NCT Number: NCT04101721
IND Number: 012462
EudraCT Number: 2019-001764-29

Regeneron Pharmaceuticals, Inc.

Clinical Study Protocol

RANDOMIZED, CONTROLLED, MULTI-CENTER STUDY TO ASSESS THE EFFICACY, SAFETY, AND TOLERABILITY OF INTRAVITREAL AFLIBERCEPT COMPARED TO LASER PHOTOCOAGULATION IN PATIENTS WITH RETINOPATHY OF PREMATURITY

Compound:	Intravitreal Aflibercept Injection
Clinical Phase:	3
Protocol Number:	VGFTe-ROP-1920
Protocol Version:	VGFTe-ROP-1920 Amendment 1
Amendment 1 Date of Issue:	*See appended electronic signature page*
Original Date of Issue:	27 Jun 2019
Medical /Study Director:	[redacted]

Regeneron Pharmaceuticals, Inc.
777 Old Saw Mill River Road
Tarrytown, NY 10591

[redacted]

AMENDMENT HISTORY

Amendment 1

The following table outlines the changes made to the protocol and the affected sections:

Change and Rationale for Change	Section Changed
Updated statistical sections to describe the Per Protocol Set (PPS) analysis, which is required for noninferiority studies. Removed reference to pooled analyses (with the Bayer sister study) as this is no longer planned.	Clinical Study Protocol Synopsis: Statistical Plan Section 11.3.1 Full Analysis Set Section 11.3.2 Per Protocol Set (new section) Section 11.4.3.1 Primary Efficacy Analysis
Clarified that collection of demographic and baseline data includes relevant maternal history.	Section 5.1 Demographic and Baseline Characteristics
Modified section for simplification and clarity.	Section 8.1 Investigational and Reference Treatments
Clarified that the hearing test will be performed once only, at any time prior to discharge from the neonatal intensive care unit (NICU).	Table 1 Schedule of Events (moved location of footnote #4) Section 9.2.1 Procedures Performed Only at the Screening/Baseline Visit
Clarified that a second central nervous system imaging will be carried out prior to discharge from the NICU.	Table 1 Schedule of Events (moved location of footnote #5) Section 9.2.1 Procedures Performed Only at the Screening/Baseline Visit
Removed description of volumes for blood sampling, as this information is referenced in other study document(s).	Section 9.1.1 Footnotes for the Schedule of Events Table (#13 and #14) Section 9.2.5 Drug Concentration and Measurements
Updated protocol to conform with recently-updated standard Pharmacovigilance text.	Section 10.1.1 General Guidelines Section 10.2.2 Serious Adverse Event Section 10.4 Notifying Health Authorities, Institutional Review Board /Ethics Committee, and Investigators
Added brief description of the RAINBOW study (in retinopathy of prematurity) for clarity.	Section 11.1 Statistical Hypothesis
Administrative Changes	Section 1 Introduction Section 3.3.1 Known and Expected Benefits Section 3.3.2 Potential Risks and Mitigation Measures Section 5.2 Efficacy Variables Section 9.2.3.1 Ophthalmic Examinations Section 9.2.4 Treatment of Overdose Section 11.6 Additional Statistical Data Handling Conventions
Minor editorial changes	Throughout

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ABR	Auditory brainstem response
ADA	Anti-drug antibody
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
AP-ROP	Aggressive posterior retinopathy of prematurity
AST	Aspartate aminotransferase
BAER	Brainstem auditory evoked response
BP	Blood pressure
BUN	Blood urea nitrogen
CONSORT	Consolidated Standards of Reporting Trials
CPK	Creatine phosphokinase
CRF	Case report form (electronic or paper)
CRO	Contract research organization
DMC	Data monitoring committee
DOB	Date of birth
DWFI	Digital wide-field imaging/images (retina or fundus)
EC	Ethics Committee
EDC	Electronic data capture
EOS	End of study
FA	Fluorescein angiography
FAS	Full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HDL	High-density lipoprotein
ICF	Informed consent form
ICH	International Council for Harmonisation
IOP	Intraocular pressure
IRB	Institutional Review Board
IVRS	Interactive Voice Response System
IVT	Intravitreal
IWRS	Interactive Web Response System
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein

NAb	Neutralizing antibody
PCSV	Potentially clinically significant value
PD	Pharmacodynamic
PK	Pharmacokinetic
PPS	Per protocol set
RBC	Red blood cell
RC	Reading center
Regeneron	Regeneron Pharmaceuticals, Inc.
ROP	Retinopathy of prematurity
SAE	Serious adverse event
SAF	Safety analysis set
SAP	Statistical analysis plan
SAS	Statistical Analysis System
SC	Subcutaneous
SOC	System organ class
SUSAR	Suspected unexpected serious adverse reaction
TEAE	Treatment-emergent adverse event
US	United States
VEGF	Vascular endothelial growth factor
VEGF-A	Vascular endothelial growth factor-A
VEGFR1	Vascular endothelial growth factor receptor 1
VEGFR2	Vascular endothelial growth factor receptor 2
WBC	White blood cell

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CLINICAL STUDY PROTOCOL SYNOPSIS

Title	Randomized, Controlled, Multi-Center Study to Assess the Efficacy, Safety, and Tolerability of Intravitreal Aflibercept Compared to Laser Photocoagulation in Patients with Retinopathy of Prematurity
Site Location(s)	Approximately 60 sites intended from North America, South America, Asia, and Europe.
Principal Investigator	Each site will have a PI. The treating physician would either be a retinal physician or pediatric ophthalmologist treating retinopathy of prematurity (ROP)
Objectives	The primary objective of the study is to assess the efficacy of aflibercept compared to laser in patients diagnosed with ROP. The secondary objectives of the study are: • To assess the need for a second treatment modality • To assess the recurrence of ROP in the study • To assess the safety and tolerability of aflibercept The exploratory objectives of the study are: • To further characterize the anatomical effects of treatment with aflibercept in patients with ROP • To further investigate the study intervention (eg, mode-of-action-related effects and/or safety) relevant to this disease process • To assess the treatment burden of aflibercept and laser • To characterize the concentrations of free and bound aflibercept in plasma over time • To describe the potential immunogenicity of aflibercept
Study Design	This is a phase 3, multicenter, randomized, 2-arm, open-label clinical study to assess the efficacy, safety, and tolerability of intravitreal (IVT) aflibercept versus laser in patients with ROP. The study consists of screening/baseline (1 or 2 visits), a treatment period (including potential retreatment and rescue treatment), and a final visit at week 52 of chronological age. The study also includes an optional fluorescein angiography (FA) sub-study, which includes optional FA at baseline, and mandatory FA at week 52 of chronological age.
Study Duration	The duration of the study for each patient is from baseline to 52 weeks of chronological age.
End of Study Definition	The end of the study as a whole is defined as the date of the last visit of the last patient in the study in all centers in all participating countries.

Population

Sample Size:	Approximately 112 patients are planned to be enrolled in a 3:1 ratio (approximately 84 in the aflibercept group and approximately 28 in the laser group).
Target Population:	The study population will consist of 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 International Classification for ROP.

Treatment(s)

Aflibercept

Dose/Route/Schedule:

Patients randomized to aflibercept will receive a single IVT injection of aflibercept 0.4 mg/0.01 mL per eligible eye at baseline.

Thereafter, if required, up to 2 additional IVT injections of aflibercept 0.4 mg/0.01 mL may be administered in each eye in case both the following retreatment criteria are met:

- Presence of ROP requiring treatment AND
- The interval since the last aflibercept IVT injection is ≥28 days

Rescue treatment with laser may be performed if 1 of the following conditions is met:

- Worsening of ROP compared to the examination immediately preceding the previous aflibercept injection, during the 27 days following the aflibercept injection
- Presence of ROP requiring treatment after the patient already received a total of 3 aflibercept injections

Laser

Patients randomized to laser will undergo treatment in each eligible eye at baseline. Treatment will be applied to the entire avascular peripheral retina. Treatment should be kept well away from the fovea. Laser ablation should be as complete as possible. It is recommended that fundus photographs (digital wide-field imaging [DWFI]) should be obtained right after administration of laser treatment to look for "skip" areas, and if found, laser should be administered to those areas prior to the investigator deeming the laser treatment as "complete." In case multiple sessions are necessary within 1 week from baseline, they will be counted as a single treatment.

Supplementary laser treatments are allowed during the study. Retreatment with laser is allowed if both of the following criteria are met:

- Presence of ROP requiring treatment

- Fundus examination reveals area for additional laser treatment as judged by the investigator (fundus photography recommended to help with adjudication)

Rescue treatment with aflibercept 0.4 mg/0.01 mL is allowed if the fundus examination reveals that laser treatment is complete as judged by the investigator and if 1 of the following conditions is met:

- Worsening of ROP compared to the most recent pre-laser examination
- Persistence of ROP requiring treatment

Patients who initiate aflibercept rescue treatment may receive additional aflibercept injections according to the aflibercept group treatment regimen.

Endpoint(s)

Primary:

The primary endpoint is the proportion of patients with absence of active ROP and of unfavorable structural outcomes at week 52 of chronological age, as determined by the Investigator. For patients with both eyes enrolled in the study, both eyes must meet the endpoint.

Unfavorable structural outcomes are defined as retinal detachment, macular dragging, macular fold, or retrolental opacity.

Secondary:

The secondary endpoints are:

- Proportion of patients requiring intervention with a second treatment modality from baseline to week 52 of chronological age
- Proportion of patients with recurrence of ROP through week 52 of chronological age
- The proportion of patients with treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs) (ocular and systemic) from baseline to week 52 of chronological age

Procedures and Assessments

The efficacy procedures/assessments include: Posterior segment assessment, indirect ophthalmoscopy, color fundus photographs using wide-field digital retinal photography, visual function, retinoscopy, and ocular motility.

The safety procedures/assessments include: ophthalmic examinations, intraocular pressure, physical examination, vital signs, clinical laboratories, and central nervous system imaging.

The study also includes pharmacokinetic, anti-drug antibody, and biomarker assessments.

Statistical Plan

Statistical Hypothesis:

This study will examine the following hypothesis for the primary efficacy variable regarding the proportion of patients with absence of active ROP and unfavorable structural outcomes at week 52 of chronological age. Statistical testing will be conducted to demonstrate the non-inferiority of the aflibercept group to the laser group, with a non-inferiority margin of 5% and a significance level of 5% (one-sided test).

H_0: $p_t = p_c - 5\%$ versus H_1: $p_t \geq p_c - 5\%$

where p_t is the true proportion of patients with absence of active ROP and unfavorable structural outcomes at week 52 of chronological age for the aflibercept group, and p_c is the true proportion of patients with absence of active ROP and unfavorable structural outcomes at week 52 of chronological age for the laser group.

The proposed non-inferiority margin of 5% is smaller than the smallest difference between laser and ranibizumab in the RAINBOW study (a phase 3 study comparing the effect of IVT ranibizumab to laser in the management of ROP), and not greater than the difference between the 2 ranibizumab doses.

Justification of Sample Size:

From the RAINBOW study, the response rate was 66.1% for the laser group and 88.1% for the 0.2 mg ranibizumab group in zone II disease. Furthermore, based on the clinical evidence for aflibercept investigator-initiated studies, the response rates range all the way 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 intravitreal aflibercept doses ranging from 0.4 mg to 1 mg. An estimated 90% response rate for the aflibercept group and 66.1% response rate for the laser group would be a reasonable assumption. It is assumed that the proportion of patients without active ROP will be in line with this. A sample size of 84 patients in the aflibercept group and 28 patients in the laser group (randomized in a 3:1 ratio) will provide 92% power for rejecting the null hypothesis at a 1-sided 5% significance level.

Statistical Methods:

Unless stated otherwise, all variables will be analyzed descriptively with appropriate statistical methods: continuous variables by sample statistics (ie, mean, standard deviation, median, quartiles, minimum, and maximum), and categorical variables by frequencies and percentages.

Efficacy Analyses:

The primary and secondary efficacy variable analyses will be conducted on the FAS. The primary analysis is a statistical evaluation of non-inferiority of aflibercept vs. laser at week 52 of chronological age, with respect to the primary efficacy variable. The non-inferiority margin is set at 5%.

Superiority testing will follow if non-inferiority is established. Sensitivity analysis of the primary efficacy endpoint will also be conducted based on the per protocol set.

If data are available from the week 40 of chronological age visit, it will be carried forward (LOCF) to the EOS visit for analysis. If the patient discontinued at or before this visit, no data will be carried forward and the patient will be considered a non-responder. For patients with both eyes enrolled in the study, both eyes must meet the endpoint. Patients with only one study eye enrolled will be responders if the respective eye responds.

The statistical analysis will be performed using the Cochran-Mantel-Haenszel method stratified by baseline ROP status. The 1-sided 95% Mantel-Haenszel confidence intervals using normal approximation of the difference of response rates between the aflibercept group and the laser group will be calculated. Aflibercept will be considered to be non-inferior to laser if the confidence interval of the difference lies entirely above -5%.

Furthermore, eyes will be considered to be non-responders if rescue treatment is given. The primary analysis for this endpoint will be based on the investigator assessment of, and sensitivity analyses based on the central RC data will be conducted.

