Ranibizumab versus laser therapy for the treatment of very low birthweight infants with retinopathy of prematurity (RAINBOW): an open-label randomised controlled trial

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Summary

Background Despite increasing worldwide use of anti-vascular endothelial growth factor agents for treatment of retinopathy of prematurity (ROP), there are few data on their ocular efficacy, the appropriate drug and dose, the need for retreatment, and the possibility of long-term systemic effects. We evaluated the efficacy and safety of intravitreal ranibizumab compared with laser therapy in treatment of ROP.

Methods This randomised, open-label, superiority multicentre, three-arm, parallel group trial was done in 87 neonatal and ophthalmic centres in 26 countries. We screened infants with birthweight less than 1500 g who met criteria for treatment for retinopathy, and randomised patients equally (1:1:1) to receive a single bilateral intravitreal dose of ranibizumab 0·2 mg or ranibizumab 0·1 mg, or laser therapy. Individuals were stratified by disease zone and geographical region using computer interactive response technology. The primary outcome was survival with no active retinopathy, no unfavourable structural outcomes, or need for a different treatment modality at or before 24 weeks (two-sided α=0·05 for superiority of ranibizumab 0·2 mg against laser therapy). Analysis was by intention to treat. This trial is registered with ClinicalTrials.gov, NCT02375971.

Interpretation Between Dec 31, 2015, and June 29, 2017, 225 participants (ranibizumab 0·2 mg n=74, ranibizumab 0·1 mg n=77, laser therapy n=74) were randomly assigned. Seven were withdrawn before treatment (n=1, n=1, n=5, respectively) and 17 did not complete follow-up to 24 weeks, including four deaths in each group. 214 infants were assessed for the primary outcome (n=70, n=76, n=68, respectively). Treatment success occurred in 56 (80%) of 70 infants receiving ranibizumab 0·2 mg compared with 57 (75%) of 76 infants receiving ranibizumab 0·1 mg and 45 (66%) of 68 infants after laser therapy. Using a hierarchical testing strategy, compared with laser therapy the odds ratio (OR) of treatment success following ranibizumab 0·2 mg was 2·19 (95% CI 0·99–4·82, p=0·051), and following ranibizumab 0·1 mg was 1·57 (95% CI 0·76–3·26); for ranibizumab 0·2 mg compared with 0·1 mg the OR was 1·35 (95% CI 0·61–2·98). One infant had an unfavourable structural outcome following ranibizumab 0·2 mg, compared with five following ranibizumab 0·1 mg and seven after laser therapy. Death, serious and non-serious systemic adverse events, and ocular adverse events were evenly distributed between the three groups.

Findings In the treatment of ROP, ranibizumab 0·2 mg might be superior to laser therapy, with fewer unfavourable ocular outcomes than laser therapy and with an acceptable 24-week safety profile.

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Introduction

Retinopathy of prematurity (ROP) is a disease of the developing retina. Mild forms of ROP resolve spontaneously with few sequelae, but severe ROP can progress to retinal detachment, vision impairment, and blindness. Ablation of the peripheral retina with cryotherapy[1] or confluent laser photocoagulation[2] reduces but does not eliminate ocular morbidity. Annually, 28 300–45 600 infants worldwide are diagnosed with irreversible visual impairment from ROP.[3]

Antivascular endothelial growth factor (anti-VEGF) agents injected into the vitreous are widely used for vasoproliferative disease and diseases associated with hyperpermeabilty in adult ophthalmic practice. They present a new opportunity for ROP treatment. To date, the largest clinical trial of anti-VEGF in preterm infants compared the efficacy of intravitreal bevacizumab with laser therapy in a defined US population.[4] Fewer retreatments occurred in the bevacizumab group, but the difference was significant only for ROP located in retina zone I (the most posterior region of the retina).[4] This study was preceded and followed by several case series,[5–7] small trials,[8–10] and two small dosing studies.[11,12] Despite the low level of evidence cited in a Cochrane review in 2018,[13] use of anti-VEGF agents for treatment of ROP in clinical practice is increasing worldwide.[14,15]

Several aspects of anti-VEGF therapy in ROP remain unanswered: ocular efficacy, the appropriate drug and dose, the need for retreatment, and the possibility of long-term systemic effects.[13–17] In light of these

Research in context

Evidence before this study

Retinopathy of prematurity (ROP) is the primary cause of blindness in 28 300–45 600 premature infants per year worldwide. Over the past 25 years, treatment with laser therapy has been able to halt the disease and prevent blindness. However, this does not eliminate ocular morbidity, which remains of concern. Uncontrolled retinal neovascularisation, which drives the clinical progression of the disease, is provoked by local secretion of vascular endothelial growth factor (VEGF) in ischaemic retinal tissue. Anti-VEGF agents could therefore be used to target uncontrolled retinal neovascularisation in ROP.

A Cochrane review published in 2018 identified five trials of bevacizumab and one trial of pegaptanib, grading the quality of the evidence as low or very low over eight outcomes followed for up to 55 weeks and mortality at 30 months of age, whether alone or in combination with laser and cryotherapy. A further search for English language publications between Jan 1, 2011, and March 31, 2018, was done using PubMed, MEDLINE, and Web of Science using the search terms "anti-VEGF", "individual agents", and "retinopathy of prematurity". Two small trials of ranibizumab were identified comprising 50 infants (in progress) and 19 infants (published).

Without research evidence of either long-term benefit or risk, and with few attempts at finding the appropriate dose, off-label anti-VEGF use has become widespread and is the first and sometimes only treatment option in many settings across the world. Several aspects of the use of anti-VEGF agents remain unanswered: ocular efficacy, the appropriate drug and dose, the need for retreatment, and the possibility of long-term systemic effects from sustained suppression of VEGF in a developing infant.

Added value of this study

RAINBOW shows that ranibizumab, an established anti-VEGF agent in adult practice, might be superior to laser therapy in infants with ROP. The use of ranibizumab seems to be associated with better eye outcomes and it has an acceptable short-term safety profile. Follow-up is underway to determine vision outcomes to 5 years of age. Systemic VEGF levels appear to be unchanged after ranibizumab. RAINBOW investigated two ranibizumab doses to collect data on anti-VEGF dose finding in ROP.

Implications of all the available evidence

Intravitreal injection of ranibizumab can be considered as a novel treatment for ROP. However, disease recurrence must be monitored closely because it is common with anti-VEGF agents and can occur later compared with laser therapy. No detectable effects on systemic VEGF levels seem advantageous for ranibizumab over other anti-VEGF agents. It remains too early to determine definitive vision outcomes or to confirm long-term systemic safety. Both will be addressed in the ongoing RAINBOW extension trial.

unanswered questions and the widespread off-label use of anti-VEGF treatment in ROP, assessment of anti-VEGF agents under defined trial conditions is urgently needed, not only in the USA and Europe but also in other countries where neonatal care can be more variable. The need is especially pressing in low-resource settings where an estimated 60% of the world's ROP occurs,[3] and anti-VEGF agents are being widely used as the primary treatment. This choice of treatment is not surprising for several reasons. Compared with laser therapy, intravitreal injection is relatively simple to administer, has substantially shorter administration time, requires fewer resources and less user expertise, and often no anaesthesia is given. However, intravitreal anti-VEGF treatment introduces a risk of intraocular infection,[18] the need for longer and more frequent follow-up,[19] retinal abnormalities after treatment,[20] and potential local and systemic developmental effects.[17]

See Online for appendix

The ranibizumab compared with laser therapy for the treatment of infants born prematurely with retinopathy of prematurity (RAINBOW) study was designed to evaluate the efficacy and safety of two doses of ranibizumab versus conventional laser therapy in a randomised open-label study that recruited infants worldwide, with a broad scope of ROP stages. Here, we report the outcomes of RAINBOW from enrolment until 24 weeks after treatment.

Methods

Study design and participants

RAINBOW is a randomised, open-label, superiority trial to assess the efficacy and safety of intravitreal ranibizumab compared with laser therapy for the treatment of ROP done in 87 neonatal and ophthalmic centres in 26 countries. Eligible infants had a birthweight less than 1500 g and a diagnosis of bilateral ROP zone I stage 1+, 2+, 3, or 3+, or zone II stage 3+, or aggressive posterior ROP (AP-ROP).[21] Zone II stage 2+ was not included because treatment is controversial in some countries.[22] Exclusion criteria included ocular and neurological comorbidities that might result in confounding visual impairment, and active ocular infection within 5 days before investigational treatment (appendix p 7). Unilateral cases in which only one eye met treatment criteria were not included.

The follow-up phase was until day 169 (24 weeks) when the infant exited the study and became eligible to join the RAINBOW extension study in which long-term ophthalmological and paediatric efficacy and safety will be studied to 5 years of age. The study was done in accordance with the Declaration of Helsinki. The study protocol was reviewed and approved by an independent ethics committee or institutional review board at each contributing centre. Parents or guardians provided written informed consent. An independent data and

safety monitoring board oversaw the safety of study participants.

