CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761355Orig1s000

NON-CLINICAL REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	761355
Supporting document/s:	1
Applicant's letter date:	12-27-2022
CDER stamp date:	12-27-2022
Product:	Eylea HD (aflibercept 8 mg)
Indication:	Neovascular (Wet) age-related macular degeneration (nAMD) Diabetic macular edema (DME) Diabetic retinopathy (DR)
Applicant:	Regeneron Pharmaceuticals, Inc
Clinical Division:	Division of Ophthalmology
Pharm/Tox Division:	Division of Pharm/Tox for Rare Diseases, Pediatrics, Urologic and Reproductive Medicine/ Specialty Medicine (DPT-RPURM/SM)
Reviewer:	María I Rivera, PhD
Pharm/Tox Supervisor/Team Leader:	Mukesh Summan, PhD acting for Lori E. Kotch
Pharm/Tox Division Director:	Mukesh Summan, PhD
Project Manager:	Michael J Puglisi

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 761355 are owned by Regeneron or are data for which Regeneron has obtained a written right of reference. Any information or data necessary for approval of BLA 761355 that Regeneron does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of BLA 761355.

TABLE OF CONTENTS

1 EXECUTIVE SUMMARY....5

1.1 INTRODUCTION....5
1.2 BRIEF DISCUSSION OF NONCLINICAL FINDINGS....5
1.3 RECOMMENDATIONS....6

2 DRUG INFORMATION....7

2.1 DRUG....7
2.2 RELEVANT INDS, NDAS, BLAS AND DMFS....7
2.3 DRUG FORMULATION....7
2.4 COMMENTS ON NOVEL EXCIPIENTS....8
2.5 COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN....8
2.6 PROPOSED CLINICAL POPULATION AND DOSING REGIMEN....9
2.7 REGULATORY BACKGROUND....9

3 STUDIES SUBMITTED....9

3.1 STUDIES REVIEWED....9
3.2 STUDIES NOT REVIEWED....10
3.3 PREVIOUS REVIEWS REFERENCED....10

4 PHARMACOLOGY....10

4.1 PRIMARY PHARMACOLOGY....10
4.3 SAFETY PHARMACOLOGY....12

5 PHARMACOKINETICS/ADME/TOXICOKINETICS....12

6 GENERAL TOXICOLOGY....12

6.2 REPEAT-DOSE TOXICITY....12

7 GENETIC TOXICOLOGY....30

8 CARCINOGENICITY....30

9 REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY....30

11 INTEGRATED SUMMARY AND SAFETY EVALUATION....30

Table of Tables

Table 1: Nominal Composition of Formulation.....8
Table 2: Formulations Used in Primary Pharmacology Study 10
Table 3: Aflibercept Formulations Evaluated in 6-Month IVT Toxicity Study in Monkeys 13
Table 4: Study Design - 6-Month IVT Toxicity Study in Monkeys.....15
Table 5: Spleen Weight Changes Observed with Formulation 2 - 6-Month IVT Toxicity Study in Monkeys 19
Table 6: Incidence and Severity of Microscopic Findings in the Nasal Cavities at Terminal Sacrifice – 6-Month IVT Toxicity Study in Monkeys.....21
Table 7: Incidence and Severity of Microscopic Findings in the Nasal Cavities at Recovery Sacrifice – 6-Month IVT Toxicity Study in Monkeys 22
Table 8: Mean TK Parameters of Free and Bound VEGF-Trap in Plasma for Aflibercept Formulation 2 – 6-Month IVT Toxicity Study in Monkeys 23
Table 9: Free VEGF Trap in Vitreous Humor - 3-Month IVT Toxicity Study in Monkeys28
Table 10: Mean Aflibercept TK parameters – 3-Month IVT Toxicity Study in Monkeys .29
Table 11: Free Aflibercept Exposure Multiples Achieved in Monkeys and Rabbits Relative to Human Exposure.....32
Table 12: Exposure Data and Safety Margins or Exposure Ratios for Free Aflibercept after Unilateral IVT Treatment with 8 mg Aflibercept/eye Regarding Safety Pharmacology Endpoints from Nonclinical Studies with IVT or Systemic Dosing of Aflibercept.....33

Table of Figures

Figure 1: Aflibercept Resolves RNV Leak After a Single IVT Dose.....11
Figure 2: Duration of RNV Leak Suppression is Related to the Aflibercept Dose 12

1 Executive Summary

1.1 Introduction

EYLEA® (aflibercept; 40 mg/mL) is an FDA approved drug product for intravitreal (IVT) use in the treatment of various retinal diseases including nAMD, DME, and DR. Aflibercept is a recombinant protein that is composed of two domains of the human VEGF cell surface receptors (VEGFR-1 and VEGFR-2) fused to the Fc region of human IgG. This recombinant molecule binds with high affinity to VEGF-A (K_D=0.36-0.76 pM) along with the related Placental Growth Factor (PlGF; K_D=29-392 pM). For most indications, the dosing recommendation is 2 mg (0.05 mL) every 4 weeks initially followed by 2 mg every 8 weeks.

Under the current marketing application, Regeneron (also the owner of EYLEA) and Bayer are codeveloping a novel drug product able to deliver a higher mg dose of aflibercept (8 mg; 114.3 mg/mL; EYLEA HD; HD aflibercept) by IVT administration. Although HD aflibercept and the approved 2 mg aflibercept share the same drug substance, HD aflibercept is a distinct product with differences in excipients/formulation and molar concentration that result in a different dosing schedule/frequency and volume of administration.

HD aflibercept is being developed for the treatment of nAMD, DME, and DR, targeting longer durability with a subsequent reduction in the number of injections per year. The recommended dose for EYLEA HD is 8 mg (0.07 mL) administered by IVT injection every 4 weeks for the first three doses, followed by 8 mg (0.07 mL) via IVT injection once every 8 to 16 weeks.

1.2 Brief Discussion of Nonclinical Findings

The nonclinical development program was designed to evaluate the in vivo pharmacology and toxicology profiles of high dose aflibercept formulation. One new in vivo pharmacology study in rabbits and 2 new repeat-dose IVT toxicity studies in monkeys with monthly administration for 3 months (total of 4 injections) and 6 months (total of 7 injections) were conducted.

The pharmacology study conducted in a chronic retinal neovascularization (RNV) model in rabbits support the hypothesis that increasing the IVT dose of aflibercept in humans will result in a longer durability of effect. Eyes that received a single IVT dose of 2 mg/eye (corresponding to a human dose of approximately 8 mg/eye based on a vitreous volume of 1.17 mL in rabbits and 4.0 mL in humans) remained complete leak-free for a longer period compared to eyes that received 500 µg/eye (corresponding to a human dose of approximately 2 mg/eye).

The 3-month IVT toxicity study evaluated the marketing formulation at a concentration of 140 mg/mL (enriched with high molecular weight species). The 6-month IVT toxicity study evaluated several aflibercept formulations at concentrations of 80 or 140 mg/mL, with Formulation 2 having a similar composition as the marketing formulation except for a higher concentration of histamine ([redacted] (b) (4) in the marketing formulation). The formulation concentrations were 2 to 3.5X higher than the approved 40 mg/mL for EYLEA®.

The nonclinical safety profile of the high dose aflibercept formulations were generally comparable to the previously established safety profile of EYLEA®. Across studies with the aflibercept high dose formulations, there were no adverse ocular effects at any dose level. Ocular findings were limited to generally mild, transient ocular inflammation (low scores of aqueous cell or flare [0.5 to 1+] and/or vitreous cell [0.5 to 1+]) consistent with findings previously reported with the EYLEA formulation in the same species. The ocular NOAEL was the high dose 7 mg/eye. When normalized for vitreal volume (2 mL in monkeys and 4 mL in humans), the ocular NOAEL is 1.8-fold higher than the intended human dose of 8 mg.

In the 6-month ocular toxicity study, the only extraocular findings observed were microscopic changes in the respiratory epithelium of the nasal turbinates. The findings included erosion and/or ulceration (minimal to marked), squamous metaplasia (minimal to moderate), and hemorrhage (minimal to slight). The findings were observed at both doses evaluated, 4 and 7 mg/eye. There was no NOAEL. Most findings were reversible or showed partial reversibility during the 12-week recovery period.

The nasal turbinate findings were noted at doses ≥ 2 mg/eye of EYLEA®, with a NOAEL of 0.5 mg/eye. These findings are acknowledged in the EYLEA® label. The Applicant is using the same language as that accepted for EYLEA® with corrected exposure margin. At the NOAEL of 0.5 mg/eye in monkeys, the systemic exposure (AUC) for free aflibercept was approximately 3 times higher than the population pharmacokinetic estimated exposure observed in humans after an IVT dose of 8 mg.

Per the Applicant, in the PULSAR (nAMD Phase 3), CANDELA (nAMD Phase 2), and PHOTON (DME Phase 2/3) studies, an analysis of treatment emergent adverse events of nasomucosal findings was conducted with no indication of a safety signal in the 8 mg aflibercept groups compared to 2 mg.

1.3 Recommendations

1.3.1 Approvability

Approval is recommended.

1.3.2 Additional Nonclinical Recommendations

None

1.3.3 Labeling

Pharmacology/Toxicology team has no edit recommendations to the proposed nonclinical sections of the label. The proposed label includes the same language as that accepted for EYLEA® with correction for exposure margins based on the population estimated AUC in humans after administration of a unilateral IVT dose of 8 mg. The exposure margins corrections are considered adequate.

2 Drug Information

2.1 Drug

Refer to nonclinical review of BLA 125387 (Rivera; available upon request to the Document Room or at Drugs@FDA).

