bound aflibercept.

Assay for free endogenous VEGF
The Quantikine ELISA kit from R&D System (ref. DVE00) was used. It is a sandwich enzyme immunoassay using one monoclonal antibody and one enzyme-linked polyclonal antibody calibrated with $VEGF_{165}$, the predominant isoform of VEGF-A (VEGF). It was demonstrated to measure specifically free endogenous VEGF.

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?

The ranges of the standard curves for all the assays described below were adequate for the purposes of determining plasma concentrations of free and VEGF-bound aflibercept, and free VEGF in the clinical studies.

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 were calibrated against their respective nominal concentrations using a 4-parameter logistic curve fit (see equation below), from which all other measurements (samples and QCs) were 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 the x-value at the inflection point of the sigmoidal curve, and B is the slope. The curve parameters were 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.

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 RLU readings of the standards were calibrated against their respective nominal concentrations using a Log-Log curve fit (see equation below), from which all other measurements (samples and QCs) were subsequently computed.

$$\text{Log10}\ (y) = A + B\ (\text{Log10}(x))$$

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

Assay for free VEGF
The assay was calibrated using a standard curve generated from nine standards: 1000, 750, 500, 350, 200, 100, 50, 30, and 15 pg/mL. The standard calibration curve was based on an unweighted parabolic regression model (log/log).

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

A summary of lower and upper limits of quantification for the free and VEGF-bound aflibercept, and free VEGF assays is shown in **Table 19**.

Table 19. Summary of Lower and Upper Limits of Quantification

Report No.	Analyte	Matrix	LLOQ (ng/mL)	ULOQ (ng/mL)
VGFT-AS-01026-SA-01V2	Free aflibercept	Plasma (CTAD)	31.3	1000
VGFT-AV-01026-PV01-01V2			15.6	1000
VGFT-AS-02016-SA-01V2	Bound aflibercept	Plasma (CTAD)	43.9	500
VGFT-AV-02016-PV01-01V2			43.9	500
DOH0457	Free VEGF	Plasma (citrate)	0.015	1

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

A summary of intra- and inter-assay accuracy and precision for the free and VEGF-bound aflibercept, and free VEGF assays is shown in **Table 20**.

Table 20. Summary of Intra- and Inter-Accuracy and Precision

Report No.	Analyte	Intra-assay accuracy (AR%)	Inter-assay accuracy (AR%)	Intra-assay precision (%CV)	Inter-assay precision (%CV)
VGFT-AS-01026-SA-01V2	Free aflibercept	103 - 112	91 - 106	3.3 - 3.8	2.4 - 10.5
VGFT-AV-01026-PV01-01V2		106 - 110	92 - 103	9.6 - 13.7	1.1 - 16.2
VGFT-AS-02016-SA-01V2	Bound aflibercept	79 - 93	80 - 110	2.0 - 4.4	1.6 - 10.0
VGFT-AV-02016-PV01-01V2		116 - 123	94 - 110	1.1 - 1.6	0.4 - 16.2
DOH0457	Free VEGF	87 - 101	96 - 101	3.4 - 17.0	6.0 - 13.0

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)?

A summary of available sample stability data for free and VEGF-bound aflibercept, and free VEGF is shown in **Table 21**.

Table 21. Summary of Sample Stability

Condition	Analyte		
	Free aflibercept	**Bound aflibercept**	**Free VEGF**
Long-term stability (-80°C)	15 months	24 months	12 months
Overnight (2°C to 8°C)	Yes	Yes	-
Room temperature	4 hours	-	-
Freeze-thaw cycles	9	10	3

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-bound aflibercept were diluted to 20% plasma (final concentrations of 80, 40, and 25 ng/mL), and included in each analysis.

Assay for free VEGF
Three positive QCs were prepared in human citrated plasma (free VEGF concentrations of 900, 300, 75 pg/mL), and included in each analysis.

2.6.6 What bioanalytical methods are used to assess the formation of the anti-product antibodies? Briefly describe the methods and assay performance including sensitivity, specificity, precision, cut point, interference and matrix, etc.

Assay for anti-aflibercept antibody
Two assays for detecting anti-product antibodies (APA) in human serum were developed: a quasi-quantitative, ELISA-based assay (PCL059) used in the Phase 1 and 2 studies with a sensitivity of 240 ng/mL and a more sensitive, titer-based, non-quantitative, bridging immunoassay (PCL2375) used in the Phase 3 studies with a sensitivity of 5.4 ng/mL.

Serum samples collected in Study ARD6122 and ARD6123 previously analyzed using the PCL059 method were reanalyzed using the bridging immunoassay (PCL2375).

ELISA assay

The quasi-quantitative ELISA assay was performed using microtiter plates coated with extra-cellular receptor domains of aflibercept to capture anti-product antibodies and a dual conjugate detection system. The assay was calibrated using a standard curve generated from seven standards prepared using a mouse monoclonal antibody specific for the R1 domain of aflibercept 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 curve. 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. A summary of assay performance is summarized in **Table 22**.

Table 22. Summary of APA Assay (PCL059) Performance

Criterion	Result
Calibration range, ng/mL	11.92-200
LLOQ, ng/mL ULOQ, µg/mL	238.4 (undiluted sample) 4 (undiluted sample)
Linearity, r^2	>0.998
Accuracy, %RE	94.53%-103.41% [a] 95.46%-103.73% [b] 97.2%-102.17% [c]
Precision, %CV	0.14%-3.41% [a] 4.07%-6.44% [b] 1.43%-1.92% [c]
Specificity	10 naïve human plasma samples yielded BLQ results
Stability	Stable up to 12 months at -80°C; after 3 freeze-thaw cycles; storage at room temperature for 4 hours or at 2-8ºC overnight

[a] Inter-assay for standards
[b] Inter-assay for QCs
[c] Intra-assay for QCs

Non-quantitative bridging immunoassay

The non-quantitative, titer-based, bridging immunoassay was performed using a mouse-anti-aflibercept antibody as the positive control and employed biotinylated- and ruthenium-labeled aflibercept as bridge components. Complexes consisting of antibody bound to both biotinylated- and ruthenium-aflibercept bind to the streptavidin-coated

plate to generate an ECL signal. Samples positive in the initial screen were then reanalyzed in the confirmation assay for a drug-specific response. Drug-treated samples (with 100 µg/mL unlabeled aflibercept) were reported as positive if they showed at least 50% reduction in the signal. Three positive QC samples, prepared with the mouse anti-aflibercept monoclonal antibody were included in each assay run: a high-QC (6,000 ng/mL), a mid-QC (600 ng/mL), and a low-QC (30 ng/mL) sample. The sensitivity of the bridging immunoassay is approximately 5.4 ng/mL in the absence of aflibercept, and approximately 25.2 ng/mL in the presence of 20 µg/mL of aflibercept. The positive QCs were shown to be stable after 10 freeze-thaw cycles, storage at room temperature for 5 hours, storage overnight at 2-8ºC, and long-term storage at -80°C for up to 24 months.

Assay for neutralizing antibody

Samples that were positive in the APA assays were further characterized by a neutralizing antibody (NAb) assay. This non-quantitative, competitive ligand binding assay uses a VEGF ELISA as an indirect method to determine the presence of neutralizing antibodies that block VEGF binding to aflibercept, and by inference bioactivity of aflibercept. There were four QCs: VEGF QC (200 ng/mL VEGF), Complex QC (500 ng/mL of aflibercept and 200 ng/mL of VEGF in approximately equimolar amounts), high-QC (8,000 ng/mL), and low-QC (1,500 ng/mL). The sensitivity of the assay is approximately 940 ng/mL in the presence of 500 ng/mL of aflibercept. The positive QCs (high-QC and low-QC) were shown to be stable after 10 freeze-thaw cycles, storage at room temperature for 4 hours, storage overnight at 2-8ºC, and long term storage at -80°C for up to 24 months. 111

3. REFERENCES

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4. DETAILED LABELING RECOMMENDATIONS

Only relevant clinical pharmacology sections are included. An underline indicates the content that was added, and the ~~strikethrough~~ indicates content removed by the Agency from the proposed draft labeling.

6 ADVERSE REACTIONS

6.2 Immunogenicity

As with all therapeutic proteins, there is a potential for immunogenicity- (b) (4)

(b) (4) Among patients who tested positive for APA and had sufficient samples for further testing, neutralizing antibodies were detected in 17 of 48 aflibercept-treated patients and in 2 of 40 placebo-treated patients.

(b) (4)

Immunogenicity data are highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody positivity in an assay may be influenced by several factors, including sample handling, timing of sample collection, concomitant

medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to ZALTRAP with the incidence of antibodies to other products may be misleading.

7 DRUG INTERACTIONS

No (b) (4) dedicated drug-drug interaction studies have been conducted for ZALTRAP (b) (4)

(b) (4)

8 USE IN SPECIFIC POPULATIONS

8.6 Hepatic impairment

(b) (4)

No dedicated clinical studies have been conducted to evaluate the effect of hepatic impairment on the pharmacokinetics of (b) (4) aflibercept.

Based on a population pharmacokinetic analysis with data from 1507 patients, aflibercept exposure in patients with mild and moderate hepatic impairment were similar to those in patients with normal hepatic function [*see Clinical Pharmacology (12.3)*].

(b) (4)

8.7 Renal impairment

(b) (4) -No dedicated clinical studies have been conducted to evaluate the effect of renal impairment on the pharmacokinetics of aflibercept.

Based on a population PK analysis with data from 1507 patients, aflibercept exposure in patients with mild, moderate, and severe renal impairment were similar to those in patients with normal renal function [*see Clinical Pharmacology (12.3)*].

(b) (4)

12 CLINICAL PHARMACOLOGY

12.3 Pharmacokinetics

(b) (4)

(b) (4) –Plasma concentrations of free and VEGF-bound aflibercept were measured using specific enzyme-linked immunosorbent assays (ELISAs)- (b) (4) Free aflibercept concentrations appear to exhibit linear pharmacokinetics in the dose range of 2-9 mg/kg. Following 4 mg/kg every two weeks intravenous administration of ZALTRAP monotherapy, the elimination half-life of free aflibercept was approximately 6 days (range 4-7 days). (b) (4) Steady state concentrations of free aflibercept were reached by the second dose. (b) (4)

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Specific Populations:
Based on a population pharmacokinetic analysis, age, race, and gender did not have a clinically meaningful effect on the exposure of free aflibercept. Patients weighing ≥100 kg had a (b) (4) increase in systemic exposure compared to patients weighing (b) (4)

(b) (4)

Hepatic Impairment
Based on a population pharmacokinetic analysis which included patients with mild (total bilirubin >1.0x-1.5x ULN and any SGOT/AST, n=63) and moderate (total bilirubin >1.5x–3x ULN and any SGOT/AST, n=5) hepatic impairment, there was no effect of total bilirubin, aspartate amino transferase and alanine amino transferase on the clearance of free aflibercept. There is no data available for patients with severe hepatic impairment (total bilirubin >3x ULN and any SGOT/AST).

(b) (4)

Renal Impairment
Based on a population pharmacokinetic analysis which included patients with mild (CL_{CR} 50-80 mL/min, n=549), moderate (CL_{CR} 30-50 mL/min, n=96), and severe renal impairment (CL_{CR} <30 mL/min, n=5), there was no clinically meaningful effect of creatinine clearance on the clearance of free aflibercept.

(b) (4)

(b) (4)

12.6 Cardiac Electrophysiology

The effect of 6 mg/kg intravenous ZALTRAP every three weeks on QTc interval was evaluated in 87 patients with solid tumors in a randomized, placebo-controlled study. No large changes in the mean QT interval from baseline (i.e., greater than 20 ms as corrected for placebo) based on Fridericia correction method were detected in the study. However, a small increase in the mean QTc interval (i.e., less than 10 ms) cannot be excluded due to limitations of the study design.

