

Comparing Alternative Ranibizumab Dosages for Safety and Efficacy in Retinopathy of Prematurity

A Randomized Clinical Trial

Andreas Stahl, MD; Tim U. Krohne, MD; Nicole Eter, MD; Isabel Oberacher-Velten, MD; Rainer Guthoff, MD; Synke Meltendorf, MD; Oliver Ehrt, MD; Sabine Aisenbrey, MD; Johann Roider, MD; Heinrich Gerding, MD; Claudia Jandeck, MD; Lois E. H. Smith, MD, PhD; Johanna M. Walz; for the Comparing Alternative Ranibizumab Dosages for Safety and Efficacy in Retinopathy of Prematurity (CARE-ROP) Study Group

[+ Supplemental content](#)

IMPORTANCE Anti-vascular endothelial growth factor (VEGF) therapies are a novel treatment option in retinopathy of prematurity (ROP). Data on dosing, efficacy, and safety are insufficient.

OBJECTIVE To investigate lower doses of anti-VEGF therapy with ranibizumab, a substance with a significantly shorter systemic half-life than the standard treatment, bevacizumab.

DESIGN, SETTING, AND PARTICIPANTS This randomized, multicenter, double-blind, investigator-initiated trial at 9 academic medical centers in Germany compared ranibizumab doses of 0.12 mg vs 0.20 mg in infants with bilateral aggressive posterior ROP; ROP stage 1 with plus disease, 2 with plus disease, or 3 with or without plus disease in zone I; or ROP stage 3 with plus disease in posterior zone II. Patients were recruited between September 2014 and August 2016. Twenty infants were screened and 19 were randomized.

INTERVENTIONS All infants received 1 baseline ranibizumab injection per eye. Reinjections were allowed in case of ROP recurrence after at least 28 days.

MAIN OUTCOMES AND MEASURES The primary end point was the number of infants who did not require rescue therapy at 24 weeks. Key secondary end points included time-to-event analyses, progression of physiologic vascularization, and plasma VEGF levels. Stages of ROP were photodocumented and reviewed by an expert committee.

RESULTS Nineteen infants with ROP were enrolled (9 [47.4%] female; median [range] postmenstrual age at first treatment, 36.4 [34.7-39.7] weeks), 3 of whom died during the study (1 in the 0.12-mg group and 2 in the 0.20-mg group). Of the surviving infants, 8 (88.9%) (17 eyes [94.4%]) in the 0.12-mg group and 6 (85.7%) (13 eyes [92.9%]) in the 0.20-mg group did not require rescue therapy. Both ranibizumab doses were equally successful in controlling acute ROP (Cochran-Mantel-Haenszel analysis; odds ratio, 1.88; 95% CI, 0.26-13.49; $P = .53$). Physiologic intraretinal vascularization was superior in the 0.12-mg group. The VEGF plasma levels were not systematically altered in either group.

CONCLUSIONS AND RELEVANCE This pilot study demonstrates that ranibizumab is effective in controlling acute ROP and that 24% of the standard adult dose (0.12 mg) appears equally effective as 40% (0.20 mg). Superior vascularization of the peripheral retina with 0.12 mg of ranibizumab indicates that the lower dose may be favorable. Unchanged plasma VEGF levels point toward a limited systemic drug exposure after ranibizumab.

TRIAL REGISTRATION clinicaltrials.gov Identifier: [NCT02134457](#) and clinicaltrialsregister.eu Identifier: 2013-002539-13.

JAMA Pediatr. 2018;172(3):278-286. doi:10.1001/jamapediatrics.2017.4838
Published online January 8, 2018.

Author Affiliations: Author affiliations are listed at the end of this article.

Group Information: CARE-ROP Study Group members are listed at the end of this article.

Corresponding Author: Andreas Stahl, MD, Eye Center, Medical Center, University of Freiburg, Killianstrasse 5, 79106 Freiburg, Germany (andreas.stahl@uniklinik-freiburg.de).

Retinopathy of prematurity (ROP) can lead to bilateral blindness in early infancy. Disturbed blood vessel growth is at the core of ROP pathophysiology. The aim of all current ROP treatments is therefore to prevent or reverse pathologic blood vessel growth while ideally fostering the expansion of physiologic retinal vasculature into the retinal periphery.¹ Both pathologic and physiologic vascular growth in the retina are driven by angiogenic growth factors, mainly vascular endothelial growth factor (VEGF).^{2,3} As a consequence, pharmacologic treatment approaches that target VEGF are being evaluated. The largest anti-VEGF study in ROP to date, the BEAT-ROP (Efficacy of Intravitreal Bevacizumab for Stage 3+ Retinopathy of Prematurity) trial,⁴ found that bevacizumab, a full-size anti-VEGF antibody, can halt the progression of severe ROP, revert pathologic angiogenic changes, and induce the progression of physiologic intraretinal vasculature.

There are, however, several concerns with regard to anti-VEGF therapy in ROP. It is, for example, known that intravitreally injected drugs leak into the systemic circulation. A single dose of intravitreally injected bevacizumab suppresses VEGF plasma levels below the limit of detection for weeks.⁵⁻⁹ It is unknown what adverse effects such systemic VEGF suppression has on organ development. Long-term systemic safety of anti-VEGF drugs is therefore an important question in ROP.^{10,11} The second unresolved question relates to anti-VEGF dosing.^{10,11} The BEAT-ROP trial⁴ used half the adult bevacizumab dose per eye. A recent study¹² found that significantly lower bevacizumab doses are effective in ROP.

The aim of this study was to evaluate, in a prospective randomized trial, the efficacy and safety of 2 doses of ranibizumab for ROP. Unlike bevacizumab, ranibizumab is an anti-VEGF antibody fragment with a systemic half-life of hours rather than days.^{13,14} The effect of ranibizumab on systemic VEGF levels is therefore expected to be limited.¹⁵ In addition, this study investigates 2 different ranibizumab doses that are both lower than 50% of the standard adult dose.

Methods

Study Design

The Comparing Alternative Ranibizumab Dosages for Safety and Efficacy in Retinopathy of Prematurity (CARE-ROP) study is a randomized, multicenter, prospective, double-blind, 2-arm, parallel-group, phase 2, investigator-initiated trial. The study was approved by the German Health Authorities (Paul Ehrlich Institute) and the ethics committees in line with the guidelines provided by the International Conference on Harmonisation Harmonized Tripartite Guideline for Good Clinical Practice and the Declaration of Helsinki. Written informed consent was obtained from all legal representatives. The trial protocol is included in [Supplement 1](#).

Study End Points

The primary end point was the number of infants without need for rescue therapy until 24 weeks after initial treatment. Rescue therapy was defined as the need for either laser photocoagulation or 0.2-mg ranibizumab reinjection within 4 weeks

Key Points

Question Can anti-vascular endothelial growth factor (VEGF) therapy in retinopathy of prematurity (ROP) be improved by using ranibizumab given at lower doses than the standard treatment, bevacizumab, thus alleviating systemic safety concerns while effectively treating disease?