1. INTRODUCTION

Retinopathy of prematurity (ROP) is a proliferative vascular retinopathy caused by an abnormal development of the vascularization of the peripheral retina in premature infants (Mutlu, 2013) (Pertl, 2015) (Salman, 2015). It mainly affects newborns with a preterm gestational age (≤32 weeks) and very low birth weight (≤1500 g). Approximately 10% to 11% of all newborn infants are born preterm (Blencowe, 2013) (Hellström, 2013). Among premature infants, the incidence of ROP ranges from 20% to 30%, increasing for younger gestational ages. The prevalence of blindness caused by ROP in developed nations ranges between 6% and 18%, while in developing nations the estimate is >20% (Hartnett-1, 2014) (Gilbert, 1997). Epidemiological studies have also identified increased risks for ROP due to genetic variants and environmental factors, such as oxygen exposure (Hartnett-2, 2014). The first wave of ROP cases, in the 1940s and 1950s, were identified in preterm infants who were exposed to high levels of supplemental oxygen in closed incubators, causing abnormal development of the vascular network in the premature retina. Stricter control of supplemental oxygen led to a decreased incidence of this condition (Hellström, 2013). An English study showed that the incidence of ROP later increased from 12.8 per 1000 low birth-weight infants in 1990 to 125.5 per 1000 low-birth-weight infants in 2011 (Painter, 2015). This second wave of ROP cases may be attributed to improved survival of the most extremely premature infants, introducing a new population of vulnerable newborns more susceptible to ROP.

Vascular endothelial growth factor (VEGF) is up-regulated under ischemic conditions. As ROP is characterized by incomplete vascularization of the retina in premature infants, it has also been associated with increased levels of VEGF. Vascular endothelial growth factor-A (VEGF-A) is a member of the VEGF family of angiogenic factors that acts through activation of the receptor tyrosine kinases vascular endothelial growth factor receptor 1 (VEGFR1) and vascular endothelial growth factor receptor 2 (VEGFR2) to increase mitosis, chemotaxis, and vascular permeability in endothelial cells. Upregulation of VEGF-A due to ischemia in the avascular retina may induce pathologic neovascularization and subsequently lead to retinal detachment and blindness, as seen in late-stage ROP (Stone, 1996) (Young, 1997). Aflibercept inhibits these effects of VEGF by acting as a soluble decoy receptor that binds VEGF-A with higher affinity than the natural receptors and thereby inhibits the binding and activation of these cognate VEGF receptors. Aflibercept also inhibits the effects of placental growth factor, which binds only to VEGFR1. Placental growth factor can synergize with VEGF-A, enhancing these processes of pathologic neovascularization, and is also known to promote leukocyte infiltration and vascular inflammation. Based on the its mode of action, aflibercept has a high potential to become an effective treatment option for the treatment of ROP, and several reports of off-label clinical use have shown positive efficacy outcomes without indicating new safety concerns (Sukgen, 2018) (Salman, 2015) (Huang, 2018).

In addition to the evidence currently available for aflibercept, the efficacy and safety of anti-VEGF agents for the treatment of ROP have been compared to laser in several case series and 2 large randomized controlled trials (BEAT-ROP and RAINBOW). The totality of the clinical evidence available thus far indicates a trend towards superior prevention of unfavorable anatomical outcomes for anti-VEGF agents compared to laser for ROP in central retinal locations (Zone I with any ROP stages without plus disease, or Zone I stage 3 without plus disease, or Zone II with

stages 2 plus or 3 plus disease), and aggressive posterior ROP (AP-ROP), and establishes the proof of concept for this indication (Mintz-Hittner, 2011) (Harder, 2013) (Hwang, 2015).

Aflibercept has been previously studied for several adult indications characterized by ocular neovascularization or increased permeability of the retinal vascular network, and is approved in multiple countries for treatment of indications such as diabetic retinopathy (DR), neovascular age-related macular degeneration, diabetic macular edema, macular edema secondary to retinal venous occlusion and choroidal neovascularization secondary to pathological myopia. Considering the consistently positive outcomes provided by aflibercept across these adult VEGF-mediated indications, it is deemed appropriate to conduct a study for a pediatric VEGF-mediated indication for the treatment of ROP.

Additional background information on the study drug and development program can be found in the Investigator's Brochure.

2. STUDY OBJECTIVES

2.1. Primary Objective

The primary objective of the study is to assess the efficacy of aflibercept compared to laser in patients diagnosed with ROP.

2.2. Secondary Objectives

The secondary objectives of the study are:

- To assess the need for a second treatment modality
- To assess the recurrence of ROP in the study
- To assess the safety and tolerability of aflibercept

2.3. Exploratory Objectives

The exploratory objectives of the study are:

- To further characterize the anatomical effects of treatment with aflibercept in patients with ROP
- To further investigate the study intervention (eg, mode-of-action-related effects and/or safety) relevant to this disease process
- To assess the treatment burden of aflibercept and laser
- To characterize the concentrations of free and bound aflibercept in plasma over time

  To describe the potential immunogenicity of aflibercept

3. HYPOTHESIS AND RATIONALE

3.1. Hypothesis

Intravitreal aflibercept dosed at 0.4 mg will achieve comparable results as assessed by the primary endpoint (absence of active ROP and of unfavorable structural outcomes) when compared to laser therapy.

3.2. Rationale

3.2.1. Rationale for Study Design

Laser photocoagulation of the peripheral avascular retina is the current standard of care for the treatment of ROP. Considering aflibercept's mode of action, and the current clinical evidence describing positive outcomes in ROP with off-label use of anti-VEGF in central retinal disease or AP-ROP, a randomized clinical trial to compare the ability of these treatments to manage active ROP and prevent the development of ocular complications is warranted.

The substantial amount of existing data describing outcomes of laser in ROP is sufficient to provide an accurate understanding of the potential benefits and limitations of this treatment modality. The purpose of the current study is primarily to collect the missing data on the outcomes of aflibercept in the treatment of ROP, and for this reason, a 3:1 randomization design is planned. Additionally, a fully-masked study of intravitreal (IVT) aflibercept compared to laser is technically unfeasible.

The inclusion and exclusion criteria allow the selection of an appropriate patient population and increase the likelihood of producing reliable and reproducible results, while guarding against exploitation of vulnerable patients. The proposed criteria are based on existing clinical knowledge and feedback from key opinion leaders involved in treatment of preterm infants with ROP.

3.2.2. Rationale for Dose Selection

Currently available clinical data (Sukgen, 2018) (Salman, 2015) (Sidorenko, 2018) consistently show promising efficacy, with no identification of major safety concerns, when using aflibercept 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). In order to limit drug exposure, the lowest dose for which positive efficacy was reported (0.4 mg/0.01 mL) was selected for this study. An injection volume of 0.01 mL is considered acceptable for IVT administration in infants.

3.3. Risk-Benefit

Aflibercept is marketed for the treatment of adult patients with several retinal diseases that are characterized by ischemia-induced upregulation of VEGF, are related to pathological neovascularization and/or vascular leakage, and can result in retinal thickening and edema, which are thought to contribute to vision loss. The efficacy and safety of IVT aflibercept used in adult patients with retinal diseases are well established, and its risk-benefit profile is considered favorable.

Inhibition of VEGF activity by aflibercept can be expected to result in therapeutic benefit in premature infants with ROP, since ROP is also characterized by the pathological development of the retinal vasculature and has been associated with VEGF upregulation. Aflibercept has demonstrated efficacy in animal models of pathological ocular neovascularization after systemic and IVT administration. No differences in the mechanism of action of aflibercept in the treatment of neovascular ocular diseases between children (eg, ROP) and adults are expected. There is a high and unmet medical need for an effective, safe, and approved treatment for preterm infants with vision-threatening ROP, a disease that is a leading cause of blindness in both developing and developed countries. Laser (for the peripheral early stage of ROP) is currently considered a standard of care, but fails to achieve a normal retinal structure in up to 25% of ROP patients with central retinal disease or AP-ROP. Additionally, laser is associated with potential risks, including irreversible loss of visual field and high myopia. Vitreoretinal surgery is reserved for advanced disease stages with retinal detachment (stages 4 and 5). Increasing clinical experience from off-label use of IVT aflibercept for central ROP suggests positive efficacy outcomes, usually after a single injection, with no major safety concerns as currently reported. Thus, aflibercept has the potential to offer improved outcomes in this high-risk, vulnerable premature newborn population with ROP.

3.3.1. Known and Expected Benefits

Aflibercept is expected to provide demonstrable anatomical benefits through regression of ROP features while allowing the vascularization to resume its usual development towards the retinal periphery, thus preventing unfavorable structural outcomes in approximately 90% of treated patients. Visual function is also expected to improve (preservation of peripheral visual field, prevention of loss of best corrected visual acuity, and lower proportion of patients developing high myopia).

Moreover, additional advantages of aflibercept over laser treatment are expected. An aflibercept injection is less time-consuming than laser (an IVT injection is often less than 30 minutes, whereas laser treatment may take 2 or more hours). Laser treatment requires general anesthesia/sedation, risks that would be largely avoided when using aflibercept. Aflibercept treatment may be given regardless of the patient's ocular anatomy (while laser treatment would be difficult in patients without adequate visualization of the retina, such as in patients with hazy corneas, iris neovascularization, or small pupils). Aflibercept treatment offers the potential for a single-injection treatment option and can be administered immediately after diagnosis and informed consent by the parent(s)/legally authorized representative(s). Bilateral cases can be treated in a single session. Upon recurrence of vision-threatening ROP, re-treatment with the same dose may be given to each treatment-requiring eye, after injection-free intervals of at least 28 days. For laser, once avascular retina ablation is complete, re-treatment may not offer additional benefits. In addition to all this, laser treatment is more susceptible to operator-dependent differences with the possibility of having significant areas of untreated retina, consequently increasing the risk of failure. Achieving adequate laser treatment depends on the experience and ability of an individual practitioner.

3.3.2. Potential Risks and Mitigation Measures

Based on the cumulative safety experience with aflibercept for the treatment of adult patients with retinal diseases, the potential risks for the study patients include those described below.

3.3.2.1. Ocular Risks

Ocular risks include intraocular inflammation (including endophthalmitis), retinal tear/ detachment, transient intraocular pressure increase, and traumatic cataract.

Patients with relevant ongoing or significant previous use of systemic steroids at immunosuppressive levels are excluded, to mitigate any potential increased risk of local or systemic infections, and to address any potential risk of worsening clinical conditions that have been historically reported in the context of steroid use in preterm infants, such as more frequent or greater incidence rates of necrotizing enterocolitis, cerebral palsy, and gastrointestinal ulcer.

3.3.2.2. Hypersensitivity/Immunogenicity

Hypersensitivity/immunogenicity (inherent to all therapeutic proteins) is addressed by exclusion of relevant patients (see Section 7.2.2).

3.3.2.3. Acute Systemic Effects

A minimum weight (800 g) at the time of study treatment (see Section 7.2.1) is required. At this minimum weight, a patient in the aflibercept group will receive 1 mg/kg when dosed bilaterally. This dose is predicted to increase systemic exposure in preterm infants when compared to adults receiving IVT treatment with aflibercept, but to remain below limits determined in adult nAMD patients after intravenous dosing of aflibercept with a maximal tolerated dose of 1 mg/kg. Patients will be monitored for any evidence of arterial hypertension, proteinuria, worsening of concomitant conditions typical for preterm infants (eg, worsening of intraventricular hemorrhage I or II, other non-ocular hemorrhages), or arterial thromboembolic events. Pharmacokinetic (PK) samples for the measurement of systemic aflibercept concentrations (both free aflibercept [the pharmacologically active form] and aflibercept bound to VEGF [the inactive form]) will be taken. The study population is vulnerable, due to their underlying prematurity, to increased risk for multi-organ comorbidities (such as bronchopulmonary dysplasia, intraventricular hemorrhage, necrotizing enterocolitis, septic conditions), and developmental delays, compared to term infants. Given the high unmet medical need, the potential to more effectively treat ROP with a single injection (or possibly up to 3 injections), and the risks associated with laser treatments, the potential lifelong benefits to children treated with aflibercept outweigh the potential risks, and the expected risk-benefit profile is favorable.

4. ENDPOINTS

4.1. Primary and Secondary Endpoints

4.1.1. Primary Endpoint

The primary endpoint is the proportion of patients with absence of active ROP and of unfavorable structural outcomes at week 52 of chronological age, as determined by the Investigator. For patients with both eyes enrolled in the study, both eyes must meet the endpoint.

Unfavorable structural outcomes are defined as retinal detachment, macular dragging, macular fold, or retrolental opacity.

4.1.2. Secondary Endpoints

The secondary endpoints are:

- Proportion of patients requiring intervention with a second treatment modality from baseline to week 52 of chronological age
- Proportion of patients with recurrence of ROP through week 52 of chronological age
- The proportion of patients with treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs) (ocular and systemic) from baseline to week 52 of chronological age

4.1.3. Exploratory Endpoints

The exploratory endpoints are:

- The number of aflibercept administrations from baseline to week 52 of chronological age
- The number of laser treatments from baseline to week 52 of chronological age
- The proportion of patients needing more than 1 aflibercept injection through week 52 of chronological age
- The proportion of patients/eyes needing more than 1 laser treatment through week 52 of chronological age
- Time to recurrence of ROP
- Time to intervention with a second treatment modality for ROP or to development of unfavorable structural outcomes
- Completion of vascularization of the peripheral retina to within 1 disc diameter of the ora serrata on FP at week 52 of chronological age
- Assessment of retinal vascularization and leakage on fluorescein angiography (FA) at week 52 of chronological age in patients participating in the FA substudy
- Time to completion of retinal vascularization

- Regression of plus disease, regression of pre-retinal vascularized ridge, and progression of retinal vascularization beyond the ridge from baseline to week 52 of chronological age
- Progression to stage 4 or 5 ROP from baseline to week 52 of chronological age
- Evaluation of visual function at week 52 of chronological age
- Time required to perform aflibercept injection or initial laser treatment
- Requirement for sedation and/or general anesthesia to complete laser or aflibercept injection
- Number of visits required up to week 52 of chronological age
- Systemic exposure to free and bound aflibercept (at expected maximum plasma concentration and during the elimination period from plasma) determined by sparse sampling
- To assess immunogenicity as incidence of treatment emergent antibodies to aflibercept in patients over time.

