REGENERON

EYLEA® (AFLIBERCEPT) INJECTION FOR RETINOPATHY OF PREMATURITY

SPONSOR BRIEFING DOCUMENT

DERMATOLOGIC AND OPHTHALMIC DRUGS ADVISORY COMMITTEE

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Definition
ADA	Anti-drug antibody
ADR	Adverse drug reaction
AMD	Age-related macular degeneration
APGAR	Appearance, Pulse, Grimace, Activity, and Respiration
AP-ROP	Aggressive posterior retinopathy of prematurity
BP	Blood pressure
CA	Chronological age
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence interval
CMH	Cochran-Mantel-Haenszel analysis method
COVID-19	Coronavirus Disease 2019
DBP	Diastolic blood pressure
DME	Diabetic macular edema
DR	Diabetic retinopathy
EU	European Union
FAS	Full analysis set
FDA	Food and Drug Administration
GA	Gestational age
ICROP	International Classification of Retinopathy of Prematurity
IDMC	Independent Data Monitoring Committee
IOP	Intraocular pressure
ITT	Intent-to-treat
IV	Intravenous
IVT	Intravitreal
mCNV	Myopic choroidal neovascularization
MedDRA	Medical Dictionary for Regulatory Activities
MEfRVO	Macular edema following retinal vein occlusion
nAMD	Neovascular (wet) age-related macular degeneration
NICU	Neonatal intensive care unit
PD	Pharmacodynamic
PK	Pharmacokinetic
PlGF	Placental growth factor
PP	Per protocol
PT	Preferred term
PWR	Pediatric Written Request
ROP	Retinopathy of prematurity
SAE	Serious adverse event
SAF	Safety analysis set
SAP	Statistical analysis plan
sBLA	Supplemental Biologics License Application
SBP	Systolic blood pressure
SE	Spherical equivalent
SOC	System organ class
SPA	Special Protocol Assessment

TE	Treatment-emergent
TE SAE	Treatment-emergent serious adverse event
TEAE	Treatment-emergent adverse event
US	United States
VEGF	Vascular endothelial growth factor

1. EXECUTIVE SUMMARY

Regeneron is seeking a supplemental indication for aflibercept (EYLEA®) 0.4 mg for the treatment of retinopathy of prematurity (ROP). Two pivotal studies provide evidence to support the efficacy and safety of aflibercept in ROP. The results build on a growing body of evidence on the effectiveness and safety of off-label use of anti-vascular endothelial growth factor (VEGF) agents, as an alternative to laser photocoagulation, in treating ROP. Off-label anti-VEGF treatment is increasingly becoming the standard of care for ROP as it spares the retina from the permanent destructive effects of laser and can be administered without the need for the long sedation and anesthesia times associated with laser therapy. Regulatory approval of aflibercept for the treatment of ROP would standardize dosing and thus provide greater consistency in treatment, ensure FDA oversight and reporting of safety, and improve access for appropriate patients in need.

1.1. Introduction

Type 1 ROP is a vision-threatening, neovascularization disease of the incompletely vascularized, immature retina of preterm infants (born < 37 weeks of gestational age [GA]) and is among the top 3 leading causes of childhood blindness worldwide (Chiang, 2004; Hellstrom, 2013). Despite the severity of ROP, laser photocoagulation is the only treatment option cleared by the Food and Drug Administration (FDA), and there are no FDA-approved pharmacological treatment options for ROP. While effective, laser photocoagulation is associated with several major challenges including the need for endotracheal intubation and/or lengthy sedation, laser availability, and requirement for specially trained surgeons who may not be readily available during the short time window when treatment is required (typically 72 hours from the time of identification of Type 1 ROP). Laser photocoagulation can require more than one session for completion and inherently damages the retina, which leads to a variable degree of loss of peripheral vision and increased risk for high myopia (Mutlu, 2013).

ROP, a two-phase disease, is initiated with delayed retinal vascular growth after premature birth (Phase I). Insufficient vascularization of the developing retina creates hypoxia, which precipitates the release of factors such as VEGF, stimulating new and abnormal blood vessel growth (Phase II). VEGF is known to be overexpressed in several retinal diseases with neovascularization, including ROP (Cao, 2010; Hellstrom, 2016; Krzystolik, 2002; Kwak, 2000; Nork, 2011). VEGF-A serves as an endothelial cell mitogen and promotes endothelial cell migration, survival, and tube formation, playing an important role in physiological vascular development, physiological angiogenesis, as well as pathological neovascularization seen in several retinal diseases including ROP. Based on the well understood mechanism of VEGF in causing pathological neovascularization of the retina, coupled with the ease of intravitreal administration of anti-VEGF treatments and their approval in other indications, anti-VEGF agents have been increasingly used off-label for the treatment of ROP (Kychenthal, 2021).

Bevacizumab is the most frequently used off-label anti-VEGF, although bevacizumab is not approved for any ophthalmologic use and is only available from compounding pharmacies. In some parts of the United States (US), the use of anti-VEGF agents has exceeded the use of retinal photocoagulation for the treatment of Type 1 ROP for several years (Nitkin, 2022; Prakalapakorn, 2021). The efficacy and safety of anti-VEGF treatments in ROP have been described in the literature (Pertl, 2015). Anti-VEGF treatments do not damage the retina and

require less time to administer than laser, and therefore substantially reduce the requirements for sedation and/or anesthesia. Anti-VEGF treatment may also provide an intermediate treatment option by allowing the normal retinal vascularization to progress as far as possible to the periphery of the retina prior to laser photocoagulation procedure, thereby minimizing the area of the retina requiring ablation.

Aflibercept is a recombinantly produced fusion protein that inhibits VEGF. Aflibercept binds to VEGF-A and placental growth factor (PlGF), thereby reducing endothelial cell proliferation, vascular leakage, and new pathological blood vessel formation.

Aflibercept (EYLEA) was initially approved by the FDA in 2011 for the treatment of neovascular (wet) age-related macular degeneration (nAMD) and has since been approved for other retinal vascular diseases (ie, macular edema following retinal vein occlusion, diabetic macular edema, and diabetic retinopathy). EYLEA is marketed in more than 113 countries. EYLEA has received marketing approval for the treatment of ROP in Japan, a positive opinion from the Committee for Medicinal Products for Human Use (CHMP) for the treatment of ROP in the EU, and is under review in the United Kingdom, Switzerland, and Brazil. The approved dose for adults is 2 mg (using a 40 mg/mL formulation), and the recommended dose for ROP is 0.4 mg (using the same 40 mg/mL formulation as adults).

More than 11 years – encompassing more than 50 million injections – of post-marketing safety information in the approved adult indications informs the safety profile and provides additional support for the use of aflibercept in preterm infants. In completed clinical trials with adults, serious adverse reactions related to the injection procedure occurred in < 0.1% of intravitreal injections with EYLEA and include endophthalmitis and retinal detachment (EYLEA Prescribing Information 2011). The most common adverse reactions (≥ 5%) were conjunctival hemorrhage, eye pain, cataract, vitreous detachment, vitreous floaters, and intraocular pressure increased.

The efficacy and safety of aflibercept for the treatment of ROP are supported by two Phase 3, multicenter, randomized, open-label studies comparing aflibercept (0.4 mg per eye) to laser photocoagulation in preterm infants with Type 1 ROP, BUTTERFLEYE and FIREFLEYE. In both studies, approximately 80% of infants treated with aflibercept met the primary endpoint, which was the absence of active ROP and unfavorable structural outcomes, without being rescued by another treatment modality. The primary hypothesis was that aflibercept would be non-inferior to laser. While the lower bound of the confidence interval (CI) did not meet the prespecified non-inferiority margin, the response rate for infants treated with aflibercept were numerically similar to the response rates for laser. Additionally, secondary and exploratory endpoint results showed that infants treated with aflibercept required treatment with a second treatment modality (eg, laser, surgery, etc.) at the same or lower frequency than laser-treated infants and required less to perform the procedure, which likely implies less time under sedation or anesthesia, which is particularly meaningful in this vulnerable patient population. The safety findings through 52 weeks chronological age (CA; ie, age from birth) from these studies are reassuring with mostly non-serious, mild, transient, and manageable treatment-emergent adverse events (TEAEs). The safety profile was as expected for a very preterm infant population, and ocular TEAEs were generally consistent with those described for the adult population treated with intravitreal (IVT) aflibercept.

Based on the unmet medical need and potential benefit of IVT aflibercept in Type 1 ROP, FDA determined that information related to the use of aflibercept in the pediatric population may produce health benefits and issued a Pediatric Written Request (PWR) to Regeneron in June 2019. In July 2019, aflibercept was granted Orphan Drug Designation based on the rarity of ROP. Regeneron worked in close collaboration with FDA to ensure acceptable study design and criteria, and all study protocols and statistical analysis plans (SAPs) were approved under a Special Protocol Assessment (SPA). In August 2022, Regeneron submitted the supplemental Biologics License Application (sBLA) for aflibercept for the treatment of ROP.

Overall, aflibercept offers important clinical and practical benefits in the treatment of preterm infants with ROP, a potentially vision-impairing disease with no approved pharmacologic agents in the US. Importantly, the efficacy comes with a favorable safety profile and an overall positive benefit/risk balance in this vulnerable pediatric population.

1.2. Background and Unmet Need

ROP is a complication of preterm birth characterized by incomplete vascularization and pathological neovascularization of the retina in infants born before 37 weeks GA. ROP is more common and severe in infants born at a low GA (≤ 32 weeks) and those with very low birth weight (≤ 1500 g [3.3 lbs]). Infants born extremely premature (≤ 28 weeks) and/or at extremely low birth weight (≤ 1,000 g) are at an even greater risk of ROP.

While rare, the incidence of ROP is increasing due to improved survival of extremely preterm infants, improved awareness of ROP, and implementation of ROP screening guidelines. The incidence of ROP ranges from 1% to 2% among all live births (Chiang, 2004; Hellstrom, 2013); however, the incidence is approximately 36% among patients screened in neonatal intensive care units (NICUs) where the infant population is typically more premature (GA ≤ 32 weeks) (Blencowe, 2013).

ROP occurs in preterm infants for several reasons. ROP is initiated in part by incomplete development of physiological retinal vascularization in its first phase (Hellstrom, 2016; Smith, 2013). At approximately 31–34 weeks post-menstrual age, an abundance of growth factors, particularly VEGF, are secreted by the ischemic retina, which leads to disorganized vascular growth. Additionally, external factors including certain medications, supplemental oxygen, bright lights, and elevated temperatures in the NICU can stimulate VEGF production (Pierce, 1996). The aberrant vasculature causes the formation of a tissue ridge between the vascular and avascular retina, which can often resolve without treatment. However, if cases of ROP that warrant prompt treatment (ie, Type 1 ROP) are left untreated, vascular growth may proliferate into the vitreous cavity, eventually causing involution of the blood vessels with cicatricial contraction, which can lead to tractional retinal detachment. The consequences of end-stage ROP include severe vision loss and blindness, and surgical treatment (retinal detachment surgery or enucleation) may have to be applied at this late stage.

Preterm infants in the NICU are routinely screened for the development and progression of ROP by an ophthalmologist trained in ROP care. ROP is classified based on the International Classification of Retinopathy of Prematurity (ICROP), which defines the location of retinal involvement and the severity. Three zones describe the location of retinal involvement (Figure 7):

- Zone I (center of the retina, surrounding the macula)
- Zone II (mid-periphery of the retina, outside the macula)
- Zone III (outer temporal crescent of the retina)

Severity consists of 5 stages:

- Stage 1 (flat white demarcation line between vascular and avascular retina)
- Stage 2 (ridge of fibrous tissue protrudes into the vitreous in the region between vascular and avascular retina)
- Stage 3 (new blood vessels and fibrous tissue grow along the ridge and often extend into vitreous)
- Stage 4 (partial retinal detachment)
- Stage 5 (total retinal detachment)

Further classification includes the detection of "plus disease" which is venous dilatation and arteriolar tortuosity of the posterior retinal vessels in at least 2 quadrants, indicating a more aggressive course at any stage.

A subtype of ROP, aggressive posterior ROP (AP-ROP), is an uncommon, severe form of ROP characterized by posterior location, prominence of plus disease, and rapid progression to retinal detachment. AP-ROP requires immediate treatment, usually within 72 hours of diagnosis. A main goal in ROP treatment is to prevent adverse structural (eg, retinal vessel dragging, retinal fold, partial or complete retinal detachment) and functional (visual acuity and visual fields) outcomes.

Type 1 ROP is defined as ROP that needs treatment and includes ROP in Zone I with Stage 1 plus, 2 plus, or 3 (with or without plus), ROP in Zone II with Stage 2 plus or 3 plus (including AP-ROP). Patients with Stage 4 or 5 ROP usually require surgery (scleral buckling or vitrectomy), and the prognosis for preserved visual function is worse than if treatment is initiated at an earlier stage.

Despite the severity of Type 1 ROP, there are limited treatment options available in the US. Standard of care treatment is laser photocoagulation therapy. Anti-VEGF treatments, most commonly bevacizumab, are frequently used off-label (Prakalapakorn, 2021). Laser and anti-VEGF treatments target a reduction in VEGF levels with the goal to regress pathological blood vessel growth and halt neovascularization (Khan, 2022).

Laser photocoagulation therapy has been used for nearly 20 years and is effective but is associated with several challenges. Laser photocoagulation burns away the peripheral ischemic retina to reduce VEGF production leading to defects in the peripheral visual field and carries a risk for high myopia (Mutlu, 2013). The ophthalmologist administering laser photocoagulation

must ensure that all ischemic retina is destroyed by the laser. If areas of ischemic retina remain (ie, "skip areas"), reactivation of ROP can occur following treatment. Laser photocoagulation typically takes 1 hour per eye to administer, is often stressful to the infant, and is usually performed under deep sedation or general anesthesia necessitating transfer of the infant from the NICU to the operating room. Additionally, laser photocoagulation requires special training to administer in centers designated for administration to neonates, which can limit access to timely care or require patients to be transferred to specialized centers.

Clinical guidelines include off-label use of IVT injections of VEGF inhibitors as an alternative option to laser photocoagulation because it does not damage the retina and is technically easier to administer (typically at the bedside under local anesthesia, with or without sedation) (Mintz-Hittner, 2011). In particular, anti-VEGF agents are useful in infants for whom laser photocoagulation is difficult or impossible (eg, those with opaque cornea or lens, vitreous haze, or poor pupil dilation). VEGF inhibitors have been previously compared to laser in 2 prospective, randomized studies: the BEAT-ROP trial (bevacizumab vs laser) and the RAINBOW trial (ranibizumab vs laser) (Mintz-Hittner, 2011; Stahl, 2019). Both studies showed positive outcomes with numerically higher response rates for anti-VEGF treatment compared to laser. In the RAINBOW study, which was designed with the same primary endpoint as BUTTERFLEYE and FIREFLEYE, but assessed at 24 weeks rather than 52 weeks, the proportion of infants with absence of active ROP and unfavorable structural outcome was 80% with ranibizumab 0.2 mg (20uL) compared to 66% with laser (p=0.051). A ranibizumab dose of 0.1 mg (10uL) was also tested with 75% of infants considered a treatment success.

There are several published reports of off-label use of aflibercept for the treatment of ROP that have shown positive efficacy without new safety concerns (Azuma, 2019; Huang, 2018; Salman, 2015; Sidorenko, 2018; Sukgen, 2019). In these studies, 80%–100% of patients treated with aflibercept at doses ranging from 0.4–1.0 mg/eye achieved favorable structural outcomes. These small studies have limitations but provide important preliminary information regarding the safety and efficacy of aflibercept in ROP.

Ranibizumab has been approved since 2019 for the treatment of patients with ROP in the European Union (EU) and Japan; however, no anti-VEGF treatments are approved for the treatment of ROP in the US. While anti-VEGF treatments are used off-label in the US, FDA approval would increase patient access, prevent complications resulting from compounding of bevacizumab (the most frequently used off-label anti-VEGF therapy) (Watson, 2021), provide guidance for prescribers and parents/caregivers through labeling and ongoing monitoring of benefit and risk through regulated pharmacovigilance system, improve the tracking of drug distribution and quality related data, improve management of all potential post-marketing actions (eg, product recall), and provide additional FDA oversight.

1.3. Development Program

The aflibercept ROP development program consists of 2 similarly designed Phase 3 interventional studies, Study 1920 (BUTTERFLEYE) and Study 20090 (FIREFLEYE), which each have long-term follow-up studies through to 5 years of age (Study 2036 [BUTTERFLEYE NEXT] and Study 20275 [FIREFLEYE NEXT], respectively). In BUTTERFLEYE the primary endpoint was assessed at Week 52 of CA. FIREFLEYE was initiated for submission in the EU with a Week 24 primary endpoint; however, for FDA submission, the data from FIREFLEYE

were combined with data from FIREFLEYE NEXT through 52 weeks CA. Therefore, all analyses shown in this briefing document were conducted on the 52-week CA endpoint for both studies.

BUTTERFLEYE and FIREFLEYE investigated the efficacy and safety of a 0.4 mg (0.01 mL) dose of aflibercept compared to laser treatment in infants with ROP. This dose was chosen based on the published data with doses ranging from 0.4 mg to 1 mg per eye (ie, 1/5 to 1/2 of the 2 mg dose approved for indications in adult patients) (Salman, 2015; Sidorenko, 2018; Sukgen, 2019). In order to limit drug exposure, the lowest dose reported (0.4 mg), which also showed positive efficacy, was selected for the studies. In addition, an injection volume of 0.01 mL is considered acceptable for IVT administration in infants.

1.4. BUTTERFLEYE and FIREFLEYE Study Designs

1.4.1. Overview of Study Design

BUTTERFLEYE and FIREFLEYE were both randomized, controlled, open-label, multicenter Phase 3 studies that assessed the efficacy, safety, and tolerability of IVT aflibercept versus laser in preterm infants with ROP (Figure 9 and Figure 11). BUTTERFLEYE was conducted at 39 study centers in 10 countries, including the US, and FIREFLEYE was conducted at 64 study centers in 27 countries.

The primary objective of the studies was to assess the efficacy of aflibercept 0.4 mg compared with laser photocoagulation at 52 weeks CA. Infants were randomized (3:1 in BUTTERFLEYE and 2:1 in FIREFLEYE) to aflibercept 0.4 mg or laser photocoagulation treatment in eligible eyes. If only one eye was treated, the other eye was kept under observation and received the same treatment as the first eye if Type 1 ROP subsequently developed. In the aflibercept groups, if required, each treated eye could receive up to 2 retreatments for a total of 3 IVT aflibercept injections. In the laser groups, additional laser sessions occurring in the first week were considered part of the initial treatment. Since laser often requires more than one session to ensure complete lasering of ischemic retina and no "skip areas," this approach was consistent with best practice. Rescue treatment was permitted with laser for infants randomized to aflibercept and rescue aflibercept for infants randomized to laser.

Infants were followed with mandatory visits at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 weeks after treatment, and at 40 weeks CA and 52 weeks CA.

Inclusion and exclusion criteria were similar in both studies. Infants were enrolled with GA at birth of ≤ 32 weeks or a birth weight ≤ 1,500 g. Weight at baseline needed to be ≥ 800 g. In accordance with ICROP guidelines, infants had to have treatment-naïve ROP Zone I Stage 1 plus, 2 plus, 3 non-plus or plus, or Zone II Stage 2 plus or 3 plus or AP-ROP (International Committee for the Classification of Retinopathy of Prematurity 2005).

The same endpoints were used in both studies. The primary efficacy endpoint was the absence of both active ROP and unfavorable structural outcomes, without being rescued by another treatment modality at 52 weeks CA based on the investigator's assessment. Secondary endpoints were the proportion of infants requiring intervention with a second treatment modality (ie, rescue treatment or any other surgical or nonsurgical treatment for ROP including IVT anti-VEGF injection, ablative laser therapy, cryotherapy, or vitrectomy) through 52 weeks CA, and the

proportion of infants with recurrence of ROP through 52 weeks CA. Additional exploratory endpoints of particular importance to patients and families included the need for sedation or general anesthesia and the time required to perform treatment.

BUTTERFLEYE and FIREFLEYE were designed as non-inferiority studies. A non-inferiority margin of 5% for the difference in response rates was prespecified. This margin was chosen conservatively, to be smaller than the difference of either active dose of ranibizumab (0.1 and 0.2 mg) versus laser in the RAINBOW study. The observed response rates in the RAINBOW study for laser, ranibizumab 0.1 mg and ranibizumab 0.2 mg were 66% (95% CI: [55%, 77%]), 75% (95% CI: [65%, 85%]) and 80% (95% CI: [71%, 89%]), respectively. Since the RAINBOW study was considered to be the proof-of-concept study for BUTTERFLEYE and FIREFLEYE, the response rates for both aflibercept and for laser were assumed to be similar to those observed in the RAINBOW study; the published reports on the response rates for aflibercept were also consistent with the observed response rate for ranibizumab arms in RAINBOW (Azuma, 2019; Huang, 2018; Salman, 2015; Sidorenko, 2018; Sukgen, 2019; Vedantham, 2019; Vural, 2019). A two-sided significance level of 0.049 was prespecified (ie, 95.1% CI for assessing non-inferiority), which included an adjustment for the Independent Data Monitoring Committee (IDMC) reviews of the data. Non-inferiority was assessed by the difference in response rates between treatments, adjusted for baseline ROP status. With these assumptions, each study had > 80% power to meet the primary hypothesis of non-inferiority.

1.4.2. Patient Populations

Baseline demographics and disease characteristics are described in Table 1. In BUTTERFLEYE, the mean (SD) weight at baseline was lower in the aflibercept group than the laser group (2,058.3 g [548.3 g] vs 2,248.1 g [725.0 g], respectively), and more infants in the aflibercept group were female than in the laser group (55.9% vs 37.0%, respectively). In FIREFLEYE, the mean (SD) weight at baseline was higher in the aflibercept group than the laser group (2,026.7 g [678.9 g] vs 1,850.9 g [546.1 g], respectively), and approximately half of infants in both groups were female (45% vs 50%, respectively). In both studies, the mean GA at birth ranged from 26.0 to 27.3 weeks, and the mean CA at randomization ranged from 9.8 to 11.1 weeks.

Table 1: Demographic Characteristics of Infants in BUTTERFLEYE and FIREFLEYE

	BUTTERFLEYE (N=120)		FIREFLEYE (N=113)	
	Aflibercept (N=93)	Laser (N=27)	Aflibercept (N=75)	Laser (N=38)
Chronological Age at Randomization (weeks), mean (SD)	9.76 (3.149)	11.09 (4.338)	10.35 (2.781)	10.17 (2.290)
Gestational Age at Birth (weeks), mean (SD)	27.34 (2.753)	27.06 (2.652)	26.48 (2.071)	25.97 (1.618)
Post-Menstrual Age at Randomization (weeks)[a], mean (SD)	37.11 (2.425)	38.15 (3.599)	36.82 (2.732)	36.14 (2.150)
Gestational Age at Birth group, n (%)				
≤ 26 weeks	38 (40.9%)	11 (40.7%)	38 (50.7%)	22 (57.9%)
> 26 weeks	55 (59.1%)	16 (59.3%)	37 (49.3%)	16 (42.1%)
Sex, n (%)				
Female	52 (55.9%)	10 (37.0%)	34 (45.3%)	19 (50.0%)
Male	41 (44.1%)	17 (63.0%)	41 (54.7%)	19 (50.0%)
Weight at Birth (g)				
Mean (SD)	991.2 (407.00)	934.1 (406.61)	881.1 (305.63)	824.6 (230.80)
Median	900.0	798.0	820.0	790.0
Weight at Baseline (g)				
Mean (SD)	2058.3 (548.28)	2248.1 (724.95)	2026.7 (678.93)	1850.9 (546.13)
Median	1948.0	2050.0	1851.0	1735.5
Race, n (%)				
White	26 (28.0%)	11 (40.7%)	55 (73.3%)	28 (73.7%)
Black or African American	6 (6.5%)	2 (7.4%)	2 (2.7%)	0
Asian	44 (47.3%)	13 (48.1%)	17 (22.7%)	9 (23.7%)
American Indian or Alaska Native	0	0	0	1 (2.6%)
Native Hawaiian or Other Pacific Islander	0	0	0	0
Multiple	NR	NR	1 (1.3%)	0
Other/Not Reported	17 (18.3%)	1 (3.7%)	0	0
Ethnicity, n (%)				
Hispanic or Latino	16 (17.2%)	4 (14.8%)	NR	NR
Not Hispanic or Latino	72 (77.4%)	22 (81.5%)	NR	NR
Unknown/Not Reported	5 (5.4%)	1 (3.7%)	NR	NR

Abbreviations: NR=not reported; SD=standard deviation.
[a] Post-menstrual age at randomization=Gestational age at birth + Chronological age at randomization.