Findings In this randomized clinical trial with 20 patients, both investigated ranibizumab doses were lower than the current standard bevacizumab dose for ROP. Both ranibizumab doses were effective in controlling acute ROP (17 of 18 per protocol treated eyes [94%] with 0.12 mg and 13 of 14 per protocol treated eyes [93%] with 0.20 mg); systemic VEGF levels remained unchanged.

Meaning Low-dose ranibizumab is effective in treating acute ROP without suppressing plasma VEGF levels.

after study treatment or the need for laser treatment at any other time point. Note that for the full analysis set, infants who died were considered as having received rescue therapy.

Reinjections of the (blinded) study medication dose could be given as part of the protocol without being considered rescue therapy if ROP activity recurred after an initial response lasting at least 28 days. The important difference between rescue and reinjection is that the need for rescue therapy indicates a VEGF nonresponse, while the need for reinjection after an initial response indicates disease recurrence when intraocular anti-VEGF drug levels decline over time.

Secondary end points were regression of plus disease and vascularized ridge, progression of peripheral intraretinal vascularization, number and kind of adverse events, changes in systemic VEGF levels, number of reinjections, number of patients progressing to stage 4 or 5, and number of patients with complete retinal vascularization.

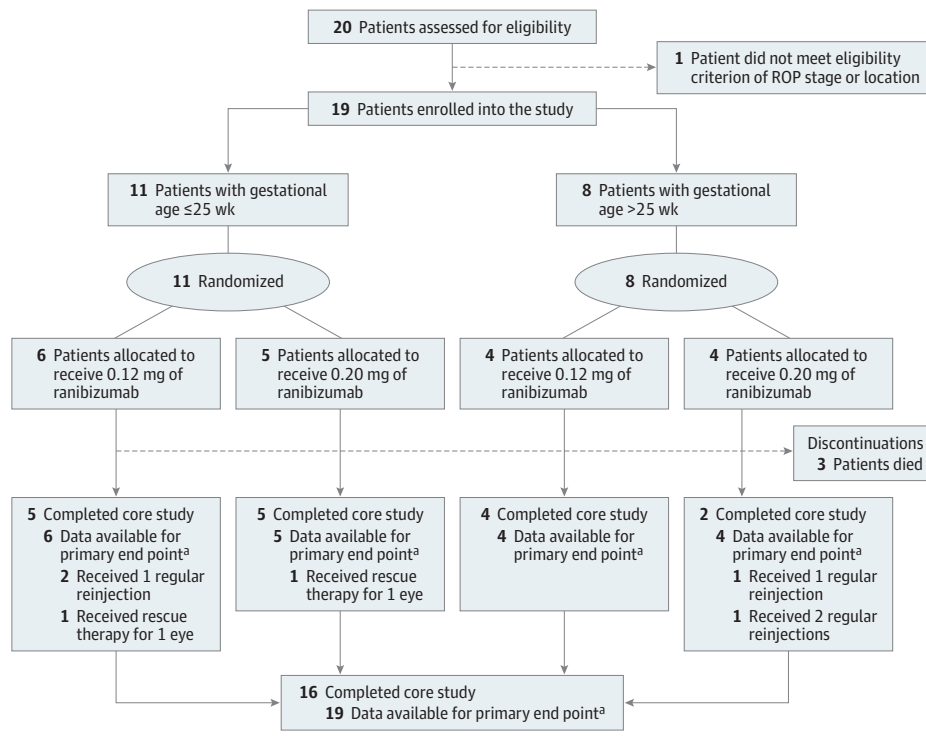
Patients

Nine academic centers in Germany were activated for CARE-ROP and 6 recruited patients. Patients with bilateral ROP in zone I (stages 1 with plus disease, 2 with plus disease, 3 with or without plus disease and aggressive posterior ROP) or posterior zone II (stage 3 with plus disease or aggressive posterior ROP) were eligible. Stages of ROP were graded according to the International Classification of Retinopathy of Prematurity.¹⁶ Inclusion and exclusion criteria are provided as eTable 1 in [Supplement 2](#). The CARE-ROP trial was a pilot study with no formal trial size calculation. Between September 5, 2014, and July 14, 2016, 20 patients were screened and 19 were enrolled ([Figure 1](#)).

RetCam Documentation and Data Safety Monitoring

RetCam (Clarity Medical Systems) photographs were acquired at baseline and throughout the study. All baseline images were evaluated by the data and safety monitoring board (DSMB) to confirm correct enrollment. If the DSMB had not approved enrollment, an infant would have been excluded from the analysis and the event would have been regarded as screening failure. In addition to confirming correct enrollment, the DSMB regularly reviewed all serious adverse events.

Figure 1. Flow of Patients in Comparing Alternative Ranibizumab Dosages for Safety and Efficacy in Retinopathy of Prematurity Study



Over a 2-year recruitment window, 20 patients were screened and 19 randomized for CARE-ROP (representing the full analysis set). All patients received the intervention as allocated. Three patients died during the course of the study. No major protocol violations were noted in the remaining infants. The per protocol set at 24 weeks thus comprises 16 infants. All infants were treated bilaterally with study medication.

^a All enrolled patients were considered for analysis of primary end point.

Randomization and Treatment

Gestational age at birth is 1 of the major risk factors for ROP.¹⁷ Infants were therefore stratified by gestational age into 2 groups (≤ 25 and > 25 weeks). Two randomization lists were created off-site at a contract research organization, and patients were randomized 1:1 using an online tool. In the low-dose group, 20 μ L of 6 mg/mL ranibizumab was administered per eye (0.12 mg [equivalent to 24% of the standard adult dose]). In the high-dose group, 20 μ L of 10 mg/mL ranibizumab was administered per eye (0.20 mg [equivalent to 40% of the standard adult dose]). Investigators and legal representatives were masked to the injected dose.

VEGF Measurements

Venous blood samples were collected following a defined protocol (eFigure 1 in Supplement 2). A buffer of citrate, theophylline, adenosine, and dipyridamole (CTAD) was used to avoid thrombolysis.^{18,19} Enzyme-linked immunosorbent assay (ELISA) measurements were performed using the Human VEGF Quantikine ELISA Kit (R&D Systems) at the Natural and Medical Sciences Institute, Reutlingen, Germany. Values below the lowest value of the ELISA standard curve (15.6 pg/mL) were replaced by half this value.

Statistical Analysis

Analysis for differences in the primary end point was performed using the Cochran-Mantel-Haenszel test, and odds ratios were estimated, adjusting for gestational age. Analyses per eye were performed assuming a stratified cluster sampling de-

sign with patients equivalent to clusters, eyes equivalent to units within clusters, and gestational age equivalent to strata, using Taylor series linearization for variance estimation. Differences per eye were analyzed using the Rao-Scott χ^2 test, and odds ratios were estimated. For percentages, (modified) Clopper-Pearson confidence intervals were calculated.