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 012462
BLA 125387

2.3 Drug Formulation

Aflibercept drug product (DP) is a sterile solution for intravitreal injection in an aqueous buffered solution, pH 5.8, containing 114.3 mg/mL aflibercept, (b) (4) (b) (4) arginine (b) (4) hydrochloride, (b) (4) histidine, (b) (4) % (w/v) sucrose, and (b) (4) % (w/v) polysorbate 20. The DP is filled into a (b) (4) glass vial with an (b) (4) stopper, and sealed with an aluminum seal cap. Each vial has a 0.07 mL withdrawable volume providing a dose of 8 mg. The nominal composition of aflibercept is summarized in Table 1.

Table 1: Nominal Composition of Formulation

Component	Function	Quality Standard	Concentration	Content per Vial (mg)[a]	Content per 0.07 mL Withdrawable Volume (mg)
Aflibercept	Active ingredient	Regeneron specification	114.3 mg/mL	(b) (4)	8.00
(b) (4) arginine (b) (4) hydrochloride	(b) (4)	USP, Ph. Eur., JP	(b) (4)		0.737
(b) (4) histidine		USP, Ph. Eur., JP	(b) (4)		0.040
L-histidine (b) (4) hydrochloride monohydrate		Ph. Eur., JP	(b) (4)		0.093
Sucrose		NF, Ph. Eur., JP	(b) (4) % (w/v)		3.50
Polysorbate 20		NF, Ph. Eur., JPE	(b) (4) % (w/v)		0.021
Water for Injection		USP, Ph. Eur., JP	QS		QS

(mg)[a] Calculations are based on a target fill volume of (b) (4) mL/vial.
JP, Japanese Pharmacopeia; JPE, Japanese Pharmaceutical Excipients; NF, National Formulary; Ph. Eur., European Pharmacopeia; QS, quantity sufficient; USP, United States Pharmacopeia; w/v, weight by volume

Source: BLA Module 3.2.P.1 Table 1, page 2

The EYLEA HD formulation composition is not the same as that in EYLEA® (aflibercept 40 mg/mL), which is formulated in an aqueous buffered solution at pH 6.2, containing (b) (4) sodium phosphate, (b) (4) sodium chloride, (b) (4)% (w/v) sucrose, and (b) (4)% (w/v) polysorbate 20 (PS20).

2.4 Comments on Novel Excipients

(b) (4) arginine and (b) (4) histidine are not listed in the FDA Inactive Ingredient Database for ocular use; they are listed for systemic use. However, (b) (4) histidine is found in approved ocular drugs (e.g., Lucentis). Both excipients were qualified in the formulations evaluated in the 3-month and 6-month ocular toxicity studies conducted to support this marketing application.

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

- Neovascular (Wet) Age-Related Macular Degeneration (nAMD)

  The recommended dose for EYLEA HD is 8 mg (0.07 mL) administered by IVT injection every 4 weeks for the first three doses, followed by 8 mg (0.07 mL) via IVT injection once every 8 – 16 weeks.

- Diabetic Macular Edema (DME)

  The recommended dose for EYLEA HD is 8 mg (0.07 mL) administered by IVT injection every 4 weeks for the first three doses, followed by 8 mg (0.07 mL) via IVT injection once every 8 – 16 weeks.

- Diabetic Retinopathy (DR)

  The recommended dose for EYLEA HD is 8 mg (0.07 mL) administered by IVT injection every 4 weeks for the first three doses, followed by 8 mg (0.07 mL) via IVT injection once every 8 – (b) (4) weeks.

2.7 Regulatory Background

- Pre-IND 141278 – Submitted 12-20-2018 – Nonclinical review (filed in DARRTS on 1-15-2019; Rivera) - Sponsor meeting held on 1-22-2019 (minutes filed in DARRTS on 2-8-2019; Puglisi)
  - The Agency agreed that no additional toxicology studies were required to support the proposed clinical development program with high dose aflibercept (with up to 8 mg of aflibercept dosed every 8 weeks after a maximum of three initial monthly doses administered chronically) with the currently approved formulation.
  - The Agency also recommended additional nonclinical data to support the safety if a new formulation is used.

- Type-B-Pre-BLA Sponsor Meeting – 9-26-2022
  - The Agency agreed it was acceptable to cross-reference applicable sections of BLA 125387 (EYLEA).

3 Studies Submitted

3.1 Studies Reviewed

- The Duration of Inhibition of Pathological Retinal Vascular Leak by Intravitreal Aflibercept is Dose-Dependent (Study No. VGFT-PH-19050-SR-01V1)
- A 3-Month Intravitreal Toxicology and Toxicokinetic Study of VEGF-Trap, Administered to Cynomolgus Monkeys Followed by a 12-week Recovery Period (Study NO. VGFT-TX-20170)

- 9-Week or 6-Month Intravitreal Toxicology and Toxicokinetic Study with Three Different Aflibercept (VEGF-Trap) Formulations in Cynomolgus Monkeys with a 12-Week Recovery Phase (Study No. GFT-TX-18169)

3.2 Studies Not Reviewed

Module 4.2.2 Analytical Methods and Validation Reports

3.3 Previous Reviews Referenced

BLA 125387 (Rivera; available upon request to the Document Room or at Drugs@FDA).

4 Pharmacology

4.1 Primary Pharmacology

The Duration of Inhibition of Pathological Retinal Vascular Leak by Intravitreal Aflibercept is Dose-Dependent (Study No. VGFT-PH-19050-SR-01V1) – A chronic retinal neovascularization (RNV) model induced by IVT injection of DL-α-aminoadipic acid (AAA) was developed and characterized in rabbits to investigate the extent and duration of antiangiogenic activity of IVT aflibercept. The formulations used have some differences from the marketing formulation, i.e., (b) (4)% sucrose instead of (b) (4)% and lack of (b) (4) arginine (Table 2).

Table 2: Formulations Used in Primary Pharmacology Study

Group	Aflibercept Conc. (mg/mL)	Lot Number	Excipients
Control	0	S18-001124	(b) (4) Histidine, pH 5.8, (b) (4)%(w/v) Sucrose, (b) (4) %(w/v) Polysorbate20
500µg	10	L18-001126	(b) (4) Histidine, pH 5.8, (b) (4)%(w/v) Sucrose, (b) (4) %(w/v) Polysorbate20
2mg	40	L18-001125	

Source: Table 1 Study Report, page 7

Male New Zealand White rabbits received single doses of 500 µg/eye or 2 mg/eye aflibercept, which the Sponsor calculated they correspond to human doses of approximately 2 mg/eye and 8 mg/eye, respectively, based on vitreous volume of 1.17 mL in rabbits and 4 mL in humans (Study Report refers to publication by Struble et al[1]). Ophthalmic evaluations of each eye, conducted prior to treatment and periodically after

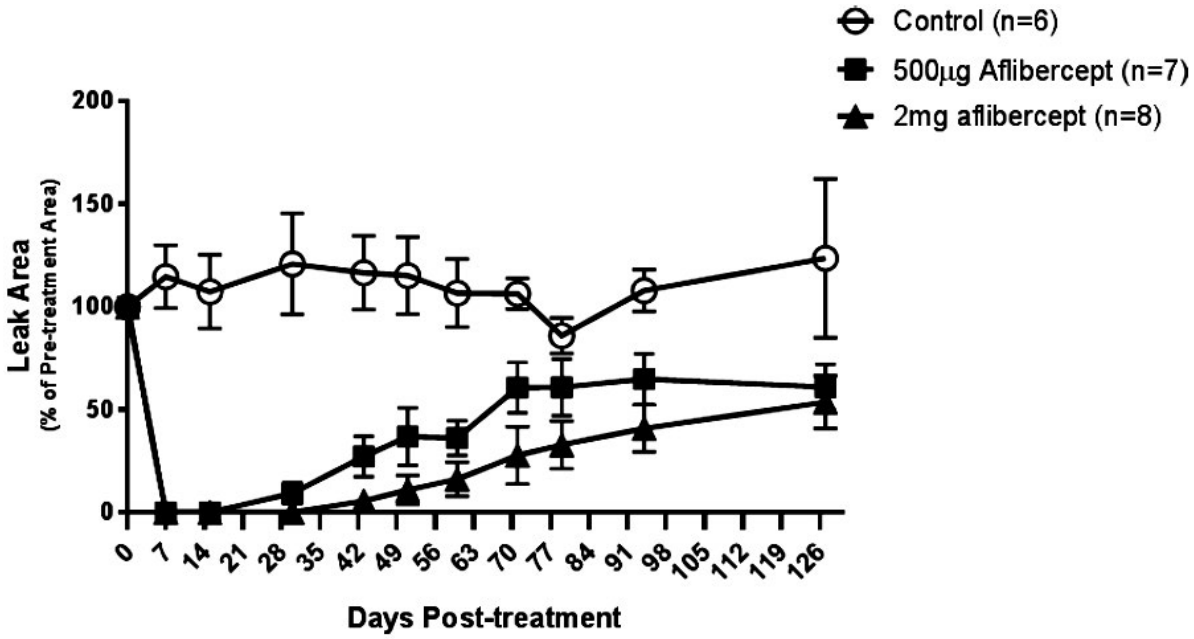
[1] Struble C, Howard S, Relph J. Comparison of ocular tissue weights (volumes) and tissue collection techniques in preclinical animal species. Poster presented at: European Association for Vision and Eye Research; October 1, 2014; Nice, France.

treatment, consisted of photographs of the eye for assessment of gross inflammation, IOP, serum and plasma collection at specific timepoints, ophthalmoscopic fundus examination (red free imaging, fluorescein angiography and optical coherence tomography). IOP and plasma PK data were not included in the Study Report.