As would be expected based on the disease, most infants (85%–95%) were treated for bilateral ROP. The majority (65%–74%) of infants had Zone II ROP in both studies (Table 2).

Table 2: Baseline ROP Staging per Investigator Assessment in BUTTERFLEYE and FIREFLEYE

	BUTTERFLEYE (N=120)		FIREFLEYE (N=113)	
	Aflibercept (N=93)	Laser (N=27)	Aflibercept (N=75)	Laser (N=38)
Laterality of treatment, n (%)[a]				
Unilateral	7 (7.5%)	4 (14.8%)	4 (5.3%)	4 (10.5%)
Bilateral	86 (92.5%)	23 (85.2%)	71 (94.7%)	34 (89.5%)
Laterality of treatment at baseline, n (%)[b]				
Unilateral	10 (10.8%)	4 (14.8%)	6 (8.0%)	5 (13.2%)
Bilateral	83 (89.2%)	23 (85.2%)	69 (92.0%)	33 (86.8%)
Number of eyes treated, number of eyes[a,c]	179	50	146	72
ROP zone, by eye, number of eyes (%)[d]				
Zone I	47 (26.3%)	13 (26.0%)	51 (34.9%)	21 (29.2%)
Stage 1, plus disease	NR	NR	2 (1.4%)	0
Stage 2, plus disease	16 (8.9%)	1 (2.0%)	6 (4.1%)	7 (9.7%)
Stage 3, no plus disease	2 (1.1%)	0	6 (4.1%)	1 (1.4%)
Stage 3, plus disease	21 (11.7%)	12 (24.0%)	27 (18.5%)	9 (12.5%)
AP-ROP	20 (11.2%)	3 (6.0%)	23 (15.8%)	8 (11.1%)
Zone II	132 (73.7%)	37 (74.0%)	95 (65.1%)	51 (70.8%)
Stage 2, no plus disease	NR	NR	0	1 (1.4%)
Stage 2, plus disease	32 (17.9%)	5 (10.0%)	17 (11.6%)	11 (15.3%)
Stage 3, plus disease	100 (55.9%)	30 (60.0%)	74 (50.7%)	37 (51.4%)
AP-ROP	8 (4.5%)	3 (6.0%)	5 (3.4%)	2 (2.8%)

Abbreviations: AP-ROP= aggressive posterior retinopathy of prematurity; NR=none reported.
[a] Included all treated eyes - including eyes for which treatment started only after the baseline visit.
[b] Included all eyes for which treatment started at the baseline visit.
[c] This row presents the denominator for calculation of percentages in rows below.
[d] ROP classification was based on the baseline value for those eyes starting treatment at baseline and at the second eye treatment visit for those eyes starting treatment after the baseline visit. Eyes with AP-ROP were not always exclusive of disease stage.

A total of 127 infants were randomized 3:1 in BUTTERFLEYE (94 aflibercept, 33 laser), and 118 infants were randomized 2:1 in FIREFLEYE (75 aflibercept, 43 laser). Of these infants, 99% of those randomized to aflibercept and 82% of those randomized to laser received treatment in BUTTERFLEYE, and 100% of those randomized to aflibercept and 88% of those randomized to laser received treatment in FIREFLEYE. Six infants in BUTTERFLEYE and 5 infants in FIREFLEYE randomized to laser were withdrawn by parent/guardian before receiving open-label treatment; only 1 infant assigned to aflibercept in BUTTERFLEYE was withdrawn by the parent/guardian prior to treatment.

In BUTTERFLEYE, 93% of aflibercept-treated infants and 79% of laser-treated infants completed the study through 52 weeks CA. In FIREFLEYE, 88% of aflibercept-treated infants and 79% of laser-treated infants completed the study through 52 weeks CA. These completion

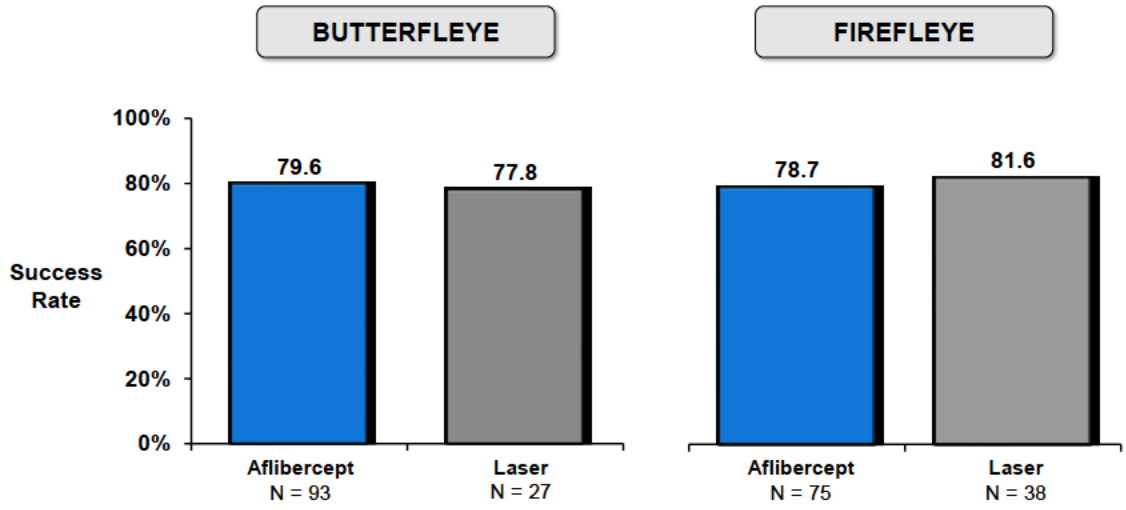
rates are notable, as the studies overcame regulatory and clinical operational challenges of recruiting preterm participants from an orphan disease population and conducting retina evaluations in person during the global Coronavirus Disease 2019 (COVID-19) pandemic.

1.5. Efficacy Findings

1.5.1. Primary Endpoint Results

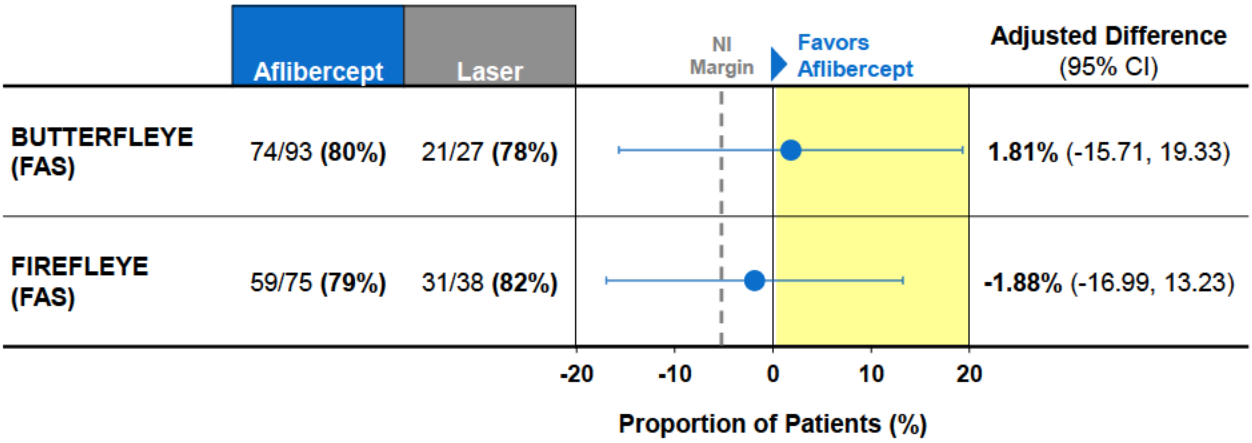
Results of the primary endpoint – proportion of infants with absence of active ROP and unfavorable structural outcomes, without being rescued by another treatment modality, at Week 52 CA – for both studies are shown in Figure 1.

Figure 1: Proportion of Infants without Active ROP and Unfavorable Structural Outcomes BUTTERFLEYE and FIREFLEYE



Numerically, the response rates for aflibercept were similar to laser, but neither study met the prespecified non-inferiority threshold of 5% for the lower bound of the 95.1% CI of the primary endpoint analysis (Figure 2).

Figure 2: Forest Plot of Primary Endpoint Results for BUTTERFLEYE and FIREFLEYE (FAS)

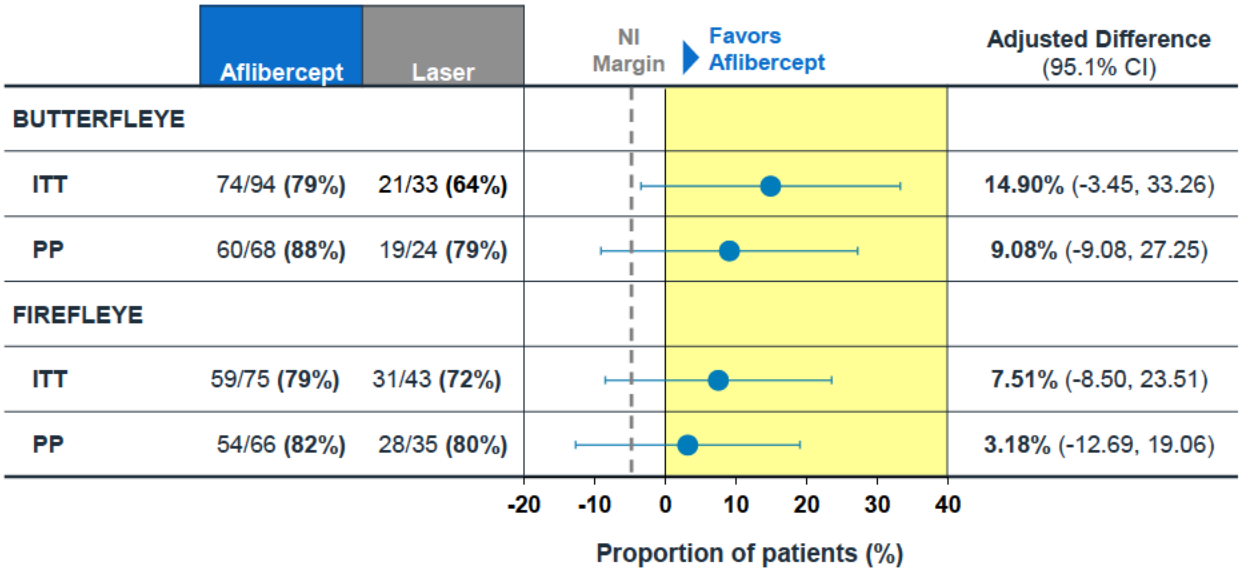


Abbreviations: CI=confidence interval; FAS=Full Analysis Set; NI=non-inferiority.

The response rate seen with aflibercept is consistent with that seen in other randomized-controlled trials on anti-VEGF therapy for the treatment of ROP (Mintz-Hittner, 2011; Stahl, 2019). In the laser groups in BUTTERFLEYE and FIREFLEYE, 78% and 82% of infants met the primary endpoint, respectively, which is remarkably higher than previously assumed based on published data from the RAINBOW study, which reported a response rate of 66% in the laser group (Stahl, 2019). The laser success rates seen in BUTTERFLEYE and FIREFLEYE were higher than the upper bound of the 95% CI for the laser success rate in RAINBOW, suggesting that the constancy assumption of the active control group was violated (Food and Drug Administration 2016) and making the prespecified non-inferiority margin more conservative than originally planned. No substantial changes in the laser technology used since the RAINBOW study was conducted are noted. However, the treating investigators in the aflibercept studies were very experienced in the treatment paradigms involving ROP, including laser photocoagulation, and these studies mandated wide-field imaging of the retina using fundus photography, which aided the treating physicians for clinical confirmation of complete administration of laser and helped identify "skip" areas of the retina that were missed in the initial laser session that could be treated with an additional laser application.

Prespecified sensitivity analyses were conducted using the All Randomized (Intent-to-treat [ITT]) population and the Per Protocol (PP) population and showed similar results to the Full analysis set (FAS) Population (Figure 3).

Figure 3: Forest Plot of Sensitivity Analyses of Primary Efficacy in BUTTERFLEYE and FIREFLEYE

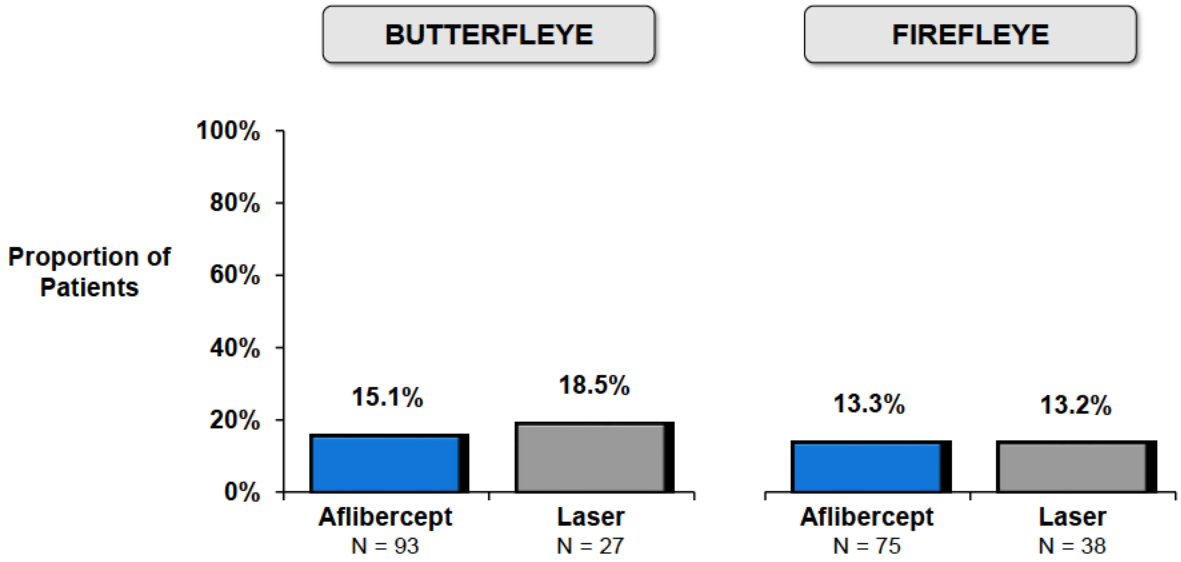


Abbreviations: ITT=intent-to-treat; PP=per protocol; CI=confidence interval; NI=non-inferiority margin.

1.5.2. Secondary Endpoint Results

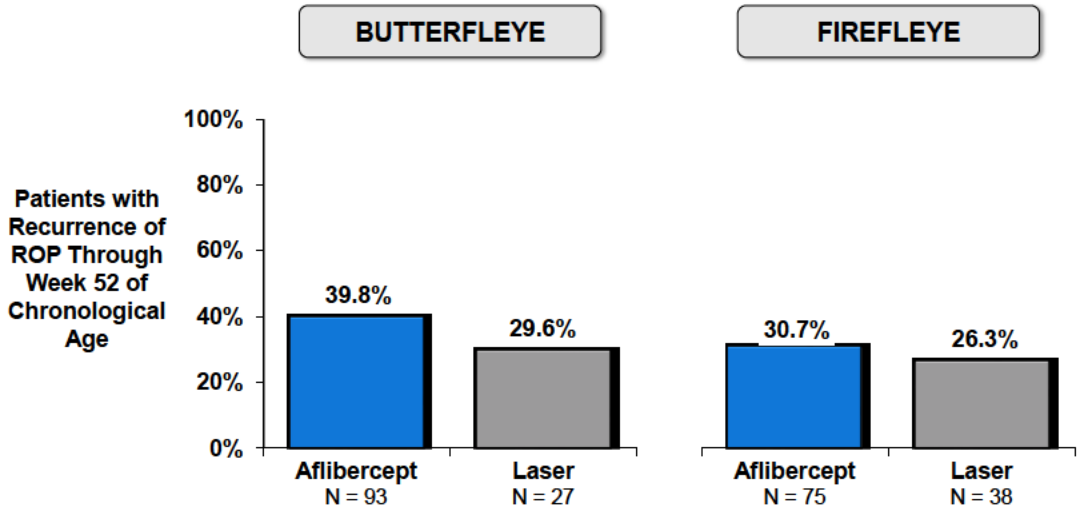
The proportion of infants in either treatment group requiring intervention with a second treatment modality through 52 weeks was comparable in the aflibercept group and the laser group in both studies, with an adjusted difference (95.1% CI): -3.66% (-19.86%, 12.54%) in BUTTERFLEYE and -0.37% (-13.57%, 12.83%) in FIREFLEYE (Figure 4). Second treatment modality was defined as laser treatment for the aflibercept group and aflibercept treatment for the laser group, as well as anti-VEGF agents other than aflibercept or any surgical intervention for the management of ROP complications for either group.

Figure 4: Proportion of Infants Requiring Intervention with Second Treatment Modality in BUTTERFLEYE and FIREFLEYE



The proportion of infants with recurrence of ROP (regardless of the need for additional treatment with either the randomized therapy or with a rescue therapy) was higher in the aflibercept groups than the laser groups (Figure 5). These results were not unexpected since aflibercept has a short pharmacologic half-life compared to the permanent effects of laser. Therefore, ROP could recur in the aflibercept group even if it was initially effective, whereas in the laser group, recurrence would more likely reflect failure to adequately ablate the avascular portion of the retina. The mean time to recurrence of ROP was 154.9 days for the aflibercept group and 123.9 days for the laser group in BUTTERFLEYE and 100.4 days for the aflibercept group and 60.3 days for the laser group in FIREFLEYE.

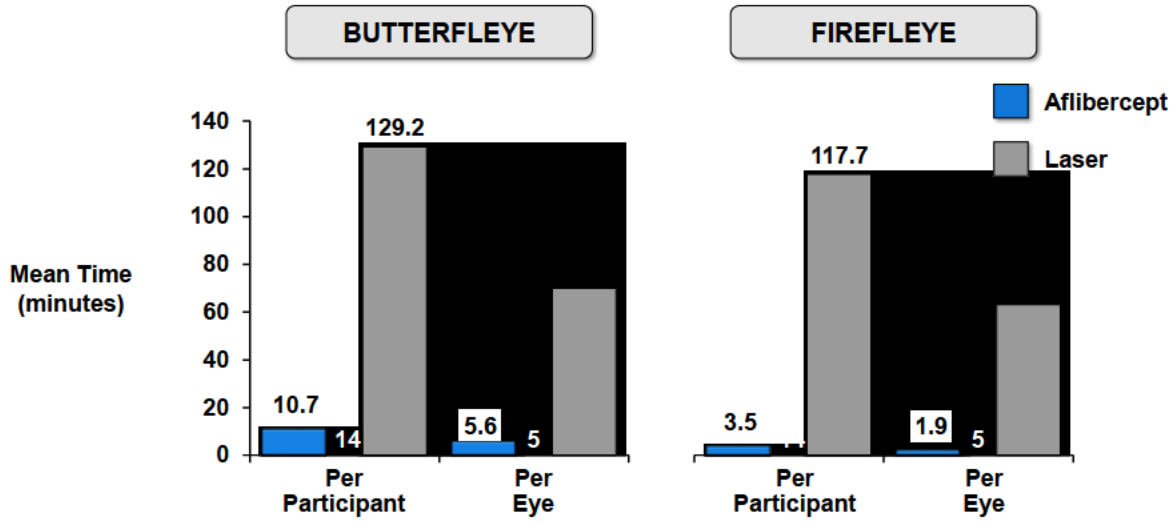
Figure 5: Proportion of Infants with Recurrence of Retinopathy of Prematurity in BUTTERFLEYE and FIREFLEYE



1.5.3. Exploratory Endpoint Results

Fewer infants in the aflibercept groups in both studies (47% and 69% in BUTTERFLEYE and FIREFLEYE, respectively) required sedation or anesthesia compared to the laser groups (78% and 87% in BUTTERFLEYE and FIREFLEYE, respectively) (Table 12 and Table 21). The time required to carry out the aflibercept treatment was also shorter than the time required to perform the laser treatment (Figure 6). Such a short amount of time taken to perform aflibercept treatment may have a significantly positive impact in reducing the time under sedation/anesthesia and the treatment burden in a very vulnerable patient population.

Figure 6: Time Required to Complete Study Procedure in BUTTERFLEYE and FIREFLEYE



1.6. Safety Findings

As demonstrated by the pooled safety analysis of BUTTERFLEYE and FIREFLEYE, aflibercept was well tolerated and demonstrated a favorable safety profile, with mostly mild and transient TEAEs and no new safety findings.

1.6.1. Exposure

Across studies, 168 infants were exposed to aflibercept and 65 received laser (Table 24). The majority (92%) of infants in both groups were treated bilaterally. The majority of infants in the aflibercept group (73% of infants and 83% of eyes) only received a single aflibercept injection per eye. In the laser group, 19% of infants in BUTTERFLEYE and 11% of infants in FIREFLEYE required > 1 laser session in the first week (which counted as initial treatment), and 4% of infants in BUTTERFLEYE and 11% of infants in FIREFLEYE received laser retreatment after Week 1. The majority of infants received no more than 2 repeat laser treatments after the end of the first week of treatment initiation.

Importantly, aflibercept has minimal short-term systemic exposure. Systemic concentrations of free aflibercept were measured in the FIREFLEYE study, with only 1 infant having a detectable concentration of free aflibercept beyond Day 28.

1.6.2. Overview of Adverse Events

A TEAE is defined as any adverse event experienced within 30 days of last treatment administration including rescue treatment. Overall, infants treated with aflibercept had a similar frequency of TEAEs compared with those treated with laser in both studies (Table 3). Fewer infants in the aflibercept group experienced treatment-emergent serious adverse events (TE SAEs) than the laser group. There were 4 deaths reported across both studies; all deaths occurred in infants in the aflibercept group, and none were considered related to treatment by the investigator. Of the 4 deaths, 2 were treatment-emergent and 2 occurred outside of the treatment period and were not considered treatment-emergent (details provided in Section 7.7 and Appendix 11.2). For context, the death rate in the RAINBOW study was 5% (Stahl, 2019).

Table 3: Summary of Treatment-Emergent Adverse Events

	BUTTERFLEYE		FIREFLEYE	
	Aflibercept N=93	**Laser N=27**	**Aflibercept N=75**	**Laser N=38**
Any AE	69 (74.2)	23 (85.2)	71 (94.7)	35 (92.1)
Any TEAE	52 (55.9)	16 (59.3)	57 (76.0)	29 (76.3)
Ocular	17 (18.3)	7 (25.9)	29 (38.7)	14 (36.8)
Non-Ocular	44 (47.3)	14 (51.9)	40 (53.3)	25 (65.8)
TEAE Leading to discontinuation	0	0	3 (4.0)	1 (2.6)
SAE	32 (34.4)	12 (44.4)	25 (33.3)	17 (44.7)
TE SAE	18 (19.4)	5 (18.5)	9 (12.0)	10 (26.3)
Ocular	6 (6.5)	3 (11.1)	6 (8.0)	3 (7.9)
Non-Ocular	12 (12.9)	2 (7.4)	5 (6.7)	7 (18.4)
AE Resulting in Death*	1 (1%)	0	3 (4%)*	0

Abbreviations: AE=adverse event; TEAE=treatment-emergent adverse event; TE SAE=treatment-emergent serious adverse event. Treatment-emergent defined as occurring within 30 days of the last treatment with a randomized therapy.
[a] Includes all AEs including TEAEs
[b] Two deaths occurred within 30 days of last treatment.