Results

Patient Disposition and Baseline Characteristics

During the 2-year study period (September 2014 to August 2016), 20 patients were screened. One infant had disease that did not meet ROP severity criteria bilaterally and was excluded, resulting in 19 infants (9 [47.4%] female; median [range] postmenstrual age at first treatment, 36.4 [34.7-39.7] weeks) randomized for the trial (10 to the 0.12-mg ranibizumab group and 9 to the 0.20-mg group). One infant in the 0.12-mg group and 2 infants in the 0.20-mg group died before the primary end point. Death occurred 101 days or more after ranibizumab treatment in all 3 infants (Figure 1 and eTable 2 in Supplement 2). A causal relationship to the study treatment was not suspected in any of the 3 deaths. Medical history and adverse events of all infants who died are provided in eTable 3 in Supplement 2. No major protocol deviations occurred during the study. All minor protocol deviations are listed in eTable 4 in Supplement 2.

Gestational age at birth and postmenstrual age at first treatment were comparable between groups (Table 1). Stage 3 ROP

with plus disease in posterior zone II was the most prevalent disease stage at baseline in both groups. Occurrence of ROP in zone I was more prevalent in the 0.12-mg ranibizumab group than in the 0.20-mg ranibizumab group (5 of 20 eyes [25.0%]

vs 3 of 18 eyes [16.7%], respectively). Apgar scores at 1, 5, and 10 minutes were comparable (eFigure 2 in Supplement 2).

Table 1. Baseline Characteristics for All Infants Randomized in Comparing Alternative Ranibizumab Dosages for Safety and Efficacy in Retinopathy of Prematurity^a

Baseline Characteristic	Receiving 0.12 mg of Ranibizumab	Receiving 0.20 mg of Ranibizumab
Infants, No.	10	9
GA at birth, median (range), wk	24.6 (22.9-29.6)	24.7 (23.1-27.3)
PMA at first treatment, median (range), wk	36.8 (34.7-39.7)	36.0 (35.1-39.6)
Infants with GA ≤25 wk, No. (%)	6 (60.0)	5 (55.6)
Female, No. (%)	4 (40.0)	5 (55.6)
Birth weight, median (range), g	555 (395-935)	595 (360-1075)
Birth length, median (range), cm	30.5 (27-35)	30.8 (26.5-35)
Birth head circumference, median (range), cm	21.5 (18.0-24.6)	22.0 (19.3-24.5)
Eyes with ROP, No.	20	18
Zone I, stage 3 with plus disease, No. (%)	5 (25.0)	3 (16.7)
Posterior zone II, stage 3 with plus disease, No. (%)	15 (75.0)	15 (83.3)
Ridge with active proliferations, median (range), clock hours	10 (5-12)	8 (3-12)
Retinal hemorrhages, median (range), clock hours	4 (2-8)	6 (2-12)

Abbreviations: GA, gestational age; PMA, postmenstrual age; ROP, retinopathy of prematurity.

^a Baseline values were comparable between the 2 groups. The most prevalent ROP stage was stage 3 with plus disease in posterior zone II. The proportion of eyes with zone I disease was higher in the 0.12-mg group.

Primary End Point and Treatment Response

The predefined primary end point was the number of infants without need for rescue therapy until 24 weeks after initial treatment. In the per protocol set, this end point was met by 8 infants (88.9%) in the 0.12-mg group and 6 infants (85.7%) in the 0.20-mg group (Table 2). When analyzed per eye, 17 of 18 eyes in the 0.12-mg per protocol group (94.4%) and 13 of 14 eyes in the 0.20-mg per protocol group (92.9%) reached 24 weeks posttreatment without need for rescue therapy (Table 2). There was no statistically significant difference between the 2 groups (Cochran-Mantel-Haenszel analysis; odds ratio, 1.88; 95% CI, 0.26-13.49; $P = .53$). The same is true in the full analysis set in which infants who died were considered as having received bilateral rescue therapy (eTable 5 in Supplement 2). Hazard ratios comparing the 2 groups were 0.89 (95% CI, 0.06-14.36) for the per protocol set and 0.75 (95% CI, 0.05-11.97) for the full analysis set (eTable 6 in Supplement 2). When both groups are combined, ranibizumab was effective in 14 of 16 surviving infants (87.5%) or 30 of 32 eyes (93.8%). No infant with gestational age greater than 25 weeks required rescue therapy. Outcomes for all eyes relative to their baseline ROP severity are shown in eTable 7 in Supplement 2. Twelve of 20 eyes in the 0.12-mg group (60.0%) and 10 of 18 eyes in the 0.20-mg group (55.6%) had no ROP at the final visit. All remaining eyes had ROP stage 1 in anterior zone II or zone III at the final visit.

Figure 2 shows example images and Kaplan-Meier plots of treatment response. Resolution of plus disease and the disappearance of active proliferations were observed early after treatment (Figure 2B and C and eFigure 3 in Supplement 2). Resolution of the ridge and complete resolution of any ROP were slower to appear, and not all eyes achieved full

Table 2. Per Protocol Set Primary Outcome Analysis per Patient and per Eye^a

Incidence Without Need for Rescue Therapy	No. (%; 95% CI)			Odds Ratio (95% CI)
	Received 0.12 mg of Ranibizumab	Received 0.20 mg of Ranibizumab	Total	
Patients	(n = 9)	(n = 7)	(n = 16)	NA
All patients	8 (88.9; 51.8-99.7)	6 (85.7; 42.1-99.6)	14 (87.5; 61.7-98.4)	1.00 ^b (0.05-22.18)
GA ≤25 wk	4 (80.0; 28.4-99.5)	4 (80.0; 28.4-99.5)	8 (80.0; 44.4-97.5)	1.00 (0.05-22.18)
GA >25 wk	4 (100.0; 39.8-100.0)	2 (100.0; 15.8-100.0)	6 (100.0; 54.1-100.0)	NA
Eyes	(n = 18)	(n = 14)	(n = 32)	NA
All patients	17 (94.4; 72.7-99.9)	13 (92.9; 66.1-99.8)	30 (93.8; 79.0-99.3)	1.31 ^b (0.05-31.77)
GA ≤25 wk	9 (90.0; 55.5-99.7)	9 (90.0; 55.5-99.7)	18 (90.0; 68.3-98.8)	1.00 (0.05-22.06)
GA >25 wk	8 (100.0) ^c	4 (100.0) ^c	12 (100.0) ^c	NA

Abbreviations: GA, gestational age; NA, not applicable.