Key findings:

- Following a single aflibercept dose of either 500 µg or 2 mg, retinal vascular leak was completely blocked at 1-week postdose (Figure 1). The leak was completely suppressed in all experimental eyes for 4 or 6 weeks after the 500 µg or 2 mg dose, respectively.
- The number of eyes in which leak was completely suppressed was significantly greater at the 2 mg dose at all sampling timepoints during the 18-week post-dosing study period (Figure 2).
- These results support the hypothesis that increasing the IVT dose of aflibercept in human patients will result in a longer durability of effect, which could allow a reduction in the number of injections per year.

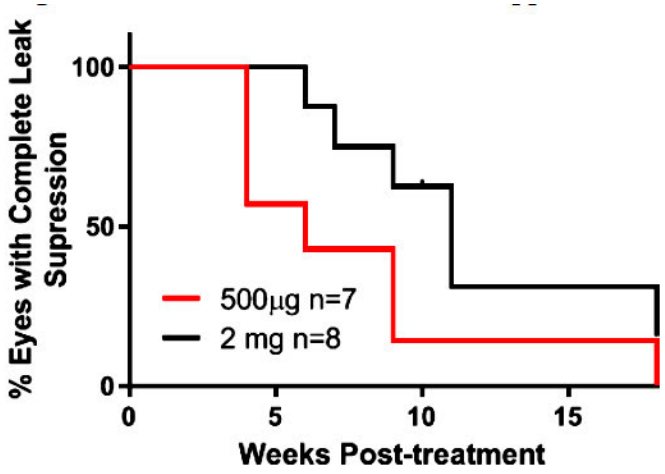
Figure 1: Aflibercept Resolves RNV Leak After a Single IVT Dose



Mean retinal vascular leak area in rabbit eyes over time, as a percentage of pre-treatment leak, following single-dose IVT administration of 500µg or 2mg aflibercept, or formulation vehicle control (n =number of eyes). All timepoints representing treated animals were significantly different from control ($p<0.05$) with the exception of the 11-week (Day 77) timepoint in the 500µg group; treated groups were not significantly different from one another.

Source: Figure 2 Study Report, page 10

Figure 2: Duration of RNV Leak Suppression is Related to the Aflibercept Dose



Time course of complete leak suppression after IVT administration of 500µg or 2mg aflibercept (n=number of eyes). The percentage of eyes with complete leak suppression is significantly greater in the 2mg aflibercept dose group ($p< 0.05$).

Source: Figure 3 Study Report, page 11

4.3 Safety Pharmacology

Safety pharmacology endpoints were incorporated in the 6-month ocular toxicity study in monkeys. No aflibercept-related effects were observed in vital signs (heart rate, body temperature, oximetry, and cageside respiration rates), blood pressure, electrocardiography, and neurological examinations after bilateral IVT doses of 4 mg/eye or 7 mg/eye. See Study VGFT-TX-18169 under Section 6 General Toxicology of this review for further details.

5 Pharmacokinetics/ADME/Toxicokinetics

No new studies were conducted with the high dose aflibercept formulation.

6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: 9-Week or 6-Month Intravitreal Toxicology and Toxicokinetic Study with Three Different Aflibercept (VEGF-Trap) Formulations in Cynomolgus Monkeys with a 12-Week Recovery Phase

Study no.: VGFT-TX-18169
Study report location: Module 4.2.3.2
\\CDSESUB1\EVSPROD\bla761355\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\vgft-tx-18169\vgft-tx-18169.pdf

Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	November 14, 2018
GLP compliance:	Yes There are some exceptions that are not considered to affect the interpretation or validity of the study.
QA statement:	Yes
Drug, lot #, and % purity:	VEGF-Trap 80 mg/mL or 140 mg/mL See Table 3 below. Overall, the % purity ranged from 98.1% to 99.1%. Note: Formulation 2 has all excipients and at similar concentrations as in the intended marketing formulation except for higher concentration of histidine ([redacted] (b) (4) in the marketing formulation).

Table 3: Aflibercept Formulations Evaluated in 6-Month IVT Toxicity Study in Monkeys

Test Article[a,b]	Storage	Lot Number	Stability	Concentration
Test Article (TA) 1: Aflibercept in [redacted] (b) (4) Histidine, pH 5.8, [redacted] (b) (4) % Sucrose, [redacted] (b) (4) % Polysorbate 20	In a refrigerator (2 to 8°C); protected from light	9040300001 9040000002	c c	80 mg/mL 140 mg/mL
Test Article (TA) 2: Aflibercept in [redacted] (b) (4) Histidine, pH 5.8, [redacted] (b) (4) Arginine, [redacted] (b) (4) % Sucrose, [redacted] (b) (4) % Polysorbate 20	In a refrigerator (2 to 8°C); protected from light	9040400001 9040100002 9040400002 9040100003	c c c c	80 mg/mL 140 mg/mL 80 mg/mL 140 mg/mL
Test Article (TA) 3: Aflibercept in [redacted] (b) (4) Histidine, pH 5.8, [redacted] (b) (4), [redacted] (b) (4) % Sucrose, [redacted] (b) (4) % Polysorbate 20	In a refrigerator (2 to 8°C); protected from light	9040500001 9040200002	c c	80 mg/mL 140 mg/mL

a Provided preformulated by the Sponsor in vials containing approximately 2.5 mL at concentrations listed (see Protocol Deviations).
b Also known as VGEF-Trap.
c Stability and purity of test article, at concentrations provided and over the duration of use (for specific lots), is demonstrated by end-of-study testing completed by the Sponsor and documented in the appendices of this report (see Certificates of Analysis).

Key Study Findings

- Monthly IVT administrations of control article or aflibercept Formulations 1, 2, and 3 at doses of 4 or 7 mg/eye were well tolerated. Formulation 2 has the same composition of the intended marketing formulation except for a higher concentration of histidine ([redacted] (b) (4), respectively).

- Findings were limited to generally mild aqueous cells (0.5+ to 1+) or aqueous flare (0.5+) and mild (0.5 to 1+) vitreous cells. The aqueous cells and/or flare were dose dependent. These findings were also observed in animals administered the control vehicles indicating there was a contribution by the vehicle or injection procedure.
- The anterior segment inflammatory response was transient (generally resolved within a week). Vitreous cells persisted through the last examination of the 12-week recovery period but generally was of 0.5+ (rarely 1+) severity.
- No striking differences in ocular findings were noted between the 3 aflibercept formulations.
- Microscopic observations in all 3 aflibercept formulations included minimal to marked erosion and/or ulceration, minimal to moderate squamous metaplasia, and minimal to slight hemorrhage of the respiratory epithelium in the nasal turbinates. A trend toward reversibility was noted for most findings at the end of the 12-week recovery period.
- The ocular NOAEL is the high dose, 7 mg/eye.
- Based on the nasal turbinates, there was no systemic NOAEL. At the low dose (4 mg/eye), the mean plasma C_{max} values for Formulation 2 ranged from 11.4 to 15.9 µg/mL for free aflibercept and 6.89 to 11.3 µg/mL for VEGF-bound aflibercept at all timepoints evaluated, respectively.

Methods

Doses:	See Table 4 below.
Frequency of dosing:	Animals in Groups 1, 3, 4, 5, 8, and 9 were dosed on Days 1, 29, and 57. Animals in Groups 2, 6, and 7 were dosed on Days 1, 29, 57, 85, 113, 141 (Cohorts 1, 2, and 4), 142 (Cohort 3), and 169.
Route of administration:	IVT (both eyes)
Dose volume:	50 µL
Formulation/Vehicle:	• (b) (4) Histidine, (b) (4)% Sucrose, (b) (4)% Polysorbate 20, pH 5.8 • (b) (4) Histidine, (b) (4) Arginine, (b) (4)% Sucrose, (b) (4)% Polysorbate 20, pH 5.8 • (b) (4) Histidine, (b) (4) (b) (4)% Sucrose, (b) (4)% Polysorbate 20, pH 5.8
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	5
Age:	26 to 38 months old
Weight:	2.0 to 2.9 kg for males and 2.1 to 3.0 kg for females
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None with an impact in the study validity or interpretation of the data

Table 4: Study Design - 6-Month IVT Toxicity Study in Monkeys

	No. of Animals[a]		Dosing Regimen		Dose Level (mg/eye)[b]		Dose Concentration (mg/mL)	
Group	Male	Female	Left Eye	Right Eye	Left Eye	Right Eye	Left Eye	Right Eye
1 (VCA 1)	5	5	Vehicle 1	Vehicle 1	0	0	0	0
2 (VCA 2)	5	5	Vehicle 2	Vehicle 2	0	0	0	0
3 (VCA 3)	5	5	Vehicle 3	Vehicle 3	0	0	0	0
4 (TA 1 Low)	5	5	Test Article 1	Test Article 1	4	4	80	80
5 (TA 1 High)	5	5	Test Article 1	Test Article 1	7	7	140	140
6 (TA 2 Low)	5	5	Test Article 2	Test Article 2	4	4	80	80
7 (TA 2 High)	5	5	Test Article 2	Test Article 2	7	7	140	140
8 (TA 3 Low)	5	5	Test Article 3	Test Article 3	4	4	80	80
9 (TA 3 High)	5	5	Test Article 3	Test Article 3	7	7	140	140

TA = Test article; VCA = Vehicle control article.

Note: Animals were divided into four cohorts as follows. Each cohort was designated as a subgroup in the data collection system (Pristima).

