Office of Clinical Pharmacology Review

BLA Number	125387 S-075
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Submission Date	8/11/2022
PDUFA Goal Date	2/11/2023
Submission Type	Efficacy Supplement (Priority Review)
Brand Name	EYLEA
Generic Name	Aflibercept
Dosage Form and Strength	Iso-osmotic solution, 40 mg/mL
Route of Administration	Intravitreal (IVT) injection
Proposed Indication	For treatment of retinopathy of prematurity (ROP)
Applicant	Regeneron Pharmaceuticals, Inc.
Associated IND	(b) (4)
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1. EXECUTIVE SUMMARY

Aflibercept (EYLEA) is a vascular endothelial growth factor (VEGF) inhibitor. EYLEA was initially approved in the United States for the treatment of neovascular (wet) age-related macular degeneration (nAMD) in November 2011. Additional approved indications include treatment of macular edema following branch retinal vein occlusion (RVO), diabetic macular edema (DME) and diabetic retinopathy (DR).

In the current supplemental BLA, the Applicant is seeking an indication for the treatment of premature infants with retinopathy of prematurity (ROP). The proposed dosing regimen is 0.4 mg (b) (4) mL) administered by intravitreal injection. The treatment is to be initiated with a single injection per eligible eye and may be given bilaterally on the same day (b) (4) The treatment interval between doses injected into the same eye should be at least (b) (4)

The clinical development program to support the ROP indication includes two phase 3 clinical studies (VGFTe-ROP-1920, 20090/20275), in which the efficacy and safety of aflibercept was compared with standard laser treatment in pediatric patients with ROP. This Clinical Pharmacology review is focused on pharmacokinetics and immunogenicity data collected from Studies VGFTe-ROP-1920 and 20090/20275.

The key review findings with specific recommendations and comments are summarized below in Table 1.

Table 1. Summary of Clinical Pharmacology Review

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	Pivotal evidence of effectiveness of aflibercept in pediatric patients with ROP was evaluated in two phase 3 studies: VGFTe-ROP-1920 and 20090. Both Studies VGFTe-ROP-1920 and 20090 were randomized, two-arm, parallel group, open-label, controlled, multicenter studies of at least 52 weeks duration with the primary efficacy endpoint being the proportion of participants with absence of active ROP and unfavorable structural outcomes (e.g., retinal detachment) at week 52 following birth. Refer to the clinical/statistical review for the benefit/risk assessment of aflibercept in pediatric patients with ROP.
General dosing instructions	The proposed dose is 0.4 mg administered by intravitreal injection. The treatment is to be initiated with a single injection per eligible eye and may be given bilaterally on the same day. In total, up to 3 injections per eye may be administered from treatment initiation up to one year of chronological age, if there are signs of disease activity. The treatment interval between doses injected into the same eye should be at least (b) (4) This dosing regimen was evaluated in Studies VGFTe-ROP-1920 and 20090.

Dosing in patient subgroups (intrinsic and extrinsic factors)	No dose adjustments based on any intrinsic or extrinsic factors are recommended.
Labeling	Refer to Section 2.3.
Bridge between the to-be-marketed and clinical trial formulations	A bridging pharmacokinetic study between the to-be-marketed and clinical trial formulation is not warranted since the to-be-marketed formulation was used in the pivotal studies.

1.1 Recommendations

The Office of Clinical Pharmacology has reviewed clinical pharmacology data submitted for BLA 125387 S-075 and has determined that there are no approval issues from a clinical pharmacology perspective. Defer to clinical/statistical review for additional benefit/risk assessment.

1.2 Post-Marketing Requirements/Commitments

None

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Pharmacology and Clinical Pharmacokinetics

Mechanism of Action

Aflibercept acts as a soluble decoy receptor that binds VEGF-A and PlGF, and thereby can inhibit the binding and activation of these cognate VEGF receptors.

Pharmacokinetics

The systemic PK of aflibercept in pediatric patients with ROP who received IVT dose of 0.4 mg aflibercept per eye either unilaterally or bilaterally were assessed in Studies VGFTe-ROP-1920 and 20090/20275. In Study VGFTe-ROP-1920, the mean concentrations of free aflibercept in plasma declined from a maximum of 583 ng/mL at day 1 to 40.6 ng/mL at day 28 in bilaterally treated patients. In unilaterally-treated patients, the mean concentrations of free aflibercept were approximately 78% lower on day 1 and similar on day 28 when compared to bilaterally-treated patients. In Study 20090/20275, the mean concentrations of free aflibercept, for all patients who were either bilaterally or unilaterally treated, declined from a maximum of 481 ng/mL at day 1 to concentrations below or close to the lower limit of quantification (LLOQ; 15.6 ng/mL) within approximately 8 weeks.

In comparison to adult patients with wet AMD who received a 2 mg IVT dose in one eye, the mean concentrations of aflibercept in pediatric patients in Studies VGFTe-ROP-1920 and 20090/20275 were higher (Table 2).

Table 2. Comparisons of systemic exposure (Cmax in ng/mL) in premature infants and adults

	Premature Infants				Adults	Adults
Study	20090/20275		VGFTe-ROP-1920		VGFT-OD-0702	PDY6656
Dose	0.4 mg IVT/eye				2 mg IVT	1 mg/kg IV
	Bilaterally-treated	Unilaterally-treated	Bilaterally-treated	Unilaterally-treated	Unilaterally-treated	
N	69	6	81	10	6	12
Free aflibercept Median (range)	249 (0-4570)	181 (29-351)	258 (0-5760)	137 (36-837)	15.0 (0-54.0)	17600 (13000 - 24700)
Adj. Bound aflibercept Median (range)	1291 (0-5887)	810 (306-1090)	1047 (0-3370)	1133 (539-1513)	193 (100-286)	1190 (989 - 1540)

Concentrations below the LLOQ (0.0156 mg/L) are set to zero for mean calculation.
Source: Table 2, Module 2.5; reviewer's analysis from Tables 4 and 9 20090-BA-01V1; reviewer's analysis from adpcrs.xpt VGFTe-ROP-1920-CP-01V1; Tables 3.4.2.2 and 3.4.2.4 VGFT-OD-0702.PK; Tables 46 and 49 PDY6656

Immunogenicity

The immunogenicity potential following unilateral or bilateral dosing with aflibercept 0.4 mg IVT injection in pediatric patients with ROP is low (less than 1%).