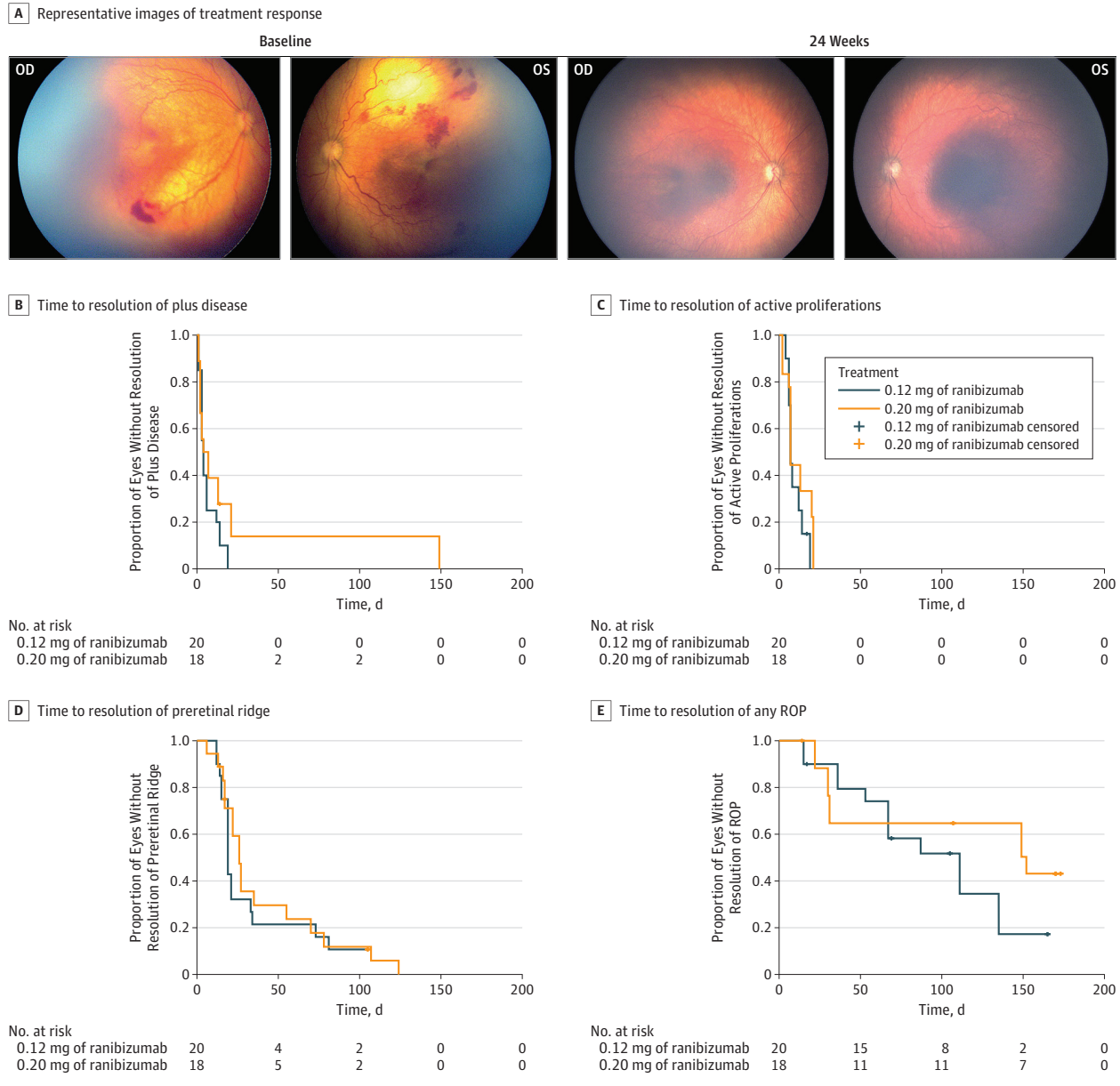
^a Number of patients and eyes without rescue therapy up to week 24. The data show effective treatment in most patients in both groups with no statistical difference between the 2 doses. On a patient level, 88.9% of per protocol treated infants in the 0.12-mg and 85.7% in the 0.20-mg group did not require rescue therapy in any eye. When analyzed per eye, 94.4% of per protocol treated eyes in the 0.12-mg group and 92.9% in the 0.20-mg group did not require rescue therapy. In the stratum of infants born after gestational week 25, no eye required rescue therapy in either group. Results for the full analysis set confirm this data and can be found in Supplement 2. For difference between

treatment groups per patient (Cochran-Mantel-Haenszel test) $P > .99$. For difference between treatment groups per eye (Rao-Scott χ^2 test) $P > .86$. For eyes, confidence limits and P values are calculated assuming a stratified cluster sampling design with patients equivalent to clusters, eyes equivalent to units within clusters, and GA equivalent to strata, using the Taylor series linearization method for variance estimation. For percentages, modified Clopper Pearson confidence limits are calculated.

^b Odds ratio estimate adjusted for GA (Mantel-Haenszel estimate).

^c 95% CIs cannot be calculated because 100% of eyes met the primary end point.

Figure 2. Treatment Response to Ranibizumab Therapy



A, Representative images of treatment response to ranibizumab. Two eyes from 1 infant are shown before baseline ranibizumab injection (left) and at 24 weeks (right). Staging of retinopathy of prematurity (ROP) at baseline was stage 3, posterior zone II, moderate plus disease in the right eye and stage 3, zone I, moderate plus disease in the left eye. Note the significant retinal hemorrhages in both eyes. At week 24, all plus disease, hemorrhages, and preretinal ridge had fully resolved. OD indicates right eye, OS, left eye.

