



# BMJ Open Study protocol for a multicentre, open-label, single-arm phase I/II trial to evaluate the safety and efficacy of ripasudil 0.4% eye drops for retinopathy of prematurity

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## ABSTRACT

**Introduction** Retinopathy of prematurity (ROP) is a vascular proliferative disorder that occurs in preterm infants. Existing treatments are only indicated in severe ROP cases due to the high invasiveness and the potential risk of irreversible side effects. We previously elucidated that ripasudil, a selective inhibitor of the Rho-associated protein kinase, has the ability to inhibit abnormal retinal neovascularisation in animal models. In addition, ripasudil eye drops (Glanatec ophthalmic solution 0.4%) have been already used for the treatment of glaucoma. Since eye drop therapy is less invasive, early intervention for ROP is possible. The purpose of this phase I/II trial is to evaluate the safety and efficacy of ripasudil eye drops for preterm infants with ROP.

**Methods and analysis** This is a multicentre, open-label, single-arm phase I/II trial. To evaluate the safety and efficacy of ripasudil as much as possible, ripasudil will be administered to all enrolled preterm infants with zone I/II, stage 1, or worse ROP. The safety and efficacy of ripasudil in treated patients will be assessed in comparison to a historical control group. Because this is the first trial of ripasudil in preterm infants, a dose-escalation study (once daily for 1 week, then two times per day for 2 weeks) will be conducted in phase I. After obtaining approval from the independent data and safety monitoring board to continue the trial after the completion of phase I, phase II will be conducted. In phase II, ripasudil eye drops will be administered two times per day for 12 weeks. The primary endpoint in phase II is also safety. Efficacy and pharmacokinetics will be evaluated as secondary endpoints.

**Ethics and dissemination** This study protocol was approved by the institutional review board at each of the participating centres. Data will be presented at international conferences and published in peer-reviewed journals.

**Trial registration numbers** NCT04621136 and JRCT2071200047.

## Strength and limitations of this study

- Ripasudil has already been used for glaucoma treatment, and no serious adverse events caused by ripasudil eye drops have been reported in adults.
- Treatment with eye drops is less invasive than existing therapies such as laser photocoagulation and intravitreal injection of anti-vascular endothelial growth factor agents.
- Phase II will only be conducted in the absence of safety issues of concern in phase I.
- The limitations of this trial are its open-label and single-arm design, as well as the small number of enrolled patients.

## INTRODUCTION

Retinopathy of prematurity (ROP) is a vision-threatening disease that occurs in preterm infants. Annually, more than 20 000 infants worldwide are diagnosed with irreversible visual impairment due to ROP.<sup>1</sup> Improvements in the management of preterm infants, such as lower oxygen saturation and postnatal nutritional intervention, have led to a decrease in the ROP onset in developed countries.<sup>2</sup> At the same time, however, improved maternal and neonatal management has also increased the survival rate of extremely preterm infants at high risk of developing severe ROP.<sup>3</sup> Younger gestational age and lower birth weight exacerbate the severity of ROP;<sup>4</sup> therefore, there is a risk that the incidence of severe ROP may increase in the future.

Preterm infants are vulnerable to stress and easily develop circulatory failure.<sup>5–8</sup> As the number of treatments for extremely preterm

infants with ROP is expected to increase, the development of a less invasive treatment option is needed. We have demonstrated that the Rho-associated protein kinase (ROCK) is activated in the mouse retina exposed to oxygen-induced retinopathy (OIR),<sup>9</sup> a widely used animal model of ROP.<sup>10</sup> We have confirmed that eye drops of ripasudil, a selective ROCK inhibitor, reduce the area of the ischaemic retina and inhibit abnormal retinal angiogenesis.<sup>9</sup> We have also shown that ROCK activated by vascular endothelial growth factor (VEGF), which is upregulated in the ROP retina, causes retinal inflammation by promoting the entry of inflammatory cells into the retina and activation of inflammatory cells.<sup>9 11</sup> These results indicate that ripasudil eye drops have the potential to inhibit VEGF-induced and cytokine-induced abnormal angiogenesis and promote normal angiogenesis.