2.2 Dosing and Therapeutic Individualization

2.2.1 General dosing

The proposed dosing regimen for aflibercept is 0.4 mg (0.01 mL of 40 mg/mL solution) administered by intravitreal injection. The treatment is to be initiated with a single injection per eligible eye and may be given bilaterally on the same day. In total, up to 3 injections per eye may be administered from treatment initiation up to one year of chronological age if there are signs of disease activity.

2.2.2 Therapeutic individualization

The Applicant has not proposed any therapeutic individualization. The available clinical pharmacology information does not warrant a need for therapeutic individualization.

2.2 Outstanding Issues

There are no outstanding issues from a clinical pharmacology perspective.

2.3 Summary of Labeling Recommendations

The applicable changes that are relevant to Clinical Pharmacology from this supplement include the addition of PD data, PK data and immunogenicity data obtained from preterm infants with ROP in sections (b) (4) 12.3 and 12.6, respectively. The labeling recommendations are shown below:

(b) (4)

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

EYLEA® (aflibercept) is currently approved for the treatment of neovascular (wet) AMD, macular edema following RVO, DME and DR. In this sBLA submission, the Applicant is seeking a new indication for ROP.

EYLEA is available in single-dose vials and pre-filled syringes. For the ROP indication, only the single-dose vial product is proposed.

The efficacy and safety of aflibercept in the treatment of ROP was evaluated in phase 3 studies, VGFTe-ROP-1920 (also referred to as BUTTERFLEYE) and 20090/20275 (also referred to as FIREFLEYE/FIREFLEYE NEXT). Both Studies VGFTe-ROP-1920 and 20090 were randomized, 2-arm (aflibercept 0.4 mg IVT per eye and laser treatment), controlled, open-label studies of at least 52-week chronological age of the patients. Study 20275 is an ongoing follow-up study to assess the long-term outcomes of patients who were treated in Study 20090. No study intervention is administered during Study 20275. The PK and immunogenicity data were collected in Studies VGFTe-ROP-1920 and 20090/20275. Exploratory PD data as measured with blood pressure was collected from Study 20090/20275.

3.2 General Pharmacology and Pharmacokinetic Characteristics

Table 3. Summary of Clinical Pharmacology and Pharmacokinetics

Mechanism of Action	Aflibercept acts as a soluble decoy receptor that binds VEGF-A and PlGF, and thereby can inhibit the binding and activation of these cognate VEGF receptors.
Active Moieties	Aflibercept

Bioanalysis	Analyses of free and bound aflibercept concentrations were performed using validated luminescence-based enzyme-linked immunosorbent assays (ELISAs).
Drug exposure at steady state following the therapeutic dosing regimen	Adult patients with wetAMD, RVO, and DME: following IVT 2 mg/eye, the mean C_{max} of free aflibercept in the plasma was 0.02 mcg/mL (range: 0 to 0.054 mcg/mL), 0.05 mcg/mL (range: 0 to 0.081 mcg/mL), and 0.03 mcg/mL (range: 0 to 0.076 mcg/mL), respectively and was attained in 1 to 3 days. The free aflibercept plasma concentrations were undetectable two weeks post-dosing in all patients. Pharmacokinetics of aflibercept were evaluated in pre-term infants with ROP at a dose of 0.4 mg aflibercept (per eye) administered unilaterally or bilaterally. In the VGFTe-ROP-1920 study, mean concentrations of free aflibercept in plasma declined from a maximum of 0.583 mcg/mL at Day 1 to 0.0406 mcg/mL at Day 28 in bilaterally treated patients. In the 20090/20275 study, mean concentrations of free aflibercept in plasma for all patients (bilateral and unilateral administration combined) declined from a maximum of 0.481 mcg/mL at Day 1 to 0.13 mcg/mL at Day 28. Concentrations of free aflibercept in plasma subsequently declined to values below or close to the lower limit of quantitation within approximately 8 weeks.
Accumulation	Adult patients with wetAMD, RVO, and DME: following IVT 2 mg/eye, the aflibercept did not accumulate in plasma when administered as repeated doses intravitreally every 4 weeks.
Distribution	Adult patients with wetAMD, RVO, and DME: The volume of distribution of free aflibercept following intravenous (I.V.) administration of aflibercept has been determined to be approximately 6L.
Metabolism/Elimination	Aflibercept is a therapeutic protein and no drug metabolism studies have been conducted. Aflibercept is expected to undergo elimination through both target-mediated disposition via binding to free endogenous VEGF and metabolism via proteolysis. The terminal elimination half-life (t1/2) of free aflibercept in plasma was approximately 5 to 6 days after I.V. administration of doses of 2 to 4 mg/kg aflibercept.
Specific Population	Renal Impairment: Pharmacokinetic analysis of a subgroup of patients (n=492) in one wet AMD study, of which 43% had renal impairment (mild n=120, moderate n=74, and severe n=16), revealed no differences with respect to plasma concentrations of free aflibercept after intravitreal administration every 4 or 8 weeks. Similar results were seen in patients in a RVO study and in patients in a DME study. No dose adjustment based on renal impairment status is needed for either wet AMD, RVO, or DME patients.
Immunogenicity	In the adult wet AMD, RVO, and DME studies, the pre-treatment incidence of immunoreactivity to EYLEA was approximately 1% to 3% across treatment groups. After dosing with EYLEA for 24-100 weeks, antibodies to EYLEA were detected in a similar percentage range of patients. There were no differences in efficacy or safety between patients with or without immunoreactivity. In the pediatric ROP studies, after unilateral or bilateral dosing with EYLEA 0.4 mg, antibodies to EYLEA were detected in less than 1% of patients for up to 12 weeks. Because of the low occurrence of ADA, the effect of these antibodies on the pharmacokinetics, safety, or effectiveness of aflibercept 0.4 mg per eye is unknown.

Impact of Immunogenicity on PK, efficacy, and Safety	Due to the low incidence of immunogenicity reported from this study, the effect of immunogenicity on the PK, safety or efficacy of aflibercept was not explored.
Pharmacodynamics	Increased blood pressure was evaluated as an exploratory PD marker of systemic anti-VEGF effects of aflibercept in the 20090/20275 study. No meaningful differences in BP were observed between the treatment groups.