B-E, Time-to-event analyses for first resolution of plus disease (B), active

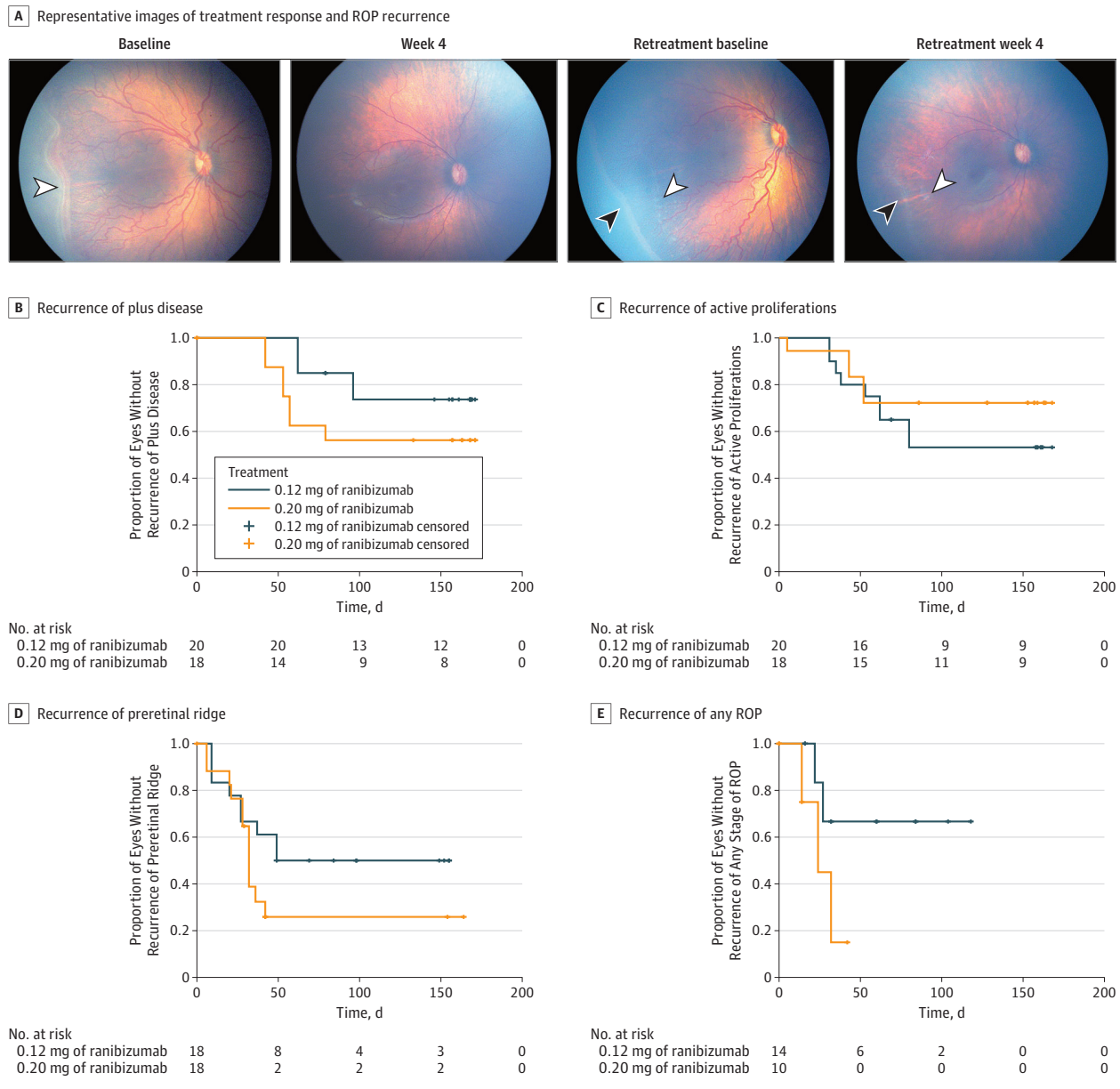
proliferation (C), preretinal ridge (D), and any ROP (E). These plots represent the time to first occurrence of the respective event and illustrate the dynamics of the initial treatment response. Later recurrences of the respective item are not captured here. Censored values represent eyes in which the respective event did not occur before the last study visit or before the first rescue or reinjection. Plus disease and active proliferations showed rapid response to treatment in almost all patients. Resolution of the preretinal ridge and all ROP stages were slower to occur.

resolution of ROP (Figure 2D and E). Importantly, all graphs in Figure 2B, C, D, and E display a time-to-event analysis for the respective parameters' first disappearance. Recurrences are not captured in this figure but are discussed separately. With regard to treatment response, both ranibizumab doses show comparable time courses. A detailed outcome list for all patients is provided in eTable 8 in Supplement 2.

Rescue Therapy

Two eyes required rescue therapy. Details are listed in eTable 9 in Supplement 2. Both rescue therapies occurred at comparable time points after baseline (14 and 17 days). Both treated eyes responded well to rescue therapy and had fully resolved ROP at the final visit. The contralateral eye of each of the 2 patients had responded to study treatment and did not require rescue therapy.

Figure 3. Recurrences of Retinopathy of Prematurity (ROP)



A, Representative images of initial response to treatment (left image) and response to retreatment after recurrence of acute ROP at 10 weeks (right image). The initial preretinal ridge is marked with a white arrow. The secondary ridge forming at the time of recurrence is marked with a black arrow. The initial ridge is fully resolved at week 4 (second image from left) but reappears together with the new ridge at the time of recurrence (second image from right). Four weeks after retreatment, both the initial and the secondary ridge have flattened but are still visible as flat demarcation lines in the vascularized retina (right image). Physiologic intraretinal blood vessels can be seen proceeding beyond the secondary ridge.

B-E, Time-to-event analyses for first recurrence of plus disease (B), active proliferation (C), preretinal ridge (D), or any ROP (E). These plots represent the time to first recurrence of the respective event. Note that recurrences can only occur in patients who had prior resolution of the respective item. Number of patients at risk and proportional values therefore vary between graphs. Time to first recurrence is calculated from time of full resolution for each item. Censored values represent eyes in which the respective event did not occur before the last study visit.

Disease Recurrence and Retreatments

Recurrences of ROP activity are common after anti-VEGF treatment.¹⁸⁻²⁰ Figure 3 displays a time-to-event analysis for recurrence of several ROP components and typical images of an eye receiving retreatment (Figure 3A). It is interesting to note that reappearance of a preretinal ridge is quite common

(Figure 3D). Recurrence of more severe signs of ROP (plus disease and active proliferations) were observed less frequently (Figure 3B and C). Recurrence of any ROP stage was more prevalent in the 0.20-mg group (Figure 3E). Two infants in each group (8 eyes [21.1%]) had recurrences that were severe enough to warrant retreatment. In all other

eyes, ROP recurrence was transient and did not require retreatment.

Retreatments after an initial response are very different entities from rescue treatments. The CARE-ROP protocol allowed retreatment if an initial treatment response of at least 4 weeks had been observed. Details of all retreatments are listed in eTable 10 in [Supplement 2](#). Mean (SD) time between initial treatment and retreatment was 87 (18) days in the 0.12-mg group and 53 (3) days in the 0.20-mg group. One infant in the 0.20-mg group required a second retreatment 71 days after the first. Final outcome at the end of the study for eyes with retreatment was either no ROP or stage 1 ROP in anterior zone II or zone III. No rescue therapy was necessary following retreatment.

Progression of Physiologic Vascularization

Only vascularized parts of the retina have the potential to contribute to visual function. In addition, progression of the physiologic retinal vasculature reduces retinal ischemia and minimizes the risk of ROP recurrence.²¹ The data in eFigure 4 in [Supplement 2](#) show that physiologic vascularization appears to proceed faster and complete vascularization is reached more frequently in eyes receiving lower anti-VEGF doses. Eleven eyes (55.0%) had full vascularization in the 0.12-mg group, and only 3 eyes (16.7%) had full vascularization in the 0.20-mg group.

VEGF Measurements and Safety Parameters

Mean VEGF levels were not reduced after ranibizumab treatment in either group (eFigure 5 in [Supplement 2](#)). A detailed list of all VEGF levels is provided in eTable 11 in [Supplement 2](#). Vital signs and growth values were documented as additional safety parameters and were comparable between groups (eFigure 6, eFigure 7, and eFigure 8 in [Supplement 2](#)). Number and type of serious adverse events were also not different between groups (eTable 12 in [Supplement 2](#)).