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5.2 Pharmacometrics Review

OFFICE OF CLINICAL PHARMACOLOGY: PHARMACOMETRIC REVIEW

1 SUMMARY OF FINDINGS

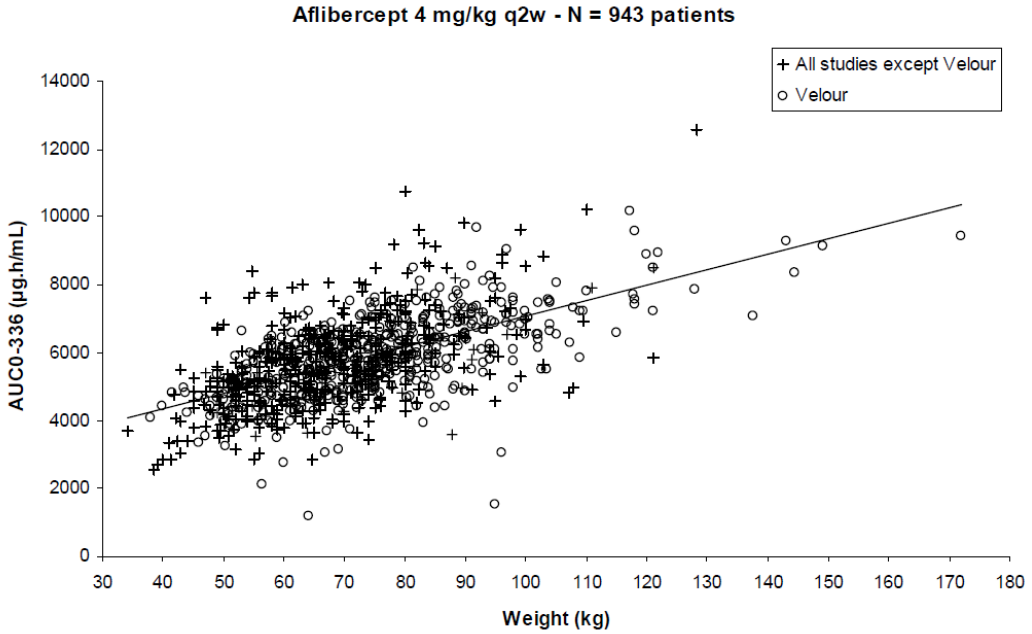
1.1 Key Review Questions

The purpose of this review is to address the following key questions.

1.1.1 Is weight-based dosing appropriate for aflibercept?

A fixed dose, rather than body weight-based dosing appears to be a preferable strategy to minimize variability in aflibercept exposure due to body weight. Population pharmacokinetic analysis revealed a shallow relationship between body weight and free aflibercept clearance. Weight-based dosing of aflibercept in the pivotal trial (4 mg/kg) therefore resulted in a strong relationship between body weight and free aflibercept exposure (AUC) (Figure 1).

Figure 1: Relationship Between Body Weight and Free Aflibercept AUC for Cycle 1

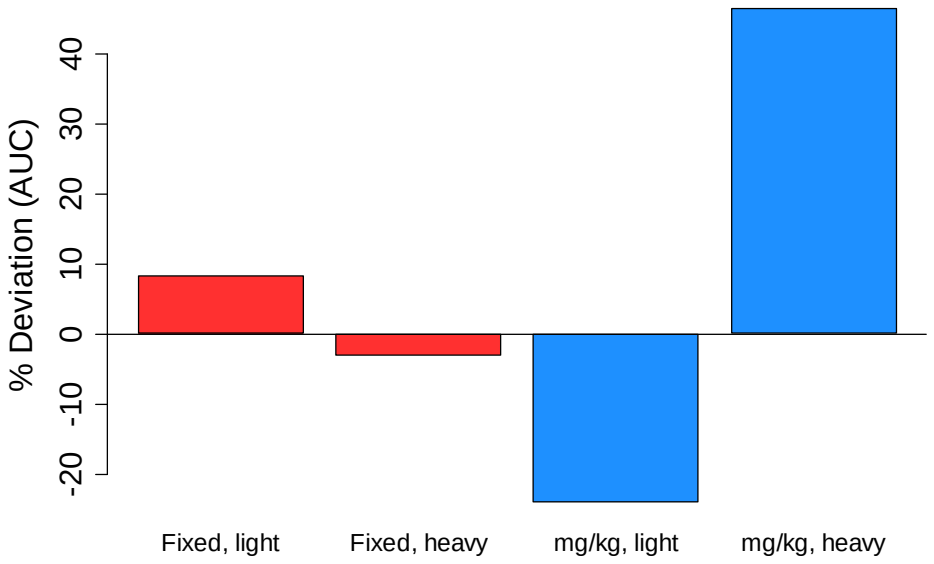


Source: Study POH0265 Report, Page 55, Figure 4.

Simulations were therefore undertaken to compare a fixed dose of 300 mg (equivalent to a 4 mg/kg dose in a 75 kg patients) to the weight-based dose (4 mg/kg). Although median AUC_{ss} is predicted to be similar between the two dosing regimens, fixed dosing clearly results in a tighter distribution of AUC_{ss} values. The %CV is predicted to drop from 35% to 28% and the 90% range is predicted to drop from 117% to 95% with fixed dosing relative to weight-based dosing. Furthermore, the deviation of exposure in heavy (body

weight ≥ 90th percentile) and light (body weight ≤ 10th percentile) patients is expected to be considerably lower under a fixed dose regimen compared to weight-based dosing (Figure 2). Given the exposure-response relationships for efficacy and safety identified in the pivotal trial, the lower deviation of exposures offered by a fixed dose has potential to reduce underexposure in lighter patients and reduce overexposure in heavier patients and therefore improve outcomes.

Figure 2: Deviation of Exposure (AUC_{ss}) from Median Body Weight Patients



1.1.2 What are the characteristics of the exposure-response relationship for efficacy?

In the pivotal trial (VELOUR), overall survival was significantly related with free and bound aflibercept exposure. For the multivariate cox proportional regression analysis using model-derived free aflibercept steady-state AUC, an increase of 1000 µg.h/mL was associated with a 21% decrease in the survival hazard rate. The multivariate analysis considered the following factors: age, gender, region, ECOG PS, prior bevacizumab, location of disease, number of metastatic organs, prior hypertension and endogenous VEGF. Similar relationships were also detected with other pharmacokinetic parameters, including C_{max}, clearance, C_{tau} on Cycle 1 and bound aflibercept clearance. Consistent results were observed for progression free survival (PFS) where an increase of 1000 µg.h/mL was associated with a decrease in the PFS hazard rate of 19%. The relationship is illustrated graphically in Figure 3 for overall survival and Figure 4 for PFS. Note that these Kaplan-Meier plots are simply stratified by AUC and do not take into account imbalances in baseline confounding factors.

Figure 3: Exposure-Response Relationship for Overall Survival in VELOUR. The exposure parameter is extrapolated free aflibercept AUC. The legend displays the median survival time [95% CI] for each quartile of drug exposure.

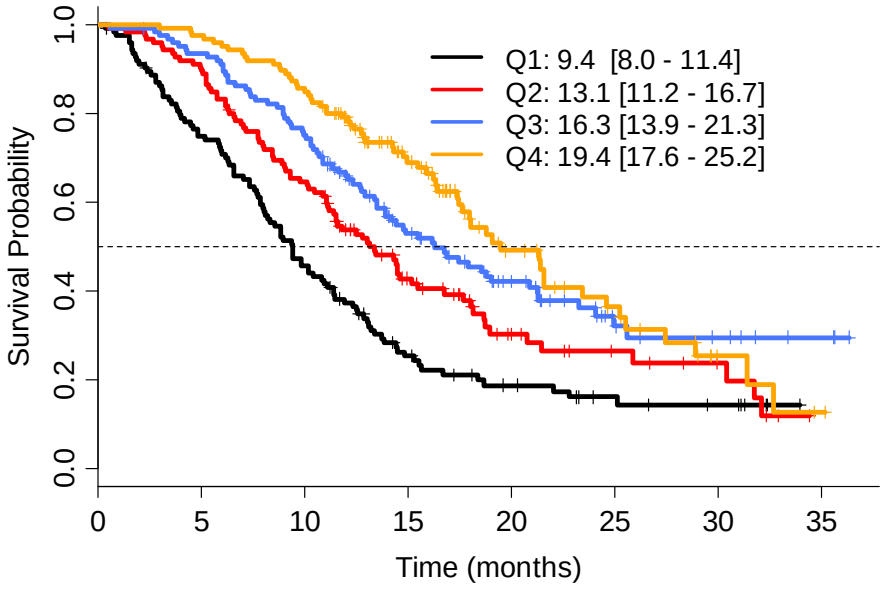
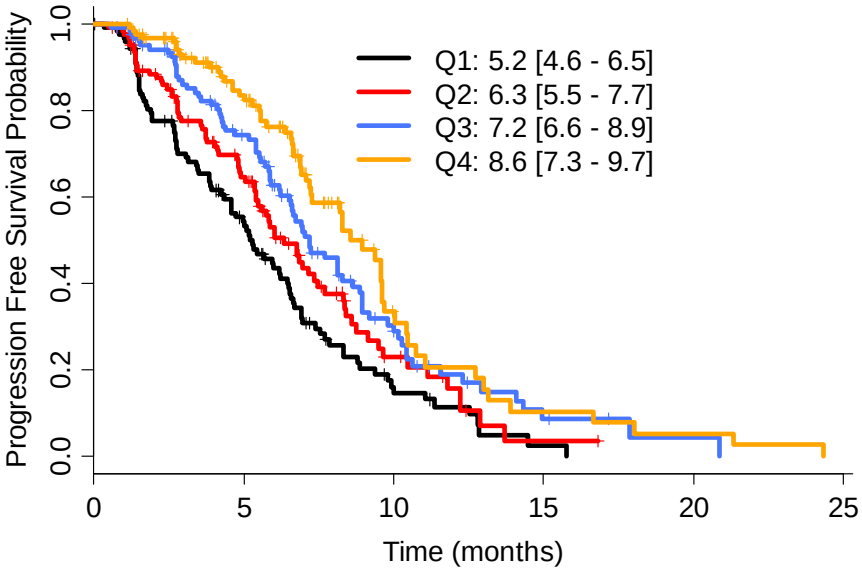


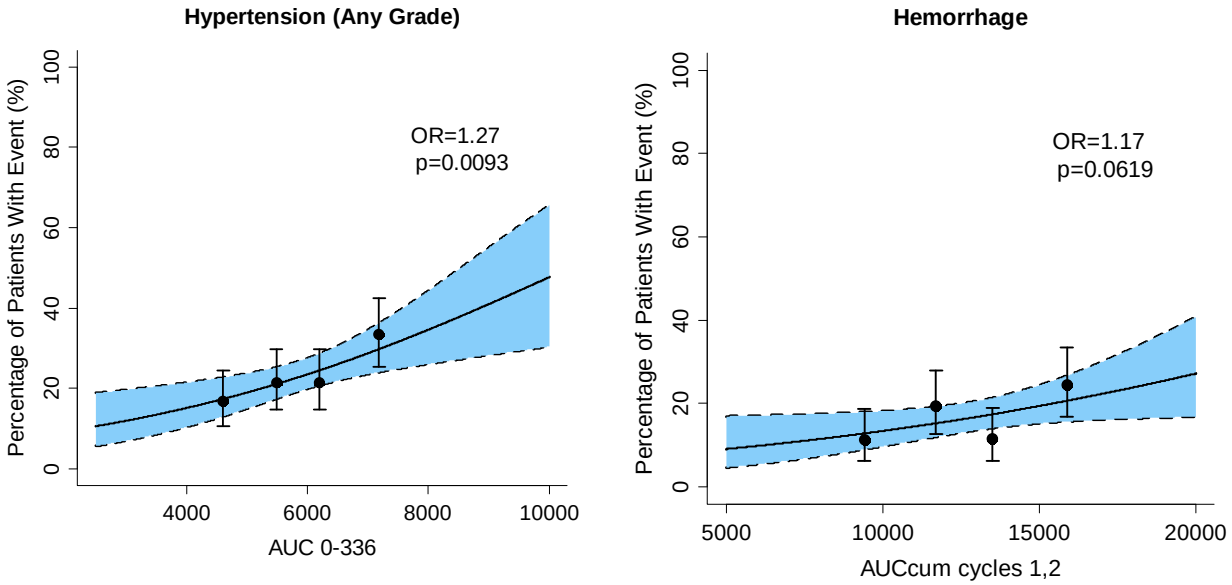
Figure 4: Exposure-Response Relationship for PFS in VELOUR. The exposure parameter is extrapolated free aflibercept AUC. The legend displays the median progression free survival time [95% CI] for each quartile of drug exposure.