1.6.3. Ocular Adverse Events

Infants treated with aflibercept experienced fewer ocular TEAEs than infants treated with laser therapy (details provided in Section 7.3). The most common ocular TEAEs for aflibercept vs laser were retinal detachment, retinal hemorrhage, and conjunctival hemorrhage, all of which occurred in $\leq$ 7% of infants in either group. Retinal detachment is a known complication of ROP and is considered an unfavorable structural outcome. Importantly, most ocular TEAEs were reported in only 1 infant in either treatment group.

Ocular TE SAEs were reported at equal or lower rates in the aflibercept groups across both studies (Table 28). Aflibercept treatment was well tolerated with few TEAEs leading to discontinuation (Section 7.3.2).

1.6.4. Non-Ocular Adverse Events

Non-ocular TEAEs occurred with a lower frequency in infants treated with aflibercept compared to laser therapy (Table 29). Most non-ocular TEAEs were consistent with underlying prematurity and included apnea/infantile apnea, bronchopulmonary dysplasia, inguinal hernia, and umbilical hernia. No non-ocular TEAEs led to treatment discontinuation, and a similar proportion of infants experienced a serious non-ocular TEAE in the aflibercept and laser groups (details provided in Section 7.4.3).

1.7. Benefit-Risk Summary

Aflibercept offers meaningful clinical and practical benefits to treat preterm infants with Type 1 ROP, a potentially severe vision-impairing disease. The principle of targeting the pathologic overexpression of VEGF in ROP with IVT aflibercept is reflected by the current off-label use of anti-VEGF treatment. Aflibercept provides a safe and effective treatment for ROP offering physicians and families an expedient option to retinal ablating laser surgery, which is associated with long-term risks of high myopia and irreversible peripheral visual field deficits. While anti-VEGF treatments are extensively used off-label, FDA approval would provide benefits including improved access, guidance for prescribers and parents/caregivers through labeling, ongoing monitoring of benefit and risk through pharmacovigilance and FDA oversight.

The aflibercept clinical development program, including the randomized, controlled studies BUTTERFLEYE and FIREFLEYE, provides evidence of the clinical benefit, safety and tolerability of aflibercept 0.4 mg for the treatment of ROP. Treatment with aflibercept 0.4 mg was associated with a response rate of 80% in BUTTERFLEYE and 79% in FIREFLEYE and meaningful benefits on clinically relevant ocular outcomes in preterm infants with ROP (absence of active ROP and unfavorable structural outcomes at 52 weeks CA). The evidence suggests a lower treatment burden for the patient and prescriber with a shorter time required for treatment administration and reduced need for general anesthesia.

Aflibercept was well tolerated in BUTTERFLEYE and FIREFLEYE studies, and treatment discontinuation rates were low (≤ 4%). TEAEs were generally mild or moderate in severity and transient. Risks of aflibercept treatment include the ocular events (retinal detachment, retinal hemorrhage, conjunctival hemorrhage, and eyelid edema) which are known from use of anti-VEGF agents and are mainly related to the IVT injection procedure. The risks are adequately described in the prescribing information for IVT aflibercept. Long-term safety data are being collected in the ongoing FIREFLEYE NEXT and BUTTERFLEYE studies where infants are followed-up through 5 years CA.

While the studies did not establish statistical non-inferiority, they also did not suggest that aflibercept was inferior to laser photocoagulation. The differences between aflibercept and laser were +1.81% in BUTTERFLEYE, 95.1% CI: [-15.71%, +19.33%], and -1.88% in FIREFLEYE, 95.1% CI: [-16.99%, +13.23%]). Thus, while non-inferiority based on the prespecified margin cannot be concluded statistically, no statistical conclusion can be made due to the fact that each study's point estimate flanks no difference. According to the FDA guidance "Non-Inferiority Clinical Trials to Establish Effectiveness," in such a situation the recommendation is to utilize clinical judgement on the benefit/risk of the different therapies (Food and Drug Administration 2016). Laser photocoagulation has known long-term complications based on its destructive

mechanism of action, including a > 50% rate of high myopia as well as diminished peripheral vision (Mutlu, 2013). Any reduction in the rate of these long-term complications would provide meaningful benefits to children who would have otherwise been treated with laser. FDA approval of aflibercept would provide clinicians with a treatment option in addition to laser that would successfully regress ROP and prevent unfavorable structural outcomes in a majority of patients.

Additionally, aflibercept has clinical utility in other scenarios, such as:

- In unstable preterm infants where transfer to another NICU/hospital may not be feasible or advisable.
- In stable preterm infants that cannot be treated with laser photocoagulation for logistical reasons (eg, no access to laser treatment within the 72-hour treatment window).
- Use as a retina-sparing modality which may delay laser administration until normal retinal vessels have grown closer to the periphery of the retina, thereby reducing the amount of laser required.

Therefore, based on the totality of evidence supporting aflibercept for the treatment of patients with severe ROP requiring treatment, the expected clinical benefits outweigh the potential risks and support that aflibercept be approved in ROP as an option for patients alongside the sole currently approved option: laser photocoagulation therapy.

2. BACKGROUND ON RETINOPATHY OF PREMATURITY (ROP)

Summary

- ROP is a rare, potentially vision-impairing and blinding retinal disease impacting preterm infants.
- Laser photocoagulation therapy is the standard of care for ROP; there are no pharmacologic agents currently FDA approved in the US to treat ROP.
- Laser photocoagulation is effective but comes with challenges, including the requirement for prolonged sedation or anesthesia, loss of peripheral vision, and potential risk of high myopia.
- An alternative, approved, simpler to administer treatment option with comparable efficacy and safety to laser would be an important advance in the treatment of ROP.

2.1. Overview of ROP

ROP is a vasoproliferative pathologic process that occurs in the incompletely vascularized, developing retina of low birth weight preterm neonates.

The pathogenesis involves 2 discrete phases: Phase 1 occurs from roughly 22 to 30 weeks post-menstrual age, and Phase 2 from roughly 31 to 44 weeks post-menstrual age. Phase 1 involves relative hyperoxia and decreased VEGF levels, whereas Phase 2 involves relative hypoxia and increased VEGF levels (Mintz-Hittner, 2011).

Incidence and severity of ROP were found to rise with degree of prematurity at birth, with low GA and low birth weight being the main risk factors (Fierson, 2013; Hartnett, 2012). The vascular changes of ROP may be mild and regress completely with time without major long-term sequelae or may increase in severity and lead to macular dragging, total retinal detachment, severe visual impairment, and lifelong blindness (Hardy, 2004).

While the overall incidence of ROP among all live births is 1%–2% (Chiang, 2004; Hellstrom, 2013), in NICUs where the infant population is typically more premature (GA $\leq$ 32 weeks), the overall incidence of ROP is approximately 36% of all infants screened for ROP (Blencowe, 2013).

2.1.1. Epidemiology

Of the approximately 28,000 infants born weighing $\leq$ 2.75 lbs (approximately 1,250 g) annually in the US, approximately 14,000–16,000 are affected by some degree of ROP. The majority (approximately 90%) of these infants have mild ROP that improves without treatment and leaves no permanent damage; however, approximately 1,100–1,500 infants annually in the US develop ROP that is severe enough to require medical treatment (The National Eye Institute 2014).

If severe ROP is left untreated or treatment is delayed, the disease will further progress. Potential complications include unfavorable structural outcomes including retinal detachment, which is associated with severe visual impairment or loss of vision. ROP is one of the top 3 leading causes of childhood blindness worldwide (Chiang, 2004; Hellstrom, 2013). The prevalence of

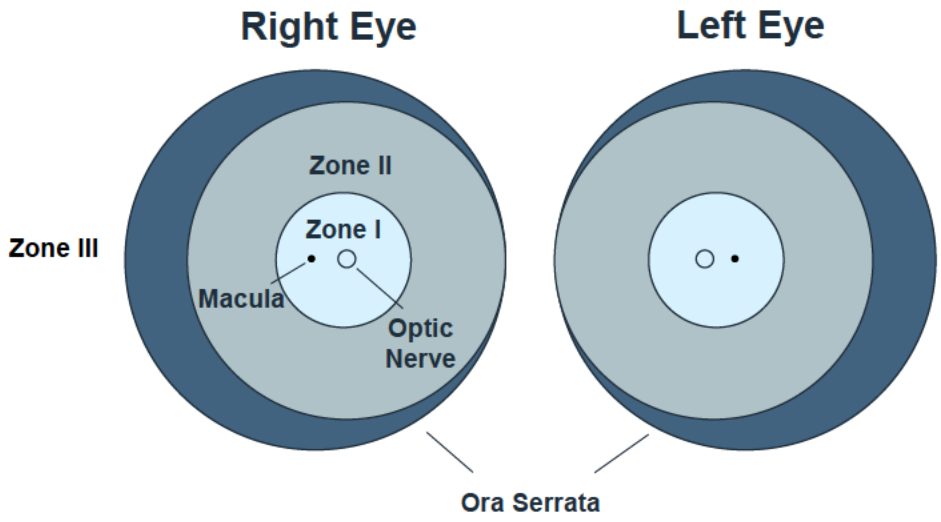
childhood blindness caused by ROP in developed nations ranges between 6% and 18%, while in developing nations the estimate is greater than 20% (Gilbert, 1997; Hartnett, 2014).

2.1.2. Diagnosis and Classification

According to the ICROP (International Committee for the Classification of Retinopathy of Prematurity 2005), the main features for the classification of ROP are:

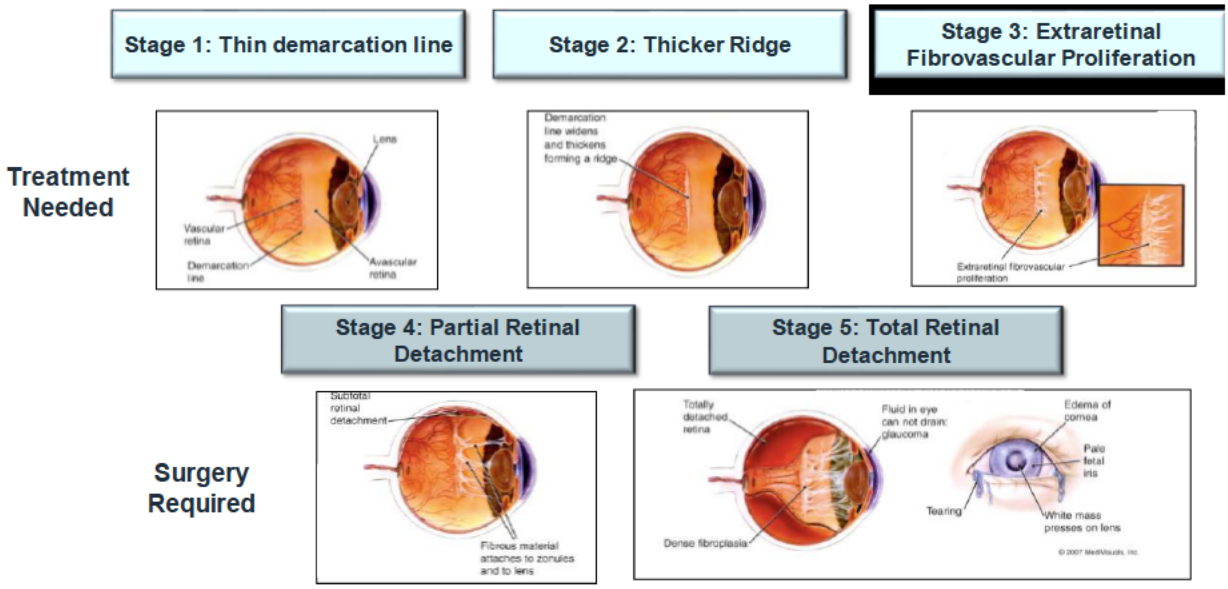
- Location of retinal involvement (with 3 concentric zones centered on the optic nerve as shown in Figure 7)
- Extent of circumferential disease (measured in number of clock hours, as illustrated in Figure 7)
- Stage of severity (Stage 1 through 5 as described in Section 1.2 and depicted in Figure 8)
- Presence of plus disease (characterized by venous dilatation and arteriolar tortuosity of the posterior retinal vessels in at least 2 quadrants and indicating more aggressive course at any stage)

Figure 7: Zones and Clock Hours to Describe the Location and Extent of ROP Based on the International Classification of ROP



Source: The International Classification of Retinopathy of Prematurity revisited, 2005

Figure 8: Stages of ROP Severity



Source: International Committee for the Classification of Retinopathy of Prematurity, 2005

A subtype called AP-ROP is an uncommon severe form of ROP characterized by posterior location, prominence of plus disease, and extremely intense vascular activation. If untreated, AP-ROP shows rapid progression (over a few days) to advanced stages.

2.2. Current Treatment Options for ROP

2.2.1. ROP Treatment Guidelines

US treatment guidelines for ROP recommend that all infants with a birth weight of ≤ 1,500 g or GA of ≤ 30 weeks and selected infants who are believed to be at risk for ROP (such as infants with hypotension requiring inotropic support, infants who received oxygen supplementation for more than a few days, or infants who received oxygen without saturation monitoring) should be screened for ROP. The ICROP (described in Section 2.1.2) should be used to classify, diagram, and record retinal findings at the time of examination.

According to the guidelines, the presence of plus disease in Zones I or II indicates that treatment, rather than observation, is appropriate (Early Treatment for Retinopathy of Prematurity Cooperative Group 2003; Hutchinson 1998). Treatment should be initiated for the following retinal findings that characterize Type 1 ROP:

- Zone I ROP: any stage with plus disease
- Zone I ROP: stage 3, no plus disease
- Zone II: stage 2 or 3 with plus disease

To minimize the risk of retinal detachment, treatment should generally be delivered within 72 hours of determination of the presence of treatable disease.

The treatment guidelines state that laser photocoagulation is currently the preferred method of ablation for ROP, and while not currently FDA-approved, "anti-VEGF treatment may hold great promise in the treatment of type 1 ROP" (Fierson, 2018). According to the guidelines, initial follow-up is recommended 3–7 days after laser photocoagulation or anti-VEGF injection to ensure that there is no need for additional treatment.

During the conduct of the aflibercept ROP studies, ICROP was updated from version 2 (2005) to 3 (2021); however, the guidelines for ROP requiring treatment have not changed as a result of these new guidelines.

2.2.2. Laser Photocoagulation

Laser therapy is considered the standard of care and is the only FDA-cleared treatment for ROP. Laser photocoagulation burns the ischemic peripheral retina where physiological vascularization has not yet developed and reduces metabolic demand and production of factors such as VEGF, thereby preventing progression of ROP lesions.

Laser treatment, while effective, has several important limitations. Laser requires adequate visualization of the retina, which may be compromised in patients with conditions such as hazy cornea. Ensuring complete laser treatment of all ischemic peripheral retina is critical to successful treatment, and incomplete laser or leaving "skip areas" can result in poor outcomes. Laser treatment may fail to achieve a physiological retinal structure in up to 25% of patients with ROP with more central disease (Mintz-Hittner, 2011).

Additionally, laser used for retinal ablation causes scarring and destruction of the evolving retinal tissue in preterm infants, thereby preventing development of normal retinal anatomy. In the BEAT-ROP study described in Section 1.2, more very high myopia was found in eyes that received laser than in eyes that received IVT bevacizumab (Geloneck, 2014). Very high myopia (≥ -8.00 D) occurred in Zone I in 3.8% of eyes that received bevacizumab and 51.4% of eyes that received laser treatment ($p < .001$). Very high myopia occurred in Zone II posterior in 1.7% of eyes that received bevacizumab and 36.4% of eyes that received laser treatment ($p < .001$). More central disease requires greater amounts of laser treatment to manage the condition. Of particular concern in this vulnerable patient population, laser photocoagulation typically requires a prolonged period of general anesthesia or deep sedation.

2.2.3. Off-label Use of Anti-VEGF Therapy

Due to the limitations of laser photocoagulation, clinical interest in alternative treatment options that are more tissue- and function-conserving has steadily increased. Based on the finding that VEGF plays a critical role in the pathophysiology of ROP (Pieh, 2008; Stone, 1996; Young, 1997), off-label drug treatment with IVT injections of VEGF inhibitors (eg, aflibercept, bevacizumab, or ranibizumab) has increased in recent years. No anti-VEGF therapies have been approved by the FDA for ROP. Bevacizumab is the most commonly used off-label anti-VEGF treatment (Prakalapakorn, 2021), although no randomized-controlled trials have been conducted with bevacizumab in this indication. Ranibizumab, a VEGF-A inhibitor, has been approved for the treatment of patients with ROP [Zone I (Stage 1+, 2+, 3 or 3+), Zone II (Stage 3+) or AP-ROP] in the EU and Japan since 2019. Aflibercept was approved in Japan for ROP in September 2022 and received a positive CHMP opinion for the treatment of ROP in the EU.

VEGF inhibitors for the treatment of ROP have been previously investigated in 2 prospective, randomized, laser-controlled studies. BEAT-ROP was the first randomized, laser-controlled clinical trial that evaluated an anti-VEGF agent, bevacizumab (not currently approved for any retinal disease), administered intravitreally in 150 patients with ROP in the US (Mintz-Hittner, 2011). RAINBOW was a prospective, randomized, controlled trial comparing ranibizumab at 2 doses (0.1 mg, 0.2 mg) to laser in 225 patients worldwide. These trials reported similarly positive outcomes with numerically higher response rates for anti-VEGF treatment compared to laser treatment at Week 54 post-menstrual age in BEAT-ROP and Week 24 in RAINBOW, although the prespecified statistical thresholds for success (i.e., superiority to laser) were not consistently reached (Hwang, 2015; Mintz-Hittner, 2011; Stahl, 2019).

Unlike laser, IVT anti-VEGF agents lead to a regression of the disease potentially with no destructive effect on the physiological retinal structure. Additionally, IVT injection procedures are typically completed in several minutes, which is significantly less time than laser, do not typically require general anesthesia, and can usually be administered at the bedside under local anesthesia. This is a critical distinction, considering the typical comorbidity profile in these preterm infants as well as the potential long-term negative sequelae associated with general anesthesia use in infancy and childhood (Reighard, 2022).

2.3. Unmet Medical Need

Despite the severity of ROP, there are no pharmacologic agents currently approved in the US, leaving only laser therapy or off-label anti-VEGF options. An alternative to laser that is less time consuming to administer, minimizes the requirement for and/or duration of general anesthesia or deep sedation, and has comparable efficacy and safety would be an important advance in the treatment of ROP.

3. AFLIBERCEPT PRODUCT DESCRIPTION

Summary

- The proposed indication for aflibercept is the treatment of patients with ROP at a recommended dose of 0.4 mg by IVT injection for up to 3 injections per eye at least 28 days apart.
- Aflibercept is a VEGF inhibitor that inactivates multiple isoforms of VEGF-A and PlGF to reduce endothelial cell proliferation, vascular leakage, and new blood vessel blood formation.

3.1. Proposed Indication and Dosing

The proposed indication for aflibercept is the treatment of patients with ROP. The recommended dose for aflibercept is 0.4 mg (0.01 mL or 10 microliters) administered by IVT injection. Treatment is 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 CA, if there are signs of recurrence. The treatment interval between doses injected into the same eye should be at least 4 weeks (at least 28 days).

3.2. Mechanism of Action

Similar to other anti-VEGF treatments, aflibercept acts as an inhibitor of VEGF. Aflibercept is a recombinantly produced fully-human fusion protein that has a molecular weight of 115,000 Daltons. Aflibercept is a specific blocker that binds and inactivates multiple isoforms of VEGF-A and PlGF. The binding of aflibercept to VEGF-A prevents its interaction with its receptors (VEGFR1 and VEGFR2), and also of PlGF to VEGFR1 on the surface of endothelial cells, reducing endothelial cell proliferation, vascular leakage, and new blood vessel blood formation.

VEGF is an essential growth factor for vascular endothelial cells that is overexpressed in several retinal diseases with neovascularization including ROP in preterm infants (Cao, 2010; Hellstrom, 2016; Krzystolik, 2002; Kwak, 2000; Nork, 2011). VEGF serves as an endothelial cell mitogen, but also promotes endothelial cell migration, survival, and tube formation. VEGF plays an important role not only for physiological vascular development and physiological angiogenesis, but also in promoting pathological angiogenesis, excessive vascular permeability, and pathological neovascularization in patients with ROP.

4. REGULATORY AND DEVELOPMENT HISTORY

Summary

- EYLEA (aflibercept) is FDA approved for several retinal disorders in adults.
- In June 2019, FDA issued a PWR to Regeneron to study aflibercept for the treatment of ROP.
- In July 2019, aflibercept was granted Orphan Drug Designation.
- All study protocols and SAPs were submitted to FDA and approved under a SPA agreement.
- The clinical development program for aflibercept for ROP included Phase 3 interventional studies – BUTTERFLEYE and FIREFLEYE – and their long-term follow-ups – BUTTERFLEYE NEXT and FIREFLEYE NEXT.
- The sBLA Application for EYLEA in ROP and Request of Pediatric Exclusivity Determination were submitted on 11 August 2022, and FDA granted the Pediatric Exclusivity for EYLEA (aflibercept) Injection on 19 October 2022.

4.1. Regulatory Background

The clinical development program for aflibercept for ROP is being conducted to support regulatory approvals worldwide and to fulfill the FDA's PWR which was issued to Regeneron in June 2019 in recognition of the important unmet medical need to produce clinical data for the treatment of this very vulnerable pediatric population. Aflibercept was also granted Orphan Drug Designation in July 2019. Study protocols and SAPs were submitted and agreed with the FDA under a SPA agreement. Regeneron submitted the sBLA for aflibercept for the treatment of ROP and in fulfillment of all terms outlined in the PWR in August 2022.

4.1.1. Currently Approved Indications for Aflibercept

In adult patients, EYLEA (aflibercept) 2 mg (in an injection volume of 0.05 mL) is approved and commercialized for the treatment of several ophthalmological diseases (primarily retinal diseases). The first marketing authorization for EYLEA was granted in the US in November 2011 for the indication of nAMD. EYLEA is currently authorized to be marketed in more than 113 countries. EYLEA's authorized indications in the US are for the treatment of adults with nAMD, macular edema following retinal vein occlusion (MEfRVO [central retinal vein occlusion and branch retinal vein occlusion]), diabetic macular edema (DME), and diabetic retinopathy (DR). Outside of the US, EYLEA has additionally been approved for myopic choroidal neovascularization (mCNV) and neovascular glaucoma (indication approved only in Japan).

Aflibercept 0.4 mg has received marketing approval for the treatment of ROP in Japan, a positive CHMP opinion for the treatment of ROP in the EU, and is under review in the United Kingdom, Switzerland, and Brazil.

4.1.2. Framework for Regulatory Approval in ROP

The totality of the data from aflibercept ROP studies fully meets the requirements outlined in the PWR issued by the FDA in June 2019, including but not limited to the following:

- 2 studies, each being a "randomized, parallel group, controlled study of at least 52 weeks duration with a 5-year follow-up and include an assessment of retinal photographs. Submission of Week 52 data is required to meet the terms of the Written Request. Five-year follow-up must be submitted to the US FDA but is not required to meet the terms of their Written Request."
- Patients to be studied:
  - Age group in which studies will be performed: Preterm infants with ROP
  - Total number of patients to be studied: In total (combined from both studies), the clinical studies will study at least 150 preterm infants with ROP who have been treated with aflibercept and followed for at least 52 weeks after birth.
- Study endpoints: The primary efficacy endpoint will be the absence of active ROP and absence of unfavorable structural outcomes at Week 52 following birth (eg, retinal detachment) and must be assessed by visualization of the retina (photographic and/or directly by investigators). Safety outcomes must include the collection of adverse experiences as outlined in the agreed-upon protocol.
- Statistical information, including power of studies and statistical assessments:
  - Patients should be randomized between active treatment and an active standard of care control. The study design may be either a superiority design or a non-inferiority design compared to an established standard of care. The SAPs must be submitted and agreed upon by the Division and include specifications for handling missing data. Demographic characteristics and adverse experiences should be summarized descriptively and compared for each treatment group.
- The aflibercept ROP studies were conducted under a SPA agreement with FDA.
- Notice that Regeneron had met the requirements of the PWR and received extension of pediatric exclusivity on 19 October 2022.