Randomisation and masking

Eligible infants were randomly assigned 1:1:1 to receive a single bilateral intravitreal dose of ranibizumab 0·2 mg, a single bilateral intravitreal dose of ranibizumab 0·1 mg, or laser therapy at baseline (day 1, treatment phase). Random allocation was done by local investigators by using web-based interactive response technology, stratified by ROP zone of the worst eye, because this characteristic might affect treatment outcome, and with blocks pre-allocated to fixed sections, combined with dynamic block allocation for the stratification of geographical region (at the level of country or site) to reflect variations in practice. Region 1 comprised countries with a neonatal mortality of less than five per 1000 livebirths in 2012, and region 2 countries with neonatal mortality rates five per 1000 or greater.[23] Neither treatment nor outcomes were masked.

Procedures

The allocated intravitreal treatment was a single dose of 0·2 mg or 0·1 mg ranibizumab applied to both eyes. Signs of disease persistence or recurrence were used by investigators to determine whether additional treatment was needed (appendix p 8). Laser treatment was administered per local practice (standard treatment). Under the protocol, in the laser therapy group supplementary treatment to skip lesions was allowed up to day 11. In the ranibizumab groups, up to two additional treatments with ranibizumab were allowed in each eye at a minimum of 28-day intervals. A switch to a different treatment method was considered as treatment failure. The retina was assessed at baseline and at each study visit up to day 169 (24 weeks). Examinations were done using indirect ophthalmoscopy and, where available, by wide-field retinal imaging. 75 (86%) of 87 study centres did retinal imaging. These images were transmitted to a central reading centre for documentation.

Patient data were captured via electronic case report forms by the investigation sites. Data validation, handling, and management were done on validated systems and in compliance with Good Clinical Practice and other applicable regulatory and national requirements.

Outcomes

The primary objective was to investigate whether intravitreal ranibizumab 0·2 mg had superior efficacy to laser therapy in the treatment of ROP, as defined by survival without active ROP, unfavourable structural outcomes, or the need for a treatment modality other than that assigned (treatment switch), in both eyes, up to 24 weeks after starting investigational treatment. Active ROP was defined as vessel dilatation (plus disease) in at least two quadrants, or extra-retinal vessels extending from the retina into the vitreous, representing potentially sight-threatening disease. Unfavourable structural outcomes included structural abnormalities that have potential effects on visual acuity: retrolental membrane obscuring the view of the posterior pole, substantial temporal retinal vessel dragging causing abnormal structural features or macular ectopia, posterior retinal fold involving the macula, or retinal detachment involving the macula.

The principal secondary objective was to investigate the efficacy of ranibizumab 0·1 mg relative to 0·2 mg or to laser therapy. Other secondary objectives were to assess time to intervention with a second modality for ROP or development of unfavourable outcome or death, recurrence of ROP requiring any post-baseline intervention up to 24 weeks, ocular and systemic safety of intravitreal ranibizumab, pharmacokinetics of intravitreal ranibizumab, and systemic VEGF levels.

Pharmacology

We adopted a sparse sampling approach to ranibizumab pharmacokinetics and VEGF pharmacodynamics, sampling within 24 h of baseline treatment, and around 14 (7–21) and 28 (22–28) days after treatment. The concentration of ranibizumab in serum samples was determined using enzyme-linked immunosorbent assay. The concentration of VEGF was determined in K3-EDTA plasma samples by an electrochemiluminescence sandwich immunoassay on samples taken before treatment and around day 14 and 28.

Statistical analysis

We assumed a failure event rate for ranibizumab 0·2 mg of 6·5% and laser therapy 42·4% in zone I, and 5·1% and 12·5% in zone II, respectively, which was similar to that observed in the BEAT-ROP trial[4] for the recurrence of ROP at 54 weeks' postmenstrual age, and that each patient had a 45% and 55% probability of ROP in zones I and II, respectively. At least 80 evaluable patients per treatment group (100 enrolled patients with an estimated dropout rate of 20%) were expected to provide more than 90% power to show superiority of ranibizumab 0·2 mg against laser therapy. Slow enrolment and recruitment challenges led the sponsor to change this target after consultation with the European Medicine Agency. 60 patients per treatment group (48 evaluable patients assuming a dropout rate of 20%) were expected to provide more than 80% power to show superiority of ranibizumab 0·2 mg against laser therapy. This minimum target replaced the original target but it was agreed that additional patients above this new target should be enrolled, until preplanned closure of enrolment on June 30, 2017, which was achieved.

The Cochran-Mantel Haenszel test was used to test the primary outcome because recruitment was stratified by zone of ROP. The prespecified level of significance was two-sided $\alpha=0·05$ for superiority of ranibizumab 0·2 mg against laser therapy. A sequential test strategy was used to minimise the risk of type 1 errors caused by multiple comparisons. Significance testing of secondary

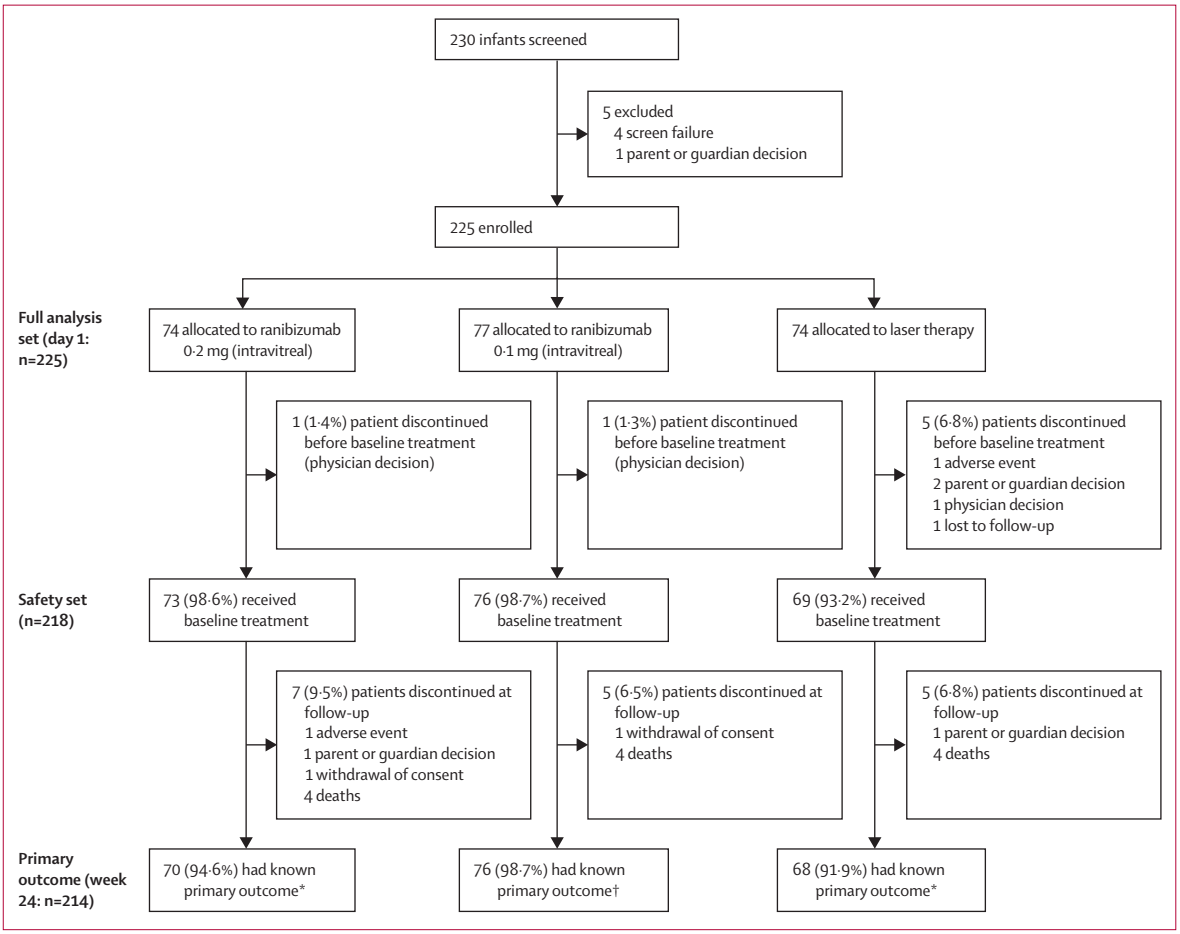

Figure 1: **CONSORT diagram detailing enrolment, randomisation, and follow-up of infants in the RAINBOW trial**
*Includes four deaths. †Includes four deaths and one treatment switch before withdrawal.

outcomes was done only if the test of the primary comparison (ranibizumab 0·2 mg *vs* laser therapy) was significant. The Mantel-Haenszel odds ratio (OR) with 95% CI is presented for each comparison. Efficacy analysis was by intention to treat per patient. This trial is registered with ClinicalTrials.gov, NCT02375971.