1.1.3 What are the characteristics of the exposure-response relationships for safety?

Incidence of hypertension and hemorrhage during the first two cycles were found to be significantly related to exposure of free aflibercept in VELOUR. The odds for experiencing hypertension increase by 27% for an increase in $AUC_{0\text{-}336}$ of 1000 µg.h/mL. For an increase of 2000 µg.h/mL in $AUC_{cum\ cycles1,\ 2}$, the odds of hemorrhage increase by 17%. The relationships are visualized in Figure 5.

Figure 5: Exposure-Response Relationship for Hypertension (left) and Hemorrhage (right) in VELOUR



1.1.4 Is the proposed dose of 4 mg/kg optimal?

The proposed dose of 4 mg/kg is acceptable because a 1.44 month survival benefit was observed in the aflibercept arm compared to placebo. However, there are reasons to suggest that the 4 mg/kg dosing regimen is not optimal:

- The magnitude of the survival benefit is modest (1.44 months). Exposure-response analysis for survival suggests that higher exposures are associated with longer survival. Similar exposure-response relationships for survival were also found in aflibercept trials which did not meet the primary endpoint: VANILLA (pancreatic cancer) and VITAL (non-small cell lung cancer). These findings suggest that higher doses or exposures may provide additional benefit to patients.
- The maximum tolerated dose for aflibercept was not reached in two dose escalation studies. Instead, the dose was selected in large part to achieve a target free/bound aflibercept ratio. Although VEGF-related toxicities (hypertension, hemorrarhage, proteinuria) were reported in VELOUR and were exposure-related, it is possible that patients who tolerate aflibercept at 4 mg/kg may be able to tolerate higher exposures.

- A fixed dose should be used to reduce variability in aflibercept exposure due to body weight and reduce the deviation in heavy (body weigh ≥ 90th percentile) and light (body weight ≤ 10th percentile) patients.

1.2 Recommendations

For future development of aflibercept, the Applicant should consider the following to optimize dosing:

- A fixed dose of aflibercept should be used.
- Strategies to individualize dosing by identifying a subset of patients who will benefit from an increase in aflibercept exposure should be explored. One possibility is to allow for an increase in aflibercept dose in patients who tolerate the starting dose. Another strategy may be to measure free aflibercept concentration and increase the dose in those patients with low exposure.

1.3 Label Statements

Labeling statements to be removed are shown in ~~red strikethrough font~~ and suggested labeling to be included is shown in underline blue font.

8.6 Hepatic impairment

No dedicated clinical studies have been conducted to evaluate the effect of hepatic impairment on the pharmacokinetics of aflibercept.

Based on a population PK analysis with data from 1507 patients, aflibercept exposure in patients with mild and moderate hepatic impairment were similar to those in patients with normal hepatic function [*see Clinical Pharmacology (12.3)*].

(b) (4)

8.7 Renal impairment

No dedicated clinical studies have been conducted to evaluate the effect of renal impairment on the pharmacokinetics of aflibercept.

Based on a population PK analysis with data from 1507 patients, aflibercept exposure in patients with mild, moderate, and severe renal impairment were similar to those in patients with normal renal function , [*see Clinical Pharmacology (12.3)*].

(b) (4)

Specific Populations: Based on a population analysis, age, race and gender did not have a clinically meaningful effect on the exposure of free aflibercept. Patients weighing ≥ 100 kg had a (b) (4) increase in drug exposure compared to patients weighing (b) (4).

(b) (4)

Hepatic Impairment

Based on a population pharmacokinetic analysis which included patients with mild (total bilirubin >1.0x –1.5x ULN and any SGOT/AST, n=63) and moderate (total bilirubin >1.5x–3x ULN and any SGOT/AST, n=5) hepatic impairment, there was no effect of total bilirubin, aspartate amino transferase and alanine amino transferase on the clearance of free aflibercept. There is no data available for patients with severe hepatic impairment (total bilirubin >3x ULN and any SGOT/AST)

(b) (4)

(b) (4)

Renal Impairment

Based on a population pharmacokinetics analysis which included patients with mild (CLCR 50 to 80 mL/min, n=549), moderate (CLCR 30 to 50 mL/min, n=96), and severe renal impairment (CLCR <30 mL/min, n=5), (b) (4)

(b) (4)

2 PERTINENT REGULATORY BACKGROUND

A Biologics License Application (BLA 125418) was submitted for aflibercept on February 3, 2010. Aflibercept is a recombinant human fusion protein designed to act as a decoy receptor to block the VEGF pathway by binding to VEGF-A, VEGF-B and P1GF and preventing these factors from activating their endogenous receptors. The Applicant is seeking an indication for aflibercept in combination with irinotecan-fluoropyrimidine-based chemotherapy for patients with metastatic colorectal (MCRC) previously treated with an oxaliplatin-containing regimen. The proposed dose is a 4 mg/kg infusion over one hour every two weeks.

3 RESULTS OF SPONSOR'S ANALYSIS

3.1 Pivotal Trial (EFC10262/VELOUR)

The efficacy of aflibercept in combination with irinotecan and fluoropyrimidine based chemotherapy in previously treated patients with MCRC was tested in one pivotal trial (EFC10262/VELOUR). A total of 1226 patients were randomized to receive either 4 mg/kg aflibercept as a one hour infusion (N=612) or placebo (N=614) in combination with 5-fluoracil plus irinotecan [FOLFIRI: irinotecan 180 mg/m^2 IV infusion over 90 minutes and leucovorin (dl racemic) 400 mg/m^2 IV infusion over 2 hours, followed by 5-FU 400 mg/m^2 IV bolus, followed by 5-FU 2400 mg/m^2 continuous IV infusion over 46 hours]. Treatment cycles were repeated every two weeks until disease progression or unacceptable toxicity. The primary endpoint was survival and assignment was stratified by the ECOG performance status (0 vs. 1 vs. 2) and according to prior therapy with bevacizumab. Pharmacokinetic samples for measurement of free and bound aflibercept were to be collected on day 1 of cycle 1, prior to, and at the end of the aflibercept infusion, then prior to administration of study treatment in each odd numbered cycle and approximately 30 and 90 days after the last aflibercept administration. The study demonstrated a significant difference in overall survival of aflibercept over placebo (HR:

0.817, p=0.0032) with median survival of 13.5 months in the aflibercept arm and 12.06 months in the placebo arm. Drug class safety events which had higher incidence rates in the aflibercept arm included hypertension (41% vs. 11%), hemorrhagic events (38% vs. 19%) and thromboembolic events (9.3% vs. 7.3%).

3.2 Population Pharmacokinetic Analysis

The Applicant conducted a series of six population pharmacokinetic analyses throughout the course of the aflibercept development program. Reviewed here is a population PK analysis combining 1007 patients from previous studies with 500 patients from VELOUR. The list of studies included in the analysis is displayed in Table 1. The aim of the analysis was to estimate the PK parameters of free and bound aflibercept administered in combination with FOLFIRI and to evaluate the influence of covariates on the PK of aflibercept.

Table 1: Database for Population PK Modeling

Study	Doses	Blood samples	Number of patients
TED6115/6116, TCD6120, TCD6118, ARD6122, ARD6123, EFC6125 (POH0253)	2-9 mg/kg q2-3 weeks	4005 (5 to 19 per patient)	433 (416 for PK of bound)
EFC10547-Vanilla (Combination with gemcitabine in patients)	4 mg/kg q2weeks	624 (3 per patient)	204 (176 for PK of bound)
EFC10261-Vital (Combination with docetaxel in patients)	6 mg/kg q3weeks	1259 (3 per patient)	370 (326 for PK of bound)
EFC10262 – Velour (Combination with Irinotecan/ 5-Fu (FOLFIRI)	4 mg/kg q2weeks	2029 (3 per patient)	500 (460 for PK of bound)

Source: Study POH0265 Report, Page 3.

A previous analysis (POH274) had found a 2-compartment model with first-order elimination from the central compartment best described free aflibercept concentrations. Inter-individual variability was described with an exponential model and residual variability was modeled with additive and proportional error components. The first order conditional estimation (FOCE) with interaction method in NONMEM Version VI was used for parameter estimation. Covariates were screened by forward addition at a p-value of 0.05. The final model was tested by deleting each covariate at a p-vale of 0.05.

A total of 589 patients in VELOUR had blood samples drawn for pharmacokinetic analysis. However, 89 (15%) patients were removed from the database for various reasons, including:

- Actual sampling times were missing (56 observations from 33 patients)
- Actual times of administration were missing, resulting in removal of concentrations after the first missing time (322 observations, 25 patients excluded)

- High aflibercept concentration before the first administration (31 observations, 7 patients excluded)
- Sampling time not documents (57 patients excluded)

This database was first used to fit the model derived from the previous analysis (POH274 final in Table 2). The correlation between CL and Q was poorly estimated so 35 outliers (WRES > 5) were removed from the database and the model was refit (POH274 final ov in Table 2). Covariates were then screened and a final model was established (POH265 full in Table 2).

The covariate analysis identified albumin (ALB), body weight (WT), serum alkaline phosphatase (ALK), creatinine clearance, total protein (TP), sex, irinotecan/LV5-FU2 in Study TCD6118, and docetaxel in Studies VITAL and TDC6120 as significant factors explaining variability in the clearance parameter. Body weight, creatinine clearance, total protein and sex were identified as significant covariates for the central volume of distribution. Final parameter estimates are listed in Table 2 and the Applicant's goodness of fit plot is presented in Figure 6. The effect of covariates on clearance and volume of distribution for the range of values in the database (5th to 95th percentiles) is presented in Table 3. Clearance increases with an increase in ALK, creatinine clearance, body weight, total protein, in males, in Study TCD 6118 and with docetaxel. Clearance decreases with an increase in ALB. There was no effect of total bilirubin, aspartate amino transferase, or alanine amino transferase on free aflibercept clearance. Central volume of distribution increases with increases in weight, creatinine clearance and in males, and decreases with an increase of total protein. Sex had the largest effect on free aflibercept pharmacokinetics with men having a 16% higher clearance and 21% higher volume of distribution. The remaining interindividual variability not explained by covariates was 28% for clearance and 20% for volume of distribution. Shrinkage for clearance and central volume of distribution were 18.5 and 46.8, respectively.

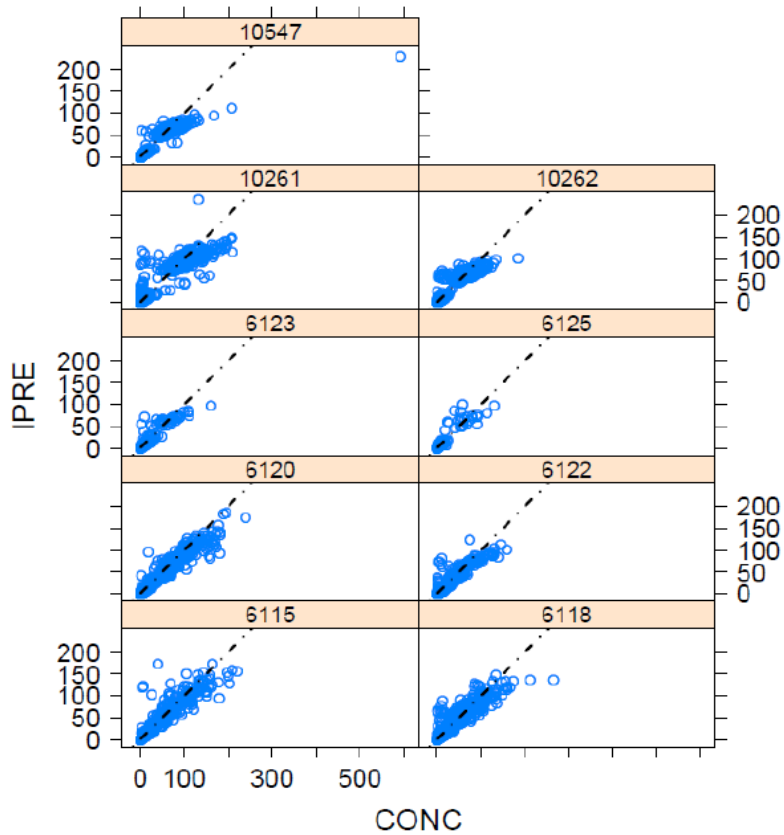
Table 2: Parameter Estimates of the Final Model