Ripasudil eye drops (Glanatec ophthalmic solution 0.4%) have already been used for the treatment of glaucoma.<sup>12 13</sup> Ripasudil is rapidly metabolised and, therefore, is less likely to accumulate in the body. Results of pharmacokinetic studies in the plasma of adults have shown that the half-life values of ripasudil and its metabolite M1 (activity is 1/6th of ripasudil) were 0.5 hour and 2.6 hours, respectively. The long-term safety of ripasudil eye drops in adults has also been examined in several studies and there have been no reports of serious adverse events (SAEs).<sup>14–16</sup>

Eye drop therapy is less invasive compared with the existing treatment options such as laser photocoagulation (LPC) and intravitreal injection of anti-VEGF agents (anti-VEGF therapy).<sup>17–19</sup> This facilitates early intervention, which is a major advantage of the eye drop therapy. The indication for LPC or anti-VEGF therapy is defined as ‘type 1 ROP (stage 2 or 3 in zone II with plus disease, stage 3 in zone I with or without plus disease or stage 1 or 2 disease in zone I with plus disease)’ due to concerns about treatment invasiveness, post-treatment complications and recurrence.<sup>17 19–23</sup> If the infant’s general condition worsens, it becomes difficult to even perform an ophthalmic examination. Because the examination to decide the indication for an LPC or anti-VEGF treatment cannot be conducted, it is possible that the disease deteriorates to type 1 ROP. By contrast, eye drop therapy can be continued even if the examination cannot be performed and the ROP progression can be prevented. To establish the early therapeutic intervention with ripasudil eye drops, the safety and efficacy of ripasudil in premature infants need to be fully ascertained.

## METHODS AND ANALYSIS

### Study design

This is a multicentre, open-label, single-arm, investigator-initiated phase I/II trial to evaluate the safety and efficacy of ripasudil in preterm infants with ROP. Phase I is conducted at Kyushu University Hospital, and phase II is conducted at Kyushu University Hospital, Hospital of the

## Box 1 Inclusion and exclusion criteria

### Inclusion criteria

1. Informed consent signed by parents or legal guardians of the patient.
2. Patients born with  $\leq 32$  weeks of gestational age and/or  $\leq 1500$  g of birth weight.
3. Patients with the following types of ROP in both eyes:
  - Zone I ROP with stage  $\geq 1$ .\*
  - Zone II ROP with stage  $\geq 1$ .\*

### Exclusion criteria

1. Patients with aggressive posterior ROP in one or both eyes.
2. Patients with type 1 ROP† in one or both eyes.
3. Patients with a confirmed or suspected chromosomal abnormality or genetic disorder.
4. History of hypersensitivity to ripasudil (including drugs with similar chemical structure) in patients or their mothers.
5. Patients or their mothers have participated in another intervention study and have used the study drug of another intervention study within 30 days prior to enrolment in this study or within 5 times the half-life of the study drug.
6. Patients with an ocular structural abnormality that can affect the evaluation of this clinical study by investigators.
7. Patients with a clinically significant neurological disease (eg, intraventricular hemorrhage/haemorrhage grade 3 or higher, periventricular leukomalacia and severe hydrocephalus with significantly increased intracranial pressure).
8. Patients with inadequate blood access.
9. Patients with complications, allergies or worsening systemic conditions that make it difficult to conduct this trial.
10. Patients judged unsuitable by investigators.

ROP, retinopathy of prematurity.

\*Zones and stages are defined according to the International Classification of Retinopathy of Prematurity Revisited.<sup>24</sup>

†Type 1 ROP means stage 2 or 3 in zone II with plus disease, stage 3 in zone I with or without plus disease or stage 1 or 2 disease in zone I with plus disease.

University of Occupational and Environmental Health and Yamaguchi University Hospital.