5. STUDY VARIABLES

5.1. Demographic and Baseline Characteristics

Baseline characteristics will include standard demography (eg, age, race, weight, height, etc.), disease characteristics, medical history, and medication history, as well as relevant maternal history, for each patient.

5.2. Efficacy Variables

The efficacy variables relevant to the primary endpoint are ROP status and ocular anatomy.

The efficacy variables relevant to the secondary endpoints are:

- Intervention with a second treatment modality
- Recurrence of ROP

The efficacy variables relevant to the exploratory endpoints are:

- Exposure:
  - Number of aflibercept injections
  - Number of laser treatments
  - Number of rescue treatments
  - Number of visits

- Time
  - To recurrence of ROP
  - To intervention with second modality or development of unfavorable structural outcomes
  - Needed to perform aflibercept injection and initial laser treatment
- Assessment of retinal vasculature and ROP status with fundus exam, FP and FA (for those participating in FA substudy), including time to completion of retinal vasculature
- Assessment of visual function
- Need for sedation and/or general anesthesia

5.3. Safety Variables

The safety variables are:

- Adverse events (TEAEs and SAEs)
- Ophthalmic examinations
- Physical examinations
- Vital signs
- Clinical laboratories
- Central nervous system imaging

5.4. Pharmacokinetic Variables

The PK variable is the concentration of free or bound aflibercept.in plasma at each time point. Samples in this study will be collected using a sparse sampling schedule (eg, only 1 blood sample for drug concentration assessment will be collected at any single clinic visit). These sampling time points are specified in Table 1.

5.5. Immunogenicity Variables

Anti-drug antibody variables will include ADA status (positive or negative), titer, and neutralizing antibody (NAb) status for analyzed samples collected at the time points specified in Table 1.

5.6. Biomarker Variables

The biomarker variables may include those relevant to diagnostic, safety, pharmacodynamics, monitoring, or potentially predictive biomarkers.

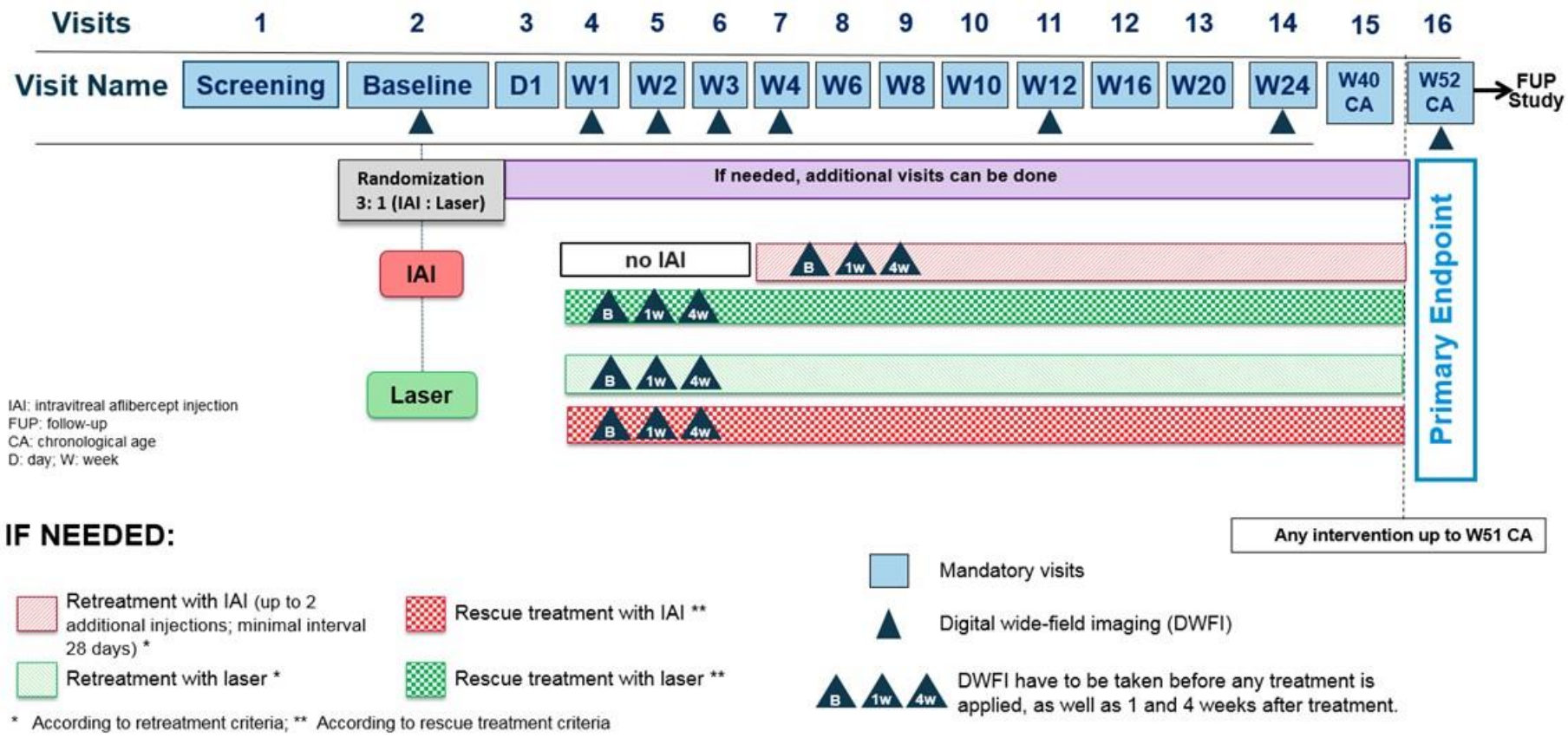
6. STUDY DESIGN

6.1. Study Description and Duration

This is 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 study consists of screening/baseline (1 or 2 visits), a treatment period (including potential retreatment and rescue treatment), and a final visit at week 52 of chronological age (Figure 1).

The study also includes an optional fluorescein angiography (FA) sub-study (Appendix 1), which includes optional FA at baseline, and mandatory FA at week 52 of chronological age.

Figure 1: Study Flow Diagram



Successful screening requires the presence of treatment-naïve ROP classified by the investigator according to the International Classification for Retinopathy of Prematurity in at least 1 eye with 1 of the following retinal findings:

- Zone I Stage 1 plus, 2 plus, 3 non-plus or 3 plus, or
- Zone II Stage 2 plus or 3 plus, or
- AP-ROP

One or both eyes can be treated according to the investigator's assessment of the study's eligibility criteria. If both eyes are eligible, they will be assigned to the same treatment group. The second eye of patients who start the study with only 1 eligible eye should be kept under observation according to the local ROP screening guidelines or at every study visit, whichever is more frequent. Second eyes that develop ROP requiring treatment during the study should receive treatment according to the randomization assignment of the first eye. If a second eye is treated within 8 weeks of the initial treatment of the first eye, the second eye will be included in the efficacy analysis.

Color fundus photography with digital wide-field images (DWFI) of the retina will be taken before any treatment is applied. These images will be submitted to a central reading center (RC) as soon as possible for confirmation of ROP staging, and if deemed eligible, treatment should be administered. However, due to the urgency of treatment for this medical condition, in situations where the investigator considers that awaiting a response from the RC and thus delaying treatment may be detrimental to the outcome, treatment is allowed to be administered immediately after the images are acquired, before availability of the RC confirmation of ROP staging. In this case, the investigator's assessment of the images will be used in stratification.

Patients will be randomized 3:1 to treatment with either aflibercept injection or laser, respectively.

Aflibercept group:

Patients randomized to aflibercept will receive a single IVT injection of aflibercept 0.4 mg/0.01 mL per eligible eye at baseline.

Thereafter, if required, up to 2 additional IVT injections of aflibercept 0.4 mg/0.01 mL may be administered in each eye in case both the following retreatment criteria are met (Figure 2):

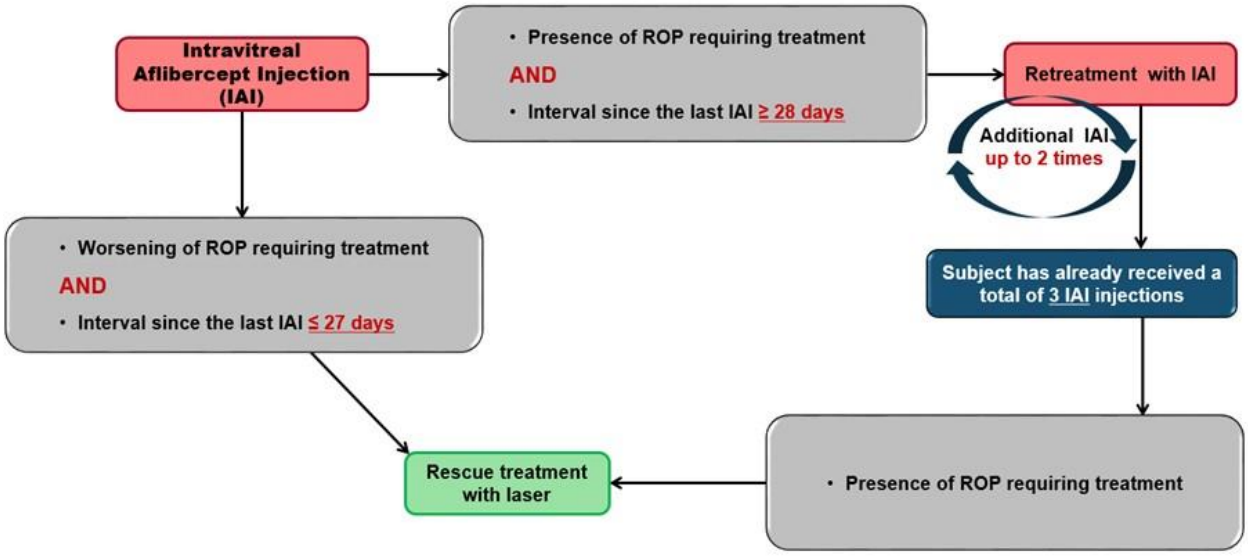
- Presence of ROP requiring treatment **AND**
- The interval since the last aflibercept IVT injection is ≥28 days

Rescue treatment with laser may be performed if 1 of the following conditions is met (Figure 2):

- Worsening of ROP compared to the examination immediately preceding the previous aflibercept injection, during the 27 days following the aflibercept injection
- Presence of ROP requiring treatment after the patient already received a total of 3 aflibercept injections

Once rescue treatment is applied, treatment with the patient's randomized treatment cannot be reinitiated.

Figure 2: Aflibercept Treatment, Aflibercept Retreatment, and Rescue Treatment



Once rescue treatment is applied, treatment with the patient's randomized treatment cannot be reinitiated.

Laser Group:

Patients randomized to laser will undergo treatment in each eligible eye at baseline. Treatment will be applied to the entire avascular peripheral retina. Treatment should be kept well away from the fovea. Laser ablation should be as complete as possible. It is recommended that fundus photographs (DWFI) should be obtained right after administration of laser treatment to look for "skip" areas, and if found, laser should be administered to those areas prior to the investigator deeming the laser treatment as "complete." In case multiple sessions are necessary within 1 week from baseline, they will be counted as a single treatment.

Supplementary laser treatments are allowed during the study. Retreatment with laser is allowed if both of the following criteria are met (Figure 3):

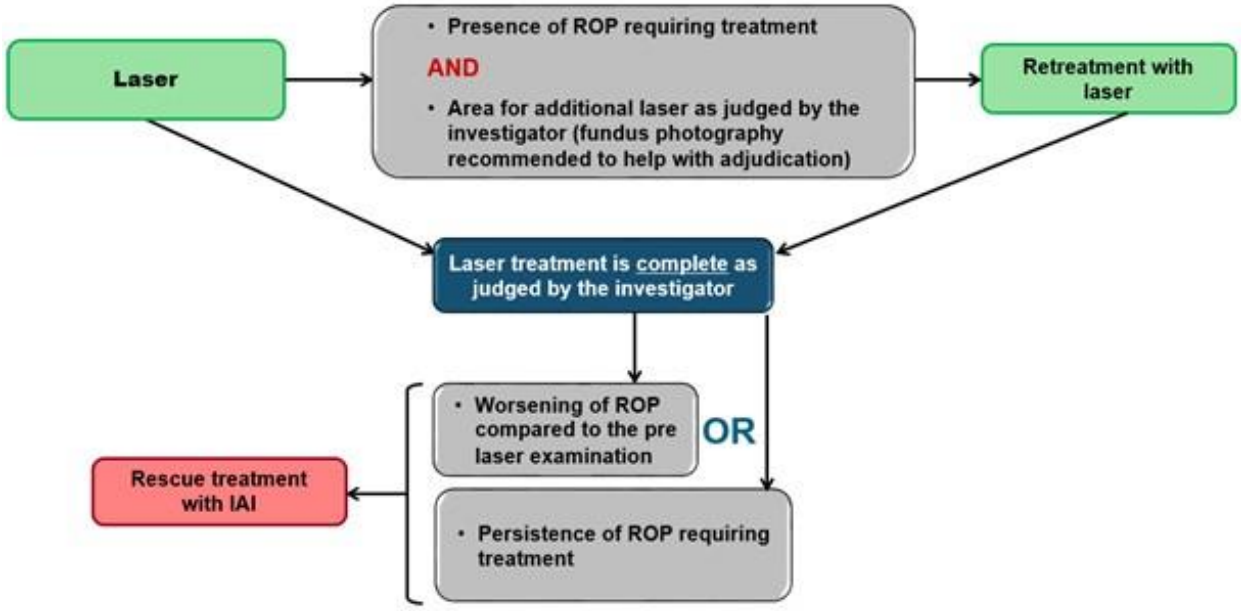
- Presence of ROP requiring treatment
- Fundus examination reveals area for additional laser treatment as judged by the investigator (fundus photography recommended to help with adjudication)

Rescue treatment with aflibercept 0.4 mg/0.01 mL is allowed if the fundus examination reveals that laser treatment is complete as judged by the investigator and if 1 of the following conditions is met (Figure 3):

- Worsening of ROP compared to the most recent pre-laser examination
- Persistence of ROP requiring treatment

Patients who initiate aflibercept rescue treatment may receive additional aflibercept injections according to the aflibercept group treatment regimen.