Model		POH274 final1 from poh274 database		POH274 final (run 1) from poh265 database		POH274 final ov (run 2) from poh265 db without outliers		POH265 full (run 21) from poh265 db without outliers	
Archive					527 386 603		527 387 015		527 387 032
Fit			**24 140.3**		**31 359.94**		**30 179.02**		**30 174.90**
Nb of patients			1007		1507		1507		1507
CL (L/h)		θ1	0.0443 (2.75)	θ1	0.0418(2.12)	θ1	0.0425(1.93)	θ1	0.0425(1.93)
	ALB	θ8	-0.570 (12.8)	θ8	-0.487(-16.6)	θ8	-0.537(-9.74)	θ8	-0.54(-9.7)
	WT	θ9	0.197 (43.9)	θ9	0.19(35.6)	θ9	0.188(34.2)	θ9	0.191(33.7)
	ALK	θ10	0.0959 (19.2)	θ10	0.0947(14.3)	θ10	0.0908(13.9)	θ10	0.0904(13.9)
	CLCR	θ11	0.140 (24.8)	θ11	0.169(17.4)	θ11	0.168(16.8)	θ11	0.17(16.5)
	TP	θ12	0.337 (29.4)	θ12	0.272(32.7)	θ12	0.314(23.6)	θ12	0.277(29)
	SEX	θ13	0.827 (3.07)	θ13	0.846(2.52)	θ13	0.842(2.29)	θ13	0.842(2.3)
	Study 6118	θ17	0.859 (3.68)	θ17	0.899(3.53)	θ17	0.883(3.35)	θ17	0.882(3.34)
	Combi docetaxel	θ20	0.945 (2.93)	θ18	0.972(2.56)	θ18	0.95(2.44)	θ18	0.949(2.44)
V1 (L)		θ2	4.45 (1.90)	θ2	4.5(1.58)	θ2	4.46(1.52)	θ2	4.47(1.55)
	WT	θ14	0.341 (21.1)	θ14	0.325(19.9)	θ14	0.349(17.4)	θ14	0.359(17.1)
	CLCR	θ15	0.159 (30.7)	θ15	0.147(27.9)	θ15	0.148(28.7)	θ15	0.149(28.3)
	TP		-		-		-	θ19	-0.168(-69.6)
	SEX	θ16	0.780 (2.65)	θ16	0.797(2.36)	θ16	0.79(2.15)	θ16	0.788(2.18)
V2 (L)		θ3	3.54 (4.89)	θ3	3.21(5.11)	θ3	3.31(4.47)	θ3	3.3(4.45)
Q (L/h)		θ4	0.0804 (11.3)	θ4	0.0742(12.7)	θ4	0.0891(12.2)	θ4	0.0891(12)
Corr CL/Q		θ7	0.735 (42.2)	θ7	0.332(136)	θ7	0.958(31.4)	θ7	0.96(31.4)
	ωCL (%)		31.8 (11.6)		30(10.2)		27.6(10.2)		27.6(10.2)
	ωV1 (%)		22.8 (24.9)		23.7(23.4)		19.8(25.2)		19.8(25.4)
	ωV2 (%)		46.8 (20.2)		51.5(19.4)		45.2(17.5)		45.2(17.7)
	σadd (µg/mL)	θ5	0.0657 (13.5)	θ5	0.0555(8.97)	θ5	0.0463(15.8)	θ5	0.0462(15.9)
	σprop (%)	θ6	34.1 (2.24)	θ6	35.1(2.09)	θ6	33.9(1.88)	θ6	33.9(1.88)
t½λ1 (h)			15.1		15.7		13.5		13.5
t½λz (h)			140		142		139		139
Vss (L)			7.99		7.71		7.77		7.77
Kel (h-1)			0.00996		0.00929		0.00953		0.00951

( ) CV of estimation; ALB: Albumin ; ALK: Alkaline phosphatase ; ALT: Alanine amino transferase; AST: Aspartate amino transferase; CLCR: Creatinine clearance; TP: Total protein; WT: Weight; CV Coefficient of variation; CL: Clearance; Kel: Elimination rate constant; Q: Inter-compartmental clearance; t½λ1: Distribution half-life; t½λz: Terminal half-life; V1: Distribution volume for central compartment; V2: Distribution volume for peripheral compartment; Vss: Steady-state volume of distribution; ω: interindividual variability ; σ: residual error; Corr: correlation; -: Not applicable

Source: Study POH0265 Report, Page 42, Table 6.

Figure 6: Individual Predictions vs. Observations (Final Model)



Goodness of fit plots by study number. IPRE: Individual predictions (µg/mL); CONC: Free aflibercept concentrations (µg/mL); : unity line.

Source: Study POH0265 Report, Page 5.

Table 3: Covariate Effects on Free Aflibercept Parameters

Covariate	Quantiles	CL (L/h)	% change $	V1 (L)	% change $
Mean patient *		0.0425		4.47	
ALBn	5% : 0.568	0.0501	**17.8**	NA	-
	95% : 0.966	0.0376	**-11.6**		
CLCR	5% : 44.6	0.0388	**-8.64**	4.13	**-7.62**
	95% : 147	0.0476	**11.9**	4.93	**10.4**
CLCR	min pop (18.2 mL/min)	0.0333	**-21.6**	3.61	**-19.2**
ALKn	5% : 0.423	0.0400	**-5.77**	NA	-
	95% : 3.24	0.0481	**13.3**		
TPn	5% : 0.736	0.0406	**-4.41**	4.59	**2.77**
	95% : 1.03	0.0446	**4.92**	4.34	**-2.87**
SEX	Female	0.0358	**-15.8**	3.52	**-21.2**
WT	5%: 49.0 kg	0.0400	**-5.80**	4.00	**-10.6**
	95% : 99.8 kg	0.0459	**7.91**	5.16	**15.4**
Study TCD6118		0.0375	**-11.8**	NA	-
Combi docetaxel		0.0403	**-5.10**	NA	-

* male, 67 kg, normalized ALB 0.769, normalized ALK 0.816, normalized TP 0.866, CLCR=75.9 mL/min

$ theoretical effect (% change with respect to the mean) of the covariate considered alone, the other covariate being set to its median value

Source: Study POH0265 Report, Page 45, Table 9.

Reviewer's Comment: The Applicant's pharmacokinetic model reasonable describes the data and is generally acceptable, although the removal of 15% of patients from the database because of data integrity issues is troubling. The further removal of 35 outliers because of large WRES values was not well supported, but did not appear to have a significant effect on parameter estimates. None of the covariates tested appeared to have a clinically meaningful effect on aflibercept clearance. Shrinkage on clearance was 18.5. Individual estimates of exposure can be used for exposure-response analyses.

3.2.1 Body Weight

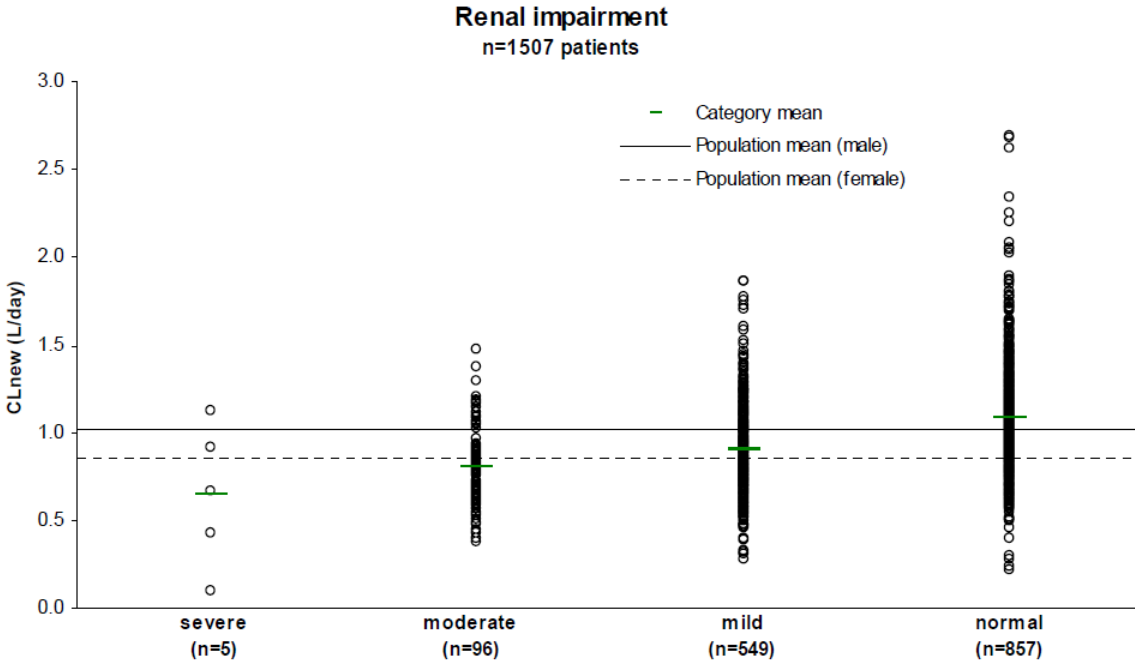
Weight has a weak relationship with aflibercept clearance. Relative to a median weight of 67 kg, free aflibercept clearance decreases by 5.8% for a weight of 49 kg and increases by 7.91% for a weight of 100 kg. In spite of the small effect of body weight on clearance, aflibercept was dosed on a per kilogram basis (4 mg/kg). The consequence is a strong relationship between body weight and exposure which is illustrated in Figure 1. The Applicant calculated a 29% increase in exposure for cycle 1 ($AUC_{0\text{-}336}$) in the group of patients weighing more than 100 kg compared to the group of patients weighing 50 to 100 kg.

Reviewer's Comment: These results suggest that body weight-based dosing may not be appropriate for aflibercept. See the reviewer's analysis for a further exploration of fixed vs. weight-based dosing strategies.

3.2.2 Renal Function

After the completion of the population pharmacokinetic report, the Applicant discovered that individual creatinine clearance values from one of the Phase III studies (VANILLA) were incorrectly derived when missing (204 patients (14% of the population)). The Applicant subsequently updated the dataset and submitted an amendment to the original report. The updated dataset did not significantly alter the finding from the original report. The effect of creatinine clearance on free aflibercept was minor; clearance decreases by 6.5% for a creatinine clearance of 48 mL/min (5th percentile of the population) and increases by 10% for a creatinine clearance of 148 mL/min (95th percentile of the population). This difference is not expected to be clinically meaningful. The relationship is illustrated in Figure 7.

Figure 7: Effect of Renal Function on Free Aflibercept Clearance



Source: Study POH0265 Report (Amendment 1), Page 28, Figure 1.

3.2.3 Bound Aflibercept

The Applicant also developed a combined model to describe free and bound aflibercept concentrations simultaneously. Free aflibercept was again described by a two compartment model. Free aflibercept in the tissue compartment was allowed to bind to VEGF through a Michaelis-Menten relationship. Bound aflibercept was then assumed to be directly eliminated through internalization. Inter-individual variability was described with an exponential model and residual variability was modeled with additive and

proportional error components. Covariate selection was not performed. Parameter estimation was performed using the SAEM algorithm in MONOLIX. Parameter estimates are displayed in Table 4.