### Eligibility criteria

After obtaining informed consent from patients’ parents or legal guardians, potential participants will be screened by investigators. The inclusion and exclusion criteria are shown in [box 1](#). The zone and stage of ROP are defined according to the International Classification of Retinopathy of Prematurity Revisited.<sup>24</sup>

### Endpoints

The primary endpoint of this study is the safety of ripasudil in preterm infants with ROP. Ophthalmic and systemic examinations, as well as clinical laboratory and physiology tests, will be performed to examine the incidence of local and systemic adverse events (AEs) and adverse drug reactions (ADRs). An AE will be registered even if the abnormality occurs in only one eye. Tolerability of ripasudil will also be assessed. The secondary endpoints are the efficacy and pharmacokinetics of ripasudil. The exploratory endpoints include the number of days until ROP worsens to type 1 ROP, the number of days

## Box 2 Evaluation items in this trial

### Primary endpoint

Proportion of patients with AEs and ADRs.

### Secondary endpoints

#### 1. Efficacy:

- Proportion of patients whose ROP worsens to type 1 ROP in one or both eyes (phase II).
- Proportion of patients with ROP remission in both eyes (phase II).

#### 2. Pharmacokinetics of ripasudil (phases I and II).

### Exploratory endpoints (phase II)

#### 1. Number of days until ROP worsens to type 1 ROP\* (phase II).

#### 2. Number of days until ROP remission† (phase II).

#### 3. Most advanced stage of ROP within the study period‡ (phase II).

ADR, adverse drug reaction; AE, adverse event; ROP, retinopathy of prematurity.

\*If both eyes reach type 1 ROP, the number of days in the eye that reached it first is used for the analysis.

†The number of days required for ROP remission in both eyes is used for the analysis.

‡If the most advanced stage is different between the left and right eye, the stage of the more advanced eye is used for the analysis.

until ROP remission and the most advanced stage of ROP within the study period. The details of each evaluation item are summarised in [box 2](#).

### Historical control

In order to collect as much information as possible on the safety and efficacy of ripasudil, all infants will receive the ripasudil eye drop therapy in this trial. The survey result of the medical records of 84 infants who underwent ROP screenings with ophthalmic examinations between April 2017 and November 2019 at the clinical trial institutions will be used as a historical control. Of the 84 patients, 27 (33%) patients developed type 1 ROP by 12 weeks after the onset of stage 1 ROP and received LPC or anti-VEGF therapy. To assess the efficacy of ripasudil, the results of this study will be compared with those of the historical control.

### Investigational drug

Glanatec ophthalmic solution 0.4% (Kowa Co., Japan) containing ripasudil hydrochloride hydrate will be used for this trial. Glanatec ophthalmic solution 0.4% was granted marketing approval for the treatment of glaucoma and ocular hypertension in Japan, Korea, Singapore and Malaysia in 2014, 2019, 2020 and 2020, respectively. The investigational drug and its safety information have been obtained from Kowa Co.

### Intervention

#### Phase I

The study schedule for infants participating from phase I is shown in [table 1](#). A total of three infants participating from phase I will receive one drop of ripasudil ophthalmic solution in both eyes, once daily for 1 week, followed by one drop in both eyes, two times per day for 2 weeks. At the end of the 3 weeks of treatment for the first

patient, a data and safety monitoring board (DSMB) will be held to decide whether the second and third patients can be enrolled into phase I. The DSMB will discuss the safety again at the end of the 3-week eye drop treatment for the third patient to determine if phase II can begin. In addition, the three infants who participate in phase I can continue to receive one drop in both eyes, two times per day for additional 9 weeks (12 weeks in total) if the investigators determine that there are no safety issues with ripasudil.

#### Phase II

The study schedule for infants participating from phase II is shown in [table 2](#). A total of 21 patients from three clinical trial institutions will receive one drop of ripasudil ophthalmic solution in both eyes, two times per day for 12 weeks. The safety and efficacy will be assessed at the end of the 12-week treatment period. The efficacy of ripasudil will be evaluated in comparison to the results of the historical control. In addition, another follow-up examination will be performed 4 weeks after the end of the treatment.