Figure 3: Laser Treatment, Laser Retreatment, and Rescue Treatment



Once rescue treatment is applied, treatment with the patient's randomized treatment cannot be reinitiated.

Both Groups:

Once rescue treatment is applied to an eye, treatment in that eye with the patient's randomized treatment cannot be reinitiated. However, the fellow eye can still receive the patient's randomized study intervention, if retreatment criteria are met.

Patients will have mandatory evaluations at regular intervals during the study at the time points specified in Table 1. Patients receiving retreatment and/or rescue treatment will have mandatory evaluations 1 day, 1 week, and 4 weeks after the retreatment/rescue treatment to a given eye.

Acquisition and submission of DWFIs (eg, RetCam) to the RC is required at baseline and at weeks 1, 2, 3, 4, 12, and 24, at week 52 of chronological age, before each retreatment and/or rescue treatment, and 1 week and 4 weeks after each retreatment/rescue treatment. Safety will be assessed through the evaluation of adverse events (AEs), ophthalmic and physical examinations (including assessment of acute tolerability post-dosing), vital signs, and laboratory tests. Pharmacokinetic evaluations will investigate plasma concentrations of free and bound aflibercept. The potential emergence of ADAs will also be evaluated by serum sampling at baseline and week 12.

Additional optional evaluation visits may be performed in accordance with the usual standard of care, as medically needed.

A phase 3b extension study is planned to assess the long-term outcomes of patients who received study intervention in this study. All treated patients will be offered an opportunity to participate until they are 5 years of age to assess ocular effects, and clinical and neurodevelopmental outcomes.

6.1.1. End of Study Definition

A patient is considered to have completed the study if he/she has completed all phases of the study including the last visit at week 52 of chronological age.

The end of the study as a whole is defined as the date of the last visit of the last patient in the study in all centers in all participating countries.

Primary completion is defined as the date of the last visit of the last patient for the primary outcome.

6.2. Planned Interim Analysis

No interim analysis is planned.

6.3. Study Committees

6.3.1. Independent Data Monitoring Committee

The aim of the safety assessments is to determine if the study shows unacceptable risks for the patients, and to discontinue the study if necessary. The assessments will be performed by an independent data monitoring committee (DMC). Detailed information regarding the DMC procedures will be explained in a separate DMC charter.

7. SELECTION, WITHDRAWAL, AND REPLACEMENT OF PATIENTS

7.1. Number of Patients Planned

Approximately 112 patients are planned to be enrolled in a 3:1 ratio (approximately 84 in the aflibercept group and approximately 28 in the laser group).

7.2. Study Population

The study population will consist of 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 International Classification for ROP.

7.2.1. Inclusion Criteria

A patient must 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 International Classification for ROP 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
4. Male or female
5. Signed informed consent from parent(s)/legally authorized representative(s) as described in Section 13.2, which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

7.2.2. Exclusion Criteria

A patient who meets any of the following criteria will be 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 neonatal intensive care unit 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 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 drug, whichever is longer

7.2.3. Rescreening of Screen Failures

Screen failures are patients for whom informed consent has been obtained but who do not subsequently enter the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure patients to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities.

Minimal information includes demography, screen failure details, eligibility criteria, and any serious pretreatment events.

In general, re-starting the defined set of screening procedures to enable the "screen failure" patient's participation at a later time point is not allowed. Thus, in general, participation of an initial "screen failure" patient at a later time point is not acceptable, except if the screening failure was triggered by missing inclusion criteria 2 and/or 3, or by meeting exclusion criteria 4, 5, 8, 9, and/or 11, or if the inclusion/exclusion criteria preventing the patient's initial attempt to participate have been changed via protocol amendment.

Under any of the above exceptions, a patient may only be re-screened once, and only within 10 days of the start of the first screening period.

To be eligible, rescreened patients must meet all selection criteria at the re-screening visit. In any case, the investigator has to ensure that the repeated screening procedures do not expose the patient to an unjustifiable health risk. Also, for rescreening, the patient's parent(s)/legally authorized representative(s) have to sign a new ICF, even if it was not changed after the patient's previous screening.

Rescreened patients should be assigned a new patient number.

7.3. Premature Withdrawal from the Study

A patient's parent(s)/legal guardian(s) has the right to withdraw the patient from the study at any time, for any reason, and without repercussion.

The investigator and/or sponsor have the right to withdraw a patient from the study if it is no longer in the interest of the patient to continue in the study, or if the patient's continuation in the study places the scientific outcome of the study at risk (eg, if a patient is not able to follow study procedures). An excessive rate of withdrawals would render the study uninterpretable; therefore, unnecessary withdrawal of patients should be avoided.

Parent(s)/legal guardian(s) of patients who are withdrawn prematurely from the study will be asked for the patient to complete the early termination visit, as described in Section 9.1.2.

7.4. Replacement of Patients

Patients prematurely discontinued from the study will not be replaced.

8. STUDY TREATMENTS

8.1. Investigational and Reference Treatments

Patients will be randomized 3:1 to receive treatment with an IVT injection of aflibercept 0.4 mg or laser.

Aflibercept (at a concentration of 40 mg/mL) will be supplied in sterile, sealed, 3 ml glass vials. The injection volume will be 10 µL (0.01 mL) and will be administered to the patients by IVT injection. Instructions on dose preparation are provided in the pharmacy manual.

See Section 6.1 for aflibercept dosing strategies.

Transpupillary conventional laser will be given following topical anesthesia, sedation, or general anesthesia, with appropriate respiratory support as required (eg, laryngeal mask, endotracheal intubation, or similar), and administered according to standard local procedures. The pupil of the eye to be treated must be dilated (mydriasis) with 2 or 3 drops of mydriatic agent(s) applied topically to the eye, according to local practice.

Laser power settings should be defined according to the investigator's practice and applicable medical standards at the site. The treatment should cover the entire avascular retina, using a confluent laser pattern and extending to the ora serrata.

See Section 6.1 for laser treatment strategies.

8.2. Rescue Treatments

For patients randomized to aflibercept, rescue treatment with laser may be performed. For patients randomized to laser, rescue treatment with aflibercept may be performed. The criteria for the allowance of rescue treatment are specified in Section 6.1. Use of rescue treatment in either eye must be recorded on the respective electronic case report form (eCRF) page.

All patients requiring rescue treatment will be counted as non-responders in the primary endpoint. Nevertheless, the patients should be followed to assess the efficacy and safety outcomes after rescue treatment.

8.3. Dose Modification and Study Treatment Discontinuation Rules

8.3.1. Dose Modification

Dose modification for an individual patient is not allowed.

8.3.2. Study Drug Discontinuation

Patients who permanently discontinue from study drug should be encouraged to remain in the study. Those who agree and do not withdraw from the study will be asked to return to the clinic for all remaining study visits per the visit schedule.

Patients who permanently discontinue from study drug and who opt to withdraw from the study will be asked to complete study assessments, per Section 9.1.2.

8.4. Method of Treatment Assignment

Eligible patients will be randomized 3:1 to receive either treatment with aflibercept or laser, respectively, stratified by ROP classification in Zone I, Zone II, or AP-ROP according to investigator assessment. If both eyes meet the inclusion criteria of the study after screening, the eye with the more severe disease will be considered for stratification.

Treatment allocation will be done according to a computer-generated randomization list specified by the sponsor's responsible statistician and provided by the sponsor's randomization management group. To prevent imbalances, the randomization result of patients who do not complete baseline treatment will be assigned to a subsequent patient once it is known that the baseline treatment was not completed.

Patients will be centrally assigned to randomized study intervention using an Interactive Voice Response System/Interactive Web Response System (IVRS/IWRS). Before the study is initiated, the telephone number and call-in directions for the IVRS and/or the log in information and directions for the IWRS will be provided to each site. The investigator will provide the IVRS/IWRS with study center identification, the patient´s date of birth (DOB), gender, and weight at birth. The complete DOB (day, month, and year) will be entered if allowed by local regulation because it is needed in order to correctly determine the participant's chronological and corrected age during the study. Because of the particular population in this study, absence of an exact DOB will cause a 30-day deviation, resulting in possible misinterpretation of results and confounding the ability to understand the outcomes, as this is usually compared with controls matched for the same corrected gestational age. Additional details are documented in the IVRS/IWRS instruction manuals. Study intervention will be dispensed at the study visits summarized in Table 1.

8.5. Masking

This is an open-label study where the study sites, the study team and the patient and patient's parent(s)/legally authorized representative(s) know the treatment the patient is being given. Potential bias will be reduced by central randomization and objective endpoints. Any aggregated treatment-specific data will be kept strictly confidential and will not be communicated to investigators.

8.6. Treatment Logistics and Accountability

8.6.1. Packaging, Labeling, and Storage

Open-label study drug will display the product lot number on the label. Each vial of study drug will be labeled per country requirements.

Study drug will be stored at the site at a temperature of 2ºC to 8ºC; storage instructions will be provided in the pharmacy manual.

8.6.2. Supply and Disposition of Treatments

Study drug will be shipped at a temperature of 2ºC to 8ºC to the investigator or designee at regular intervals or as needed during the study. At specified time points during the study (eg, interim site monitoring visits), at the site close-out visit, and following drug reconciliation and documentation by the site monitor, all opened and unopened study drug will be destroyed/ returned to the sponsor or designee.

8.6.3. Treatment Accountability

All drug accountability records must be kept current.

The investigator must be able to account for all opened and unopened study drug. These records should contain the dates, quantity, and study medication

- dispensed to each patient
- returned from each patient (if applicable), and
- disposed of at the site or returned to the sponsor or designee.

All accountability records must be made available for inspection by the sponsor and regulatory agency inspectors; photocopies must be provided to the sponsor at the conclusion of the study.

8.6.4. Treatment Compliance

Study intervention will be administered by a qualified ophthalmologist. Details of aflibercept injection and of the laser procedure will be recorded in the eCRF (eg, time required, type of anesthesia, presence or absence of endotracheal intubation, treatment site).

All drug compliance records must be kept current and made available for inspection by the sponsor and regulatory agency inspectors.

8.7. Concomitant Medications

All medications the patient received prior to visit 1 and any medication or vaccine (including any sedation, anesthesia, eye drops used for the study procedures, blood-derived products, prescription or over-the-counter medicines, probiotics, vitamins, and herbal supplements) that the patient is receiving at the time of enrollment or receives during the study should be recorded along with:

- The reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

The use of medications, recreational drugs, or similar substances during pregnancy will be recorded on the Maternal Prior Medication section on the eCRF at screening (eg, alcohol, tobacco, antibiotics, corticosteroids, vaccination, supplements). For breastfeeding mothers, all maternal medication or similar products taken after the umbilical cord clamping will be recorded in the Maternal Concomitant Medication section of the eCRF only if the transfer of these products into human milk is feasible according to the investigator's judgment. The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

8.7.1. Prohibited Medications

Exposure to any IVT or systemic anti-VEGF agent is not allowed in patients or in mothers who are breastfeeding.

8.7.2. Permitted Medications

The use of phototherapy lamp for treating jaundice is allowed. Appropriate eye protection must be provided to the patient. The use of phototherapy for jaundice will be documented in the eCRF, including the use of eye protection.

The use of oxygen supplementation will be recorded in the corresponding eCRF section with special consideration of the method, concentration, duration of administration and the patient´s oxygen saturation by pulse oximetry once the supplementation is stable. In the last visit of the study, it will be recorded if oxygen supplementation is required. Standard of care treatment for other conditions affecting patients is permitted.

8.8. Post-study Treatments

A phase 3b extension study is planned to assess the long-term outcomes of patients who received study intervention in this study and accept participation in the extension study. No study treatment will be required or provided by the sponsor in the extension study. In case any treatment for ROP is required during the extension study, the site's standard of care should be administered. Patients will undergo safety and efficacy assessments until 5 years of age.

The baseline visit of the extension study can be conducted concomitantly with the week 52 chronological age visit of this study.

9. STUDY SCHEDULE OF EVENTS AND PROCEDURES

9.1. Schedule of Events

Study assessments and procedures are presented by study period and visit in Table 1.

