Recurrence of Retinopathy of Prematurity After Intravitreal Ranibizumab Monotherapy: Timing and Risk Factors

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Purpose. To investigate timing and risk factors of recurrent retinopathy of prematurity (ROP) after intravitreal ranibizumab (IVR) monotherapy.

Methods. Fifty eyes (the more severe eye) of 50 infants treated with IVR monotherapy for type 1 ROP were studied retrospectively. The mean follow-up time was 31 weeks after IVR. Recurrent ROP (recurrence of extraretinal fibrovascular proliferation [EFP]) was determined by RetCam wide-angle fundus imaging and binocular indirect ophthalmoscopy. Risk time of recurrence was estimated by Kaplan-Meier survival analysis with recurrence as the endpoint. Time-varying recurrence hazard rate was determined using the hazard function of life-table analysis. The risk factors of recurrence were explored by logistic regression analysis.

Results. Recurrence of ROP occurred in 32 (64%) of 50 eyes at 7.9 ± 2.7 weeks after IVR. Most of recurrence (94%) occurred in 2.5 to 12.0 weeks following IVR treatment. The recurrence hazard rate reached its maximum at 8 weeks. Recurrence affecting the initial site of EFP occurred significantly earlier than recurrence only at the new vascular advancing edge (4.5 ± 1.4 weeks versus 9.1 ± 2.0 weeks after IVR, $P < 0.001$). The independent risk factors of recurrence included extensive retinal neovascularization ($P = 0.005$) and oxygen requirement after IVR ($P = 0.016$).

Conclusions. Recurrence of type 1 ROP should be carefully watched in a long-term follow-up after IVR monotherapy, particularly in the first 12 weeks after IVR and for those with extensive retinal neovascularization or prolonged oxygen therapy.

Keywords: retinopathy of prematurity, ranibizumab, recurrence, neovascularization

Retinopathy of prematurity (ROP) is a vasoproliferative disorder and a major cause of blindness in premature birth infants.[1] Type 1 ROP may require treatment to prevent macular dragging and tractional retinal detachment.[2] Etiology of ROP is multifactorial, whereas interplay of oxygen and vascular endothelial growth factor (VEGF) levels is proven to play the key roles in the two phases of ROP: relative hyperoxia and decreased levels of VEGF in phase 1 with delayed physiologic retinal vascular development, followed by relative hypoxia and increased levels of VEGF in phase 2 with vasoproliferation.[3,4] By directly halting the VEGF molecules in phase 2 ROP, intravitreal injection of anti-VEGF agents, either with bevacizumab (Avastin; Genentech, Inc., San Francisco, CA, USA) or ranibizumab (Lucentis; Genentech, Inc.) were effective in treating type 1 ROP.[5–9]

The main advantages of anti-VEGF treatment over conventional laser photocoagulation include promoting rapid regression of acute-phase ROP (neovascularization and plus disease), allowing potentials for retinal vascularization, approaching eyes with a rigid pupil, and a lower chance of unfavorable outcomes in type 1 ROP in zone I or posterior zone II.[5,6,8] Compared with bevacizumab, ranibizumab is a smaller molecule with a much shorter systemic half-life and a shorter time of VEGF suppression, and it is used to treat ROP in fragile preterm infants with the purpose of being a safer option.[6,10,11]

However, the effect of anti-VEGF therapy may be transient in VEGF suppression.[10,12,13] Recurrence of extraretinal fibrovascular proliferation (EFP) after a single treatment of an anti-VEGF agent, either with bevacizumab or ranibizumab, was reported in type 1 ROP, possibly resulting macular dragging or retinal detachment.[5,14–18] Therefore, timely detection as well as management of recurrence has become a major concern in anti-VEGF therapy for ROP. There is a scarcity of evidence to guide clinicians on the time course of recurrence, major risk time, time-related characteristics, length of follow-up, and risk factors for ROP recurrence after intravitreal ranibizumab (IVR) monotherapy. Better knowledge of these data will aid clinicians in the optimal use of IVR in ROP treatment as well as a timely management of ROP recurrence after IVR.

In the present study, we reported our experience with IVR monotherapy for type 1 ROP. The timing and risk factors of recurrence were studied and discussed.

Patients and Methods

This retrospective study was conducted in accordance with the tenets of the Declaration of Helsinki. Study protocols were approved by the hospital ethics committee, and informed

consent to receive off-label IVR treatment for ROP and to participate into the study were obtained from all legal guardians of the patients.

