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Title Page

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Short Title: Aflibercept for ROP – IVT injection versus laser therapy

Acronym: FIREFLEYE

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Role: Global Clinical Leader

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Protocol Amendment Summary of Changes Table**Amendment 01 (23 JUN 2020)**

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union.

Overall Rationale for the Amendment:

The current amendment aims at collecting additional pharmacokinetic (PK) data after Week 4 over the course of the study to further characterize the PK profile in those premature infants who were treated with aflibercept, document the further elimination of free (pharmacologically active) aflibercept and bound aflibercept from plasma, and provide estimates of the elimination half-life. The amendment is supported by the study's Steering Committee and Data Monitoring Committee.

CCI

To collect the aforementioned additional PK data, a total of 3 PK samples (0.6 mL each) after Week 4 will be added, i.e. at Weeks 8, 12 and 24 (end of the study). The consecutive additional blood draw volumes are well in line with the limits set by respective regulatory guidance ([Recommendations of the expert group on clinical trials for the implementation of Regulation \[EU\] No 536/2014, 2017](#)) and recommendations on blood draw volume limits in children ([Howie, 2011](#)). The addition of the 3 PK samples introduces new blood draws for PK data collection at two timepoints (Weeks 8 and 24). At Week 12, the additional blood draw for PK analyses can be taken in the context of the already scheduled blood draw at the Week 12-visit.

Section # and Name	Description of Change	Brief Rationale
Section 1.3 Schedule of Activities (SoA)	Added PK sampling at Visits 9, 11 and 14 (EOS) at Weeks 8, 12 and 24, respectively.	To further characterize the elimination of free and bound aflibercept from plasma, and provide estimates of the elimination half-life.
Section 8.5 Pharmacokinetics	Changed the total blood volume collected for PK analysis from 1.8 mL to 3.6 mL; Changed the time period for PK evaluations from “after the first IVT injection up to 4 weeks thereafter” to “after the first IVT injection up to 24 weeks thereafter.”	Additional 3 PK samples of 0.6 mL each will be added to the Schedule of Activities. These additional PK samples will be taken at Weeks 8, 12 and 24. Blood volumes associated with these additional PK samples are well in line with the respective regulatory guidance (Recommendations of the expert group on clinical trials for the implementation of Regulation [EU] No 536/2014, 2017) and recommendations on blood draw volume limits in children (Howie, 2011).
	Added “Pharmacokinetics in patients who receive repeated injections will be evaluated up to the time point prior to the second injection. In these patients,	Clarified PK evaluation for patients receiving repeated injections of aflibercept

Section # and Name	Description of Change	Brief Rationale
	concentration data for sampling times beyond the second injection will be evaluated exploratively, considering total dose and potential accumulation. ”	
Section 9.5 Interim Analysis	Added how to deal with the final PK sample results that come in later for the initial database release	To ensure the planned initial database release as availability of results for PK samples from Week 24 takes longer.
Title Page	GCL Changed from PPD to PPD	Study personnel update
Sponsor Signatory	Sponsor Signatory page removed and relevant information was stated on Title Page.	To follow the e-signature process
Section 2.2 Introduction	“Zone I with any ROP stages without plus disease, or Zone I stage 3 without plus disease, or ...” changed to “Zone I with any ROP stages with plus disease, or Zone I stage 3 without plus disease, or...”	Correction of typo
Section 4.1 Overall Design	Specified that pharmacokinetic evaluation will be performed in subjects treated with aflibercept by adding “ In subjects treated with aflibercept, ” to the beginning of “Pharmacokinetic evaluations will investigate plasma concentrations of free and bound aflibercept.”	To better orient investigators that such laboratory assessment is done only in aflibercept-treated subjects, and to harmonize with the respective information given in the SoA.
Section 4.1 Overall Design	In the figure for Aflibercept arm : “Worsening of ROP requiring treatment And Interval since the last AFL injection < 27 days” changed to “.....Interval since the last AFL inject ≤27 days”	Correction of typo
Section 8 Study Assessments and Procedures	Added details for the blood volume taken for study-related safety assessments as “for analysis of study drug (PK, in blood plasma), and ADA (in blood serum) in conjunction....”	For clarification

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1. Protocol Summary

1.1 Synopsis

Protocol Title: Open-label, randomized, two-arm, controlled study to assess the efficacy, safety, and tolerability of intravitreal (IVT) aflibercept compared to laser photocoagulation in patients with retinopathy of prematurity (ROP)

Short Title: Aflibercept for ROP – IVT injection versus laser therapy

Rationale: Currently available clinical data indicate potential advantages of the use of IVT anti-VEGF agents, including aflibercept, compared to laser photocoagulation for treatment of ROP, and caused a gradual but steady increase in off-label use of IVT anti-VEGF agents in this condition. However, currently available prospective and longer-term data on the use of aflibercept for the treatment of ROP are still insufficient. The efficacy of IVT aflibercept in a well-characterized population of patients with ROP still needs to be evidenced by data from a prospective clinical trial. Knowledge of the benefit-risk profile for this population needs to be improved, and the pharmacokinetic parameters of aflibercept need to be more fully characterized taking into account the characteristics and limitations of this premature newborn population. These data are planned to be generated in a clinical development program including this randomized, phase 3, multicenter, 2-arm, open-label clinical study to assess the efficacy, safety, and tolerability of IVT aflibercept in subjects with ROP.

Objectives and Endpoints:

Objectives	Endpoints
Primary	
To assess the efficacy of aflibercept in subjects diagnosed with retinopathy of prematurity (ROP) in comparison to laser	Primary endpoint: <ul style="list-style-type: none"> Proportion of patients with absence of active ROP and unfavorable structural outcomes at 24 weeks after starting study treatment Secondary endpoints addressing primary objective: <ul style="list-style-type: none"> Requirement for intervention with a second treatment modality from baseline to Week 24 Recurrence of ROP from baseline to Week 24 To explore new Retinopathy of Prematurity Activity Scale proposed by the International Neonatal Consortium
Secondary	
To assess the safety and tolerability of aflibercept To assess the treatment burden of aflibercept and laser To describe the systemic exposure to aflibercept	Secondary endpoints addressing secondary objectives: <ul style="list-style-type: none"> Number of aflibercept administrations from baseline to Week 24 Number of laser treatments from baseline to Week 24 Proportion of participants with ocular TEAEs and SAEs from baseline to Week 24 Proportion of participants with systemic TEAEs and SAEs from baseline to Week 24 Systemic exposure to free aflibercept (at expected maximum plasma concentration and during elimination period from plasma) determined by sparse sampling Presence of anti-drug antibodies before and 12 weeks after aflibercept injection

Overall Design:**Intervention Model:**

Parallel

Primary Purpose:

Treatment

Number of Arms: 2

Masking: No masking

Number of Participants:

A minimum of 102 preterm infants are planned to be randomized to achieve at least 102 subjects evaluable for the primary analysis.

The determination of sample size is described in Section 9.2.

Intervention Groups and Duration:

Subjects will be randomized 2:1 to receive treatment with an IVT injection of aflibercept 0.4 mg/0.01 mL (approximately 68 subjects) or laser photocoagulation (approximately 34 subjects). Study duration must be planned for at least 24 weeks in the study protocol.

The study comprises the following study phases:

- Screening and initial treatment (may be conducted at the same visit or within 10 days)
- Continued observation and retreatment and/or rescue treatment as needed (from Week 1 to Week 23)
- Final observation (Week 24; can occur between Weeks 25 and 27 for subjects treated between Weeks 21 and 23)

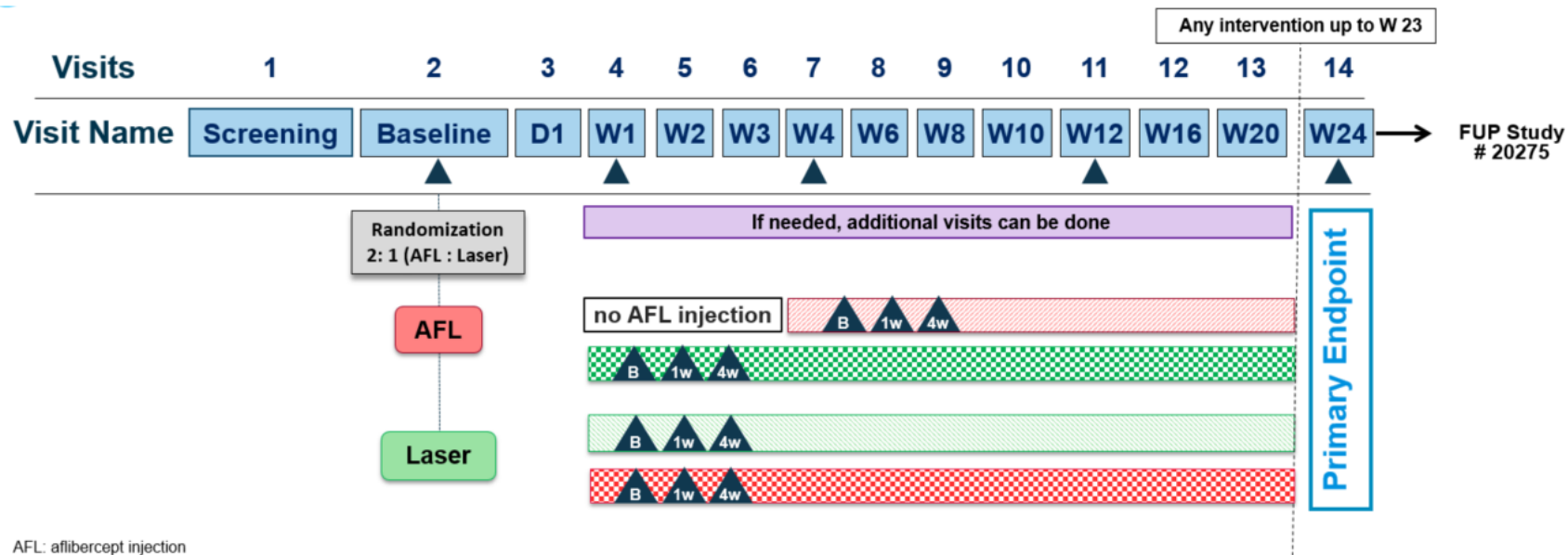
One or both eyes can be treated according to the investigator's assessment of the study's eligibility criteria. The second eye of subjects who start the study with only one eligible eye should be kept under observation according to the local ROP screening guidelines or at every study visit, whichever 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.