Table 1: Schedule of Events

Visit Number[1]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16 EOS	Retreatment or rescue treatment follow-up visits			
Visit Name[1]	**SCR**	**BL**	**D1**	**W1**	**W2**	**W3**	**W4**	**W6**	**W8**	**W10**	**W12**	**W16**	**W20**	**W24**	**W40 CA**	**W52 CA**	**TRT Visit**	**D1 FUP**	**1W FUP**	**4W FUP**
Visit Window	**--**	**--**	**+2D**	**±3D**	**±3D**	**±3D**	**+7D**	**±7D**	**±7D**	**±7D**	**±7D**	**±7D**	**±7D**	**±7D**	**±10D**	**±10D**	**--**	**+2D**	**±3D**	**+7D**
Informed consent	X																			
In/exclusion criteria	X	X																		
Enroll (IVRS/IWRS)		X																		
Demographic	X																			
Medical history[2]	X																			
Prior/concomitant medication[3]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Body weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Head circumference and body length	X								X			X		X	X	X				
Hearing test	X[4]																			
CNS imaging	X[5]																			
Physical exam[6]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs[7]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Anterior segment examination[8]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Binocular indirect ophthalmoscopy[9]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Tonometry[10]		X															X			
Digital wide-field retinal imaging[11]		X		X	X	X	X				X			X		X	X		X	X
Fluorescein angiography (for optional sub-study)[12]		X														X				
Blood sample for ADA (aflibercept group only)[13]		X									X									
Blood sample for PK (aflibercept group only)[14]			X		X		X													

Visit Number[1]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16 EOS	Retreatment or rescue treatment follow-up visits			
Visit Name[1]	**SCR**	**BL**	**D1**	**W1**	**W2**	**W3**	**W4**	**W6**	**W8**	**W10**	**W12**	**W16**	**W20**	**W24**	**W40 CA**	**W52 CA**	**TRT Visit**	**D1 FUP**	**1W FUP**	**4W FUP**
Visit Window	**--**	**--**	**+2D**	**±3D**	**±3D**	**±3D**	**+7D**	**±7D**	**±7D**	**±7D**	**±7D**	**±7D**	**±7D**	**±7D**	**±10D**	**±10D**	**--**	**+2D**	**±3D**	**+7D**
Hematology, clinical chemistry, urinalysis[15]	X																			
Urine protein test[16]		X	X		X		X													X
Aflibercept injection (for patients randomized to active)[17]		X															X			
Laser treatment (for patients randomized to control)[18]		X															X			
Visual function														X		X				
Refraction																X				
Ocular extrinsic motility														X		X				

Abbreviations CA = chronological age

9.1.1. Footnotes for the Schedule of Events Table

1. Additional visits may be performed depending on the investigator's assessment of the patient's response to treatment, as part of local standard of care. Visits 1 and 2 can be on same day or within 10 days of each other. If treatment cannot take place at visit 2, it can be administered within 3 days of visit 2. For Visits 3 to 14, the intervals are based on the date of initial treatment. The intervals of the rescue/retreatment follow-up visits are based on the date of rescue/retreatment. A retreatment/rescue follow-up visit may be combined with a regularly scheduled visit. If a patient's second eye qualifies for treatment during the study, both eyes can follow the visit schedule of the first eye after the retreatment/rescue treatment follow-up visit schedule is completed for the second eye.
2. Maternal and patient medical history will be recorded.
3. Includes recording of oxygen supplementation. At screening, includes any of the medications listed in Section 8.7 given to mother during pregnancy and breastfeeding.
4. Hearing test to be performed once, at any time prior to discharge from neonatal intensive care unit (NICU).
5. Imaging is not required at screening if results from an imaging exam performed within the previous 10 days are available and there are no new neurological sign/symptoms. A second CNS imaging is to be carried out prior to discharge from the NICU.
6. Includes cardiovascular, respiratory, gastrointestinal, and neurological systems according to local general practice and aiming to evaluate overall health.
7. Temperature, blood pressure (before study treatment, if applicable), respiratory, and pulse rate.
8. Can be done using indirect ophthalmoscopy or portable slit lamp.
9. The pupils must be sufficiently dilated to allow examination of all ROP features. Unfavorable structural outcome is defined as retinal detachment, macular dragging, macular fold, or retrolental opacity.
10. Only in patients receiving aflibercept. The IOP will be measured in both eyes prior to the injection, and at least once post-injection (only in treated eyes).
11. Baseline imaging will be done prior to study treatment on the treatment day or up to 2 days prior to treatment. Imaging at week 24 and/or week 52 of chronological age may not be required if the following conditions are met: the reading center assessment of morphological outcomes on the week 12 image is in agreement with indirect ophthalmoscopy performed by the investigator, and the patient shows poor tolerance for the imaging procedure, preventing acquisition of images with adequate quality. Cases of rescue/retreatment require additional imaging before any treatment (on the treatment day or up to 2 days prior to treatment) as well as 1 week and 4 weeks following rescue/ retreatment.
12. Optional at baseline; mandatory at week 52 of chronological age (visit 16).

13. Only applicable for patients treated with aflibercept at baseline. One blood sample will be taken at baseline prior to dosing. At week 12, another blood sample will be taken prior to dosing to detect anti-drug antibodies (ADAs) and, if applicable, the occurrence of potential neutralizing antibodies (NAb) (if collection is not possible, the week 12 sample can be taken at week 16).
14. Only applicable for patients treated with aflibercept at baseline. One blood sample each for plasma concentrations of study drug will be taken on day 1 (approximately 24 hours after dosing), day 14, and day 28. Blood pressure must be measured before the PK sample is taken. If a patient's second eye is deemed eligible for treatment and is treated with aflibercept at visits 3, 5, or 7, the PK sample is taken before treatment.
15. A sample is not needed if results from laboratory tests within 8 days prior to screening are available and there was no change in the clinical situation from the time of the sample to the screening visit.
16. The baseline sample can be taken up to 2 days prior to baseline. The day 1 sample will be approximately 24 hours after study treatment.
17. The injection should be performed in both eyes on the same day, if applicable. After initial treatment, each study eye may receive up to 2 additional treatments (with a minimum interval of 28 days between injections) only if retreatment criteria are met. After retreatment, the retreatment/rescue treatment follow-up visits are also required. Blood pressure must be measured before the injection. Details of each injection will be recorded in the eCRF.
18. Multiple sessions performed within 1 week after baseline to complete the procedure will be counted as a single treatment. Each study eye may receive retreatment only if the retreatment criteria are met. After retreatment, the retreatment/rescue treatment follow-up visits are also required. Blood pressure must be measured before laser.

9.1.2. Early Termination Visit

Patients who are withdrawn from the study before the primary endpoint visit (week 52 of chronological age) will be asked to return to the clinic: once for an early termination visit consisting of the end of study assessments described in Table 1.

9.1.3. Unscheduled Visits

All attempts should be made to keep patients on the study schedule. Unscheduled visits may be necessary to repeat testing following abnormal laboratory results, for follow-up of AEs, management and/or follow-up of procedures performed in the observation phase of the study, or for any other reason, as warranted.

Unscheduled visits for observation follow the list of assessments used for visit 8 (week 6). In case treatment is required, the assessments listed in the retreatment visit will be performed.

9.2. Study Procedures

9.2.1. Procedures Performed Only at the Screening/Baseline Visit

Procedures performed only at the screening/baseline visits are: Informed consent, inclusion/ exclusion criteria, demographics, medical history, and collection of clinical laboratory samples (Table 1).

9.2.2. Efficacy Procedures

All ophthalmic evaluations will be conducted according to the schedule detailed in Table 1.

Posterior segment assessment to confirm ROP staging or resolution will be evaluated by the investigator by binocular indirect ophthalmoscopy or wide-field digital retinal photography (eg, RetCam, Phoenix ICON Camera).

Indirect ophthalmoscopy will be performed according to local medical practice and applicable medical standards at the site (eg, usually using a head-mounted light source and a 25- 28, or 30-diopter lens). For this examination, the pupil of the eye must be dilated (mydriasis) with topical application of mydriatic eye drops to the eye.

Clinically significant abnormal findings will be reported as AEs in the eCRF.

Color fundus photographs using wide-field digital retinal photography have to be taken at the time points specified in Table 1.

All images taken by wide-field digital retinal imaging photography during the study will be submitted to a central reading center to confirm the ROP staging or resolution, and analysis of efficacy data. All images are part of the source data for the study and must be retained by the investigator site. A detailed protocol for color fundus photographs image acquisition and transmission can be found in the respective manual.

9.2.2.1. Other Ocular Assessments

With all ophthalmological examinations, abnormalities of the retina or optic nerve as well as unfavorable ocular structural outcomes in each eye will be assessed. All of the following ocular assessments are performed bilaterally at the time points specified in Table 1.

Visual function will be 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.

Monocular and binocular evaluation of visual function has to be performed.

If the patient is not able to cooperate with the above methods, another suitable method (eg, Visual evoked potentials) can be used to evaluate visual function.

Cycloplegic refraction will be measured with retinoscopy in each eye and reported separately for each eye.

Ocular motility tests of both eyes (binocular testing) will be assessed to investigate the integrity of the extrinsic ocular muscles and their nerves (eg, Cover test or Hirschberg test).

9.2.3. Safety Procedures

9.2.3.1. Ophthalmic Examinations

Assessments of ocular safety will include ophthalmologic assessment of the anterior and posterior segment (eg, by indirect ophthalmoscopy), and measurement of intraocular pressure (IOP), only in patients receiving aflibercept, at the time points specified in Table 1.

All ophthalmic examinations are to be conducted in both eyes, unless otherwise indicated.

Intraocular pressure will be measured only in patients receiving aflibercept using a portable tonometer (eg, Tono-Pen, Perkin's Tonometer, or other locally approved device). The same method of IOP measurement should be used in each patient throughout the study. Intraocular pressure will be measured in both eyes prior to the injection, and only in the study eye(s) post-injection. A local anesthetic may be topically applied to the eye(s) being tested (eg, 1 drop of oxybuprocain).

9.2.3.2. Physical Examination

A routine physical examination will assess cardiovascular, respiratory, gastrointestinal, and neurological systems and will follow the standard practice of the site. The assessment will be based on the clinical judgment of the physician and aim to evaluate the overall health of the baby. Weight, body length, and head circumference will be measure as specified in Table 1.

9.2.3.3. Vital Signs

Temperature, heart rate, respiratory rate, and blood pressure (BP) will be measured according to the local medical practice and regulations, at the time points specified in Table 1. Study staff trained in the assessment of infants will perform these assessments. Blood pressure and heart rate should be assessed with a completely automated device, appropriate for use in infants. Blood pressure must be measured before any study treatment is administered and before PK sampling, if applicable.

Clinically significant abnormal findings will be reported as AEs in the eCRF.

9.2.3.4. Hearing Test

The preferred hearing test will be a brainstem auditory evoked response (BAER), also referred to as an auditory brainstem response (ABR), using electrodes placed on the child's head and ears to generate a response. The choice of tool will consider the child's age, degree of cooperation, and available resources. The child also should be comfortable with the testing situation. Hearing will be assessed once, as specified in Table 1.

9.2.3.5. Laboratory Testing

The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the eCRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those that are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the patient's condition.

All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 4 weeks after the last dose of study intervention should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator.

- If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified and the sponsor notified
- If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in patient management or are considered clinically significant by the investigator (eg, SAE or AE or dose modification), then the results must be recorded in the eCRF.

Laboratory (hematology, chemistry, and urinalysis) analyses will be performed and reviewed at screening (visit 1). A sample is not needed if results from laboratory tests within 8 days prior to screening are available and there was no change in the clinical situation from the time of the sample to the screening visit.

In order to avoid discomfort and reduce the probability of urinary tract infections, collection bags will be used to collect urine samples if possible.

Additional samples, including ADA samples, may be collected at any time during the study as determined necessary by the investigator or required by local regulations. This might be true for patients receiving rescue treatment with aflibercept.

Samples for laboratory testing will be collected at visits according to Table 1. Tests will include:

Blood Chemistry

Sodium	Total protein, serum	Total bilirubin
Potassium	Creatinine	Uric acid
Chloride	Blood urea nitrogen (BUN)	Creatine phosphokinase (CPK)
Carbon dioxide	Aspartate aminotransferase (AST)	
Calcium	Alanine aminotransferase (ALT)	
Glucose	Alkaline phosphatase	
Albumin	Lactate dehydrogenase (LDH)	

Hematology

Hemoglobin	Differential:
Hematocrit	Neutrophils
Red blood cells (RBCs)	Lymphocytes
White blood cells (WBCs)	Monocytes
Red cell indices	Basophils
Platelet count	Eosinophils

Urinalysis

Color	Glucose	RBC
Clarity	Blood	Hyaline and other casts
pH	Bilirubin	Bacteria
Specific gravity	Leukocyte esterase	Epithelial cells
Ketones	Nitrite	Crystals
Protein	WBC	Yeast

Abnormal Laboratory Values and Laboratory Adverse Events

All laboratory values must be reviewed by the investigator or authorized designee.

Significantly abnormal test results that occur after start of treatment must be repeated to confirm the nature and degree of the abnormality. When necessary, appropriate ancillary investigations should be initiated. If the abnormality fails to resolve or cannot be explained by events or conditions unrelated to the study medication or its administration, the Medical/Study Director must be consulted.

The clinical significance of an abnormal test value, within the context of the disease under study, must be determined by the investigator.

Criteria for reporting laboratory values as an AE are provided in Section 10.1.1.

9.2.3.6. Central Nervous System Imaging

Central nervous system ultrasound examinations will be performed by a well-experienced examiner, at the time points specified in Table 1. The minimum source documentation will include electronic or paper documentation (medical report). Other modalities of imaging (eg, magnetic resonance imaging) can be used instead of ultrasound if the alternative test was planned independently of the study.

9.2.4. Treatment of Overdose

For this study, any instance where the investigator assumes that a single IVT dose of more than 0.4 mg (0.01 mL) was administered will be considered an overdose. Overdosing with increased injection volume may increase IOP. In these cases, evaluation of IOP and central retinal artery perfusion should be performed immediately after the injection and monitored until normalized. If there is severe elevation of IOP causing disruption of central retinal artery perfusion, immediate measures to reduce IOP and restore central retinal artery perfusion (eg, performance of an anterior chamber paracentesis) should be considered.

Additionally, after ensuring that IOP and central retinal artery perfusion are in a safe range, the investigator should:

1. Contact the Medical Monitor immediately
2. Closely monitor the patient for any further AE/SAE and laboratory abnormalities

3. Obtain a plasma sample for PK analysis if requested by the Medical Monitor (determined on a case-by-case basis)
4. Document the quantity of the excess administered overdose in the eCRF

Decisions regarding dose interruptions or modifications will be made by the investigator in consultation with the Medical Monitor based on the clinical evaluation of the patient.