3.3 Clinical Pharmacology Review Questions

3.3.1 Does the Clinical Pharmacology Program Provide Supportive Evidence of Effectiveness?

Aflibercept is administered via IVT route, and the site of action is the eye; therefore, the systematic exposure is not expected to impact treatment efficacy, and thus does not provide pivotal or supportive evidence for the effectiveness of IVT aflibercept.

3.3.2 Is the Proposed Dosing Regimen Appropriate for the General Patient Population for Which the Indication is Being Sought?

The proposed dosing regimen, aflibercept 0.4 mg (0.01 mL of 40 mg/mL solution) administered by intravitreal injection, which is to be initiated with a single injection per eligible eye and may be given bilaterally on the same day, for up to 3 injections per eye in total, for up to one year of chronological age, if there are signs of disease activity, was assessed in preterm infants with ROP in Study VGFTe-ROP-1920 and Study 20090/20275. In both studies, the primary efficacy endpoint was the proportion of participants with absence of both active ROP and unfavorable structural outcomes at 52 weeks chronological age based on the investigator's assessment. The primary analysis was a statistical evaluation of non-inferiority of aflibercept versus laser at week 52 of chronological age, with respect to the primary efficacy variable. Non-inferiority of aflibercept to laser could not be established at a significance level of 0.0245 (1-sided) for the primary endpoint for each study.

At the proposed IVT dosing regimen, the systemic exposure of free aflibercept in pediatric patients with ROP is higher than that in adults given 2 mg IVT but less than that in adults given 1 mg/kg IV (Table 2).

For additional benefit/risk assessment, defer to the clinical/statistical review for details.

3.3.5 Immunogenicity

In Study VGFTe-ROP-1920, immunogenicity samples were collected at baseline and Week 12 to determine the presence of anti-aflibercept antibodies in serum. No treatment-emergent/treatment-boosted ADA responses were observed through Week 12.

In Study 20090/20275, immunogenicity samples were collected at baseline and Week 12 to determine the presence of anti-aflibercept antibodies. Treatment-emergent ADA was reported

in one patient (1%) in the aflibercept group at Week 12, and the ADA titer was low (1:30). No neutralizing antibody response was observed in this patient.

Due to the low incidence of anti-drug antibody, the impact of ADA on the PK, safety or efficacy of aflibercept was not explored.

4. APPENDICES

4.1 Summary of Bioanalytical Method Validation and Performance

Analyses of free and bound aflibercept concentrations were performed using validated luminescence-based enzyme-linked immunosorbent assays (ELIZAs). The free aflibercept ELISA measures the systemic concentrations of aflibercept that are not in complex with VEGF. The bound aflibercept ELISA measures the systemic concentrations of aflibercept that are bound to VEGF forming a VEGF-aflibercept complex. The bound aflibercept complex consisted of 71.7% aflibercept and 28.3% human VEGF165 based on the molecular weight of each component. Therefore, the concentration of the bound aflibercept complex was multiplied by 0.717 to yield the concentration of adjusted bound aflibercept. Total aflibercept was calculated by summing the plasma concentrations of free and adjusted bound aflibercept.

The method validation of a bioanalytical method for the quantitative measurement of free aflibercept and bound aflibercept in human plasma is summarized in Table 4 and Table 5, respectively. Samples from Studies VGFTe-ROP-1920 and 20090 were analyzed using these methods.

Incurred sample reanalysis (ISR) was conducted for samples obtained from Study 20090. For free aflibercept analysis, 35 out of 37 samples passed the ISR with a passing rate of 94.6%. For bound aflibercept analysis, 34 out of 36 samples passed the ISR with a passing rate of 94.4%.

Table 4. Validation Summary of a Bioanalytical Method for the Quantitative Measurement of Free Aflibercept in Human Plasma

Study No. and Title	VGFT-AV-19095, Validation of a Bioanalytical Method for the Quantitative Measurement of Free VEGF Trap in Human Plasma (Lithium Heparin)
Analyte	VEGF Trap
Capture Reagent	Human VEGF165
Detection Reagent	Mouse anti-VEGF Trap mAb (REGN19) + Goat Anti-Mouse IgG Fcγ - HRP
Assay Range Neat human plasma (ULOQ - LLOQ) 10% human plasma (Assay MRD = 1:10)	 1000 – 15.6 ng/mL 100 – 1.56 ng/mL
Linearity %AR 80 - 120% (Standard 1 and 7: 75 - 125%)	 99 – 101%

CV% Conc ≤ 20% (Standard 1 and 7: ≤ 25%)	1%
Inter-Assay Accuracy and Precision %AR CV% Conc Total Error ≤ 30% (ULOQ and LLOQ ≤ 40%)	 94 – 98% 3 – 5% 5 – 10%
Intra-Assay Accuracy and Precision %AR CV% Conc Total Error ≤30% (ULOQ and LLOQ ≤ 40%)	 90 – 106% 0 – 5% 1 – 13%
Matrix Interference At least 80% of the naïve individual samples must be < LLOQ	100% (10 of 10 samples BLQ)
Selectivity at LLOQ (15.6 ng/mL) and HQC (700 ng/mL) %AR 75 - 125%, CV% Conc ≤ 25% for at least 80% of the LLOQ-spiked naïve samples %AR 80 - 120%, CV% Conc ≤ 20% for at least 80% of the HQC-spiked naïve samples	 83% – 105%, 0 – 2% (10 of 10 samples) 95% – 106%, 0 – 3% (10 of 10 samples)
Selectivity at LLOQ (15.6 ng/mL) and HQC (700 ng/mL) – Hemolyzed Samples %AR 75 - 125%, CV% Conc ≤ 25% for at least 80% of the LLOQ-spiked naïve samples %AR 80 - 120%, CV% Conc ≤ 20% for at least 80% of the HQC-spiked naïve samples	 77-86%, 1-5% (5 of 5 samples) 86-87%, 0-2% (5 of 5 samples)
Dilution Recovery (High Level Quality Controls) %dAR 80 - 120% CV% Conc ≤ 20% Final CV%Conc≤20%	 94 – 106% 0 – 3% 3 – 5 %
Robustness (Standards, Quality Controls) %AR 80 - 120% (Standard 1, 7, ULOQ and LLOQ 75 - 125%) CV% Conc ≤ 20% (Standard 1, 7, ULOQ and LLOQ: ≤ 25%)	 98 – 103% 0 – 3%
Target Interference %AR 80 - 120%, CV% Conc ≤ 20% (HQC without VEGF165) %AR 80 - 120%, CV% Conc ≤ 20% (MQC without VEGF165) Free VEGF165 must be BLQ	 110%, 2% 107%, 1% BLQ
Short-term Analyte Stability (4°C 24 hours, 6 hours Room Temperature, Freeze/Thaw - 8 Cycles) %AR 80 - 120%	 86 – 99%
Long-term Analyte Stability (-20°C Freezer) %AR 80–120%	At least 24 months 83–102%
Long-term Analyte Stability (-80°C Freezer) %AR 80–120%	At least 24 months 81–98%

