TABLE 1. Demographics of Infants with Retinopathy of Prematurity

Patient	Sex	Eye/Stage	Intravitreal Bevacizumab (mg)	Gestational Age (Weeks)	Body Weight at Birth (g)	Postmenstrual Age at Intravitreal Bevacizumab (Weeks)	Body Weight at Intravitreal Bevacizumab (g)	Time of Intravitreal Bevacizumab to Vitrectomy (Days)
1	Male	Right/5	—	24	753	—	1820	Not applicable
		Left/4A	0.5			36		2
2	Male	Right/4B	—	23	611	—	2490	1
		Left/4A	0.5			39		1
3	Female	Right/4A	0.5	23	492	37	1354	3
		Left/4A	0.5			37		Not applicable
4	Male	Right/5	0.5	24	332	38	1384	2
		Left/4A	0.5			38		2
5	Male	Right/4A	0.5	23	686	41	2098	2
		Left/3	0.5			41		Not applicable
6	Female	Right/3	0.5	26	826	33	1214	Not applicable
		Left/3	0.5			33		Not applicable
7	Female	Right/3	0.25	25	768	41	2600	Not applicable
		Left/4B	—			—		0
8	Male	Right/3	0.25	27	454	51	2151	Not applicable
		Left/3	0.25			51		Not applicable
9	Male	Right/3	0.25	26	828	35	1476	Not applicable
		Left/3	0.25			35		Not applicable
10	Female	Right/3	0.25	23	472	38	940	6
		Left/5	0.25			38		6
11	Male	Right/3	0.25	27	1042	32	1398	Not applicable
		Left/3	0.25			32		Not applicable

temic effects.[14,15] These adverse effects (for example, systemic thrombotic events and hypertension) are similar to the ones reported after intravenous administration of bevacizumab for cancer treatments. Although no systemic adverse event has been reported after intravitreal bevacizumab in eyes with ROP,[3,8,9,13] the serum concentration of bevacizumab after intravitreal bevacizumab has not been determined.

Thus, the purpose of this study was to determine the serum concentrations of bevacizumab and VEGF in ROP infants who received intravitreal bevacizumab.

METHODS

THE FUNDUS OF INFANTS WITH ROP WAS EXAMINED WITH a slit lamp and contact lens (Volk Quad Pediatric Lens; Volk Optical Inc, Mentor, Ohio, USA) under general anesthesia. During the examinations, fundus photographs and fluorescein angiograms were taken with a RetCam 120 digital fundus camera (Clarity Medical Systems, Inc, Pleasanton, California, USA). The stage of the ROP was based on the International Classification of Retinopathy of Prematurity.[16] The ROP eyes were also classified into 3 groups according to the vascular activity: highly vascular-active ROP, moderately vascular-active ROP, and mildly vascular-active ROP.[17] The eyes with highly vascular-active ROP initially received 0.25 mg or 0.5 mg of intravitreal bevacizumab and underwent 23-gauge pars plicata vitrectomy without cannula system. The surgery was performed within 1 week after the injection when considered to be necessary.[3]

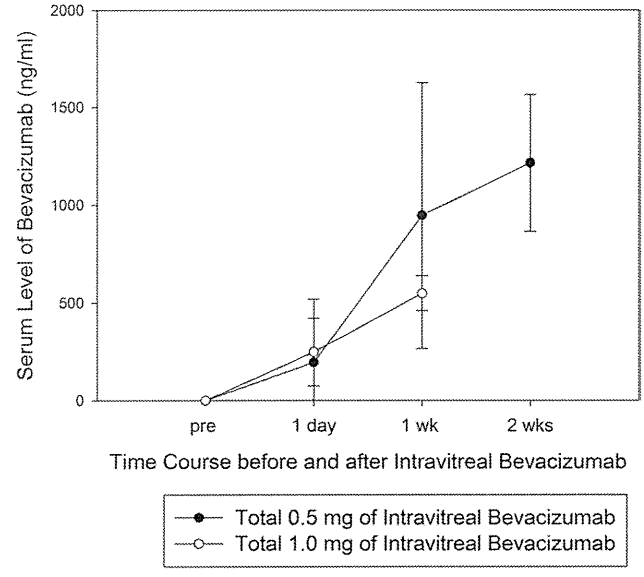

FIGURE 1. Time course of serum level of bevacizumab in infants with retinopathy of prematurity who received intravitreal bevacizumab. The abscissa represents the time before and after intravitreal bevacizumab and the ordinate represents the serum level of bevacizumab.

TABLE 2. Serum Levels of Bevacizumab (Avastin) and Vascular Endothelial Growth Factor in Infants With Retinopathy of Prematurity

Eye/Stage	Total Dosage of Intravitreal Bevacizumab (mg)	Serum Level of Bevacizumab (ng/mL)				Serum Level of Vascular Endothelial Growth Factor (pg/mL)			
		Before Intravitreal Bevacizumab	1 Day After Intravitreal Bevacizumab	1 Week After Intravitreal Bevacizumab	2 Weeks After Intravitreal Bevacizumab	Before Intravitreal Bevacizumab	1 Day After Intravitreal Bevacizumab	1 Week After Intravitreal Bevacizumab	2 Week Intrav Bevaci
Right/5 Left/4A	0.5	0	23	NA	NA	NA	NA	NA	N
Right/4B Left/4A	0.5	0	31	81	NA	NA	NA	NA	N
Right/4A Left/4A	1.0	0	206	665	NA	NA	NA	NA	N
Right/5 Left/4A	1.0	0	396	513	NA	NA	NA	NA	N
Right/4A Left/3	1.0	0	19	560	NA	NA	NA	NA	N
Right/3 Left/3	1.0	0	372	453	NA	NA	NA	NA	N
Right/3 Left/4B	0.25	0	11	113	NA	418	303	301	N
Right/3 Left/3	0.5	0	33	1204	NA	603	227	106	N
Right/3 Left/3	0.5	0	36	610	844	1140	515	208	12
Right/3 Left/5	0.5	0	841	1905	1255	2110	433	331	43
Right/3 Left/3	0.5	0	209	928	1542	2660	533	337	23

serum levels of bevacizumab and vascular endothelial growth factor in the blank cells could be not measured because of the limited sample volumes.