9.2.5. Drug Concentration and Measurements

Since aflibercept levels are systemically demonstrable after IVT administration, systemic concentrations of study drug will be collected and described. The PK evaluations will support the safety evaluation. The objectives of the PK evaluations are to:

- Describe the individual concentrations of free and bound aflibercept at several time points after the first IVT injection up to 4 weeks thereafter
- Explore the potential influence of demographic and other factors on systemic aflibercept concentrations
- Explore the relationship of systemic exposure and blood pressure

PK sampling schedule (only applicable for patients treated with aflibercept at baseline)

One blood sample each for plasma concentrations of study drug will be taken according to the time points in Table 1 after BP is measured.

If the second eye needs to be treated with aflibercept on a day with PK sample collection, the PK sample should be collected first.

To ensure accuracy of the PK analyses, it is critical to accurately record the exact date and time (24-hour clock) of all blood samples taken on the eCRF, as well as the exact time of the study intervention administration.

Details about the collection, processing, storage and shipment of samples will be provided separately in the laboratory manual. No capillary blood is allowed for PK samples.

Additional samples may be collected at any time during the study as determined necessary by the investigator or required by local regulations.

9.2.6. Immunogenicity Measurements and Samples

Samples for ADA assessments will be collected at time points listed in Table 1.

9.2.7. Biomarker Procedures

Blood pressure as a safety biomarker will be measured using an age-appropriate monitor at the time points shown in Table 1. When applicable, measurements will be taken before study treatment, and before PK sampling.

Only a very limited amount of blood is collected; leftovers (if any) may be used for additional research on study drug and/or disease. Blood volume will not be increased for these research purposes.

In addition to the biomarkers described above, further biomarkers related to the mode of action or the safety of aflibercept and similar drugs may be examined. The same applies to further biomarkers deemed relevant to diseases of the eye and associated health problems. These investigations may include eg, diagnostic, safety, pharmacodynamics, monitoring, or potentially predictive biomarkers.

10. SAFETY EVALUATION AND REPORTING

10.1. Recording and Reporting Adverse Events

10.1.1. General Guidelines

The investigator must promptly record all clinical events occurring during the study data collection period (see Section 11.4.5.1) from the time of signing the ICF or the first dose, to the end of the treatment period. Medical conditions that existed or were diagnosed prior to the signing of the Informed Consent will be recorded as part of medical history. Abnormal laboratory values and vital signs observed at the time of Informed Consent should also be recorded as medical history. Any subsequent worsening (ie, any clinically significant change in frequency and/or intensity) of a pre-existing condition that is temporally associated with the use of the study drug should also be recorded as an AE.

At each visit, the investigator will determine whether any AEs have occurred by evaluating the patient. Adverse events may be directly observed, reported spontaneously by the patient's parent/legal guardian, or by questioning the patient's parent/legal guardian at each study visit. Patient's parent/legal guardian should be questioned in a general way, without asking about the occurrence of any specific symptoms. The Investigator must assess all AEs to determine seriousness, severity, and causality, in accordance with the definitions in Section 10.2. The Investigator's assessment must be clearly documented in the site's source documentation with the Investigator's signature. The Investigator should follow up on SAEs (and AESIs) until they have resolved or are considered clinically stable; AEs should be followed until they are resolved or last study visit, whichever comes first.

Always report the diagnosis as the AE or SAE term. When a diagnosis is unavailable, report the primary sign or symptom as the AE or SAE term with additional details included in the narrative until the diagnosis becomes available. If the signs and symptoms are distinct and do not suggest a common diagnosis, report them as individual entries of AE or SAE.

Laboratory results, vital signs, and other diagnostic results or findings should be appraised by the Investigator to determine their clinical significance. Isolated abnormal laboratory results, vital sign findings, or other diagnostic findings (ie, not part of a reported diagnosis) should be reported as AEs if they are symptomatic, lead to study drug discontinuation, dose reduction, require corrective treatment, or constitute an AE in the investigator's clinical judgment.

For events that are serious due to hospitalization, the reason for hospitalization must be reported as the serious adverse event (diagnosis or symptom requiring hospitalization). A procedure is not an AE or SAE, but the reason for the procedure may be an AE or SAE. Pre-planned (prior to signing the Informed Consent Form) procedures, treatments requiring hospitalization for

pre-existing conditions that do not worsen in severity, and admission for palliative or social care should not be reported as SAEs (see Section 10.2 for Definitions).

For deaths, the underlying or immediate cause of death should always be reported as an SAE.

Any SAE that may occur subsequent to the reporting period (end of the treatment period) that the Investigator assesses as related to study drug should also be reported.

All AEs and SAEs are to be reported according to the procedures in Section 10.1.3.

10.1.2. Reporting Procedure

All events (serious and non-serious) must be reported with investigator's assessment of the event's seriousness, severity, and causality to the (when applicable: blinded) study drug. For SAEs, a detailed narrative summarizing the course of the event, including its evaluation, treatment, and outcome should be provided on the AE CRF. Specific or estimated dates of event onset, treatment, and resolution should be included, when available. Medical history, concomitant medications, and laboratory data that are relevant to the event should also be summarized in the narrative. For fatal events, the narrative should state whether an autopsy was or will be performed, and include the results if available. Information not available at the time of the initial report must be documented in a follow-up report. Source documents (including hospital or medical records, diagnostic reports, etc.) will be summarized in the narrative on the AE CRF, and retained at the study center and available upon request.

Urgent safety queries must be followed up and addressed promptly. Follow-up information and response to non-urgent safety queries should be combined for reporting to provide the most complete data possible within each follow-up.

10.1.3. Events that Require Expedited Reporting to Sponsor

The following events also require reporting to the sponsor (or designee) within 24 hours of learning of the event:

- **SAEs**
- **AESIs:** No AESIs have been defined for this study

10.2. Definitions

10.2.1. Adverse Event

An AE is any untoward medical occurrence in a patient administered a study drug which may or may not have a causal relationship with the study drug. Therefore, an AE is any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease which is temporally associated with the use of a study drug, whether or not considered related to the study drug (ICH E2A Guideline. Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, Oct 1994).

10.2.2. Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose:

- Results in **death** – includes all deaths, even those that appear to be completely unrelated to study drug (eg, a car accident in which a patient is a passenger).
- Is **life-threatening** – in the view of the investigator, the patient is at immediate risk of death at the time of the event. This does not include an AE that had it occurred in a more severe form, might have caused death.
- Requires in-patient **hospitalization** or **prolongation of existing hospitalization**. In-patient hospitalization is defined as a hospital admission (any duration) or an emergency room visit for longer than 24 hours. Prolongation of existing hospitalization is defined as a hospital stay that is longer than was originally anticipated for the event, or is prolonged due to the development of a new AE as determined by the investigator or treating physician.
- Results in persistent or significant **disability/incapacity** (substantial disruption of one's ability to conduct normal life functions).
- Is a **congenital anomaly/birth defect**
- Is an **important medical event** - Important medical events may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the patient or may require intervention to prevent one of the other serious outcomes listed above (eg, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse).

Criteria for Serious Sight-Threatening Ocular Adverse Events

Criteria for serious sight-threatening ocular AEs include the following:

- AE causes a decrease in VA to the level of light perception or worse.
- AE requires surgical intervention (eg, vitreous tap or biopsy with IVT injection of anti-infectives, laser or retinal cryopexy with gas) to prevent permanent loss of sight.
- AE is associated with severe intraocular inflammation (ie, 4 + anterior chamber cell/flare or 4 + vitritis)
- In the opinion of the investigator, AE may require medical intervention to prevent permanent loss of sight

Criteria for reporting SAEs must be followed for these events.

10.2.3. Severity

The severity of AEs will be graded according to the following scale:

Mild: Does not interfere in a significant manner with the patient's normal functioning level. It may be an annoyance. Prescription drugs are not ordinarily needed for relief of symptoms, but may be given because of personality of the patient.

Moderate: Produces some impairment of functioning but is not hazardous to health. It is uncomfortable or an embarrassment. Treatment for symptom may be needed.

Severe: Produces significant impairment of functioning or incapacitation and is a definite hazard to the patient's health. Treatment for the symptom may be given and/or the patient may be hospitalized.

If a laboratory value is considered an AE, its severity should be based on the degree of physiological impairment the value indicates.

10.2.4. Causality

The investigator must provide causality assessment as whether or not there is a reasonable possibility that the drug caused the adverse event, based on evidence or facts, his/her clinical judgment, and the following definitions. The causality assessment must be made based on the available information and can be updated as new information becomes available.

Include the following when applicable:

The following factors should be considered when assessing causality:

- Temporal relationship: time to onset vs time drug was administered
- Nature of the reactions: immediate vs. long term
- Clinical and pathological features of the events
- Existing information about the drug & same class of drugs
- Concomitant medications
- Underlying and concurrent illnesses
- Response to dechallenge (drug discontinuation) or dose reduction
- Response to rechallenge (re-introduction of the drug) or dose increase, when applicable
- Patient's medical and social history

Causality to the study drug (including study drug administration):

- Related:
  - The AE follows a reasonable temporal sequence from study drug administration, and cannot be reasonably explained by the nature of the reaction, patient's clinical (eg, disease under study, concurrent diseases, concomitant medications), or other external factors.

  or

  - The AE follows a reasonable temporal sequence from study drug administration, and is a known reaction to the drug under study or its class of drugs, or is predicted by known pharmacology.

- Not Related:
  - The AE does not follow a reasonable sequence from study drug administration, or can be reasonably explained by the nature of the reaction, patient's clinical state (eg, disease under study, concurrent diseases, and concomitant medications) or other external factors.

Causality to the study conduct (protocol specified procedure):

- Related:
  - The AE follows a reasonable temporal sequence from a protocol specified procedure, and cannot be reasonably explained by the nature of the reaction, patient's clinical (eg, disease under study, concurrent diseases, concomitant medications), or other external factors.
- Not Related:
  - The AE does not follow a reasonable sequence from a protocol specified procedure, or can be reasonably explained by the nature of the reaction, patient's clinical state (eg, disease under study, concurrent diseases, and concomitant medications) or other external factors.

10.3. Safety Monitoring

The investigator will monitor the safety of study patients at his/her site(s) as per the requirements of this protocol and consistent with current Good Clinical Practice (GCP). Any questions or concerns should be discussed with the sponsor in a timely fashion. The sponsor will monitor the safety data from across all study sites. The Medical/Study Director will have primary responsibility for the emerging safety profile of the compound, but will be supported by other departments (eg, Pharmacovigilance; Risk Management; Biostatistics and Data Management). Safety monitoring will be performed on an ongoing basis (eg, individual review of SAEs) and on a periodic cumulative aggregate basis.

10.4. Notifying Health Authorities, Institutional Review Board /Ethics Committee, and Investigators

During the study, the sponsor and/or the CRO will inform health authorities, IECs/IRBs, and the participating investigators of any SUSARs (Suspected Unexpected Serious Adverse Reactions) occurring in other study centers or other studies of aflibercept, as appropriate per local reporting requirements. In addition, the sponsor and/or CRO will comply with any additional local safety reporting requirements.

Upon receipt of the sponsor's notification of a SUSAR that occurred with the study drug, the investigator will inform the Institutional Review Board (IRB)/Ethics Committee (EC) unless delegated to the sponsor.

Event expectedness for aflibercept is assessed against the Reference Safety Information for ROP in the current Investigator's Brochure that is effective for expedited safety reporting.

At the completion of the study, the sponsor will report all safety observations made during the conduct of the trial in the Clinical Study Report to health authorities and ECs/IRB as appropriate.

11. STATISTICAL PLAN

This section provides the basis for the statistical analysis plan (SAP) for the study. The SAP will be finalized prior to the end of the study. The final SAP will be issued before the database lock.

11.1. Statistical Hypothesis

This study will examine the following hypothesis for the primary efficacy variable regarding the proportion of patients with absence of active ROP and unfavorable structural outcomes at week 52 of chronological age. Statistical testing will be conducted to demonstrate the non-inferiority of the aflibercept group to the laser group, with a non-inferiority margin of 5% and a significance level of 5% (one-sided test).

H_0: $p_t \leq p_c$ - 5% versus H_1: $p_t \geq p_c$ - 5%

where p_t is the true proportion of patients with absence of active ROP and unfavorable structural outcomes at week 52 of chronological age for the aflibercept group, and p_c is the true proportion of patients with absence of active ROP and unfavorable structural outcomes at week 52 of chronological age for the laser group.

The proposed non-inferiority margin of 5% is smaller than the smallest difference between laser and ranibizumab in RAINBOW (a phase 3 study comparing the effect of IVT ranibizumab to laser in the management of ROP), and not greater than the difference between the two ranibizumab doses.

11.2. Justification of Sample Size

From the RAINBOW study, the response rate was 66.1% for the laser group and 88.1% for the 0.2 mg ranibizumab group in zone II disease. Furthermore, based on the clinical evidence for the aflibercept investigator-initiated studies (Salman, 2015) (Huang, 2018) (Sukgen, 2018) (Sidorenko, publication in progress), the response rates range all the way 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. An estimated 90% response rate for the aflibercept group and 66.1% response rate for the laser group would be a safe assumption. It is assumed that the proportion of patients with absence of active ROP will be in line with this.

A sample size of 84 patients in the aflibercept group and 28 patients in the laser group (randomized in a 3:1 ratio) will provide 92% power for rejecting the null hypothesis at a 1-sided 5% significance level.

11.3. Analysis Sets

11.3.1. Full Analysis Set

The full analysis set (FAS) will include all randomized patients who received any study treatment. The analysis on the FAS will be performed according to the treatment assigned at baseline (as randomized).

11.3.2. Per Protocol Set

The per protocol set (PPS) includes all patients in the FAS who had no important protocol deviations. Final determinations of the PPS will be made before database lock. Analysis of the PPS will be performed according to the treatment actually received (as treated). The PPS will be used for sensitivity analysis of the primary endpoint.