4.2. Clinical Development Program

The clinical development program for aflibercept for ROP consists of two Phase 3 interventional studies and their respective 5-year long-term follow-up studies (Table 4). In these studies, a combined 168 preterm infants with ROP were randomized to aflibercept and treated with the 0.4 mg dose from the time of first administration up to 52 weeks CA, of which a combined 153 infants (87 infants from BUTTERFLEYE and 66 infants from Study FIREFLEYE/FIRELEYE NEXT) reached the 52-week CA endpoints. Infants will be followed through 5 years CA to assess long-term safety, efficacy, and developmental outcomes.

Table 4: Overview of Studies in Aflibercept Pediatric Clinical Development Program

Study Number	Study Description
BUTTERFLEYE (Study 1920) *Ongoing*	• Phase 3, open-label, randomized, two-arm study to assess the efficacy, safety, and tolerability of IVT aflibercept compared to laser photocoagulation in treatment-naïve infants with ROP at 52 weeks CA. Infants must have been ≤ 32 weeks GA at birth or birth weight ≤ 1500 g and had to weigh > 800 g on the day of treatment. • Study duration: up to 52 weeks CA. • 3:1 randomization stratified by ROP classification in Zone I, Zone II, or AP-ROP. • Primary endpoint: Proportion of infants with absence of active ROP and unfavorable structural outcomes at 52 weeks CA, as determined by the investigator, after starting study treatment. • FAS Population: 120 infants (93 aflibercept, 27 laser).
BUTTERFLEYE NEXT (Study 2036) *Ongoing*	• Phase 3b observational study to evaluate the long-term outcomes of infants who received treatment for ROP in BUTTERFLEYE. • Follow-up to 5 years CA.
FIREFLEYE (Study 20090) *Completed*	• Phase 3, open-label, randomized, two-arm study to assess the efficacy, safety, and tolerability of IVT aflibercept compared to laser photocoagulation in treatment-naïve infants with ROP (GA at birth ≤ 32 weeks or birth weight ≤ 1500 g). • Study duration: 24 weeks (or up to Week 27 in the event of follow-up visits for infants treated after Week 21). • Primary endpoint: Proportion of infants with absence of active ROP and unfavorable structural outcomes at 24 weeks after starting study treatment. • FAS Population: 113 infants (75 aflibercept, 38 laser).
FIREFLEYE NEXT (Study 20275) *Ongoing*	• Phase 3b observational study to evaluate the long-term outcomes of infants who received treatment for ROP in FIREFLEYE. • Follow-up to 5 years CA. • For the report with data at 52 weeks CA: 100 infants (66 aflibercept, 34 laser).

Abbreviations: AP-ROP=Aggressive posterior retinopathy of prematurity; CA=chronological age; FAS=full analysis set; GA=gestational age; IVT=intravitreal; ROP=retinopathy of prematurity.

5. CLINICAL PHARMACOLOGY

Summary

- The pharmacokinetics (PK), pharmacodynamics (PD), and immunogenicity of aflibercept were assessed in pediatric patients with ROP who received IVT dose of 0.4 mg aflibercept (per eye) either unilaterally or bilaterally.
- The proposed pediatric dose is equivalent to 20% of the adult dose of 2 mg and supported by evidence in the literature where doses of up to 1 mg have been used.
- There was no evidence of treatment-related changes in blood pressure (BP).
  - Increases in systolic blood pressure (SBP) and diastolic blood pressure (DBP) were similar across the aflibercept and laser treatment groups and aligned with expectations for organ maturation and body weight gain.
  - An exploratory PK/PD analysis revealed no difference in SBP or DBP between treatment groups.

5.1. Pharmacokinetics

In comparison to aflibercept exposure in adults, mean concentrations of free and adjusted bound aflibercept in plasma in BUTTERFLEYE and FIREFLEYE/FIRELEYE NEXT were higher than those in adults with nAMD who received a 2 mg IVT dose (in one eye; Study VGFT-OD-0702), but generally lower than those observed following intravenous (IV) administration of 1 mg/kg (Study VGFT-OD-0305), a dose that was associated with a change from baseline in 24 hour mean SBP and DBP of approximately 5 mmHg (Study PDY6656 in healthy volunteers; Table 5).

Table 5: Maximum Concentration (ng/ml) of Free and Adjusted Bound Aflibercept in Plasma in Preterm and Adult Patients

Parameter	Adults			Preterm	
	VGFT-OD-0702 (n=6)	VGFT-OD-0305 (n=7)	PDY6656 (n=12)	FIREFLEYE (n=75)	BUTTERFLEYE (n=84)
Dose	2 mg IVT	1 mg/kg IV	1 mg/kg IV	0.4 mg IVT	0.4 mg IVT
C_{max} Free aflibercept, Mean (range)	19.3 (0–54.0)	14,300 (10,300–17,300)[a]	18,200 (13,000–24,700)	481 (< LLOQ–4,570)	583 (0–5,760)
C_{max} Adjusted bound aflibercept, Mean (range)	186 (100–286)	1,790 (1120–3,480)[a]	1,210 (989–1,540)	1,336 (< LLOQ–5,887)	1,030 (0–3,370)

Abbreviations: IVT= intravitreal; IV: intravenous; LLOQ=lower limit of quantitation.
[a] within first dosing interval.

5.2. Pharmacodynamics

Increased BP is a known PD marker of systemic anti-VEGF effects in adults. In BUTTERFLEYE and FIREFLEYE, BP was measured prior to any blood sample collections, and if possible, prior to any other intervention, from screening up to Week 52 CA. Mean SBP and DBP in BUTTERFLEYE and FIREFLEYE are presented in Section 7.5.1. The relationship between BP and aflibercept concentrations in plasma was evaluated for the first 4 weeks after the start of treatment in both studies. There was no evidence of an exposure-response relationship with increasing concentrations of free aflibercept and change from baseline in SBP or DBP.

Exploratory analysis of the influence of GA on the change from baseline in SBP and DBP revealed no relevant differences between aflibercept and laser treatment. Similarly, the relationship of baseline body weight and change from baseline in SBP and DBP provided no relevant differences between aflibercept and laser treatment.

In summary, against the background of challenging measurement conditions in the NICU setting, and despite a high variability of BP values in both treatment groups, there were no discernible differences in change in BP between infants treated with aflibercept IVT versus laser. The increases of BP over time were reflective of organ maturation and body weight gain in the range expected for this population of preterm infants.

5.3. Rationale for Dose Selection

The existing aflibercept formulation, 40 mg/mL vial, is considered suitable for treating preterm infants with ROP. The application of 10 µL is equivalent to 20% of the volume applied to an adult (50 µL). The adult eye has approximately 4 mL of vitreous volume, while preterm infant eyes have a vitreous volume of approximately 1 mL (Hartnett, 2015). Thus, excipient and aflibercept concentrations in the vitreous after IVT injection will be similar in preterm infants and adults.

Available clinical data (Salman, 2015; Sidorenko, 2018; Sukgen, 2019) consistently show promising efficacy, with no identification of major safety concerns, when using aflibercept pediatric doses ranging from 0.4 mg to 1 mg per eye (ie, 20%–50% of the 2 mg dose approved for adults). In order to limit drug exposure in this vulnerable pediatric population, the lowest dose reported (0.4 mg), which also showed positive efficacy, was selected for the studies.

The proposed dose - a single dose of 0.4 mg (in an injection volume of 0.01 mL) per injection and eye, with up to 2 additional injections per eye based on prespecified retreatment criteria - was used in the Phase 3 studies, which demonstrated clinically meaningful efficacy and a positive safety profile in infants with ROP.

6. CLINICAL EFFICACY

Summary

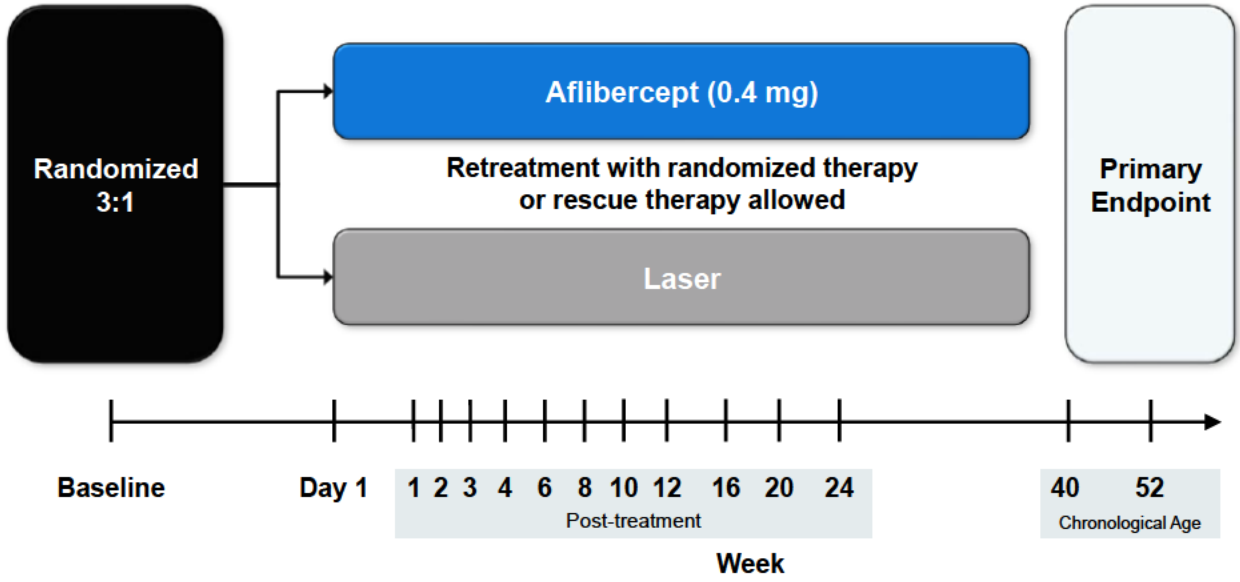
- The efficacy data demonstrate that aflibercept provides a clinically meaningful benefit in the treatment of ROP in preterm infants, with less treatment burden than laser therapy.
- BUTTERFLEYE and FIREFLEYE were very similar Phase 3, multicenter, randomized, 2-arm, open-label studies that assessed the efficacy and safety of IVT aflibercept 0.4 mg versus laser.
- In both studies, more infants completed aflibercept treatment than laser therapy at 52 weeks CA (93% vs 79% in BUTTERFLEYE, respectively, and 88% and 79% in FIREFLEYE, respectively).
- The primary endpoint for each study did not reach the prespecified non-inferiority threshold of -5% for the lower bound of the 95.1% CI in the FAS; however, the results observed are meaningful with successful outcomes numerically similar (77.8–81.6%) between arms and studies.
  - Importantly, the laser groups in BUTTERFLEYE and FIREFLEYE exceeded historic expectations from the RAINBOW study, which was the basis for the prespecified 5% non-inferiority margin.
- Comparable proportions of infants in both studies required a second treatment modality.
- Less ROP recurrence was observed with laser treatment compared to aflibercept, with an adjusted difference of 10% in BUTTERFLEYE and 3.6% in FIREFLEYE, which was expected per the mechanism of action of each treatment.
- Compared to laser-treated infants, fewer aflibercept-treated infants needed sedation or anesthesia in both studies, and the mean time to perform the aflibercept injection was much shorter than the time to perform the laser treatment (≤ 11 minutes for aflibercept and ≥ 110 minutes for laser), which is particularly meaningful for these vulnerable patients.

6.1. BUTTERFLEYE (Study VGFTe-ROP-1920)

6.1.1. Study Design

BUTTERFLEYE was a global, Phase 3, multicenter, randomized, open-label study to assess the efficacy, safety, and tolerability of IVT aflibercept versus laser in infants with ROP. Eligible patients were randomized (3:1) to IVT injection of aflibercept 0.4 mg or laser at 39 study centers in 10 countries. The study consisted of a screening/baseline, a treatment period (including potential retreatment and rescue treatment), and a final visit at Week 52 (CA; Figure 9).

Figure 9: BUTTERFLEYE Study Design



Eligibility and Randomization

Eligible patients included male and female preterm infants with treatment-naïve ROP Zone I Stage 1 plus, 2 plus, 3 non-plus or plus, or Zone II Stage 2 plus or 3 plus, or AP-ROP according to the ICROP 2005 in at least 1 eye.

One or both eyes could have been treated per investigator's assessment of the study's eligibility criteria. In the case of treatment-requiring unilateral disease, the non-study eye was kept under observation according to the local ROP screening guidelines or at every study visit, whichever was more frequent. Non-study eyes that developed ROP requiring treatment during the study received treatment according to the randomization assignment of the initial study eye. If a patient's second eye qualified for treatment during the study, after the retreatment/rescue treatment schedule was completed for the second eye, both eyes could follow the visit schedule for the first eye.

Study Interventions Administered

Infants randomized to aflibercept received a single IVT injection of aflibercept 0.4 mg/10 µL per eligible eye at baseline. Thereafter, if required, up to 2 additional IVT injections of aflibercept 0.4 mg/10 µL could have been administered at least 4 weeks apart in each treatment-requiring eye when both of the prespecified retreatment criteria were met (presence of ROP requiring treatment and the interval since the last aflibercept IVT injection was ≥ 28 days).

For infants randomized to aflibercept, rescue treatment with laser was permitted if 1 of the prespecified criteria were met: worsening of ROP compared to the examination before the previous injection during the 27 days following that IVT aflibercept injection OR presence of ROP requiring treatment after the infant already received a total of 3 aflibercept injections.

For infants randomized to laser treatment, transpupillary conventional laser ablative therapy was conducted per standard of care at the investigational site. In case multiple laser sessions were necessary within 1 week from baseline, they were counted as a single treatment. If additional

laser treatment was administered after 1 week from baseline treatment, it was counted as a laser retreatment.

Rescue treatment with aflibercept was allowed if the fundus examination revealed laser treatment was complete and if 1 of the prespecified criteria were met: worsening of ROP compared to the most recent pre-laser examination OR persistence of ROP requiring treatment.

6.1.1.1. Key Enrollment Criteria

Patients had to meet the following criteria at screening and baseline to be eligible for inclusion in the study:

1. GA at birth ≤ 32 weeks or birth weight ≤ 1500 g
2. Patients with treatment-naïve ROP classified according to the ICROP in at least one eye as:
   - Zone I Stage 1 plus, or 2 plus, or 3 non-plus or 3 plus, or
   - Zone II Stage 2 plus or 3 plus, or
   - AP-ROP
3. Weight at baseline (day of treatment) ≥ 800 g (Note that at this minimum weight, a patient in the aflibercept group would receive 1 mg/kg when dosed bilaterally. This dose was predicted to remain below limits determined in adult nAMD patients after IV dosing aflibercept of a maximum tolerated dose of 1 mg/kg.)

Patients who met any of the following criteria were excluded from the study:

1. Known or suspected chromosomal abnormality, genetic disorder, or syndrome
2. Previous exposure to any IVT or systemic anti-VEGF agent, including maternal exposure during pregnancy and/or during breastfeeding
3. Clinically significant neurological disease (eg, intraventricular hemorrhage Grade 3 or higher, periventricular leukomalacia, congenital brain lesions significantly impairing optic nerve function, severe hydrocephalus with significantly increased intracranial pressure)
4. Pediatric conditions rendering the infant ineligible for study intervention at baseline or for repeated blood draws as evaluated by a NICU specialist and a study ophthalmologist
5. Presence of active ocular infection within 5 days of the first treatment
6. Advanced stages of ROP with partial or complete retinal detachment (ROP Stage 4 and Stage 5)
7. ROP involving only Zone III
8. Ocular abnormalities that may interfere with the administration of study intervention or assessment of the study primary endpoint
9. Postnatal treatment with oral or intravenous corticosteroids at an equivalent dose of prednisone ≥ 1 mg/kg/day for > 2 weeks within 14 days of the first study intervention

10. Previous surgical or nonsurgical treatment for ROP (IVT anti-VEGF injection, ablative laser therapy, cryotherapy, and vitrectomy)
11. Participation of the infant or the mother in other clinical trials requiring administration of investigational treatments (other than vitamins and minerals) at the time of screening, or within 30 days or 5 half-lives of administration of the previous study drug, whichever is longer

6.1.1.2. Endpoints

Primary Endpoint

The primary efficacy endpoint was the proportion of infants with absence of both active ROP and unfavorable structural outcomes at 52 weeks CA based on the investigator's assessment. Active ROP was ROP (according to the inclusion criterion) requiring treatment and unfavorable structural outcome was defined as retinal detachment, macular dragging, macular fold, or retrolental opacity.

Secondary Endpoints (Hierarchical)

1. Proportion of infants requiring treatment intervention with a second treatment modality through Week 52 CA:

   A second treatment modality for ROP was allowed, which could include rescue treatment or any other surgical or nonsurgical treatment for ROP (eg, IVT anti-VEGF injection other than study drug, ablative laser therapy [for the aflibercept group], or any surgical intervention for the management of ROP complications). In the event this was not rescue treatment this second treatment modality was captured as concomitant medication or surgery after study start.

2. Proportion of infants with recurrence of ROP through Week 52 CA:

   Recurrence of disease was defined as the reappearance of the disease requiring further treatment (including retreatment or rescue), where both "presence of ROP" and "presence of active ROP requiring treatment" were marked as "Yes," after initial regression. Initial regression was defined as absence of ROP or ROP treatment not required for active ROP, ie, presence of ROP is marked as "No" or the presence of active ROP requiring treatment is marked as "No" at any post-baseline visit.

Exploratory Endpoints

- Time to recurrence of ROP (as defined above)
- Time required to perform treatment
- Requirement for sedation and/or general anesthesia to complete laser or aflibercept injection
- Visual function evaluated using a methodology appropriate for the age and development status of the child, including evaluation of fixation (eg, central, steady, and maintained), and fixing and following a 5-cm toy. If the infant was not able to cooperate with these methods, another suitable method (eg, visual evoked potentials) may have been used to evaluate visual function.

6.1.1.3. Statistical Methods

Determination of Sample Size

From the RAINBOW study comparing the effect of IVT ranibizumab to laser in the management of ROP (Stahl, 2019), the response rate was 66.2% for the laser group and 88.1% for the 0.2 mg ranibizumab group in Zone II disease. Anecdotal clinical evidence from aflibercept investigator-initiated studies suggested that the response rate was up to 100% (with at least 2 studies demonstrating a 100% response rate in terms of favorable anatomic structural outcomes/prevention of unfavorable anatomic structural outcomes) with IVT aflibercept doses ranging from 0.4 mg to 1 mg. Therefore, an estimated 90% response rate for the aflibercept group and 66.2% response rate for the laser group was considered a reasonable assumption. A sample size of 84 infants in the aflibercept group and 28 infants in the laser group (randomized in a 3:1 ratio) would provide 86% power for rejecting the null hypothesis at a 1-sided 2.5% significance level. Additionally, FDA stated that at least 150 premature infants with ROP treated with aflibercept and followed for at least 52 weeks after birth across 2 studies would be adequate.

Patient Populations

The primary efficacy analyses were conducted on the FAS population, which comprised randomized patients who initiated treatment. Patients were analyzed as assigned in their initial randomization. Safety analyses were conducted in the Safety Analysis Set (SAF), which comprised patients who initiated treatment; in the event that any patient received the wrong randomized therapy, such a patient would be analyzed based on the actual treatment received. Consistent with FDA guidance for non-inferiority studies, an additional PP population was defined which included only those patients in the FAS who had no validity findings or important deviations that could have affected the primary efficacy variable.

Efficacy Analyses

The primary analysis was a statistical evaluation of non-inferiority of aflibercept versus laser at Week 52 CA, with respect to the primary efficacy variable. A non-inferiority margin of 5% for the difference in response rates was prespecified. The non-inferiority margin was set at 5%, which was smaller than the smallest difference between laser and ranibizumab in the RAINBOW study (Stahl, 2019) and not greater than the difference between the 2 ranibizumab doses. The overall success rate for laser in the RAINBOW study was 66.2% (95% CI: 55.0% to 77.4%), while the success rate for a putative placebo (which has little to no efficacy) was assumed to be near 0%. The proposed non-inferiority margin of 5% preserved at least 90.9% of the control treatment effect based on the lower bound (55%) of the CI of the laser success rate in RAINBOW.

Sensitivity analysis of the primary efficacy endpoint was conducted based on the All Randomized (ITT) and PP populations.

If the patient data were not available at the Week 52 CA visits, then data available from the Week 40 CA visit were used to impute the Week 52 CA visit for analysis. If the patient discontinued before the Week 40 CA visit, the patient was considered a non-responder. For patients with both eyes enrolled in the study, both eyes must have met the endpoint for the

patient to be deemed a responder. Patients with only 1 study eye enrolled were responders if the enrolled eye responded.

The statistical analysis was performed using the Cochran-Mantel-Haenszel analysis method (CMH) stratified by baseline ROP status. The 2-sided 95.1% Mantel-Haenszel CIs (reflecting an alpha adjustment of 0.001 for the IDMC assessments) using normal approximation of the difference of response rates between the aflibercept group and the laser group were calculated. Aflibercept was considered to be non-inferior to laser if the CI of the difference lay entirely above -5%.

Eyes were also considered to be non-responders if rescue treatment was given or if a second treatment modality was administered. The primary analysis for this endpoint was based on the investigator assessment.

Missing Data

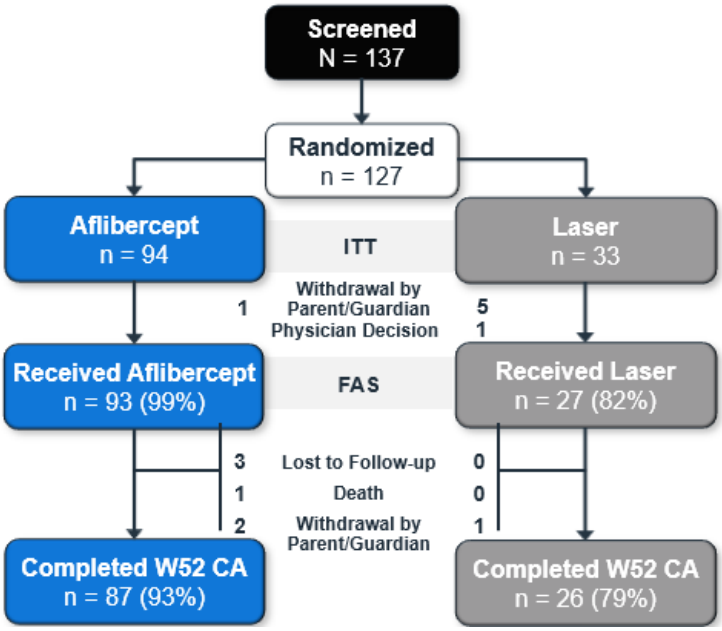
If data were not available at the Week 52 CA visits, then available data from the Week 40 CA visit were used to impute Week 52 CA visit for analysis. Any patient who was enrolled but discontinued before the Week 40 CA visit was considered a non-responder, and no data were imputed. If patients discontinued the study due to TEAEs between Week 40 and Week 52, then these patients were considered as non-responders. For participants with both eyes enrolled in the study, both eyes must have met the endpoint. Participants with only 1 study eye enrolled were responders if the respective eye responded.