A chart review was conducted in 59 consecutive patients who were referred to the neonatal intensive care units and underwent IVR monotherapy for type 1 ROP from June 2013 to December 2015 at Xinhua Hospital affiliated with the Shanghai Jiao Tong University School of Medicine, in Shanghai, China. Severe forms of type 1 ROP (stage 3 ROP with plus disease [stage 3 + ROP] or aggressive posterior ROP [AP-ROP] in zone I or posterior zone II) associated with poor systemic conditions in these patients were indications for primary IVR treatment, considering the advantages of anti-VEGF therapy over laser photocoagulation and the systemic safety of IVR treatment.[5,11] A total of 50 of the 59 patients were enrolled in this study. There were four pairs of twins and only one patient with more severe ROP was selected in this study, because data from twins were considered relevant. An additional five patients were ineligible due to rapid progression to retinal detachment without initial resolution of acute-phase ROP ($n = 1$), incomplete clinical data ($n = 2$), and prophylactic laser ablation to the avascular retina for reasons of persistent vascular arrest and early discharge from the hospital ($n = 2$).

Diagnosis and Classification of ROP

Retinopathy of prematurity was diagnosed and classified according to the International Classification of ROP.[19] Hybrid zone I and posterior zone II ROP were defined as zone I ROP. Type 1 ROP was defined as any stage of ROP with plus disease or stage 3 ROP in zone I, or stage 2 or 3 ROP with plus disease in zone II, based on the Early Treatment for Retinopathy of Prematurity Randomized Trial study.[2]

Intravitreal Ranibizumab Treatment and Follow-up

An intravitreal injection with 0.25 mg/0.025 mL of ranibizumab was performed through the pars plicata into the vitreous cavity with a 30-gauge needle inserted 1.0 mm posterior to the limbus of both eyes under topical anesthesia. Patients were followed-up at 3 days, and then weekly or biweekly or monthly after IVR treatment. The endpoint of follow-up was complete involution of acute-phase ROP (neovascularization and plus disease) with vascularization of zone III, but not necessarily with vessels reaching temporal ora seratta.[19,20] Extensive follow-up was individually tailored according to their response to treatment. The wide-angle fundus imaging system RetCam (Clarity Medical Systems, Pleasanton, CA, USA) was used to record fundus images of serial examinations at each visit pre- and postinjection. All fundus images were independently reviewed by two retinal specialists. If there was a discrepancy between the image reviewers, the images were reviewed again with both evaluators present to render a consensus. Binocular indirect ophthalmoscopy with scleral indentation was performed as needed. Fluorescein angiography (FA) under general anesthesia was performed to detect retinal vascular development and confirm ROP recurrence if indicated.

Recurrence Requiring Retreatment

Retinopathy of prematurity recurrences requiring an additional treatment after IVR monotherapy were defined as the reappearance of EFP in eyes after an initial resolution of acute-phase ROP.[5,21] Return of plus disease was not necessarily required, but an important sign showing the activity of recurrence. Recurrences were retreated with diode laser ablation to the avascular retina. Reappearance of a line or a ridge was observed until either recurrence of EFP or final involution of ROP.

Outcome Measurements and Statistical Analysis

The main outcome measurements were timing and risk factors of ROP recurrence after IVR monotherapy. Any recurrence in one or both eyes was defined as a ROP recurrence. Descriptive statistics were used to characterize the study population, including means and SDs or median value (range) for continuous variables, frequencies and proportions for categorical variables. Time of recurrence and related fundus characteristics were analyzed according to serial retinal examinations after IVR by the RetCam wide-angle fundus imaging system and binocular indirect ophthalmoscopy. Time-varying recurrence hazard rate was estimated using the hazard function of life-table analysis. Recurrence-free survival was estimated by the Kaplan-Meier method with earliest recurrence needing a retreatment as the endpoint. Any difference in recurrence-free survival was evaluated with a log-rank test. Patients who had involution of ROP without recurrence at the last visit were censored for the purpose of data analysis. Potential risk factors obtained from clinical charts were the patient's sex, birth weight (BW), gestational age (GA), postmenstrual age (PMA) of IVR treatment, clock hours (30° sectors) of preexisting retinal neovascularization, zone of ROP, patient number of comorbidities including anemia (hemoglobin <110 g/L), sepsis (positive blood culture), congenital heart disorder, intraventricular hemorrhage, neonatal respiratory distress syndrome (NRDS), bronchopulmonary dysplasia (BPD), asphyxia, and the number of patients requiring oxygen support before IVR, and oxygen requirement after IVR treatment before involution. These clinical factors were included in univariate analysis, excluding the items occurring less frequently ($n \leq 10$). A logistic multivariate regression backward stepwise model was constructed to identify independent risk factors from variables demonstrating statistically significant associations ($P < 0.05$) with risks of ROP recurrence by univariate analysis. Statistical analyses were performed using SPSS 22.0 for Windows (SPSS, Inc., Chicago, IL, USA). A $P < 0.05$ (2-sided) was considered significant for all tests.