Retreatment(s) with the subject's randomized treatment, or rescue treatment (laser for the aflibercept arm; aflibercept for the laser arm) are allowed if the specified criteria are met during the 23-week treatment period.

For the efficacy analysis, only data collected up to Week 24 will be considered. All data collected up to Week 27 will be included in the safety analyses. This applies to subjects who receive retreatment or rescue treatment between Weeks 21 and 23, as their last follow-up visit will be performed 4 weeks after treatment.

Data Monitoring Committee: Yes

1.2 Schema



AFL: aflibercept injection
FUP: follow-up; D: day; W: week

IF NEEDED:

- Retreatment with AFL (up to 2 additional injections; minimal interval 28 days) *
- Rescue treatment with AFL **
- Retreatment with laser *
- Rescue treatment with laser **

* According to retreatment criteria; ** According to rescue treatment criteria

1.3 Schedule of Activities (SoA)

Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14 EOS	Retreatment or rescue treatment follow-up visits				Additional visits may be performed depending on the investigator's assessment of the subject'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 subject'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.
Visit Name	SCR	BL	D1	W1	W2	W3	W4	W6	W8	W10	W12	W16	W20	W24	TRT Visit	D1	1 W FUP	4 W FUP	
Visit Window	--	--	+2D	±3D	±3D	±3D	+7D	±7D	±7D	±7D	±7D	±7D	±7D	±7D	--	+2D	±3D	+7D	
Informed consent	X																		
In/exclusion criteria	X	X																	
Enroll (IVRS/IWRS)		X																	
Demographic	X																		
Medical history	X																	Maternal and subject medical history will be recorded.	
Prior/concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Includes recording of oxygen supplementation. At screening, include any of the medications listed in Section 6.5 given to mother during pregnancy and breastfeeding.
AEs		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	
Head circumference and body length	X								X			X		X					
CNS imaging	X																		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.
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Includes cardiovascular, respiratory, gastrointestinal, and neurological systems according to local general practice and aiming to evaluate overall health.

Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14 EOS	Retreatment or rescue treatment follow-up visits				Additional visits may be performed depending on the investigator's assessment of the subject'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 subject'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.
Visit Name	SCR	BL	D1	W1	W2	W3	W4	W6	W8	W10	W12	W16	W20	W24	TRT Visit	D1	1 W FUP	4 W FUP	
Visit Window	--	--	+2D	±3D	±3D	±3D	+7D	±7D	±7D	±7D	±7D	±7D	±7D	±7D	--	+2D	±3D	+7D	
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Temperature, blood pressure (before study treatment, if applicable), respiratory and pulse rate.
Anterior segment examination	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Can be done using indirect ophthalmoscopy or portable slit lamp.
Binocular indirect ophthalmoscopy	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	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.
Tonometry		X													X				Only in subjects receiving aflibercept: IOP will be measured in both eyes prior to the injection. IOP will be measured at least once postinjection (only in treated eyes) as described in Section 10.2.

Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14 EOS	Retreatment or rescue treatment follow-up visits				Additional visits may be performed depending on the investigator's assessment of the subject'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 subject'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.
Visit Name	SCR	BL	D1	W1	W2	W3	W4	W6	W8	W10	W12	W16	W20	W24	TRT Visit	D1	1 W FUP	4 W FUP	
Visit Window	--	--	+2D	±3D	±3D	±3D	+7D	±7D	±7D	±7D	±7D	±7D	±7D	±7D	--	+2D	±3D	+7D	
Digital wide-field retinal imaging		X		X			X				X			X	X		X	X	Baseline imaging will be done prior to study treatment on the treatment day or up to 2 days prior to treatment. W24 imaging may not be required if the following conditions are met: the reading center assessment of morphological outcomes on the W12 image is in agreement with indirect ophthalmoscopy performed by the investigator, and the subject 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.
Blood sample for ADA (afibercept arm only)		X									X								One 0.6-mL blood sample will be taken at baseline prior to dosing. At W12 one 1.1-mL blood sample will be taken to detect anti-drug antibodies (ADAs) and, if applicable, the occurrence of potential neutralizing antibodies (NAB) (if collection is not possible, the W12 sample can be taken at W16).

Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14 EOS	Retreatment or rescue treatment follow-up visits				Additional visits may be performed depending on the investigator's assessment of the subject'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 subject'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.
Visit Name	SCR	BL	D1	W1	W2	W3	W4	W6	W8	W10	W12	W16	W20	W24	TRT Visit	D1	1 W FUP	4 W FUP	
Visit Window	--	--	+2D	±3D	±3D	±3D	+7D	±7D	±7D	±7D	±7D	±7D	±7D	±7D	--	+2D	±3D	+7D	
Blood sample for PK (aflibercept arm only)			X		X		X		X ¹		X ¹			X ¹					Only applicable for subjects treated with aflibercept at baseline. One blood sample (0.6 mL each) for plasma concentrations of study drug will be taken on Day 1 (~24 hours after dosing), Day 14, Day 28, and Weeks 8, 12 and 24. Blood pressure must be measured before the PK sample is taken. If a subject's second eye is deemed eligible for treatment and is treated with aflibercept at Visits 3, 5, 7, 9, 11 and 14 (EOS), the PK sample is taken before treatment. ¹ PK samples at Weeks 8, 12 and 24 are optional for patients with consent signed prior to the effectiveness date of the current amendment (Amendment 01).
Hematology, clinical chemistry, urinalysis	X																		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.
Urine protein test		X	X		X		X											X	The baseline sample can be taken up to 2 days prior to baseline. The Day 1 sample will be ~24 hours after study treatment.

Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14 EOS	Retreatment or rescue treatment follow-up visits				Additional visits may be performed depending on the investigator's assessment of the subject'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 subject'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.
Visit Name	SCR	BL	D1	W1	W2	W3	W4	W6	W8	W10	W12	W16	W20	W24	TRT Visit	D1	1 W FUP	4 W FUP	
Visit Window	--	--	+2D	±3D	±3D	±3D	+7D	±7D	±7D	±7D	±7D	±7D	±7D	±7D	--	+2D	±3D	+7D	
Aflibercept injection (for subjects randomized to active)		X													X				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 (see Section 4.1). 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.
Laser treatment (for subjects randomized to control)		X													X				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 (see Section 4.1). After retreatment, the retreatment/rescue treatment follow-up visits are also required. Blood pressure must be measured before laser.
Visual function														X					
Refraction														X					
Ocular extrinsic motility														X					

2. Introduction

2.1 Study Rationale

Currently available clinical data indicate potential advantages of the use of IVT anti-VEGF agents, including aflibercept, compared to laser photocoagulation for treatment of ROP, and caused a gradual but steady increase in off-label use of IVT anti-VEGF agents in this condition. However, currently available prospective and longer-term data on the use of aflibercept for the treatment of ROP are still insufficient. The efficacy of IVT aflibercept in a well-characterized population of patients with ROP still needs to be evidenced by data from a prospective clinical trial. Knowledge of the benefit-risk profile for this population needs to be improved, and the pharmacokinetic parameters of aflibercept need to be more fully characterized taking into account the characteristics and limitations of this premature newborn population. These data are planned to be generated in a clinical development program including this randomized, phase 3, multicenter, 2-arm, open-label clinical study to assess the efficacy, safety, and tolerability of IVT aflibercept in subjects with ROP.

2.2 Background

Retinopathy of prematurity is a proliferative vascular retinopathy caused by an abnormal development of the vascularization of the peripheral retina in premature infants (Mutlu and Sarici, 2013; Pertl et al, 2015; Salman and Said, 2015). It affects mainly newborns with a preterm gestational age (≤ 32 weeks) and very low birth weight (≤ 1500 g). ROP remains a major cause of childhood blindness globally. The prevalence of childhood blindness caused by ROP in developed nations ranges between 6% and 18%, while in developing nations the estimate is above 20% (Hartnett, 2014; Gilbert et al, 1997). Approximately 10% to 11% of all newborn infants are born preterm (Blencowe et al, 2013; Hellström et al, 2013). Among premature infants, the incidence of ROP ranges from 20% to 30%, increasing for younger gestational ages. Epidemiological studies have also identified increased risks for ROP due to genetic variants and environmental factors, such as oxygen exposure (Hartnett et al, 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 et al, 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 et al, 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. VEGF-A is a member of the VEGF family of angiogenic factors and acts through activation of receptor tyrosine kinases VEGFR1 and 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 et al, 1996; Young et al, 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 inhibiting 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 drug's 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 safety concerns (Sukgen and Kocluk, 2018; Salman and Said, 2015; Huang et al, 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 photocoagulation 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 photocoagulation for ROP in central retinal locations (Zone I with any ROP stages with 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 established the proof of concept for this indication (Mintz-Hittner et al, 2011; Harder et al, 2013; Hwang et al, 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 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.

A detailed description of the chemistry, pharmacology, efficacy, and safety of aflibercept is provided in the Investigator's Brochure.

2.3 Benefit/Risk Assessment

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 is 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 benefit-risk 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 been shown to have demonstrable 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 in children (eg, ROP) and adults are expected. There is a high and unmet medical need for an effective, safe and tolerable approved treatment for preterm infants with vision-threatening ROP, a disease that is a leading cause of blindness in developing and developed countries. Laser photocoagulation (for 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 subjects with central retinal disease or AP-ROP. Additionally, laser photocoagulation is associated with potential risks of substantial patient impact, 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 treatment session, with no major safety concerns as currently reported. Re-administration is rare. Thus, aflibercept has the potential to offer improved outcomes in this high risk, vulnerable premature newborn population with ROP.