### AE and ADR reporting

The investigators will collect and record all AEs that occur in patients during the study and follow-up periods. ADR is defined as: (1) the AE disappears after treatment is discontinued, (2) the AE recurs after treatment is restarted, (3) no other risk factor can explain the occurrence of this AE, (4) a correlation between the dose and duration of the investigational drug administration and the AE occurrence can be established and (5) there is no rational basis that other treatments are responsible for the onset of this AE. The investigators will determine the AE severity according to the International Neonatal Consortium (INC) Neonatal AE Severity Scale V.1.0.<sup>25</sup> AEs will be defined as SAEs in the following cases: (1) death of the patient, (2) the occurrence of AEs that pose a risk of death, (3) the occurrence of AEs that leave the patient permanently disabled or (4) the occurrence of AEs that require hospitalisation or an extended period of hospitalisation.

### Rescue treatment

When the ROP exacerbates to the indicated stage of existing treatments (ie, type 1 ROP), ripasudil eye drops will be discontinued and the rescue treatment (LPC or anti-VEGF therapy) will be performed.

### Dose change and stop criteria

When it is determined that AEs of concern have occurred due to the investigational drug, the investigator will discontinue the drug administration or switch to once-daily administrations of ripasudil. Ripasudil administration will be discontinued in the following cases: (1) the investigators decide it is difficult to continue the clinical trial due to worsening of other diseases, (2) the investigators or DSMB decide it is difficult to continue the clinical trial due to AEs that do not disappear with withdrawal

**Table 1** Study schedule for patients participating from phase I

	Study period															
	Entry	Study period													Follow-up	Cessation
Visit	V1*	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14		
Timing (D=day, W=week)	D0	D1	D2	D4	D7	D8	W2	W3	W4	W6	W8	W10	W12	W16		
Entry																
Informed consent	○															
Eligibility screen	○	○														
Registration		○														
Intervention																
Phase I		○	○	○	○†	○	○	○	○	○	○	○	○	○		
Phase II								○‡	○	○	○	○	○	○		
Assessments																
Vital signs	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Height, weight and head circumference	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Slit-lamp examination	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Fundus examination	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Fundus camera	○									○	○	○	○	○	○	○
CBC/blood chemistry test	○															
Pharmacokinetics			○			○		○								
Urinalysis	○				○				○							
Imaging evaluation of the CNS	○															
AEs and ADRs		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○

\*V1 and V2 can be performed on the same day.

†After approval of DSMB, the dose of the eye drops will be increased from one to two times per day.

‡The principal investigator decides whether to continue the trial.

ADR, adverse drug reaction; AE, adverse event; CBC, complete blood cell count; CNS, central nervous system; DSMB, data and safety monitoring board.

**Table 2** Study schedule for patients participating from phase II

Visit	Entry		Study period										Follow-up		Cessation
	V1*	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	–	
Timing (D=day, W=week)	D0	D1	D2	D4	W1	W2	W3	W4	W6	W8	W10	W12	W16	–	
<b>Entry</b>															
Informed consent	○														
Eligibility screen	○	○													
Registration		○													
<b>Assessments</b>															
Vital signs	○	○	○	○	○	○	○	○	○	○	○	○	○	○	
Height, weight and head circumference	○							○		○			○	○	
Slit-lamp examination	○	○	○	○	○	○	○	○	○	○	○	○	○	○	
Fundus examination	○	○			○		○	○	○	○	○	○	○	○	
Fundus camera	○											○	○	○	
CBC/blood chemistry test	○												○	○	
Pharmacokinetics†		○	○	○	○	○	○	○	○	○	○				
Urinalysis	○				○			○					○	○	
Imaging evaluation of the CNS	○												○	○	
AEs and ADRs		○	○	○	○	○	○	○	○	○	○	○	○	○	

\*V1 and V2 can be performed on the same day.

†Blood samples for pharmacokinetic studies will be collected 3 times at any point during the study period. Blood will be drawn at any point up to 4 hours after ripasudil administration.

ADR, adverse drug reaction; AE, adverse event; CBC, complete blood cell count; CNS, central nervous system.

or reduction of the dose of the investigational drug, (3) parents or legal guardians of the patients request to discontinue the clinical trial or withdraw the consent and (4) the treatment progress of the patients is impossible to confirm due to the discontinuation of hospital visits or other reasons.