Blood samples were collected before and 1 day, 1 week, and 2 weeks after the intravitreal bevacizumab. This schedule was based on the data from animal experiments demonstrating that the maximum blood level of bevacizumab is achieved about 1 to 2 weeks after the intravitreal bevacizumab.[18–20] The blood samples were collected in sterile tubes by an anesthesiologist or a neonatologist and centrifuged at 5000 rpm for 10 minutes until a clear separation between serum and the cell components was seen. The serum was transferred to sterile tubes and stored at −80 C until the assay.

The serum concentration of bevacizumab was measured with an enzyme-linked immunosorbent assay (ELISA) kit (Protein Detector ELISA Kit; Kirkegaard & Perry Laboratories, Inc, Gaithersburg, Maryland, USA), according to the manufacturer's protocol and also according to an earlier report with slight modifications.[21] Briefly, microwell plates (Immuno 96 MicroCell solid plates; Nunc, Roskilde, Denmark) were coated with recombinant human $VEGF_{165}$ (PeproTech, Rocky Hill, New Jersey, USA) at a concentration of 1.0 μg/mL for 1 hour at room temperature (100 μL/well). After blocking the wells to reduce nonspecific binding, 100 μL of each sample and different concentrations of the standard were added to the plates. A standard curve was prepared with bevacizumab ranging from 1 ng/mL to 5000 ng/mL. The bound bevacizumab was made visible with 0.1 μg/mL of horseradish peroxidase–goat anti-human IgG (H+L) conjugate prepared by the ELISA kit. The optical density was determined at 405 nm with an absorption spectrophotometer ($ARVO_{MX}$; PerkinElmer Japan, Kanagawa, Japan). The background absorbance was subtracted from all values. This assay measures the free bevacizumab, and all measurements were performed twice according to the manufacturer's recommendation.

The serum concentration of VEGF was measured with an ELISA kit for human anti-VEGF (R & D Systems, Minneapolis, Minnesota, USA) according to the manufacturer's protocol. The anti-VEGF kit can detect the 121 and 165 isoforms of VEGF. The minimum detectable level of the test was 9.0 pg/mL for VEGF. The optical density was determined at 450 nm with the absorption spectrophotometer with the correction wavelength set at 540 nm. The assay was also performed in duplicate.

Statistical analyses were performed using the SPSS software (Sigma Stat; Systat Software, Inc, San Jose, California, USA). Data are presented as the means and standard deviations. If the data were normally and equally distributed, 1-way repeated-measures analysis of variance was used to compare 3 or more matched groups, followed by the Holm-Sidak method to detect significant differences between each set of data. If the data were not normally or equally distributed, Friedman repeated-measures analysis of variance on ranks was performed to compare 3 or more matched groups, followed by Dunn's method to detect significant differences between each set of data. The significance of differences between 2 groups was determined by *t* tests if the data were normally and equally distributed and by the Mann-Whitney rank sum test if not normally distributed. The correlation between 2 parameters was determined by the Spearman rank order correlation because the residuals were not normally distributed with constant variance. A *P* value less than .05 was considered to be statistically significant.

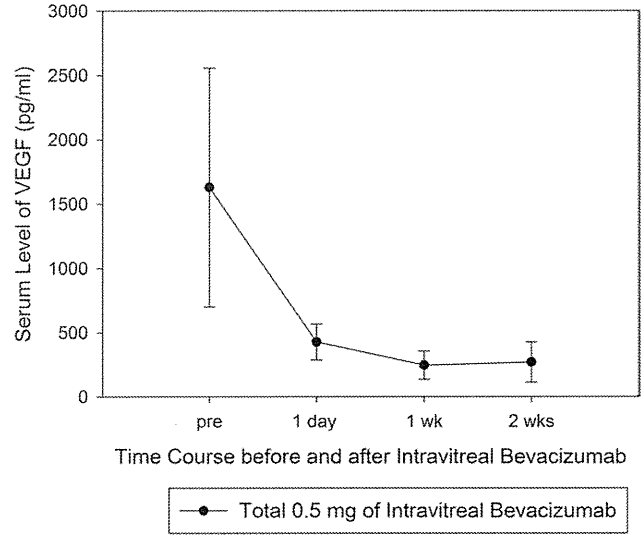

FIGURE 2. Time course of serum level of vascular endothelial growth factor (VEGF) in infants with retinopathy of prematurity who received a total of 0.5 mg of intravitreal bevacizumab. The abscissa represents the time before and after intravitreal bevacizumab and the ordinate represents the serum level of VEGF.

RESULTS

ELEVEN INFANTS (4 GIRLS AND 7 BOYS) WITH HIGHLY VASCULAR-active ROP were studied. The demographics of the patients are summarized in Table 1. Three patients received intravitreal bevacizumab in 1 eye and the other 8 received intravitreal bevacizumab in both eyes. The mean gestational age of the infants was 25 weeks (range, 23–27 weeks), and the mean body weight at birth was 660 grams (range, 332–1042 grams). All of the infants had received laser photocoagulation of the peripheral avascular retina before the intravitreal bevacizumab. The mean postmenstrual age of the infants at the time of intravitreal bevacizumab was 38 weeks (range, 32–51 weeks), and the mean body weight at the time of the intravitreal bevacizumab was 1720 grams (range, 940–2600 grams). In Patients 6, 8, 9, and 11 with stage 3 ROP, vitrectomy was not performed after the intravitreal bevacizumab because of the reduction of vascular activities. In the remaining patients, vitrectomy was performed 0 to 6 days after the intravitreal bevacizumab.

The average serum levels of bevacizumab before and 1 day, 1 week, and 2 weeks after a total of 0.5 mg of

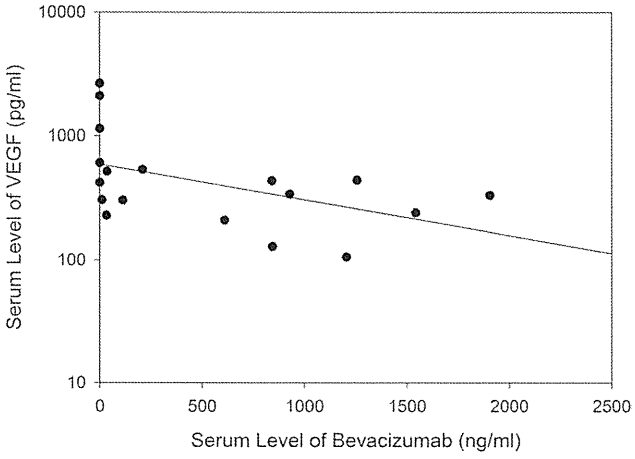

FIGURE 3. Correlation between bevacizumab and vascular endothelial growth factor (VEGF) levels in the serum of patients with retinopathy of prematurity (ROP). The abscissa represents the serum levels of bevacizumab and the ordinate represents the VEGF level in ROP infants. Statistical analyses were performed by Spearman rank order correlation ($r = -0.575$, $P = .0125$).

intravitreal bevacizumab were 0 ng/mL, 195 ± 324 ng/mL, 946 ± 680 ng/mL, and 1214 ± 351 ng/mL, respectively (Figure 1, Table 2). In Patients 2, 8, 9, 10, and 11, the serum bevacizumab levels were significantly different ($P = .008$) before and 1 day and 1week after the intravitreal bevacizumab, and the serum bevacizumab level at 1 week after the intravitreal bevacizumab was significantly higher ($P < .05$) than that before the intravitreal bevacizumab.