11.3.3. Safety Analysis Set

The safety analysis set (SAF) includes all randomized patients who received any study treatment (active or laser); it is based on the treatment actually received (as treated). Treatment compliance/administration and all clinical safety variables will be analyzed using the SAF.

11.3.4. Pharmacokinetic Analysis Set

The PK analysis population includes all patients who received any study drug and who had a at least 1 non-missing result following the first dose of study drug.

11.3.5. Immunogenicity Analysis Set

The ADA analysis set includes all patients who received any study drug and had at least 1 non-missing ADA result following the first dose of study drug.

11.4. Statistical Methods

Unless stated otherwise, all variables will be analyzed descriptively with appropriate statistical methods: continuous variables by sample statistics (ie, mean, standard deviation, median, quartiles, minimum, and maximum), and categorical variables by frequencies and percentages.

All statistical analyses will be performed using Statistical Analysis System (SAS); the version used will be specified in the SAP.

11.4.1. Patient Disposition

The following will be provided:

- The total number of screened patients: met the inclusion criteria regarding the target indication and signed the ICF
- The total number of randomized patients: received a randomization number
- The total number of patients who discontinued the study, and the reasons for discontinuation

- The total number of patients who discontinued from study treatment, and the reasons for discontinuation
- A listing of patients treated but not randomized, patients randomized but not treated, and patients randomized but not treated as randomized
- A listing of patients prematurely discontinued from treatment, along with reasons for discontinuation

11.4.2. Demography and Baseline Characteristics

Demographic and baseline characteristics will be summarized descriptively by treatment group, and by all patients combined.

11.4.3. Efficacy Analyses

11.4.3.1. Primary Efficacy Analysis

The primary efficacy variable analysis will be conducted on the FAS. The primary analysis is a statistical evaluation of non-inferiority of aflibercept vs. laser at week 52 of chronological age, with respect to the primary efficacy variable (the proportion of patients with absence of active ROP and of unfavorable structural outcomes). The non-inferiority margin is set at 5%. Superiority testing will follow if non-inferiority is established. Sensitivity analysis of the primary endpoint will also be conducted based on the PPS.

If data are available from the week 40 of chronological age visit, it will be carried forward (LOCF) to the EOS visit for analysis. If the patient discontinued at or before this visit, no data will be carried forward and the patient will be considered a non-responder. For patients with both eyes enrolled in the study, both eyes must meet the endpoint. Patients with only one study eye enrolled will be responders if the respective eye responds.

The statistical analysis will be performed using the Cochran-Mantel-Haenszel method stratified by baseline ROP status. The 1-sided 95% Mantel-Haenszel confidence interval using normal approximation of the difference of response rates between the aflibercept group and the laser group will be calculated. Aflibercept will be considered to be non-inferior to laser if the confidence interval of the difference lies entirely above -5%.

Furthermore, eyes will be considered to be non-responders if rescue treatment is given. The primary analysis for this endpoint will be based on the investigator assessment of, and sensitivity analyses based on the central RC data will be conducted.

11.4.3.2. Secondary and Exploratory Efficacy Analyses

The efficacy variables will be analyzed using the same method as for the analysis of the primary efficacy variable.

11.4.4. Control of Multiplicity

If the primary endpoint is declared significant, a hierarchical testing procedure will be used for the analysis of the secondary endpoints to control the overall alpha error rate at the 0.05 level. The order of the endpoints for hierarchical testing will be specified in the SAP.

11.4.5. Safety Analysis

Safety variables will be summarized on the SAF through week 52 of chronological age for both treatment groups.

11.4.5.1. Adverse Events

Definitions

For safety variables, 3 observation periods are defined:

- The pretreatment period is defined as the time from signing the ICF to before the first dose of study drug.
- The treatment period is defined as the day from first dose of study drug to the last dose of study drug.
- The post-treatment period is defined as the time after the last dose of study drug.

A TEAE is defined as an event (or an exacerbation of a preexisting event during the treatment period) that is observed or reported after the first administration of study drug, and no later than 30 days after last administration of study drug.

Analysis

All AEs reported in this study will be coded using the currently available version of the Medical Dictionary for Regulatory Activities (MedDRA®). Coding will be to lowest level terms. The verbatim text, the preferred term (PT), and the primary system organ class (SOC) will be listed.

Summaries of all TEAEs by treatment group, and by ocular study eye, ocular fellow eye, and non-ocular TEAEs, will include:

- The number (n) and percentage (%) of patients with at least 1 TEAE by SOC and PT
- TEAEs by severity (according to the grading scale outlined in Section 10.2.3), presented by SOC and PT
- TEAEs by relationship to drug or injection procedures (related, not related), presented by SOC and PT

Deaths and other SAEs will be listed and summarized by treatment group.

Treatment-emergent adverse events leading to permanent treatment discontinuation will be listed and summarized by treatment group.

11.4.5.2. Ocular Safety

Ocular safety variables (eg, IOP measurements) will be analyzed descriptively at their scheduled visit, including changes from baseline. All ocular safety parameters will be summarized using descriptive statistics. Information from fundus photography and FA (including the substudy) may also be used to assess ocular safety.

11.4.5.3. Other Safety

Vital Signs

Vital signs (temperature, pulse, blood pressure, and respiration rate) will be summarized by baseline and change from baseline to each scheduled assessment time with descriptive statistics.

Laboratory Tests

Laboratory test results will be summarized by baseline and change from baseline to each scheduled assessment time with descriptive statistics.

Number and percentage of patients with a potentially clinically significant value (PCSV) at any post-randomization time point will be summarized for each clinical laboratory test for all patients and separately for patients in whom the PCSV criterion was normal or missing at baseline.

Shift tables based on baseline normal/abnormal and other tabular and graphical methods may be used to present the results for laboratory tests of interest.

Listings will be provided with flags indicating the out of laboratory range values.

11.4.5.4. Treatment Exposure

Exposure to study drug will be examined for each patient. The total number of treatments administered to each patient and the duration of treatment will be analyzed and summarized using descriptive statistics by treatment group in the SAF and FAS populations

11.4.5.5. Treatment Compliance

Compliance with protocol defined study medication will be calculated as follows:

Treatment compliance = (number of received injections through a given week)/(number of planned injections during the period of participation in the study through the given week) x 100%.

11.4.6. Pharmacokinetics

11.4.6.1. Analysis of Drug Concentration Data

The plasma concentrations of free and bound aflibercept over time will be summarized by descriptive statistics.

No formal statistical hypothesis testing will be performed.

11.4.6.2. Pharmacokinetics and Pharmacokinetics/Pharmacodynamics Analyses

Population PK and exposure-response analyses for blood pressure may be performed, as appropriate, and presented in separate reports.

11.4.7. Analysis of Immunogenicity Data

Anti-drug antibody status (negative or positive) and titer over the study duration may be classified as follows:

- Treatment-emergent ADA response, defined as any post-dose positive ADA assay response when the baseline results are negative

- Treatment-boosted ADA response, defined as any post-dose positive ADA assay response that is 4-fold over baseline titer levels when baseline is positive in the ADA assay
- Maximum ADA titer values
  - Low (titer <1,000)
  - Moderate (1,000≤ titer ≤10,000)
  - High (titer >10,000)
- NAb status (positive or negative) for samples that are positive in the ADA assay

Listings of ADA positivity and titers presented by patient, time point, and study treatment received will be provided. Incidence of treatment-emergent and treatment-boosted ADA will be assessed as absolute occurrence (N) and percent of patients (%), grouped by study treatment received.

The influence of ADAs on individual PK profiles will be evaluated. Assessment of impact of ADA on safety and efficacy may be provided.

11.4.8. Analysis of Biomarker Data

Biomarker exploratory analyses will be described in the SAP.

11.5. Interim Analysis

No interim analysis is planned.

11.6. Additional Statistical Data Handling Conventions

The following analysis and data conventions will be followed:

Definition of baseline:

- The baseline assessment is defined as the latest valid pre-dose assessment

General rules for handling missing data:

- Rules for handling missing data for efficacy assessments are described in the efficacy analysis section. Additional details will be provided in the SAP.
- AE variables

  For some AEs it is important to determine whether the AE started before or after the first active aflibercept injection. If the AE start date is partially missing, it will be imputed by the latest possible date (considering other available data, e.g., stop date) to be conservative.
- Prior/concomitant medication

  For the tabulation of prior and concomitant medication, partially missing start dates of the medication will be imputed by the earliest possible time point, partially missing stop dates will be imputed by the latest possible time point

- No imputations for missing laboratory data, vital sign data, or physical examination data will be made.

Unscheduled assessments

- Assessments taken outside of protocol allowable windows will be displayed according to the CRF assessment recorded by the investigator. Unscheduled assessments will not be included in the summaries.

11.7. Statistical Considerations Surrounding the Premature Termination of a Study

If the study is terminated prematurely, only those parameters required for the development program and/or reporting to regulatory authorities will be summarized. Investigator and sponsor responsibilities surrounding the premature termination of a study are presented in Section 15.1.

12. QUALITY CONTROL AND QUALITY ASSURANCE

In accordance with ICH E6, the sponsor is responsible for quality assurance to ensure that the study is conducted and the data generated, recorded, and reported in compliance with the protocol, GCP, and any applicable regulatory requirement(s). The planned quality assurance and quality control procedures for the study are described in this section.

12.1. Data Management and Electronic Systems

12.1.1. Data Management

A data management plan specifying all relevant aspects of data processing for the study (including data validation [quality-checking], cleaning, correcting, releasing) will be maintained and stored at Regeneron (Sponsor).

A medical coding plan will specify the processes and the dictionary used for coding. All data coding (eg, AEs, baseline findings, medication, medical history/surgical history/ophthalmic history) will be done using internationally recognized and accepted dictionaries.

The CRF data for this study will be collected with an electronic data capture (EDC) system.

12.1.2. Electronic Systems

Electronic systems that may be used to process and/or collect data in this study will include the following:

- IVRS/IWRS system – randomization, study drug supply
- EDC system – data capture – Medidata Rave
- Statistical Analysis System (SAS) – statistical review and analysis
- Pharmacovigilance safety database

12.2. Study Monitoring

12.2.1. Monitoring of Study Sites

The study monitor and/or designee (eg, contract research organization [CRO] monitor) will visit each site prior to enrollment of the first patient, and periodically during the study. This study will use the principles of risk-based monitoring (ICH). This means that the number of visits for any given site may vary based on site risk indicators. The investigator must allow study-related monitoring.

The study monitors will perform ongoing source data review to verify that data recorded in the CRF by authorized site personnel are accurate, complete, and verifiable from source documents, that the safety and rights of patients are being protected, and that the study is being conducted in accordance with the current approved protocol version and any other study agreements, ICH GCP, and all applicable regulatory requirements.

12.2.2. Source Document Requirements

Investigators are required to prepare and maintain adequate and accurate patient records (source documents). The site is responsible to ensure quality within their records and systems and are accountable for ensuring that all source data and CRF data are timely, accurate and complete.

The investigator must keep all source documents on file with the CRF (throughout this protocol, CRF refers to either a paper CRF or an electronic CRF). Case report forms and source documents must be available at all times for inspection by authorized representatives of the sponsor and regulatory authorities.

12.2.3. Case Report Form Requirements

Study data obtained in the course of the clinical study will be recorded on electronic Case Report Forms (CRFs) within the EDC system by trained site personnel. All required CRFs must be completed for each and every patient enrolled in the study. The investigator must ensure the accuracy, completeness, and timeliness of the data reported to the sponsor in the CRFs. After review of the clinical data for each patient, the investigator must provide an electronic signature. A copy of each patient CRF casebook is to be retained by the investigator as part of the study record and must be available at all times for inspection by authorized representatives of the sponsor and regulatory authorities.

Corrections to the CRF will be entered in the CRF by the investigator or an authorized designee. All changes, including date and person performing corrections, will be available via the audit trail, which is part of the EDC system. For corrections made via data queries, a reason for any alteration must be provided.

12.3. Audits and Inspections

This study may be subject to a quality assurance audit or inspection by the sponsor or regulatory authorities. Should this occur, the investigator is responsible for:

- Informing the sponsor of a planned inspection by the authorities as soon as notification is received, and authorizing the sponsor's participation in the inspection

- Providing access to all necessary facilities, study data, and documents for the inspection or audit
- Communicating any information arising from inspection by the regulatory authorities to the sponsor immediately
- Taking all appropriate measures requested by the sponsor to resolve the problems found during the audit or inspection

Documents subject to audit or inspection include but are not limited to all source documents, CRFs, medical records, correspondence, ICFs, IRB/EC files, documentation of certification and quality control of supporting laboratories, and records relevant to the study maintained in any supporting pharmacy facilities. Conditions of study material storage are also subject to inspection. In addition, representatives of the sponsor may observe the conduct of any aspect of the clinical study or its supporting activities both within and outside of the investigator's institution.

In all instances, the confidentiality of the data must be respected.

12.4. Study Documentation

12.4.1. Certification of Accuracy of Data

A declaration assuring the accuracy and content of the data recorded on the CRF/eCRF must be signed electronically by the investigator. This signed declaration accompanies each set of patient final CRFs/eCRFs that will be provided to the sponsor.

12.4.2. Retention of Records

The investigator must retain all essential study documents, including ICFs, source documents, investigator copies of CRFs, and drug accountability records for at least 15 years following the completion or discontinuation of the study, or longer, if a longer period is required by relevant regulatory authorities. The investigator must obtain written approval from the sponsor before discarding or destroying any essential study documents during the retention period following study completion or discontinuation. Records must be destroyed in a manner that ensures confidentiality.

If the investigator's personal situation is such that archiving can no longer be ensured, the investigator must inform the sponsor (written notification) and the relevant records will be transferred to a mutually agreed-upon destination.