Cohort 1: All males in Groups 1, 3, 4, 5, 8, and 9 designated for recovery sacrifice (Day 85 of the recovery phase; Day 1 of the recovery phase started on Day 64 of the dosing phase) and all males in Groups 2, 6, and 7 designated for recovery sacrifice (Day 86 of the recovery phase; Day 1 of the recovery phase started on Day 176 of the dosing phase)

Cohort 2: All females in Groups 1, 3, 4, 5, 8, and 9 designated for recovery sacrifice (Day 85 of the recovery phase; Day 1 of the recovery phase started on Day 64 of the dosing phase) and all females in Groups 2, 6, and 7 designated for recovery sacrifice (Day 85 of the recovery phase; Day 1 of the recovery phase started on Day 176 of the dosing phase)

Cohort 3: All males in Groups 1, 3, 4, 5, 8, and 9 designated for terminal sacrifice (Day 64 of the dosing phase) and all males in Groups 2, 6, and 7 designated for terminal sacrifice (Day 176 of the dosing phase)

Cohort 4: All females in Groups 1, 3, 4, 5, 8, and 9 designated for terminal sacrifice (Day 64 of the dosing phase) and all females in Groups 2, 6, and 7 designated for terminal sacrifice (Day 176 of the dosing phase)

a Animals in Groups 1, 3, 4, 5, 8, and 9 designated for terminal sacrifice (three animals/sex/group) were sacrificed on Day 64 of the dosing phase. Animals in Groups 2, 6, and 7 designated for terminal sacrifice (three animals/sex/group) were sacrificed on Day 176 of the dosing phase. Animals in Groups 1, 3, 4, 5, 8, and 9 designated for recovery sacrifice (two animals/sex/group) underwent at least 12 weeks of recovery and were sacrificed on Day 85 of the recovery phase. Animals in Groups 2, 6, and 7 designated for recovery sacrifice (two animals/sex/group) underwent at least a 12-week recovery phase and were sacrificed on Day 85 (females; Cohort 2) or 86 (males; Cohort 1) of the recovery phase.

b Dose levels were based on a dose volume of 50 µL/eye/dose.

Observations and Results

Mortality (2X/day)

None

Clinical Signs (Cageside observations: predose and daily; Detailed observations: predose, Day 1, weekly, and on day of sacrifice)

Ocular-related clinical observations included squinting (Formulation 3 only) and pupil dilation. Pupil dilation was observed in both controls and all 3 test article formulations and was considered procedure related (related to the use a mydriatic for ophthalmic examinations). Squinting eyes was noted on Day 36 for one animal administered 4 mg/eye and one animal administered 7 mg/eye of Formulation 3. The low frequency of the finding suggests it was not test article related.

Body Weights (Predose and weekly during the dosing and recovery phase)

No test article related effects in body weight or body weight gain

Feed Consumption (Predose and daily during dosing and recovery phases; qualitative as part of clinical observations)

No test article related effects

Vital signs (Heart rate, body temperature, pulse oximetry, and cageside respiration rates once during the predose phase, once during Weeks 9 [all groups] and 25 [Groups 2, 6, and 7 only] of the dosing phase, and once during Week 12 of the recovery phase)

No test article-related effects

Blood Pressure (Predose, once on Days 2, 7, 15, 29 [prior to dosing, as applicable], 30, 35, 43, 56, 64 [prior to necropsy], and 176 [Groups 2, 6, and 7; prior to necropsy] of the dosing phase; and on Day 85 [prior to necropsy] of the recovery phase)

No test article-related effects

Electrocardiogram Examinations (Once during the predose phase, on Day 58 [Groups 1, 3, 4, 5, 8, and 9 only] or Day 170 [Groups 2, 6, and 7 only] of the dosing phase, and during Week 12 of the recovery phase)

No test article-related effects

Neurological examination (Predose phase, once during Weeks 9 [all groups] and 25 [Groups 2, 6, and 7 only] of the dosing phase, and once during Week 12 of the recovery phase)

The head and eyes were examined for unusual orientation and movements; reflexes were tested (including menace, pupillary, palpebral, limb flexor, and patellar), and muscle tone of unrestrained limbs was evaluated. No test article related effects were noted.

Ophthalmoscopy (Multiple assessments from Days 3 to 176 of the dosing phase and Days 8 to 85/86 of the recovery phase; indirect ophthalmoscopy and slit-lamp biomicroscopy)

In control groups, anterior segment inflammation manifested by mild (0.5+ to 1+) aqueous cell was observed in eyes administered Vehicle Control Article 3 (11 instances) or Vehicle Control Article 1 (one instance) or Vehicle Control Article 2 (one instance).

Intravitreous administration of 4 or 7 mg/eye of aflibercept Formulations 1, 2 or 3 were well tolerated but associated with a transient dose-related anterior segment inflammatory response generally mild in nature (aqueous cell 0.5+ to 1+) with some occurrences of moderate (2+) at the high dose for all 3 formulations (and one instance of 3+ in Formulation 3 high dose). Mild (0.5+) aqueous flare (visible protein) was observed one eye administered 4 mg/eye/dose of VEGF-Trap Formulation 2 and in one or more eyes administered 7 mg/eye/dose of VEGF-Trap Formulation 1, 2, or 3.

The anterior segment inflammatory response generally resolved by the next examination (with one exception in an eye administered 4 mg/eye/dose of either aflibercept Formulation 2 or aflibercept Formulation 3 where aqueous cell resolved by Day 22 of the dosing phase) and was less frequent after the second (and subsequent doses) than after the first dose.

Posterior segment findings were limited to mild (0.5 to 1+) vitreous cell except for a single instance of moderate (2+) vitreous cell in an eye administered 7 mg/eye/dose of Aflibercept Formulation 1 or Aflibercept Formulation 2. These findings were also observed in animals administered the control vehicles indicating there was a contribution by the vehicle or injection procedure.

Mild (usually 0.5+ but rarely 1+) vitreous cell persisted through the last examination of the recovery phase (Days 85/86) in all groups except for vehicle Group 2 in which vitreous cell was last observed on Day 78 of the recovery phase.

IOP (In conjunction with ophthalmic examinations)

No test article-related effects

Full-field ERG (Predose phase, once during Weeks 9 [all groups] and 25 [Groups 2, 6, and 7] of the dosing phase, and once during Weeks 6 and 12 of the recovery phase)

No test article related effects

Ocular Photography and FA (Predose phase, once during Weeks 9 [all groups] and 25 [Groups 2, 6, and 7] of the dosing phase, and once during Weeks 6 and 12 of the recovery phase)

No test article related effects

Hematology and Coagulation (Predose, on Days 64/65 [all groups] and 176 [Groups 2, 6, and 7] of the dosing phase, and on Day 85 of the recovery phase)

Minimal but statistically significant increases in mean red blood cell mass parameters (RBC, hemoglobin and/or hematocrit %) were observed on Day 64/65 compared to concurrent controls values in females administered 4 and/or 7 mg/eye Formulation 1. The values were comparable to baseline values, and therefore, the

changes appear unrelated to the test article. Hematocrit % was also minimally but statistically significantly increased in males administered 7 mg/eye Formulation 2. The value was within baseline range across groups and again, appears unrelated to the test article.

Clinical Chemistry (Predose, on Days 64 [all groups] and 176 [Groups 2, 6, and 7] of the dosing phase, and on Day 85 of the recovery phase)

No test article-related effects

Urinalysis (Predose, on Days 64 [all groups] and 176 [Groups 2, 6, and 7] of the dosing phase, and on Day 85 of the recovery phase)

No test article-related effects

Gross Pathology (Day 64 [3 animals/sex in Groups 1, 3, 4, 5, 8, and 9] and Day 176 [3 animals/sex in Groups 2, 6, and 7] of the dosing phase; Day 85/86 of the recovery phase)

No test article-related effects

Organ Weights (adrenals, brain, cervix, epididymis, heart, kidney, liver, ovaries, pituitary gland, prostate, seminal vesicles, spleen, testes, thymus, thyroid/parathyroid, uterus)

At terminal sacrifice on Day 176, a statistically significant decrease in absolute and relative (organ:body weight and organ:brain weight ratios) spleen weights were observed in females administered Formulation 2 (Table 5). No difference was noted at end of the 12-week recovery period. The decrease was not dose dependent. The tissue was not evaluated microscopically. A similar but not statistically significant decrease was observed at both doses of Formulation 3 (non-dose dependent) at the end of the 12-week recovery period. The spleen was not identified as a target at higher plasma concentrations in the IV toxicology studies conducted for Eylea in monkeys. Therefore, the spleen weight changes appear related to biological variability and not related to the test article.

Table 5: Spleen Weight Changes Observed with Formulation 2 - 6-Month IVT Toxicity Study in Monkeys

Group/ Subgroup/ Sex		Terminal Body Weight (g)	Spleen Unadjusted (g)	Spleen Body Weight (%)	Spleen Brain Weight (%)
2/3,4 /F	Mean	2766.7	3.156	0.1142	4.6212
	SD	152.75	0.2271	0.00754	0.71342
	N	3	3	3	3
	P(overall)	0.0463	0.0012	0.0035	0.0081
6/3,4 /F	Mean	2400.0	1.706	0.0709	2.5769
	SD	100.00	0.3178	0.01105	0.38502
	N	3	3	3	3
	P(v2)	0.0301*	0.0008*	0.0025*	0.0061*
7/3,4 /F	Mean	2566.7	2.118	0.0827	3.0594
	SD	152.75	0.2151	0.00944	0.45111
	N	3	3	3	3
	P(v2)	0.2073	0.0047*	0.0116*	0.0208*
	Statistics	A	A	A	A

* P<=0.05
A = ANOVA and Dunnett's

Source: Excerpt Study Report, Anatomic Pathology Report Table 5.2

At the recovery sacrifices, decreased uterus/cervix weight was noted in females at 4 or 7 mg/eye Formulation 3 (~40-50%) and at 7 mg/eye Formulation 1 (~14-20%). No effect was observed for Formulation 2 (more comparable to the marketing formulation). The decrease was not statistically significant and had no correlating microscopic observations. Per a statement in the Study Report, no differences in sexual maturity were observed among groups. Given the lack of a microscopic correlate, the finding, if test article related, is not considered adverse. The proposed label contains adequate warning for potential reproductive toxicity, as was done for the approval of Eylea.