Supplementary Oxygen

eTable 13 in [Supplement 2](#) shows oxygen supplementation before and after study enrollment. The need for supplementary oxygen was comparable for both groups before treatment, but infants in the 0.20-mg ranibizumab group required longer oxygen supplementation after treatment than infants in the 0.12-mg group, with a median of 83 vs 19 days of oxygen by nasal cannula. Target oxygen ranges were comparable (eTable 13 in [Supplement 2](#)).

Discussion

To our knowledge, the CARE-ROP study is the first randomized clinical trial comparing 2 different doses of ranibizumab for ROP. Control of ROP without need for rescue therapy was achieved in 14 of 16 surviving infants (87.5%) (30 of 32 eyes [93.8%]), and there was no significant difference between the 2 dose groups regarding primary end point outcomes. The study compared 40% vs 24% of the standard adult ranibizumab dose. Both doses are lower than the 50% adult bevacizumab dose that is most frequently used in off-label ROP therapy.^{17,22} Our results demonstrate that ranibizumab is

effective in controlling acute ROP and that 24% of the standard adult ranibizumab dose appears to be as effective as 40%.

In each study group, 1 eye showed insufficient response to ranibizumab treatment and required rescue therapy. Both eyes receiving rescue therapy showed fully resolved ROP at week 24. Unlike their respective contralateral eyes, the 2 eyes that received rescue therapy did not achieve full retinal vascularization to the ora serrata. This is because laser treatment was applied as rescue therapy. Laser therapy renders further vascular development as well as visual function impossible in the treated areas. This is particularly relevant when central parts of the retina are affected (ie, ROP in zone I or posterior zone II). After anti-VEGF therapy, in contrast, physiologic vascular growth can resume its course toward the ora serrata.^{23,24}

Achieving full retinal vascularization is highly desirable after anti-VEGF treatment for ROP because it maximizes the retinal area that is able to contribute to visual function. In addition, a fully vascularized retina is less likely to generate recurrences of acute ROP triggered by VEGF overexpression from avascular areas. In the 0.12-mg ranibizumab group, full vascularization was achieved in 11 eyes (55.0%) vs only 3 eyes (16.7%) in the 0.20-mg group. This is in line with data from pre-clinical animal models showing that higher anti-VEGF doses can impede physiologic vascularization.²³ Assessment of peripheral vascularization was performed by ophthalmoscopy in our study. Fluorescein angiograms were not performed.

Four infants (8 eyes [21.1%]) in our study showed recurrence of ROP requiring retreatment. This proportion is higher than in the BEAT-ROP population but comparable to several other studies.^{4,17,25,26} Recurrence of ROP was equally distributed across the 2 dose groups. It is important to note that retreatments are very different in nature from rescue therapy. Retreatments imply an initial response to the first dose of ranibizumab that is then followed by recurrence of acute ROP at a later time. Retreatments were an integral part of the CARE-ROP protocol. The idea behind allowing anti-VEGF retreatments is that they allow an anti-VEGF dose titration: most eyes will be sufficiently treated with 1 (low) anti-VEGF dose, while others will require 1 or more reinjections (spread over several months). From a pharmacodynamic point of view, this approach may be preferable over a single high-dose anti-VEGF bolus being applied uniformly to all eyes. Final outcomes for eyes receiving retreatment in our study were either no ROP or stage 1 ROP at 24 weeks after baseline. Because ROP recurrences are possible beyond week 24,^{18-20,27} all eyes without full intraretinal vascularization are being followed up beyond the primary end point. It is important to note that re-appearance of a demarcation line or preretinal ridge without active proliferations or plus disease is quite frequent after anti-VEGF therapy. These stages are often self-limiting and generally do not require retreatment unless active proliferations, visible traction, or plus disease occur.

Beyond being effective, any pharmacologic ROP treatment must demonstrate ocular and systemic safety. No anti-VEGF substance is currently approved for ROP treatment, and reliable clinical safety data are rare. Nevertheless, anti-VEGF therapy is being used with increasing frequency.¹⁷ To our knowledge, this study is the first to systematically report safety

parameters from a double-blind prospective clinical anti-VEGF trial in ROP. Overall, 3 patients died in CARE-ROP. None of the 3 deaths was suspected to be related to the study treatment. All deaths occurred at least 101 days (14 weeks) after ranibizumab treatment, and none of these infants had received more than the baseline ranibizumab injections. Two of the 3 infants who died had no sign of active ROP at their last visit, and 1 infant had even achieved complete vascularization to the ora serrata bilaterally. There were no deaths among infants who required multiple injections. Serious and nonserious adverse events were distributed evenly between the 2 study groups. Long-term functional outcome parameters from the CARE-ROP population will become available after the scheduled follow-up examinations at 1, 2, and 5 years after treatment. These will entail both ophthalmologic as well as standardized pediatric examinations.

An important aspect regarding systemic safety stems from the fact that a single intravitreal anti-VEGF injection can suppress systemic VEGF levels for weeks.^{5,28} The implications of such long-term systemic VEGF suppression on organ development are unknown. We hypothesized that ranibizumab may be advantageous in this regard because ranibizumab has a systemic half-life of hours vs days for bevacizumab.¹⁵ To measure free plasma VEGF we used a CTAD buffer, which was

shown to be superior over ethylenediaminetetraacetic acid (EDTA) in preventing thrombolysis.^{29,30} Our VEGF values are therefore generally lower compared with other studies and reflect only free plasma VEGF, unaffected by VEGF from thrombolysis. In both our groups, there was no sustained suppression of systemic VEGF levels. These results demonstrate that systemic VEGF suppression is less prevalent after ranibizumab than it is after bevacizumab.

Limitations

Limitations of our study include the small number of patients, a relatively early primary end point (with scheduled 5-year follow-up), and the fact that doses lower than 0.12 mg were not evaluated.

Conclusions

Combined, the presented data provide evidence that ranibizumab is effective in treating ROP in 2 different doses that are both lower than the current anti-VEGF standard dose. Physiologic intraretinal vascularization to the ora serrata was favorable in the 0.12-mg vs the 0.20-mg group, and systemic VEGF was not suppressed in either group.

ARTICLE INFORMATION

Accepted for Publication: September 26, 2017.

Published Online: January 8, 2018.
doi:10.1001/jamapediatrics.2017.4838

Open Access: This article is published under the JN-OA license and is free to read on the day of publication.