Table 5. Validation Summary of a Bioanalytical Method for the Quantitative Measurement of Bound Aflibercept in Human Plasma

Study No. and Title	GFT-AV-19128, Validation of a Bioanalytical Method for the Quantitative Measurement of Bound VEGF Trap in Human Plasma (Lithium Heparin)
Analyte	VEGF165:VEGF Trap Complex (Bound VEGF Trap)
Capture Reagent	Goat anti-human $VEGF_{165}$ pAb
Detection Reagent	Mouse anti-VEGF Trap mAb (REGN19) + Goat Anti-Mouse IgG Fcγ - HRP
Assay Range Neat human plasma (ULOQ - LLOQ) 10% human plasma (Assay MRD = 1:10)	 2000 - 31.3 ng/mL 200 - 3.13 ng/mL
Linearity %AR 80 - 120% (Standard 1 and 7: 75 - 125%) CV% Conc ≤ 20% (Standard 1 and 7: ≤ 25%)	 99-101% 1-2%
Inter-Assay Accuracy and Precision %AR CV% Conc Total Error ≤ 30% (ULOQ and LLOQ ≤ 40%)	 96-108% 4-7% 6-15%
Intra-Assay Accuracy and Precision %AR CV% Conc Total Error ≤30% (ULOQ and LLOQ ≤ 40%)	 91-118% 1-6% 2-20%
Matrix Interference At least 80% of the naïve individual samples must be < LLOQ	 100% (10 of 10 samples BLQ)
Selectivity at LLOQ (31.3 ng/mL) and HQC (1500 ng/mL) %AR 75 - 125%, CV% Conc ≤ 25% for at least 80% of the LLOQ-spiked naïve samples %AR 80 - 120%, CV% Conc ≤ 20% for at least 80% of the HQC-spiked naïve samples	 100-114%, 0-6% (10 of 10 samples) 101-113%, 1-3% (10 of 10 samples)
Selectivity at LLOQ (31.3 ng/mL) and HQC (1500 ng/mL) – Hemolyzed Samples %AR 75 - 125%, CV% Conc ≤ 25% for at least 80% of the LLOQ-spiked naïve samples %AR 80 - 120%, CV% Conc ≤ 20% for at least 80% of the HQC-spiked naïve samples	 95-104%, 0-3% (5 of 5 samples) 88-93%, 1-2% (5 of 5 samples)
Dilution Recovery (High Level Quality Controls) %dAR 80 - 120% CV% Conc ≤ 20% Final CV%Conc≤20%	 95-109% 0-4% 1 – 4%

Robustness (Standards, Quality Controls) %AR 80 - 120% (Standard 1, 7, ULOQ and LLOQ 75 - 125%) CV% Conc ≤ 20% (Standard 1, 7, ULOQ and LLOQ: ≤ 25%)	 97-115% 0-3%
Short-term Analyte Stability (4°C 24 hours, 6 hours Room Temperature, Freeze/Thaw - 8 Cycles) %AR 80 - 120%	 91-103%
Long-term Analyte Stability (-20°C Freezer) %AR 80–120%	At least 24 months 81–113%
Long-term Analyte Stability (-80°C Freezer) %AR 80–120%	At least 24 months 85–114%

4.2 Summary of Key Study Designs of the Pivotal Clinical Studies

Study Identifier	Key Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects (All Randomized)	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status
VGFTe- ROP-1920	Primary objective: To assess the efficacy of aflibercept compared to laser in patients diagnosed with retinopathy of prematurity (ROP). Secondary objectives: To assess the need for a second treatment modality. To assess the recurrence of ROP in the study. To assess the safety and tolerability of aflibercept.	Phase 3, multicenter, randomized, 2-arm, open- label study.	Intravitreal (IVT) aflibercept injection (IAI) 0.4 mg/0.01 mL or laser photocoagulation.	Total N=127	Treatment-naïve participants with ROP at 52 weeks of chronological age. Participants must have been of ≤32 weeks gestational age at birth or birth weight ≤1500 g.	Treatment at baseline; retreatment/ rescue treatment based on prespecified treatment	Complete for final analysis.
			0.4 mg/0.01 mL IAI: single IVT injection of aflibercept 0.4 mg/0.01 mL per eligible eye at baseline. Up to 2 additional IVT injections of aflibercept 0.4 mg/0.01 mL could have been administered in each treatment requiring eye if prespecified retreatment criteria were met. Rescue treatment with laser was performed if prespecified conditions were met.	0.4 mg/0.01 mL IAI: 94			
			Laser Photocoagulation: Laser treatment in each eligible eye at baseline. Supplementary laser treatments were allowed during the study. Retreatment with laser was allowed if prespecified criteria were met. Rescue treatment with aflibercept 0.4 mg/0.01 mL was allowed if the laser	Laser Photo-coagulation: 33			