Histopathology (Eyes, eyelids, optic nerve, extraorbital muscles [superior rectus], lacrimal glands, nasal turbinates, gross lesions, and selected systemic tissues)

Adequate Battery – Yes, main targets (bones, kidney, adrenals, ovary, uterus, and nasal cavity) and other target tissues (heart, brain, duodenum, and colon) for EYLEA® were evaluated.

Peer Review: Yes

No test article-related findings were observed in the ocular tissues. Minimal or slight mononuclear and/or mixed cell infiltrates were observed in the conjunctiva, ciliary body, eyelids, lacrimal glands, extraorbital muscles, and nasal turbinates in controls and/or aflibercept formulations. The finding was still present in most ocular tissues at the end of recovery.

Microscopic observations in all 3 aflibercept formulations included minimal to marked erosion and/or ulceration, minimal to mild (one incidence of moderate) squamous metaplasia, and minimal to slight hemorrhage of the respiratory epithelium in the nasal turbinates (Table 6).

At the terminal sacrifices, the incidence and severity of the observations in animals administered Formulation 1 or 3 were similar. The incidence and, often the severity, was greater for Formulation 2 than with the other formulations. However, this reviewer agrees with the conclusion in the Study Report that the increase may be related to longer treatment duration for Formulation 2. No dose-response relationship was evident for any formulation.

Table 6: Incidence and Severity of Microscopic Findings in the Nasal Cavities at Terminal Sacrifice – 6-Month IVT Toxicity Study in Monkeys

Sex	Male			Female		
Dose Level (mg Aflibercept/eye)	0	4	7	0	4	7
Number examined	3	3	3	3	3	3
	Aflibercept Formulation 1					
Group Number	1	4	5	1	4	5
Nasal Turbinates						
Erosion, respiratory epithelium						
Minimal	1	0	0	0	0	1
Slight	0	1	1	0	0	0
Moderate	0	1	0	0	1	0
Ulcer, respiratory epithelium						
Minimal	0	1	0	0	0	0
Moderate	0	0	0	0	1	0
Metaplasia, squamous cell						
Minimal	0	0	1	0	0	0
Slight	0	1	0	0	0	0
Hemorrhage						
Minimal	0	1	1	0	0	0
	Aflibercept Formulation 2					
Group Number	2	6	7	2	6	7
Nasal Turbinates						
Erosion, respiratory epithelium						
Minimal	0	0	0	0	1	1
Slight	0	1	2	0	0	0
Moderate	0	1	0	0	1	0
Marked	0	0	1	0	0	1
Ulcer, respiratory epithelium						
Slight	0	0	1	0	0	0
Moderate	0	0	0	0	1	0
Marked	0	0	1	0	0	1
Metaplasia, squamous cell						
Minimal	0	0	1	0	1	0
Slight	0	3	1	0	0	1
Moderate	0	0	1	0	0	0
Hemorrhage						
Minimal	0	1	2	0	1	0
	Aflibercept Formulation 3					
Group	3	8	9	3	8	9
Nasal Turbinates						
Erosion, respiratory epithelium						
Minimal	0	1	2	0	1	1
Slight	0	1	0	0	0	0
Moderate	0	0	0	0	1	1
Ulcer, respiratory epithelium						
Minimal	0	0	1	0	0	0
Moderate	0	0	0	0	1	1
Metaplasia, squamous cell						
Minimal	0	1	1	0	1	2
Slight	0	1	0	0	0	0
Hemorrhage						
Slight	0	0	0	0	0	1

At the recovery sacrifices (Table 7), erosion and/or squamous metaplasia persisted in males administered Formulations 1, 2, or 3, and in females administered Formulation 2. The overall lower incidence of most of these findings (compared to the terminal sacrifices) and the absence of ulceration at the recovery sacrifices was

indicative of a trend toward reversibility. Squamous cell metaplasia was still moderate for Formulation 2, indicating no recovery.

Table 7: Incidence and Severity of Microscopic Findings in the Nasal Cavities at Recovery Sacrifice – 6-Month IVT Toxicity Study in Monkeys

Sex	Male			Female		
Dose Level (mg/eye)	0	4	7	0	4	7
Number examined	2	2	2	2	2	2
	Aflibercept Formulation 1					
Group Number	1	4	5	1	4	5
Nasal Turbinates						
Erosion, respiratory epithelium						
Moderate	0	1	0	0	0	0
Metaplasia, squamous cell						
Slight	0	1	0	0	0	0
	Aflibercept Formulation 2					
Group Number	2	6	7	2	6	7
Nasal Turbinates						
Erosion, respiratory epithelium						
Minimal	0	0	2	0	0	0
Slight	0	1	0	0	0	1
Metaplasia, squamous cell						
Moderate	0	1	0	0	0	1
	Aflibercept Formulation 3					
Group	3	8	9	3	8	9
Nasal Turbinates						
Erosion, respiratory epithelium						
Minimal	0	1	0	0	0	0

A publication (Chamanza et al.[2]) was provided in the Study Report demonstrating that squamous metaplasia in the nasal cavity (severity grades of minimal to moderate) is a common background finding for cynomolgus monkeys. Because the finding was not present in any control monkeys in the current study, it was considered test article related but not adverse.

Toxicokinetics (Days 1, 29, 57, and 169 of the dosing phase and Days 36, 57, 78, and 85 of the recovery phase; predose and up to 504 hours postdose)

Per summary information in the study report, based on totality of the data, no marked difference in free and bound VEGF-Trap concentrations or kinetics was observed between the 3 formulations tested.

The TK report focused on Formulation 2 as it is the formulation evaluated in the clinical trials.

Free VEGF-Trap C_{max} and AUC values increased in a dose-proportional manner. Exposure to free VEGF-Trap in plasma was observed for at least 3 weeks in densely

[2] Chamanza R, Taylor I, Gregori1 M, et al (2016) Normal anatomy, histology, and spontaneous pathology of the nasal cavity of the cynomolgus monkey (Macaca fascicularis). Toxicol Pathol; 44(5):636-654

sampled intervals for 90% (18/20) of animals; by the end of the 4-week interval, animals had minimal or undetectable concentrations.

Continuous exposure to bound VEGF-Trap in plasma was observed in all but 2 animals during the 6-month treatment period and the 12-week recovery period. Exposure was comparable across the 4 and 7 mg/eye doses, likely indicating saturation of bound VEGF-Trap in circulation at both dose levels.

No drug accumulation was observed for free or bound VEGF-Trap. No significant gender differences were observed.

The mean TK parameters are shown in the table below.

Table 8: Mean TK Parameters of Free and Bound VEGF-Trap in Plasma for Aflibercept Formulation 2 – 6-Month IVT Toxicity Study in Monkeys

Parameter	Unit	Dose	Free VEGF-Trap						Bound VEGF-Trap					
			4 mg/eye Formulation 2			7 mg/eye Formulation 2			4 mg/eye Formulation 2			7 mg/eye Formulation 2		
			N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
C_{max}	µg/mL	1	10	15.9	3.07	10	26.3	6.48	10	6.89	0.849	10	8.29	1.75
		2	10	12.3	2.94	10	20.7	5.40	10	10.2	1.72	10	10.9	2.87
		3	10	12.8	1.84	10	22.1	6.07	10	10.9	2.16	8	11.9	2.15
		7	10	11.4	3.43	10	19.5	5.39	10	11.3	2.58	8	11.4	2.40
C_{max}/Dose	(µg/mL)/mg	1	10	1.98	0.384	10	1.88	0.463	10	0.861	0.106	10	0.592	0.125
		2	10	1.54	0.367	10	1.48	0.386	10	1.27	0.215	10	0.778	0.205
		3	10	1.60	0.230	10	1.58	0.434	10	1.36	0.270	8	0.853	0.154
		7	10	1.43	0.429	10	1.40	0.385	10	1.41	0.323	8	0.812	0.171
AUC_{tau}[a]	day•(µg/mL)	1	10	164	31.4	10	282	86.6	10	140	14.3	10	152	16.2
		2	10	136	38.3	10	238	86.4	10	248	44.9	8	290	58.3
		7	4	143	24.7	4	286	65.0	4	300	28.6	4	312	93.6
AUC_{rec}		7	4	70.6	18.6	4	167	57.2	4	420	59.0	4	554	235
AUC_{tau}/Dose	(day•µg/mL)/mg	1	10	20.5	3.92	10	20.2	6.19	10	17.5	1.79	10	10.9	1.16
		2	10	17.0	4.79	10	17.0	6.17	10	31.0	5.61	8	20.7	4.16
		7	4	17.9	3.08	4	20.4	4.64	4	37.5	3.58	4	22.3	6.68
AUC_{rec}/Dose		7	4	8.83	2.32	4	11.9	4.09	4	52.5	7.38	4	39.6	16.8
C_{trough}	µg/mL	1	10	BLQ	NC	10	0.295	0.335	10	6.18	1.23	10	7.37	2.43
		2	10	BLQ	NC	10	0.316	0.353	10	8.16	1.99	8	9.93	2.82
		6	10	BLQ	NC	10	0.240	0.308	10	9.11	2.48	8	9.71	2.91

* Concentration values considered to be ADA impacted or an outlier were excluded from data analysis (Table 24).
[a] When dose 7 was the last dose, the dosing interval extended into the recovery period from 7 days post dose. Since there was no TK sampling at 28 days post dose, the reported AUC_{tau} was an extrapolated AUC_{0-28}.
ADA, Anti-drug antibody; AUC, Area under the concentration-time curve; AUC_{rec}, AUC calculated during the recovery period (from 7 days after last dose until recovery sacrifice); AUC_{0-28}, AUC computed from time zero to 28 days post dose; AUC_{tau}, AUC calculated during the dosing interval; BLQ, Below the limit of quantitation (<0.157 µg/mL for free VEGF-Trap and < 0.0391 µg/mL for bound VEGF-Trap); C_{max}, Peak concentration; C_{trough}, Last concentration in a dosing interval; N, Number of animals; NC, Not calculated; SD, Standard deviation
Note: Animals received bilateral intravitreal dosing.