The average serum bevacizumab levels before and 1 day and 1 week after a total of 1.0 mg of intravitreal bevacizumab were 0 ng/mL, 248 ± 174 ng/mL, and 548 ± 89 ng/mL, respectively (Figure 1, Table 2). In Patients 3 through 6, the serum bevacizumab levels were significantly different ($P = .005$) before and 1 day and 1 week after the intravitreal bevacizumab. The serum bevacizumab level at 1 week after the intravitreal bevacizumab was significantly higher ($P < .05$) than that before the intravitreal bevacizumab. The differences in the serum bevacizumab levels after a total of 0.5 mg and 1.0 mg of intravitreal bevacizumab were not significant at any time points.

The average serum concentrations of VEGF before and 1 day, 1 week, and 2 weeks after a total of 0.5 mg of intravitreal bevacizumab were 1628 ± 929 pg/mL, 427 ± 140 pg/mL, 246 ± 110 pg/mL, and 269 ± 157 pg/mL, respectively (Figure 2, Table 2). The serum VEGF level in Patients 1 through 6 could not be measured because the volumes of the samples collected were too small. In Patients 8 through 11, who received a total of 0.5 mg of intravitreal bevacizumab, the serum VEGF levels were significantly different ($P = .005$) before and 1 day and 1 week after the intravitreal bevacizumab. The serum VEGF level at 1 week after the intravitreal bevacizumab was significantly lower ($P < .05$) than that before the intravitreal bevacizumab.

The correlation of serum levels of bevacizumab and VEGF was investigated in Patients 7 through 11, who received a total of 0.25 mg or 0.5 mg of intravitreal bevacizumab. The results showed that there was a significant negative correlation between the 2 levels ($r = -0.575$, $P = .0125$; Figure 3).

DISCUSSION

OUR RESULTS SHOWED THAT THE SERUM BEVACIZUMAB level was significantly higher at 1 week after than before the intravitreal bevacizumab in the ROP infants who received a total of 0.5 mg or 1.0 mg of intravitreal bevacizumab. In addition, the serum VEGF level was significantly lower at 1 week after than before the intravitreal bevacizumab in the ROP infants who underwent a total of 0.5 mg of intravitreal bevacizumab. Our results showed that there was a significant negative correlation between the serum levels of bevacizumab and VEGF in the ROP infants who received a total of 0.25 mg or 0.5 mg of intravitreal bevacizumab.

With regard to the serum bevacizumab level, animal experiments showed that the blood level of bevacizumab peaked at about 1 to 2 weeks after intravitreal bevacizumab.[18–20] In our patients, the serum level of bevacizumab was significantly increased 1 week after the intravitreal bevacizumab after a total of 0.5 mg or 1.0 mg of intravitreal bevacizumab, and the highest serum level of bevacizumab was achieved 2 weeks after the intravitreal bevacizumab after a total of 0.5 mg of intravitreal bevacizumab.

An in vitro experiment using human umbilical vein endothelial cells demonstrated that about 500 ng/mL of bevacizumab was able to completely block the VEGF activities.[22] Our results showed that the average serum level of bevacizumab exceeded 500 ng/mL at 1 week after the intravitreal bevacizumab in ROP infants who received a total of 0.5 mg or 1.0 mg of intravitreal bevacizumab. These results account for the decreased serum levels of VEGF after intravitreal bevacizumab in ROP infants. The results of an in vivo experiment (interview form for bevacizumab, Chugai Oncology, Tokyo, Japan; available only in Japanese) showed that a once-weekly intravenous injection of 2 mg/kg of bevacizumab in young macaque monkeys did not induce any obvious side effects 26 weeks after the beginning of the injections. The in vivo experiment demonstrated that the bevacizumab concentration in the serum 1 week after 1 intravenous injection of 2 mg/kg bevacizumab was over 10 μg/mL, which is much higher than the maximum serum level of bevacizumab in our study. Fortunately, the patients in our study did not show any systemic adverse events as far as our neonatologists

(K.W. and H.A.) could determine. However, a careful long-term study is necessary.

Our results also showed that the VEGF level significantly decreased 1 week after intravitreal bevacizumab, and the VEGF level was significantly correlated negatively with the serum bevacizumab level. These results suggest that bevacizumab escapes from the vitreous into the systemic circulation and reduces the VEGF concentrations in ROP infants after the intravitreal bevacizumab.

Data regarding the safe range of VEGF serum concentrations in premature infants with ROP have not been reported, although the systemic levels of VEGF in infants with or without ROP have been investigated.[23,24] Villegas-Becerril and associates[23] reported that at 4 to 6 weeks after birth, the mean serum VEGF concentration in premature babies with ROP was 708 pg/mL, which was significantly higher than the 511 pg/mL in premature babies without ROP. Pieh and associates[24] reported that the median plasma level of VEGF in ROP infants was 904 pg/mL at 32 weeks and 344 pg/mL at 36 weeks of postmenstrual age, and that in infants without ROP was 658 pg/mL at 32 weeks and 437 pg/mL at 36 weeks of postmenstrual age. The differences in the VEGF levels between ROP and non-ROP infants at both 32 and 36 weeks were not significant.[24]

The average serum VEGF level before the intravitreal bevacizumab in our ROP infants (Patients 7 through 11), whose average gestational age was 26.0 weeks, was 1386 pg/mL at an average postmenstrual age of 39.8 weeks. After the intravitreal bevacizumab, the average serum VEGF level was comparable to those of the 2 reports[23,24] for both ROP and non-ROP infants. Thus, the intravitreal bevacizumab did not induce an extreme inhibition of VEGF activities in ROP infants, although the serum VEGF level was significantly reduced 1 week after the 0.5 mg of intravitreal bevacizumab in ROP infants. Further studies are needed to determine the safe range of VEGF in ROP infants in order to establish the appropriate dose of intravitreal bevacizumab in ROP infants.