Results

Subjects

Patient demographics are listed in Table 1. The mean follow-up time was 31 weeks after IVR (24 to 70 weeks). No ocular complications related to intravitreal injection were noted. No major systemic side effects, such as infection, deteriorated respiratory condition, or death occurred. Retinopathy of prematurity recurrence requiring retreatment occurred in 64% of the enrolled patients (31 of 50 with bilateral and 1 with unilateral ROP recurrence). All underwent additionally laser treatment besides initial IVR monotherapy. A 25-gauge lens-sparing vitrectomy was performed to remove fibrosis proliferation in the nasal quadrant in one eye at 4 weeks after laser retreatment (43 weeks at PMA). All recurrences were resolved at the last visit without adverse anatomic outcomes like macular ectopia, dragged-disc, or retinal detachment. The remaining 18 patients had involution of ROP associated with vascularization of zone III (five eyes of 4 patients without vessels reaching temporal ora serrata) and required no retreatment. Considering that consistency of recurrence or not in both eyes was observed in 49 of 50 patients, one eye with more severe ROP from each patient was selected for subsequent analysis. The selected eyes consisted of 45 eyes

Table 1. Demographic and Ocular Characteristics of Patients With ROP

Variables	All Patients, $n = 50$
Sex (% male)	29 (58)
BW, g, mean ± SD (range)	1369 ± 338 (650–2000)
GA, wk, mean ± SD (range)	29.4 ± 1.7 (25.3–32.0)
PMA of IVR, wk, mean ± SD (range)	35.5 ± 1.3 (32.0–38.0)
Stage, *n*	
Stage 3+	45
AP	5
Zone of ROP	
Symmetric, *n* (%)	46 (92)
Zone I	17
Posterior zone II	29
Asymmetric, *n* (%)	
Zone I in one eye/posterior zone II in the other	4 (8)
Extent of neovascularization, clock hour, mean ± SD (median; range)	9 ± 2 (9; 5–12)
Major neonatal comorbidities, *n* (%)	
Pulmonary status	
NRDS	11 (22)
BPD	17 (34)
Asphyxia	8 (16)
Oxygen requirement	
Before IVR (CPAP and artificial ventilation)	38 (76)
After IVR	20 (40)
Types	
CPAP	5
Mask or incubation at FiO_2 33%	15
Cumulative time after IVR, d, median (range)	6 (3–33)
Sepsis	9 (18)
Anemia	26 (52)
Congenital heart defect	21 (46)
Intraventricular hemorrhage	10 (20)
Congenital hypothyroidism	9 (18)

CPAP, continuous positive airway pressure; FiO_2, Fraction of inspiration O_2.

with stage 3 + ROP in zone I or posterior zone II and 5 eyes with AP-ROP.

Timing of Recurrence

Time points of postinjection status in 50 selected eyes are summarized in Table 2. Rapid resolution of plus disease and neovascularization was induced in 49 (98%) and 46 (92%) eyes, respectively. Advancing of retinal vessels was observed in 46 eyes (92% of all).

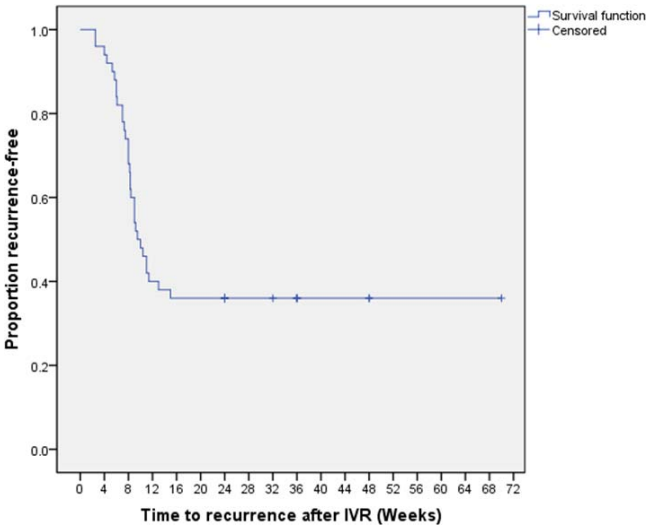


Figure 1. Kaplan-Meier recurrence-free survival curve of ROP treated with IVR. A steep slope of the curve indicates a sharp decrease of recurrence-free survival between 2.5 and 12.0 weeks after IVR.