			treatment was judged complete by the investigator and prespecified criteria were met. Participants who initiated aflibercept rescue treatment could receive additional aflibercept injections according to the aflibercept group treatment regimen.				
20090/ 20275	Primary objective: Assess the efficacy of aflibercept in subjects diagnosed with ROP in comparison to laser through 52 weeks Secondary objectives: To assess the safety and tolerability of aflibercept. To assess the treatment burden of aflibercept and laser. To describe the systemic exposure to aflibercept.	Phase 3, multicenter, open-label, randomized, two-arm study (20090) and a phase 3b 5-year follow-up study with no treatment (only data through week 52 CA) (20275)	IAI 0.4 mg/0.01 mL or laser photocoagulation.	Total N=118	Treatment- naïve participants with ROP (gestational age at birth ≤32 weeks or birth weight ≤1500 g).	Treatment at baseline; re-treatment/ rescue treatment based on prespecified treatment	Complete for final analysis. Final CSR This CSR includes pooled data from Study 20090 and from Study 20275 through 52 weeks CA.
			Study 20090: 0.4 mg/0.01 mL IAI: single IVT injection of aflibercept 0.4 mg/0.01 mL per eligible eye at baseline. Up to 2 additional IVT injections of aflibercept 0.4 mg/0.01 mL could have been administered in each treatment requiring eye if prespecified retreatment criteria were met. Study 20275: No study treatment was administered Rescue treatment with laser was performed if prespecified conditions were met.	0.4 mg/0.01 mL IAI: 75			
			Study 20090: Laser Photocoagulation: Laser treatment in each eligible eye at baseline. If multiple sessions were necessary within 1 week from baseline, they were counted as single treatment. Study 20275: No study treatment was administered Retreatment with laser was allowed if prespecified criteria were met.	Laser Photo-coagulation: 43			

CA=chronological age; CSR=clinical study report; IAI=Intravitreal aflibercept injection; IVT=intravitreal; Regeneron=Regeneron Pharmaceuticals, Inc.; ROP=retinopathy of prematurity

4.3 Key Pharmacokinetics and Immunogenicity Data from the Pivotal Clinical Studies

4.3.1 Study VGFTe-ROP-1920

In Study VGFTe-ROP-1920, free, adjusted bound (0.717*bound aflibercept) and total aflibercept (sum of adjusted bound aflibercept and free aflibercept) concentrations in patients that received unilateral or bilateral injections were evaluated. PK samples were collected on Day 1 (approximately 24 hours after dosing), Day 14 and Day 28. The aflibercept concentrations in patients who received unilateral aflibercept injections at any time or bilateral aflibercept injections at anytime are presented in Table 6 and Table 7, respectively. Free and bound aflibercept concentrations were debatable for up to 28 days (last sample collection) following unilateral or bilateral aflibercept injections. The mean peak concentrations of free aflibercept were approximately 4.4-fold higher in patients who received bilateral injections (0.573 mg/L) than in patients who received unilateral aflibercept injections (0.130 mg/L). The mean peak concentrations of adjusted bound and total aflibercept were similar in patients who received bilateral injections or unilateral injections.

Table 6. Summary of Concentrations of Free, Adjusted Bound, and Total Aflibercept in Plasma by Nominal Time in Patients with ROP with Unilateral Injections from Study VGFTe-ROP-1920

	Concentrations of Aflibercept (mg/L)					
	Free		Adjusted Bound		Total	
Sampling Time Post First Dose (Day)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
1	7	0.130 (0.224)	7	0.0831 (0.0646)	7	0.213 (0.281)
14	7	0.105 (0.0576)	7	0.881 (0.330)	7	0.986 (0.386)
28	7	0.0409 (0.0254)	7	0.975 (0.387)	7	1.02 (0.411)

n = Number of patients contributing to each timepoint; SD = Standard deviation
Note: Adjusted Bound Aflibercept = 0.717*Bound Aflibercept. Total Aflibercept = sum of Adjusted Bound Aflibercept + Free Aflibercept.

Source: Table 4, VGFTe-ROP-1920-CP-01V1

Table 7. Summary of Concentrations of Free, Adjusted Bound, and Total Aflibercept in Plasma by Nominal Time in Patients with ROP with Bilateral Injections at Any Time from Study VGFTe-ROP-1920

Sampling Time Post First Dose (Day)	Concentrations of Aflibercept (mg/L)					
	Free		Adjusted Bound		Total	
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
1	81	0.573 (0.877)	81	0.150 (0.131)	81	0.723 (0.938)
14	77	0.169 (0.287)	77	1.06 (0.602)	77	1.23 (0.829)
28	75	0.0413 (0.0521)	76	1.03 (0.650)	75	1.07 (0.693)

n = Number of patients contributing to each timepoint; SD = Standard deviation
Note: Adjusted Bound Aflibercept = 0.717*Bound Aflibercept. Total Aflibercept = sum of Adjusted Bound Aflibercept + Free Aflibercept. Three patients who initially received treatment for one eye at baseline and then received treatment for the second eye (before the Day 28 sample collection) were included.

Source: Table 6, VGFTe-ROP-1920-CP-01V1

For immunogenicity assessment, samples were collected at baseline and Week 12 to determine the presence of anti-aflibercept antibodies in serum. No treatment-emergent or treatment-boosted ADA responses were observed in any participant who received unilateral or bilateral aflibercept injections as shown in Table 8 below.

Table 8. Summary of ADA Status, ADA Category, and Maximum Titer Category by Unilateral and Bilateral Injections in Patients with ROP from Study VGFTe-ROP-1920

ADA Status/Category Max. Titer Category	Aflibercept		Overall
	Unilateral n (%)	Bilateral n (%)	n (%)
ADA			
ADA Analysis Set	7 (100%)	75 (100%)	82 (100%)
Negative	7 (100%)	75 (100%)	82 (100%)
Pre-existing Immunoreactivity	0	0	0
Treatment-Boosted Response	0	0	0
Treatment-Emergent Response	0	0	0
TE & TB Maximum Titer Category			
Low (<1,000)	0	0	0
Moderate (1,000 to 10,000)	0	0	0
High (>10,000)	0	0	0

n = Number of patients; TE = Treatment-emergent; TB = Treatment-boosted
Note: Percentages are based on ADA analysis set. Three patients who initially received treatment for one eye at baseline and then received treatment for the second eye (before the Day 28 sample collection) were included in the bilateral group.