Role of the funding source

The funder of the study had full access to data collection, analysis, and interpretation and was involved in the writing of the manuscript, as well as the decision to submit. The authors had full access to the data, which are owned by Novartis, and had final responsibility for the decision to submit for publication.

Results

Between Dec 31, 2015, and June 29, 2017, 87 centres in 26 countries enrolled infants (appendix p 10). 225 infants were enrolled into RAINBOW and were randomly allocated to one of the three study groups. Of these infants, 218 received baseline treatment (day 1), and 201 completed the study (figure 1).

The median gestational age for participants at birth was 26 weeks (range 23–32). Gestational age was slightly lower in the ranibizumab 0·2 mg group than in the other two groups and lower in region 1 than in region 2. Most other baseline characteristics were well balanced between study groups (table 1).

Categorisation of ROP was similarly distributed across the treatment groups at baseline (appendix p 11). ROP was present in zone I in 86 (38%) infants and in zone II in 138 (61%) infants. Most infants had stage 3+ disease, 135 (60%) in zone II and 37 (16%) in zone I. AP-ROP was present in 30 (13%) of 225 infants, 29 of whom had zone I disease.

The primary efficacy outcome was known for 214 infants (figure 1). Treatment success occurred in 45 (66%) of 68 infants who received laser therapy (table 2) and in 56 (80%) of 70 infants who received ranibizumab 0·2 mg (OR compared with laser therapy 2·19, 95% CI 0·99–4·82, p=0·051); hence, other significance testing was not undertaken. Treatment success occurred in 57 (75%) of 76 infants who received ranibizumab 0·1 mg (OR compared with laser therapy 1·57, 95% CI 0·76–3·26;

	Ranibizumab 0·2 mg (n=74)	Ranibizumab 0·1 mg (n=77)	Laser therapy (n=74)
Geographical region*			
Region 1	45 (61%)	45 (58%)	44 (60%)
Region 2	29 (39%)	32 (42%)	30 (41%)
Sex			
Male	33 (45%)	37 (48%)	37 (50%)
Female	41 (55%)	40 (52%)	37 (50%)
Mother's race or ethnic origin			
White	43 (58%)	45 (58%)	45 (61%)
Black	0	4 (5%)	3 (4%)
Asian	27 (37%)	22 (29%)	23 (31%)
Other	4 (5%)	6 (8%)	3 (4%)
Gestational age (weeks)			
Overall	25 (23–32)	26 (23–32)	26 (23–32)
Region 1	24 (23–30)	25 (23–29)	24 (23–31)
Region 2	28 (24–32)	28 (24–32)	28 (24–32)
Gestational age category (weeks)			
≤24	32 (43%)	22 (29%)	29 (39%)
>24 to <27	18 (24%)	21 (27%)	17 (23%)
≥27	24 (32%)	34 (44%)	28 (37%)
Birthweight (g)			
Mean (SD)	791 (244)	886 (299)	831 (284)
Z score mean (SD)	−0·49 (1·18)	−0·52 (1·04)	−0·56 (1·14)
Plurality			
Singleton	52 (70%)	55 (71%)	48 (65%)
Multiple	18 (24%)	18 (23%)	18 (24%)
Postmenstrual age at baseline treatment (weeks)			
Age	36·7 (30·3–51·9)	36·9 (31·9–54·9)	36·6 (30·6–55·3)

Data are n (%) or median (range), unless otherwise indicated. Plurality data were missing for 4 (5%) patients who received ranibizumab, 4 (5%) patients who received ranibizumab, and 8 (11%) patients who received laser therapy. *Neonatal mortality in geographical region was <5 per 1000 livebirths in region 1 and >5 per 1000 livebirths in region 2.[23]

Table 1: **Demographics and baseline characteristics**

	Ranibizumab 0·2 mg	Ranibizumab 0·1 mg	Laser therapy
Patients entered	74	77	74
Patients with known primary outcome*	70	76	68
Treatment success†	56 (80%)	57 (75%)	45 (66%)
Reason for not meeting primary objective‡			
Active ROP present§	0	3	0
Unfavourable structural outcome¶	1	5	7
Treatment switch‖	11	13	18
Death of infant	4	4	4
Prespecified subgroup analyses			
Primary outcome by ROP zone			
Zone 1	19/28 (68%)	21/30 (70%)	14/23 (61%)
Zone 2	37/42 (88%)	36/46 (78%)	31/45 (70%)
Primary outcome by gestation			
≤24 weeks	22/29 (76%)	17/22 (77%)	12/27 (44%)
>24 to <27 weeks	16/18 (89%)	18/21 (86%)	9/15 (60%)
≥27 weeks	18/23 (78%)	22/33 (67%)	24/26 (92%)
Primary outcome by region**			
Region 1	36/42 (86%)	37/45 (82%)	26/42 (62%)
Region 2	20/28 (71%)	20/31 (64%)	19/26 (73%)
Primary outcome by infant sex			
Male	28/33 (85%)	26/36 (72%)	19/35 (54%)
Female	28/37 (76%)	31/40 (78%)	26/33 (79%)

Data are n (%) or n/N (%), unless otherwise indicated. ROP=retinopathy of prematurity. *See figure 1. †Alive and without treatment switch and unfavourable structural outcome or active ROP at day 169. ‡Infants can have multiple events. §Three infants with active ROP at day 169 had vessel dilatation (plus disease) but no extraretinal vessels. ¶Unfavourable structural outcome in either eye, any one of: retrolental membrane obscuring the view of the posterior pole; substantial temporal retinal vessel dragging causing abnormal structural features or macular ectopia; posterior retinal fold involving the macula; and retinal detachment involving the macula. ‖Treatment switch is the intervention for ROP with a treatment modality other than the modality of the first investigational treatment (this includes additional standard of care treatment such as vitrectomy). **Neonatal mortality was <5 per 1000 livebirths in region 1 and >5 per 1000 livebirths in region 2.[23]

Table 2: **Primary efficacy outcome and prespecified subgroup analysis**

OR compared with ranibizumab 0·2 mg 1·35, 95% CI 0·61–2·98).

Three infants in the ranibizumab 0·1 mg group had active ROP at week 24, comprising plus disease in two or more quadrants but with no extra-retinal vessels extending from the retina. No patients in the other groups had active disease. One infant had an unfavourable structural outcome following ranibizumab 0·2 mg, compared with five following ranibizumab 0·1 mg and seven after laser therapy (appendix p 12).

Because of minor baseline differences in key variables, a single confirmatory post-hoc exploratory logistic regression analysis for the primary outcome adjusting for three potential confounders—gestational age, geographical region, and infant sex—was done; the OR in favour of ranibizumab 0·2 mg versus laser treatment was 2·32 (95% CI 1·04–5·16).

Overall, treatment success was higher for all three groups in zone II than in zone I, and the gradient of effect remained in favour of ranibizumab (table 2). Among the two ranibizumab groups, full peripheral vascularisation assessed by indirect ophthalmoscopy occurred by day 169 in 28 (38%) infants in the ranibizumab 0·2 mg group and 21 (27%) infants in the ranibizumab 0·1 mg group.

61 infants received additional post-baseline treatments: 23 (31%) in the ranibizumab 0·2 mg group, 24 (31%) in the ranibizumab 0·1 mg group, and 14 (19%) in the laser therapy group (figure 2). In the laser group this was in addition to the treatment of skip lesions in 11 infants before day 11 but including one infant who received laser therapy on day 12. Including treatment to skip lesions, 22 (30%) infants in the laser group needed additional post-baseline treatment (laser therapy or switch to ranibizumab). 12 infants in each of the ranibizumab groups had one or more ranibizumab retreatments and achieved a successful primary outcome. The median