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 subjects. **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, **improvements of treatment convenience in ROP patients** are expected, such as the advantage of being less time consuming than laser (an IVT injection is often less than 30 minutes, whereas laser treatment may take 2 or more hours), potentially less risky by avoiding general anesthesia/sedation, and is not dependent on the ocular anatomy to be performed (on the contrary, laser treatment would be difficult in patients without adequate visualization of the retina, such as subjects with hazy corneas, iris neovascularization, or small pupils). Aflibercept treatment offers 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 larger areas of untreated retina, increasing the risk of failure. Achieving adequate laser treatment may depend on the experience of an individual practitioner.

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 subjects include:

Ocular risks (intraocular inflammation including endophthalmitis, retinal tear/detachment, transient intraocular pressure increase, traumatic cataract). Specific guidance on the appropriate use and administration of aflibercept, in order to optimize the technique of IVT injection in the premature eye and reduce injection procedure-related complications is given in Section 10.2.

Subjects with relevant ongoing or significant previous use of immunosuppressive exposure levels of systemic steroids are excluded as well, 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.

Hypersensitivity/immunogenicity (inherent to all therapeutic proteins) is addressed by exclusion of relevant patients (see exclusion criteria in Section 5.2).

Acute systemic effects: A minimal weight at the time of study treatment (see Section 5.1) is requested. Subjects will be monitored for any evidence of arterial hypertension, proteinuria, worsening of concomitant conditions typical for preterm infants, such as worsening of intraventricular hemorrhage I or II, other non-ocular hemorrhages, or arterial thromboembolic

events. Pharmacokinetic 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, 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 or few injections, and the risks associated with laser treatments, the potential lifelong benefits to children treated with aflibercept outweigh the potential risks, and the expected benefit-risk profile is favorable.

For details of study procedures, dose, and study design justification see Section [4](#).

3. Objectives and Endpoints

Objectives	Endpoints
Primary	
To assess the efficacy of aflibercept in subjects diagnosed with ROP in comparison to laser	<p>Primary endpoint:</p> <ul style="list-style-type: none"> Proportion of patients with absence of active ROP and unfavorable structural outcomes at 24 weeks after starting study treatment * <p>Secondary endpoints addressing primary objective:</p> <ul style="list-style-type: none"> Requirement for intervention with a second treatment modality from baseline to Week 24 Recurrence of ROP from baseline to Week 24 * To explore new Retinopathy of Prematurity Activity Scale proposed by the International Neonatal Consortium
Secondary	
<p>To assess the safety and tolerability of aflibercept</p> <p>To assess the treatment burden of aflibercept and laser</p> <p>To describe the systemic exposure to aflibercept</p>	<p>Secondary endpoints addressing secondary objectives:</p> <ul style="list-style-type: none"> Number of aflibercept administrations from baseline to Week 24 Number of laser treatments from baseline to Week 24 Proportion of participants with ocular TEAEs and SAEs from baseline to Week 24 Proportion of participants with systemic TEAEs and SAEs from baseline to Week 24 Systemic exposure to free aflibercept (at expected maximum plasma concentration and during elimination period from plasma) determined by sparse sampling Presence of anti-drug antibodies before and 12 weeks after aflibercept injection
Other pre-specified	
<p>To characterize further aspects of the effect of aflibercept in the treatment of ROP</p> <p>To further investigate the study intervention and similar drugs (ie, mode-of-action-related effects and/or safety) and to further investigate pathomechanisms deemed relevant to diseases of the eye and associated health problems</p>	<ul style="list-style-type: none"> Evaluation of visual function at Week 24 Time required to perform treatment Requirement for sedation or general anesthesia Requirement for treatment with more than one aflibercept injection Time to intervention with a second treatment modality for ROP or development of unfavorable structural outcomes * Time to recurrence of ROP * Regression of plus disease, regression of pre-retinal vascularized ridge and progression of retinal vascularization beyond the ridge from baseline to Week 24 * Progression to Stage 4 or 5 ROP from baseline to Week 24* Completion of vascularization of the peripheral retina to within one disc diameter of the ora serrata at Week 24 * Time to completion of vascularization * Number of visits required up to Week 24 Systemic exposure to total aflibercept determined by sparse sampling Various biomarkers (eg, diagnostic, safety, pharmacodynamics, monitoring, or potentially predictive biomarkers)

* The primary analysis for these endpoints will be based on the investigators' assessments.

Note: Unfavorable structural outcome is defined as retinal detachment, macular dragging, macular fold, or retrolental opacity.

4. Study Design

4.1 Overall Design

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 photocoagulation in subjects with ROP. The study consists of screening/baseline (1 or 2 visits), a 23-week treatment period (including retreatment and rescue treatment), and a final visit at Week 24 (up to Week 27 for subjects treated after Week 21).

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 one eye with one 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. The second eye of subjects who start the study with only one 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.

Color fundus photography with digital wide-field retinal images 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. Subjects who received treatment and are not confirmed by the RC to meet the inclusion criteria will continue being followed in the study and their efficacy and safety outcomes will be collected, however, their data will not be in the modified full analysis set (mFAS) or the per protocol set (PPS).

Subjects will be randomized 2:1 to treatment with either aflibercept injection or laser photocoagulation, respectively.

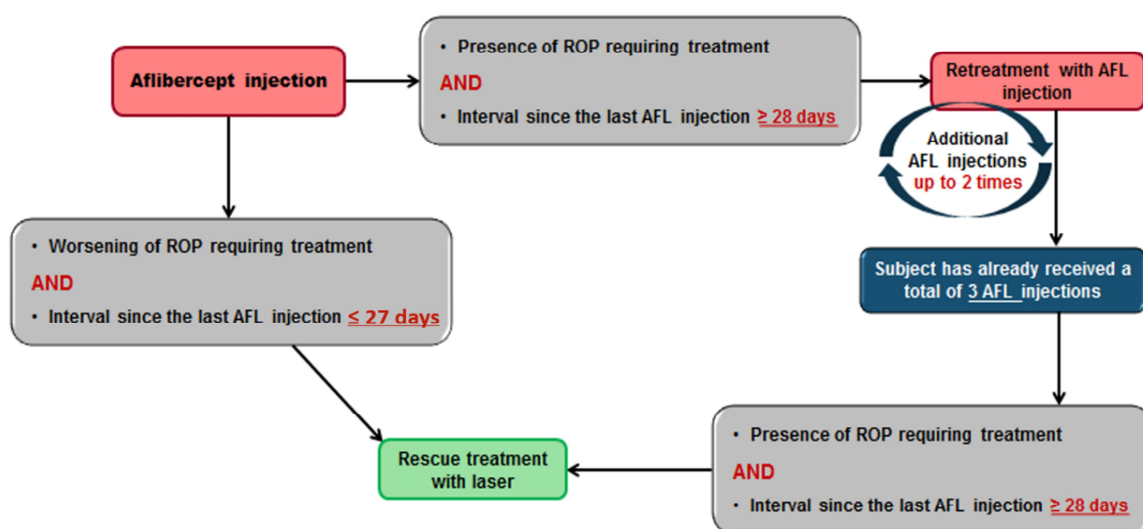
Aflibercept arm

Subjects 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 retreatment criteria are met:

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

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

- Worsening of ROP compared to the examination before the previous injection during the 27 days following that IVT aflibercept injection
- Presence of ROP requiring treatment after the subject already received a total of 3 aflibercept injections and the interval since the last IVT injection is 28 or more days



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

Laser photocoagulation arm

Subjects randomized to laser photocoagulation will undergo treatment in each eligible eye at baseline. Laser ablation should be as complete as possible as judged by the investigator. In case multiple sessions are necessary within 1 week from baseline, they will be counted as a single treatment. Treatment will be applied to the entire avascular peripheral retina. Treatment should be kept well away from the fovea.

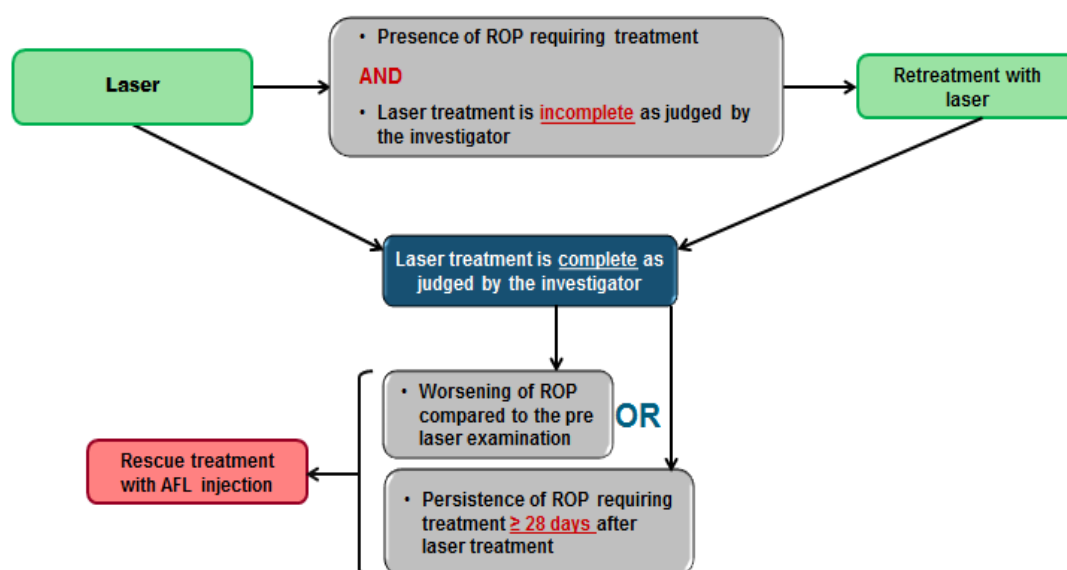
Supplementary laser treatments are allowed during the study. Retreatment with laser is allowed if both of the following criteria are met:

- Presence of ROP requiring treatment
- Fundus examination reveals laser treatment is incomplete as judged by the investigator

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

- Worsening of ROP compared to the prelaser examination
- Persistence of ROP requiring treatment 28 or more days after laser treatment

Subjects who initiate aflibercept rescue treatment will thereafter be managed according to the aflibercept arm treatment regimen.