### Safety evaluation

The incidence of AEs and ADRs and tolerability of ripasudil will be evaluated up to 3 weeks in phase I and up to 12 weeks in phase II. Particular attention will be paid to the occurrence of AEs and ADRs that have a significant negative impact on circulatory and respiratory status, neurodevelopment and general condition.

### Efficacy evaluation

In phase II, as shown in [box 2](#), (1) the proportion of patients whose ROP worsens to type 1 ROP in one or both eyes and (2) the proportion of patients with ROP remission in both eyes in ripasudil-treated patients will be compared with the historical control group. The following exploratory endpoints will be also evaluated: (1) number of days until ROP worsens to type 1 ROP in patients receiving the rescue treatment, (2) number of days until ROP remission and (3) the most advanced stage of ROP

within the study period in patients presenting ROP remission without treatment. The outcome of each patient at the end of the treatment period (12 weeks after ripasudil administration) will be used for statistical analyses. Fundus photographs taken at study entry and at the end of the treatment period will be confirmed by the independent review committee to assess the validity of the zones and stages determined by the investigators.

### Pharmacokinetics

To examine the concentration of ripasudil and its metabolite M1, blood samples will be collected from patients in phases I and II. In phase I, the minimum plasma concentration ( $C_{min}$ ) at three points of each patient will be investigated to confirm the drug accumulation in the body. If aldehyde oxidase activity (metabolic enzyme of ripasudil) in preterm infants is low, there is a risk of ripasudil accumulation in the body of preterm infants. Therefore, we planned to measure the plasma concentration of ripasudil and M1 at 12 hours and 24 hours after administration. In phase II, population pharmacokinetics will be conducted with sparse sampling of each patient to obtain the profiles of absorption, distribution, metabolism and excretion. As shown in [table 1](#), blood will be collected in

phase I at visit 3 (24 hours after drug administration), visit 6 (12 hours after drug administration) and visit 8 (at the end of the treatment). In phase II, blood will be collected at any three points from visit 2 to 11 (table 2). The timing can be any time up to 4 hours after administration. Since the patient is a preterm baby, it is difficult to perform multiple blood collections from the same patient at short intervals. Therefore, blood samples will be obtained from multiple patients at different time points. Pharmacokinetic analysis will be performed using non-linear mixed effects modelling. We will estimate the contribution of covariates such as gestational age, postnatal age, postconceptional age and body weight.

For pharmacokinetic analysis, 800  $\mu$ L of blood will be collected from the subject. Peripheral blood samples are obtained by venipuncture. Non-nutritive sucking, facilitated swaddling and holding will be performed for relieving pain during sample collections.

Eye drops cannot be administered properly, especially to babies who are crying or fighting. Therefore, nurses and parents are instructed not to force eye drops into crying babies, but to apply eye drops when they are resting. In the case of failure to apply the drops correctly due to eyelid closure or other reasons, we will allow only one additional application within 30 min. We will keep a record of the time of eye drop application and share this information with pharmacokinetic analysis specialists. After the patients are discharged from the hospital, the parents will be asked to record the eye drop time in a logbook to monitor and confirm the drug administration.

### Trial period

Phase I was conducted between October 2020 and March 2021. Phase II started from April 2021 and will end in July 2022. The registration of patients for phase II will be closed in April 2022.

### Sample size

A total of 24 patients (3 in phase I and 21 in phase II) will be enrolled in this trial. Based on the past medical records, the number of new subjects per month who were considered to be eligible for this clinical trial was eight. Assuming a dropout rate of about 10% for medical record screening, a consent acquisition rate of approximately 35% and a dropout rate of about 15% after inclusion, approximately five cases were expected to be included during the planned registration period of phase I. In phase II, the number of newly eligible patients is expected to be approximately 95 and a total of approximately 25 cases can be expected to be included during the registration period.

### Statistical analysis

The following items will be tabulated as demographic and other baseline characteristics: institution, sex, gestational age, postmenstrual age, birth weight, presence of comorbidities, Apgar scores (1 min/5 min), stage and zone of ROP and presence of ROP plus disease.