There are some limitations in this study. The number of patients was limited mainly because of the small number of infants with severe ROP. The number of blood samples at various time points was also limited because of the technical difficulties in obtaining blood samples from low-birth weight infants. These limitations made the statistical analyses difficult, and there is a possibility that the serum bevacizumab level may reach its maximal point more than 2 weeks after the intravitreal bevacizumab. Another limitation is that all of the infants had received laser photocoagulation to the peripheral avascular retina before the intravitreal bevacizumab. The laser photocoagulation may break down the retinal barrier.[25] Thus, although bevacizumab is a large molecule so that it has difficulty in escaping from the eye,[13] there is a possibility that the retinal photocoagulation led to the higher systemic levels of bevacizumab. In addition, the eyes with preoperative intravitreal bevacizumab received vitrectomy in Patients 1, 2, 3, 4, 5, and 10. Thus, the possible role of vitrectomy should be determined by either allowing the systemic diffusion of bevacizumab by opening the eye or decreasing it by washing out the intravitreal bevacizumab.

In conclusion, the serum levels of bevacizumab and VEGF were determined in vascularly active ROP infants who received intravitreal bevacizumab. The results suggest that bevacizumab escapes from the vitreous into systemic circulation and could suppress the VEGF concentration in infants with ROP after intravitreal bevacizumab. Although no systemic adverse events were observed in our patients, continued extensive evaluation of infants is warranted for possible effects after intravitreal bevacizumab in ROP patients.

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Biosketch

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Long-term efficacy and safety of ranibizumab administered *pro re nata* in Japanese patients with neovascular age-related macular degeneration in the EXTEND-I study

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ABSTRACT.

Purpose: **To evaluate the long-term efficacy and safety of ranibizumab administered *pro re nata* (PRN) in Japanese patients with choroidal neovascularization secondary to age-related macular degeneration during the extension phase of the EXTEND-I study.**

Methods: **EXTEND-I, an open-label, multicenter, Phase I/II study comprised: a single-injection (Group A); a multiple-injection (Groups A and B; the latter consisted of patients who did not participate in the single-injection phase); and an extension phase. In the extension phase, a PRN regimen of ranibizumab (0.3 or 0.5 mg) guided by monthly best-corrected visual acuity (BCVA) score and other ophthalmic examinations was employed. The efficacy variables included the mean BCVA change from Month 12 to the last visit in Group B. Safety was assessed in all patients.**

Results: **In the extension phase, efficacy was assessed only in Group B patients. The number of ranibizumab injections per year in the 0.3 and 0.5 mg Group B patients was 4.19 and 4.27, respectively. The mean BCVA change (SD) from Month 12 to the last visit was −3.6 (14.82) letters for 0.3 mg (n = 28) and −2.2 (7.92) letters for 0.5 mg groups (n = 33) in Group B. Conjunctival haemorrhage and nasopharyngitis were the most commonly reported adverse events. Of the 13 serious adverse events reported, cerebral infarction (two incidences) was suspected to be study-drug related.**

Conclusions: ***Pro re nata*** **regimen of ranibizumab guided by monthly BCVA and other ophthalmic examinations appears effective in sustaining the BCVA gained with 12 monthly injections while reducing the number of injections during the extension phase. Ranibizumab was well tolerated during the extension phase.**

Key words: age-related macular degeneration – best-corrected visual acuity score – efficacy – individualized flexible interval regimen – Japanese patients – PRN – ranibizumab – safety – subfoveal choroidal neovascularization

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Introduction

Age-related macular degeneration (AMD) is a leading cause of vision loss in the elderly population. Of the two types of AMD, the wet form caused by choroidal neovascularization (CNV) is mainly responsible for AMD-related vision loss (Bressler 2004). According to the Hisayama study (prospective cohort study in Japan), the prevalence of neovascular AMD in residents aged 50 years or older was 0.67% in 1998, which was lower than that observed in the Caucasians (Oshima et al. 2001). However, another recent study (The Funagata study) in Japanese residents aged 35 years or older suggested that the prevalence of neovascular AMD in Japanese men was similar to that seen in the Caucasian men (Kawasaki et al. 2008).

Current evidence points to the role of vascular endothelial growth factor (VEGF) in CNV proliferation, and hence agents that block its activity are considered as a suitable therapeutic intervention in the management of this form of AMD (Ferrara et al. 2006; Waisbourd et al. 2007). Ranibizumab (Lucentis®; Novartis Pharma AG, Basel, Switzerland and Genentech Inc, South San Francisco, CA, USA)

is a humanized monoclonal antibody fragment that inhibits active forms of VEGF-A, the main factor responsible for CNV proliferation and vascular permeability (Ferrara et al. 2006, 2007). Benefits of ranibizumab treatment in improving best-corrected visual acuity (BCVA) have been shown in the Caucasian population (Brown et al. 2006, 2009; Rosenfeld et al. 2006; Mitchell et al. 2010). Ranibizumab is currently approved in the United States, European Union, Japan and several other countries. EXTEND-I was the first study in Japanese patients that showed the safety and efficacy of monthly ranibizumab treatment (12-month results) during multiple-injection phase in terms of BCVA gain, reduction in total area of leakage from CNV plus retinal pigment epithelium staining and foveal retinal thickness, which were consistent with the pivotal studies performed in the Caucasian population (Tano & Ohji 2010). After the patients had completed the 12-month multiple-injection phase, all patients who provided written consent and were eligible based on the inclusion and exclusion criteria of the extension phase had the opportunity to continue to receive the 'individualized flexible interval regimen' [namely, *pro re nata* (PRN), as needed] until the approval of ranibizumab in Japan. This also provided a means to assess its long-term safety and efficacy. The PRN regimen was expected to maintain the improved visual acuity (VA) with less frequent injections in the extension phase. Current treatment guidelines in Europe recommend three initial monthly dosing followed by a maintenance phase, wherein the ranibizumab administration is decided based on monthly BCVA observation (Holz et al. 2010; Mitchell et al. 2010). This recommendation is based mainly on the results of the ranibizumab pivotal randomized phase III studies, namely MARINA (Rosenfeld et al. 2006) and ANCHOR (Brown et al. 2006) with monthly ranibizumab treatment. In these studies, the improvement of the BCVA score had stabilized (almost reached a plateau) by Month 3, and further increase in BCVA was minimal during the subsequent monthly treatments. On the other hand, in another pivotal randomized Phase IIIb study, PIER, quarterly treatment regimen could not maintain the improvement in BCVA score that was obtained by the three initial monthly injections (Regillo et al. 2008). However, there were also patients who maintained their gain in BCVA score during the quarterly regimen.