Recurrent EFP posterior to zone III developed in 32 eyes at 7.9 ± 2.7 weeks (2.5 to 15.0 weeks) after IVR, or 43.1 ± 3.3 weeks at PMA (37.5 to 50.8 weeks). Cumulative recurrence rates at 4, 8, 12, and 16 weeks after IVR were 6% (3/50), 32% (16/50), 60% (30/50), and 64% (32/50), respectively. Major risk time of recurrence was 2.5 to 12.0 weeks after IVR and the recurrence-free survival curve declined the most rapidly during this period with an extremely steep slope covering 94% recurrence (30/32). Subsequently, the curve continued downward, albeit with a gentler slope, reaching a plateau by 15 weeks (Fig. 1). The hazard rate curve by life-table analysis showed that the major recurrence surge peaked at 8 weeks after IVR (Fig. 2).

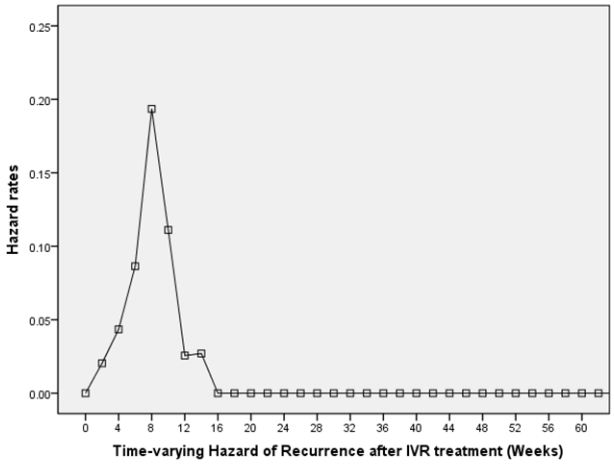
Distribution of recurrence sites over post-IVR time are shown in Figure 3. Recurrence involving original sites of retinal neovascularization occurred in eight eyes at 4.5 ± 1.4 weeks after IVR (2.5 to 7.0 weeks) (Fig. 4). Associated presentations were inadequate resolution of acute-phase ROP with ($n = 2$) or without increased fibrosis formation ($n = 2$), and persistence of avascular retina after initial resolution ($n = 4$). Recurrent EFP at a new vascular advancing edge occurred in 24 eyes at a significantly ($P < 0.001$, independent samples *t*-test) later time, with a mean of 9.1 ± 2.0 weeks after IVR (6.0 to 15.0 weeks). Return of pre-plus or plus disease was observed in 12 eyes (Fig. 5).

Risk Factors

A total of 12 potential factors entered the univariate analysis, and 4 were found statistically associated with ROP recurrence

Table 2. Time Points of Recurrence Status After IVR Monotherapy

Status	Eyes, *n* (%)	Mean Time After IVR ± SD (range)	Mean PMA ± SD (range)
Receiving IVR	50 (100)		35.5 ± 1.3 (32.0–38.0)
Initial resolution			
Plus	49 (98)	1.5 ± 0.6 (0.5–3.0)	37.0 ± 1.4 (33.0–39.8)
Neovascularization	46 (92)	3.4 ± 1.1 (1.0–6.0)	38.9 ± 1.6 (35.0–42.0)
Advancing of vessels	46 (92)		
Recurrence	32 (64)	7.9 ± 2.7 (2.5–15.0)	43.1 ± 3.3 (37.5–50.8)
Complete involution	18 (36)	15.4 ± 3.4 (11.0–23.0)	51.5 ± 3.5 (47.0–59.4)



Time after IVR (wk)	2	4	6	8	10	12	14	16
Eyes at risk of recurrence	50	48	44	37	25	20	19	18
Hazard rates (%)	2	4	9	19	11	3	3	0

FIGURE 2. Time-varying hazard rate curves by life-table analysis for recurrence of ROP treated with IVR. A recurrence surge reaches a maximum of 19% hazard rate at 8 weeks after IVR.

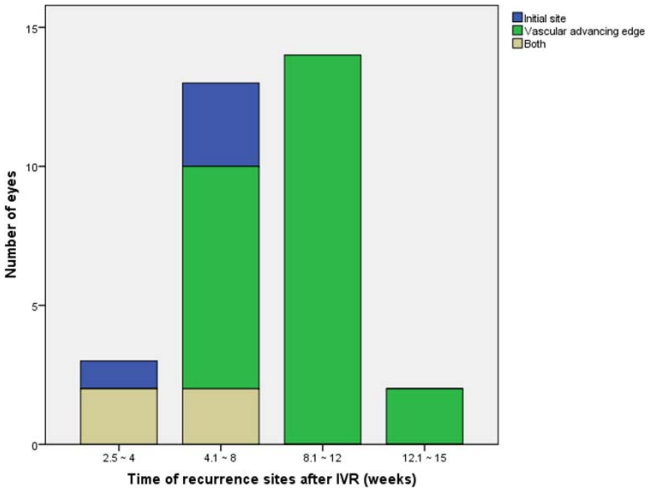


FIGURE 3. Histograms showing timing of different recurrence sites after IVR.