For safety, the number and percentage of cases with AEs will be calculated. We will evaluate the efficacy of ripasudil by comparing the results of the ripasudil-treated and the historical control groups on the following five items:

1. Proportion of patients whose ROP worsens to type 1 ROP in one or both eyes.
2. Proportion of patients with ROP remission in both eyes.
3. Number of days until ROP worsens to type 1 ROP in patients receiving the rescue treatment.
4. Number of days until ROP remission.
5. The most advanced stage of ROP within the study period.

The analysis method for each of these items is shown below:

1. The normal approximation to the binomial distribution will be used to assess whether the proportion of patients who have reached type 1 ROP in the ripasudil-treated group is smaller than the proportion in the historical control group.
2. The normal approximation to the binomial distribution will be used to assess whether the proportion of ripasudil-treated patients with ROP remission is larger than the corresponding proportion in the historical control group.
3. If both eyes reach type 1 ROP, the time in the first eye to reach type 1 ROP is used for the analysis. The Kaplan-Meier method will be used to plot a survival curve and calculate the median, as well as the 25th and 75th percentile points, for the time to reach type 1 ROP. The two-sided 80% and two-sided 95% CIs will be calculated using the method of Brookmeyer and Crowley, respectively. The same analysis will be performed for the historical control group.
4. The time to ROP remission in both eyes will be used in this analysis. The Kaplan-Meier method will be used to create a survival curve and calculate the median, as well as the 25th and 75th percentile points, for the time to reach ROP remission. The two-sided 80% and two-sided 95% CIs will be calculated using the method of Brookmeyer and Crowley, respectively. The same analysis will be performed for the historical control group.
5. The proportions of stages that progressed the most in the study period will be calculated. If the most advanced stages of both eyes are different, the worse will be adopted for this analysis. Then, the percentage of subjects who fall into the following categories will be evaluated to be less than the percentage of the historical control group using the normal approximation method of binomial distribution:
  1. Progression from stage 1 to stage 2 or higher (subjects with stage 1 disease in both eyes at baseline).
  2. Progression from stage 1 to stage 3 (subjects with stage 1 disease in both eyes at baseline).
  3. Progression from stage 2 to stage 3 (subjects with stage 2 disease in the more severely affected eye at baseline).

In addition, the two-sided 80% and two-sided 95% CIs of the relevant number of subjects in the ripasudil eye drop and historical control groups will be calculated using the Wilson method.

### Patient and public involvement

Patients' parents and/or the public were not involved in the design of this study.

### ETHICS AND DISSEMINATION

This trial will be performed according to the ethical principles originating from the Declaration of Helsinki and the Good Clinical Practice guidelines. This trial was approved by the institutional review board of each institution. Informed consent will be obtained from parents or legal guardians of each patient before the registration. All data will be collected by investigators confidentially. We will present the results of this trial in international peer-reviewed journals and at international and national conferences. Approval for this study has been obtained from the IRB of Kyushu University Hospital (Approval No. 2020–767).

### DISCUSSION

The development of a less invasive treatment modality for ROP is urgently needed. The ultimate goal of this study is to expand the indication of Glanatec ophthalmic solution to ROP. This phase I/II will collect the information necessary to conduct phase III. The primary endpoint of this trial is safety. While the safety of ripasudil has been documented in adults,<sup>14–16</sup> it is not well evaluated in preterm infants. We are concerned about the occurrence of possible ADRs as described below.

Because the circulating blood volume of preterm infants is lower than that of adults, the plasma concentration of ripasudil in preterm infants needs to be evaluated. The concentration of ripasudil will not exceed the safety range that has been identified in non-clinical toxicity tests. Thus, it is unlikely that ripasudil eye drops will cause systemic negative effects in premature infants. However, respiratory depression and hypothermia can occur when the plasma concentration of ripasudil exceeds the safety range assessed in non-clinical studies using rats. We, therefore, should particularly pay attention to changes in vital signs.