Source: Table 7, VGFTe-ROP-1920-CP-01V1

4.3.2 Study 20090/20275

In Study 20090/20275, PK samples were collected at 6 time points after dosing: at Day 1, Week 2, Week 4, Week 8, Week 12 and Week 24. The free and adjusted bound aflibercept concentrations in all patients with ROP are summarized in Table 9 and Table 10, respectively. The maximum plasma concentration of free aflibercept was observed at Day 1 and declined thereafter as shown in Figure 1. The concentrations of free aflibercept were almost all below LLOQ within approximately 8 weeks after dosing. The concentrations of adjusted bound aflibercept increased from Day 1 to Week 4 and decreased thereafter up to Week 24.

Table 9. Summary of Free Aflibercept Concentrations in All Patients with ROP from Study 20090

Time	N total	N ≥ LLOQ [a]	Arith. Mean ± SD (ng/mL)	Range (ng/mL)
Week 0/Day 1	75	66	481 ± 885	<LLOQ – 4570
Week 2	66	60	219 ± 359	<LLOQ – 2750
Week 4	68	54	133 ± 205	<LLOQ – 923
Week 8	3	1	N.C.	<LLOQ – 16.1
Week 12	7	1	N.C.	<LLOQ – 194
Week 24	14	0	N.C.	<LLOQ

LLOQ=15.6 ng/mL

[a]Values below LLOQ were substituted by 0 for arithmetic statistics.

Abbreviations: Arith.= arithmetic, LLOQ=lower limit of quantification, N=number of subjects, N.C.=not calculable, SD=standard deviation.

Source: Table 30, CSR BAY 86-5321/ 20090 & 20275 (52 Week Chronological Age)

Table 10. Summary of Adjusted Bound Aflibercept Concentrations in All Patients with ROP from Study 20090

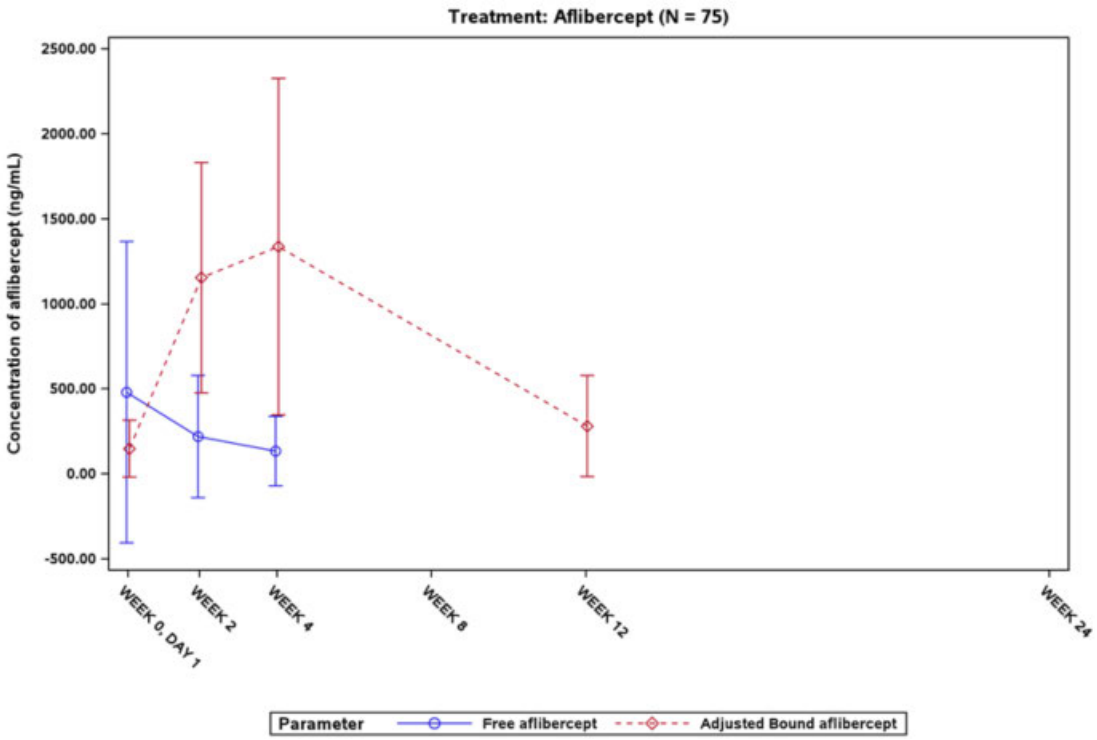
Time	N total	N ≥ LLOQ	Arith. Mean ± SD (ng/mL)	Range (ng/mL)
Week 0/Day 1	75	66	149 ± 166	<LLOQ – 968
Week 2	65	60	1154 ± 677	<LLOQ – 2646
Week 4	67	61	1336 ± 990	<LLOQ – 5887
Week 8	3	1	N.C.	<LLOQ – 1090
Week 12	7	5	281 ± 297	<LLOQ – 803
Week 24	14	9	N.C.	<LLOQ – 457

LLOQ = 22.4 ng/mL

Abbreviations: LLOQ=lower limit of quantification, N=number of subjects, N.C.=not calculable, SD=standard deviation

Source: Table 33, CSR BAY 86-5321/ 20090 & 20275 (52 Week Chronological Age)

Figure 1. Mean Concentrations (±SD) of Free and Adjusted Bound Aflibercept in Plasma from Study 20090



Values below LLOQ were substituted by 0.
Source: Figure 3, CSR BAY 86-5321/ 20090 & 20275

For immunogenicity assessment, immunogenicity samples were collected at baseline prior to aflibercept dosing and at 12 weeks after dosing from a total of 75 subjects. Samples with a confirmed positive response in the ADA assay were then analysed for neutralizing anti-aflibercept antibodies. Treatment-emergent ADA was reported in one patient (1%) in the aflibercept group at Week 12. The ADA titer was low (1:30) and no neutralizing antibody response was observed in this patient.