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

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

Subjects will have mandatory evaluations at regular intervals during the study at the time points specified in the SoA. Subjects 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 digital wide-field retinal images (eg, RetCam) to the RC is required at baseline and at Weeks 1, 4, 12, and 24, and before each retreatment and rescue treatment, and after 1 and 4 weeks of retreatment/rescue treatment. Safety will be assessed through the evaluation of AEs, ophthalmic and physical examinations (including assessment of acute tolerability post-dosing), vital signs, and laboratory tests. In subjects treated with aflibercept, pharmacokinetic evaluations will investigate plasma concentrations of free and bound aflibercept. The potential emergence of antidrug antibodies (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.

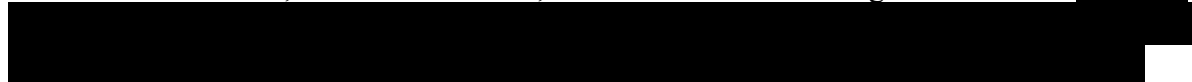
A Phase 3b study (Study 20275; extension study) is planned to assess the long-term outcomes of patients who received study intervention in this study. All treated patients must be offered participation in a follow-up Study 20275 until they are 5 years of age to assess ocular effects, clinical and neurodevelopmental outcomes. CCI

[REDACTED] See Section 6.7 for details.

4.2 Scientific Rationale for Study Design

Laser photocoagulation of the peripheral avascular retina is the current standard of care treatment for ROP. Considering aflibercept's mode of action, and the current clinical evidence describing positive outcomes of 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 necessary.

A fully masked study of IVT aflibercept compared to laser photocoagulation is technically not feasible since laser treatment produces burns that are readily detectable to the grader who assesses treatment outcomes. Additionally, the substantial amount of already existing data describing outcomes of laser photocoagulation 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 2:1 randomization design was defined. CCI



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

4.3 Justification for Dose

Currently available clinical data ([Sukgen and Kocluk, 2018](#); [Salman and Said, 2015](#); [Sidorenko, 2018](#)) consistently shows promising efficacy, with no identification of major safety concerns, when using aflibercept doses ranging from 0.4 mg to 1.0 mg per eye (ie, 1/5 to 1/2 of the 2 mg dose 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.

4.4 End of Study Definition

A subject is considered to have completed the study if he/she has completed all phases of the study including the last visit at Week 24 (or up to Week 27 in the event of follow-up visits).

The end of the study as a whole is defined as the date of the last visit of the last subject in the study in all centers in all participating countries (EU and non-EU).

Primary completion

The primary completion is defined as the date of the last visit of the last subject for the primary outcome.

Subjects who are treated in this study must be offered participation in a follow-up Study 20275, which will include evaluation of visual function and overall neurological development.

5. Study Population

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

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 aggressive posterior retinopathy of prematurity (AP-ROP) according to the International Classification for ROP.

5.1 Inclusion Criteria

Subjects are eligible to be included in the study only if all of the following criteria are met:

Age

1. Gestational age at birth ≤ 32 weeks or birth weight ≤ 1500 g

Type of Participant and Disease Characteristics

2. Subjects 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

Weight

3. Weight at baseline (day of treatment) ≥ 800 g

Sex

4. Male or female

Informed Consent

5. Signed informed consent from parent(s)/legally authorized representative(s) as described in Section 10.1.3, which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

5.2 Exclusion Criteria

Subjects are excluded from the study if any of the following “per subject” criteria are met. A potential study eye is excluded from the study if any of the “per eye” criteria are met:

Medical Conditions – per subject

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

Medical Conditions – per eye

6. Advanced stages of ROP with partial or complete retinal detachment (ROP Stages 4 and 5)
7. ROP involving only Zone III
8. Ocular abnormalities that may interfere with the administration of study intervention or assessment of the study primary endpoint

Prior/Concomitant Therapy – per subject

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

Prior/Concomitant Therapy – per eye

10. Previous surgical or nonsurgical treatment for ROP (IVT anti-VEGF injection, ablative laser therapy, cryotherapy, and vitrectomy)

Prior/Concurrent Clinical Study Experience

11. Participation of the subject 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

5.3 Lifestyle Considerations

No restrictions during the study are required.

5.4 Screen Failures

Screen failures are subjects 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 subjects 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” subject’s participation at a later time point is not allowed. Thus, in general, participation of an initial “screen failure” subject 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 in-/exclusion criteria preventing the subject’s initial attempt to participate have been changed via protocol amendment.

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

To be eligible, rescreened subjects 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 subject to an unjustifiable health risk. Also, for re-screening, the subject’s parent(s)/legally authorized representative(s) has to sign a new informed consent form, even if it was not changed after the subject’s previous screening.

Rescreened subjects should be assigned a new subject number.

6. Study Intervention

Study intervention is defined as any investigational intervention(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study subject according to the study protocol.

6.1 Study Intervention(s) Administered

Intervention Name	Aflibercept	Laser photocoagulation
Type	Drug	Procedure/surgery
Use	Experimental	Active comparator
Dose formulation	solution in a sterile glass vial	Not applicable
Unit dose strengths	mg (mL)	Not applicable
Dosage Level(s)	0.4 mg (0.01 mL)	Not applicable
Route of Administration	IVT injection	Transpupillary conventional laser ablative therapy
Packaging and Labeling	Study intervention will be provided in vials. Each vial will be labeled as required per country requirement.	Not applicable
Current/Former Name(s) or Alias(es)	BAY 86-5321	Not applicable

6.1.1 Medical Devices

The medical device (not manufactured by or for Bayer) provided for use in this study is the 18-gauge filter needle. Ancillary components required (30-gauge injection needle and Luer-lock 1-mL syringe) may also be provided. The ancillary medical devices provided for use in this study are CE marked or FDA cleared according to the regulatory requirements specific for the country where the study site is located.

Medical device incidents, including those resulting from malfunctions of the device, must be detected, documented, and reported by the investigator throughout the study (see Section 8.3.6).

6.2 Preparation/Handling/Storage/Accountability

Only subjects enrolled in the study may receive study intervention and only authorized site staff may supply or administer study intervention.

The investigator is responsible for study intervention accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

Further guidance and information for the final disposition/destruction of unused study drugs are provided in the Investigator Site File.

Aflibercept

The study drug will be supplied in kits that include the following:

- Sterile study drug in sealed glass vials (2 mL) with a withdrawable volume of 0.1 mL (ie, 10-fold the intended injection volume)
- Filter needle (18-gauge)

Other ancillary components required for the administration of aflibercept (ie, 30-gauge injection needle and a 1-mL Luer-lock syringe) may also be supplied to the study sites.

Aflibercept must be stored in a secure and monitored (manual or automated) area with access limited to the investigator and authorized site staff. Aflibercept vials are to be stored in the refrigerator (2°C to 8°C) and must not be frozen. The vial must be kept in the outer carton to protect from light. The investigator or designee must confirm that the appropriate temperature conditions have been maintained during transit and any discrepancies are reported and resolved before use.

When aflibercept vials are removed from the refrigerator, the solution should be inspected visually and it should have no evidence of turbidity. If particulates, cloudiness, or discoloration are visible, the vial must not be used. Prior to use, the unopened vial may be stored at room temperature (below 25°C) for up to 24 hours.

Details on the administration of aflibercept IVT injection and postinjection procedures can be found in Section 10.2.

Laser photocoagulation

Transpupillary conventional laser photocoagulation 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 previously 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.

The location of study intervention should follow medical standards at each site (eg, bedside in the NICU or in a separate operating room following local practice).

6.3 Measures to Minimize Bias: Randomization and Masking

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

Eligible subjects will be randomized 2:1 to receive either treatment with aflibercept or laser photocoagulation, respectively, stratified by Japanese and Non-Japanese sites as well as 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. For preventing imbalances, the randomization result of subjects who do not complete baseline treatment will be assigned to a subsequent subject once it is known that the baseline treatment was not completed.

Subjects will be centrally assigned to randomized study intervention using an Interactive Voice/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 subject'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 causing misinterpretation of results and the impossibility 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 the SoA.

6.4 Study Intervention 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).

6.5 Prior and Concomitant Therapy

All medications the subject 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 subject 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 phototherapy lamp for treating jaundice is allowed. Appropriate eye protection must be provided to the subject. 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 subject's oxygen saturation by pulse oximetry once the supplementation is stable. In the last visit of the trial, it will be recorded whether oxygen supplementation is required.

Exposure to any IVT or systemic anti-VEGF agent is not allowed.

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.

Anti-VEGF agents are not allowed in mothers who are breastfeeding.

6.5.1 Rescue Medicine

For subjects randomized to aflibercept, rescue treatment with laser may be performed. For subjects randomized to laser, rescue treatment with aflibercept may be performed. The criteria for the allowance of rescue treatment is specified in Section 4.1. Use of rescue treatment in either eye must be recorded on the respective eCRF page.

All subjects requiring rescue treatment will be counted as missing the primary endpoint. Nevertheless, the subjects should be followed to assess the efficacy and safety outcomes after rescue treatment.

6.6 Dose Modification

Not applicable. Only the 0.4 mg/0.01mL dose of aflibercept will be used for all treatments.

6.7 Intervention after the End of the Study

A Phase 3b study (Study 20275; extension study) is planned to assess the long-term outcomes of subjects who received study intervention in this study and accept participation in the extension study. No study treatment will be required in this study, or provided by the sponsor. In case any treatment for ROP is required during Study 20275, the site's standard of care should be administered. Subjects will undergo safety and efficacy assessments until 5 years of age.

The baseline visit of Study 20275 can be conducted concomitantly with the Week 24 visit or the last follow-up visit, whichever is later, or at any later point between this date and ≤ 1 month after the date when the subject is 1 year of chronological age.

7. Discontinuation of Study Intervention and Participant Discontinuation/Withdrawal

7.1 Discontinuation of Study Intervention

Study intervention discontinuation can be triggered by the subject's parent(s)/legally authorized representative(s) or by the treating physician.

Subjects in which retreatment or rescue treatment are planned at any time during the study but not administrated will be considered a temporarily discontinuation of study intervention. If study intervention is temporarily discontinued, it can be restarted at any time during the study.