The extension phase of this study was initiated, therefore, to investigate whether ranibizumab administered PRN based on monthly BCVA scores and other ophthalmic examinations at two consecutive visits could maintain the improvement in BCVA scores. The reduction in dosing frequency was expected to reduce the risk of adverse events (AEs) associated with the intravitreal injection procedure in the elderly population as well as to address the difficulties in treating AMD through monthly injection of ranibizumab in a clinical setting.

Based on the 6-month interim results of the extension phase with PRN regimen as well as the 6- and 12-month interim analyses of monthly multiple-injection phase of this study, and the results of pivotal studies in the Caucasian population, ranibizumab was approved in Japan in January 2009. This paper presents the final data on long-term efficacy (in terms of BCVA) and safety of ranibizumab with PRN regimen from whole period of the extension phase of EXTEND-I.

Methodology

Study design

EXTEND-I was an open-label, multicentre, Phase I/II study comprising three phases: a single-injection phase, a multiple-injection phase and an extension phase (Fig. 1). The single-injection phase (Group A) was designed to sequentially evaluate the safety of intravitreal injections of 0.3 and 0.5 mg ranibizumab (six patients treated with each dose). The patients who successfully completed the single-dose phase (i.e., did not experience a Grade-3 targeted AE) could enter a multiple-injection phase wherein they received the same dose for an additional 11 months. The 12-month multiple-injection phase (Groups A and B; the latter consisted of patients who did not partic-

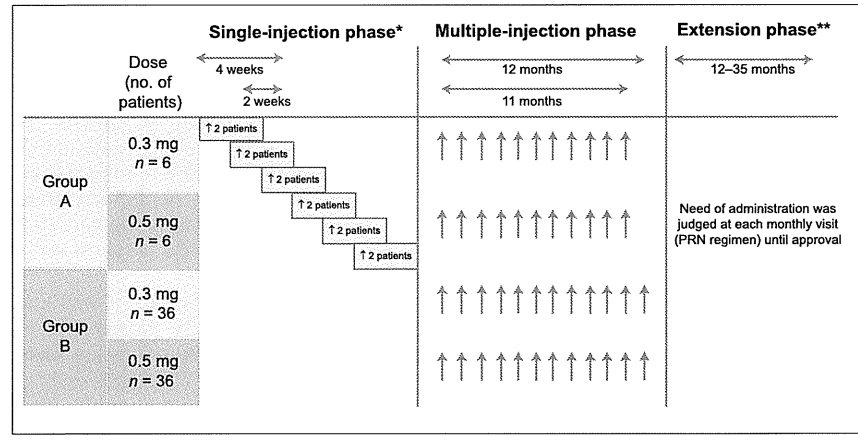

Fig. 1. EXTEND-I treatment schedule. *Upon completion of the single-dose phase, patients in Group A were eligible to enter the multiple-injection phase, which began ≥4 weeks after the final visit of the single-injection phase. Multiple injections did not begin until both doses were shown to be well tolerated in all cohorts. **Upon completion of the multiple dose phase, based on prespecified inclusion/exclusion criteria, patients could enter the extension phase. For the extension phase, treatment as per *pro re nata* regimen; dose same as core phase; retreatment at the monthly visit if loss of >5 letters in best-corrected visual acuity (BCVA) on two consecutive visits (except unscheduled visits), considering other ophthalmic examinations, such as slit-lamp examination, ophthalmoscopy, fundus photography, fluorescein angiography and optical coherence tomography, the investigator decided whether ranibizumab treatment would be performed. Similarly, if the BCVA score decreased on two consecutive visits (except unscheduled visits) by ≤5 letters using ETDRS-like visual acuity chart, a decision was taken whether treatment could be withheld. In any case, other ophthalmic examinations were taken into consideration. For the extension phase, the number of patients for Group A was 3 in the 0.3 mg group and 6 in the 0.5 mg group; the number of patients for Group B was 28 in the 0.3 mg group and 33 in the 0.5 mg group.

ipate in the single-injection phase) evaluated the safety and efficacy of both doses administered as monthly intravitreal injections in two parallel groups of 0.3 mg dose and 0.5 mg dose (Tano & Ohji 2010). The multiple-injection phase was followed by an extension phase in which the ranibizumab (0.3 or 0.5 mg) administration was on a PRN basis, but assessments were carried out on a monthly basis. If the BCVA score decreased at two consecutive visits (except unscheduled visits) by >5 letters, considering other ophthalmic examinations, such as slit-lamp examination and ophthalmoscopy for safety, fundus photography, fluorescein angiography and optical coherence tomography for efficacy, the investigator decided whether ranibizumab treatment would be administered although no specific retreatment criteria were provided for fundus photography, fluorescein angiography and optical coherence tomography and were at the discretion of the investigators. Similarly, if the BCVA score decreased at two consecutive visits (except unscheduled visits) by ≤ 5 letters, in conjunction with other ophthalmic examinations, a decision was taken whether the treatment could be withheld.

This study was conducted in accordance with the Declaration of Helsinki, International Conference on Harmonization Good Clinical Practice (GCP) guidelines and Japanese GCP. The study was approved by Institutional Review Boards at each study centre. All patients provided written informed consent before participating in the study and the extension. The trial is registered with clinicaltrials.gov (NCT00275821).

Inclusion and exclusion criteria

All patients with subfoveal CNV secondary to AMD who completed the multiple-injection phase in either of the ranibizumab groups (Groups A or B), provided written consent and met all of the inclusion criteria set at the beginning of the study (Tano & Ohji 2010) were eligible to enrol in the extension phase. Patients were allowed to participate in the extension phase regardless of the time elapsed between the exit visit of the multiple-injection phase and the participation in the extension phase.

Patients were excluded from the extension phase if they had received any anti-angiogenic drugs (bevacizumab, pegaptanib, ranibizumab, anecortave acetate, corticosteroids or protein kinase C inhibitors) or participated in any other clinical study of an investigational drug during the period from the exit visit of the multiple-injection phase to participation in the extension phase. However, as the extension phase was not started on the day of the exit visit from the multiple-injection phase, photodynamic therapy with verteporfin was allowed for the study eye during the transition period.