(Table 3). In multivariate regression analysis, two factors were identified significantly associated with ROP recurrence: larger extent of preexisting retinal neovascularization ($P = 0.005$) and oxygen requirement after IVR ($P = 0.016$). Every 1 clock-hour increase in the extent of retinal neovascularization increased the risk of recurrence by 2-fold. Patients who were more dependent on oxygen after IVR had an 11-fold increased risk of ROP recurrence compared with those independent of oxygen after IVR.

Kaplan-Meier recurrence-free survival with log-rank test was performed to compare the survival rates between eyes with preexisting retinal neovascularization $\geq$9 clock hours ($n = 24$) and those with neovascularization <9 clock hours ($n = 26$, subgrouped by the median 9 clock hours of preexisting retinal neovascularization of 50 eyes). Eyes with preexisting retinal neovascularization $\geq$9 clock hours had a significantly lower 24-week recurrence-free survival rate than those with <9 clock hours (15% and 58%, $P = 0.008$) (Fig. 6).

Discussion

In the present study, IVR was effective in curing type 1 ROP in posterior zones. Intravitreal ranibizumab induced rapid resolution of acute-phase ROP in most eyes, a part of which had complete involution of ROP with zone III vascularization. Intravitreal ranibizumab also provided an opportunity for laser retreatment of a less vascularly active retina, reducing the possibility of adverse outcomes.

However, our study showed that recurrence of ROP may occur after IVR monotherapy. The major recurrence risk period was from 2.5 to 12.0 weeks postinjection, with its risk peak at 8 weeks. The risk time not only covers the previously reported recurrence time[15,17,18] of 4 to 8 weeks after injection or approximately 41 to 42 weeks' PMA, but also indicates a prolonged follow-up with special vigilance in the specific risk time. According to our results, a follow-up by 8 weeks after IVR or 42 weeks at PMA could detect only half of the recurrences. However, a follow-up by 12 weeks after IVR, the endpoint of risk time, would be effective to observe 94% of recurrences. Meanwhile, no involution of ROP was observed before 11-week postinjection time in our study. Thus, an extremely vigorous follow-up in the first 12 weeks after IVR is indispensable for timely identification of ROP recurrence.

Besides the risk time of ROP recurrence, our study was also trying to determine an optimal follow-up length after IVR monotherapy. It was recently reported that ROP recurrence occurred at approximately 37 to 69 weeks' PMA after intravitreal bevacizumab (IVB) monotherapy.[5,14,16] The mean interval between IVB and retreatment ranged from 14.4 to 19.2 weeks.[5,14,16] Thus, a follow-up to at least 70 weeks' PMA until complete involution of ROP with vascularization of zone III was recommended for patients treated with IVB monotherapy.[16] Although there were single case reports[22,23] of very late ROP recurrence after IVB monotherapy or repeated injections, lack of frequent observations in eyes with persistent avascularity in peripheral zone II and zone III after IVB would account for the unfavorable outcomes. Our study also recommends a post-IVR follow-up until complete involution of ROP with vascularization of zone III to observe any possible recurrence, and the length of follow-up should be tailored according to individual response to IVR treatment. In our experience, ROP recurrence occurred before 15 weeks following IVR or 51 weeks at PMA. Earlier recurrence after IVR monotherapy than after IVB monotherapy may be related to the shorter half-life in vitreous of ranibizumab than that of bevacizumab (2.88 vs. 4.32 days, in animal models).[10,11,13] Meanwhile, complete involution of ROP was observed no later than 24 weeks postinjection or 60 weeks at PMA. According to our data, a post-IVR follow-up longer than 24 weeks after IVR monotherapy or 60 weeks at PMA would be much better than a shorter follow-up in management of ROP recurrence.