4.3.3 Pharmacodynamics

In Study 20090, BP was evaluated as an exploratory PD marker of systemic anti-VEGF effects of aflibercept as increases in both SBP and DBP have been observed with administration of drugs that inhibit or interfere with the BEGF signaling pathway[1]. BP measurements were taken from screening up to week 24. The SBP measurements at each visit and the change from baseline for the laser treatment group and aflibercept treatment group are summarized in Table 11 and Table 12, respectively. The DBP measurements at each visit and the change from baseline for the laser treatment group and aflibercept treatment group are summarized in Table 13 and Table 14, respectively. The mean increase in SBP over 24 weeks was 13.1 mmHg and 10.2

[1] Robinson ES, et al. Hypertension Induced by VEGF Signaling Pathway Inhibition: Mechanisms and Potential Use as a Biomarker. Semin Nephrol. 2010 Nov; 30(6): 591–601.

mmHg for the laser treatment group and aflibercept treatment group, respectively. The mean increase in DBP over 24 weeks was 7.9 mmHg for both treatment groups. No meaningful differences in the change of BP were observed between the treatment groups.

The SBP was positively correlated to gestational age (

Figure 2) and body weight (

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Figure 3), suggesting that the observed increases in BP could be due to increased body weight and organ maturation in premature infants.

Table 11. Summary and Change from Baseline in Systolic Blood Pressure (mmHg) from the Laser Treatment Group

Laser treatment group		Value at Visit		Change from Baseline	
Visit	n	Mean	SD	Mean	SD
BASELINE	38	75.4	12.00	N/A	N/A
WEEK 0, DAY 1	37	77.5	11.23	2.1	13.94
WEEK 1	37	79.4	12.79	3.4	12.90
WEEK 2	37	77.6	14.09	2.2	18.48
WEEK 3	37	82.2	15.52	6.3	16.92
WEEK 4	37	82.7	14.42	7.4	15.87
WEEK 6	34	80.4	11.61	3.8	15.67
WEEK 8	30	83.6	12.75	8.8	14.84
WEEK 10	30	85.4	11.38	10.1	14.99
WEEK 12	30	81.1	12.56	5.3	19.55
WEEK 16	34	84.1	14.28	8.4	17.69
WEEK 20	30	81.7	14.78	5.9	17.48

WEEK 24	36	88.8	15.06	13.1	17.67

Source: adapted from Table 14.3.5.1, CSR BAY 86-5321/ 20090 & 20275

Table 12. Summary and Change from Baseline in Systolic Blood Pressure (mmHg) from the Aflibercept Treatment Group

Aflibercept treatment group		Value at Visit		Change from Baseline	
Visit	n	Mean	SD	Mean	SD
BASELINE	74	76.5	12.54	N/A	N/A
WEEK 0, DAY 1	73	77.8	12.55	1.0	11.44
WEEK 1	71	79.4	13.17	3.3	17.51
WEEK 2	71	80.8	12.50	4.8	14.88
WEEK 3	71	79.9	12.75	3.6	16.76
WEEK 4	70	81.3	13.39	5.0	14.60
WEEK 6	70	82.8	12.05	6.3	15.62
WEEK 8	65	82.2	13.15	6.2	16.51
WEEK 10	65	82.5	13.37	7.2	16.81
WEEK 12	69	85.3	13.30	8.3	16.43
WEEK 16	68	84.2	13.18	7.8	17.98
WEEK 20	62	85.5	10.95	9.8	14.54
WEEK 24	66	86.8	13.04	10.2	14.66

Source: adapted from Table 14.3.5.1, CSR BAY 86-5321/ 20090 & 20275

Table 13. Summary and Change from Baseline in Diastolic Blood Pressure (mmHg) from the Laser Treatment Group

Laser treatment group		Value at Visit		Change from Baseline	
Visit	n	Mean	SD	Mean	SD
BASELINE	38	44.9	10.85	N/A	N/A
WEEK 0, DAY 1	37	43.1	8.03	-1.2	11.10
WEEK 1	37	43.4	9.45	-1.9	10.34
WEEK 2	37	43.8	8.57	-0.5	13.56
WEEK 3	37	45.9	11.56	0.6	11.47
WEEK 4	37	46.1	12.55	1.8	15.55
WEEK 6	34	44.9	9.01	-1.2	11.12
WEEK 8	30	46.0	8.86	2.1	12.99
WEEK 10	30	49.2	10.54	3.3	14.68
WEEK 12	29	47.2	8.02	1.3	14.46
WEEK 16	34	50.4	11.04	4.7	14.85
WEEK 20	30	48.6	12.65	3.8	16.00
WEEK 24	36	52.5	14.03	7.9	16.64

Source: adapted from Table 14.3.5.2, CSR BAY 86-5321/ 20090 & 20275

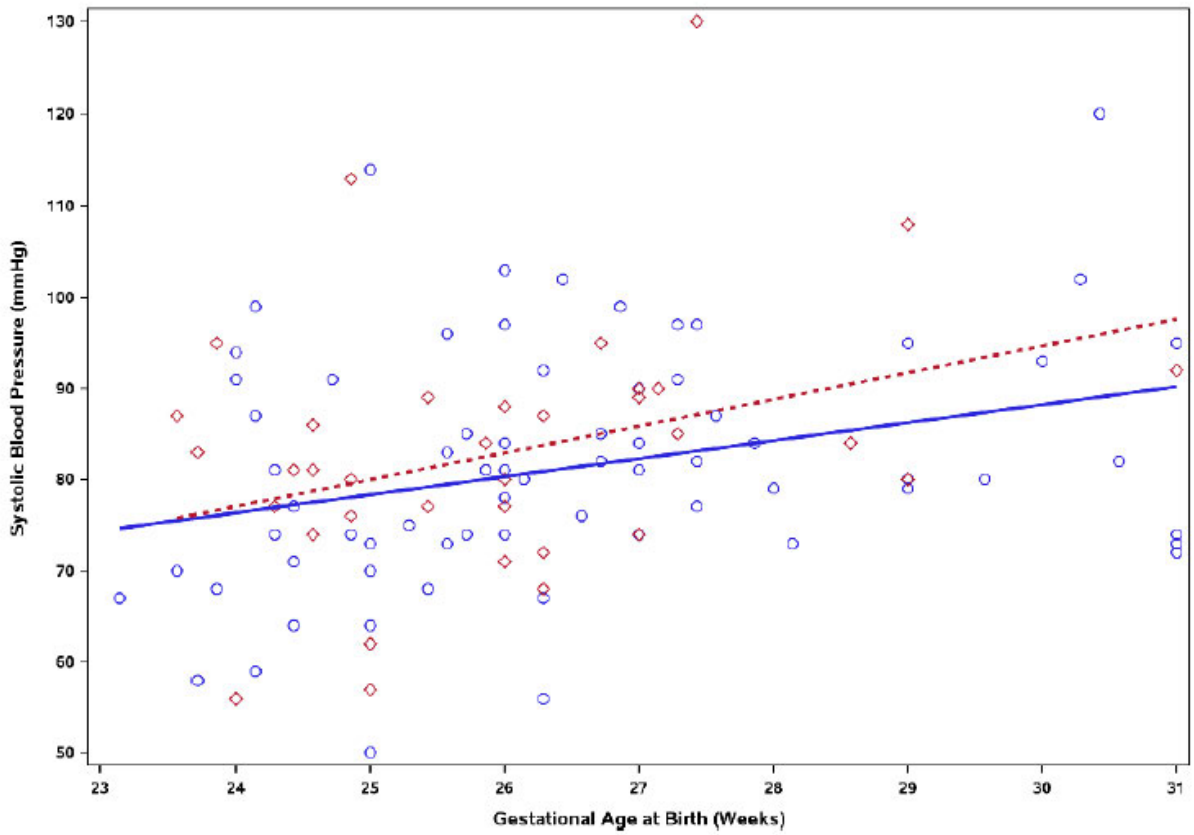
Table 14. Summary and Change from Baseline in Diastolic Blood Pressure (mmHg) from the Aflibercept Treatment Group