Table 4: Parameter Estimates of the Free and Bound Aflibercept Model

Fixed effects		Interindividual variability	Residual variability		
Parameter	Estimate (RSE%)	ω (%) (RSE%)		σa (µg/mL) (RSE%)	σp(%) (RSE%)
CLf (L/h)	0.0366 (1)	31.7 (3)	Free	0.0359 (6)	33.4 (1)
V_p (L)	4.02 (4)	65.8 (4)	Bound	0.347 (2)	9.8 (3)
Q (L/h)	0.0667 (7)	40.7 (23)	-	-	-
V_t (L)	3.96 (1)	27.2 (5)	-	-	-
V_b (L)	4.02 (=Vp)	-	-	-	-
V_{max} (mg/h)	0.0317 (2)	14.1 (8)	-	-	-
K_m (µg/mL)	1.71 (6)	38.4 (27)	-	-	-
k_{int} (h-1)	0.00189 (1)	19.7 (6)	-	-	-
Corr CL/Q	0.649 (21)	-	-	-	-

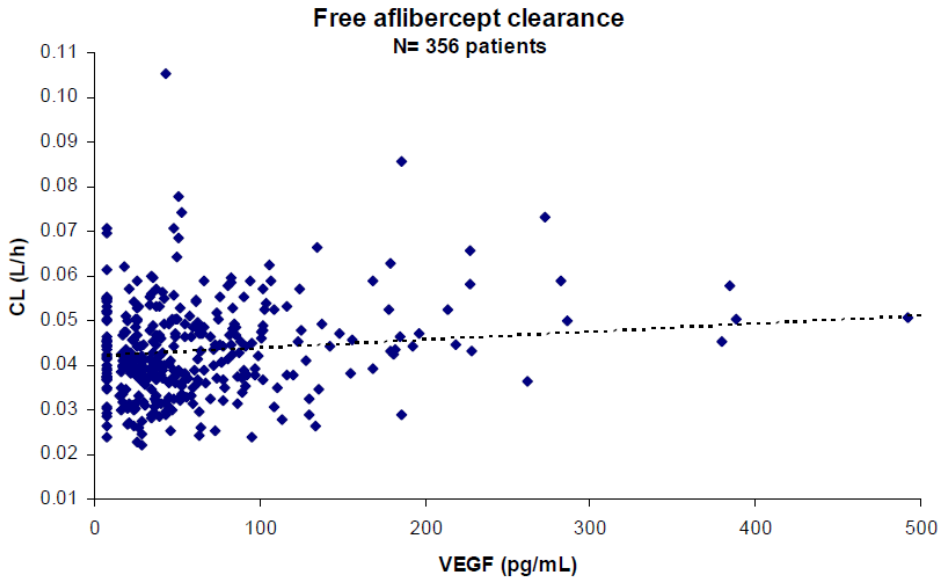
V_p=V_b*R with R fixed to 1.
CLf: Clearance of free aflibercept from central compartment (CL=kel*Vp)
Q: Intercompartmental clearance of free aflibercept (Q=ktp*Vt=kpt*Vp)
V_p: Central volume of distribution of free aflibercept
V_t: Peripheral volume of distribution of free aflibercept
V_b: Volume of distribution of bound aflibercept
k_{int}: First order rate constant of bound aflibercept internalization
Corr: Correlation
ω: Interindividual variability
σ: Residual error; a: additive; p: proportional
RSE: Relative standard error
-: Not applicable

Source: Study POH0265 Report, Page 51, Table 15.

3.2.4 Endogenous VEGF

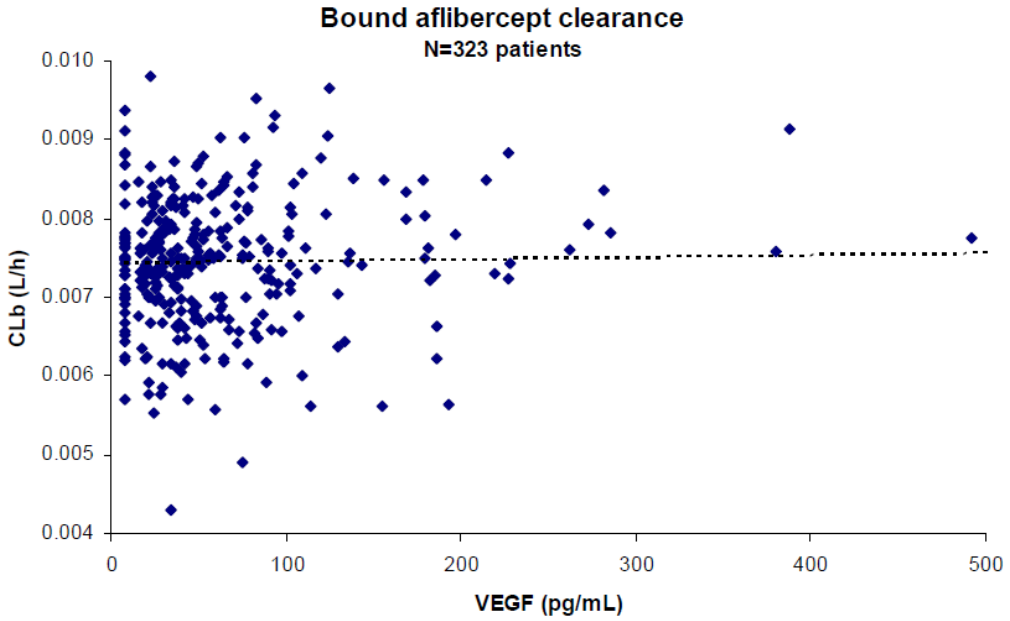
Baseline endogenous VEGF was measured in 356 (71%) patients with PK data in VELOUR. The Applicant performed graphical analysis to explore potential relationships between baseline endogenous VEGF and aflibercept pharmacokinetics. Plots of free (Figure 8) and bound (Figure 9) aflibercept clearance vs. baseline VEGF do not show a strong relationship.

Figure 8: Free Aflibercept Clearance vs. Baseline Endogenous VEGF. The dotted line represents the regression line. Six subjects with extreme VEGF levels (>500 pg/mL) have been removed from the plot to facilitate interpretation of the relationship.



Source: Study POH0265 Report, Page 63, Figure 12.

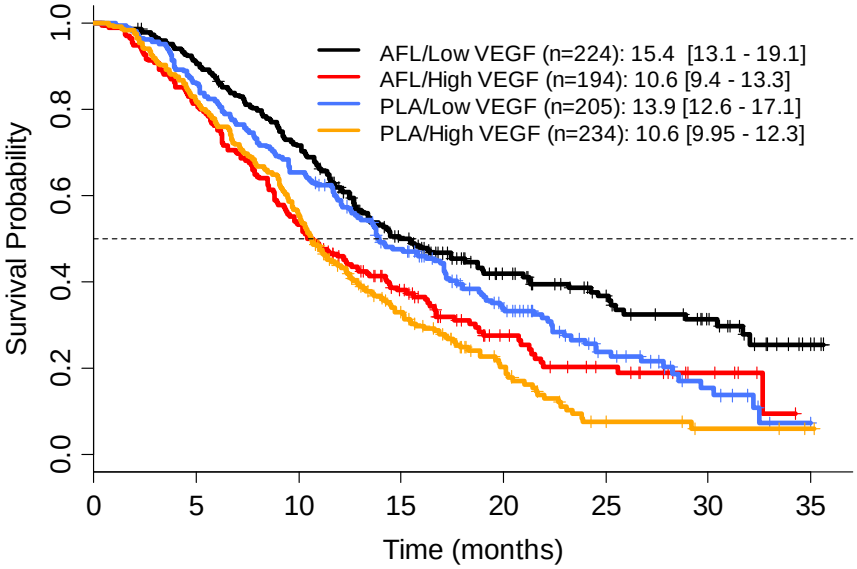
Figure 9: Bound Aflibercept Clearance vs. Baseline Endogenous VEGF. The dotted line represents the regression line. Six subjects with extreme VEGF levels (>500 pg/mL) have been removed from the plot to facilitate interpretation of the relationship.



Source: Study POH0265 Report, Page 64, Figure 13.

Baseline endogenous VEGF, however, was prognostic of overall survival in VELOUR (Figure 10).

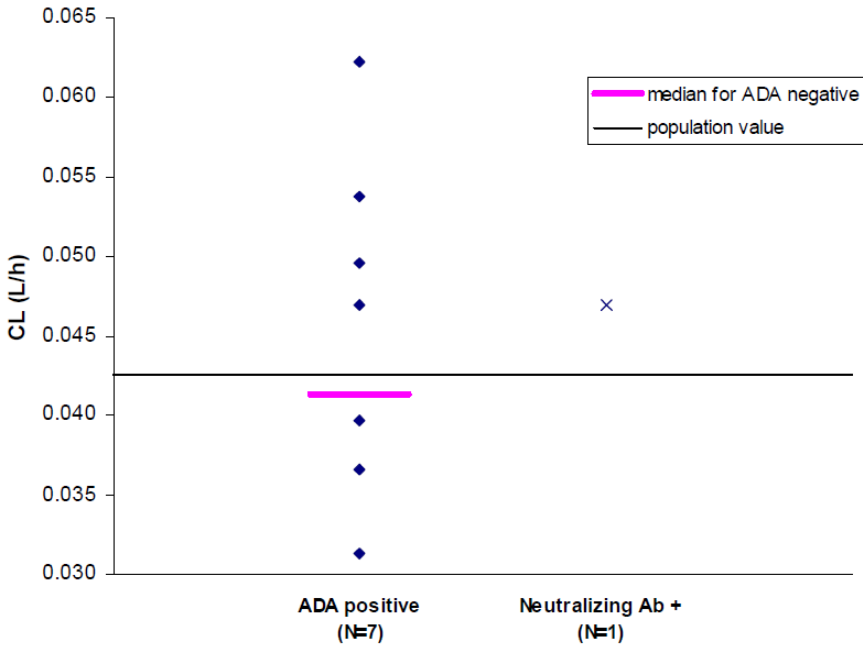
Figure 10: Relationship Between Endogenous Baseline VEGF and Overall Survival in Aflibercept (AFL) and Placebo (PLA) Patients in VELOUR. Low VEGF and High VEGF refer to endogenous VEGF values below and above the median, respectively. The legend shows the median survival time [95% CI]



3.2.5 Effect of Immunogenicity

A total of 8 patients in the PK database were positive in the anti-drug antibody (ADA) assay. Of these 8, one was positive before drug administration and one was positive in the neutralizing antibody assay. In this limited sample, an effect of immunogenicity on posthoc estimates of aflibercept could not be detected (Figure 11).

Figure 11: Free Aflibercept Clearance vs. Immunogenicity from VELOUR



Source: Study POH0265 Report, Page 67, Figure 16.

3.3 Exposure-Response Analysis

The Applicant performed exposure-response analyses to explore the relationship between PK parameters and safety and efficacy endpoints in VELOUR. The following model-derived PK parameters were used in the analyses:

- Free Aflibercept Clearance
- Bound Aflibercept Clearance
- AUC (free aflibercept) of first cycle ($AUC_{0\text{-}336}$)
- AUC (free aflibercept) extrapolated (steady-state)
- C_{max} (free aflibercept) of first cycle
- Cumulative AUC of free aflibercept up to last aflibercept administration +90 days or date of death of cutoff date (whichever came first), divided by the number of cycles (AUC_{cumOS})
- Cumulative AUC of free aflibercept up to the last aflibercept administration +90 days or date of progression or cutoff date (whichever came first) divided by the number of cycles (AUC_{cumPFS})
- Cumulative AUC of free aflibercept over the first two cycles ($AUC_{cum\ cycles1,\ 2}$)

There were a total of 500 (82%) aflibercept-treated patients in VELOUR with free aflibercept PK parameters and 460 (75%) with bound aflibercept PK parameters.

3.3.1 Exposure-Efficacy Analysis

The relationship between efficacy and PK parameters was first analyzed by a univariate proportional hazards regression model. Multivariate analyses were then conducted using the following covariates: age, gender, region ECOG PS, prior bevacizumab, location of primary disease, prior hypertension and number of metastatic organs involved. An additional multivariate analysis also included endogenous VEGF as a covariate. Inclusion of VEGF reduced the sample size to 356 because not all patients were sampled for baseline VEGF. A stepwise procedure was used for inclusion and deletion of covariates at a significance level of 0.15. Overall survival (OS) and progression free survival (PFS) were the efficacy endpoints analyzed.

Overall survival was significantly related with all free and bound PK parameters tested (Table 5). For the multivariate analysis using AUC extrapolated, an increase of 1000 µg.h/mL was associated with a 21% decrease in the survival hazard rate. The relationship remained significant when endogenous VEGF was included in the model. Similar results were observed for PFS (Table 6).

Table 5: Summary of Exposure-Efficacy Analysis for OS in VELOUR

EFFICACY ENDPOINTS		Free aflibercept (N=500)	Bound aflibercept (N=460)
Overall survival	Univariate analyses	Cmax (per 10µg/mL)	Clearance (per 1mL/h)
		HR=0.821 95%CI=(0.735,0.918) p=0.0005	HR= 1.320 95%CI= (1.153,1.510) p <.0001
		Clearance (per 10 mL/h)	
		HR= 1.052 95%CI= (1.010,1.096) p= 0.0147	
		AUCextrapolated (per 1000µg.h/mL)	
		HR = 0.803 95%CI= (0.754,0.855) p <0.0001	
		AUCcum OS (per 1000 µg.h/mL)	
		HR = 0.869 95%CI= (0.818,0.924) p <0.0001	
		AUC0-336 (per 1000 µg.h/mL)	
		HR = 0.750 95%CI= (0.684,0.822) p <0.0001	
	Multivariate analysis	AUCextrapolated (per 1000µg.h/mL)	Clearance (per 1mL/h)
		HR = 0.788 95%CI= (0.736,0.844) p <0.0001	HR= 1.394 95%CI= (1.213,1.603) p <0.0001
	Multivariate analysis adding endogenous VEGF as a covariate	N=356	N=323
		AUCextrapolated (per 1000µg.h/mL)	Clearance (per 1mL/h)
		HR = 0. 779 95%CI= (0.718,0.845) p <0.0001	HR= 1.343 95%CI= (1.140,1.583) p =0.0004

[*] Multivariate analysis included age, gender, region ECOG PS, prior bevacizumab, location of primary disease, prior hypertension and number of metastatic organs involved

Source: Study EFC10262 Report, Page 1359, Table 116.