Author Affiliations: Eye Center, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany (Stahl, Walz); Department of Ophthalmology, University of Bonn, Bonn, Germany (Krohne); Department of Ophthalmology, University of Muenster Medical Center, Muenster, Germany (Eter); Department of Ophthalmology, University of Regensburg, Regensburg, Germany (Oberacher-Velten); Department of Ophthalmology, Faculty of Medicine, University of Dusseldorf, Dusseldorf, Germany (Guthoff); Department of Ophthalmology, Otto von Guericke University, Magdeburg, Germany (Meltendorf); Department of Ophthalmology, Ludwig-Maximilian University of Munich, Munich, Germany (Ehrt); University Eye Hospital, Eberhard Karls University of Tuebingen, Tuebingen, Germany (Aisenbrey); Department of Ophthalmology, University of Kiel, University Medical Center, Kiel, Germany (Roeder); Augenzentrum Pallas Kliniken, Olten, Switzerland (Gerding); Artemis Eye Clinic, Frankfurt, Germany (Jandeck); Department of Ophthalmology, Harvard Medical School, Boston Children's Hospital, Boston, Massachusetts (Smith); Department of Pharmacology and Toxicology, University of Regensburg, Regensburg, Germany (Walz).

Author Contributions: Dr Stahl had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Stahl, Eter, Oberacher-Velten, Roeder, Walz.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Stahl, Walz.

Critical revision of the manuscript for important intellectual content: Stahl, Krohne, Eter,

Oberacher-Velten, Guthoff, Meltendorf, Ehrt, Aisenbrey, Roeder, Gerding, Jandeck, Smith.

Obtained funding: Stahl.

Administrative, technical, or material support: Stahl, Krohne, Eter, Oberacher-Velten, Guthoff, Aisenbrey, Roeder, Gerding, Walz.

Study supervision: Stahl, Krohne, Oberacher-Velten, Guthoff, Roeder, Gerding, Jandeck, Smith, Walz.

Conflict of Interest Disclosures: None reported.

Funder/Sponsor: The University Hospital Freiburg is the study sponsor. Novartis Pharma GmbH, Germany, provided financial support and study medication.

Role of the Funder/Sponsor: University Hospital Freiburg took on all roles of study conduct and manuscript preparation as the sponsor of this investigator-initiated trial. Novartis Pharma GmbH was not involved in the design and conduct of the study or collection, management, analysis, and interpretation of the data; Novartis Pharma GmbH was able to review the manuscript prior to submission but was not involved in preparation or approval of the manuscript or the decision to submit the manuscript for publication.

Additional Contributions: Monika Schwager, PhD, and Benedikt Steger (Winicker Norimed GmbH, a contract research organization) provided statistical data analysis. University Hospital Freiburg compensated Winicker for their services. CROLLL GmbH provided clinical trial monitoring and trial coordination in the role of a contract research organization and was compensated by University Hospital Freiburg accordingly.

CARE-ROP Study Group Members:

The CARE-ROP Study Group members are as follows: University of Freiburg, Ophthalmology: Anima Bühler, MD, Moritz Daniel, Susanne Felzmann, Nicolai Gross, MD, Stefanie Horn, MD, Wolf Lagrèze, MD, Fanni Molnár, MD, Claudia Müller, Sabine Reichl, MD, Charlotte Reiff, MD, Olga Richter, MD, Andreas Stahl, MD, Milena Stech, MD; University of Freiburg, Neonatology: Roland Hentschel, MD, Dimitria Stavropoulou, MD, Juliane Tautz, MD; University of Bonn, Ophthalmology: Kerstin Bartsch, Jennifer Braunstein, MD, Ralf Brinken, Christian Karl Brinkmann, MD, Joanna Czauderna, Wiebke Dralle, MD, Martin Gliem, Arno Goebel, MD, Philipp Heymer, MD, Martina Hofmann, Frank G. Holz, MD, Tim Krohne, MD, David Kupitz, MD, Philipp Müller, MD, Michael Petrak, MD, Eva Janine Schmitz, MD, Steffen Schmitz-Valckenberg, MD, Moritz Schröder, MD, Julia Steinberg, MD, Julia Supé; University of Bonn, Neonatology: Evelyn Kant, MD, Diana Kunze, MD, Andreas Müller, MD; University of Münster, Ophthalmology: Adeline Adorf, Anne Alex, MD, Florian Alten, MD, Christoph R. Clemens, MD, Nicole Eter, MD, Silvia Falkenau, Caroline Friedhoff, Desiree Sandra Loos, MD, Natasa Mihailovic, MD, Julia Termühlen, Constantin Uhlig, MD; University of Münster, Neonatology: Isabell Hörnig-Franz, MD, Esther Rieger-Fackeldey, MD, Maria Tekaat, Claudius Werner; University of Regensburg, Ophthalmology: Mathias Altmann, MD, Theresa Barth, MD, Christiane Blecha, MD, Sabine Brandl-Rühle, Horst Helbig, MD, Karsten Hufendiek, MD, Herbert Jägle, MD, Julia Konrad, MD, Eva Kopetzky, MD, Fabian Lehmann, MD, Isabel Oberacher-Velten, MD; Barmherzige Brüder Hospital Regensburg, Neonatology: Annette Keller-Wackerbauer, MD, Jochen Kittel, MD, Hugo Segerer, MD; University of Dusseldorf, Ophthalmology: Phillip Ackermann, Jemina Benga, Rainer Guthoff, MD, Tanja Guthoff, MD, Elena Kleinert, Ertan Mayatepek, MD, Stefan Schrader,

MD, Magdalena Völker, MD; University of Düsseldorf, Neonatology: Thomas Höhn, MD, Klaus Lohmeier, MD, Hemmen Sabir, MD; University of Duisburg, Neonatology: Francisco Brevis, Tina Mönig, MD, Simone Schwarz, MD; University of Magdeburg, Ophthalmology: Angela Ehmer, Synke Meltendorf, MD, Claudia Schuart, MD; University of Magdeburg, Neonatology: Stefan Avenarius, MD, Ralf Böttger, MD; University of Magdeburg, Pharmacy: Christoph Apel, Anne Bergmann, Karsten Herrmann, Franziska Ockert-Schön, Sabine Wegener; Ludwigs-Maximilian University Munich, Ophthalmology: Oliver Ehrht, MD, Martin Nentwich, MD, Angelika Pressler, Günther Rudolph, MD; Ludwigs-Maximilian University Munich, Neonatology: Orsolya Genzel-Boroviczeny, MD, Susanne Schmidt, MD; Hauner'sches Kinderspital Munich, Neonatology: Hans-Georg Münch, MD, Claude Thilmany, MD; University of Tübingen, Ophthalmology: Sabine Aisenbrey, MD, Anna Bruckmann, MD, Spyridon Dimopoulos, MD, Ulrike Hagemann, Werner Inhoffen, PhD, Michael Partsch, MD, Merle Schrader, MD, Daniela Süsskind, MD, Michael Völker, MD; University of Tübingen, Neonatology: Anja Bialkowski, MD, Ingo Müller-Hansen, MD; University of Kiel, Ophthalmology: Andrea Gerberth, Heike Christine Hasselbach, MD, Solveig Lindemann, MD, Konstantine Purtskhvanidze, MD, Yvonne Raffel, Johann Roeder, MD, Greta Schröder, MD, Beke Szymank, Jan Tode, MD; University of Kiel, Neonatology: Meike Bendiks, MD, Simon Modlich, MD.