In case there is a decision against retreatment or rescue treatment, subjects will be considered a permanent discontinuation of study intervention. If study intervention is permanently discontinued, further treatment is at the investigator's discretion.

Study intervention discontinuation must not lead to study discontinuation of a subject.

If study intervention is permanently discontinued, the subject should continue in the study on an observational basis (eg, for study examinations only) for safety evaluation.

7.2 Participant Discontinuation/Withdrawal from the Study

A subject may be withdrawn from the study at any time at the request of the parent(s)/legally authorized representative(s), or at the discretion of the investigator for safety, behavioral, compliance, or administrative reasons. This is expected to be uncommon.

Any surgical treatment for ROP other than study interventions is considered a prohibited treatment, and subject must be withdrawn from the study.

At the time of discontinuing from the study, if possible, an end of study (EOS) visit should be conducted, as shown in the SoA. See the SoA for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

The participant will be permanently discontinued from the study intervention and from the study at that time.

If the parent(s)/legally authorized representative(s) withdraw consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

7.3 Lost to Follow-Up

A subject will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and the study site is unable to contact the subject's parent(s)/legally authorized representative(s).

The following actions must be taken if a subject fails to return for a required study visit:

- The site must attempt to contact the subject's parent(s)/legally authorized representative(s) and reschedule the missed visit as soon as possible and counsel the subject's parent(s)/legally authorized representative(s) on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject's parent(s)/legally authorized representative(s) wishes to have the subject continue in the study.
- Before a subject is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the subject's parent(s)/legally authorized representative(s) (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's parent[s]/legally authorized representative[s] last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.
- Should the subject's parent(s)/legally authorized representative(s) continue to be unreachable, the subject will be considered lost to follow-up.

8. Study Assessments and Procedures

Study procedures and their timing are summarized in the SoA. Protocol waivers or exemptions are not allowed.

Subjects should be seen for all visits on the designated day, with an allowed "visit window" as indicated in the SoA.

Safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the subject should continue or discontinue study intervention.

Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.

All screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.

The blood volume taken for study-related safety assessments, for analysis of study drug (PK, in blood plasma), and ADA (in blood serum) in conjunction with non-study, safety-related assessments should be kept to a minimum, at the discretion of the investigator and applying local regulations.

For subjects randomized to aflibercept: in order to monitor subjects for the potential development of ADA, one 0.6-mL blood sample for determination of ADA serum titers will be taken at baseline prior to aflibercept dosing (Visit 2), and one 1.1-mL blood sample at 12 weeks after dosing (Visit 11), which can also be used to detect the occurrence of potential neutralizing antibodies (NAB). If it is not possible to obtain an ADA sample at Visit 11, it can be collected at Visit 12. A total of approximately 1.7 mL of blood will be collected for ADA measurement in serum during the first 12 weeks after treatment.

For the collection of PK data, one blood sample (0.6 mL each) will be taken at 6 time points after dosing: at Day 1 (Visit 3), Week 2 (Visit 5), Week 4 (Visit 7), Week 8 (Visit 9), Week 12 (Visit 11) and Week 24 (Visit 14 / EOS)¹.

The time and date of samplings must be documented. Details are provided in the sample handling manual.

For PK and ADA, the total sample volume was specified to a minimum of 3.6 mL (Section 8.5) and 1.7 mL respectively. No capillary blood is allowed for PK and ADA samples.

Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples. Study blood samples should be performed in combination with regular blood draws to avoid an additional puncture if possible.

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 re-treatment visit will be performed.

All results will be recorded in the eCRF.

8.1 Efficacy Assessments

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

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 20- or 28-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 the SoA.

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.

¹ PK samples at Weeks 8, 12 and 24 are optional for patients with consent signed prior to the effectiveness date of the current amendment (Amendment 01).

8.1.1 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 ocular assessments have to be performed bilaterally.

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 subject is not able to cooperate with the above methods, an other 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).

8.2 Safety Assessments

Planned time points for all safety assessments are provided in the SoA.

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

Care should be taken to use the same equipment for all examinations of a certain subject whenever possible.

8.2.1 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 subjects receiving aflibercept).

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

IOP will be measured only in subjects 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 subject throughout the study. IOP will be measured in both eyes prior to the injection and postinjection only in the study eye(s) according to the directions in Section 10.2. A local anesthetic may be topically applied to the eye being tested (eg, 1 drop of oxybuprocain).

8.2.2 Physical Examinations

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 the SoA.

8.2.3 Vital Signs

Temperature, pulse rate, respiratory rate, and blood pressure will be measured according to the local medical practice and regulations. Study staff trained in the assessment of infants will perform these assessments. Blood pressure and pulse measurements 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.

8.2.4 Clinical Safety Laboratory Assessments

Section 10.3 lists the clinical laboratory tests to be performed and the SoA specifies the timing.

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 subject'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 subject 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/NAB 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 subjects receiving rescue treatment with aflibercept.

8.2.5 Central Nervous System Imaging

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

8.3 Adverse Events and Serious Adverse Events

The definitions of an AE or SAE can be found in Section 10.4.

AEs will be reported by the subject's treating physician, caregiver, surrogate, or the subject's legally authorized representative(s) or health care professional not involved in the study.

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE. They remain responsible for following up SAEs, or AEs considered related to the study intervention or study procedures, or those that caused the subject to discontinue the study intervention or the study.

8.3.1 Time Period and Frequency for Collecting AE and SAE Information

All AEs/SAEs will be collected from the start of study intervention at the time points specified in the SoA (Section 1.3).

Medical occurrences that begin before the start of study intervention but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the eCRF not the AE section. Any relevant maternal medical occurrences during pregnancy until the delivery of the newborn that may have impact on the subject's health will be recorded on the Maternal Medical History section of the eCRF (eg, urinary tract infections, TORCH infections, high blood pressure, preeclampsia).

All SAEs will be recorded and reported to the sponsor or designee immediately and under no circumstances should this exceed 24 hours, as indicated in Section 10.4. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AEs or SAEs after the conclusion of study participation at Week 24 or at the follow-up, whichever is later. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the investigator must promptly notify the sponsor.

8.3.2 Method of Detecting AEs and SAEs

The methods of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Section 10.4.

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject's parent(s)/legally authorized representative(s) is the preferred method to inquire about AE occurrences.

8.3.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the subject is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is given in Section 10.4.

8.3.4 Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to the sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a study intervention under clinical investigation are met.

The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs), and investigators.

Investigator safety reports must be prepared for suspected unexpected serious adverse reactions according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.

An investigator who receives an investigator safety report describing an SAE or other specific safety information (eg, summary or listing of SAEs) from the sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

8.3.5 Pregnancy

Not applicable.

8.3.6 Medical Device Incidents (Including Malfunctions)

A filter needle is provided for use in this study. In order to fulfill regulatory reporting obligations worldwide, the investigator is responsible for the detection and documentation of events meeting the definitions of incident or malfunction that occur during the study with such devices.

Definitions of a medical device incident/AE/SAE and device deficiency can be found in Section 10.5.

NOTE: Incidents fulfilling the definition of an AE/SAE will also follow the processes outlined in Section 8.3.3 and Section 10.4.

8.3.6.1 Time Period for Detecting Medical Device Incidents

Medical device incidents or malfunctions of the device identified, documented, and reported during all periods of the study in which the medical device is used.

If the investigator learns of any incident at any time after a subject has been discharged from the study, and such incident is considered reasonably related to a medical device provided for the study, the investigator will promptly notify the sponsor.

The method of documenting medical device incidents is provided in Section 10.5.

8.3.6.2 Follow-up of Medical Device Incidents

All medical device incidents involving an AE will be followed and reported in the same manner as other AEs (see Section 8.3.3). This applies to all subjects, including those who discontinue study intervention.

The investigator is responsible for ensuring that follow-up includes any supplemental investigations as indicated to elucidate the nature and/or causality of the incident.

New or updated information will be recorded on the originally completed form with all changes signed and dated by the investigator.

8.3.6.3 Prompt Reporting of Medical Device Incidents to Sponsor

Medical device incidents will be reported to the sponsor within 24 hours after the investigator determines that the event meets the protocol definition of a medical device incident.

The Medical Device Incident Report Form will be sent to the sponsor by facsimile transmission. If unavailable, then telephone, overnight mail or courier service should be utilized.

The same individual will be the contact for the receipt of medical device reports and SAE.

8.3.6.4 Regulatory Reporting Requirements for Medical Device Incidents

The investigator will promptly report all incidents occurring with any medical device provided for use in the study in order for the sponsor to fulfill the legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.

The investigator, or responsible person according to local requirements (eg, the head of the medical institution), will comply with the applicable local regulatory requirements relating to the reporting of incidents to the IRB/IEC.

8.4 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 performance of an anterior segment 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 subject 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 subject.

8.5 Pharmacokinetics

Since aflibercept is systemically demonstrable after IVT administration, systemic concentrations of study drug will be collected and described. The PK evaluations will support the safety evaluation. The objective 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 24 weeks thereafter²
- 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 subjects treated with aflibercept at baseline)

One 0.6-mL blood sample for plasma concentrations of study drug will be taken according to the time points in SoA after BP is measured. A total of 3.6 mL of blood will be collected for PK analysis during the study. See the SoA in Section 1.3 for details.

² PK samples at Weeks 8, 12 and 24 are optional for patients with consent signed prior to the effectiveness date of the current amendment (Amendment 01).

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.

PK evaluation and reporting

Concentrations of free and bound aflibercept will be listed by sample time point, and descriptive statistics will be applied. No formal PK parameters will be evaluated.

Decline of aflibercept concentrations after the first injection will be described. Drug concentration listings will be further grouped by potentially influencing factors (ie, renal impairment as determined by serum creatinine values, concomitant medications, body weight, ethnicity) and evaluated by means of descriptive statistics.

Pharmacokinetics in patients who receive repeated injections will be evaluated up to the time point prior to the second injection. In these patients, concentration data for sampling times beyond the second injection will be evaluated exploratively, considering total dose and potential accumulation.