Table 6: Summary of Exposure-Efficacy Analysis for PFSin VELOUR

EFFICACY ENDPOINTS		Free aflibercept (N=500)	Bound aflibercept (N=460)
Progression free survival	Univariate analyses	Clearance (per 10 mL/h)	Clearance (per 1mL/h)
		HR = 1.118 95%CI= (1.069,1.168) p <0.0001	HR = 1.229 95%CI= (1.065,1.417) p =0.0048
		AUC extrapolated (per 1000 µg.g/mL)	
		HR = 0.822 95%CI= (0.768,0.879) p <0.0001	
		AUC0-336 (per 1000 µg.h/mL)	
		HR = 0.779 95%CI=(0.702,0.865) p <0.0001	
		AUCcum PFS (per 1000 µg.h/mL)	
		HR = 0.890 95%CI= (0.839,0.944) p = 0.0001	
	Multivariate analysis	AUC extrapolated (per 1000 µg.g/mL)	Clearance (per 1mL/h)
		HR = 0.814 95%CI= (0.761,0.872) p <0.0001	HR = 1.234 95%CI= (1.069,1.426) p = 0.0042
	Multivariate analysis adding endogenous VEGF as a covariate	N=356	N=323
		AUC extrapolated (per 1000 µg.g/mL)	Clearance (per 1mL/h)
		HR = 0.833 95%CI= (0.769,0.901) p <0.0001	HR = 1.149 95%CI= (0.977,1.352) p = 0.0926

[*] Multivariate analysis included age, gender, region ECOG PS, prior bevacizumab, location of primary disease, prior hypertension and number of metastatic organs involved

Source: Study EFC10262 Report, Page 1359, Table 116.

3.3.2 Exposure-Safety Analysis

The relationship between safety and PK parameters (excluding AUC_{cumPFS} and AUC_{cumOS}) was analyzed with a univariate logistic regression model if the number of events was ≥ 5. Patients with safety events were defined as having experienced the events (any grade, unless specified otherwise) at least once during the first two cycles of treatment. Multivariate analysis was also performed as described for the exposure-efficacy analysis. The safety endpoints considered were:

- Hypertension
- Dysphonia
- Proteinuria grade ≥ 2
- Venous thromboemolic event
- Hemorrhage
- Renal failure events (grade 3 or 4 renal failure, grade 3 or 4 serum creatinine increase from laboratory data, or calculated creatinine clearance < 30 mL/min)
- Diarrhea (grade 3-4)
- Headache (grade 3-4)
- Stomatitis and ulceration
- Infection and infestation

Modeling was not performed for arterial thromboembolic events and headache because there were less than 5 events in the PK population. No significant exposure-safety relationships were identified for dysphonia, venous thromboembolic event, renal failure, diarrhea, stomatitis and ulceration, and infection and infestation. The occurrence of hypertension was significantly related to PK parameters of free aflibercept and remained significant in a multivariate analysis and with VEGF as a covariate in the model. The odds for experiencing hypertension increase by 27% for an increase in $AUC_{0\text{-}336}$ of 1000 µg.h/mL.

Table 7: Summary of Exposure-Safety Analysis for Hypertension in VELOUR

ADVERSE EVENTS (Any grade)		Free aflibercept (N=500)	Bound aflibercept (N=460)
HYPERTENSION	Univariate analyses	Nb of patients with AE (C1&C2/All) = 114/202	Nb of patients with AE (C1&C2/All) =104 / 186
		Cmax (per 10µg/mL) OR=1.201 95%CI=(1.005,1.434) p=0.0437	Clearance (per 1mL/h) p= 0.9765
		AUCcum cycles 1,2 (per 2000 µg.h/mL) OR= 1.191 95%CI= (1.029,1.378) p= 0.0193	
		AUCextrapolated (per 1000µg.h/mL) OR=1.163 95%CI=(1.039,1.301) p= 0.0086	
		AUC0-336 (per 1000µg.h/mL) OR=1.312 95%CI=(1.097,1.568) p=0.0029	
	Multivariate analysis	AUC0-336 (per 1000µg.h/mL) OR= 1.274 95%CI= (1.062,1.529) p= 0.0093	No modelling is done since no PK parameter was significant at the 10% level
	Multivariate analysis adding endogenous VEGF as a covariate	N=356 AUC0-336 (per 1000µg.h/mL) OR= 1.264 95%CI= (1.013,1.577) p= 0.0385	No modelling is done since no PK parameter was significant at the 10% level

Source: Study EFC10262 Report, Page 1361, Table 117.

The occurrence of hemorrhage was also significantly related to free afliberecept AUC extrapolated and $AUC_{cum\ cycles1,\ 2}$ and remained significant in a multivariate analysis and with VEGF as a covariate in the model. For an increase of 2000 µg.h/mL in $AUC_{cum\ cycles1,\ 2}$, the odds of hemorrhage increase by 17% (Table 8).

Table 8: Summary of Exposure-Safety Analysis for Hemorrhage in VELOUR

ADVERSE EVENTS (Any grade)		Free aflibercept (N=500)	Bound aflibercept (N=460)
HAEMORRHAGE	Univariate analyses	Nb of patients with AE (C1& C2/All) = 83/189	Nb of patients with AE (C1&C2/All) =78 / 180
		AUCcum Cycles 1,2 (per 2000µg.h/mL) OR= 1.191 95%CI= (1.012,1.402) p= 0.0351	Clearance (per 1mL/h) p= 0.2157
		AUC extrapolated (per 1000 µg.g/mL) OR= 1.130 95%CI= (0.996,1.281) p= 0.0577	
	Multivariate analysis	AUCcum Cycles 1,2 (per 2000µg.h/mL) OR=1.171 95%CI= (0.992,1.382) p= 0.0619	No modelling is done since no PK parameter was significant at the 10% level
	Multivariate analysis adding endogenous VEGF as a covariate	N=356 AUCcum Cycles 1,2 (per 2000µg.h/mL) OR= 1.198 95%CI=(0.991,1.447) p= 0.0615	No modelling is done since no PK parameter was significant at the 10% level

Source: Study EFC10262 Report, Page 1363, Table 117.

For proteinuria, a significant relationship was only found for $AUC_{cum\ cycles1,\ 2}$ with an unexpected finding that the odds of experiencing proteinuria decrease with an increase in $AUC_{cum\ cycles1,\ 2}$. Only 15 of 500 patients experienced proteinuria grade ≥ 2 during the first two cycles. Bound aflibercept clearance was only found to be of borderline significance for diarrhea and venous thromboembolic event.

Reviewer's Comment: Exposure-response analyses performed by the Applicant were pre-specified in the protocol and are acceptable.

4 REVIEWER'S ANALYSIS

4.1 Introduction

During the course of the review, the reviewers noted a strong relationship between body weight and aflibercept exposure (Figure 1). This is an unexpected finding if the goal of weight-based dosing is to reduce variability in exposure due to body weight. The variability in exposure introduced by body weight might be of little concern if there were no relationships between free aflibercept exposures and either safety or efficacy. The Applicant, however, identified exposure-response relationships for both safety and survival. Furthermore, the VELOUR trial showed a relatively small drug effect. This implies that dosing may potentially be optimized by reducing the variability introduced by the weight-based dosing regimen proposed by the Applicant.

4.2 Objectives

Analysis objectives are:

1. Compare weight-based dosing to fixed dosing.

4.3 Methods

4.3.1 Data Sets

Data sets used are summarized in Table 9.

Table 9. Analysis Data Sets

Study Number	Name	Link to EDR
EFC10262	adatte.xpt	\\cbsap58\m\eCTD_Submissions\STN125418\0000\m5\datasets\efc10262\analysis
EFC10262	adpp.xpt	\\cbsap58\m\eCTD_Submissions\STN125418\0000\m5\datasets\efc10262\analysis
EFC10262	adpat.xpt	\\cbsap58\m\eCTD_Submissions\STN125418\0000\m5\datasets\efc10262\analysis
EFC10262	ades.xpt	\\cbsap58\m\eCTD_Submissions\STN125418\0000\m5\datasets\efc10262\analysis
EFC10262	adsl.xpt	\\cbsap58\m\eCTD_Submissions\STN125418\0000\m5\datasets\efc10262\analysis

EFC10262	velourpf.xpt	\\cbsap58\m\eCTD_Submissions\STN125418\0000\m5\datasets\poh0265\analysis
EFC10262	velourdf.xpt	\\cbsap58\m\eCTD_Submissions\STN125418\0000\m5\datasets\poh0265\analysis

4.3.2 Software

R Version 2.13.1 was used for the analyses.

4.3.3 Body-Weight vs. Fixed Dosing

Monte Carlo simulations were conducted to explore the difference between body-weight based dosing and fixed dosing of aflibercept. Clearance values were simulated for 1000 patients by bootstrapping (with replacement) weight values from patients enrolled in VELOUR and calculating free aflibercept clearance according to the final population PK model. The simulation assumed 27.6% interindividual variability for clearance, as derived from the population PK model. Individual patient exposure (AUC_{SS}) was calculated as Dose/CL. Dose was defined as either weight based (4 mg/kg) or fixed (300 mg). The fixed dose corresponds to a typical 75 kg patient receiving the 4 mg/kg dose.

Simulated AUC_{ss} values were grouped into three populations based on body weight: normal (body weight between 10th and 90th percentiles), light (body weight $\leq 10^{th}$ percentile) and heavy (body weight $\geq 90^{th}$ percentile). The 90% range of exposure was defined as:

$$90\%\ \text{Range} = \frac{AUC_{ss}(95th) - AUC_{ss}(5th)}{AUC_{ss}(\text{median of normal})} \times 100\%$$

where $AUC_{ss}(95^{th})$ and $AUC_{ss}(5^{th})$ are the 95th and 5th percentiles, respectively, of the simulated AUC_{ss} in all patients and AUC(median of normal) is the median simulated AUC_{ss} in the normal population. The difference between fixed and bodyweight-based dosing was then calculated as:

Difference = 90% Range (fixed) – 90% Range (body-weight based)

Finally, the deviation of exposure for heavy and light populations from the normal population was defined as:

$$\%\ \text{deviation} = \frac{AUC_{ss}(\text{median of extreme}) - AUC_{ss}(\text{median of normal})}{AUC_{ss}(\text{median of normal})} \times 100\%$$

where extreme refers to either the light or heavy population.

The results of the simulation are displayed in Table 10. Although median AUC_{ss} remained similar between the two dosing regimens, fixed dosing clearly results in a

Table 10: Predicted Aflibercept Exposure for Fixed (300 mg) and Weight-Based (4 mg/kg) Dosing

	AUC_{ss} (µg.h/mL) Fixed Dosing	AUC_{ss} (µg.h/mL) Weight-Based Dosing
Minimum	3174	2650
5th Percentile	4368	3859
Median	6872	6618
Mean	7126	7005
95th Percentile	10884	11618
Maximum	16124	22532
% CV	28%	35%
90% Range	95%	117%
Difference	-32%	-

The deviation of exposure in heavy and light patients is illustrated in Figure 2. The percent deviation for fixed dosing is much smaller than for weight-based dosing, suggesting that a fixed dose would lead to less potential for under-exposure in light subjects or over-exposure in heavy subjects. This is especially relevant in light of the exposure-response relationships for efficacy and safety observed for aflibercept.