6.1.2. Patient Population

6.1.2.1. Patient Disposition

After screening, 94 infants were randomized to aflibercept 0.4 mg, and 33 infants were randomized to laser. One infant randomized to aflibercept and 6 infants randomized to open-label laser were withdrawn before receiving any study intervention (Figure 10). Six additional aflibercept-treated infants and 1 additional laser-treated infant discontinued from the study. In total, 87 (93%) infants in the aflibercept group and 26 (79%) infants in the laser group completed the Week 52 CA visit at the time of data cut-off.

Figure 10: BUTTERFLEYE: Patient Disposition



Abbreviations: W=week; CA=chronological age; FAS=Full analysis set; ITT=Intent-to-treat.

6.1.2.2. Demographics and Baseline Characteristics

Demographics were generally balanced between groups, and differences in baseline body weight, race, and sex were not considered to impact efficacy or safety in either treatment group (Table 6). Infants in the aflibercept group weighed less than those in the laser group (mean [SD] 2,058.3 g [548.3 g] vs 2,248.1 g [725.0 g], respectively), fewer infants were white (28.0% vs 40.7%, respectively), and more were female (55.9% vs 37.0%, respectively). This study enrolled infants from Europe, Asia, North America, and South America. The mean GA at birth was 27 weeks, with 41% of infants in both groups in the ≤ 26 weeks category.

Baseline medical characteristics are shown in Table 7, including the proportion of infants in the aflibercept group and laser group that required oxygen supplementation at baseline (37.6% vs 29.6%, respectively) or had a history of sepsis (54.8% vs 55.6%, respectively), necrotizing enterocolitis (17.2% vs 11.1%, respectively), intraventricular hemorrhage (37.6% vs 29.6%, respectively), or bronchopulmonary dysplasia (49.5% vs 59.3%, respectively).

A total of 179 eyes (aflibercept group) and 50 eyes (laser group) were treated, and most infants were treated bilaterally (Table 8). The majority of infants in both treatment groups were classified by investigators as having eyes with Zone II ROP (73.7%, aflibercept group and 74.0%, laser group).

Table 6: BUTTERFLEYE: Baseline Demographics

	Aflibercept (N=93)	Laser (N=27)
Chronological Age at Randomization (weeks), mean (SD)	9.76 (3.149)	11.09 (4.338)
Gestational Age at Birth (weeks), mean (SD)	27.34 (2.753)	27.06 (2.652)
Post-Menstrual Age at Randomization (weeks)[a], mean (SD)	37.11 (2.425)	38.15 (3.599)
Gestational Age at Birth group, n (%)		
≤ 26 weeks	38 (40.9%)	11 (40.7%)
> 26 weeks	55 (59.1%)	16 (59.3%)
Sex, n (%)		
Female	52 (55.9%)	10 (37.0%)
Male	41 (44.1%)	17 (63.0%)
Weight at Birth (g)		
Mean (SD)	991.2 (407.00)	934.1 (406.61)
Median	900.0	798.0
Weight at Baseline (g)		
Mean (SD)	2058.3 (548.28)	2248.1 (724.95)
Median	1948.0	2050.0
Race, n (%)		
White	26 (28.0%)	11 (40.7%)
Black or African American[b]	6 (6.5%)	2 (7.4%)
Asian	44 (47.3%)	13 (48.1%)
American Indian or Alaska Native	0	0
Native Hawaiian or Other Pacific Islander	0	0
Other/Not Reported	17 (18.3%)	1 (3.7%)
Ethnicity, n (%)		
Hispanic or Latino	16 (17.2%)	4 (14.8%)
Not Hispanic or Latino	72 (77.4%)	22 (81.5%)
Unknown/Not Reported	5 (5.4%)	1 (3.7%)

Abbreviations: SD=standard deviation.
[a] Post-menstrual age at randomization=Gestational age at birth + Chronological age at randomization.
[b] Within US sites, there were 22.2% Black or African American infants in the aflibercept group and 28.6% in the laser group.

Table 7: BUTTERFLEYE: Baseline Medical Characteristics

	Aflibercept (N=93)	Laser (N=27)
APGAR score 5 min after birth, n (%)		
0–4	8 (8.6)	6 (22.2)
5–7	35 (37.6)	9 (33.3)
8–10	30 (32.3)	7 (25.9)
APGAR score category 1 min after birth, n (%)		
0–4	35 (37.6)	15 (55.6)
5–7	40 (43.0)	9 (33.3)
8–10	11 (11.8)	3 (11.1)
APGAR score category 5 min after birth, n (%)		
0–4	8 (8.6)	6 (22.2)
5–7	35 (37.6)	9 (33.3)
8–10	30 (32.3)	7 (25.9)
Required O_2 supplementation at baseline, n (%)	35 (37.6)	8 (29.6)
History of sepsis, n (%)	51 (54.8)	15 (55.6)
History of necrotizing enterocolitis, n (%)	16 (17.2)	3 (11.1)
History of intraventricular hemorrhage, n (%)	35 (37.6)	8 (29.6)
History of bronchopulmonary dysplasia	46 (49.5)	16 (59.3)

Abbreviations: APGAR=Appearance, Pulse, Grimace, Activity, and Respiration; min=minutes; O_2=oxygen.

Table 8: BUTTERFLEYE: Summary of Baseline ROP Staging per Investigator Assessment

	Aflibercept (N=93)	Laser (N=27)
Laterality of eyes treated, n (%)[a]		
Unilateral	7 (7.5%)	4 (14.8%)
Bilateral	86 (92.5%)	23 (85.2%)
Laterality of eyes treated at baseline, n (%)[b]		
Unilateral	10 (10.8%)	4 (14.8%)
Bilateral	83 (89.2%)	23 (85.2%)
Number of eyes treated, n[a,c]	179	50
ROP zone, by eye, n (%)[d]		
Zone I	47 (26.3%)	13 (26.0%)
Stage 2, plus disease	16 (8.9%)	1 (2.0%)
Stage 3, no plus disease	2 (1.1%)	0
Stage 3, plus disease	21 (11.7%)	12 (24.0%)
AP-ROP	20 (11.2%)	3 (6.0%)
Zone II	132 (73.7%)	37 (74.0%)
Stage 2, plus disease	32 (17.9%)	5 (10.0%)
Stage 3, plus disease	100 (55.9%)	30 (60.0%)
AP-ROP	8 (4.5%)	3 (6.0%)

Abbreviations: AP-ROP=Aggressive posterior ROP; ROP=retinopathy of prematurity.
[a] Included all treated eyes - including eyes for which treatment started only after the baseline visit.
[b] Included all eyes for which treatment started at the baseline visit.
[c] This row presents the denominator for calculation of percentages in rows below.
[d] ROP classification was based on the baseline value for those eyes starting treatment at baseline and at the second eye treatment visit for those eyes starting treatment after the baseline visit. Eyes with AP-ROP were not always exclusive of disease stage.

6.1.3. Primary Endpoint Results: Absence of Active ROP and Unfavorable Structural Outcomes at 52 Weeks (Chronological Age)

The majority of infants in both groups met the primary endpoint criteria, absence of active ROP and unfavorable structural outcomes at 52 weeks CA based on the investigator's assessment; 79.6% in aflibercept vs 77.8% for laser. The adjusted difference (95.1% CI) was 1.81% (-15.71%, 19.33%) in the prespecified FAS; the lower bound of the CI for treatment difference was below the prespecified non-inferiority margin of -5% (Table 9). While non-inferiority of aflibercept to laser was not established with the 5% margin, a numerically larger proportion of infants in the aflibercept group met the primary endpoint compared to those in the laser group.

Table 9: BUTTERFLEYE: Proportion of Infants with Absence of Active ROP and Unfavorable Structural Outcomes at Week 52 CA (FAS)

Treatment	Infants with absence of active ROP and unfavorable structural outcomes at Week 52 of age	Adjusted difference (%) (95.1% CI) [a]
Aflibercept (n=93)	74/93 (79.6%)	1.81 (-15.71, 19.33)
Laser (n=27)	21/27 (77.8%)	

Abbreviations: CI=confidence interval; FAS=full analysis set; ROP=retinopathy of prematurity.
[a] Difference with CI was calculated using Mantel-Haenszel weighting scheme adjusted by baseline ROP status.
Note: Analysis was based on investigator assessment.
If the patient data were not available at EOS visit, then data available from the Week 40 CA visit were used to impute Week 52; if the patient discontinued before the Week 40 CA visit, the patient was considered a non-responder. If patients discontinued the study due to TEAEs between Week 40 and Week 52, then these patients were considered as non-responders.
Patients were considered as non-responders if rescue treatment (including any second treatment modality) was given in the study eye(s).
For patients with both eyes enrolled in the study, both eyes must have met the endpoint. Patients with only one study eye enrolled were responders if the respective eye responded.

6.1.3.1. Sensitivity Analyses of Primary Endpoint

The primary efficacy endpoint was analyzed using the All Randomized (ITT) and PP populations in sensitivity analyses.

In the All Randomized (ITT) population, there was a higher proportion of infants in the aflibercept group who met the primary endpoint criteria (ie, without active ROP or unfavorable structural outcomes) compared with the laser group (78.7% and 63.6%, respectively) with an adjusted difference (95.1% CI) of 14.90% (-3.45, 33.26) (Table 10).

In the PP population, 88.2% of infants in the aflibercept group and 79.2% of infants in the laser group met the primary endpoint criteria, with an adjusted difference (95.1% CI) of 9.08% (-9.08, 27.25).

Observed Case, Central Reading Center (FAS), and Central Reading Center (observed values) supplementary analyses consistently showed higher numerical success rates for aflibercept vs laser, but with a lower bound of the CI of less than -5%.

Table 10: BUTTERFLEYE: Sensitivity and Supplementary Analysis of Proportion of Infants with Absence of Active ROP and Unfavorable Structural Outcomes at Week 52 CA

Treatment	Infants with absence of active ROP and unfavorable structural outcomes at Week 52 of age	Adjusted difference (%) (95.1% CI) [a]
All Randomized (Intent-to-Treat) Patients [b,c]		
Aflibercept (n=94)	74/94 (78.7%)	14.9 (-3.5, 33.3)
Laser (n=33)	21/33 (63.6%)	
Per Protocol Set [b,c]		
Aflibercept (n=68)	60/68 (88.2%)	9.08 (-9.1, 27.3)
Laser (n=24)	19/24 (79.2%)	
Observed Case (Full Analysis Set)[c, e]		
Aflibercept (n=93)	72/87 (82.8%)	2.35 (-14.8, 19.5)
Laser (n=27)	21/26 (80.8%)	
Central Reading Center (Full Analysis Set) [b,d]		
Aflibercept (n=93)	50/93 (53.8%)	5.10 (-15.7, 25.9)
Laser (n=27)	13/27 (48.1%)	
Central Reading Center (Observed values) [d,e]		
Aflibercept (n=59)	50/59 (84.7%)	11.03 (-10.5, 32.6)
Laser (n=17)	13/17 (76.5%)	

Abbreviations: CA=chronological age; CI=confidence interval; EOS=end of study; ROP= Retinopathy of prematurity; TEAE=treatment-emergent adverse event.
[a] Difference with CI was calculated using Mantel-Haenszel weighting scheme adjusted by baseline ROP status.
[b] If the patient data were not available at EOS visit, then data available from the Week 40 CA visit were used to impute Week 52; if the patient discontinued before the Week 40 CA visit, the patient was considered a non-responder. If patients discontinued the study due to TEAEs between Week 40 and Week 52, then these patients were considered as non-responders.
[c] Analysis was based on investigator assessment.
[d] Analysis is based on Central Reading Center assessment.
[e] Only observed, non-censored, and gradable values were used for analysis.
Note: Patients were considered as non-responders if rescue treatment (including any second treatment modality) was given in the study eye(s).
For patients with both eyes enrolled in the study, both eyes must have met the endpoint. Patients with only one study eye enrolled were responders if the respective eye responded.

6.1.3.2. Baseline ROP Status of Primary Endpoint

Subgroup analyses by baseline ROP status are provided in Table 11. Results are consistent across subgroups. Response rates for both treatments were lower in infants with Zone I ROP and AP-ROP than in Zone II ROP.

Table 11: BUTTERFLEYE: Proportion Analysis of Infants with Absence of Active ROP and Unfavorable Structural Outcomes at Week 52 CA by ROP Status Subgroup (FAS)

Baseline ROP Status	Treatment	Infants with absence of active ROP and unfavorable structural outcomes at Week 52 of age	Adjusted Difference (%) (95.1% CI) [a]
Zone I	Aflibercept (n=16)	11/16 (68.8%)	8.75 (-40.04, 57.54)
Zone I	Laser (n=5)	3/5 (60.0%)	
Zone II	Aflibercept (n=68)	56/68 (82.4%)	-2.65 (-20.81, 15.52)
Zone II	Laser (n=20)	17/20 (85.0%)	
AP-ROP	Aflibercept (n=9)	7/9 (77.8%)	27.78 (-46.98, 100.00)
AP-ROP	Laser (n=2)	1/2 (50.0%)	

Abbreviations: CA=chronological age; EOS=end of study; FAS=Full analysis set; ROP=Retinopathy of prematurity; TEAE=treatment-emergent adverse event.
[a] Difference with CI was calculated using Mantel-Haenszel weighting scheme.
Note: Analysis was based on investigator assessment.
If the patient data were not available at EOS visit, then data available from the Week 40 CA visit were used to impute Week 52; if the patient discontinued before the Week 40 CA visit, the patient was considered a non-responder. If patients discontinued the study due to TEAEs between Week 40 and Week 52, then these patients were considered as non-responders.
Patients were considered as non-responders if rescue treatment (including any second treatment modality) was given in the study eye(s).
For patients with both eyes enrolled in the study, both eyes must have met the endpoint. Patients with only one study eye enrolled were responders if the respective eye responded.

6.1.4. Secondary Endpoints

6.1.4.1. Requirement of Second Treatment Modality

The proportion of infants in either treatment group requiring intervention with a second treatment modality was numerically lower in the aflibercept group compared to the laser group (15.1% versus 18.5%, respectively) with an adjusted difference (95.1% CI): -3.66% (-19.86%, 12.54%).

6.1.4.2. Recurrence of ROP Through Week 52 (Chronological Age)

The proportion of infants in either treatment group with recurrence of ROP was 39.8% in the aflibercept group compared to 29.6% in the laser group with an adjusted difference (95.1% CI): 10.1 (-9.8, 30.0).

The mean time to recurrence of ROP was 154.9 days for the aflibercept group and 123.9 days for the laser group, and there was no statistically significant difference between treatment groups for time to recurrence of ROP. At the time of this analysis, most events for each treatment group were censored, and median time to recurrence was not estimable.

6.1.5. Exploratory Endpoints

6.1.5.1. Requirements for Sedation or General Anesthesia

The requirement for sedation or general anesthesia for aflibercept treatment was less frequent compared to laser treatment (Table 12). Additionally, a considerably higher proportion of

treatment sessions in the aflibercept group (51.4%) did not require sedation or general anesthesia to complete the study procedure than in the laser group (22.0%). The need for each infant to receive sedation or general anesthesia to complete the study procedure at least once was also considerably less frequent in the aflibercept group compared to the laser group: 47.3% versus 77.8%, respectively.

Table 12: BUTTERFLEYE: Requirement for Sedation or General Anesthesia to Complete Laser or Aflibercept Injection (FAS)

	Aflibercept	Laser
Total number of treatment sessions[a]	214	59
Study treatment needed sedation or general anesthesia to complete study procedure		
Sedation	31/214 (14.5%)	20/59 (33.9%)
General anesthesia	73/214 (34.1%)	26/59 (44.1%)
Neither	110/214 (51.4%)	13/59 (22.0%)
Infant needed sedation or general anesthesia to complete study procedure at least once		
Yes	44/93 (47.3%)	21/27 (77.8%)
No	49/93 (52.7%)	6/27 (22.2%)

Abbreviations: FAS=Full Analysis Set.
[a] This row presents the denominator for calculation of percentages in subrows immediately below.
Note: Does not include rescue treatment.

6.1.5.2. Time Required to Perform Treatment

Infants who received laser treatment had substantially longer treatment durations than those who received aflibercept. The mean (SD) time to perform study treatment was 10.7 (17.24) minutes per infant for the aflibercept group and 129.2 (95.63) minutes per infant for the laser group (Table 13).

Table 13: BUTTERFLEYE: Time Required to Perform Aflibercept Injection or Initial Laser Treatment (FAS)

	Aflibercept	Laser
Time to perform study treatment per infant (min) [a]		
n	89	26
Mean (SD)	10.7 (17.24)	129.2 (95.63)
Median	4.0	104.5
Time to perform study treatment per eye (min) [b]		
n	168	48
Mean (SD)	5.6 (8.81)	70.0 (48.71)
Median	2.0	47.5

Abbreviations: FAS=Full analysis set; SD=standard deviation.
[a] Durations for bilaterally treated patients and for multiple laser sessions within 7 days of initiation of laser treatment (per protocol considered as one session) were added prior to summarization.
[b] Multiple laser sessions within 7 days of initiation of laser treatment (per protocol considered as one session) for the same eye were added prior to summarization.
Note: The values summarized in this table are for the initial treatments only.

6.1.5.3. Visual Function

Most eyes in the aflibercept and laser groups demonstrated central fixation (approximately 87% overall) and were able to fix and follow a 5-cm toy (approximately 89% overall) (Table 14).

For cycloplegic refraction, the refractive spherical equivalent at Week 52 CA is displayed and was calculated as: sphere + ½ cylinder. The mean spherical equivalent (SD) was 0.5 (4.84) diopters in the aflibercept group and -1.0 (3.02) diopters in the laser group.

In both treatment groups, the majority of eyes had neither manifest strabismus (74.7% overall) nor nystagmus in the primary position of gaze (87.3% overall).

Table 14: BUTTERFLEYE: Visual Function Results for Aflibercept or Laser Treatment (FAS)

	Aflibercept	Laser
Number of treated eyes [a,b]	179	50
Fixation (central, steady, and maintained)		
Yes	153/179 (85.5%)	47/50 (94.0%)
No	8/179 (4.5%)	1/50 (2.0%)
Fixing and following a 5-cm toy		
Yes	158/179 (88.3%)	46/50 (92.0%)
No	3/179 (1.7%)	2/50 (4.0%)
Number of eyes whose visual function cannot be assessed with fixation [a]	2/179 (1.1%)	0/50
Visual function assessment via visual evoked potentials		
Normal	2/2 (100%)	0/0
Abnormal	0/2	0/0

	Aflibercept	Laser
Cycloplegic refraction, spherical equivalent (D)		
N[c]	145	46
Mean (SD)	0.5 (4.84)	-1.0 (3.02)
Median	0.3	-0.1
Q1: Q3	-0.8: 1.0	-2.3: 1.0
Min: Max	-6: 53	-13: 3
Ocular motility tests		
Assessment of ocular palsy with affected nerves		
Yes	1/179 (0.6%)	4/50 (8.0%)
No	156/179 (87.2%)	40/50 (80.0%)
Unknown	6/179 (3.4%)	4/50 (8.0%)
Assessment of strabismus		
Normal	130/179 (72.6%)	41/50 (82.0%)
Abnormal	20/179 (11.2%)	5/50 (10.0%)
Unknown	13/179 (7.3%)	2/50 (4.0%)
Assessment of nystagmus		
Normal	156/179 (87.2%)	44/50 (88.0%)
Abnormal	5/179 (2.8%)	4/50 (8.0%)
Unknown	2/179 (1.1%)	0/50

Abbreviations: CA=chronological age; D=diopter; FAS=Full analysis set; Q1=first quartile; Q3=third quartile; SD=standard deviation.
[a] This row presents the denominator for calculation of percentages in sub-rows below.
[b] Number of eyes treated included all eyes treated at baseline and eyes for which treatment started after the baseline visit and before the Week 8 visit.
[c] Not all eyes underwent cycloplegic refraction.
Note: Eyes treated refers to any study treatment, either with aflibercept or with laser treatment.
For the respective assessments, only eyes that were evaluated at the Week 52 CA visit were included.
16 aflibercept patients and 2 laser patients did not undergo visual function evaluation.

6.2. FIREFLEYE (Study 20090)/FIREFLEYE NEXT (Study 20275)

6.2.1. Study Design

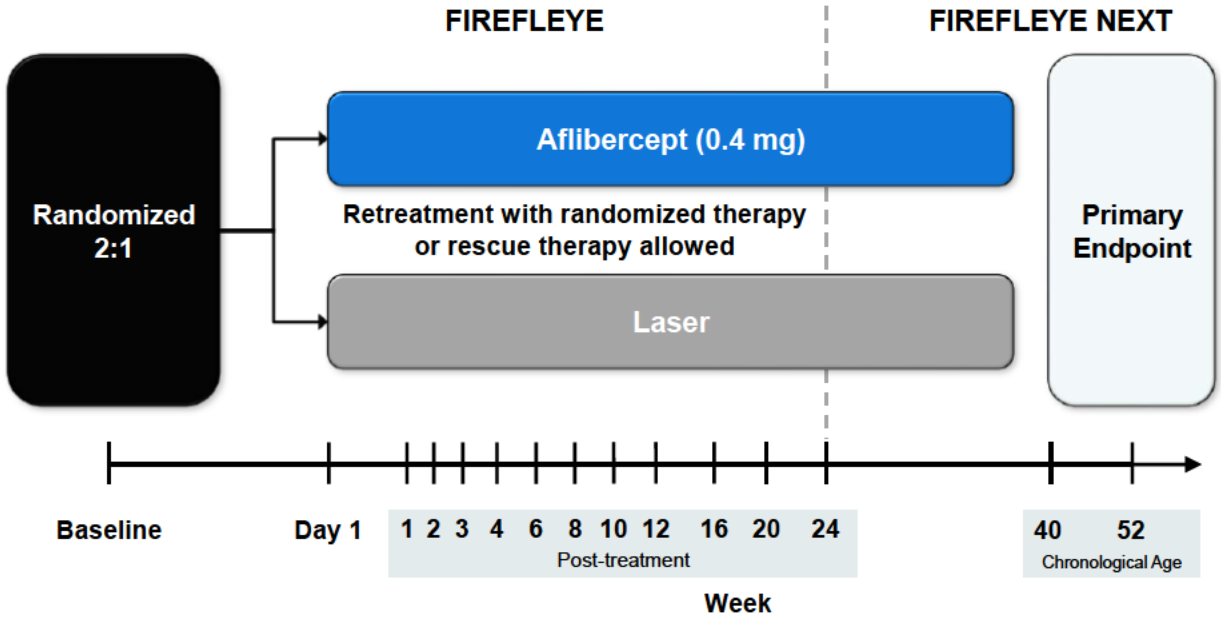
FIREFLEYE (Study 20090) was a global, Phase 3, multicenter, open-label, randomized, 2-arm controlled study to assess the efficacy, safety, and tolerability of IVT aflibercept versus laser in infants with ROP. Eligible patients were randomized 2:1 to receive either aflibercept or laser treatment at 64 study centers in 27 countries. The study consisted of screening/baseline (performed either the same day or within 10 days of each other), a treatment period (including potential retreatment and rescue treatment), and a final visit at Week 24 (up to Week 27 for infants treated after Week 21). Study duration was planned for at least 24 weeks in the study protocol (Figure 11).

FIREFLEYE NEXT (Study 20275) is an ongoing Phase 3b, multicenter study to assess the long-term outcomes of infants previously diagnosed with ROP who were treated in the completed

FIREFLEYE. No study intervention is administered during this follow-up study, and any potential treatments are decided by the treating physician, according to local standards of care.

For this submission, infants were followed through FIREFLEYE and through the Week 52 CA visit in FIREFLEYE NEXT, and results are thus presented.

Figure 11: FIREFLEYE Study Design



Eligibility and Randomization

Eligible patients included male and female preterm infants with treatment-naïve ROP Zone I Stage 1 plus, 2 plus, 3 non-plus or plus, or Zone II Stage 2 plus or 3 plus, or AP-ROP according to the ICROP 2005 in at least 1 eye.