13. ETHICAL AND REGULATORY CONSIDERATIONS

13.1. Good Clinical Practice Statement

It is the responsibility of both the sponsor and the investigator(s) to ensure that this clinical study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with the ICH guidelines for GCP and applicable regulatory requirements.

13.2. Informed Consent

The principles of informed consent are described in ICH guidelines for Good Clinical Practice.

The ICF used by the investigator must be reviewed and approved by the sponsor prior to submission to the appropriate IRB/EC. A copy of the IRB/EC -approved ICF and documentation of approval must be provided to the sponsor before study drug will be shipped to the study site.

It is the responsibility of the investigator or designee (if acceptable by local regulations) to obtain written informed consent from each patient's parent(s) or legal guardian(s) prior to the patient's participation in the study and after the aims, methods, objectives, and potential hazards of the study have been explained to the fullest possible extent in language that the patient's parent(s) or legal guardian(s) can understand. The ICF should be signed and dated by the patient's parent(s) or legal guardian(s) and the same investigator or designee who explained the ICF.

Local law must be observed in deciding whether 1 or both parents/guardians consent is required.

If the patient's parent(s) or legal guardian(s) can write but cannot read, the ICF will be read to them before signing their name on the ICF. If the patient's parent(s) or legal guardian(s) can understand but can neither write nor read, the ICF will be read to them in the presence of an impartial witness, who will sign and date the ICF to confirm that informed consent was given.

If only 1 parent or guardian signs the consent form, the investigator must document the reason the other parent or guardian did not sign. The patient's parent/legal guardian may also be required to sign and date the ICF, as determined by the IRB/EC and in accordance with the local regulations and requirements.

- Patient's parent(s)/legal guardian(s) who can write but cannot read will have the assent form read to them before writing their name on the form.
- Patient's parent(s)/legal guardian(s) who can understand but who can neither write nor read will have the ICF read to them in presence of an impartial witness, who will sign and date the ICF to confirm that informed consent was given.

The original ICF must be retained by the investigator as part of the patient's study record, and a copy of the signed ICF must be given to the patient's parent(s) or legal guardian(s).

If new safety information results in significant changes in the risk/benefit assessment, or if there are significant changes to the study procedures, the ICF must be reviewed and updated appropriately. All study patients' parent(s) or legal guardian(s) must be informed of the new information and provide their written consent if they wish the patient to continue in the study. The original signed revised ICF must be maintained in the patient's study record and a copy must be given to the patient's parent(s) or legal guardian(s).

13.3. Patients' Confidentiality and Data Protection

The investigator must take all appropriate measures to ensure that the anonymity of each study patient will be maintained. Patients should be identified by a patient identification number and date of birth, on CRFs or other documents submitted to the sponsor. Documents that will not be submitted to the sponsor (eg, signed ICF) must be kept in strict confidence.

The patient's and investigator's personal data, which may be included in the sponsor database, will be treated in compliance with all applicable laws and regulations. The sponsor shall take all appropriate measures to safeguard and prevent access to this data by any unauthorized third party.

13.4. Institutional Review Board/Ethics Committee

An appropriately constituted IRB/EC, as described in ICH guidelines for GCP, must review and approve:

- The protocol, ICF, and any other materials to be provided to the patient's parent(s)/legal guardian(s) (eg, advertising) before any patient may be enrolled in the study
- Any amendment or modification to the study protocol or ICF before implementation, unless the change is necessary to eliminate an immediate hazard to the patient, in which case the IRB/EC should be informed as soon as possible
- Ongoing studies on an annual basis or at intervals appropriate to the degree of risk

In addition, the IRB/EC should be informed of any event likely to affect the safety of patients or the continued conduct of the clinical study.

A copy of the IRB/EC approval letter with a current list of the IRB/EC members and their functions must be received by the sponsor prior to shipment of drug supplies to the investigator. The approval letter should include the study number and title, the documents reviewed, and the date of the review.

Records of the IRB/EC review and approval of all study documents (including approval of ongoing studies) must be kept on file by the investigator.

13.5. Clinical Study Data Transparency

Final study results will be published on a public clinical trial website according to applicable local guidelines and regulations. Treatment codes will be disseminated to each investigation site thereafter.

14. PROTOCOL AMENDMENTS

The sponsor may not implement a change in the design of the protocol or ICF without an IRB/EC-approved amendment. Where required per local legislation, regulatory authority approval will also be sought.

15. PREMATURE TERMINATION OF THE STUDY OR CLOSEOUT OF A SITE

15.1. Premature Termination of the Study

The sponsor has the right to terminate the study prematurely. Reasons may include efficacy, safety, or futility, among others. Should the sponsor decide to terminate the study, the investigator(s) will be notified in writing.

15.2. Closeout of a Site

The sponsor and the investigator have the right to closeout a site prematurely.

Investigator's Decision

The investigator must notify the sponsor of a desire to closeout a site in writing, providing at least 30 days' notice. The final decision should be made through mutual agreement with the sponsor. Both parties will arrange the closeout procedures after review and consultation.

Sponsor's Decision

The sponsor will notify the investigator(s) of a decision to closeout a study site in writing. Reasons may include the following, among others:

- The investigator has received all items and information necessary to perform the study, but has not enrolled any patient within a reasonable period of time
- The investigator has violated any fundamental obligation in the study agreement, including but not limited to, breach of this protocol (and any applicable amendments), breach of the applicable laws and regulations, or breach of any applicable ICH guidelines
- The total number of patients required for the study are enrolled earlier than expected

In all cases, the appropriate IRB/EC and Health Authorities must be informed according to applicable regulatory requirements, and adequate consideration must be given to the protection of the patients' interests.

16. CONFIDENTIALITY

Confidentiality of information is provided as a separate agreement.

17. FINANCING AND INSURANCE

Financing and insurance information is provided as a separate agreement.

18. PUBLICATION POLICY

Publication rights and procedures will be outlined in a separate clinical study agreement.

19. REFERENCES

Blencowe H, Lee AC, Cousens S, et al. Preterm birth-associated neurodevelopmental impairment estimates at regional and global levels for 2010. Pediatr Res 2013;74 Suppl 1:17-34.

Gilbert C, Rahi J, Eckstein M, et al. Retinopathy of prematurity in middle-income countries. The Lancet 1997;350(9070):12-14.

Harder BC, Schlichtenbrede FC, Von Baltz S, et al. Intravitreal bevacizumab for retinopathy of prematurity: refractive error results. Am J Ophthalmol 2013 Jun;155(6):1119-1124.

Hartnett ME. Vascular endothelial growth factor antagonist therapy for retinopathy of prematurity. Clin Perinatol 2014;41:925-943.

Hartnett ME, Morrison MA, Smith S, et al. Genetic variants associated with severe retinopathy of prematurity in extremely low birth weight infants. Invest Ophthalmol Vis Sci 2014 Oct;55(10):6194-6203.

Hellström A and Smith LEH. Dammann O. Retinopathy of prematurity. Lancet 2013;382(9902):1445-1457.

Huang CY, Lien R, Wang NK, et al. Changes in systemic vascular endothelial growth factor levels after intravitreal injection of aflibercept in infants with retinopathy of prematurity. Graefes Arch Clin Exp Ophthalmol 2018;256, 479-487.

Hwang CK, Baker HG, Hutchinson AK, et al. Outcomes after intravitreal bevacizumab versus laser photocoagulation for retinopathy of prematurity: a 5-year retrospective analysis. Ophthalmology 2015 May;122(5):1008-1015.

Mintz-Hittner HA, Kennedy KA, and Chuang AZ. Efficacy of intravitreal bevacizumab for stage 3+ retinopathy of prematurity. NEJM 2011;364(7):603-15.

Mutlu FM and Sarici SU. Treatment of retinopathy of prematurity: a review of conventional and promising new therapeutic options. Int J Ophthalmol 2013;6:228-236.

Painter SL, Wilkinson AR, Desai P, et al. Incidence and treatment of retinopathy of prematurity in England between 1990 and 2011: database study. Br J of Ophth 2015;99(6):807.

Pertl L, Steinwender G, Mayer C, et al. A Systematic Review and Meta-Analysis on the Safety of Vascular Endothelial Growth Factor (VEGF) Inhibitors for the Treatment of Retinopathy of Prematurity. PLoS One. 2015;10:e0129383.

Salman AG and Said AM. Structural, visual and refractive outcomes of intravitreal aflibercept injection in high-risk prethreshold type 1 retinopathy of prematurity. Ophth Res 2015;53:15-20.

Sidorenko EE. Treatment of retinopathy of prematurity with aflibercept in Russia. Presented at: 17th International Conference on Clinical and Experimental Ophthalmology. 2018 Oct 01-03. Moscow, Russia. Available from: https://ophthalmology.conferenceseries.com/abstract/2018/treatment-of-retinopathy-of-prematurity-with-aflibercept-in-russia

Sidorenko Pulication in progress, Azuma Oral Communication

Stone J, Chan-Ling T, Pe'er J, et al. Roles of Vascular Endothelial Growth Factor and Astrocyte Degeneration in the Genesis of Retinopathy of Prematurity. Invest Ophthalmol Vis Sci 1996;37:290-99.

Sukgen EA and Kocluk Y. Comparison of clinical outcomes of intravitreal ranibizumab and aflibercept treatment for retinopathy of prematurity. Graefe's Archive for Clinical and Experimental Ophthalmology. Revised 2018 Apr 16. Available from: https://doi.org/10.1007/s00417-018-4168-5.

Young TL, Anthony DC, Pierce E, et al. Foley E, Smith LE. Histopathology and vascular endothelial growth factor in untreated and diode laser-treated retinopathy of prematurity. J AAPOS. 1997;1:105-10.

20. INVESTIGATOR'S AGREEMENT

I have read the attached protocol: Randomized, Controlled, Multi-Center Study to Assess the Efficacy, Safety, and Tolerability of Intravitreal Aflibercept Compared to Laser Photocoagulation in Patients with Retinopathy of Prematurity, and agree to abide by all provisions set forth therein.

I agree to comply with the current International Council for Harmonisation Guideline for Good Clinical Practice and the laws, rules, regulations, and guidelines of the community, country, state, or locality relating to the conduct of the clinical study.

I also agree that persons debarred from conducting or working on clinical studies by any court or regulatory agency will not be allowed to conduct or work on studies for the sponsor or a partnership in which the sponsor is involved. I will immediately disclose it in writing to the sponsor if any person who is involved in the study is debarred, or if any proceeding for debarment is pending, or, to the best of my knowledge, threatened.

This document contains confidential information of the sponsor, which must not be disclosed to anyone other than the recipient study staff and members of the IRB/EC. I agree to ensure that this information will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the sponsor.

_____ _____

(Signature of Investigator) (Date)

_____

(Printed Name)

APPENDIX 1. FLUORESCEIN ANGIOGRAPHY SUB-STUDY

Fluorescein Angiography Sub-Study

This is an exploratory analysis for fluorescein angiography (FA) conducted as a sub-study to protocol VGFTe-ROP-1920. Investigational sites that are participating in VGFTe-ROP-1920 may choose to participate in this optional, exploratory FA sub-study. In order to participate, sites must have the ability and prior experience to perform FAs on the patient/study population identified as part of VGFTe-ROP-1920. These specifically include experience in performing FA in pre-term infants using a digital fiber optic contact FA in patients treated with vascular endothelial growth factor inhibitors for retinopathy of prematurity.

A. Sub-study Objective

The objective of this sub-study is to explore the use of FA to assess vascular leakage, non-perfusion, ischemia and the development of the normal retinal vasculature in the population under study pre- and post-treatment. It will involve the injection/administration of intravenous/oral fluorescein dye into the patient, followed by taking wide-angle photographs and angiographic images of the retina in both eyes. As FA has the ability to image vessels and leakage accurately, it may provide additional information related to treatment response and on the disease state beyond that achievable with Color Fundus Photography Imaging. This will also help us generate a Non-Perfusion Index (NPI). The NPI is the ratio of non-perfused (i.e. ischemic) retina to perfused retina as seen on FA.

B. Number of Patients and Sites

Patients will be enrolled at the investigator's discretion at sites that have access to FA imaging. There will be no cap on the number of patients or sites that may participate.

C. Timing of Fluorescein Angiography

FA will be performed prior to administration of very first study treatment (optional yet desired to compare NPI change) and at the week 52 of chronological age visit (mandatory) (Table 1). Additional FA may be performed at the investigator's discretion in conjunction with all the other scheduled study visits for the main study, as well as at any other treatment intervention.

Detailed instructions for procedure, image collection and transmission will be provided in the study procedure manual.

SIGNATURE OF SPONSOR'S RESPONSIBLE OFFICERS

(Medical/Study Director, Regulatory Representative, Clinical Study Lead, and Biostatistician)

To the best of my knowledge, this report accurately describes the planned conduct of the study.

Study Title: Randomized, Controlled, Multi-Center Study to Assess the Efficacy, Safety, and Tolerability of Intravitreal Aflibercept Compared to Laser Photocoagulation in Patients with Retinopathy of Prematurity

Protocol Number: VGFTe-ROP-1920

Protocol Version: VGFTe-ROP-1920 Amendment 1

See appended electronic signature page

Sponsor's Responsible Medical/Study Director

See appended electronic signature page

Sponsor's Responsible Regulatory Liaison

See appended electronic signature page

Sponsor's Responsible Clinical Study Lead

See appended electronic signature page

Sponsor's Responsible Biostatistician

Signature Page for VV-RIM-00083535 v1.0

ESig Approval	[redacted]00
ESig Approval	[redacted]2019 21:04:37 GMT+0000
ESig Approval	[redacted]2019 21:13:21 GMT+0000
ESig Approval	[redacted]9 22:30:51 GMT+0000

Signature Page for VV-RIM-00083535 v1.0 Approved