Evaluation of the data will be reported separately and attached to the final clinical study report as an appendix. Fundamental results and conclusions of the PK evaluation will also be included in the main text of the final clinical study report.

8.6 Pharmacodynamics

Pharmacodynamic parameters are not evaluated in this study.

8.7 Genetics

Pharmacogenetic investigations are not planned.

8.8 Biomarkers

Biomarkers for efficacy will not be evaluated.

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

Urine protein will also be measured. The date and time of urine sampling must be documented.

8.8.1 Other Biomarkers

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.

The additional biomarker results may be reported separately.

8.9 Medical Resource Utilization and Health Economics

Health Economics/Medical Resource Utilization and Health Economics parameters are not evaluated in this study.

9. Statistical Considerations

9.1 Statistical Hypotheses

The study will provide estimates of efficacy and success will be concluded based on the posterior probability from the Bayesian analysis using the following success criterion:

Success criterion defined as “response probability for aflibercept is greater than the one for laser minus 5 percentage points with at least 95% probability.”

This can be interpreted as non-inferiority hypothesis with a non-inferiority margin of 5 percentage points.

9.2 Sample Size Determination

The sample size rationale is based on the primary efficacy endpoint “absence of active ROP and unfavorable structural outcomes at 24 weeks after starting study treatment” and the success criterion that the response probability for aflibercept is greater than the one for laser minus 5 percentage points with at least 95% probability.

With at least 102 subjects evaluable for the primary analysis in the full analysis set (FAS; see Section 9.3 for definition) and a randomization ratio of 2:1 (active:control) to receive either treatment with aflibercept or laser photocoagulation (corresponds to 68 and 34 subjects, respectively), the defined success criterion will be achieved with a power of 81% under following assumptions:

- The laser response probability for the study is similar to historic data: A Bayesian meta-analytical prediction based on BEAT-ROP and RAINBOW resulted in a posterior predictive distribution described by a beta(34.7, 13.8) distribution. For the power simulations, the true response probability for laser photocoagulation in this trial was drawn from this distribution for each simulation run.
- And the response probability for aflibercept is 15 percentage points higher than for laser, but not higher than 95%.

Respective power simulations were performed using package rjags ([Plummer, 2016](#)) in the statistical software R ([R Core Team, 2016](#)).

9.3 Populations for Analyses

For purposes of analysis, the following populations are defined:

Population	Description
Full analysis set (FAS)	All subjects who received any type of study treatment, and had a baseline and at least one post-baseline assessment of efficacy
Modified full analysis set (mFAS)	All subjects with central reading center positive confirmed disease stages meeting the inclusion criteria who completed baseline treatment, had a baseline and at least one post-baseline central reading center assessment of efficacy.
Per protocol set (PPS)	All subjects in the mFAS who have no validity findings or important deviations that could affect the primary efficacy variable. The status of each subject, with regard to protocol deviations, will be determined by the sponsor and documented before database release.
Safety analysis set (SAF)	All subjects who received any type of study treatment

The primary efficacy variable will be analyzed using the FAS, mFAS, and the PPS, where the FAS analysis is considered to be the primary one. The secondary and explorative efficacy variables will be analyzed using the FAS and mFAS, where in all cases the analyses with the mFAS and PPS are considered as supportive. Safety variables will be analyzed based on the SAF.

9.4 Statistical Analyses

The statistical analysis plan (SAP) will be developed and finalized before database release and will describe the subject populations to be included in the analyses, and procedures for accounting for missing, unused, and spurious data. This section is a summary of the planned statistical analyses of the primary and secondary endpoints.

Data of the second eye (of subjects who have started the study with only one eligible eye) will be included in the efficacy and safety analyses if treated before or on Visit 9 (≤ 8 weeks from baseline), whereas second eyes treated after Visit 9 (> 8 weeks from baseline) will be included in the safety analysis only.

9.4.1 Efficacy Analyses

Endpoint	Statistical Analysis Methods
Primary: Proportion of patients with absence of active ROP and unfavorable structural outcomes at 24 weeks after starting study treatment.	Primary analysis: The data to be analyzed by a Bayesian statistical model. (detail below)
Secondary: <ul style="list-style-type: none"> Requirement for intervention with a second treatment modality from baseline to Week 24 Recurrence of ROP from baseline to Week 24 Number of aflibercept administrations from baseline to Week 24 Number of laser treatments from baseline to Week 24 To explore new Retinopathy of Prematurity Activity Scale proposed by the International Neonatal Consortium * 	The first two secondary endpoints will be analyzed using Bayesian statistical modelling as described below. The number of aflibercept administrations and laser treatments as well as the ROP activity scale will be analyzed descriptively. No hypothesis testing will be conducted for the secondary endpoints.
Other pre-specified: <ul style="list-style-type: none"> Evaluation of visual function at Week 24 Time required to perform treatment Requirement for sedation or general anesthesia Requirement for treatment with more than one aflibercept injection Time to intervention with a second treatment modality for ROP or development of unfavorable structural outcomes Time to recurrence of ROP Regression of plus disease, regression of pre-retinal vascularized ridge and progression of retinal vascularization beyond the ridge from baseline to Week 24 Progression to Stage 4 or 5 ROP from baseline to Week 24 Completion of vascularization of the peripheral retina to within one disc diameter of the ora serrata at Week 24 Time to completion of vascularization Number of visits required up to Week 24 	The analysis of all other prespecified endpoints will be described in the SAP finalized before database release.

*[Smith et al, 2018](#)

The primary endpoint is the proportion of patients with absence of active ROP and unfavorable structural outcomes at 24 weeks after starting study treatment.

For this endpoint, active ROP is defined as ROP requiring treatment (according to the inclusion criteria). Unfavorable structural outcome is defined as retinal detachment, macular dragging, macular fold, or retrolental opacity.

Furthermore, eyes are considered non-responders if rescue treatment was given.

The primary analysis for this endpoint will be based on the investigator assessment of ROP; sensitivity analyses based on the central reading center data will be conducted.

The primary endpoint will be analyzed using a Bayesian statistical model with a non-informative prior probability distribution for the response probability for a single eye (p). One

eye or both eyes of a patient to be included into the analysis to determine the primary endpoint on a patient level, if treated and meeting the inclusion criteria.

As it is assumed that in most subjects both eyes will be treated, this will be accounted for by using a bivariate model including a correlation coefficient ρ with an informative prior distribution allowing positive values only. The model is based on following distribution assumptions:

$l_i \sim \text{Bernoulli}(p)$: response of subject i in left eye

$r_i \sim \text{Bernoulli}(p)$: response of subject i in right eye

l_i and r_i are correlated with correlation coefficient ρ

$p \sim \text{beta}(1,1)$ – non-informative prior for the response probability in one eye

$\rho \sim \text{beta}(1,1)$ – prior for the correlation between the two eyes of one subject (allowing only a positive correlation)

Bivariate probability distribution:

left eye / right eye	Response	No response	
Response	$p^2 + \rho p(1-p)$	$p(1-p)(1-\rho)$	p
No response	$p(1-p)(1-\rho)$	$(1-p)(1-p(1-\rho))$	$1-p$
	p	$1-p$	

Based on this model the primary endpoint “Proportion of patients with absence of active ROP and unfavorable structural outcomes at 24 weeks after starting study treatment,” (eg, response on a subject level, i.e. $p^2 + \rho p(1-p)$) will be derived for each of the 2 treatment groups.

The efficacy of aflibercept and laser photocoagulation will be characterized by the resulting posterior distribution for the probability of response. 90% credible intervals to be provided for the probability of response.

In addition mean, median, and mode of the posterior distributions for the response probabilities of the 2 treatments as well as for the difference in response probabilities between the treatments will be provided together with 90% two-sided credible intervals.

Success of this study will be concluded, if the response probability for aflibercept is greater than the one for laser minus 5 percentage points with at least 95% probability.

This is the case if the lower limit of the one-sided 95% credible interval for the treatment difference (aflibercept – laser photocoagulation) is greater than -5%.

As non-informative prior distributions are used for the analysis this success criterion corresponds to a frequentist non-inferiority test with a non-inferiority margin of 5 percentage points and a significance test of 5% (one-sided test).

This success criterion is reasonable since in historical studies, differentiation between laser and anti-VEGF in unfavorable structural outcomes has not been consistently demonstrated when treating ROP in Zone II. In addition, laser photocoagulation requires burning and destroying retina where vessels have not yet developed, whereas anti-VEGF agents allow further development of retinal vascularization and potential future functional benefits such as

improved visual fields. Furthermore, laser photocoagulation is associated with development of high myopia. The proposed non-inferiority (NI) margin of 5% is smaller than the smallest difference between laser and ranibizumab in RAINBOW, and not greater than the difference between the two ranibizumab doses. With the proposed sample size, numerically better response (~10 percentage points) needs to be observed for aflibercept compared to laser to meet the success criterion and the NI test can be seen as a “relaxed” superiority test.

If the success criterion is met, in a second step superiority of aflibercept over laser photocoagulation is evaluated by comparing the lower limit of the 95% one-sided credible interval with 0.

Handling of missing data for the primary endpoint:

The handling of subjects with missing data for the primary endpoint is outlined in the following. Further details will be provided in the SAP.

The following rules apply only for eyes not having had an unfavorable structural outcome or rescue treatment before dropping out, as in these cases the eye would always be considered as non-responding.

If at the last visit before dropping out the subject had no active ROP and Zone II was completely vascularized (aflibercept subjects) or laser treatment was completed (laser subjects) the respective eye will be considered as responding.

Otherwise, if the subject dropped out at or after week 16, the last ROP staging before dropping out will be carried forward and used for determining the response in this eye. If the subject dropped out before week 16, the missing information will be imputed as follows:

If there was a clear documentation that the subject dropped out due to lack of efficacy, the respective eye will be considered as non-responding; otherwise, a multiple imputation approach will be used based on the subject’s treatment group and the initial staging (Zone I versus II versus AP-ROP).

For the primary and secondary efficacy endpoints, subgroup analyses by zones (Zone I/II/AP-ROP) will be performed.