In clinical trials conducted prior to approval, several ocular ADRs have been reported in adults. The most common ADR is conjunctival hyperemia (69.0%, 457 out of 662 patients), which is caused by vasodilation due to ROCK inhibition.<sup>26</sup> This hyperemia is transient and has been known to appear within 1 hour after instillations and persist for 1–3 hours.<sup>27 28</sup> The next common ADRs are blepharitis and allergic conjunctivitis. In a trial with two times per day doses over 52 weeks, their incidences were 17.8% (63 out of 354) and 15.3% (54 out of 354), respectively. On the other hand, the incidence rates of

these unwanted effects in an 8-week study were 0.3% (1 out of 308) and 0.3% (1 out of 308), respectively. In a postmarketing survey (mean observation period: 96±27 days), the incidences of conjunctival hyperemia, blepharitis and allergic conjunctivitis were 4.0% (122/3058), 0.8% (24/3058) and 1.4% (42/3058), respectively. These results indicate that the incidences of these ADRs are not expected to be high in this trial with its 12-week study period. However, preterm infants secrete less tear fluid than full-term infants,<sup>29</sup> which may lead to prolonged retention of ripasudil in the conjunctival sac. If so, there is a risk of an increased incidence of ocular ADRs. In adults, blepharitis and allergic conjunctivitis were both reversible and disappeared following treatment discontinuation. Therefore, if the onset of these ADRs is observed in this trial, the dose should be reduced or the drug discontinued immediately.

In a non-clinical study using dogs, lens opacity was observed along the suture line following administration of ripasudil eye drops at a high concentration for 13 weeks. The non-toxic dose in dogs is 10 times the clinical dose, so there is a sufficient safety range. However, the epithelial barrier function is weak in newborns.<sup>30</sup> Instilled drugs are either absorbed through the corneal or non-corneal route, and ripasudil is considered to be mainly absorbed through the corneal route.<sup>31</sup> Since the epithelial barrier function of the cornea and conjunctiva is reduced in newborns, the ripasudil concentration in the anterior chamber is possibly elevated. Okumura *et al* reported that ROCK inhibition promotes the adhesion of corneal endothelial cells, inhibits apoptosis and increases the number of proliferating cells.<sup>32</sup> A slit-lamp examination will be performed at all visits to exclude the occurrence of corneal endothelial abnormalities as well as a cataract. To examine the effect of ripasudil on ocular development, the fundus photographs will be evaluated by an independent image evaluation committee and the refractive values will be measured at the end of the trial.

The prevalence of ROP is low<sup>1</sup>; thus, the main limitation of this trial is, besides the lack of a placebo group, the small number of enrolled patients. To evaluate the safety and efficacy of ripasudil as much as possible, all patients will receive the investigational drug. The primary endpoint of this trial is safety. We aim to obtain the most information on safety as possible from a small population. If necessary, stratified analysis by gestational age or birth weight will be conducted. Another limitation is that OIR, which was used in the efficacy test of ripasudil, is not a disease model that fully reflects the pathology of human ROP. In this model, exposure to continuous hyperoxia (constant exposure of 75% O<sub>2</sub>) for 5 days from postnatal day 7 causes vaso-obliteration and relative hypoxia due to withdrawal from hyperoxia induces vasoproliferation.<sup>10</sup> In contrast, in humans, oxygen is often administered immediately after birth, and concentrations as high as 75% are not administered because high oxygen administration is associated with ROP severity and lung damage. Both the American Academy of Pediatrics and European

consensus guidelines recommend a target SpO<sub>2</sub> in the range of 90%–95%, and the minimum concentration of oxygen needed to maintain this range is given to preterm infants.<sup>33 34</sup> In addition, pathological angiogenesis occurs in OIR, but this disappears spontaneously with time. Therefore, OIR is a disease model that can only reproduce stage 2 of human ROP. Although the efficacy of investigational drugs for ROP is mainly tested using OIR, the results may not be completely extrapolated to human ROP. OIR has been widely used worldwide for ROP studies, but it has these limitations.

If we can confirm the safety and efficacy of ripasudil for ROP in this trial, we will continue to prepare for a phase III study. In parallel with this study, we also plan to conduct an observational study to verify the long-term safety of ripasudil. This study will follow the development of visual function and neurodevelopment in children up to the age of 3 years.

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