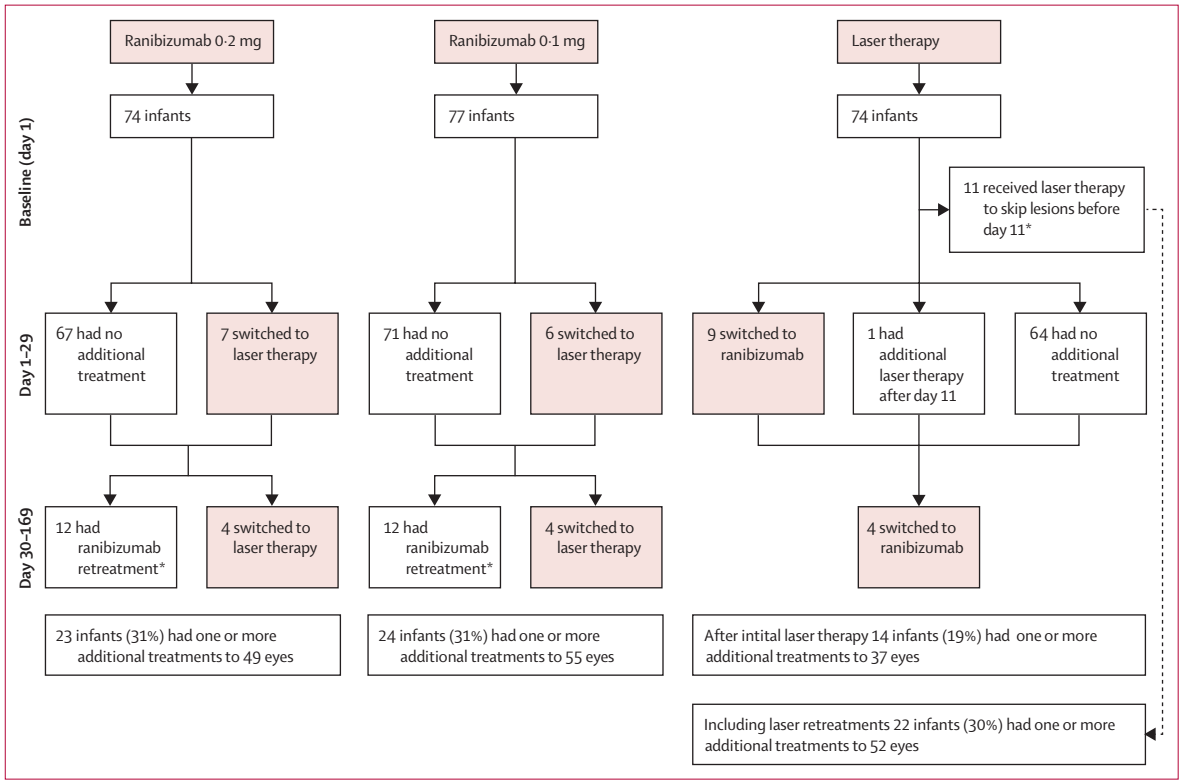

Figure 2: **Progress of infants with retinopathy of prematurity through additional post-baseline study treatments**
Flow chart shows the number of infants receiving at least one additional treatment (ranibizumab or laser therapy), either allowed in the protocol (up to 29 days when ranibizumab retreatment was first allowed) or up to 169 days, and treatment switches, which contributed to the primary outcome (shaded pink). Infants might have received one or more additional treatments in one or both eyes. Laser treatments to skip lesions were allowed as part of the baseline treatment but are included because they represent extra treatment episodes. *Allowed in protocol.

interval between baseline and first retreatment was 55 days (range 29–111) in the ranibizumab 0·2 mg group and 57 days (30–128) in the ranibizumab 0·1 mg group.

Death, serious adverse events (AEs), and non-serious systemic AEs were evenly distributed among the three groups (appendix pp 12–14). All events were from complications of preterm birth. Four deaths occurred in each group, including one infant who was treated with ranibizumab 0·1 mg in whom the investigator suspected that death was related to the study drug or procedure. The infant died at home following a feed within 24 h of treatment and was classified as fatal respiratory failure (appendix p 12). Serious and non-serious ocular AEs were similarly distributed between the three groups (appendix pp 14–15).

One infant (ranibizumab 0·2 mg) had a moderate cataract in one eye noted on day 28, thought to be lens damage by the injecting needle. Cataract surgery was not done during the study.

One infant (ranibizumab 0·1 mg) developed unilateral endophthalmitis and orbital infection 6 days after intravitreal injection. This infant was described as having bilateral conjunctivitis and left periocular staphylococcal infection 11 days before ranibizumab injection and treated with antibiotics. Infection was deemed resolved by the investigator before study entry, and prophylactic topical antibiotic was given to the left eye for 3 days immediately before intravitreal injection. Ipsilateral conjunctivitis and orbital infection presented 4 days after injection and endophthalmitis was evident at day 6. Vitrectomy was done at 8 weeks. The retinal structural outcome was unfavourable.

There were no significant differences between the study groups in body length, head circumference, knee to heel length, weight, or blood pressure from baseline to days 85 or 169 (appendix p 16).

Median serum ranibizumab and plasma VEGF levels within 24 h of, and 14 days and 28 days after, intravitreal injection are shown in the appendix p 16. Serum ranibizumab levels decayed from peak levels immediately after baseline to much lower levels after 28 days. Plasma VEGF levels were variable and there was no clear evidence of plasma VEGF suppression or of differences between the three treatment groups (figure 3). Detailed population pharmacokinetics of ranibizumab and related VEGF pharmacodynamics will be reported elsewhere.

Discussion

In RAINBOW, ranibizumab was an effective and well tolerated treatment for ROP in a range of settings around

the world. The proportion of infants with successful outcome was greater following intravitreal ranibizumab 0·2 mg compared with laser therapy and does not exclude superiority of ranibizumab over laser therapy.[24] In particular, ranibizumab 0·2 mg resulted in fewer eyes with unfavourable structural outcomes than did ranibizumab 0·1 mg or laser therapy. A ranibizumab dose of 0·1 mg offered no advantage over the 0·2 mg dose. Further safety data and functional outcomes to 5 years of age will be ascertained during the ongoing extension study. The observed systemic safety profile was as expected in a preterm population, and ocular adverse events were consistent with the established profile for ranibizumab in adults.

RAINBOW included ROP in all zone II locations, including mid and anterior zone II. The efficacy of ranibizumab was similar to laser in both zone I and zone II disease. In all three treatment groups, the highest risk of unfavourable outcome occurred in zone I disease, as has been reported in other trials.[1,4,25] Switching to alternative forms of treatment was frequent in all three groups. This might have been partly caused by the study design because the rules for additional treatments were set at a low threshold to minimise the risk of visual impairment and applied to both laser therapy and ranibizumab groups.

Our trial had several limitations. Care was provided by clinicians from a wide range of settings and experience to mimic routine clinical practice. Instructions for the administration of ranibizumab were provided but not training in the use of fundoscopy to determine the primary outcome, and not all centres had access to retinal photography. The trial was open label, not masked, and used no placebo. Decisions on retreatment were made on an individual basis and retreatment with ranibizumab restricted to intervals of 28 days. Clinician preference for one treatment could lead to biased decisions to re-treat. The study was limited by slow enrolment, which led to a modified recruitment target and reduced power. It is unclear why the significance of the primary comparison was marginal. Retrospectively, it is difficult to ascribe this result either to treatment effects being similar or to an inadequate sample size.

Although RAINBOW and the BEAT-ROP trial[4] both evaluated anti-VEGF agents, direct comparisons should be approached with caution as the two trials have very different protocols. RAINBOW included all forms of ETROP type 1 ROP[2] apart from zone II stage 2+ from an international group of hospitals. By contrast, the BEAT-ROP trial included only stage 3+ disease in zone I and posterior zone II in a single region. In RAINBOW, outcomes were evaluated per patient 24 weeks after first treatment (at a mean age of 60 weeks' [SD 4·4] postmenstrual age), whereas in BEAT-ROP ascertainment was by each eye to 54 weeks' postmenstrual age (and occurred between 50 to 70 weeks' postmenstrual age). RAINBOW used a composite primary outcome that included survival without active ROP, unfavourable structural outcomes, or treatment switch. By contrast BEAT-ROP used recurrence of retinal neovascularisation requiring retreatment. Therefore, the two studies are not directly comparable.

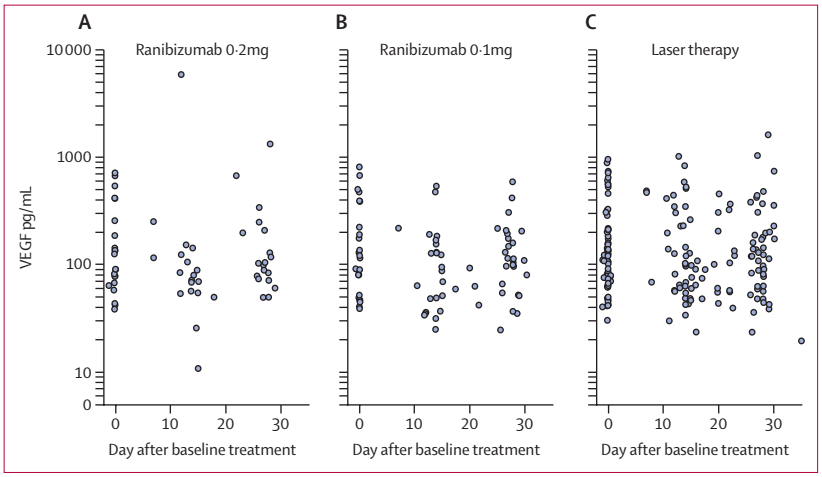

Figure 3: **Vascular endothelial growth factor levels following study entry to 35 days for each treatment group**
(A) Ranibizumab 0·2 mg. (B) Ranibizumab 0·1 mg. (C) Laser therapy.