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9.4.2 Safety Analyses

All safety analyses will be performed on the SAF. All safety variables including ocular and systemic TEAEs and SAEs from baseline to Week 24 and the presence of ADAs before and 12 weeks after the baseline aflibercept injection will be analyzed descriptively using summary statistics or frequency tables as appropriate. Thereby, TEAE is defined as AE that is observed or reported after the first and not later than 30 days after the last administration of study treatment.

All AEs will be coded using Medical Dictionary for Regulatory Activities (MedDRA). The latest available version at the time of coding will be used. The results will be summarized, at a minimum, on the level of system organ class and preferred term. Data will also be summarized according to intensity and investigator’s causality assessment.

9.4.3 Other Analyses

PK and biomarker exploratory analyses (see Section 8.5 and 8.8) will be described in the SAP. Results of further bioanalytical analyses will be presented separately from the main CSR (see Section 8.5).

9.5 Interim Analyses

No interim analysis is planned. However, safety assessments will be continuously performed.

Database release will occur after all subjects have completed the Week 24 visit or have dropped out of the study. In case of the scenario where a subject has a 30-day follow-up visit after Week 24 or the final PK sample results come in later and these data cannot be cleaned in time for the initial data release, they will be included in a planned re-release of the database.

9.5.1 Data Monitoring Committee (DMC)

The aim of the safety assessments are to determine if the study shows unacceptable risks for the subjects, and to discontinue the study if necessary. The assessments will be performed by an independent DMC. Detailed information regarding the DMC procedures will be explained in a separate DMC charter.

10. Supporting Documentation and Operational Considerations

10.1 Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
 - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
 - Applicable ICH Good Clinical Practice (GCP) Guidelines
 - Applicable laws and regulations
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (eg, advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study subjects.
- The investigator will be responsible for the following:
 - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
 - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
 - Providing oversight of the study conduct at the site and adherence to all applicable requirements (21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies, and local regulations)

10.1.2 Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

10.1.3 Informed Consent Process

The investigator or his/her qualified representative will explain the nature of the study to the subject's parent(s)/legally authorized representative(s), including that participation is voluntary, and answer all questions regarding the study.

The subjects' parent(s)/legally authorized representative(s) will be required to sign a statement of informed consent that meets the requirements of all applicable regulations (eg, 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act [HIPAA]) and the IRB/IEC or study center.

The medical record must include a statement that written informed consent was obtained before the subject was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

If the ICF is revised during the study, re-consent must be obtained, if applicable.

A copy of the ICF(s) must be provided to the parent(s)/legally authorized representative(s).

A new ICF is required if a subject is rescreened.

10.1.4 Data Protection

Subjects will be assigned a unique identifier by the sponsor. Any subject records or datasets that are transferred to the sponsor will contain the identifier only; subject names or any information that would make the subject identifiable will not be transferred.

The subject's parent(s)/legally authorized representative(s) must be informed that the subject's personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained.

The subject's parent(s)/legally authorized representative(s) must be informed that the subject's medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

10.1.5 Committees Structure

An independent **central reading center** (RC) will evaluate the digital wide-field retinal images.

The sponsor may decide to institute a **Steering Committee** to guide the trial in all aspects of safety and efficacy and must ensure that all relevant information is provided by investigators. The composition of the committee, the functional roles, and responsibilities will be specified in its charter.

10.1.6 Dissemination of Clinical Study Data

Result summaries of Bayer's sponsored clinical trials in drug development Phases 2, 3, and 4 and Phase 1 studies in subjects are provided in the Bayer Trial Finder application after

marketing authorization approval in line with the position of the global pharmaceutical industry associations laid down in the "Joint Position on the Disclosure of Clinical Trial Information via Clinical Trial Registries and Databases." In addition, results of clinical drug trials will be provided on the publicly funded website www.ClinicalTrials.gov and EU Clinical Trials Register in line with the applicable regulations.

Bayer commits to sharing upon request from qualified scientific and medical researchers subject-level clinical trial data, study-level clinical trial data, and protocols from clinical trials in subjects for medicines and indications approved in the United States (US) and European Union (EU) on or after 01 JAN 2014 as necessary for conducting legitimate research.

All Bayer-sponsored clinical trials are considered for publication in the scientific literature irrespective of whether the results of the clinical trials are positive or negative.

10.1.7 Data Quality Assurance

- All subject data relating to the study will be recorded in an eCRF unless transmitted to the sponsor or designee electronically (eg, laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by electronically signing the eCRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The sponsor assumes accountability for actions delegated to other individuals (eg, Contract Research Organizations).
- Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for 25 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

10.1.8 Source Documents

- Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- The site must implement processes to ensure availability of all required source documentation. A source document checklist (not part of this protocol) will be used at the site to identify the source data for key data points collected and the monitor will work with the site to complete this. It is the expectation of the sponsor that all data have source documentation available at the site.

- Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- Definition of what constitutes source data can be found in ICH-GCP guidelines E6(R2) § 1.51, 1.52.

10.1.9 Study and Site Closure

The sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of subjects by the investigator
- Discontinuation of further study intervention development

10.2 Appendix 2: Aflibercept IVT Administration

IVT injections must be carried out according to medical standards and applicable guidelines by a qualified ophthalmologist experienced in administering IVT injections. After opening the vial, all preparation steps have to take place under aseptic conditions.

The supplied kit includes a vial of aflibercept 40 mg/mL and an 18-gauge filter needle. The procedure also requires ancillary components including a 30-gauge injection needle with up to 13 mm length and a 1-mL Luer-lock syringe.

Aflibercept is formulated as a sterile liquid to a final concentration of 40 mg/mL. The volume of injection is 10 µL (0.01 mL) for the 0.4 mg dose of aflibercept. The study drug will be withdrawn using aseptic technique through the filter needle attached to the syringe. The filter needle will be discarded after withdrawal of the vial contents and is not used for IVT injection. The injection needle is attached to the syringe and the contents should be expelled until the plunger is aligned with the line that marks 0.01 mL on the syringe. Special care should be taken to ensure accurate alignment of the plunger with the 0.01 mL dose mark, and use of magnification equipment such as surgical microscope or an indirect ophthalmoscopy lens is recommended. The injection of aflibercept must be completed within 2 hours of the start of dose preparation.

The IVT injection 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 procedures for IVT injection adapted for ROP infants. Administration is recommended to be carried out in an inpatient surgical room, or in a clean room (as defined by local infection control regulations). In case both eyes require treatment, the procedures should be kept as independent as possible. Each vial should only be used for the treatment of a single eye. Each eligible eye will receive an IVT injection

of 0.4 mg (0.01 mL) of aflibercept. This drug administration protocol is based on recommendations from the Euretina Expert Consensus ([Grzybowski et al, 2018](#)). Detailed recommendations are provided below.

1. Apply topical anesthetic.
2. Apply povidone-iodine ophthalmic solution to eyelid margins, eyelashes, and conjunctival surface.

Povidone iodine is the agent of choice for subjects without an iodine allergy. Of note, subjects with an **iodine allergy must receive an agent other than povidone iodine**. A disinfecting agent must be used; however, this can be povidone iodine or another agent, according to local clinical standard and the investigator's decision.

3. Place 1 or 2 drops of povidone-iodine (or alternative agent for subjects with iodine allergy) ophthalmic solution on the ocular surface at the intended injection site.
4. Drape.
5. Apply an additional drop of povidone-iodine (or alternative agent for subjects with iodine allergy) ophthalmic solution to the site of injection.
6. Sterilized forceps and calipers (speculum) should be used to stabilize the globe and measure the injection site; the entry site of the needle for the IVT injection should be 1.0-2.0 mm from the limbus, usually in the inferotemporal quadrant. Other quadrants can be used depending on easy of access.
7. Insert needle at marked injection point. The relative size of the lens in premature infants is larger than in adults, therefore the needle should be directed to the posterior pole, to avoid damaging the lens with the needle. The needle should only be introduced to the depth require to administer the injection (eg, half the length of a 13 mm needle). It should not be completely introduced into the eye to avoid potential trauma to the posterior pole.
8. Gently inject study drug.
9. As the needle is withdrawn, a sterile cotton tip applicator should be rolled over the entry site to minimize the risk of drug reflux. This should be held in place for 10 seconds.

After the injection, any unused product must be discarded.

10.2.1 Use of Topical Antibiotic Agents

The use of topical antibiotics as prophylaxis in IVT injections, both in the preparation and postinjection varies considerably between different practices. There is no consensus on the use of topical antibiotics, the agent to be used and the dose to be administered, especially in pediatric settings. Additionally, treatment should performed be as soon as possible, and the injection date should not be postponed to allow antibiotic prophylaxis. If time allows, it is recommended that a broad spectrum topical antibiotic be used as part of the preparation for the IVT injection, as prophylaxis, in the days immediately preceding and following the injection.

Suggested use:

The injection date should be as early as possible, and not be postponed to allow any prophylaxis. Cases of infection (eg, conjunctivitis) must be adequately treated before the injection is performed. In case the injection date allows, treatment with 1 to 2 drops of antibiotic to both eyes 3 times a day can be initiated up to 3 days before the injection day.

On the injection day, as part of the preparation for injection, instill 1 to 2 drops to the eye 1 hour before the injection, and another drop 15 minutes before the injection.

After the injection, administer 1 to 2 drops of antibiotic to the treated eye(s). Subsequently, administer 1 to 2 drops of the topical antibiotic to the injected eye(s), 3 times a day, for an additional 3 days.

10.2.2 Postinjection Examinations

No further special precautions are required for a subject who has had an uneventful recovery from IVT injection. In the days following the IVT injection, physicians and nursing staff should be instructed to report any symptoms suggestive of endophthalmitis (eg, redness, irritation of the eye, ocular discharge, lid swelling, photophobia) without delay.

Cases of postinjection AEs requiring additional monitoring should remain under observation and be treated according to the investigator's medical judgment.

Optic nerve and retina

Visualize the optic nerve to verify reperfusion of the central retinal artery in the immediate postinjection period. Verify that the retina is attached and that there is no new intraocular hemorrhage.