One or both eyes could have been treated per investigator's assessment of the study's eligibility criteria. In the case of treatment-requiring unilateral disease, the non-study eye was kept under observation according to the local ROP screening guidelines or at every study visit, whichever was more frequent. Non-study eyes that developed ROP requiring treatment during the study received treatment according to the randomization assignment of the initial study eye. If a patient's second eye qualified for treatment during the study, after the retreatment/rescue treatment schedule was completed for the second eye, both eyes could follow the visit schedule for the first eye.

Study Interventions Administered

Patients randomized to aflibercept received a single IVT injection of aflibercept 0.4 mg/10 µL per eligible eye at baseline. Thereafter, if required, up to 2 additional IVT injections of aflibercept 0.4 mg/10 µL could have been administered at least 4 weeks apart in each treatment-requiring eye when both of the prespecified retreatment criteria were met (presence of ROP requiring treatment and the interval since the last aflibercept IVT injection was $\geq$ 28 days).

For patients randomized to aflibercept, rescue treatment with laser was permitted if 1 of the prespecified criteria was met: worsening of ROP compared to the examination before the previous injection during the 27 days following that IVT aflibercept injection OR presence of ROP requiring treatment after the patient already received a total of 3 aflibercept injections and at least 28 days have passed since the last injection.

For patients randomized to laser treatment, transpupillary conventional laser ablative therapy was conducted per standard of care at the investigational site. In case multiple laser sessions were necessary within 1 week from baseline, they were counted as a single treatment. If additional laser treatment was administered after 1 week from baseline treatment, it was counted as a laser retreatment.

Rescue treatment with aflibercept was allowed if the fundus examination revealed laser treatment was complete and if 1 of the prespecified criteria were met: worsening of ROP compared to the most recent pre-laser examination OR persistence of ROP requiring treatment and at least 28 days have passed since the last laser treatment.

6.2.1.1. Key Enrollment Criteria

Patients had to meet the following criteria at screening and baseline to be eligible for inclusion in the study:

1. Gestational age at birth ≤ 32 weeks or birth weight ≤ 1500 g
2. Patients with treatment-naïve ROP classified according to the ICROP in at least one eye as:
   - Zone I Stage 1 plus, or 2 plus, or 3 non-plus, or 3 plus, or
   - Zone II Stage 2 plus or 3 plus, or
   - AP-ROP
3. Weight at baseline (day of treatment) ≥ 800 g

Patients who met any of the following criteria were excluded from the study:

1. Known or suspected chromosomal abnormality, genetic disorder, or syndrome
2. Previous exposure to any IVT or systemic anti-VEGF agent, including maternal exposure during pregnancy and/or during breastfeeding
3. Clinically significant neurological disease (eg, intraventricular hemorrhage Grade 3 or higher, periventricular leukomalacia, congenital brain lesions significantly impairing optic nerve function, severe hydrocephalus with significantly increased intracranial pressure)
4. Pediatric conditions rendering the infant ineligible for study intervention at baseline or for repeated blood draws as evaluated by a NICU specialist and a study ophthalmologist
5. Presence of active ocular infection within 5 days of the first treatment
6. Advanced stages of ROP with partial or complete retinal detachment (ROP Stages 4 and 5)

7. ROP involving only Zone III
8. Ocular abnormalities that may interfere with the administration of study intervention or assessment of the study primary endpoint
9. Postnatal treatment with oral or intravenous corticosteroids at an equivalent dose of prednisone ≥ 1 mg/kg/day for > 2 weeks within 14 days of the first study intervention
10. Previous surgical or nonsurgical treatment for ROP (IVT anti-VEGF injection, ablative laser therapy, cryotherapy, and vitrectomy)
11. Participation of the patient or the mother in other clinical trials requiring administration of investigational treatments (other than vitamins and minerals) at the time of screening, or within 30 days or 5 half-lives of administration of the previous study intervention, whichever was longer

All treated patients from FIREFLEYE were included in FIREFLEYE NEXT if the patient was < 13 months CA and had a signed informed consent from a legally authorized representative. Patients were excluded from the study if they had a medical condition preventing participation in the study or performance of study procedures.

6.2.1.2. Endpoint Definitions

Primary Endpoint

The primary endpoint was defined as the proportion of infants with absence of active ROP and unfavorable structural outcomes at 52 weeks of CA after starting study treatment, based on the investigator's assessment.

Secondary Endpoints (Hierarchical)

1. Proportion of infants requiring treatment intervention with a second treatment modality through Week 52 CA
2. Proportion of infants with recurrence of ROP through Week 52 CA

Exploratory Endpoints

- Time to recurrence of ROP (as defined above)
- Time required to perform treatment
- Requirement for sedation and/or general anesthesia to complete laser or aflibercept injection
- Visual function evaluated using a methodology appropriate for the age and development status of the child, including evaluation of fixation (eg, central, steady, and maintained), and fixing and following a 5-cm toy. If the infant was not able to cooperate with these methods, another suitable method (eg, visual evoked potentials) may have been used to evaluate visual function.

6.2.1.3. Statistical Methods

Determination of Sample Size

From the RAINBOW study comparing the effect of IVT ranibizumab to laser in the management of ROP (Stahl, 2019), the response rate was 66.2% for the laser group and 88.1% for the 0.2 mg ranibizumab group in Zone II disease. Anecdotal clinical evidence from aflibercept investigator-initiated studies suggested that the response rate was up to 100% (with at least 2 studies demonstrating a 100% response rate in terms of favorable anatomic structural outcomes/prevention of unfavorable anatomic structural outcomes) with IVT aflibercept doses ranging from 0.4 mg to 1 mg. Therefore, an estimated 90% response rate for the aflibercept group and 66.2% response rate for the laser group was considered a reasonable assumption. A sample size of 68 patients in the aflibercept group and 34 patients in the laser group (randomized in a 2:1 ratio) would provide 90% power for rejecting the null hypothesis at a 1-sided 2.5% significance level. Additionally, FDA stated that at least 150 premature infants with ROP treated with aflibercept and followed for at least 52 weeks after birth across 2 studies would be adequate.

Patient Populations

The primary efficacy analyses were conducted on the FAS population, which comprised randomized patients who initiated treatment. Patients were analyzed as assigned in their initial randomization. Safety analyses were conducted in the SAF, which comprised patients who initiated treatment; in the event that any patient received the wrong randomized therapy, such a patient would be analyzed based on the actual treatment received. Consistent with FDA guidance for non-inferiority studies, an additional PP population was defined which included only those patients in the FAS who had no validity findings or important deviations that could have affected the primary efficacy variable.

Efficacy Analyses

The primary analysis was a statistical evaluation of non-inferiority of aflibercept versus laser at Week 52 CA, with respect to the primary efficacy variable. The non-inferiority margin was set at 5%, which was smaller than the smallest difference between laser and ranibizumab in the RAINBOW study (Stahl, 2019) and not greater than the difference between the 2 ranibizumab doses. The overall success rate for laser in the RAINBOW study was 66.2% (95% CI: 55.0% to 77.4%), while the success rate for a putative placebo (which has little to no efficacy) was assumed to be near 0%. The proposed non-inferiority margin of 5% preserved at least 90.9% of the control treatment effect based on the lower bound (55%) of the CI of the laser success rate in RAINBOW.

Sensitivity analysis of the primary efficacy endpoint was conducted based on the All Randomized (ITT) and PP populations.

If the patient data were not available at the Week 52 CA visits, then data available from the Week 40 CA visit were used to impute the Week 52 CA visit for analysis. If the patient discontinued before the Week 40 CA visit, the patient was considered a non-responder. For patients with both eyes enrolled in the study, both eyes must have met the endpoint for the patient to be deemed a responder. Patients with only 1 study eye enrolled were responders if the enrolled eye responded.

The statistical analysis was performed using the CMH stratified by baseline ROP status. The 2-sided 95.1% Mantel-Haenszel CIs (reflecting an alpha adjustment of 0.001 for the IDMC

assessments) using normal approximation of the difference of response rates between the aflibercept group and the laser group were calculated. Aflibercept was considered to be non-inferior to laser if the CI of the difference lay entirely above -5%.

Eyes were also considered to be non-responders if rescue treatment was given or if a second treatment modality was administered. The primary analysis for this endpoint was based on the investigator assessment.

Missing Data

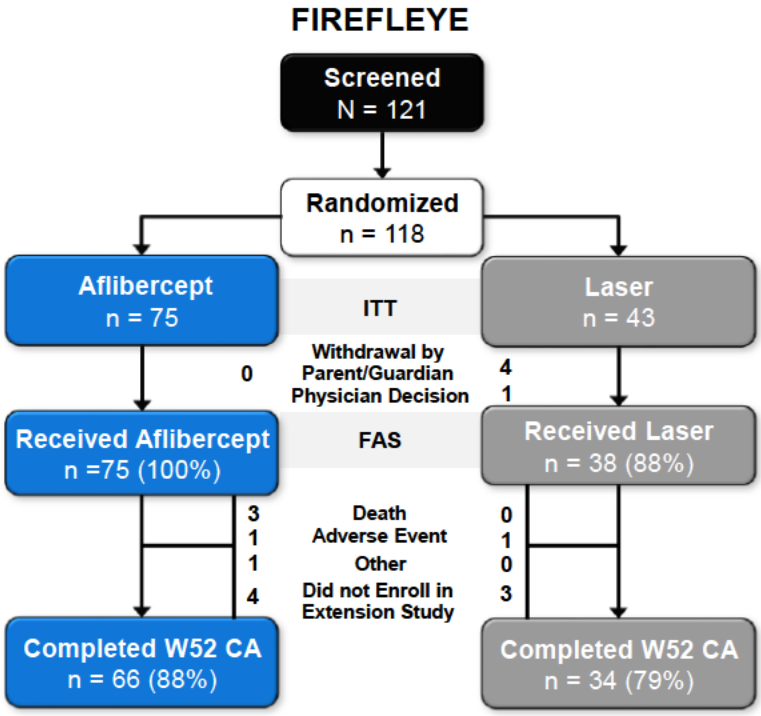
If data were not available at the Week 52 CA visits, then available data from the Week 40 CA visit were used to impute Week 52 CA visit for analysis. Any patient who was enrolled but discontinued before the Week 40 CA visit was considered a non-responder, and no data were imputed. If patients discontinued the study due to TEAEs between Week 40 and Week 52, then these patients were considered as non-responders. For participants with both eyes enrolled in the study, both eyes must have met the endpoint. Participants with only 1 study eye enrolled were responders if the respective eye responded.

6.2.2. Patient Population

6.2.2.1. Patient Disposition

In FIREFLEYE, infants were randomized 2:1 to aflibercept (75 infants) and laser (43 infants; Figure 12). Five infants randomized to laser were withdrawn before receiving any study intervention due to their legally authorized representative(s) or physician's decision against laser. Three infants received treatment but discontinued early from FIREFLEYE; however, they enrolled in the follow-up study (FIREFLEYE NEXT) and had data at Week 52 CA and, therefore, were not considered as discontinued in the analyses. In each study group, the following number of infants completed the Week 52 CA visit: 34 (79.1%) laser, 66 (88.0%) aflibercept.

Figure 12: FIREFLEYE Patient Disposition



Abbreviations: W=week; CA=chronological age; FAS=Full analysis set; ITT=Intent-to-treat.

6.2.2.2. Demographics and Baseline Characteristics

Demographics were generally balanced between groups, and differences in baseline body weight, race, and sex were not considered to impact efficacy or safety in either treatment group (Table 15). Infants in the aflibercept group weighed more than those in the laser group (mean [SD] 2,026.7 g [678.93 g] vs 1,850.9 g [546.13 g], respectively). Overall, there were slightly more male infants than female in the study (53.1% vs 46.9%). Across both groups, the GA at birth ranged from 23 to 31 weeks (median 26 weeks), with approximately 53% in the ≤ 26 weeks category.

Baseline medical characteristics are shown in Table 16. The majority (77%) of the infants had an APGAR Score of 5 or higher when measured 5 minutes after birth. The mean (SD) post-menstrual age at randomization was 36.6 (2.6) weeks. Oxygen supplementation at baseline was required by 60% of infants. Most (90.3%) infants were treated bilaterally at baseline: 144 eyes (aflibercept) and 71 eyes (laser). The majority of eyes in both treatment groups were classified by investigators as having Zone II ROP (65%, aflibercept; 71%, laser; Table 17), which presented with Stage 3 plus disease (50.9%) or Stage 2 plus disease (12.8%).

Table 15: FIREFLEYE Baseline Demographics (SAF)

Baseline Characteristic	Aflibercept (N=75)	Laser (N=38)
Chronological Age at Randomization (weeks), mean (SD)	10.35 (2.781)	10.17 (2.290)
Gestational Age at Birth (weeks), mean (SD)	26.48 (2.071)	25.97 (1.618)
Post-Menstrual Age at Randomization (weeks) [a], mean (SD)	36.82 (2.732)	36.14 (2.150)
Gestational Age at Birth group, n (%)		
≤ 26 weeks	38 (50.7)	22 (57.9)
> 26 weeks	37 (49.3)	16 (42.1)
Sex, n (%)		
Female	34 (45.3)	19 (50.0)
Male	41 (54.7)	19 (50.0)
Weight at Birth (g), mean (SD)	881.1 (305.63)	824.6 (230.80)
Baseline weight (g), mean (SD)	2026.7 (678.93)	1850.9 (546.13)
Race, n (%)		
White	55 (73.3)	28 (73.7)
Black or African American	2 (2.7)	0
Asian	17 (22.7)	9 (23.7)
American Indian or Alaska Native	0	1 (2.6)
Native Hawaiian or Other Pacific Islander	0	0
Multiple	1 (1.3)	0

Abbreviations: SAF=safety analysis set, SD=standard deviation.
[a] Post-menstrual age at randomization=Gestational age at birth + Chronological age at randomization.

Table 16: FIREFLEYE Baseline Medical Characteristics (SAF)

Baseline Characteristic	Aflibercept (N=75)	Laser (N=38)
APGAR score category 1 min after birth, n (%)		
0–4	36 (48.0)	22 (57.9)
5–7	27 (36.0)	12 (31.6)
8–10	8 (10.7)	3 (7.9)
APGAR score category 5 min after birth, n (%)		
0–4	11 (14.7)	6 (15.8)
5–7	32 (42.7)	19 (50.0)
8–10	27 (36.0)	9 (23.7)
O_2 supplementation at baseline, n (%)	45 (60.0)	23 (60.5)
History of sepsis, n (%)	32 (42.7)	15 (39.5)
History of necrotizing enterocolitis, n (%)	15 (20.0)	5 (13.2)
History of intraventricular hemorrhage, n (%)	19 (25.3)	16 (42.1)
History of bronchopulmonary dysplasia	49 (65.3)	29 (76.3)

Abbreviations: APGAR=Appearance, Pulse, Grimace, Activity, and Respiration; O_2=oxygen; SAF=safety analysis set.

Table 17: FIREFLEYE: Summary of Baseline ROP Staging per Investigators Assessment (SAF)

	Aflibercept (N=75)	Laser (N=38)
Laterality of eyes treated, n (%) [a]		
Unilateral	4 (5.3)	4 (10.5)
Bilateral	71 (94.7)	34 (89.5)
Laterality of eyes treated at baseline, n (%) [b]		
Unilateral	6 (8.0)	5 (13.2)
Bilateral	69 (92.0)	33 (86.8)
Number of eyes treated, n [a,c]	146	72
ROP Zone by eye [d]		
Zone I	**51 (34.9)**	**21 (29.2)**
Stage 1, plus disease	2 (1.4)	0
Stage 2, plus disease	6 (4.1)	7 (9.7)
Stage 3, no plus disease	6 (4.1)	1 (1.4)
Stage 3, plus disease	27 (18.5)	9 (12.5)
AP-ROP	23 (15.8)	8 (11.1)
Zone II	**95 (65.1)**	**51 (70.8)**
Stage 2, no plus disease	0	1 (1.4)
Stage 2, plus disease	17 (11.6)	11 (15.3)
Stage 3, plus disease	74 (50.7)	37 (51.4)
AP-ROP	5 (3.4)	2 (2.8)

Abbreviations: AP-ROP= aggressive posterior ROP; ROP=retinopathy of prematurity; SAF=safety analysis set.
[a] Includes all treated eyes - including eyes for which treatment started only after the baseline visit.
[b] Includes all eyes for which treatment started at the baseline visit.
[c] This row presents the denominator for calculation of percentages in rows below.
[d] ROP classification is based on the baseline value for those eyes starting treatment at baseline and at the second eye treatment visit for those eyes starting treatment after the baseline visit.
Note: Values for AP-ROP within respective zones are not exclusive of the stage or plus disease status, if applicable.

6.2.3. Primary Endpoint Results: Absence of Active ROP and Unfavorable Structural Outcomes at 52 Weeks (Chronological Age)

The majority of infants in both groups met the primary endpoint criteria, absence of active ROP and unfavorable structural outcomes at 52 weeks CA based on the investigator's assessment. The adjusted difference (95.1% CI) was -1.88% (-16.99%, 13.23%) in the FAS; the lower bound of the CI for treatment difference was below the prespecified non-inferiority margin of -5% (Table 18). Therefore, non-inferiority of aflibercept to laser could not be established at a significance level of 0.0245 (1-sided). Despite missing this threshold, the results observed are meaningful with successful outcomes numerically similar between arms and studies.

Again, the success rates seen in the laser group are notably higher than previously assumed based on published data from the RAINBOW study (see details in Section 6.1.1.3).

Table 18: FIREFLEYE: Proportion Analysis of Infants with Absence of Active ROP and Unfavorable Structural Outcomes at Week 52 CA (FAS)

Treatment	Infants with absence of active ROP and unfavorable structural outcomes at Week 52 of age	Adjusted difference (%) (95.1% CI)[a]
Aflibercept (N=75)	59/75 (78.7%)	-1.88% (-16.99%, 13.23%)
Laser (N=38)	31/38 (81.6%)	

Abbreviations: CA=chronological age; CI=confidence interval; EOS=end of study; FAS=full analysis set; ROP= Retinopathy of prematurity; TEAE=treatment-emergent adverse event.
[a]Difference with CI was calculated using Mantel-Haenszel weighting scheme adjusted by baseline ROP status.
Endpoint was based on investigator assessment.
Note: If the patient data were not available at EOS visit, then data available from the Week 40 CA visit were used to impute Week 52; however, if the patient discontinued before the Week 40 CA visit, the patient was considered a non-responder. If patients discontinued the study due to TEAEs between Week 40 and Week 52, then these patients were considered as non-responders.
Patients were considered as non-responders if rescue treatment (includes any second treatment modality) was given in the study eye(s).
For patients with both eyes enrolled in the study, both eyes must meet the endpoint. Patients with only one study eye enrolled were responders if the respective eye responded.

6.2.3.1. Sensitivity Analyses of Primary Endpoint

The primary efficacy variable was also analyzed using the ITT and PP Populations as sensitivity analyses. As observed for the FAS, there was negligible difference between the 2 treatment groups with the lower bound of the CI less than -5% (Table 19). In the ITT Population, there was a higher proportion of infants in the aflibercept group without active ROP or unfavorable structural outcomes compared to the laser group (78.7% and 72.1%, respectively) with an adjusted difference (95.1% CI) of 7.51% (-8.50%, 23.51%).

In the PP Population, 81.8% of infants in the aflibercept group and 80.0% of infants in the laser group met the primary endpoint, with an adjusted difference (95.1% CI) of 3.18% (-12.69%, 19.06%).

A supplementary analysis using observed cases only (ie, infants who completed Week 52 CA) showed that, in both treatment groups, a vast majority of infants met the primary endpoint: 89.4% of infants in the aflibercept group and 91.2% of infants in the laser group, with an adjusted difference (95.1% CI) of -0.45% (-12.25%, 11.34%).

Table 19: FIREFLEYE: Sensitivity Analysis of Proportion of Infants with Absence of Active ROP and Unfavorable Structural Outcomes at Week 52 CA

Treatment	Infants with absence of active ROP and unfavorable structural outcomes at Week 52 of age	Adjusted difference (%) (95.1% CI)[a]
All Randomized Patients		
Aflibercept (N=75)	59/75 (78.7%)	7.51 (-8.50, 23.51)
Laser (N=43)	31/43 (72.1%)	
Per Protocol Set		
Aflibercept (N=66)	54/66 (81.8%)	3.18 (-12.69, 19.06)
Laser (N=35)	28/35 (80.0%)	
Observed Case (Full Analysis Set)		
Aflibercept (N=75)	59/66 (89.4%)	-0.45 (-12.25, 11.34)
Laser (N=38)	31/34 (91.2%)	

Abbreviations: CA=chronological age; CI=confidence interval; EOS=End of Study; ROP= Retinopathy of prematurity; TEAE=treatment-emergent adverse event.
[a]Difference with CI is calculated using Mantel-Haenszel weighting scheme adjusted by baseline ROP status.
Endpoint was based on investigator assessment.
Note: If the patient data were not available at EOS visit, then data available from the Week 40 CA visit were used to impute Week 52; however, if the patient discontinued before the Week 40 CA visit, the patient was considered a non-responder. If patients discontinued the study due to TEAEs between Week 40 and Week 52, then these patients were considered as non-responders.
Patients were considered as non-responders if rescue treatment (includes any second treatment modality) is given in the study eye(s).
For patients with both eyes enrolled in the study, both eyes must meet the endpoint. Patients with only one study eye enrolled were responders if the respective eye responded.

6.2.3.2. ROP Status at Baseline: Primary Endpoint

Subgroup analyses did not reveal meaningful differences in response based on baseline ROP status (Table 20). Response rates for both treatments were lower in infants with Zone I ROP and AP-ROP than in Zone II ROP.

Table 20: FIREFLEYE: Proportion Analysis of Infants with Absence of Active ROP and Unfavorable Structural Outcomes at Week 52 CA by ROP Status Subgroup (FAS)

Baseline ROP Status	Treatment	Infants with absence of active ROP and unfavorable structural outcomes at Week 52 of age	Difference (%) (95.1% CI)[a]
ZONE I	Aflibercept (N=15)	10/15 (66.7%)	9.52% (-34.41%, 53.45%)
ZONE I	Laser (N=7)	4/7 (57.1%)	
Zone II	Aflibercept (N=46)	39/46 (84.8%)	-3.68% (-19.83%, 12.47%)
Zone II	Laser (N=26)	23/26 (88.5%)	
AP-ROP	Aflibercept (N=14)	10/14 (71.4%)	-8.57% (-51.06%, 33.91%)
AP-ROP	Laser (N=5)	4/5 (80.0%)	

Abbreviations: AP-ROP= Aggressive posterior ROP; CA=chronological age; CI=confidence interval; EOS=End of Study; FAS=full analysis set; ROP=retinopathy of prematurity; TEAE=treatment-emergent adverse event.
[a] Difference with CI was calculated using Mantel-Haenszel weighting scheme.
Endpoint was based on investigator assessment.
Note: If the patient data were not available at EOS visit, then data available from the Week 40 CA visit were used to impute Week 52; if the patient discontinued before the Week 40 CA visit, the patient was considered a non-responder. If patients discontinued the study due to TEAEs between Week 40 and Week 52, then these patients were considered as non-responders. Patients were considered as non-responders if rescue treatment (includes any second treatment modality) was given in the study eye(s). For patients with both eyes enrolled in the study, both eyes must have met the endpoint. Patients with only one study eye enrolled were responders if the respective eye responded.

6.2.4. Secondary Endpoints

6.2.4.1. Requirement of Second Treatment Modality

The proportion of infants in either treatment group requiring intervention with a second treatment modality was approximately 13% in both treatment groups with an adjusted difference (95.1% CI) of -0.37% (-13.57%, 12.83%).

6.2.4.2. Recurrence of ROP Through Week 52 (Chronological Age)

The proportion of infants in either treatment group with recurrence of ROP was similar (30.7% in the aflibercept group compared to 26.3% in the laser group) with an adjusted difference (95.1% CI) of 3.64% (-13.54%, 20.82%).

The mean time to recurrence of ROP was 60.3 days for the laser group and 100.4 days for the aflibercept group. There was no statistically significant difference between treatment groups for time to recurrence of ROP. At the time of this analysis, most events for each treatment group were censored and median time to recurrence was not estimable.