5 LISTING OF ANALYSES CODES AND OUTPUT FILES

File Name	Description	Location in \\cdsnas\pharmacometrics\
run100.mod	Final Population PK Model (NONMEM control file)	\Reviews\Ongoing PM Reviews\Aflibercept_BLA125418_KMK\PK Analyses\Final Model
run100.csv	Final Population PK Dataset	\Reviews\Ongoing PM Reviews\Aflibercept_BLA125418_KMK\PK Analyses\Final Model
run100.lst	Final Population PK Model (Output File)	\Reviews\Ongoing PM Reviews\Aflibercept_BLA125418_KMK\PK Analyses\Final Model
make.AUCextrvsOS_NoPlacebo.R	Exposure-Response Analysis (OS)	Reviews\Ongoing PM Reviews\Aflibercept_BLA125418_KMK\ER Analyses\Efficacy
make.AUCexvsPFS_NoPlacebo.R	Exposure-Response Analysis (PFS)	Reviews\Ongoing PM Reviews\Aflibercept_BLA125418_KMK\ER

		Analyses\Efficacy
make hypertension.R	Exposure-Safety Analysis (Hypertension)	Reviews\Ongoing PM Reviews\Aflibercept_BLA125418_KMK\ER Analyses\Safety
make hemorrhage.R	Exposure-Safety Analysis (Hemorrhage)	Reviews\Ongoing PM Reviews\Aflibercept_BLA125418_KMK\ER Analyses\Safety
make.BW.R	Fixed vs. Weigh-Based Dosing Simulations	Reviews\Ongoing PM Reviews\Aflibercept_BLA125418_KMK\PK Analyses

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This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

RUBY LEONG
07/06/2012

KEVIN M KRUDYS
07/06/2012

CHRISTINE E GARNETT
07/09/2012

HONG ZHAO
07/09/2012
I concur.

Office of Clinical Pharmacology

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA/BLA Number	125418	**Brand Name**	Zaltrap™
OCP Division (I, II, III, IV, V)	V	**Generic Name**	Aflibercept
Medical Division	DOP2	**Drug Class**	Fusion protein
OCP Reviewer	Ruby Leong	**Indication(s)**	Metastatic colorectal cancer previously treated with an oxaliplatin-containing regimen
OCP Team Leader	Hong Zhao	**Dosage Form**	25 mg/mL solution (100 mg/4 mL and 200 mg/8 mL single-use vials)
Pharmacometrics Reviewer	Kevin Krudys	**Dosing Regimen**	4 mg/kg every 2 weeks (Q2W) in combination with irinotecan-fluoropyrimidine-based chemotherapy
Date of Submission	February 3, 2012	**Route of Administration**	Intravenous (IV) infusion over 1 hour
Estimated Due Date of OCP Review	June 8, 2012	**Sponsor**	Sanofi-Aventis
Medical Division Due Date	July 7, 2012	**Priority Classification**	Priority
PDUFA Due Date	August 4, 2012		

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X	8		Free VEGF (1); Free aflibercept (2); Bound aflibercept (2); Anti-aflibercept antibodies (2); Anti-aflibercept neutralizing antibodies (1)
I. Clinical Pharmacology				
Mass balance:	n/a			
Isozyme characterization:	n/a			
Blood/plasma ratio:	n/a			
Plasma protein binding:	n/a			
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:		2		PDY6655, PDY6656
multiple dose:				
Patients-				
single dose:				
multiple dose:		10		TED6113, TED6114, TED6115, TED6116, TCD6117, TCD6118, TCD6119, TCD6120, TCD6121, TCD10173
Dose proportionality -				
fasting / non-fasting single dose:	n/a			

fasting / non-fasting multiple dose:	n/a			
Drug-drug interaction studies -				PopPK
In-vivo effects on primary drug:	n/a			
In-vivo effects of primary drug:	n/a			
In-vitro:	n/a			
QT studies -		1		TES10897
Subpopulation studies -				
ethnicity:				PopPK
gender:				PopPK
pediatrics:	n/a			
geriatrics:				PopPK
renal impairment:				PopPK
hepatic impairment:				PopPK
PD -				
Phase 2:	n/a			
Phase 3:	n/a			
PK/PD -				
Phase 1 and/or 2, proof of concept:	n/a			
Phase 3 clinical trial:		3		EFC10547, EFC10261, EFC10262
Population Analyses -				
		6		POH0251, POH0253, POH0263, POH0262, POH0274, POH0265
II. Biopharmaceutics				
Absolute bioavailability	n/a			
Relative bioavailability -	n/a			
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -	n/a			
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies	n/a			
Bio-waiver request based on BCS	n/a			
BCS class	n/a			
Dissolution study to evaluate alcohol induced dose-dumping	n/a			
III. Other CPB Studies				
Genotype/phenotype studies	n/a			
Chronopharmacokinetics	n/a			
Pediatric development plan				Requested pediatric waiver
Literature References	X			
Total Number of Studies		30		

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?			X	
2	Has the applicant provided metabolism and drug-drug interaction information?			X	
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?			X	
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?	X			
5	Has a rationale for dose selection been submitted?	X			
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?	X			

7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	X			
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?	X			
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
	Data				
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?			X	
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			X	
	Studies and Analyses				
11	Is the appropriate pharmacokinetic information submitted?	X			
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?	X			
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?	X			
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?	X			
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			X	
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			X	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?	X			
	General				
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	X			
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?			X	

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE?

If the NDA/BLA is not fileable from the clinical pharmacology perspective, state the reasons and provide comments to be sent to the Applicant.

The application is fileable from a clinical pharmacology perspective.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

No potential review issues were identified.

Reviewing Clinical Pharmacologist Date

Team Leader/Supervisor Date

Clinical Pharmacology - BLA Filing Memorandum

BLA:	125418	**IND:** 9948
Compound:	Aflibercept	
Applicant:	Sanofi-Aventis	
Filing Date:	April 3, 2012	
Reviewer:	Ruby Leong, Pharm.D.	

Background and Mechanism of Action

Aflibercept (Vascular Endothelial Growth Factor [VEGF] Trap), is a recombinant human soluble fusion protein, consisting of the extracellular domains of VEGF receptors 1 and 2 (VEGFR-1 and VEGFR-2) fused to the Fc portion of IgG_1. It is a dimeric glycoprotein with a molecular weight of 115 kDa.

According to the applicant, aflibercept acts as a soluble decoy receptor that binds to VEGF-A with higher affinity than its native receptors, as well as to related ligands, placenta growth factor (PlGF) and VEGF-B to form a stable, inert complex. As a ligand trap, aflibercept prevents VEGFR activation, blocking endothelial cell proliferation and new blood vessel formation. The applicant states that aflibercept exerts direct anticancer activity and potentiates the anticancer activity of chemotherapy agents through inhibition of proliferation of endothelial cells, antiangiogenic activity, vascular constriction, vascular normalization, direct effects on tumor cell function, offsetting effects of chemotherapy induction on VEGF levels, and inhibition of VEGF repression of dendritic cell function.

The applicant is seeking approval of intravenous (IV) administration of aflibercept at 4 mg/kg every 2 weeks (Q2W) in combination with irinotecan/fluoropyrimidine-based chemotherapy for patients with metastatic colorectal cancer (mCRC) previously treated with an oxaliplatin-containing regimen.

Rationale for Dose Selection

Preclinical studies suggested that maintaining a free/bound aflibercept ratio above 1 throughout the dosing interval would maximize binding of endogenous VEGF and maintain VEGF levels <20 pg/mL (near the median value of 17 pg/mL in healthy subjects). At doses >2 mg/kg Q2W, the mean ratio of free/bound aflibercept trough concentrations was >1 in all single agent and combination Phase 1 studies. The applicant states that the proposed dosing regimen of aflibercept 4 mg/kg IV Q2W was initially evaluated in single agent Phase 1 studies TED6115 and TED6116, and identified as a safe and biologically active dose for further evaluation as a single agent in Phase 2 studies. In the Phase 1 combination study (TCD6118) with 5-fluorouracil, leucovorin, and irinotecan (FOLFIRI), aflibercept doses from 2 to 6 mg/kg Q2W were investigated and best overall response was evaluated at the selected dosing regimen of 4 mg/kg IV Q2W in an extension cohort (similar to a single arm Phase 2 trial). According to the applicant, results from single agent studies and Study TCD6118 supported the proposed dosing regimen of aflibercept 4 mg/kg IV Q2W in the pivotal EFC10262/VELOUR trial.

Effectiveness in Clinical Trials

The proposed indication is Zaltrap™ (aflibercept), in combination with irinotecan/fluoropyrimidine-based chemotherapy for mCRC patients previously treated with an oxaliplatin-containing regimen. The proposed indication is based primarily on the overall survival (OS) of previously treated mCRC patients in the pivotal trial EFC10262/VELOUR, a randomized, double-blind trial comparing the efficacy of FOLFIRI with and without aflibercept. The efficacy analyses were based on all randomized patients (intent-to-treat [ITT] population: 612 patients in the aflibercept arm and 614 patients in the placebo arm). The applicant stated that the study met its primary endpoint with a stratified hazard ratio (HR) of 0.817 ([95.34% CI: 0.713, 0.937]; p = 0.0032), an 18.3% reduction in the risk of death. The median OS was 13.50 months versus 12.06 months in the aflibercept and placebo treatment arms, respectively. The Kaplan-Meier curves show a separation of the curves starting at approximately 6 months with continued and increasing separation. Median follow up was 22 months. As of the data cutoff date of February 7, 2011 for OS analysis, there were a total of 863 deaths (70.4% of 1226 patients enrolled).

Safety Evaluation in Clinical Trials

The applicant reports that the safety of aflibercept has been evaluated in 1216 patients in the pivotal trial EFC10262/VELOUR (612 aflibercept-treated patients) and in a total of 2073 aflibercept-treated patients from 5 single agent Phase 1 or Phase 2 studies, 5 combination Phase 1 studies, and two supportive Phase 3 studies in metastatic pancreatic cancer and NSCLC.

In summary, the addition of aflibercept to FOLFIRI increases the incidence of AEs associated with VEGF blockade including hypertension, dysphonia, proteinuria, and hemorrhage (epistaxis, gastrointestinal bleeding, hemoptysis), and less frequent but potentially severe AEs such as arterial and venous thromboembolic events (ATE and VTE), osteonecrosis, and gastrointestinal perforation and fistula. Aflibercept also increases the frequency and severity of background chemotherapy toxicities including stomatitis, decreased weight, infections, diarrhea, palmar plantar erythrodysesthesia syndrome, neutropenia, and neutropenic complications.

Human Pharmacokinetic Data

Aflibercept was initially administered subcutaneously in 2 single agent Phase 1 dose-finding trials (TED6113, TED6114). However, the SC route of administration was discontinued due to the high volume of injection required for the formulated aflibercept concentration of 25 mg/mL. Subsequently, only IV administration was used in all clinical studies.

The PK of IV aflibercept have been evaluated as a single agent in the dose range of 0.3 to 7 mg/kg IV Q2W and in combination with various chemotherapies in the dose range of 2 to 6 mg/kg Q2W and 2 to 9 mg/kg Q3W. Free aflibercept exposure increased slightly more than dose-proportionally between 1 to 2 mg/kg, then increased approximately proportionally between 2 to 9 mg/kg. T_{max} of free aflibercept is reached at the end of infusion (1 hour). Following administration of aflibercept 4 mg/kg Q2W, the accumulation ratio for free aflibercept was 1.2 with steady state reached by the sixth dose (approximately 70 days). Volume of distribution ($V_{d,ss}$) was estimated by the population PK approach to be 7.8 L, slightly greater than blood compartment. Free and bound aflibercept exhibits non linear PK due to saturable high-affinity binding of aflibercept to endogenous VEGF at higher doses. In cancer patients, mean free aflibercept clearance decreased from 1.95 to 1.13 L/day in the dose range of 0.3 to 2 mg/kg and

then remained stable over the dose range of 2 to 9 mg/kg (0.9 to 1.3 L/day). Mean free aflibercept terminal half-life ($t_{1/2}$) increased from 1.7 to 3.76 days in the dose range of 0.3 to 2 mg/kg and then remained stable over the dose range of 2 to 9 mg/kg (5 to 7 days). Elimination of VEGF-bound aflibercept is slower with an apparent clearance of 0.182 L/day and a $t_{1/2}$ of 15 days.

Applicant's Population Pharmacokinetic (popPK) Analyses

The applicant reports that the PK of aflibercept is best described by a structural model involving two compartments for free aflibercept and one for bound aflibercept, with a Michaelis-Menten type binding of free aflibercept to VEGF from the peripheral compartment. Free aflibercept in plasma distributes first to tissues then binds to VEGF to form the complex. The bound aflibercept is assumed to be directly eliminated through internalization and not to any appreciable extent through reversible dissociation to re-generate free aflibercept and free VEGF.