In the ETROP randomised trial,[25] 9·1% of eyes had unfavourable structural outcomes after laser treatment, which compares to 1·43% of infants in RAINBOW following ranibizumab 0·2 mg and 10% of infants following laser therapy. The efficacy of ranibizumab with fewer unfavourable structural outcomes compared with laser therapy needs to be weighed against the two serious ocular complications—cataract and endophthalmitis—that each occurred in one eye in this study. To minimise risk, any form of periocular infection just before a planned intravitreal injection, even if treated, should be regarded as an exclusion criterion.

In RAINBOW, additional treatments were administered throughout the follow-up period. Regular follow-up eye examinations are therefore required after ranibizumab treatment. Median time to recurrence requiring additional treatment was 8 weeks in this study, indicating that this time frame is particularly important during follow-up examinations, but much later recurrences after anti-VEGF therapy can also occur.[26]

Ranibizumab is a fully humanised monoclonal antigen-binding 48kDa Fab antibody fragment.[27] In adults, the serum half-life of ranibizumab is approximately 9 days after intravitreal injection, probably due to slow egress from the vitreous.[28] RAINBOW is the first study to measure ranibizumab pharmacokinetics in preterm infants. Serum ranibizumab levels fell slowly with detectable but much reduced levels at day 29. By contrast, bevacizumab, a 149 kDa monoclonal antibody, has a serum half-life of 21 days after intravitreal injection in preterm infants.[29]

VEGF-mediated angiogenesis is essential for the development of a number of tissues,[30,31] but little is known about the effects of VEGF suppression in preterm

infants. We measured plasma rather than serum VEGF levels because this approach avoids VEGF release from platelets during thrombolysis and better reflects true free VEGF.[32] There was no clear evidence of plasma VEGF suppression compared with infants in the laser therapy group or of differences over time between the three treatment groups in RAINBOW, confirming findings in CARE-ROP.[11] By contrast, following intravitreal bevacizumab in preterm infants serum VEGF levels were substantially reduced for many weeks compared with baseline or those treated with laser therapy.[29,33]

In summary, ranibizumab 0·2 mg was as effective and safe in the treatment of active ROP as laser therapy, might be superior, and was associated with better short-term ocular outcomes. Compared with laser treatment, the potential for procedural complications and the need for regular clinical follow-up after ranibizumab treatment must be balanced against fewer adverse structural outcomes. On Sept 4, 2019, ranibizumab received approval for retinopathy of prematurity in the EU from the European Commission (European Medicines Agency).

Contributors

AS, DL, AF, BF, JDR, MFC, and NM in conjunction with Novartis conceived and designed the study. Novartis funded and fully managed the study. AS, DL, AF, BF, JDR, MFC, and NM formed the Protocol Steering Committee. The statistical analysis plan was agreed a priori by all authors and undertaken by QZ. All contributors had full access to the data. NM and AS wrote the first draft of the manuscript and DL, AF, BF, JDR, MFC, JL, ML, RM, and QZ equally contributed. All authors agreed the final version of the manuscript. The authors alone are responsible for the views expressed in this Article and they do not necessarily represent the views, decisions, or policies of the bodies with which they are affiliated.

Independent Data & Safety Monitoring Committee

Prof Antonio Capone (Chair), Dr Alay S Banker, Dr Kevin J Carroll, Professor Nicoline Schalij-Delfos, and Prof Umberto Simeoni.

Declaration of interests

AS, DL, AF, JDR, MFC, and NM received personal fees from Novartis during the design and execution of the trial. AS, DL, AF, BF, JDR, MFC, and NM received travel reimbursement from Novartis during the design and execution of the trial. AS declares personal fees from Novartis, Bayer, Allergan, Recordati Rare Diseases, and Boehringer Ingelheim outside the submitted work. AF declares personal fees from Recordati Rare Diseases outside the submitted work. MFC declares personal fees from Clarity Medical Systems and Inteleretina outside the submitted work. NM declares personal fees from Shire and GlaxoSmithKline outside the submitted work. JL, ML, RM, and QZ are employees of Novartis. NM receives a proportion of funding from the Department of Health's National Institute for Health Research Biomedical Research Centres funding scheme at University College London Hospitals/University College London. MFC is supported by the National Institutes of Health (grant P30EY10572) and by unrestricted departmental funding from Research to Prevent Blindness.

Data sharing

Study results have been disclosed as required by corresponding regulations and are publicly available on ClinicalTrials.gov, the EU clinical trial registry, and the Novartis clinical trial results websites.

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: An international randomized controlled open label trial of ranibizumab compared with laser therapy for the treatment of very low birthweight infants with retinopathy of prematurity.

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METHODS

1.1 Secondary Objectives

- To evaluate whether intravitreal ranibizumab 0·2mg has superior efficacy to intravitreal ranibizumab 0·1 mg in the treatment of ROP as measured by the absence of active ROP and absence of unfavorable structural outcomes in both eyes 24 weeks after starting investigational treatment, as assessed by the investigator
- To evaluate whether intravitreal ranibizumab 0·1mg has superior efficacy to laser therapy in the treatment of ROP as measured by the absence of active ROP and absence of unfavorable structural outcomes in both eyes 24 weeks after starting investigational treatment, as assessed by the investigator;
- To evaluate the time to intervention with a second modality for ROP or development of unfavorable structural outcome or death;
- To evaluate the recurrence of ROP receiving any post-baseline intervention at 24 weeks or before;
- To evaluate the ocular and systemic safety of intravitreal ranibizumab 0·1mg and 0·2mg in the treatment of ROP as assessed by ocular examination, monitoring of AEs throughout the study, and by the assessment of length, weight, head circumference and lower leg length at Baseline, Day 85, and Day 169;
- To evaluate the systemic pharmacokinetics of intravitreal ranibizumab in patients with ROP, as evaluated by sparse-sampling population PK methods;
- To evaluate the effects of investigational treatment on systemic VEGF levels in patients with ROP, as evaluated by sparse-sampling population concentration-response methods;
- To assess the number of ranibizumab administrations needed in the treatment of patients with ROP.

1.2 Exclusion criteria

Patients fulfilling any of the following criteria prior to receiving the first investigational treatment were not eligible for inclusion in the study. No additional exclusions were to be applied by the Investigator, in order to ensure that the study population would be representative of all eligible patients.

Investigational treatment not clinically appropriate for the following patients:

- Have ROP disease characteristic in either eye other than that defined for inclusion at the time of the first investigational treatment;
- Have a history of hypersensitivity (either the patient or the mother) to any of the investigational treatments or to drugs of similar chemical classes.
- Risk of confounding efficacy and/or safety assessments in the following patients:
    - Have received any previous surgical or nonsurgical treatment for ROP (e.g., ablative laser therapy or cryotherapy, vitrectomy.
    - Have been previously exposed to any intravitreal or systemic anti-VEGF agent (either the patient or the mother during this child's pregnancy);
    - Have used (either the patient or the mother) other investigational drugs as part of another clinical study (other than vitamins and minerals) within 30 days or within 5 half-lives of the other investigational drug, whichever is longer;
    - Have ocular structural abnormalities that are assessed by the Investigator to have a clinically significant impact on study assessments;
    - Have active ocular infection within 5 days before or on the day of first investigational treatment;
    - Have a history of hydrocephalus requiring treatment;
    - Have a history of any other neurological conditions that are assessed by the Investigator to have a significant risk of severe impact on visual function;
    - Have any other medical conditions or clinically significant co-morbidities or personal circumstances that are assessed by the Investigator to have a clinically relevant impact on study participation, any of the study procedures, or on efficacy assessments (e.g., poor life expectancy, pupil not able to be adequately dilated, unable to comply with the visit schedule).

1.3 Administration of treatments (Ranibizumab 0·1mg or 0·2mg)

Patients randomized to receive ranibizumab 0·1 mg or 0·2 mg received a single dose of intravitreal ranibizumab to each eye on Day 1.

For patients who received initial ranibizumab treatment, re-treatment with ranibizumab for either eye occurred for worsening of ROP at least 28 days after the previous ranibizumab treatment in that eye. Up to 2 re-treatments with ranibizumab per eye to treat ROP recurrence was allowed. The dose used was the same as the dose to which the patient had been randomized. Only the eye with worsening of ROP was re-treated. If both eyes had these signs, then both eyes were re-treated. Ranibizumab re-treatment was not administered to an eye

that had developed stage 4 or 5 ROP. The development of any complications of ROP, assessed by the Investigator as not suitable
for treatment with ranibizumab or laser, was managed as appropriate.