Aflibercept treatment group		Value at Visit		Change from Baseline	
Visit	n	Mean	SD	Mean	SD

BASELINE	44.1	9.55	N/A	N/A	44.1
WEEK 0, DAY 1	43.9	9.85	-0.3	11.28	43.9
WEEK 1	45.9	10.65	2.2	14.15	45.9
WEEK 2	45.8	12.39	2.2	13.83	45.8
WEEK 3	45.3	9.45	1.3	14.19	45.3
WEEK 4	47.1	10.87	3.3	13.53	47.1
WEEK 6	45.9	10.01	2.1	14.65	45.9
WEEK 8	49.3	11.68	5.8	14.97	49.3
WEEK 10	49.1	10.86	6.1	13.70	49.1
WEEK 12	49.7	11.66	5.5	15.27	49.7
WEEK 16	51.0	11.10	7.0	14.85	51.0
WEEK 20	51.1	9.39	8.0	12.22	51.1
WEEK 24	51.5	11.04	7.9	14.04	51.5

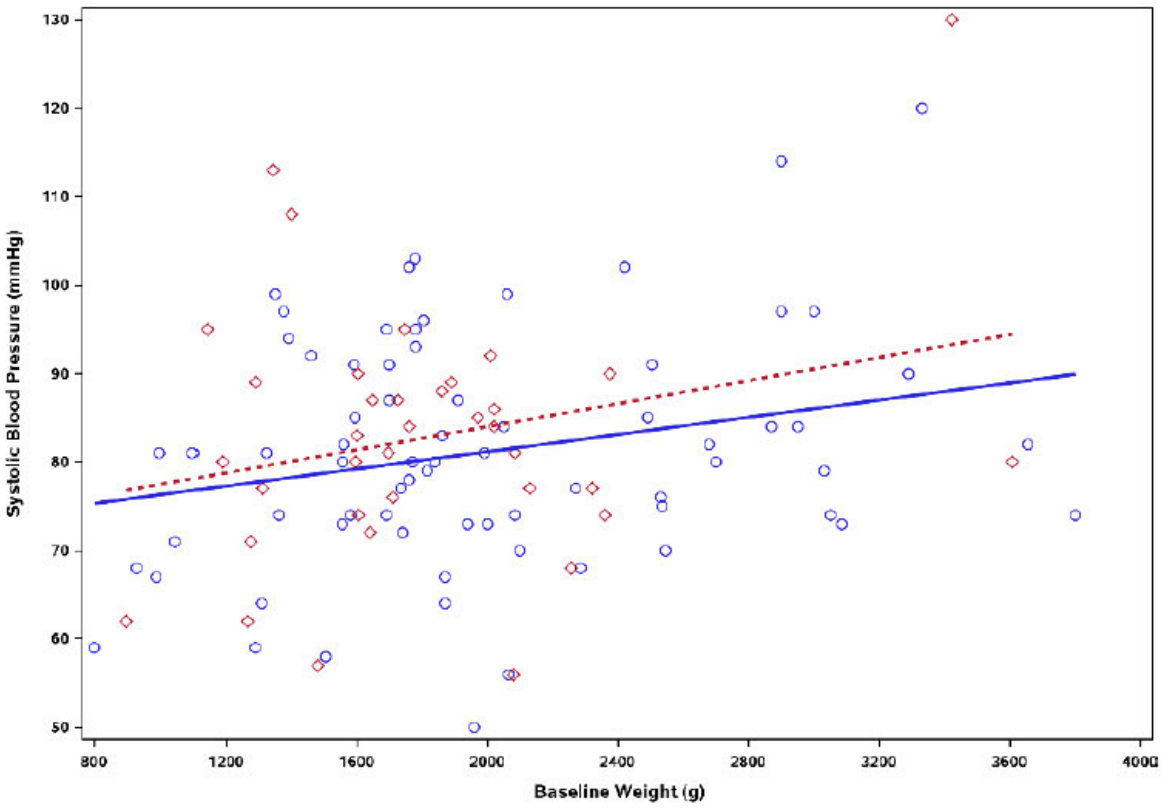
Source: adapted from Table 14.3.5.2, CSR BAY 86-5321/ 20090 & 20275

Figure 2. Scatterplot for Gestational Age (weeks) versus Systolic Blood Pressure (mmHg) Measured at Week 4 Following First Treatment, by Treatment Group



Squares: individual observations laser group, circles: individual observations aflibercept group
Dotted line: regression line for laser; straight line: regression line aflibercept
Source: Figure 5, CSR BAY 86-5321/ 20090 & 20275

Figure 3. Scatterplot for Systolic Blood Pressure (mmHg) Measured at Week 4 and Baseline Body Weight, by Treatment Group



Squares: individual observations laser group, circles: individual observations aflibercept group
Dotted line: regression line for laser; straight line: regression line aflibercept
Source: Figure 6, CSR BAY 86-5321/ 20090 & 20275

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