Efficacy assessments

The efficacy variables of the extension phase included mean change from Month 12 in BCVA score of the study eye using ETRDS chart (at a starting distance of 2 m) at the last visit of the extension phase for Group B patients only. Group A patients were not included as they were not assessed for efficacy, but only for safety throughout the study. The other efficacy variables included the proportion of patients at the last visit with a BCVA score loss <15 letters, and ≥ 30 letters, or a BCVA score gain of ≥ 15 letters in the study eye. Proportion of patients with BCVA <34 letters, approximate Snellen equivalent of 20/200 or worse, were also evaluated (ETDRS charts at a starting distance of 2 m). In the extension phase, colour fundus photography, fluorescein angiography and optical coherence tomography were performed in accordance with the routine procedures specified at each study site.

Safety assessments

All safety evaluations were based on the enrolled population (Groups A and B) of the extension phase. Safety assessments consisted of recording the frequency of the treatment collecting all AEs, serious adverse events (SAEs), with their severity, and relationship to study drug. It also included monitoring of haematology, serum chemistry, urinalysis and regular assessments of vital signs. Grade 3 targeted AEs (Tano & Ohji 2010), intraocular inflammation, myocardial infarction and stroke and AEs potentially related to systemic VEGF inhibition were analysed separately. Serum samples for the evaluation of immunoreactivity to ranibizumab (antiranibizumab antibodies) were obtained from patients prior to study administration at Month 23 and the last visit for Group A patients, and Month 24 and the last visit for Group B patients. At the last visit as well as at early termination, the assessments were performed if at least 6 months had passed since the previous measurement, on or after Month 11 for Group A patients and Month 12 for Group B patients. The last measurement in the multiple-injection phase of the study was performed at Month 11 for Group A and Month 12 for Group B.

Statistical analysis

The patient population included all enrolled patients in the extension phase. This population was used for all analyses in Groups A and B. All efficacy data presented were for observed cases without the last observation carried forward method.

Descriptive statistics of the number of injections, duration of exposure and reason of injection were presented for the enrolled population. The duration of treatment varied for each patient in the extension phase. To reduce a possible bias because of the patients who discontinued early without injection, the number of injections per year was calculated as $365.25 \times$ sum of total number of injections in the group/duration of the PRN regimen for the respective group. The number of injections per year was calculated for the respective group and not per patient. Duration of the PRN regimen was the date of the last potential treatment visit minus the date of Month 11 visit (the last treatment visit of multiple-injection phase) plus 1.

All efficacy analyses were based on the study eye. Descriptive statistics (mean, median, standard deviation, standard error, minimum and maximum) of the change from baseline (the single-injection phase of Group A and the multiple-injection phase of Group B), Month 11 and Month 12 in Group B were performed by treat-

ment and visit. The 95% confidence intervals based on *t*-distributions and p-values based on paired *t*-tests were determined for the change from baseline. Exact 95% confidence intervals were calculated for the proportion of patients with the specified response rates.

Results

Patients

Overall, 70 patients at 11 sites participated in the extension phase from 20 March 2007 to 20 January 2009: 9 in Group A (3 and 6 in the 0.3 and 0.5 mg dose groups, respectively) and 61 in Group B (28 and 33 in the 0.3 and 0.5 mg dose groups, respectively) as shown in Table 1. In Group A, a total of seven patients were not discontinued in the extension phase. Two patients in the 0.3 mg dose group withdrew from the study, as their condition did not further require the study drug. In Group B, 22 patients in the 0.3 mg dose group and 21 patients in the 0.5 mg dose groups were not discontinued in the extension phase. Six patients in the 0.3 mg dose group and 12 patients in the 0.5 mg dose group withdrew from the extension study. The maximum number of patients discontinued as they did not require the study drug because of improvement in VA (n = 9, two in the 0.3 mg dose group and seven in the 0.5 mg dose group); other reasons being AEs (n = 4, two in each dose group), withdrawal of consent (n = 4, one in the 0.3 mg dose group and three in the 0.5 mg dose group) and protocol violation (n = 1, one in the 0.3 mg dose group). None of the AEs leading to study discontinuation was thought to be related to the study drug.

The mean duration of treatment (standard deviation, SD) during the extension phase was 1.70 (0.35) years in the 0.3 mg group and 1.93 (0.09) years in the 0.5 mg dose group in Group A (Table 1). In Group B patients, the mean duration of treatment was 1.45 (0.33) years and 1.36 (0.39) years in the 0.3 and 0.5 mg dose groups, respectively.

The baseline demographic and ocular characteristics of enrolled patients at the start of the extension phase are given in Table 2. The mean (SD) BCVA score of the study eye at the start of the extension phase was 59.1 (11.69) letters and 59.8 (15.07) letters in the 0.3 and 0.5 mg dose groups of Group B, respectively. Overall, approximate Snellen equivalent VA of almost all patients was better than 20/200 except for two patients in the 0.5 mg dose group.

Of the 61 patients in Group B, approximately 90% (25/28 and 27/33 in the 0.3 mg and the 0.5 mg dose groups, respectively, Table 3) completed Month 24 from the baseline of the multiple-injection phase of the study, i.e., these patients received treatment of ranibizumab with PRN for 12 months in the extension phase. The duration of treatment of each patient in the extension phase varied with respect to the study entry and the longest was 35 months from baseline for the 0.3 mg dose group (n = 1). For the 0.5 mg dose group, the longest was 34 months (n = 1), as shown in Fig. 2.

Table 1. Patient disposition in the extension phase.

Disposition/patients studied	Group A Ranibizumab 0.3 mg	Group A Ranibizumab 0.5 mg	Group B Ranibizumab 0.3 mg	Group B Ranibizumab 0.5 mg
Patients (n %)				
Enrolled	3 (100.0)	6 (100.0)	28 (100.0)	33 (100.0)
Not discontinued	1 (33.3)	6 (100.0)	22 (78.6)	21 (63.6)
Discontinued	2 (66.7)	0 (0.0)	6 (21.4)	12 (36.4)
Main cause of discontinuation				
Adverse event (s)	0 (0.0)	0 (0.0)	2 (7.1)	2 (6.1)
Patient's condition does not requires study drug	2 (66.7)	0 (0.0)	2 (7.1)	7 (21.2)
Protocol violation	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)
Patient withdrew consent	0 (0.0)	0 (0.0)	1 (3.6)	3 (9.1)
Mean duration, years, of the extension phase (SD)	1.70 (0.35)	1.93 (0.09)	1.45 (0.33)	1.36 (0.39)

Table 2. Baseline demographics of enrolled patients and ocular characteristics (study eye) at the start of the extension phase.