6.2.5. Exploratory Endpoints

6.2.5.1. Requirements for Sedation and General Anesthesia

The requirement for sedation to complete the study procedure was similar for the 2 treatment groups; however, the requirement for general anesthesia for aflibercept treatment was less frequent compared to laser treatment (43.6% vs 64.0%, respectively; Table 21).

The need for each infant to receive sedation or general anesthesia to complete the study procedure at least once was also less frequent in the aflibercept group compared to the laser group: 69.3% versus 86.8%, respectively.

Table 21: FIREFLEYE: Requirement for Sedation or General Anesthesia to Complete Laser or Aflibercept Injection (FAS)

	Aflibercept	Laser
Total number of sessions[a]	172	89
Study treatment needed sedation or general anesthesia to complete study procedure		
Sedation	47/172 (27.3%)	19/89 (21.3%)
General anesthesia	75/172 (43.6%)	57/89 (64.0%)
Neither	50/172 (29.1%)	13/89 (14.6%)
Infant needed sedation or general anesthesia to complete study procedure at least once		
Yes	52/75 (69.3%)	33/38 (86.8%)
No	23/75 (30.7%)	5/38 (13.2%)

Abbreviations: FAS=full analysis set.
[a] This row presents the denominator for calculation of percentages in subrows immediately below.
Note: Does not include rescue treatment.

6.2.5.2. Time Required to Perform Treatment

Infants who received laser treatment had substantially longer treatment durations than those who received aflibercept. The mean (SD) time to perform study treatment was 3.5 (5.18) minutes per infant for the aflibercept group and 117.7 (86.15) minutes per infant for the laser group (Table 22).

Table 22: FIREFLEYE: Time Required to Perform Aflibercept Injection or Initial Laser Treatment (FAS)

	Aflibercept	Laser
Time to perform study treatment per infant (min)[a]		
n	73	38
Mean (SD)	3.5 (5.18)	117.7 (86.15)
Time to perform study treatment per eye (min)[b]		
n	140	71
Mean (SD)	1.9 (2.62)	63.0 (44.03)

Abbreviations: FAS=full analysis set; SD=standard deviation, min=minutes.
[a] Durations for bilaterally treated patients and for multiple laser sessions within 7 days of initiation of laser treatment (per protocol considered as one session) were added prior to summarization.
[b] Multiple laser sessions within 7 days of initiation of laser treatment (per protocol considered as one session) for the same eye were added prior to summarization.
Note: The values summarized in this table are for the initial treatments only.

6.2.5.3. Visual Function

Most eyes in the aflibercept and laser groups demonstrated central fixation (approximately 85% overall) and were able to fix and follow a 5-cm toy (approximately 86% overall) (Table 23).

For cycloplegic refraction, the refractive spherical equivalent (SE) at Week 52 CA is displayed and was calculated as: sphere + half cylinder. The mean SE (SD) was -0.2 (2.78) diopters in the aflibercept group and, slightly higher at -1.1 (3.30) diopters in the laser group.

In both treatment groups, the majority of eyes had neither manifest strabismus (75% overall) nor nystagmus in the primary position of gaze (86% overall).

Table 23: FIREFLEYE: Evaluation of Visual Function at Week 52 CA (FAS)

	Aflibercept	Laser
Number of eyes treated [a] [b]	146	72
Fixation (central, steady, and maintained)		
Yes	123/146 (84.2%)	63/72 (87.5%)
No	4/146 (2.7%)	1/72 (1.4%)
Fixing and following a 5-cm toy		
Yes	124/146 (84.9%)	63/72 (87.5%)
No	3/146 (2.1%)	1/72 (1.4%)
Number of eyes whose visual function cannot be assessed with fixation [a]	4/146 (2.7%)	12/72 (16.7%)
Visual function assessment via visual evoked potentials		
Normal	4/4 (100%)	10/12 (83.3%)
Abnormal	0/4	2/12 (16.7%)
Cycloplegic refraction, spherical equivalent (D)		
N	117	59
Mean (SD)	-0.2 (2.78)	-1.1 (3.30)
Ocular motility tests		
Assessment of ocular palsy with affected nerves		
Yes	1/146 (0.7%)	0/72
No	125/146 (85.6%)	64/72 (88.9%)
Unknown	1/146 (0.7%)	0/72
Assessment of strabismus		
Normal	109/146 (74.7%)	54/72 (75.0%)
Abnormal	17/146 (11.6%)	10/72 (13.9%)
Unknown	1/146 (0.7%)	0/72
Assessment of nystagmus		
Normal	125/146 (85.6%)	62/72 (86.1%)
Abnormal	2/146 (1.4%)	2/72 (2.8%)

Abbreviations: CA=chronological age; D=diopter; FAS=full analysis set; SD=standard deviation.
[a] This row presents the denominator for calculation of percentages in subrows below.
[b] Number of eyes treated includes all eyes treated at baseline and eyes for which treatment started after the baseline visit and before the Week 8 visit.
Note: Patient or eyes treated refers to any study intervention, either with aflibercept or with laser treatment.

6.3. Efficacy Summary

Efficacy results from the Phase 3 Studies based on 120 treated infants (93 aflibercept, 27 laser) in BUTTERFLEYE and 113 (75 aflibercept, 38 laser) in FIREFLEYE suggest that the high treatment response after baseline treatment with aflibercept 0.4 mg is robust and sustained at 52 weeks CA. The vast majority of infants from the aflibercept group (79.6% in BUTTERFLEYE and 78.7% in FIREFLEYE) met the primary endpoint criteria, demonstrating both robust efficacy of the drug in the treatment of ROP and also showing numerically similar efficacy to infants treated with laser. This response rate for aflibercept is in keeping with other randomized-

controlled trial data on anti-VEGF therapy for the treatment of ROP (Mintz-Hittner, 2011; Stahl, 2019). The studies did not satisfy the non-inferiority hypothesis, which required that the lower bound of the 95.1% CI be greater than -5%. These results should be interpreted with caution, however, since the success rates seen in the laser group are higher than previously assumed based on published data from the RAINBOW study. The RAINBOW study reported a response rate in the laser group of 66.2% (95% CI: 55.0%–77.4%), compared to 77.8% in BUTTERFLEYE, raising the possibility that the constancy assumption of the active control group was violated. Had the rate observed in the RAINBOW study been replicated in this study, the non-inferiority criterion would have been met. Despite not meeting the statistical criteria, the results of both treatments were numerically similar, and conclusions must therefore be drawn based on a clinical assessment of the benefit/risk of the 2 treatments.

The proportion of infants in either treatment group requiring intervention with a second treatment modality was numerically lower in the aflibercept group compared to the laser group (15.1% vs 18.5%, respectively) in BUTTERFLEYE while the proportion of infants in either treatment group requiring intervention with a second treatment modality was similar in FIREFLEYE (approximately 13% of infants in each treatment group).

The proportion of infants with recurrence of ROP was 39.8% for the aflibercept group and 29.6% for the laser group in BUTTERFLEYE while the proportion of infants with recurrence of ROP was 30.7% for the aflibercept group and 26.3% for the laser group in FIREFLEYE. It is important to consider that disease recurrence by itself may not necessarily imply a medical condition requiring additional treatment. Cases of disease reactivation can be transient. In general, recurrences of ROP activity after anti-VEGF treatment are not uncommon and are also more common than after laser, based on different mechanisms of action of anti-VEGF agents and laser. Clinicians treating ROP are aware of the risk of reactivation and recommendations for follow-up are described in published guidelines. By mechanism of action, anti-VEGF therapy, when compared to laser, causes disease regression to occur faster but also has a higher likelihood of disease reactivation. These observations are not unexpected and are related to the fact that, unlike laser, anti-VEGF agents are not destructive and therefore, while they do bind to VEGF, they do not prevent the production of VEGF. With decreasing anti-VEGF levels in the eye, newly produced VEGF from any avascular areas may be associated with reactivation of severe ROP, which is why the studies allowed up to 3 treatments with aflibercept. However, it should be noted that most eyes only required a single aflibercept injection. In contrast, after complete radical tissue ablation of the ischemic/avascular retina by laser, only minimal amounts of VEGF if any can be produced by the laser-ablated retina, which renders reactivation of active and severe ROP unlikely (Fleck, 2022).

Aflibercept treatment was also associated with less need for sedation or general anesthesia compared to laser procedure in both studies. The time required to carry out the aflibercept treatment was shorter than the time required to perform the laser treatment. Such a short amount of time taken to perform aflibercept treatment may have a significantly positive impact in reducing the time and hence the treatment burden in a very sensitive patient population. It should be noted that, in order to perform a complete laser treatment, approximately 11% and 19% of infants in the laser group in FIREFLEYE and BUTTERFLEYE, respectively, required additional laser within the first week of their initial laser treatment, subjecting these vulnerable patients to even more time under anesthesia. Lastly, assessment of visual function revealed a mean

refractive error (SE) of 0.5 D in the aflibercept group and -1.0 D in the laser group in BUTTERFLEYE while in FIREFLEYE it was -0.2 D in the aflibercept group and -1.1D in the laser group.

To conclude, treatment with aflibercept 0.4 mg was associated with a high response rate of 78.7% in FIREFLEYE and 79.6% in BUTTERFLEYE and meaningful benefits on several clinically relevant ocular outcomes in preterm infants with ROP, as indicated by the rates of absence of active ROP and unfavorable structural outcomes at 52 weeks CA after baseline treatment. The evidence also suggests a lower treatment burden for the infant, including a shorter duration of treatment and lower need for general anesthesia.

7. CLINICAL SAFETY

Summary

- Aflibercept was well tolerated, with mostly mild and transient TEAEs observed in infants with Type 1 ROP and no new safety findings were identified. The clinical development program demonstrated a favorable safety profile.
- A similar proportion of infants in the aflibercept group and laser group experienced TEAEs in both studies.
  - Most TEAEs were reported in only 1 infant in a treatment group.
  - No new adverse drug reactions (ADRs) were identified for the infant population, and not all known ADRs for aflibercept were reported in the Phase 3 studies.
- Infants treated with aflibercept experienced fewer serious or severe (Grade ≥ 3) ocular and non-ocular TEAEs than infants treated with laser therapy.
- TEAEs leading to discontinuation were infrequent (≤ 2% in each group).
- Deaths were infrequent and deemed unrelated to study treatment by investigators.

7.1. Treatment Exposure

Across both studies, 168 infants were treated with aflibercept (93 infants in BUTTERFLEYE and 75 infants in FIREFLEYE), and 65 infants were treated with laser (27 infants in BUTTERFLEYE and 38 infants in FIREFLEYE). Exposure is summarized in Table 24 and Table 25, respectively.

In BUTTERFLEYE, 93 infants received treatment with aflibercept in a total of 179 eyes. Among the 179 eyes treated with aflibercept, 83% received a single injection, 14% received 2 injections (baseline and 1 retreatment), and 3% received 3 injections (baseline and 2 retreatments). Thirteen aflibercept infants (14%) required laser rescue treatment; 4 infants received 1 laser treatment, 8 infants received 2 laser treatments, and 1 infant received 5 laser treatments. In the laser group, 4 infants (15%) received 2 aflibercept injections as rescue treatment.

In FIREFLEYE, 75 infants received treatment with aflibercept in a total of 146 eyes. Among the 146 eyes treated with aflibercept, 82% received a single injection and 18% received 2 injections (baseline and 1 retreatment). Five aflibercept infants (7%) required laser rescue treatment: 3 infants received 1 laser treatment and 2 infants received 2 laser treatments. In the laser group, among the 38 infants, 3 (8%) received rescue treatment with 2 aflibercept injections, and 1 (3%) received rescue treatment with 3 aflibercept injections.

Additionally, 19% of infants in BUTTERFLEYE and 4% of infants in FIREFLEYE in the laser group required retreatment within the first week, translating into the need for additional sedation/general anesthesia, the need to transport infants to the operating room multiple times, and additional resources including operating room time.

Table 24: Summary of Aflibercept Exposure in Study Eye by Treatment Group (Pooled SAF)

Treated, n (%)	BUTTERFLEYE		FIREFLEYE	
	Aflibercept (N=93)	Laser (N=27)	Aflibercept (N=75)	Laser (N=38)
Number of infants treated	93	27	75	38
Number of aflibercept administrations per infant				
0	0	23 (85.2)	0	34 (89.5)
1	7 (7.5)	0	4 (5.3)	0
2	67 (72.0)	4 (14.8)	55 (73.3)	3 (7.9)
3	8 (8.6)	0	6 (8.0)	1 (2.6)
4	8 (8.6)	0	10 (13.3)	0
5	1 (1.1)	0	0	0
6	2 (2.2)	0	0	0
Number of eyes treated	179	50	146	72
Number of aflibercept administrations per eye[a]				
0	0	42 (84.0)	0	64 (88.9)
1	149 (83.2)	8 (16.0)	120 (82.2)	7 (9.7)
2	25 (14.0)	0	26 (17.8)	1 (1.4)
3	5 (2.8)	0	0	0

* Including retreatment
Note: Infants in the laser group that received aflibercept did so as rescue treatment.

Table 25: Summary of Laser Exposure in Study Eye by Treatment Group (Pooled SAF)

Treated, n (%)	BUTTERFLEYE		FIREFLEYE	
	Aflibercept (N=93)	Laser (N=27)	Aflibercept (N=75)	Laser (N=38)
Number of infants treated	93	27	75	38
Number of laser treatments per infant				
0	80 (86.0)	0	70 (93.3)	0
1	4 (4.3)	4 (14.8)	3 (4.0)	4 (10.5%)
2	8 (8.6)	22 (81.5)	2 (2.7)	30 (78.9%)
3	0	0	0	1 (2.6%)
4	0	1 (3.7)	0	2 (5.3%)
5	1 (1.1)	0	0	0
6	0	0	0	1 (2.6%)
Number of eyes treated	179	50	146	72
Number of laser treatments per eye[a]				
0	158 (88.3)	0	139 (95.2)	0
1	18 (10.1)	48 (96.0)	7 (4.8)	65 (90.3%)
2	2 (1.1)	2 (4.0)	0	5 (6.9%)
3	1 (0.6)	0	0	2 (2.8%)

* Including retreatment
Note: Infants in the aflibercept group that received laser did so as rescue treatment.

7.2. Overview of Adverse Events

The incidence of all TEAEs was comparable in the aflibercept and laser groups in BUTTERFLEYE (56% vs 59%, respectively) and FIREFLEYE (76% in both groups) (Table 26). In BUTTERFLEYE, 19% of infants in both groups experienced TE SAEs, and a numerically lower proportion of infants experienced TE SAEs in the aflibercept group (12%) compared to laser (26%) in FIREFLEYE.

The proportion of infants with any ocular TEAE in the treated eye was lower in aflibercept group (18%) compared to laser (26%) in BUTTERFLEYE and was similar in both groups in FIREFLEYE (39% vs 37%, respectively). Non-ocular TEAEs were also numerically lower in the aflibercept group than laser in BUTTERFLEYE (47% vs 52%) and FIREFLEYE (53% vs 66%, respectively).

The majority of TEAEs in both groups were mild to moderate in both studies. TEAEs leading to discontinuation of study drug were experienced by 3 infants in the aflibercept group and 1 infant in the laser group in FIREFLEYE and no infant in BUTTERFLEYE. There were 4 AEs resulting in death in the aflibercept group, 2 of which occurred within 30 days of last treatment (ie,

treatment-emergent). None of the deaths were considered related to treatment (details provided in Section 7.7).

Table 26: Summary of Treatment-Emergent Adverse Events by Treatment Group

	BUTTERFLEYE		FIREFLEYE	
Infants with, n (%)	**Aflibercept (N=93)**	**Laser (N=27)**	**Aflibercept (N=75)**	**Laser (N=38)**
Any TEAEs	52 (55.9)	16 (59.3)	57 (76.0)	29 (76.3)
Any ocular TEAE in study eye	17 (18.3)	7 (25.9)	29 (38.7)	14 (36.8)
Any ocular TEAE in fellow eye	0	0	1 (1.3)	0
Any non-ocular TEAE	44 (47.3)	14 (51.9)	40 (53.3)	25 (65.8)
Maximum severity for any TEAE				
Mild	18 (19.4)	5 (18.5)	32 (42.7)	13 (34.2)
Moderate	23 (24.7)	7 (25.9)	16 (21.3)	10 (26.3)
Severe	11 (11.8)	4 (14.8)	8 (10.7)	5 (13.2)
Any TE SAE	18 (19.4)	5 (18.5)	9 (12.0)	10 (26.3)
Any ocular TE SAE in study eye	6 (6.5)	3 (11.1)	6 (8.0)	3 (7.9)
Any ocular TE SAE in fellow eye	0	0	0	0
Any non-ocular TE SAE	12 (12.9)	2 (7.4)	5 (6.7)	7 (18.4)
Any TEAE leading to discontinuation of study drug	0	0	3 (4.0)	1 (2.6)
AE leading to death	1 (1.1)	0	3 (4.0)	0

Abbreviations: AE=adverse event; SAF=safety analysis set; TEAE=treatment-emergent adverse event; TE SAE=treatment-emergent serious adverse event.

7.3. Ocular Adverse Events

7.3.1. Ocular Adverse Events in the Study Eye

Overall, the number of infants who experienced at least 1 ocular TEAE was similar in the aflibercept group compared to the laser group across studies (Table 27). Ocular TEAEs in the study eye reported in ≥ 5% of infants in any treatment group were retinal hemorrhage, retinal detachment, conjunctival hemorrhage, conjunctivitis, and eyelid edema.

Table 27: Summary of Ocular Treatment-Emergent Adverse Events in Study Eye(s) in ≥ 5% of Infants in Either Group (Pooled SAF)

	BUTTERFLEYE		FIREFLEYE	
Preferred Term, n (%)	Aflibercept (N=93)	Laser (N=27)	Aflibercept (N=75)	Laser (N=38)
Number of infants with any ocular TEAE	17 (18.3)	7 (25.9)	29 (38.7)	14 (36.8)
Retinal detachment	6 (6.5)	2 (7.4)	4 (5.3)	2 (5.3)
Conjunctival hemorrhage	5 (5.4)	0	4 (5.3)	0
Retinal hemorrhage	3 (3.2)	1 (3.7)	5 (6.7)	5 (13.2)
Conjunctivitis	0	0	3 (4.0)	4 (10.5)
Eyelid edema	0	1 (3.7)	2 (2.7)	3 (7.9)

Abbreviations: TEAE=treatment-emergent adverse event.
Note: The percentage was based on the number of infants in each treatment group as denominator.

7.3.2. Ocular Adverse Events Leading to Discontinuation

In BUTTERFLEYE, none of the infants in either group experienced ocular TEAEs leading to discontinuation of study drug. In FIREFLEYE, 3 (4.0%) infants in the aflibercept group and 1 (2.6%) infant in the laser group discontinued treatment due to ocular TEAEs, which included retinal detachment (aflibercept: 2 [2.7%]; laser: 1 [2.6%]) and ROP (aflibercept: 1 [1.3%]; laser: 0 [0.0%]).

7.3.3. Ocular Serious Adverse Events

The frequency of ocular TE SAEs was similar across groups in both studies (Table 28). Ocular TE SAEs in ≥ 2 infants in either group were retinal detachment, vitreous hemorrhage, and retinal hemorrhage. There were no ocular TE SAEs reported in the fellow eye.

Table 28: Ocular Treatment-Emergent Serious Adverse Events Reported in > 1 Infant in Either Group

	BUTTERFLEYE		FIREFLEYE	
Preferred Term, n (%)	Aflibercept (N=93)	Laser (N=27)	Aflibercept (N=75)	Laser (N=38)
Any ocular TE SAE	6 (6.5)	3 (11.1)	6 (8.0)	3 (7.9)
Retinal detachment	6 (6.5)	2 (7.4)	3 (4.0)	2 (5.3)
Vitreous hemorrhage	2 (2.2)	0	1 (1.3)	0
Retinal hemorrhage	0	0	2 (2.7)	0

Abbreviations: TE SAE=treatment-emergent serious adverse event.

7.4. Non-Ocular Adverse Events

7.4.1. Common Non-Ocular Adverse Events

Non-ocular TEAEs occurred in a lower proportion of infants in the aflibercept group than the laser group in both studies (Table 29). The most commonly occurring non-ocular TEAEs

reported in ≥ 5% of infants in either group were bronchopulmonary dysplasia, apnea/infantile apnea, inguinal hernia, umbilical hernia, gastroesophageal reflux disease, and constipation. Generally, these TEAEs and their frequency are known to be associated with underlying prematurity of the newborn, and the numerical imbalances in individual preferred terms (PTs) are not suggestive of a new safety signal.

Table 29: Summary of Non-ocular Treatment-Emergent Adverse Events in ≥ 5% of Infants in Either Group

	BUTTERFLEYE		FIREFLEYE	
Primary System Organ Class Preferred Term, n (%)	**Aflibercept (N=93)**	**Laser (N=27)**	**Aflibercept (N=75)**	**Laser (N=38)**
Infants with any non-ocular TEAE	44 (47.3)	14 (51.9)	40 (53.3)	25 (65.8)
Bronchopulmonary dysplasia	6 (6.5)	0	2 (2.7)	0
Inguinal hernia	6 (6.5)	2 (7.4)	2 (2.7)	1 (2.6)
Umbilical hernia	5 (5.4)	0	2 (2.7)	3 (7.9)
Apnea	2 (2.2)	4 (14.8)	2 (2.7)	3 (7.9)
Gastroesophageal reflux disease	3 (3.2)	2 (7.4)	1 (1.3)	1 (2.6)
Anemia	3 (3.2)	0	1 (1.3)	2 (5.3)
Anemia neonatal	3 (3.2)	0	0	2 (5.3)
Bacterial disease carrier	1 (1.1)	1 (3.7)	0	2 (5.3)
Constipation	1 (1.1)	3 (11.1)	0	0
Oxygen saturation decreased	1 (1.1)	2 (7.4)	3 (4.0)	0
Hemorrhage subcutaneous	0	0	0	3 (7.9)
Infantile apnea	0	0	0	2 (5.3)

Abbreviations: TEAE=treatment-emergent adverse event.
Note: The percentage is based on the number of infants in each treatment group as denominator.

7.4.2. Non-Ocular Adverse Events Leading to Discontinuation

There were no non-ocular TEAEs leading to treatment discontinuation in either group.

7.4.3. Non-Ocular Serious Adverse Events

Non-ocular TE SAEs occurred in more infants in the aflibercept group in BUTTERFLEYE and in more infants in the laser group in FIREFLEYE. Non-ocular TE SAEs reported in ≥ 2 infants in either group were apnea, bronchiolitis, and inguinal hernia, and pneumonia. No events were considered related to study treatment.

Table 30: Non-ocular Serious Adverse Events Occurring in ≥ 2 Infants

	BUTTERFLEYE		FIREFLEYE	
Preferred Term, n (%)	Aflibercept (N=93)	Laser (N=27)	Aflibercept (N=75)	Laser (N=38)
Any non-ocular TE SAE	12 (12.9)	2 (7.4)	5 (6.7)	7 (18.4)
Apnea	2 (2.2)	2 (7.4)	0	1 (2.6)
Bronchiolitis	0	0	2 (2.7)	1 (2.6)
Inguinal hernia	2 (2.2)	0	0	0
Pneumonia	1 (1.1)	0	1 (1.3)	0
Infantile apnea	0	0	0	2 (5.3)

Abbreviations: TE SAE=treatment-emergent serious adverse event.

7.5. Additional Safety Topics of Interest for Aflibercept

7.5.1. Blood Pressure

Systolic Blood Pressure

In BUTTERFLEYE, both groups had an age-appropriate rise in the mean (SD) SBP from baseline (aflibercept 76.2 ± 11.36 mmHg vs laser 78.8 ± 13.29 mmHg) over time through 52 weeks CA (aflibercept 99.7 ± 14.64 mmHg vs laser 100.9 ± 15.75 mmHg).