Source: Study Report, Toxicokinetic Report, Table 1, page 941

Anti-Drug Antibody Sample (Multiple timepoints from Day 1 to Day 260)

In the 4 and 7 mg/eye Formulation 2 groups, postdose positive ADA responses determined by immunoassay were observed in 1/10 (Day 15) and 2/10 animals (Day 57 and Day 64), respectively. The 2 animals receiving 7 mg/eye dose demonstrated an

apparent ADA impact on free and bound VEGF-Trap concentrations (impacted concentrations from these animals were excluded from mean concentrations or TK analysis).

In Formulation 1, ADA response was observed in 1/10 and 4/10 animals at 4 and 7 mg/eye, respectively. In Formulation 3, ADA response was observed in 2/10 and 1/10 animals at 4 and 7 mg/eye, respectively.

Dosing Solution Analysis

All dose formulations were provided by the Sponsor as ready-to-use formulations. Within 6 weeks after the last dosing for Groups 1, 3, 4, 5, 8, and 9 and within 7 days after the last dosing for Groups 2, 6, and 7, two unopened vials of each test and vehicle control article from each lot used were analyzed. End-of dosing stability analyses reveal that all formulations of each test article (Formulations 1, 2, and 3) were of the appropriate purity prior to study initiation (98.1% to 99.1%) and following the end of dosing establishing stability (97.4% to 98.9%)

Study title: A 3-month Intravitreal Toxicology and Toxicokinetic Study of VEGF-Trap, Administered to Cynomolgus Monkeys Followed by a 12-week Recovery Period

Study no.:	VGFT-TX-20170
Study report location:	Module 4.2.3.2 \\CDSESUB1\EVSPROD\bla761355\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\vgft-tx-20170\vgft-tx-20170.pdf
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 19, 2021
GLP compliance:	Yes There are some exceptions that are not considered to affect the interpretation or validity of the study.
QA statement:	Yes
Drug, lot #, and % purity:	VEGF-Trap Test Article Formulation 1 (REGN3 140 mg/mL, 6% HMWS), lot no. 9049300001, 93.7% pure Test Article Formulation 2 (REGN3 140 mg/mL,10% HMWS), lot no. 9049300002, 90.3%% pure

Note: This study evaluated the clinical formulation enriched with high molecular weight species (HMWS).

Key Study Findings

- Monthly IVT administrations of control article, 5.6 mg/eye/dose VEGF-Trap Formulation 1 (5.6 mg/eye/dose + 6% HMWS) or Formulation 2 (5.6 mg/eye/dose + 10% HMWS) were well tolerated.
- Findings were limited to sporadic instances of mild (0.5 to 1+) aqueous cells or flare and more frequent instances of mild (0.5 to 1+) vitreous cells compared to control group. Vitreous cell resolved by Day 85 of the recovery phase.
- Findings were comparable between both formulations.
- No systemic toxicities were observed based on clinical signs, body weight, food consumption, and gross necropsy. At the high dose, the mean plasma C_{max} values for Formulation 1 and Formulation 2 were 7.83 and 7.47 µg/mL for bound aflibercept and 21.8 and 16.9 µg/mL for free aflibercept, respectively, on Day 85.

Methods

Doses:	0, 5.6 mg/eye/dose + 6% HMWS, 5.6 mg/eye/dose + 10% HMWS
Frequency of dosing:	Days 1, 29, 57, and 85
Route of administration:	IVT (both eyes)
Dose volume:	40 µL/dose/eye
Formulation/Vehicle:	VEGF-Trap Placebo (b) (4) histidine, (b) (4) arginine, (b) (4)% sucrose, (b) (4)% polysorbate 20, pH 5.8).
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	5 males/group Three animals/sex/group were designated as terminal animals (Day 92), and 2 animals/sex/group were designated as recovery animals (Day 85).
Age:	26 to 42 months old
Weight:	2.2 to 3.0 kg
Satellite groups:	None
Unique study design:	The purpose of this study was to evaluate the ocular toxicity and TK of the intended VEGF-Trap clinical formulation enriched with approximately 6% and 10% HMWS. Normalized by vitreous volume, the rabbit dose used is approximately 2X the intended clinical dose of 8 mg.
Deviation from study protocol:	None with an impact on study validity or data interpretation

Observations and Results

Mortality (2X/day)

None

Clinical Signs (Cageside observations: predose and daily; Detailed observations: predose, Day 1, weekly, and on day of sacrifice)

Ocular-related clinical observations included squinting (also in one control), eye rubbing, and/or discharge were noted on Day 1 in some animals in both test article formulations. The transient nature indicates the findings were unrelated to the test article. Dilated pupils were observed in controls and both test article formulations at most weekly intervals up to Week 64. The finding was considered procedure related (use a mydriatic for ophthalmic examinations).

Body Weights (Predose, weekly during the dosing and recovery phase)

No test article-related effects

Feed Consumption (Predose and daily during dosing and recovery phases; qualitative)

No test article-related effects

Ophthalmoscopy (Predose and on Days 3, 8, 15, 22, 29 [predose], 31, 36, 43, 50, 57 [predose], 59, 64, 71, 78, 85 [predose], 87, and 92 of the dosing phase; and on Days 8, 15, 29, 43, 57, 71, and 85 of the recovery phase; indirect ophthalmoscopy and slit-lamp biomicroscopy)

Sporadic instances of mild (0.5 to 1+) aqueous cell or flare were noted in control and both test article formulations.

Frequent instances of mild (0.5 to 1+) vitreous cell were observed in control and both test article formulations. The finding was comparable for VEGF-Trap Formulation 1 and Formulation 2 and of slightly greater frequency and or severity (more eyes graded 1+) than those observed in eyes administered control article. Vitreous cell resolved by Day 85 of the recovery phase.

IOP (In conjunction with ophthalmic examinations)

No test article-related effects

ERG/VEP (Predose, once during Week 13 of the dosing phase, and once during Week 12 of the recovery phase)

No test article-related effects

Retinal Photography (Predose, once during Week 13 of the dosing phase, and once during Week 12 of the recovery phase)

No test article-related findings

Fluorescein Angiography (Predose, once during Week 13 of the dosing phase, and once during Week 12 of the recovery phase)

No test article-related findings

Gross Pathology (Day 92 of the dosing phase and Day 85 of the recovery phase)

No test article-related findings

Organ Weights (adrenals, brain, epididymis, heart, kidney, liver, pituitary gland, prostate, seminal vesicles, spleen, testis, thymus, thyroid/parathyroid)

No test article-related findings

Histopathology (Eyes, eyelids, lacrimal glands, nasal turbinates, nasopharynx, optic nerve, mandibular and parotid lymph nodes, and gross lesions; systemic tissues were collected and preserved but not evaluated)

Adequate Battery – Yes, the objective of the study was to evaluate local (eye) safety.

Peer Review – Yes

Histological Findings

No test article-related finding was observed. Minimal mononuclear and/or mixed cell infiltrates were observed in the ciliary body, eyelids, and nasal turbinates in control and/or test article formulations.

Vitreous Fluid Free VEGF-Trap Analysis (at scheduled sacrifices; left eye)

Free VEGF-Trap concentrations in vitreous fluid were detectable in all animals (6/6) receiving both VEGF-Trap formulations at terminal necropsy (Table 9) and were undetectable in all animals (4/4) at recovery necropsy.

Free VEGF-Trap exposure in vitreous fluid at terminal necropsy for all animals receiving VEGF-Trap Formulation 1 and Formulation 2 was remarkably greater compared to exposure in plasma (see Table 9).

Table 9: Free VEGF Trap in Vitreous Humor - 3-Month IVT Toxicity Study in Monkeys

Dose (mg/eye)	0 (Control)	5.6 (~6% HMW Species)[a]	5.6 (~10% HWM Species)[b]
Sex	Male	Male	Male
Number of Animals at Study Start	5	5	5
Day 92 (terminal necropsy)	BLQ	460	560
Day 176 (recovery necropsy)	BLQ	BLQ	BLQ

Units: µg/mL
BLQ <3.13 ng/mL
Source: Excerpt from Table 2.6.7.7.B, Module 2.6.7 Toxicology Written Summary, page 27

Toxicokinetics (Days 1, 29, and 57, and 85 of the dosing phase and once on Days 36, 57, 78, and 85 of the recovery phase at the following timepoints for free and bound VEGF-trap)

Phase	Phase Day	Time Points[a]
Dosing	1, 29, 57	Predose and 4, 8, 24, 48, 72, 168, 336, and 504 hours postdose
Dosing	85	4, 8, 24, 48, 72, and 168 hours postdose
Recovery	36, 57, 78, and 85	Once

Continuous exposure to free VEGF-Trap in plasma was maintained for at least 3 weeks in densely sampled intervals for 80% (4/5) of animals in the VEGF-Trap formulation 1 dose group and 100% (5/5) of animals in the VEGF-Trap formulation 2 dose group; by the end of the 4-week interval, animals had minimal or undetectable concentrations in both groups. All animals from both formulation groups had minimal or undetectable free VEGF-Trap concentrations during the recovery period.