Characteristic	Category/statistic	Group A Ranibizumab 0.3 mg N = 3	Group A Ranibizumab 0.5 mg N = 6	Group B Ranibizumab 0.3 mg N = 28	Group B Ranibizumab 0.5 mg N = 33
Gender – n (%)	Male	3 (100.0)	5 (83.3)	19 (67.9)	28 (84.8)
	Female	0 (0.0)	1 (16.7)	9 (32.1)	5 (15.2)
Age, years	Mean (SD)	68.0 (10.15)	72.0 (4.82)	69.8 (8.72)	70.2 (7.83)
Race (%)	Asian	3 (100.0)	6 (100.0)	28 (100.0)	33 (100.0)
Best-corrected visual acuity score	Mean (SD)	72.0 (4.58)	58.5 (15.66)	59.1 (11.69)	59.8 (15.07)
	Range	68–77	42–77	39–80	36–85
Approximate Snellen equivalent n (%)	Median	40.0	70.0	71.5	63.0
	20/200 or worse	0 (0.0)	0 (0.0)	0 (0.0)	2 (6.1)
	Better than 20/200 but worse than 20/40	1 (33.3)	3 (50.0)	20 (71.4)	20 (60.6)
	20/40 or better	2 (66.7)	3 (50.0)	8 (28.6)	11 (33.3)
Intraocular pressure (mmHg)	Mean (SD)	13.3 (1.53)	14.2 (3.06)	13.5 (2.92)	13.7 (3.09)
	Range	12–15	9–18	8–20	9–23

Data of ocular characteristics are based on Month 11 visit in Group A and Month 12 visit in Group B.
N = number of enrolled patients, n = number of patients.

Table 3. Summary of patient exposure to ranibizumab for 12 months (from Month 12 to Month 24) in the extension phase (Group B, enrolled patients).

Cumulative number of injections	Ranibizumab 0.3 mg (N = 28)	Ranibizumab 0.5 mg (N = 33)
Month 24		
n	25	27
Mean (SD)	4.1 (4.12)	3.9 (4.63)
Range	0–13	0–13
0	7	9
1–2	3	8
3–6	9	2
7–9	2	3
10–12	3	4
13	1	1
Number of injections per Year	4.19	4.27

The number of injections per year is calculated as: 365.25 × total number of injections/duration of the *pro re nata* (PRN) regimen.
Number of injections per year is calculated for total group, not per patient.
Duration of the PRN regimen: date of last potential treatment visit – date of Month 11 visit + 1.
N = number of enrolled patients, n = number of patients.

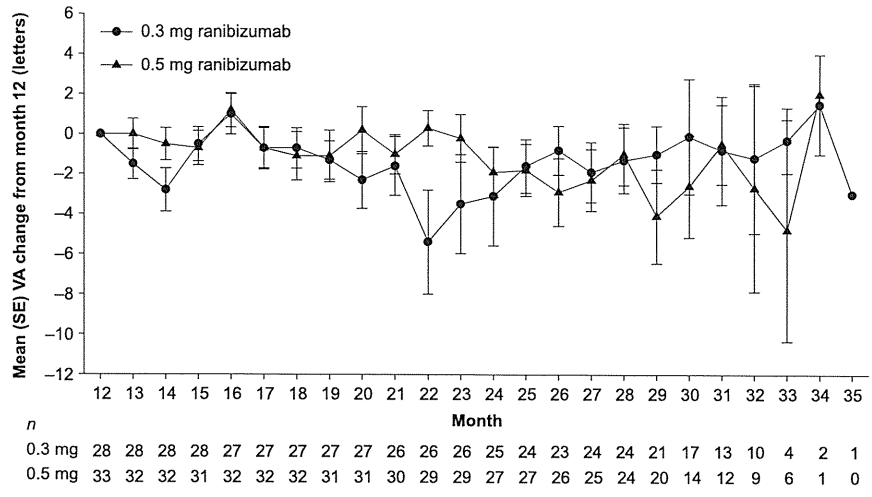

Fig. 2. Mean change from Month 12 (the start of Extension phase) in best-corrected visual acuity score (±SE) of study eye by visit during extension phase (Group B patients).

The exposure to ranibizumab in the extension phase of Group B is shown in Table 3. At Month 24, the patients had been treated with the PRN regimen for 12 months in the extension phase, and hence the maximum achievable number of injections by this visit was 13. The injection frequency of ranibizumab for individual patient varied from 0 to 13 times for this 12 months in the extension phase. The estimated number of injections per year in the extension phase was 4.19 and 4.27 in the 0.3 and 0.5 mg dose groups in Group B, respectively.

Efficacy

The mean change (SD) from Month 12 in BCVA score of the study eye to the last visit in the extension phase was −3.6 (14.82) letters in the 0.3 mg group and −2.2 (7.92) letters in the 0.5 mg group of Group B using the PRN regimen (Table 4). Furthermore, the mean change (SD) from baseline in BCVA score of the study eye to the last visit in the extension phase was 7.5 (19.12) letters in the 0.3 mg group and 7.7 (13.02) letters in the 0.5 mg group (p = 0.0475 for the 0.3 mg dose group and p = 0.0019 for the 0.5 mg dose group) (Table 4). Overall, the improvement in BCVA score at Month 12 by monthly ranibizumab injection was sustained throughout the extension phase with the PRN regimen (Fig. 2).

Table 5 shows the proportion of patients with respect to VA outcome at the last visit in the extension phase. The proportion of patients who lost < 15 letters from baseline in BCVA in the study eye was 85.7% (24/28) and 97.0% (32/33) in the 0.3 and 0.5 mg dose groups, respectively. Nine patients each in the 0.3 mg (32.1%) and 0.5 mg (27.3%) dose groups gained ≥15 letters from the baseline. One patient each in the 0.3 mg (3.6%) and 0.5 mg (3.0%) dose groups lost ≥30 letters from the baseline. The proportion of patients with approximate Snellen equivalent of 20/200 or worse was 14.3% (4/28) and 6.1% (2/33) in the 0.3 and 0.5 mg groups, respectively.

The mean time to first retreatment in the extension phase since Month 11 of the multiple-injection phase (when the last monthly injection was done) in Group B was 218.5 days (range: 29–512 days) for the 0.3 mg group and 255.6 days (range: 29–571 days) for the 0.5 mg group.