1.4 Conventional laser ablation therapy

Patients randomized to laser therapy received laser treatment to each eye on Day 1. Treatment was applied with near-confluent laser burn spacing (i.e., 0.5 to 1 burn-width apart) to the entire retina peripheral to the ROP lesion. In some cases, limited targeted laser ablation posterior to the ROP lesion, but immediately adjacent to it, was appropriate. Treatment was kept well away from the fovea. Laser ablation was as complete as possible. Multiple supplementary laser treatments were allowed for both eyes until 3 days after the Day 8 assessment and such treatments were considered part of the complete laser treatment. At the Day 2, Day 4 and Day 8 assessments, the investigator decided if supplementary laser treatment was necessary for any eye (e.g., to fill skip lesions, re-treat under-treated areas). Supplementary laser treatment was performed within the next 3 days of the investigator's decision to treat again. No further supplemental laser treatments were allowed for the patient after Day 11.

1.5 Definition of worsening disease that required further treatment, and actions taken

For the purpose of determining if a patient fulfilled the criteria for Ranibizumab re-treatment or switching of investigational treatment, the following definitions were used:

- Unchanged – No change in ROP disease activity (considering the stage and extent of ROP disease, and the severity and extent of plus disease characteristics);
- Minimally improved – A reduction in ROP disease activity (considering the stage and extent of ROP disease, and the severity and extent of plus disease characteristics), but which is minimal in the opinion of the investigator;
- Worsened - An increase in ROP disease activity (considering the stage and extent of ROP disease, and the severity and extent of plus disease characteristics).

1.6 Patients who received ranibizumab as initial treatment

For patients who received initial ranibizumab treatment, switch over to laser treatment occurred in the below situations of unsatisfactory response. Only the eye with ROP that fulfilled the below criteria was treated with laser. If both eyes fulfilled the criteria, then both eyes were treated with laser:

- ROP that remained unchanged or had worsened at the Day 4 assessment compared to before treatment;
- ROP that had only minimally improved, was unchanged, or had worsened at the Day 8 assessment compared to before treatment;
- ROP that had worsened compared to the previous assessment, any time after the Day 8 assessment and up to 27 days after the previous ranibizumab treatment in that eye;

Laser therapy was done within 3 days of the ROP fulfilling the above criteria. No further investigational ranibizumab treatments were administered to the eye after it had been switched to laser therapy.

In the case of ROP that worsened, compared to the previous assessment, at least 28 days after the previous ranibizumab treatment in that eye, ranibizumab re-treatment was administered (up to 2 re-treatments per eye) in that eye and this was not considered to be rescue treatment. For patients who received ranibizumab re-treatment, switch to laser therapy was done if the response to ranibizumab was unsatisfactory. If additional treatment was required for an eye that had already received two re-treatments of ranibizumab, the patient was treated with switch to laser or to Standard of Care therapy at the investigator's discretion. If additional treatment was required for an eye that had already been switched over to laser therapy, the patient was treated with Standard of Care therapy at the investigator's discretion. Ranibizumab treatment was not administered to an eye that had developed stage 4 or 5 ROP.

1.7 Patients who received laser therapy as initial treatment

For patients who received initial laser therapy, switch over to ranibizumab 0·2mg treatment occurred in the below situations of unsatisfactory response. Only the eye with ROP that fulfilled the below criteria was treated. If both eyes fulfilled the criteria, then both eyes were treated.

- ROP that had worsened at the Day 8 assessment compared to before treatment and provided laser treatment was complete as judged by the investigator;
- ROP that was only minimally improved, unchanged, or worsened at the Day 15 assessment compared to before treatment;

- ROP that had worsened, compared to the previous assessment, any time after the Day 15 assessment; ranibizumab 0·2mg treatment was done within 3 days of the ROP fulfilling the above criteria.

After receiving this first switched ranibizumab 0·2mg treatment, re-treatment with ranibizumab 0·2mg for the eye was done for worsening of ROP, compared to the previous assessment, at least 28 days after the previous ranibizumab treatment in that eye. Up to 2 such re-treatments with ranibizumab per eye to treat worsening of ROP was allowed. Only the eye with ROP that worsened was re-treated. If both eyes had these signs, then both eyes were re-treated. Ranibizumab treatment was not administered to an eye that had developed stage 4 or 5 ROP. If additional treatment was required for an eye that had already received 2 re-treatments of ranibizumab, the patient was treated with Standard of Care therapy at the investigator's discretion. If additional treatment was required for an eye that had received ranibizumab within the last 27 days, the eye was treated with Standard of Care therapy at the investigator's discretion.

1.8 Sequential hierarchical analysis (from Protocol)

A three-step sequential testing procedure will be used for primary (ranibizumab 0.2 mg against laser) and two key secondary comparisons (ranibizumab 0.1 mg against laser and ranibizumab 0.2 mg against 0.1 mg). Under this testing procedure, the primary comparison will be conducted at the first step followed sequentially by the two key secondary comparisons if the primary comparison is statistically significant. If the efficacy comparison at any step is not statistically significant, the remaining efficacy comparisons will be assessed descriptively. Otherwise the comparison will continue to the next step. All hypotheses will be tested at a pre-specified level of significance (two sided $\alpha = 0.05$). This testing procedure controls familywise type I error rate at a pre-specified level of significance because, for each hypothesis, testing is conditional upon rejecting all hypotheses earlier in the sequence.

The null hypotheses being tested at each step are:
Step 1: (primary comparison) H01: π Ranibizumab 0.2 mg - π Laser = 0 versus the alternative hypothesis HA1: π Ranibizumab 0.2 mg - π Laser $\neq$ 0 where π Treatment arm is the unknown proportion of patients with absence of active ROP and absence of unfavorable structural outcomes in both eyes 24 weeks after starting investigational treatment, as assessed by the Investigator in the relevant treatment arm.
If hypothesis H01 is rejected at the 2-sided 5% significance level and ranibizumab 0.2 mg is concluded to be superior compared to laser then proceed to the next step. Otherwise stop and assess key secondary comparisons (ranibizumab 0.1 mg against laser, and ranibizumab 0.2 mg against 0.1 mg) descriptively.
Step 2: (first key secondary comparison) H02: π Ranibizumab 0.1 mg - π Laser = 0 versus the alternative hypothesis HA2: π Ranibizumab 0.1 mg - π Laser $\neq$ 0
If hypothesis H02 is rejected at the 2-sided 5% significance level and ranibizumab 0.1 mg is concluded to be superior compared to laser then proceed to the next step. Otherwise stop and assess second key secondary comparison (ranibizumab 0.2 mg against 0.1 mg) descriptively.
Step 3: (second key secondary comparison) H03: π Ranibizumab 0.2 mg - π Ranibizumab 0.1 mg = 0 versus the alternative hypothesis HA3: π Ranibizumab 0.2 mg - π Ranibizumab 0.1 mg $\neq$ 0 If ranibizumab 0.2 mg is not concluded to be superior compared to 0.1 mg or hypothesis H03 is accepted at the 2-sided 5% significance level then the comparison between ranibizumab 0.2 mg against 0.1 mg will be assessed descriptively.

The comparisons will be performed using the stratified Cochran-Mantel-Haenszel test for binomial proportions. Stratification will be based on ROP zone at baseline as used for randomization. Mantel-Haenszel odds ratios and their 95% confidence intervals will also be presented.

- Superiority of ranibizumab 0.2 mg over laser will be concluded if hypothesis H01 is rejected (at the two-sided 5% significance level) and Mantel-Haenszel odds ratio of ranibizumab 0.2 mg to laser is in favor of ranibizumab 0.2 mg with regard to the success of the primary efficacy variable.
- Superiority of ranibizumab 0.1 mg over laser will be concluded if hypothesis H02 is rejected (at the two-sided 5% significance level), and Mantel-Haenszel odds ratio of ranibizumab 0.1 mg to laser is in favor of ranibizumab 0.1 mg.
- Superiority of ranibizumab 0.2 mg over 0.1 mg will be concluded if hypothesis H03 is rejected (at the two-sided 5% significance level) and Mantel-Haenszel odds ratio of ranibizumab 0.2 mg versus 0.1 mg is in favor of ranibizumab 0.2 mg.