Continuous exposure to adjusted bound VEGF-Trap in plasma was maintained in all animals throughout the treatment and recovery periods.

No apparent drug accumulation was observed for free or adjusted bound VEGF-Trap.

No significant differences in free and adjusted bound VEGF-Trap concentrations or kinetics were observed between the two formulations.

The mean TK parameters are summarized in the table below.

Table 10: Mean Aflibercept TK parameters – 3-Month IVT Toxicity Study in Monkeys

A. Bound Aflibercept

Dose (mg/eye)	0 (Control)	5.6 (~6% HMW Species)[a]	5.6 (~10% HWM Species)[b]
Sex	Male	Male	Male
Number of Animals at Study Start	5	5	5
Dosing Period			
Toxicokinetics (Adjusted Bound Aflibercept)[c]:			
AUC_{tau} (day•[µg/mL])			
Day 1[d]	NA	110	113
Day 29[d]	NA	173	177
Day 57[e]	NA	151	144
Day 85[f]	NA	50.3	46.5
C_{max} (µg/mL)			
Day 1	BLQ	5.42	5.59
Day 29	BLQ	7.28	7.37
Day 57	BLQ	8.00	7.69
Day 85	BLQ	7.83	7.47
C_{trough} (µg/mL)[g]			
Day 1	BLQ	4.29	4.21
Day 29	BLQ	6.14	5.57
Day 57	BLQ	7.55	7.40
Day 85	BLQ	7.73	7.26

B. Free Aflibercept

Dose (mg/eye)	0 (Control)	5.6 (~6% HMW Species)[a]	5.6 (~10% HWM Species)[b]
Sex	Male	Male	Male
Number of Animals at Study Start	5	5	5
Toxicokinetics (Free Aflibercept):			
AUC_{tau} (day•[µg/mL])			
Day 1[d]	NA	186	194
Day 29[d]	NA	227	214
Day 57[e]	NA	254	209
Day 85[f]	NA	126	101
C_{max} (µg/mL)			
Day 1	BLQ	19.9	20.2
Day 29	BLQ	21.7	20.1
Day 57	BLQ	23.5	18.6
Day 85	BLQ	21.8	16.9
C_{trough} (µg/mL)[g]			
Day 1	BLQ	0.125	0.103
Day 29	BLQ	0.340	0.242
Day 57	BLQ	1.55	1.67
Day 85	BLQ	17.4	13.7

[a] Referred to as Formulation 1 in the study report.
[b] Referred to as Formulation 2 in the study report.
[c] Bound aflibercept concentrations in monkey plasma were further multiplied with an adjustment factor of 0.717 to generate adjusted bound concentrations (ie, adjusted bound = bound × 0.717).
[d] AUC_{tau} dose 1 and 2 interval is up to 28 days postdose.
[e] AUC_{tau} dose 3 interval is up to 21 days postdose.
[f] AUC_{tau} dose 4 interval is up to 7 days postdose.
[g] C_{trough} corresponds to 28 days postdose for doses 1 and 2, 21 days postdose 3, and 7 days postdose 4.

Source: Excerpt from Table 2.6.7.7.B, Module 2.6.7 Toxicology Written Summary, pages 25 and 26

Anti-Drug Antibody Sample (Days 1, 15, 29, 43, 57, 85, and 92 [prior to necropsy, as applicable] of the dosing phase and on Days 29, 57, and 85 of the recovery phase; prior to dosing on Days 1, 29, 57, and 85 of the dosing phase)

All animals in the VEGF-Trap formulation 1 dose group were ADA-negative, and 20% (1/5) of animals in the VEGF-Trap formulation 2 dose group exhibited a weak ADA-positive response (1.2-fold from prestudy counts) only on Day 57 predose. At the same timepoint, a similar weak ADA positive response was observed in one control animal (1.34-fold from prestudy counts). Therefore, the positive result of the VEGF-Trap formulation 2 animal appears questionable.

Dosing Solution Analysis

Test article and control article formulations were prepared and dispensed once on each day of dosing by (b) (4) according to Sponsor-provided instructions. Only fresh (unopened) vials were used at each preparation. Test article purity was 93.7% prior to study start and 93.7% following completion of the dosing phase for lot # 9049300001 and 90.2% prior to study start and 90.3% following completion of the dosing phase for lot # 9049300002.

7 Genetic Toxicology

Refer to nonclinical review of BLA 125387 (Rivera; available upon request to the Document Room or at Drugs@FDA).

8 Carcinogenicity

Refer to nonclinical review of BLA 125387 (Rivera; available upon request to the Document Room or at Drugs@FDA).

9 Reproductive and Developmental Toxicology

Refer to nonclinical review of BLA 125387 (Rivera; available upon request to the Document Room or at Drugs@FDA).

11 Integrated Summary and Safety Evaluation

The ocular findings of the monkey 3-month and 6-month ocular toxicity studies with aflibercept high dose formulations were generally comparable to those previously observed with EYLEA®. No adverse ocular findings were observed. Ocular findings were limited to aqueous cell/flare and vitreous cells generally of minimal to slight severity.

In the 6-month ocular toxicity study in monkeys, 3 aflibercept formulations at concentrations of 80 mg/mL and 140 mg/mL (compared to 40 mg/mL for EYLEA®) were evaluated at IVT doses of 4 and 7 mg/eye. Formulation 2 had the same composition as the marketing formulation except for a higher concentration of histidine (b) (4) mM, respectively).

Systemically, microscopic changes in the respiratory epithelium of the nasal turbinates were observed int the 6-month ocular toxicity study. The findings included erosion and/or ulceration (minimal to marked), squamous metaplasia (minimal to moderate), and hemorrhage (minimal to slight). The findings were observed at both doses of aflibercept evaluated, 4 and 7 mg/eye. There was no NOAEL. Most findings were reversible or showed partial reversibility during the 12-week recovery period.

The nasal turbinate findings were noted at doses ≥ 2 mg/eye of EYLEA®, with a NOAEL of 0.5 mg/eye. As noted by the Applicant, similar, albeit more severe, lesions in the nasal cavity have been noted in systemic toxicity studies following repeated IV administration of EYLEA®. At the NOAEL of 0.5 mg/eye in monkeys (Study VGFT-TX-05011, see Table 11 below), the systemic exposure (AUC) for free aflibercept was approximately 3-fold higher than the population pharmacokinetic estimated exposure observed in humans after an IVT dose of 8 mg. Per the Applicant, an analysis of treatment emergent adverse events of nasomucosal findings was conducted in several clinical trials with no indication of a safety signal in the 8 mg aflibercept groups compared to 2 mg. The nasal turbinate findings are acknowledged in the EYLEA® label, and the Applicant maintained the language in the proposed label of EYLEA HD with a corrected exposure margin.

In the 3-month monkey ocular toxicity study, the Applicant evaluated the ocular safety of the marketing formulation enriched with 6% or 10% high molecular weight species (HMWS) at an aflibercept dose of 5.6 mg/eye IVT. These HMWS had no significant impact on the ocular safety profile. In addition, no effects were observed in the nasal turbinates. The Applicant believes the lower dosing volume, 40 µL/eye may have an impact on the nasal turbinate finding as these were observed at a dosing volume of 50 µL/eye. This hypothesis needs further investigation.

Free aflibercept exposure multiples based on NOAEL and/or LOAEL from the most pivotal toxicology studies are provided in Table 11. This reviewer has no objection for the calculation of the exposure margins.

Table 11: Free Aflibercept Exposure Multiples Achieved in Monkeys and Rabbits Relative to Human Exposure

Toxicology Study	Species/ Admin Route/ Dosing Frequency	NOAEL/ LOAEL	Free Aflibercept			
			Exposure in Animals		Estimated Exposure Multiples Based on Unilateral 8 mg dosing[a]	
			C_{max} (µg/mL)	AUC (µg•hr/mL)	Based on C_{max}	Based on AUC
Repeat-Dose Toxicity Studies via IVT Administration						
8-month study; aflibercept (EYLEA formulation) (EYLEA BLA 125387; Module 4.2.3.2, VGFT-TX-05011)	Cynomolgus monkey Bilateral IVT; Q4W	NOAEL: 0.5 mg/eye[b]	0.802	160	5.21	3.29
		LOAEL: 2 mg/eye[c]	6.49	1394	42.1	28.6
6-month study; high dose aflibercept (VGFT-TX-18169)	Cynomolgus monkey Bilateral IVT; Q4W	NOAEL: ND LOAEL: 4 mg/eye[d]	12.3	3264	79.9	67.0
3-month study; high dose aflibercept enriched with ~ 6% and 10% HMW species (VGFT-TX-20170)	Cynomolgus monkey Bilateral IVT; Q4W	NOAEL: 5.6 mg/eye (6%)[e]	21.7	5448	141	112
		NOAEL: 5.6 mg/eye (10%)[e]	20.1	5136	131	105
Repeat-Dose Toxicity Studies via IV Administration						
6-month study (including fertility); aflibercept (EYLEA BLA 125387; Module 4.2.3.2, VGFT-TX-05009)	Cynomolgus monkey IV infusion; QW for first 15 weeks, then Q2W	NOAEL: ND LOAEL: 3 mg/kg[f]	93.4	4416	606	90.7
Embryo-Fetal Development Studies via IV and SC Administration						
EFD study; aflibercept (EYLEA BLA 125387; Module 4.2.3.5.2, VGFT-TX-06002)	Rabbit IV infusion; GD 6, 9, 12, 15, and 18	EFD LOAEL: 3 mg/kg[g]	56.1	1935	364	39.7
EFD study; aflibercept (EYLEA BLA 125387; Module 4.2.3.5.2, VGFT-TX-11034)	Rabbit SC injection; GD 1, 7, and 13	EFD LOAEL: 0.1 mg/kg[h]	0.259	43.2	1.68	0.887

[a] Exposure multiples were generated using the ratios of the free aflibercept C_{max} or AUC values determined in the toxicology studies and the human free aflibercept $C_{max,\ week\ 8\text{-}12}$ (0.154 mg/L) and $AUC_{week\ 8\text{-}12}$ (2.03 mg•day/L; 48.7 µg•hr/mL) mean overall values reported in VGFT-PK-22159 Table 21.