Recurrence of ROP after anti-VEGF therapy manifests as progressing EFP, associated with fibrosis along vessel endings, large anastomosis, and persistence of anterior vascularization.[14,16] Besides previous descriptions, we found that recurrence affecting initial neovascularization occurred approximately within the first 7 weeks after IVR, which was significantly earlier than recurrence at a new vascular advancing edge. Thus, we suggest that clinicians watch for any signs of recurrence affecting initial site of neovascularization in eyes with poor resolution of acute-phase ROP, increased fibrosis formation, and persistent avascular retina, especially in this period. These presentations may imply a suboptimal response of retina to a rapidly decreased VEGF level after IVR, possibly portending an early recurrence.[24] At a later period,

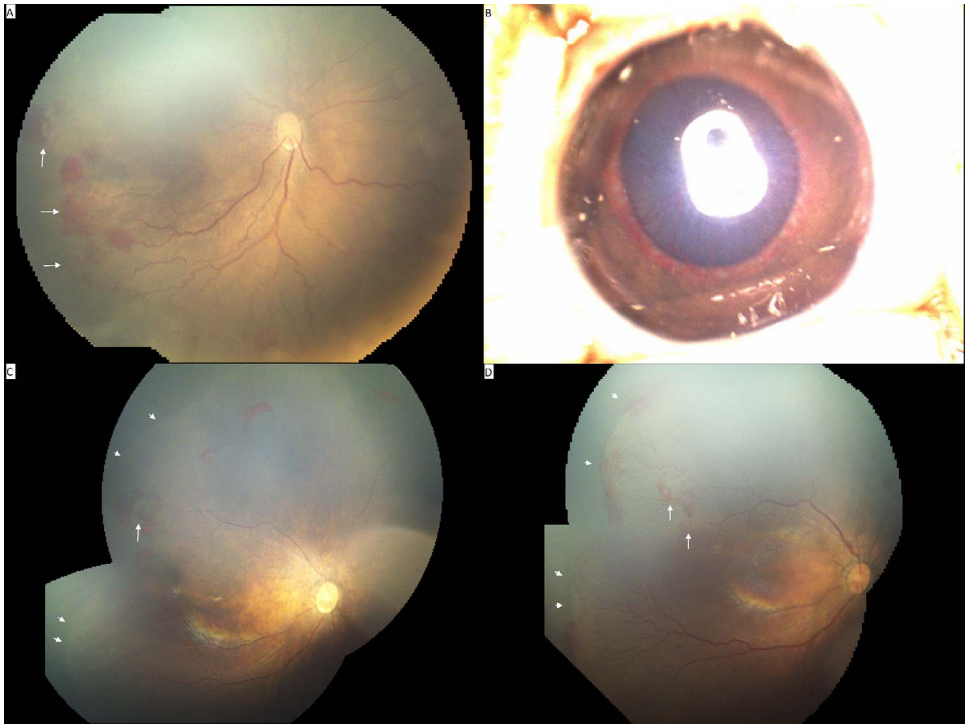


FIGURE 4. Montage fundus images and anterior segment image of the right eye of one patient (male, BW 1300 g, GA 30 weeks, AP-ROP in posterior zone II) with recurrence involving both initial site of neovascularization (*arrows* for initial vascular endings) and the new vascular advancing edge (*arrowheads*) after primary IVR. (**A**) Fundus image demonstrating extensive retinal neovascularization networks, through hazy media, with preretinal hemorrhage, at 34 weeks' PMA immediately before IVR. (**B**) Engorged iris vessels and anterior tunica vasculosa lentis before IVR. (**C**) Fundus image at 5.4 weeks after IVR showing incomplete resolution of acute-phase of ROP and advancing of retinal vessels. (**D**) Fundus image at 7.0 weeks after IVR showing recurrent EFP associated with return of preretinal hemorrhage. The *vertical arrows* highlighted the recurrence of the initial site of retinal neovascularization.