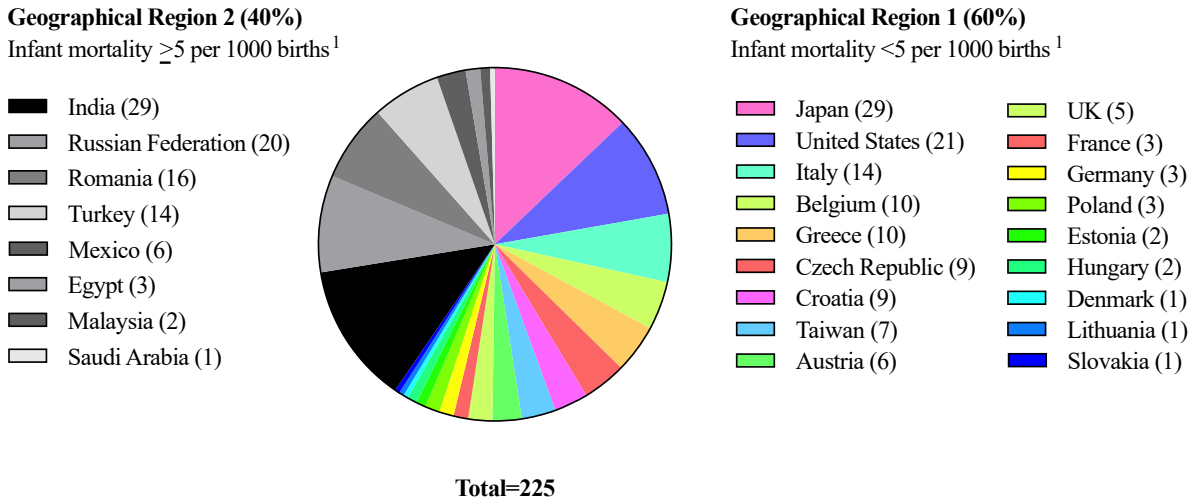
1.9 Ranibizumab PK and VEGF pharmacodynamics sampling.
Systemic ranibizumab concentration. For patients who receive initial ranibizumab treatment and with an odd patient identification number, blood samples for the determination of ranibizumab concentrations will be collected at the following time points: Within24 hours after the first administration of ranibizumab; At Day 15, with an

allowed time window of 7 to 21days after the first administration of ranibizumab; At Day 29, with an allowed time window of 22 to 28days after the first administration of ranibizumab.

Systemic vascular endothelial growth factor level. For patients who receive initial ranibizumab treatment and with an even patient identification number and for all patients who receive initial laser therapy, blood samples for the determination of systemic VEGF levels (i.e., pharmacodynamics) will be collected at the following time points: Before the first investigational treatment; At Day 15, with an allowed time window of 7 to 21days after the first investigational treatment; At Day 29, with an allowed time window of 22 to 28 days after the first investigational treatmen

Figures

Figure S1: Infant Enrolment by Country and Region; Mortality groups are derived from data in **World Health Statistics 2014: WHO available from the following web address (accessed on 8 March 2019)** http://apps.who.int/iris/bitstream/handle/10665/112738/9789240692671_eng.pdf;jsessionid=A60B920F2CC95F44769825BE02B10541?sequence=1.



Tables

Table S1: Baseline ROP characteristics as defined by the examining ophthalmologist. Infants classification based on worse eye

Characteristic	Ranibizumab 0·2mg	Ranibizumab 0·1mg	Laser therapy
ROP Zone			
ROP Zone I	28 (37·8%)	30 (39·0%)	28 (37·8%)
ROP Zone II	46 (62·2%)	46 (59·7%)	46 (62·2%)
Not specified [(a)]	0	1 (1·3%)	0
ROP classification			
Zone I AP-ROP	10 (13·5%)	10 (13·0%)	9 (12·2%)
Zone II AP-ROP	0	0	1 (1·4%)
Zone I stage 3+	12 (16·2%)	14 (18·2%)	11 (14·9%)
Zone I stage 3	3 (4·1%)	4 (5·2%)	1 (1·4%)
Zone I stage 2+	3 (4·1%)	1 (1·3%)	5 (6·8%)
Zone I stage 1+	0	1 (1·3%)	2 (2·7%)
Zone II stage 3+	46 (62·2%)	45 (58·4%)	44 (59·5%)
Zone II stage 3 [(b)]	0	1 (1·3%)	0
Zone II stage 2+ [(b)]	0	0	1 (1·4%)
Not specified [(a)]	0	1 (1·3%)	0

(a) This infant was discontinued after randomization without receiving treatment.

(b) Enrolled by investigator, out of protocol but remained in study and included in intention to treat analysis

Table S2: Causes of unfavorable structural outcomes

Ranibizumab 0·2mg group:

- One infant with substantial temporal retinal vessel dragging causing abnormal structural features/macular ectopia AND posterior retinal fold involving the macula in BOTH eyes.

Ranibizumab 0·1mg group:

- Five infants had an unfavorable structural outcome in one eye
  - Two had substantial temporal retinal vessel dragging causing abnormal structural features/macular ectopia;
  - Three had retinal detachment involving the macula

Laser therapy group:

- One infant had substantial temporal retinal vessel dragging causing abnormal structural features/macular ectopia in BOTH eyes.
- Six infants had an unfavorable structural outcome in one eye:
  - Two had substantial temporal retinal vessel dragging causing abnormal structural features/macular ectopia;
  - One had posterior retinal fold involving the macula;
  - Three had retinal detachment involving the macula.

Table S3: Causes of Death

Infant number	Treatment group	Primary cause of death	Contributory cause of death	Interval between baseline treatment and death
1	Ranibizumab 0·2mg	Aspiration	-	22 days
2	Ranibizumab 0·2mg	Bronchopulmonary dysplasia	Pneumothorax	51 days
3	Ranibizumab 0·2mg	Sepsis caused by klebsiella	-	27 days
4	Ranibizumab 0·2mg	Acute bronchopneumonia	Toxic-septic shock	138 days
5	Ranibizumab 0·1mg	Severe NEC	Cardiogenic shock	37 days
6	Ranibizumab 0·1mg	Septicaemia, cause not identified	-	9 days
7	Ranibizumab 0·1mg	Renal failure	Hyperkalaemia	9 days
8	Ranibizumab 0·1mg	Respiratory failure (a)	-	1 day
9	Laser therapy	Bronchopulmonary dysplasia	Sepsis	6 days
10	Laser therapy	Pulmonary vein stenosis	-	91 days
11	Laser therapy	Hepatic failure	Renal failure	26 days
12	Laser therapy	Cardiac arrest	Sepsis	19 days

4 of the 12 deaths were in Region 1, and 8 were in Region 2. (a) This infant died at home on the day following treatment.

Table S4: Non-ocular adverse events. Number (%) of patients regardless of study treatment or procedure relationship (greater than or equal to 3% in any arm) by preferred term

Preferred term	Ranibizumab 0·2 mg N =73, n (%)	Ranibizumab 0·1 mg N = 76, n (%)	Laser N = 69, n (%)
Number of patients with any non-ocular AE	62 (84·9)	62 (81·6)	53 (76·8)
Pyrexia	9 (12·3)	6 (7·9)	4 (5·8)
Dermatitis diaper	8 (11·0)	6 (7·9)	4 (5·8)
Nasopharyngitis	7 (9·6)	7 (9·2)	4 (5·8)
Upper respiratory tract infection	6 (8·2)	3 (3·9)	1 (1·4)
Anaemia	5 (6·8)	8 (10·5)	5 (7·2)
Gastro oesophageal reflux disease	5 (6·8)	6 (7·9)	5 (7·2)
Pneumonia	5 (6·8)	1 (1·3)	8 (11·6)
Bronchopulmonary dysplasia	4 (5·5)	5 (6·6)	5 (7·2)
Cough	4 (5·5)	2 (2·6)	1 (1·4)
Diarrhoea	4 (5·5)	2 (2·6)	1 (1·4)
Inguinal hernia	4 (5·5)	2 (2·6)	2 (2·9)
Urinary tract infection	4 (5·5)	2 (2·6)	2 (2·9)
Bronchiolitis	3 (4·1)	4 (5·3)	2 (2·9)
Bronchitis	3 (4·1)	3 (3·9)	2 (2·9)
Bronchospasm	3 (4·1)	0	1 (1·4)
Escherichia urinary tract infection	3 (4·1)	1 (1·3)	0
Rhinitis	3 (4·1)	0	2 (2·9)
Viral infection	3 (4·1)	1 (1·3)	1 (1·4)
Anaemia neonatal	2 (2·7)	4 (5·3)	1 (1·4)
Bradycardia	2 (2·7)	5 (6·6)	1 (1·4)
Flatulence	2 (2·7)	3 (3·9)	2 (2·9)
Vomiting	2 (2·7)	5 (6·6)	4 (5·8)
Apnoea	1 (1·4)	6 (7·9)	3 (4·3)
Constipation	1 (1·4)	3 (3·9)	2 (2·9)
Necrotising colitis	1 (1·4)	3 (3·9)	1 (1·4)
Pneumonia bacterial	1 (1·4)	0	3 (4·3)
Respiratory failure	1 (1·4)	4 (5·3)	1 (1·4)
Sepsis	1 (1·4)	1 (1·3)	5 (7·2)
Malnutrition	0	3 (3·9)	0
Osteopenia	0	4 (5·3)	2 (2·9)
Pneumonia aspiration	0	3 (3·9)	2 (2·9)
Umbilical hernia	0	3 (3·9)	2 (2·9)

Adverse events (AEs) with start date on or after the date of first study treatment administration are counted.

Preferred terms are sorted in descending frequency of AEs in the Ranibizumab 0·2mg arm.

A subject with multiple AEs is counted only once in the "number of subjects" row.

A subject with multiple AEs with the same preferred term is counted only once for that preferred term.