[b] The mean C_{max} and $AUC_{0\text{-}28days}$ (AUC from 6.66 µg•day/mL reported value) at 0.5 mg/eye after the 7th dose (EYLEA BLA 125387; Module 4.2.3.2, VGFT-TX-05011).

[c] The mean C_{max} and $AUC_{0\text{-}28days}$ (AUC from 58.1 µg•day/mL reported value) at 2 mg/eye after the 7th dose (EYLEA BLA 125387; Module 4.2.3.2, VGFT-TX-05011).

[d] The mean C_{max} and $AUC_{0\text{-}28days}$ (AUC from 136 µg•day/mL) at 4 mg/eye after the 2nd dose of formulation 2 (VGFT-TX-18169, Appendix 9.5 Toxicokinetic Report, Table 1); the C_{max} and $AUC_{0\text{-}28days}$ values after the 2nd dose were selected, since after the 7th dose the dosing interval extended into the recovery period and there was no 28 day sample taken.

[e] The mean C_{max} and $AUC_{0\text{-}28days}$ (AUCs from 227 and 214 µg•day/mL reported values) for material enriched with approximately 6% and 10% HMW species at 5.6 mg/eye after the 2nd dose (VGFT-TX-20170, Appendix 9.5 Toxicokinetic Report, Table 1); the 2nd dose was chosen since TK was measured up to 28 days postdose, while after the 3rd and 4th dose it was only measured up to 21 or 7 days post dose, respectively).

[f] The mean C_{max} and $AUC_{0\text{-}168h}$ (AUC from 184 µg•day/mL reported value) at week 21 at 3 mg/kg IV in EYLEA BLA 125387; Module 4.2.3.2, VGFT-TX-05009.

[g] The mean C_{max} and $AUC_{0.5\text{-}72h}$ after the injection on GD 18 (5th dose) at 3 mg/kg IV in EYLEA BLA 125387; Module 4.2.3.5.2, VGFT-TX-06002.

[h] The mean C_{max} after the 3rd dose (GD 13) and cumulative $AUC_{0\text{-}20\ days}$ at 0.1 mg/kg SC during the dosing period (SC injections on GD 1, 7, 13) calculated based on AUC values of 17.5 and 11.3 µg•h/mL reported after the 1st and 3rd dose (GD 1 and GD 13) in EYLEA BLA 125387; Module 4.2.3.5.2, VGFT-TX-11034. Calculation: $AUC_{0\text{-}20\ days}$ cumulative = $AUC_{0\text{-}144h}$ GD 1 + $AUC_{0\text{-}144h}$ GD 7 (approximated as [$AUC_{0\text{-}144h}$ GD 1 + $AUC_{0\text{-}168h}$ GD 13]/2) + $AUC_{0\text{-}168h}$ GD13 ≈ 17.5 µg•h/mL + (17.5 µg•h/mL + 11.3 µg•h/mL)/2 + 11.3 µg•h/mL = 17.5 µg•h/mL + 14.4 µg•h/mL + 11.3 µg•h/mL = 43.2 µg•h/mL

AUC, Area under the concentration-time curve; AUC_{tau}, AUC calculated during the dosing interval; C_{max}, Peak concentration; EFD, Embryo-fetal development; GD, Gestation day; HMW, High-molecular weight; IV, Intravenous; IVT, Intravitreal; LOAEL, Lowest-observed-adverse-effect level; ND, Not determined; NOAEL, No-observed-adverse-effect level; QW, Once weekly; Q2W, Once every 2 weeks; Q4W, Once every 4 weeks; SC, Subcutaneous; TK, Toxicokinetic

The Applicant noted that exposure multiples calculated based on the Phase 2 and Phase 3 studies of HD aflibercept demonstrated high margins of exposure for the NOEL for respiratory and CNS functions as well as venous and arterial thrombus formulation, while smaller safety margins were observed for the effects on systolic and diastolic blood pressures and wound healing (Table 12). Based on these data, blood

pressure measurements were included in the clinical studies of HD aflibercept. Per the Applicant, in the 44-week Phase 2 CANDELA study and through week 48 of the Phase 3 PULSAR and PHOTON studies, adverse events pertaining to hypertension were similar between the groups, and no clinically relevant increase in systolic/diastolic BP were seen in the studies.

Table 12: Exposure Data and Safety Margins or Exposure Ratios for Free Aflibercept after Unilateral IVT Treatment with 8 mg Aflibercept/eye Regarding Safety Pharmacology Endpoints from Nonclinical Studies with IVT or Systemic Dosing of Aflibercept

Study/Endpoint	Dose	Free C_{max} (µg/mL)	Safety Margins/Exposure Ratios Based on Free C_{max}
Human exposure			
Model-prediction exposures ($C_{max,8\text{-}12weeks}$) after IVT injections of 8 mg aflibercept in study eye on weeks 0, 4, and 8 in patients with nAMD or DME[a] VGFT-PK-22159, Table 21	8 mg IVT unilateral	0.154	NA
Safety Pharmacology studies:	***Testing integrated in an IVT toxicology study in monkeys on the new 8 mg aflibercept formulation***		
NOEL for cardiovascular, respiratory, and CNS function tested in a toxicological IVT monkey study utilizing the high dose aflibercept formulation[b] (VGFT-TX-18169)	7 mg/eye IVT bilateral (every 4 weeks)	20.7	134
Safety Pharmacology studies:	***Testing in systemic nonclinical studies in rats, rabbits, and monkeys***		
NOEL for respiratory and CNS function tested in a toxicological IV monkey study (EYLEA BLA 125387; Module 4.2.3.2,VGFT-TX-05009)	30 mg/kg BW IV (once weekly up to 15 weeks, then every 2 weeks)	653	4240
NOEL for the potential of thrombosis formation in the rabbit (EYLEA BLA 125387; Module 4.2.1.3, VGFT-TX-06012)	30 mg/kg BW IV (day -6, -3 and 1)	1060	6883
Blood pressure in telemetered mice & rats	Single dose		
NOEL rat	0.15 mg/kg BW SC	1.00	6.50
LOAEL rat (↑ by 4/3 mmHg syst./diastolic.)	0.5 mg/kg BW SC	2.26	14.7
LOAEL mouse* (↑ by 14/12 mmHg systolic/diastolic)	2.5 mg/kg BW SC	11.6	75
(EYLEA BLA 125387; Module 4.2.1.2, VGFT-MX-08018)			
Incisional/excisional wound healing in the rabbit LOAEL (delays in wound healing)* (EYLEA BLA 125387; Module 4.2.1.3, VGFT-TX-06010 and VGFT-TX-06011)	0.3 mg/kg BW IV (once weekly, 4 treatment occasions)	6.11	39.7

[a] In the Phase 2b study CANDELA and the 2Phase 3 studies PHOTON and PULSAR the initial 3 IVT doses were given at an interval of 4 weeks as a loading dose, while longer treatment intervals were applied later. As a conservative approach, the population pharmacokinetic model-predicted exposures for free aflibercept following 3 initial IVT doses of aflibercept in patients with nAMD or DME after the third dose was used for the calculation of exposure multiples (VGFT-PK-22159, Table 21).

[b] The mean C_{max} at 7 mg/eye in VGFT-TX-18169, Appendix 9.5 Toxicokinetic Report, Table 1; after the second dose (exposure after the second dose was selected for calculation of exposure multiples in safety pharmacology and toxicology, since after the seventh dose the dosing interval extended into the recovery period and there was no 28 day sample taken. Therefore, AUC_{tau} was an extrapolated $AUC_{0\text{-}28days}$.).

BW, Body weight; C_{max}, Peak concentration; CNS, Central nervous system; DME, Diabetic macular edema; IV, Intravenous; IVT, Intravitreal; LOAEL, Lowest-observed-adverse-effect level; MoEs, Multiples of exposure; NA, Not applicable; nAMD, Neovascular age-related macular degeneration; NOAEL, No-observed-adverse-effect level; NOEL, No-observed-effect level; SC, Subcutaneous; *, No NOAEL identified

Source: Module 2.6.2 Pharmacology Written Summary, Table 1

In conclusion, the nonclinical safety profile of the high dose aflibercept formulations evaluated was comparable to the previously established safety profile of EYLEA®. Pharmacology/Toxicology team supports approval of EYLEA HD for treatment of neovascular (Wet) age-related macular degeneration (nAMD), diabetic macular edema (DME) and diabetic retinopathy (DR).

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

MARIA I RIVERA
06/13/2023 04:33:22 PM

MUKESH SUMMAN
06/13/2023 09:02:17 PM