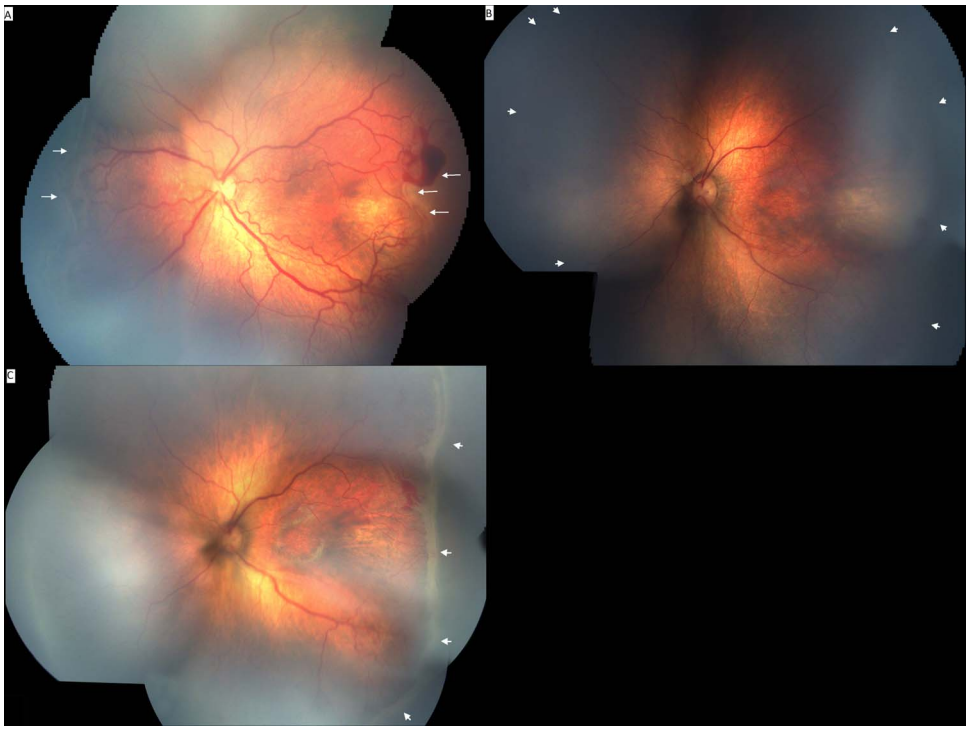


FIGURE 5. Montage fundus images of the left eye of one patient (male, BW 1245 g, GA 27 weeks, stage 3+ zone I ROP) with recurrence at a new vascular advancing edge (*arrows* for initial vascular endings, *arrowheads* for new vascular edge) after primary IVR injection. (**A**) Fundus image showing extensive retinal neovascularization with preretinal hemorrhage at 34 weeks' PMA immediately before IVR. (**B**) Fundus image at 5 weeks after IVR showing resolution of acute-phase of ROP and advancing of retinal vessels. (**C**) Fundus image at 9 weeks after IVR showing recurrent EFP associated with return of plus disease and preretinal hemorrhage.

Table 3. Univariate and Multivariate Logistic Regression Analysis of Risk Factors of Recurrent ROP

	Patients With Recurrence		Univariate Analysis		Multivariate Analysis	
Risk Factors	**Yes, $n = 32$ (%)**	**No, $n = 18$ (%)**	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
Clock hours of preexisting retinal neovascularization	10 ± 2	7 ± 2	1.979 (1.317–2.974)	0.001	2.011 (1.231–3.284)	0.005
Oxygen requirement after IVR	18 (56)	2 (11)	10.286 (2.020–52.364)	0.005	10.964 (1.554–77.365)	0.016
Oxygen requirement before IVR	28 (87)	10 (55)	5.600 (1.380–22.724)	0.016	3.212 (0.567–18.204)	0.187
PMA of IVR, wk	35.2 ± 1.3	36.0 ± 1.1	0.555 (0.324–0.954)	0.033	0.550 (0.270–1.118)	0.099
BW, g	1287 ± 306	1516 ± 351	0.998 (0.996–1.000)	0.027		
GA, wk	29.1 ± 1.7	30.0 ± 1.7	0.722 (0.501–1.041)	0.081		
Zone I	16 (50)	5 (28)	0.385 (0.111–1.332)	0.132		
Sex, male	21 (65)	8 (44)	0.419 (0.129–1.366)	0.149		
NRDS	9 (28)	2 (11)	3.130 (0.595–16.459)	0.178		
BPD	13 (40)	4 (22)	2.395 (0.642–8.931)	0.193		
Congenital heart defect	15 (47)	6 (33)	1.765 (0.531–5.865)	0.354		
Anemia	18 (56)	8 (44)	1.607 (0.502–5.141)	0.424		

CI, confidence interval; OR, odds ratio.

more attention should be given to recurrence at the vascular advancing edge rather than merely examining the posterior pole. Additionally, IVR monotherapy potentially allows for retinal vascularization in eyes with ROP, but there are possibilities for extreme preterm infants inherently having problems of full retinal vascularization owing to arrested advancing of retinal vascular precursors.[3,25] Thus, we treated all ROP recurrences with laser ablation instead of additional IVR, which may not promote retinal vascularization by repeated intravitreal injections.