Intraocular pressure

Immediately following the IVT injection, subjects should be monitored for elevation in IOP. Appropriate monitoring may consist of tonometry and/or a check for perfusion at the optic nerve head. Sterile equipment for anterior chamber paracentesis should be available, to allow immediate performance if required (refer to further guidance below). If a tonopen is used to check pressure, a clean tonopen condom should be placed on the tip before taking each measurement. Similarly, if a rebound tonometry device is used, a new, clean tip should be used. If applanation tonometry is used, the applanator tip should be swabbed with alcohol and let to dry before using it to measure IOP. If required, IOP may be lowered by pharmaceutical or surgical intervention. Treatment should be initiated whenever IOP is increased to the extent that the central retinal artery remains closed for more than 1 to 2 minutes.

Paracentesis should be used only in extreme circumstances when the degree of pressure elevation poses an imminent and irreversible threat to vision. In the rare situation when a paracentesis is warranted, IOP should be recorded before and after the procedure. A 0.1-mL paracentesis may be performed using a 30-gauge needle if judged necessary by the investigator.

IOP must be measured in injected eyes approximately 30 minutes following the injection if not done immediately postinjection or when the measurement was abnormal immediately postinjection. An abnormal value will be monitored closely until it returns to normal. If IOP was measured immediately after the injection and the value was normal, it does not have to be repeated.

Record all IOP measurements and related treatments in the source documents and the appropriate pages of the eCRF.

10.3 Appendix 3: Clinical Laboratory Tests

The tests detailed in [Table 10–1](#) will be performed and reported by the local laboratory.

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

Positive results of any prenatal congenital infectious disease screening test (eg, toxoplasmosis, syphilis, varicella-zoster, parvovirus B19, rubella, cytomegalovirus [CMV], and herpes infections), if available will be recorded as medical history. The results of any serological test performed in order to confirm these diseases will be also recorded.

Table 10–1: Protocol-Required Safety Laboratory Assessments

Laboratory Assessments	Parameters			
Hematology	Platelet count		White blood cell count Differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils) if possible	
	Red blood cell count			
	Hemoglobin			
	Hematocrit			
Clinical Chemistry	Blood urea nitrogen	Potassium	Aspartate aminotransferase (AST)/serum glutamic-oxaloacetic transaminase (SGOT)	Total bilirubin
	Creatinine	Sodium	Alanine aminotransferase (ALT)/serum glutamic-pyruvic transaminase (SGPT)	Total protein albumin
	Glucose (nonfasting)			
Routine Urinalysis	Specific gravity pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase Microscopic examination (if blood or protein is abnormal)			
Other Screening Tests	Any congenital infectious disease test performed during pregnancy that may lead to a subject infection should be recorded as medical history.			

Investigators must document their review of each laboratory safety report.

10.4 Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.4.1 Definition of AE

AE Definition
<ul style="list-style-type: none"> An AE is any untoward medical occurrence in a subject or clinical study subject, associated with the use of study intervention, whether or not considered related to the study intervention. NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) associated with the use of study intervention.
Events Meeting the AE Definition
<ul style="list-style-type: none"> Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the

medical and scientific judgment of the investigator.

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication.
- “Lack of efficacy” or “failure of expected pharmacological action” per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfil the definition of an AE or SAE.

Events NOT Meeting the AE Definition

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments that are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject’s condition.
 - The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject’s condition.
 - Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE.
 - Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
 - Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
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10.4.2 Definition of SAE

An SAE is defined as any untoward medical occurrence that, at any dose:

a. Results in death

b. Is life-threatening

- The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization

- In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
- Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in persistent disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) that may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Other situations:

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
- Examples of such events include invasive or malignant cancers, 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.

10.4.3 Recording and Follow-Up of AE and/or SAE

AE and SAE Recording

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- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory reports, diagnostics reports) related to the event.
 - The investigator will record all relevant AE/SAE information in the eCRF.
 - It is not acceptable for the investigator to send photocopies of the subject's medical records to the sponsor in lieu of completion of the AE/SAE eCRF pages.
 - There may be instances when copies of medical records for certain cases are requested by the sponsor. In this case, all subject identifiers, with the exception of the subject number, will be redacted on the copies of the medical records before submission.
 - The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.
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Assessment of Intensity

- The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:
 - Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
 - Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
 - Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.
 - An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.
-

Assessment of Causality

- The investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE.
 - A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
 - The investigator will use clinical judgment to determine the relationship.
 - Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
 - The investigator will also consult the Investigator's Brochure (IB) and/or Product Information, for marketed products, in his/her assessment.
 - For each AE/SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
 - There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to the sponsor. However, **it is**
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very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data.

- The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a subject dies during participation in the study or during a recognized follow-up period, the investigator will provide the sponsor with a copy of any post mortem findings including histopathology.
- New or updated information will be recorded in the originally completed eCRF.
- The investigator will submit any updated SAE data to the sponsor within 24 hours of receipt of the information.

10.4.4 Reporting of SAEs

SAE Reporting to the Sponsor via an Electronic Data Collection Tool

- The primary mechanism for reporting an SAE to the sponsor will be the electronic data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section).
- Contacts for SAE reporting can be found in the investigator site file.

SAE Reporting to the Sponsor via Paper CRF

- Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the sponsor.
 - In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.
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- Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames.
 - Contacts for SAE reporting can be found in the investigator site file.
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10.5 Appendix 5: Medical Device Incidents: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

The detection and documentation procedures described apply to the sponsor medical devices provided for use in the study (see Section 6.1.1).

Medical Device Incident/SAE/AE Definitions

- **An incident** is any malfunction or deterioration in the characteristics or performance of a device made available on the market, including use-error due to ergonomic features as well as any inadequacy in the information supplied by the manufacturer and any undesirable side effect.
 - **An AE** is any untoward medical occurrence, unintended disease or injury or any untoward clinical signs, including an abnormal laboratory finding, in subjects, users or other persons, in the context of a clinical investigation, whether or not related to the investigational device.
 - **An SAE** is any AE that leads to any of the following:
 - (a) death
 - (b) serious deterioration in the health of the subject, that resulted in any of the following:
 - (i) life-threatening illness or injury
 - (ii) permanent impairment of a body structure or a body function
 - (iii) hospitalization or prolongation of subject hospitalization
 - (iv) medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function
 - (v) chronic disease
 - (c) fetal distress, fetal death or a congenital physical or mental impairment or birth defect
 - **A device deficiency** is any inadequacy in the identity, quality, durability, reliability, safety or performance of an investigational device, including malfunction, use errors or inadequacy in information supplied by the manufacturer.
 - Not all incidents lead to death or serious deterioration in health. The nonoccurrence of such a result might have been due to other fortunate circumstances or to the intervention of health care personnel.
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Examples of Incidents

- A subject, user, caregiver, or healthcare professional is injured as a result of a medical device failure or its misuse.
- A subject's study intervention is interrupted or compromised by a medical device failure.
- A misdiagnosis due to medical device failure leads to inappropriate treatment.
- A subject's health deteriorates due to medical device failure.

Medical Device Incident Documenting

- Any medical device incident/device deficiency occurring during the study will be documented in the subject's medical records, in accordance with the investigator's normal clinical practice, and on the Device Event Form of the eCRF and on the Product Technical Complaint form for any technical issues identified with the device.
- For incidents fulfilling the definition of an AE or an SAE, the appropriate AE/SAE CRF page will be completed as described in Section 10.4. The CRF will be completed as thoroughly as possible and signed by the investigator before transmittal to the sponsor or designee.
- It is very important that the investigator provides his/her assessment of causality (relationship to the medical device provided by the sponsor) at the time of the initial AE or SAE report and describes any corrective or remedial actions taken to prevent recurrence of the incident.
- A remedial action is any action other than routine maintenance or servicing of a medical device where such action is necessary to prevent recurrence of an incident. This includes any amendment to the device design to prevent recurrence.

10.6 Appendix 6: Abbreviations

ADA	anti-drug antibodies
AE	adverse event
AFL	aflibercept
AG	Aktiengesellschaft
ALT	alanine aminotransferase
AP-ROP	aggressive posterior retinopathy of prematurity
AST	aspartate aminotransferase
ATE	arterial thrombotic event
BEAT-ROP	Bevacizumab Eliminates the Angiogenic Threat of Retinopathy of Prematurity trial
BL	Baseline
BP	blood pressure
BW	body weight
CE	Conformité Européenne
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CMV	Cytomegalovirus

CNS	central nervous system
CNV	choroidal neovascularization
CONSORT	Consolidated Standards of Reporting Trials
CRF	case report form
CSR	clinical study report
D	day(s)
DMC	Data Monitoring Committee
DOB	date of birth
ECG	Electrocardiogram
eCRF	electronic case report form
EOS	end of study
EU	European Union
EudraCT	European Clinical Trials Database
FAS	full analysis set
FDA	Food and Drug Administration
FUP	follow-up
GCP	Good Clinical Practice
HIPPA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IOP	intraocular pressure
IRB	Institutional Review Board
IVRS	Interactive Voice Response System
IVT	Intravitreal
IWRS	Interactive Web Response System
MedDRA	Medical Dictionary for Regulatory Activities
mFAS	modified full analysis set
MRI	magnetic resonance imaging
NAB	neutralizing antibodies
NI	non-inferiority
NICU	neonatal intensive care unit
PK	pharmacokinetic(s)
PPS	per protocol set
RAINBOW Study	RANibizumab Compared With Laser Therapy for the Treatment of INfants BOrn Prematurely With Retinopathy of Prematurity
RC	reading center
ROP	retinopathy of prematurity
SAE	serious adverse event
SAF	safety analysis set
SAP	statistical analysis plan
SCR	Screening
SGOT	serum glutamic-oxaloacetic transaminase
SGPT	serum glutamic-pyruvic transaminase (SGPT)
SoA	schedule of activities
TEAE	treatment-emergent adverse event
TORCH	toxoplasma gondii, other viruses, rubella, cytomegalovirus, and herpes simplex

TRT	Treatment
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
w/wk	Week

10.7 Appendix 7: Protocol Amendment History

The Protocol Amendment Summary of Changes Table for the current amendment is located directly before the Table of Contents (TOC).

11. References

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