In FIREFLEYE, both groups had an age-appropriate rise in the mean (SD) SBP from baseline (aflibercept 76.5 ± 12.5 mmHg vs laser 75.4 ± 12.0 mmHg) over time through 52 weeks CA (aflibercept 94.0 ± 12.8 mmHg vs laser 89.2 ± 12.7 mmHg).

Diastolic Blood Pressure

In BUTTERFLEYE, both groups had an age-appropriate rise in the mean (SD) DBP from baseline (aflibercept 43.1 ± 9.80 mmHg versus laser 46.1 ± 13.08 mmHg) over time through 52 weeks CA (aflibercept 61.6 ± 12.38 mmHg versus laser 61.4 ± 18.56 mmHg).

In FIREFLEYE, both groups had an age-appropriate rise in the mean (SD) DBP from baseline (aflibercept 44.1 ± 9.6 mmHg vs laser 44.9 ± 10.9 mmHg) over time through 52 weeks CA (aflibercept 56.5 ± 10.9 mmHg vs laser 53.0 ± 12.3 mmHg).

Immunogenicity

No treatment-emergent or treatment-boosted anti-drug antibody (ADA) responses were observed in any participant who received unilateral or bilateral aflibercept injections in BUTTERFLEYE. Treatment-emergent ADA was reported in 1 infant in the aflibercept group in FIREFLEYE at Week 12, and the ADA titer was low (1:30). No neutralizing antibody response was observed in this participant.

7.6. Adverse Drug Reactions

No new ADRs specific for the ROP population were identified. The proposed prescribing information states that the adverse reactions of retinal detachment, conjunctival hemorrhage, injection site hemorrhage, intraocular pressure (IOP) increased, and eyelid edema were reported

in more than 1 infant in either BUTTERFLEYE or FIREFLEYE. Adverse reactions established for adult indications (ie, conjunctival hemorrhage, eye pain, cataract, vitreous detachment, vitreous floaters, and IOP increased) are considered applicable to preterm infants with ROP, though not all were observed in the Phase 3 studies. The data in Table 31 reflect adverse reactions observed in 168 preterm infants with ROP randomized to aflibercept and treated with the 0.4 mg dose per eye in the 2 clinical studies (BUTTERFLEYE and FIREFLEYE/NEXT).

Table 31: Adverse Reactions in BUTTERFLEYE and FIREFLEYE

	Baseline to 52 Weeks CA			
	BUTTERFLEYE		FIREFLEYE	
Adverse Reactions, %	Aflibercept (N=93)	Laser (N=27)	Aflibercept (N=75)	Laser (N=38)
Retinal detachment	6.5%	7.4%	5.3%	5.3%
Conjunctival hemorrhage	5.4%	0	5.3%	0
Injection site hemorrhage	0	0	4.0%	0
Intraocular pressure increased	0	0	4.0%	0
Corneal epithelium defect	1.1%	0	0	0
Eyelid edema	0	3.7%	2.7%	7.9%
Corneal edema	0	0	1.3%	2.6%
Lenticular opacities	0	0	1.3%	0

Abbreviations: CA=chronological age.

7.7. Deaths

There were 4 AEs resulting in death reported in 168 infants treated with aflibercept (1 death in BUTTERFLEYE and 3 in FIREFLEYE), for an incidence rate of 1 death per 42 infants in the aflibercept group and 0 deaths in the 65 infants treated with laser. Of the 4 deaths, 2 were treatment-emergent (ie, occurred within 30 days of the last dose). None of these cases were deemed by the investigators or sponsors as related to investigational drug treatment. The causes of death were consistent with complications of prematurity. For context, the rate of death reported in the RAINBOW study was 5% (Stahl, 2019). The mortality rate observed in the current studies also falls within the expected mortality rate for this population based on the scientific literature (Ely, 2022; MacDorman, 2014; Mantagos, 2017; Manuck, 2016; Siffel, 2022; Stahl, 2019; Stoll, 2015; World Health Organization 2020). Detailed narratives of these events are provided in Appendix 11.2.

Table 32: Deaths Occurring in BUTTERFLEYE and FIREFLEYE

AE Leading to Death	Sex/ gestational age at birth (weeks)	Birth Weight (grams)	Time to onset of AE after last treatment (days)	Time to death since last treatment (days)	Chronological age at death (days)	Medical History at baseline
BUTTERFLEYE						
Multiple organ dysfunction syndrome	F/24.7	620	28	58	146	Patent Ductus Arteriosus, Pulmonary hypertension, Respiratory failure in newborn, Chronic lung disease and respiratory failure, Necrotizing enterocolitis, intestinal obstruction, parenteral nutrition, CMV infection, bacterial test positive, thrombocytopenia, skin disorder, adrenal insufficiency
FIREFLEYE						
Bronchopulmonary dysplasia and pneumothorax	F/23w 6d	445	57	59	214	Infantile Apnea, Bronchopulmonary dysplasia, Anemia, hypocarnitinemia, abdominal distension, extremity contracture, osteoporosis
Bronchiolitis	F/24w 1d	640	52	56	115	Neonatal respiratory distress syndrome, Bronchopulmonary dysplasia, Interstitial pulmonary emphysema, Several episodes of neonatal sepsis, anemia
Bronchopulmonary dysplasia	M/26	790	28	28	153	Atrial septal defect, Perinatal brain damage, Bronchopulmonary dysplasia, Respiratory failure, Apnea, Severe anemia, jaundice, dystonia, leukocyturia

Abbreviations: AE=adverse event; CMV=cytomegalovirus.

7.8. Post-marketing Safety Experience

Cumulative data from post-marketing reporting of aflibercept use in patients, as of 15 May 2022, comprised a total of 25,668 TE SAE reports received from worldwide solicited and spontaneous sources. The most frequent TE SAEs fell into the Medical Dictionary for Regulatory Activities (MedDRA) SOC Eye disorders, followed by events in the General disorders and administration site conditions and Injury, poisoning and procedural complications MedDRA system organ classes (SOCs), reflecting the known safety profile in the aflibercept-treated adult population. The post-marketing safety data are consistent with the established safety profile of aflibercept in adult patients treated for approved indications as outlined in the current prescribing information.

Concerning the off-label use of aflibercept in patients with ROP, as of 15 May 2022, a total of 67 cases reporting 53 non-serious and 14 serious events were reported. Of these 67 reports, 29 were spontaneous and 38 were from the literature reports. No new or unexpected safety findings were identified; the TEAEs reflect the underlying prematurity and comorbidity profile of these vulnerable preterm infants with ROP.

7.9. Safety Summary

Treatment with intraocular aflibercept injection was well tolerated. No new or unexpected findings were identified in terms of ocular safety and systemic safety.

The ocular safety profile comprises predominantly injection procedure-related TEAEs, mostly of a mild and transient nature, as well as ocular events compatible with the underlying disease of ROP. No intraocular inflammation was reported.

The non-ocular TEAEs reported were consistent with the complications of prematurity. In total, 4 deaths (1% in BUTTERFLEYE and 4% in FIREFLEYE) were reported in the aflibercept-treated group, and none were considered related to treatment. The mortality rate reported in this study is lower than that observed in the 0.1 mg and 0.2 mg IVT ranibizumab groups in the RAINBOW study (5.3% and 5.5%, respectively) over a 24-week study period (Stahl, 2019) and in the scientific literature for this patient population.

In terms of systemic drug exposure measured in plasma, maximum concentrations (mean) of free aflibercept were reached at Week 0/Day 1 and declined to non-quantifiable levels in almost all patients 8 weeks after initial bilateral dosing. Bound aflibercept increased within 4 weeks and decreased thereafter up to week 24. Drug concentrations, either in free or bound form, were not associated with any TEAEs. Only a single infant developed ADAs in these studies.

Growth (body length, weight, and head circumference) through Week 52 were comparable between aflibercept and laser groups with age-related increases in both groups. Additionally, 2-year follow-up data from the RAINBOW study showed comparable outcomes in growth and neurocognitive development parameters between ranibizumab and laser groups (Stahl, 2019).

Overall, no new safety signals were observed in this population of preterm infants with Type 1 ROP who received mostly bilateral, same-day injections of 0.4 mg aflibercept per eye. Aflibercept treatment was well tolerated in infants with ROP requiring treatment. Ocular TEAEs were generally consistent with the established profile for aflibercept in adults.

8. POST-MARKETING PLAN

EYLEA is expected to have low systemic exposure following IVT injection however the possibility of systemic effects cannot be entirely excluded in preterm infants with an immature, and often impaired, blood-retinal barrier.

To address a gap in knowledge about long-term safety of IVT aflibercept in neonates requiring treatment for ROP, safety, efficacy, and developmental outcomes are being monitored in the ongoing BUTTERFLEYE NEXT and FIREFLEYE NEXT studies in which patients will be evaluated annually through their fifth birthday. Assessments will include visual function, ophthalmic examination, physical examination including vital signs, and assessment of development using standard scales. Regeneron has committed to providing the data from these studies to regulatory agencies and will seek to publish the data in the medical literature.

9. BENEFIT-RISK CONCLUSIONS

ROP is a rare, potentially vision-impairing and blinding retinal disease impacting preterm infants. The goal of treatment for ROP is to prevent blindness and preserve as much normal vision as possible. Despite the severity, there are no pharmaceutical agents currently approved in the US to treat these vulnerable infants. Laser photocoagulation is the only FDA-cleared treatment for ROP, and although it is effective, laser photocoagulation requires prolonged sedation or general anesthesia and has potential long-term complications. Due to the limitations of laser photocoagulation, anti-VEGF treatments including aflibercept have been increasingly used off-label with promising efficacy and safety; however, no anti-VEGF treatments are currently FDA approved for the treatment of ROP.

Aflibercept is an anti-VEGF injection that has been FDA approved since 2011 for the treatment of several retinal disorders in adults. Regeneron is seeking a supplemental indication for aflibercept for the treatment of ROP based on the efficacy and safety findings from the BUTTERFLEYE and FIREFLEYE/FIREFLEYE NEXT studies. The benefits observed throughout the development program are comparable to laser photocoagulation therapy, without the associated long-term complications. Importantly, the positive efficacy comes with a favorable benefit-risk profile supported by evidence from BUTTERFLEYE and FIREFLEYE through 52 weeks of CA and underpinned by more than 11 years of FDA-approved use in adult indications.

Efficacy results from BUTTERFLEYE and FIREFLEYE provide evidence of the clinical benefit of aflibercept 0.4 mg for the treatment of ROP. Aflibercept 0.4 mg showed a high rate of treatment success defined as absence of active ROP and unfavorable structural outcomes at 52 weeks CA and was achieved with mostly a single and no more than 3 injections per eye. The success rates for infants treated with aflibercept (79.6% and 78.7%) were numerically similar to the success rates for infants treated with laser (77.8% and 81.6%), although the primary statistical hypothesis for non-inferiority was not demonstrated in either study. The success rates in infants treated with aflibercept were similar to those observed for the ranibizumab-treated infants in the RAINBOW study. However, the observed laser response rates in BUTTERFLEYE and FIREFLEYE were higher than in published randomized-controlled studies on the efficacy of laser for the treatment of ROP, such as the BEAT-ROP (58%–88%, depending on zone) and RAINBOW (66%) studies (Mintz-Hittner, 2011; Stahl, 2019).

Compared to laser, treatment with aflibercept was associated with a much shorter duration of intervention and generally lower need for sedation or general anesthesia. The ease of treatment – aflibercept can be administered at the bedside in less than 10 minutes – must be considered in the benefit/risk profile of treatment. Aflibercept would enable earlier treatment of vascular proliferation and could postpone laser treatment in those infants who still need it to allow for growth of normal vasculature.

Evidence for the safety and tolerability of aflibercept 0.4 mg in the treatment of preterm infants with ROP is also based on data from the BUTTERFLEYE (93 aflibercept, 27 laser) and FIREFLEYE (75 aflibercept, 38 laser) at 52 weeks CA. Aflibercept was well tolerated with a favorable safety profile, with expected, mostly mild, and transient TEAEs. The safety profile of aflibercept 0.4 mg was also consistent with the established safety profile of aflibercept 2 mg in adult patients with retinal diseases; no new safety concerns or ADRs for preterm infants with

ROP have been identified with 52-week study data. Key risks considered for the benefit-risk assessment include the ocular events observed in these studies. These ocular risks are known from the established use of aflibercept in adult patients, are mainly related to the IVT injection procedure, and are adequately addressed in the prescribing information. Safety data beyond 52 weeks CA are being collected in the ongoing BUTTERFLEYE NEXT and FIREFLEYE NEXT where participants are followed-up until 5 years CA. Long-term safety is managed via routine pharmacovigilance and risk minimization measures.

Based on the totality of evidence supporting aflibercept for the treatment of infants with severe ROP, the expected clinical benefits outweigh the potential risks and establish that aflibercept is a good choice for treatment as an option alongside the only currently approved option: laser therapy. The studied patient population is representative of the target population of infants with ROP in medical need of therapeutic alternatives to laser photocoagulation. Currently, no pharmaceutical agent is approved in the US for the treatment of ROP, which represents an important unmet need in this patient population. The data support a new therapeutic indication for the treatment of preterm infants with ROP. Aflibercept provides a clinically meaningful benefit in the treatment of ROP in preterm infants, with less treatment burden than laser therapy, while reducing the risk of long-term complications such as high myopia and loss of peripheral vision. Approval of aflibercept for treating ROP would be an important step toward meeting the unmet medical need to provide a safe, effective, easily administered, FDA-approved treatment that can be given at the bedside for preterm infants with vision-threatening ROP.

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11. APPENDICES

11.1. Ophthalmological Assessments

Ophthalmological assessments at 52 weeks CA included visual function, refraction, ocular extrinsic motility, binocular indirect ophthalmoscopy, and anterior segment examination. With all ophthalmological examinations, abnormalities of the retina or optic nerve as well as unfavorable ocular structural outcomes in each eye are assessed. All ocular assessments were performed bilaterally, unless otherwise stated. Note: IOP was measured in both eyes prior to the injection only in patients receiving aflibercept. IOP was measured at least once post-injection (only in treated eyes).

- **Visual function** was evaluated using a methodology appropriate for the age and development status of the child, including evaluation of fixation (eg, central, steady, and maintained), and fixing and following a 5 cm toy. If the patient was unable to cooperate with these methods, another suitable method (eg, VEP) may have been used to evaluate visual function.
- **Cycloplegic refraction** was measured with retinoscopy and reported separately for each eye.
- **Ocular motility tests** assessed the integrity of the extrinsic ocular muscles and their nerves (eg, Cover test, Hirschberg test, or other).
- **Stereopsis testing** was assessed by appropriate test according to each patient and age (eg, TNO, Lang, or other). Stereopsis will be carried out in FIREFLEYE only at the end of the study (5-year visit), and thus is beyond the scope of this report.
- **Dilated binocular indirect ophthalmoscopy** was performed in each eye to evaluate the ocular posterior segment (eg, indirect ophthalmoscopy, indirect slit lamp biomicroscopy).
- **Biomicroscopy**, preferably using slit lamp, was performed in each eye to evaluate the anterior segment structures and ocular adnexa.
- **Visual fields** are carried out in FIREFLEYE only at the end of study (5-year visit). When performed, automated testing is recommended (eg, Humphrey Field Analyzer).

11.2. Death Narratives

1. A 88-days-old female was enrolled in VGFTe-ROP-1920, a Phase 3, multicenter, randomized, 2-arm, open-label clinical study to assess the efficacy, safety, and tolerability of IVT aflibercept versus laser in patients with ROP. The patient experienced a fatal serious event of Multiple organ dysfunction syndrome starting on study Day 29.

The patient was born with a GA at birth of 24.7 weeks. The patient weighed 620 g and measured 29 cm at birth. Relevant medical history included low birth weight (620 g), Feeding issues (on total parenteral nutrition, intestinal failure), Respiratory failure in newborn; Hypoglycemia, Status post ligation and division of ductus arteriosus; Oliguria; Low thyroxine (T4) level, risk for impaired skin integrity; Necrotizing enterocolitis, Thrombocytopenia, Fluid overload, Electrolyte abnormality; Chronic lung disease; Chronic respiratory failure; Hypoxemia; Pulmonary

hypertension; Cytomegalovirus infection; Metabolic acidosis; Acute respiratory acidosis; Unspecified adrenocortical insufficiency; Bowel obstruction, and abnormal Urinalysis (reported bacteria out of range). At baseline, the patient was reported with ROP zone I stage 3+ in the right and left eye.

The patient received bilateral treatment with aflibercept on Day 88 CA. The patient was not retreated with aflibercept.

On study day -61, the patient had an abdominal process thought to be necrotizing enterocolitis but did not have surgery at that time. It was reported that the patient's gut never functioned well thereafter. On study day -15, the patient underwent an exploratory laparotomy, which showed massive adhesions of the bowel. An ileostomy and mucous fistula were placed. On study day -7, the patient had a wound dehiscence, which was packed, and ultimately, several entero-cutaneous fistulas developed.

On study Day 29, the patient experienced multiple system failure (MedDRA PT: Multiple organ dysfunction syndrome) of severe intensity, which the investigator considered serious as it was fatal. On the same day, the patient also experienced non-serious events of heart murmur (MedDRA PT: Cardiac murmur) and bilateral breath fine crackles (MedDRA PT: Rales). On an unknown date, the patient developed Klebsiella infection with positive cultures in blood, urine, and tracheal aspirate, along with thrombocytopenia and clinical decompensation. The patient developed worsening anasarca and inability to ventilate, with continued high pressures on the ventilator even with peak inspiratory pressure limit increased up to 70. Respiratory acidosis continued to worsen with pH of < 6.8 and oxygen saturation fell to around 50%. The patient's condition had deteriorated significantly and was beyond the point of recovery. It was discussed to limit any further care that would cause suffering, and the patient's mother agreed that code medications and chest compressions were considered futile. The patient's heart rate continued to decrease, and on study Day 59 (Day 126 CA) at 12:39 PM, the patient died due to multiple system organ failure. At the time of the patient's death, the outcome of the event of bilateral breath fine crackles was reported as unknown and the outcome of the event of heart murmur was reported as not recovered/resolved. No autopsy was performed.

The investigator considered the event not related to aflibercept, not applicable to photocoagulation, and not related to study conduct or study procedures. The sponsor assessed the event as not related to aflibercept.

2. A 71-day-old female patient was enrolled in Study 20090, a Phase 3, multicenter, open-label, randomized, 2-arm, controlled study to assess the efficacy, safety, and tolerability of intravitreal (IVT) aflibercept compared to photocoagulation in patients with ROP. The patient experienced 2 fatal serious events of Bronchopulmonary dysplasia starting on Day 142 CA and Pneumothorax starting on Day 143 CA.

The patient was born with a GA at birth of 23 weeks, 6 days. The patient weighed 445 g and measured 27 cm at birth. Relevant non-ocular medical history included: Anemia of Prematurity, Apnea of Prematurity and Bronchopulmonary dysplasia (ongoing at study entry). At baseline, the patient was reported with ROP zone I stage 3+ in the right eye and ROP zone II stage 2+ in the left eye.

The patient was first treated with aflibercept bilaterally on Day 72 CA. The patient was retreated 2 additional times, in the right eye on Day 142 CA and left eye on Day 156 CA.

The patient required intubation 141 days after initial treatment and 57 days after the last treatment with aflibercept, and on Day 212 CA, the patient was diagnosed with an exacerbation of bronchopulmonary dysplasia (MedDRA PT: Bronchopulmonary dysplasia) of severe intensity, which the investigator considered serious as it was life threatening and resulted in death. Remedial treatments administered intravenously included Adrenaline (epinephrine), Dexart (dexamethasone sodium phosphate), red blood cells-leukocytes reduced (red blood cells, concentrated), and Albumin (albumin human). The patient received resuscitation on the same day.

On the next day, Day 213 CA, the patient experienced a tension pneumothorax (MedDRA PT: Pneumothorax) of severe intensity, which the investigator considered serious as it was life threatening and resulted in death. The investigator reported the need of a thoracostomy with the use of a thoracostomy tube.

The patient reportedly died on Day 214 CA. An autopsy was not performed. The investigator considered the events not related to aflibercept, not related to photocoagulation, not related to IVT injection, and not related to protocol-required procedures because the event was attributable to high ventilation pressure under the management of the patient with a ventilator. The sponsor assessed both events as not related to aflibercept.

3. A 61-day-old female patient was enrolled in Study 20090, a Phase 3, multicenter, open- label, randomized, 2-arm, controlled study to assess the efficacy, safety, and tolerability of IVT aflibercept compared to photocoagulation in patients with ROP. The patient experienced a fatal serious event of Bronchiolitis on study Day 53.

The patient was born with a GA at birth of 24 weeks, 1 day. The patient weighed 640 g and measured 32 cm at birth. Relevant non-ocular medical history included: interstitial pulmonary emphysema, bronchopulmonary dysplasia and several episodes of confirmed or suspected neonatal sepsis. At baseline, patient was reported with bilaterally AP-ROP zone I stage 3+ in the right and left eye.

The patient received bilateral treatment with aflibercept at Day 59 CA. The patient was not retreated with aflibercept.

The patient experienced bronchiolitis (MedDRA PT: Bronchiolitis) of severe intensity 52 days after the last aflibercept injection (Day 111 CA); the event was considered serious as it required or prolonged hospitalization and resulted in death. The patient was hospitalized and was transferred to another hospital on the same day.

Although resuscitation attempts were performed, the event was fatal; the patient died on Day 115 CA.

The blood sample showed a positive Mycoplasma Pneumoniae Immunoglobulin M (IgM) of 13.0 AU/ml (normal range: 0–9.9 AU/ml). The Immunoglobulin G (IgG) was reported as negative. In addition, on the same sample day, Herpes Simplex IgM positive and IgG negative

were informed on blood. The investigator confirmed that M. Pneumoniae was the infectious agent of the bronchiolitis.

The investigator considered the event not related to aflibercept, not related to photocoagulation, not related to IVT injection, and not related to protocol-required procedures. The sponsor assessed the event as not related to aflibercept.

4. A 90-day-old male patient was enrolled in Study 20090, a Phase 3, multicenter, open-label, randomized, 2-arm, controlled study to assess the efficacy, safety, and tolerability of IVT aflibercept compared to photocoagulation in patients with ROP. The patient experienced a fatal serious event of Bronchopulmonary dysplasia on study Day 61.

The patient was born with a GA at birth of 26 weeks. The patient weighed 790 g and measured 35 cm at birth. Relevant non-ocular medical history included: respiratory insufficiency, severe anemia and bronchopulmonary dysplasia. At baseline, the patient was reported with bilaterally ROP zone II stage 3+ in the right and left eye.

The patient was first treated with aflibercept bilaterally on study Day 93. The patient was retreated in the right eye on study Day 125.

Six days after the last aflibercept injection, the patient experienced retina detachment (MedDRA PT: retinal detachment) in the right eye of moderate intensity, considered serious as it resulted in persistent or significant disability and incapacity. ROP assessment revealed progression of ROP in the right eye to zone II stage 4A+ and regression of ROP in the left eye to zone III stage 1. Aflibercept was withdrawn, and the outcome was reported as recovering/resolving. The patient discontinued the study treatment, and underwent a surgical procedure [Lensvitrshvartectomy], where extracapsular lens extraction and vitrectomy were performed simultaneously. The investigator considered the event not related to aflibercept, not related to photocoagulation, not related to IVT injection, and not related to protocol-required procedures. The sponsor assessed the event as not related to aflibercept.

On Day 153 CA (28 days after the last aflibercept injection), the patient experienced bronchopulmonary dysplasia, decompensation (MedDRA PT: bronchopulmonary dysplasia) of severe intensity; the event was considered serious as it resulted in death. The action taken with aflibercept and photocoagulation was not applicable. The event was fatal; the patient died on the same day. The investigator considered the event not related to aflibercept, not related to photocoagulation, not related to IVT injection, and not related to protocol- required procedures. The sponsor assessed the event as not related to aflibercept and photocoagulation.