The mechanisms of recurrent ROP after intravitreal anti-VEGF therapy may involve multiple factors.[3,16] To identify patients at high risk of recurrence can assist in making personalized strategies for follow-up and management. In this study of IVR monotherapy, larger extent of preexisting retinal neovascularization (especially ≥9 clock hours) and post-IVR oxygen requirement were found to be independent risk factors for recurrence. Extent of retinal neovascularization has been an important predictor for prognosis of ROP since the Cryotherapy for Retinopathy of Prematurity study,[26] in which five contiguous clocks or eight interrupted clocks of stage 3 + zone

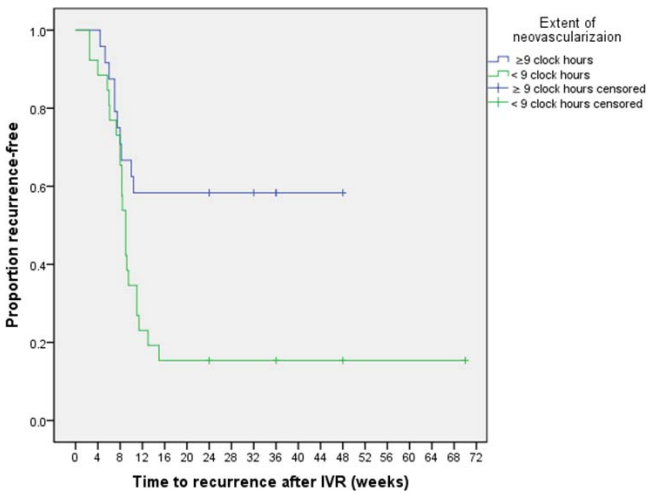


Figure 6. Kaplan-Meier recurrence-free survival curves with recurrent ROP after IVR as the endpoint. Eyes with preexisting retinal neovascularization ≥9 clock hours had a significantly lower 24-week recurrence-free survival rate compared with those with <9 clock hours (15% and 58%, $P = 0.008$, log-rank test).

II ROP was associated with a high risk of adverse anatomic outcomes. In a study[27] focusing on spontaneous involution of ROP, stage 3+ ROP extending more than 5 clock hours has been demonstrated to be independently associated with delayed involution. Also, AP-ROP being identified as a risk factor for recurrence after IVB in a recent study[16] supports our results, because retinal neovascularization in AP-ROP involves all four quadrants of retina. Another independent factor for ROP recurrence in our study is oxygen requirement after IVR, implying systemic and ocular hypoxia. When neovascularization regresses after an anti-VEGF therapy in phase 2 ROP, there may be a transient time of increased hypoxia of retina until anterior vascularization of avascular zones is completed.[3] A relatively systemic hypoxia during this period may exacerbate hypoxia of the retina, hamper retinal maturation, and induce relapse of VEGF levels in the retina, all of which increase the risk of recurrent neovascularization.[3,16,17] Additionally, IVR treatment at earlier PMA and oxygen requirement before IVR were found to be significantly associated with ROP recurrence in our univariate analysis but not in multivariate analysis. Therefore, we recommend more caution be taken in patients with independent risk factors of recurrent ROP during the post-IVR follow-up. Other risk factors of ROP recurrence after an anti-VEGF therapy reported previously include lower BW and extended duration of hospitalization, which are to some extent identical with our finding.[16] In our experience, infants with extremely low BW tend to be affected by severe ROP and be dependent on oxygen. Meanwhile, infants with an extended duration of hospitalization due to respiratory disorders are susceptible to be more oxygen-dependent.

The recurrence rate was substantial in our study, partly because we enrolled only patients with severe forms of type 1 ROP and poor systemic conditions. Our results cannot be generalized to other subforms of type 1 ROP. In fact, the recurrence rate after IVR monotherapy varied from 0% to more than 80% in previous retrospective studies with different inclusion criteria, recurrence definitions, and follow-up schedules.[6,15,17,18,28,29] Thus, a prospective study to investigate the exact incidence of recurrence after IVR monotherapy for different severity levels of ROP is required.

Major limitations of this study included its retrospective nature of collecting data for recurrence and FA not available in most cases. To minimize bias in patient selection, we excluded from this study the cases having rapid deterioration of ROP after IVR or prophylactic laser treatment for reasons other than recurrent EFP after IVR. Other limitations were a relatively

small sample being followed-up by no more than 70 weeks, and not enrolling recurrences after IVB for direct comparisons.

In conclusion, this study demonstrated that IVR is beneficial for type 1 ROP in posterior zones. Recurrence of ROP requiring treatment may be anticipated predominantly during a 15-week period after IVR monotherapy with a major risk time of 2.5 to 12.0 weeks and the recurrence surge peaks at 8 weeks. Recurrence involving initial site of neovascularization occurs significantly earlier than recurrence at a new vascular advancing edge. Thus, we suggest that physicians commence a long-term follow-up to observe any signs of recurrent ROP with special vigilance in the risk period after IVR, as well as in high-risk patients.

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