The applicant's population PK (popPK) analysis included data from 12 clinical studies and the following covariates: demographic factors (age, race, gender, weight), intrinsic factors (hepatic and renal function as assessed by serum albumin, serum alkaline phosphatase, total bilirubin, aspartate amino transferase, alanine amino transferase, total protein, creatinine clearance) and extrinsic factors (concomitant chemotherapy agents) on free aflibercept PK.

According to the applicant, gender was the most significant covariate for explaining the inter-individual variability of free aflibercept with a 15.5% higher clearance and a 20.6% higher volume of distribution in males than in females. However, no difference in AUC was noted between male and female patients, possibly due to weight-based dosing leading to higher total dose in men than in women. The minor effect of weight on free aflibercept clearance and volume of distribution resulted in 29% higher AUC in the >100 kg patients than 50-100 kg patients. No effect of race or age was identified.

Patients with low serum albumin concentrations (≤0.568 x upper limit of normal [ULN]) or high concentration of alkaline phosphatase (≥3.24 x ULN) had an 18.7% and 12.9% increase in free aflibercept clearance, respectively. The effect of creatinine clearance on aflibercept clearance was of limited magnitude with a decrease of 6.48% for a value of 47.8 mL/min.

The applicant states that based on PK data from 549 mild, 96 moderate and 5 severe renal impairment patients, dose adjustment is not necessary in patients with renal impairment. Based on limited data in 63 mild and 5 moderate hepatic impairment patients, dose adjustment is not necessary in patients with mild and moderate hepatic impairment. There is no data in patients with severe hepatic impairment.

Drug-Drug Interaction

No specific drug-drug interaction studies have been conducted with aflibercept. PK evaluations between aflibercept and combination therapies have been evaluated based on Phase 1 cross-study comparisons and historical data or literature. The applicant reports that aflibercept has no impact on the PK of oxaliplatin, cisplatin, 5-FU, irinotecan/SN38, docetaxel, pemetrexed, gemcitabine, and erlotinib. Based on these data, no impact of aflibercept on the FOLFIRI regimen used in EFC10262/VELOUR is expected.

The effect of combination therapies on the PK of aflibercept was investigated via popPK analysis. Following co-administration with irinotecan/LV5-FU2, FOLFOX4, gemcitabine, erlotinib, docetaxel, cisplatin, and pemetrexed, free and bound aflibercept concentrations were comparable to those observed in single agent studies. However, there was a trend for lower free aflibercept clearance in some combination studies as compared to monotherapy. The greatest effect was an 11.3% decrease in clearance when combined with irinotecan/LV5-FU2 in the TCD6118 trial, but no effect was found for the FOLFIRI combination in the pivotal EFC10262/VELOUR trial. A 4.7% decrease of clearance was observed for the docetaxel combination.

Applicant's Exposure-Response Analyses for Efficacy and Safety
Exposure-response analyses were conducted using PK data from the three Phase 3 trials. The applicant reports that a significant relationship was observed between free and bound aflibercept PK parameters and OS and progression-free survival (PFS). A decrease in free and bound aflibercept clearance and increase in free aflibercept exposure (AUC, C_{max}) was correlated with increased OS and PFS.

According to the applicant, occurrence of hypertension was correlated with free aflibercept PK parameters. A decrease in CL or increase in free aflibercept exposure (AUC, C_{max}) was associated with an increased rate of hypertension in all three Phase 3 trials. A correlation between occurrence of hemorrhage and free aflibercept PK parameters (AUC extrapolated and cumulative AUC at cycles 1 and 2) was found only in the EFC10262/VELOUR trial. Proteinuria (grade ≥2 during cycles 1 and 2) was found to be correlated with increased free aflibercept C_{max} in EFC10261/VITAL. No relationship was observed in any of the three Phase 3 trials for the occurrence of dysphonia, venous thromboembolic event, and renal failure. Bound aflibercept clearance was not found significant for any safety endpoint.

Human Pharmacodynamic Data

Endogenous VEGF levels were measured during PDY6655/PDY6656 studies in healthy subjects, and at baseline in the Phase 3 trials to assess any impact on the PK of aflibercept and to determine K_D *in vivo*. The applicant reports that no clear relationship was observed between free aflibercept clearance and baseline endogenous VEGF levels.

Cardiovascular pharmacodynamics was investigated in study PDY6656, a randomized, double blind, placebo-controlled, sequential ascending dose study. According to the applicant, a statistically significant increase in 24-hour mean systolic blood pressure was observed after single aflibercept IV doses of 1, 2 and 4 mg/kg. The increase in 24-hour mean systolic blood pressure observed in patients treated with 4 mg/kg was long lasting (+5.47 mmHg at 6 weeks). Similarly, the highest increase in 24-hour mean diastolic blood pressure was observed 2 weeks after administration: +6.17, +8.34 and +14.55 mmHg placebo-corrected values for the 1, 2 and 4 mg/kg doses, respectively. The sponsor states that the mechanism of hypertension is not renin-dependent, as shown by the fact that plasma renin and aldosterone concentrations decreased inversely to the changes in blood pressure without relationship to dose. The severity of vascular pharmacodynamic effects was generally lower after SC as compared to IV administration, consistent with the lower bioavailability of free aflibercept with SC administration.

QTc Evaluation
A dedicated QT clinical trial (TES10897/QUTIE) was conducted in which 87 patients were randomized to receive aflibercept (6 mg/kg) or placebo for at least 3 cycles in combination with docetaxel every 3 weeks. The primary analysis was QTcF change from baseline over the interval 1 hour post-dose (end of 1-hour infusion) to 3 hours post-dose (2 hours after end of 1-hour infusion) on Cycle 3, Day 1 (or on Cycle 1, Day 1 if Cycle 3 was not available). The sponsor reports that the least square mean difference of the change from baseline versus placebo (Cycle 1 or Cycle 3) for QTcF was +3.8 ms (90% CI: -1.6, 9.2). The QTcF largest mean difference for change from baseline was higher at cycle 3 compared to cycle 1: +8.4 msec (upper bound 90% CI of +16 msec) versus +1.7 msec (upper bound 90% CI of +5.7 msec), respectively. Of note, the evaluable population at cycle 3 consisted of only 28 patients versus 41 patients at Cycle 1. The upper bound of the 90% CI for the largest mean difference for QTcF was below 20 msec (at Cycle 1 or Cycle 3). The applicant concludes that aflibercept 6 mg/kg does not affect ventricular repolarization in humans to an extent that would require substantial risk-benefit evaluation.

Immunogenicity

Serum samples for immunogenicity assessment were collected in all patients enrolled during aflibercept clinical development. In Phase 1 and Phase 2 studies, samples were collected at baseline, during treatment, and 30 and 60 or 90 days after last aflibercept treatment. In Phase 3 studies, samples were collected at baseline, then every cycle (EFC10547/VANILLA) or every other cycle (EFC10261/VITAL and EFC10262/VELOUR) and at 30 and 90 days after last aflibercept treatment.

The sponsor reports that overall, 63/1671 (3.8%) aflibercept-treated patients and 35/1105 (3.2%) placebo-treated patients exhibited a positive low titer assay response at any time during treatment. These patients demonstrated low titers (<250), and in most cases the assay responses fluctuated above and below the assay cutpoint, resulting in both positive and negative responses at different timepoints. This pattern of low titer positive responses at baseline or in placebo-treated patients, with no significant increase in titer in subsequent samples, suggests the presence of pre-existing immunoreactivity. Pre-existing immunoreactivity can potentially be due to the presence of elevated serum levels of rheumatoid factor that can cause a positive response in the bridging immunoassay. The applicant states that given the similar level of low titer ADA assay responses in the aflibercept-treated patients as in placebo-treated patients, it is most likely that positive assay responses observed in the aflibercept-treated patients are due to high assay background levels and not drug induced antibody response. Positive neutralizing anti-aflibercept antibodies were observed in 17 (1.3%) aflibercept-treated patients and 2 (0.2%) placebo-treated patients, respectively. The applicant states that there is no evidence of an impact of positive anti-aflibercept antibodies on aflibercept PK, efficacy, or safety.

Analytical Assays

According to the applicant, free aflibercept (not bound to VEGF) and bound aflibercept (the inert VEGF:aflibercept complex) were quantified in plasma of healthy volunteers and patients with validated bioanalytical ELISA methods that had adequate sensitivity, and good within- and between-run accuracy and precision. The long-term stability of free and bound aflibercept in frozen human plasma was assessed and Incurred Sample Reanalysis (ISR) was successful.

Free aflibercept
According to the applicant's summary, the free aflibercept ELISA assay that was initially developed and validated for the clinical program was later reformatted and validated to change the detection system and allow for automation of the assay. This method employed a microtiter plate coated with human $VEGF_{165}$ and aflibercept as a standard. Aflibercept captured on the $VEGF_{165}$ coated plate was then detected using a mouse anti-human VEGFR-1 monoclonal antibody (P10G1F6). A goat anti-mouse IgG antibody conjugated to Horseradish Peroxidase was added to bind the mouse monoclonal antibody. The assay was initially developed and validated using a peroxidase substrate (3, 3', 5, 5'- tetramethyl-benzidine, [TMB]) and then modified to include a seventh nonzero standard and to utilize a luminol-based substrate specific for peroxidase (instead of a TMB-based substrate). The limit of quantitation (LOQ) for free aflibercept in plasma was initially 31.3 ng/mL (for TED6115/TED6116), then 15.6 ng/mL for other trials. Samples are stable out to at least 9 freeze-thaw cycles and long-term stability of free aflibercept was demonstrated for up to 15 months at -80°C.

Bound aflibercept (VEGF:aflibercept complex)
According to the applicant's summary, the bound aflibercept ELISA assay that was initially developed and validated for the clinical program was later reformatted and validated to change the detection system and allow for automation of the assay. This method employed a microtiter plate coated with anti-human $VEGF_{165}$ antibody and human $VEGF_{165}$:aflibercept complex as a standard. Bound aflibercept captured on the plate was then detected using a mouse anti-human VEGFR-1 monoclonal antibody (P10G1F6), followed by a peroxidase-conjugated goat anti-mouse IgG, Fc specific antibody. The assay was initially developed and validated using TMB, and then modified to utilize a luminol-based substrate specific for peroxidase. The limit of quantitation for VEGF:aflibercept complex in plasma was 43.9 ng/mL. However, since VEGF:aflibercept complex contains one molecule of endogenous VEGF (MW=45390 g/mol) and one molecule of aflibercept (MW=115000 g/mol), VEGF-bound aflibercept concentrations were expressed as free aflibercept equivalents for PK analyses using the ratio of molecular weights between free aflibercept and VEGF:aflibercept complex. Consequently, the corrected (normalized to 0.717) LOQ value for bound aflibercept used in PK analyses was 31.5 ng/mL. Samples are stable out to at least 10 freeze-thaw cycles. Bound aflibercept in human plasma was also shown to be stable at -70°C to -90°C for up to 24 months.

Free endogenous VEGF
The Quantikine ELISA kit from R&D System was used to quantify free endogenous VEGF. According to the applicant's summary, it is a sandwich enzyme immunoassay using one monoclonal antibody and one enzyme-linked polyclonal antibody calibrated with $VEGF_{165}$. It was demonstrated to specifically measure free endogenous VEGF. The LOQ for free endogenous VEGF in plasma was 15 pg/mL. Free VEGF was stable in human citrated plasma for up to 3 freeze/thaw cycles and 12 months of storage at -20°C or at -80°C.

Anti-aflibercept binding and neutralizing antibodies
Two methods were developed and validated to detect anti-aflibercept antibodies in human serum. The first assay was a sandwich ELISA used in Phase 1 studies. The second assay was a more sensitive bridging immunoassay that was used in Phase 2 and Phase 3 studies. A comparison study of both methods demonstrated that the bridging immunoassay (PCL2375) was more

sensitive than the original ELISA assay. A method for detecting neutralizing anti-aflibercept antibodies was also developed and validated.

Applicant's Proposed Labeling Statements

6 ADVERSE REACTIONS

6.2 Immunogenicity

(b) (4)

4 Page(s) of Draft Labeling has been Withheld in Full immediately following this page as B4 (CCI/TS)

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

RUBY LEONG
03/30/2012

HONG ZHAO
03/30/2012
I concur.