Table S5: Non-ocular serious adverse events (≥ 2% in any arm)

Preferred term	Ranibizumab 0·2mg N = 73, n (%)	Ranibizumab 0·1mg N = 76, n (%)	Laser N = 69, n (%)
Total	**24 (32·9)**	**24 (31·6)**	**22 (31·9)**
Pneumonia	4 (5·5)	0	2 (2·9)
Brain oedema	2 (2·7)	0	0
Bronchiolitis	2 (2·7)	4 (5·3)	0
Bronchopulmonary dysplasia	2 (2·7)	2 (2·6)	2 (2·9)
Incarcerated inguinal hernia	2 (2·7)	0	0
Inguinal hernia	1 (1·4)	2 (2·6)	0
Apnoea	0	2 (2·6)	2 (2·9)
Cardio-respiratory arrest	0	2 (2·6)	0
Diarrhoea	0	2 (2·6)	0
Nasopharyngitis	0	2 (2·6)	0
Necrotising colitis	0	3 (3·9)	0
Perinatal brain damage	0	0	2 (2·9)
Respiratory failure	0	3 (3·9)	1 (1·4)
Sepsis	0	1 (1·3)	2 (2·9)
Vomiting	0	1 (1·3)	2 (2·9)

Adverse events (AEs) with start date on or after the date of first study treatment administration are counted.
Preferred terms are sorted in descending frequency of AEs in the Ranibizumab 0·2mg arm.
A subject with multiple AEs is counted only once in the "number of subjects" row.
A subject with multiple AEs with the same preferred term is counted only once for that preferred term.

Table S6: Ocular adverse events: Number (%) of patients regardless of study treatment or procedure relationship (greater than or equal to 2% in any arm) by preferred term

Preferred term	Ranibizumab 0·2mg N = 73, n (%)	Ranibizumab 0·1mg N = 76, n (%)	Laser N = 69, n (%)
Number of patients with any ocular AEs	22 (30·1)	31 (40·8)	23 (33·3)
Conjunctival haemorrhage	6 (8·2)	6 (7·9)	2 (2·9)
Retinal haemorrhage	6 (8·2)	10 (13·2)	7 (10·1)
Retinopathy of prematurity	2 (2·7)	2 (2·6)	4 (5·8)
Conjunctivitis	1 (1·4)	6 (7·9)	3 (4·3)
Conjunctival hyperaemia	0	0	2 (2·9)
Corneal opacity	0	0	2 (2·9)
Eye haemorrhage	0	2 (2·6)	1 (1·4)
Vitreous haemorrhage	0	4 (5·3)	0

Adverse events (AEs) with start date on or after the date of first study treatment administration are counted.
Preferred terms are sorted in descending frequency of AEs in the Ranibizumab 0·2mg arm.
A subject with multiple AEs is counted only once in the "number of subjects" row.
A subject with multiple AEs with the same preferred term is counted only once for that preferred term.

Table S7: Ocular serious adverse events (any)

Preferred term	Ranibizumab 0·2mg N = 73, n (%)	Ranibizumab 0·1mg N = 76, n (%)	Laser N = 69 n (%)
Total	**4 (5·5)**	**1 (1·3)**	**4 (5·8)**
Retinopathy of prematurity	2 (2·7)	1 (1·3)	3 (4·3)
Cataract	1 (1·4)	0	0
Nystagmus	1 (1·4)	0	0
Conjunctivitis	0	0	1 (1·4)
Endophthalmitis (a)	0	1 (1·3)	0
Exophthalmos (a)	0	1 (1·3)	0
Eye disorder (a)	0	1 (1·3)	0
Orbital infection (a)	0	1 (1·3)	0

Adverse events (AEs) with start date on or after the date of first study treatment administration are counted.

Preferred terms are sorted in descending frequency of AEs in the Ranibizumab 0·2mg arm.

A subject with multiple AEs is counted only once in the "number of subjects" row.

A subject with multiple AEs with the same preferred term is counted only once for that preferred term.

Note (a) These AEs all occurred in the same eye

Table s8: Summary of clinically relevant ocular adverse events

Number of infants	Ranibizumab 0·2mg N = 73	Ranibizumab 0·1mg N = 76	Laser therapy N = 69
Any ocular adverse events	22 (30·1%)	31 (40·8%)	23 (33·3%)
Specific ocular adverse events			
Endophthalmitis *	0	1 (1·3%)	0
Cataract *	1 (1·4%)	0	0
Vitreous haemorrhage	0	4 (5·3%)	0
Retinal haemorrhage	6 (8·2%)	10 (13·2%)	7 (10·1%)
Corneal opacity	0	0	2 (2·9%)
Conjunctival haemorrhage	6 (8·2%)	6 (7·9%)	2 (2·9%)
Conjunctivitis	1 (1·4%)	6 (7·9%)	3 (4·3%)
Conjunctival hyperaemia	0	0	2 (2·9%)

Ocular adverse events with a start date on or after baseline study treatment; all clinically relevant serious adverse events (*) and non-serious events occurring in ≥2% participants are listed. Infants may have had more than one adverse event

Table S9: Mean change from baseline in vital signs (Body length, head circumference, weight, knee to heel length and blood pressure) at Day 85 and Day 169

	Ranibizumab 0·2 mg N = 73	Ranibizumab 0·1 mg N = 76	Laser N = 69
Mean change from baseline in vital signs (body length [cms], head circumference [cms] and knee to heel length [cms] at Day 85 and Day 169			
Day 85/ body length, mean ± SD, (n)	10·1 ± 2·59 (n = 61)	11·0 ± 3·33 (n = 63)	11·1 ± 3·65 (n = 60)
Day 169/ body length, mean ± SD, (n)	18·7 ± 3·28 (n = 62)	18·6 ± 3·66 (n = 62)	19·0 ± 4·50 (n = 57)
Day 85/ head circumference, mean ± SD, (n)	6·9 ± 1·96 (n = 62)	6·5± 2·31 (n = 62)	7·2 ± 2·13 (n = 59)
Day 169/ head circumference, mean ± SD, (n)	10·4 ± 2·12 (n = 62)	10·3 ± 2·56 (n = 62)	10·6 ± 2·61 (n = 57)
Day 85/ knee to heel length, mean ± SD, (n)	2·9 ±1·90 (n = 55)	3·1 ± 1·65 (n = 51)	3·1 ± 2·02 (n = 52)
Day 169/ knee to heel length, mean ± SD, (n)	5·4 ± 2·86 (n = 53)	5·1 ±2·19 (n = 52)	5·3 ± 2·15 (n = 52)
Mean change from baseline in vital signs (weight [g]) at Day 85 and Day 169			
Day 85/ weight, mean ± SD, (n)	2198·9 ± 615·68 (n = 63)	2149·9 ± 754·27 (n = 66)	2182·7 ± 612·70 (n = 60)
Day 169/ weight, mean ± SD, (n)	3794·3 ± 782·48 (n = 62)	3716·7 ± 897·15 (n = 62)	3826·0 ± 882·17 (n = 60)
Mean change from baseline in vital signs (BP [mmHg]) at Day 85 and Day 169			
Day 85/ diastolic BP, mean ± SD, (n)	8·1 ± 14·66 (n = 47)	7·0 ± 14·25 (n = 46)	9·8 ± 16·95 (n = 41)
Day 85/ systolic BP, mean ± SD, (n)	6·3 ± 15·85 (n = 47)	9·4 ± 15·73 (n = 46)	15·5 ± 16·85 (n = 41)
Day 169/ diastolic BP, mean ± SD, (n)	11·5 ± 15·60 (n = 45)	11·8 ± 14·42 (n = 46)	14·7 ± 17·05 (n = 40)
Day 169/ systolic BP, mean ± SD, (n)	10·2 ± 16·15 (n = 45)	11·2 ±13·45 (n = 46)	17·7 ± 19·35 (n = 40)

BP, blood pressure; SD, standard deviation

Table S10: Serum ranibizumab levels following intraocular injection in very low birthweight infants and plasma VEGF levels in all three trial groups in RAINBOW.

	Ranibizumab 0·2mg	Ranibizumab 0·1mg	Laser therapy
Serum ranibizumab, pg/mL (a)			
Day 1 median (IQR)	7,820 (2,000-23,200) n=49	4,350 (382-12,100) n=46	-
Day 15 median (IQR)	4,440 (2,450-8,130) n=45	3,400 (2,515-5,215) n=36	-
Day 29 median (IQR)	1,070 (705-1,730) n=31	566 (303-1,060) n=24	-
Plasma VEGF, pg/mL (b)			
Day 1 median (IQR)	136 (78-414) n=17	130 (81-388) n=21	136 (79-288) n=46
Day 15 median (IQR)	71.8 (54-124) n=21	67 (37-156) n=26	86.1 (56-230) n=44
Day 29 median (IQR)	89 (74-105) n=13	140 (97-209) n=18	123 (63-181) n=30

(a) Sampled within 24 hours after initial injection; (b) Sampled within 24 hours before initial injection or first laser treatment