REFERENCES

- Chen J, Stahl A, Hellstrom A, Smith LE. Current update on retinopathy of prematurity: screening and treatment. *Curr Opin Pediatr*. 2011;23(2):173-178.
- Smith LE, Hard AL, Hellström A. The biology of retinopathy of prematurity: how knowledge of pathogenesis guides treatment. *Clin Perinatol*. 2013;40(2):201-214.
- Hellström A, Smith LE, Dammann O. Retinopathy of prematurity. *Lancet*. 2013;382(9902):1445-1457.
- Mintz-Hittner HA, Kennedy KA, Chuang AZ; BEAT-ROP Cooperative Group. Efficacy of intravitreal bevacizumab for stage 3+ retinopathy of prematurity. *N Engl J Med*. 2011;364(7):603-615.
- Sato T, Wada K, Arahori H, et al. Serum concentrations of bevacizumab (avastin) and vascular endothelial growth factor in infants with retinopathy of prematurity. *Am J Ophthalmol*. 2012;153(2):327-333, e1.
- Kong L, Bhatt AR, Demny AB, et al. Pharmacokinetics of bevacizumab and its effects on serum VEGF and IGF-1 in infants with retinopathy of prematurity. *Invest Ophthalmol Vis Sci*. 2015;56(2):956-961.
- Avery RL, Castellarin AA, Steinle NC, et al. Systemic pharmacokinetics and pharmacodynamics of intravitreal aflibercept, bevacizumab, and ranibizumab. *Retina*. 2017;37(10):1847-1858.
- Wu W-C, Shih C-P, Lien R, et al. Serum vascular endothelial growth factor after bevacizumab or ranibizumab treatment for retinopathy of prematurity. *Retina*. 2017;37(4):694-701.
- Hong YR, Kim YH, Kim SY, Nam GY, Cheon HJ, Lee SJ. Plasma concentrations of vascular endothelial growth factor in retinopathy of prematurity after intravitreal bevacizumab injection. *Retina*. 2015;35(9):1772-1777.
- Avery RL. Bevacizumab (Avastin) for retinopathy of prematurity: wrong dose, wrong drug, or both? *J AAPOS*. 2012;16(1):2-4.
- Darlow BA, Ells AL, Gilbert CE, Gole GA, Quinn GE. Are we there yet? Bevacizumab therapy for retinopathy of prematurity. *Arch Child Fetal Neonatal Ed*. 2013;98(2):F170-F174.
- Wallace DK, Kraker RT, Freedman SF, et al; Pediatric Eye Disease Investigator Group (PEDIG). Assessment of lower doses of intravitreal bevacizumab for retinopathy of prematurity: a phase 1 dosing study. *JAMA Ophthalmol*. 2017;135(6):654-656.
- Lu JF, Bruno R, Eppler S, Novotny W, Lum B, Gaudreault J. Clinical pharmacokinetics of bevacizumab in patients with solid tumors. *Cancer Chemother Pharmacol*. 2008;62(5):779-786.
- Xu L, Lu T, Tuomi L, et al. Pharmacokinetics of ranibizumab in patients with neovascular age-related macular degeneration: a population approach. *Invest Ophthalmol Vis Sci*. 2013;54(3):1616-1624.
- Krohne TU, Holz FG, Meyer CH. Pharmacokinetics of intravitreally administered VEGF inhibitors [in German]. *Ophthalmologe*. 2014;111(2):113-120.
- International Committee for the Classification of Retinopathy of Prematurity. The International Classification of Retinopathy of Prematurity revisited. *Arch Ophthalmol*. 2005;123(7):991-999.
- Walz JM, Bemme S, Pielan A, et al. The German ROP Registry: data from 90 infants treated for retinopathy of prematurity. *Acta Ophthalmol*. 2016;94(8):e744-e752.
- Hu J, Blair MP, Shapiro MJ, Lichtenstein SJ, Galasso JM, Kapur R. Reactivation of retinopathy of prematurity after bevacizumab injection. *Arch Ophthalmol*. 2012;130(8):1000-1006.
- Wong RK, Hubschman S, Tsui I. Reactivation of retinopathy of prematurity after ranibizumab treatment. *Retina*. 2015;35(4):675-680.
- Snyder LL, Garcia-Gonzalez JM, Shapiro MJ, Blair MP. Very late reactivation of retinopathy of prematurity after monotherapy with intravitreal bevacizumab. *Ophthalmic Surg Lasers Imaging Retina*. 2016;47(3):280-283.
- Sapieha P, Joyal J-S, Rivera JC, et al. Retinopathy of prematurity: understanding ischemic retinal vasculopathies at an extreme of life. *J Clin Invest*. 2010;120(9):3022-3032.
- Pertl L, Steinwender G, Mayer C, et al. A systematic review and meta-analysis on the safety of vascular endothelial growth factor (VEGF) inhibitors for the treatment of retinopathy of prematurity. *PLoS One*. 2015;10(6):e0129383.
- Lutty GA, McLeod DS, Bhutto I, Wiegand SJ. Effect of VEGF trap on normal retinal vascular development and oxygen-induced retinopathy in the dog. *Invest Ophthalmol Vis Sci*. 2011;52(7):4039-4047.
- Hartnett ME. Vascular endothelial growth factor antagonist therapy for retinopathy of prematurity. *Clin Perinatol*. 2014;41(4):925-943.
- Hwang CK, Hubbard GB, Hutchinson AK, Lambert SR. Outcomes after intravitreal bevacizumab versus laser photocoagulation for retinopathy of prematurity: a 5-year retrospective analysis. *Ophthalmology*. 2015;122(5):1008-1015.
- Kaakour AH, Hansen ED, Aziz HA, Young RC, Berrocal AM. Changing treatment patterns of ROP at a tertiary medical center between 2002 and 2012. *Ophthalmic Surg Lasers Imaging Retina*. 2015;46(7):752-754.
- Chan JJ, Lam CP, Kwok MK, et al. Risk of recurrence of retinopathy of prematurity after initial intravitreal ranibizumab therapy. *Sci Rep*. 2016;6:27082.
- Hoerster R, Muether P, Dahlke C, et al. Serum concentrations of vascular endothelial growth factor in an infant treated with ranibizumab for retinopathy of prematurity. *Acta Ophthalmol*. 2013;91(1):e74-e75.
- Walz JM, Boehringer D, Deissler HL, et al. Pre-analytical parameters affecting vascular endothelial growth factor measurement in plasma: identifying confounders. *PLoS One*. 2016;11(1):e0145375.
- Avery RL, Castellarin AA, Steinle NC, et al. Systemic pharmacokinetics following intravitreal injections of ranibizumab, bevacizumab or aflibercept in patients with neovascular AMD. *Br J Ophthalmol*. 2014;98(12):1636-1641.