Safety

In Group A patient population (n = 9), two of three (66.7%) patients in the 0.3 mg dose group and three of six (50.0%) patients in the 0.5 mg dose group experienced at least one ocular AE in the study eye during the extension phase. In Group B, 20 of 28 (71.4%) patients in the 0.3 mg dose group and 18 of 33 (54.5%) patients in the 0.5 mg dose group experienced at least one ocular AE in the study eye during the extension phase. The most common ocular AE in the study eye in Group B was conjunctival haemorrhage. Other frequent ocular AEs included retinal haemorrhage, retinal detachment and increased intraocular pressure (Table 6). Two patients in the 0.3 mg dose group of Group B experienced Grade 3 targeted AEs (intraocular inflammation, reduced VA, increased intraocular pressure, vitreous haemorrhage, retinal tear or detachment, and retinal haemorrhage). One patient experienced retinal detachment, retinal haemorrhage and vitreous haemorrhage in the study eye, and the other patient experienced retinal haemorrhage in the fellow eye.

One patient in the 0.3 mg dose group of Group B experienced iritis in the study eye among the ocular AEs defined under the group of intraocular inflammation (iritis, iridocyclitis, vitritis, uveitis, hypopyon and anterior chamber inflammation). Two kinds of ocular AEs in six patients of Group B were suspected to be study-drug related: increased intraocular pressure (two patients in the 0.3 mg dose group and three patients in the 0.5 mg dose group) and retinal haemorrhage (one patient in the 0.5 mg dose group).

Table 4. Mean change from baseline in best-corrected visual acuity score of the study eye at the last visit in the extension phase (Group B, enrolled patients).

Visual acuity (letters)	Ranibizumab 0.3 mg $N = 28$	Ranibizumab 0.5 mg $N = 33$
Baseline		
Mean (SD)	47.9 (12.59)	50.0 (10.38)
Month 12 (start of extension phase)		
Mean (SD)	59.1 (11.69)	59.8 (15.07)
Last visit		
Mean (SD)	55.4 (17.14)	57.6 (15.36)
Change from baseline		
Mean (SD)	7.5 (19.12)	7.7 (13.02)
95% CI of the mean*	0.1, 14.9	3.0, 12.3
p-value†	0.0475	0.0019
Change from Month 12		
Mean (SD)	−3.6 (14.82)	−2.2 (7.92)
95% CI of the mean*	−9.4, 2.1	−5.0, 0.6
p-value†	0.2042	0.1186

Observed values are presented. Patients must have values at both Month 12 and last visit to be included. Baseline value is defined as the last available measurement prior to the first injection in the multiple-injection phase of the study. End of study differed between the patients and this was more evident from Month 30. Month 35 was the longest analysis point.
N = number of enrolled patients.
* Derived from t-distribution.
† Derived from paired t-test.

Nonocular AEs were observed in four patients (44.4%) in Group A (two each in the 0.3 and 0.5 mg dose groups), 19 patients (67.9%) in the 0.3 mg group and 24 patients (72.7%) in the 0.5 mg group in Group B. Nasopharyngitis was the most common AE in Group B patients (Table 6).

Adverse events potentially related to systemic VEGF inhibition were observed in four patients (14.3%) and two patients (6.1%) in the 0.3 and 0.5 mg dose groups of Group B, respectively. One patient in each dose group experienced cerebral infarction; three patients (0.3 mg dose group) and one patient (0.5 mg dose group) experienced hypertension. In Group A, AEs potentially related to systemic VEGF inhibition were observed in two patients in the 0.3 mg dose group (blood pressure increased and haematuria in one patient and hypertension in another patient).

Nonocular AEs suspected to be related to study drug were cerebral infarction, dementia and hypertension (one patient each) in 0.3 mg group, cerebral infarction and malaise (one patient each) in 0.5 mg dose group.

There were no deaths during the extension phase. Serious adverse events were reported for one of three (33.3%) patients in the 0.3 mg dose group and one of six (16.7%) patients in the 0.5 mg dose group in Group A, four patients (14.3%) in the 0.3 mg dose group and seven patients (21.2%) in the 0.5 mg dose group of Group B. Summary of ocular and nonocular SAEs is shown in Table 7. Of the SAEs, cerebral infarction (one patient each in the 0.3 and 0.5 mg dose groups of Group B) was suspected to be related to study drug and resolved with medical treatment in both patients. Four patients (two patients each from both dose groups) in Group B discontinued from the study because of SAEs. These SAEs that led to discontinuation were, however, not suspected to be study-drug related.

During the extension phase, immunoreactivity to ranibizumab (anti-ranibizumab antibodies) was not detected in patients of Group A; however, it was detected in two patients in the 0.3 mg dose group and one patient in the 0.5 mg dose group of Group B in the extension phase. In one patient in the 0.3 mg dose group, immunoreactivity to ranibizumab was detected at Month 12 (for the first time) and at study completion visit, but not at Month 24. In another patient in the 0.3 mg dose group, immunoreactivity to ranibizumab was detected at Month 24 (for the first time) and at study completion visit. In the 0.5 mg dose group, immunoreactivity to ranibizumab was detected in one patient at Month 12 (for the first time), Month 24 and at study completion visit. Of the three patients, AEs were reported in two patients. One patient in the 0.3 mg dose group experienced mild iritis as ocular AE and moderate glaucomatocyclitic crises as ocular SAE in the study eye as well as mild back injury and fall as nonocular AE. Iritis, back injury and fall were resolved without treatment and glaucomatocyclitic crises were resolved with medical treatment. One patient in the 0.5 mg dose group experienced both of conjunctival hyperaemia and intraocular pressure increased in the study eye, and both events were mild and resolved without treatment. All these events, except for intraocular pressure increased, were not suspected to be study-drug related.

Discussion

EXTEND-I was the first study with ranibizumab in Japanese patients with primary or recurrent subfoveal CNV secondary to AMD. The 6-month results indicated that monthly ranibizumab treatment significantly improved BCVA scores at Month 6 compared with baseline; the mean change (SD) observed was of +8.1 (12.65) letters and +9.0 (9.62) letters in BCVA score in the 0.3 and 0.5 mg dose groups, respectively. The improved BCVA scores at Month 6 were maintained until Month 12 by monthly treatment; the mean change (SD) observed was of +9.5 (12.79) letters and +10.5 (11.14) letters in BCVA score in the 0.3 and 0.5 mg dose groups, respectively. Monthly intravitreal injections of ranibizumab were shown to be safe and well tolerated over 12 months in Japanese patient population (Tano & Ohji 2010).

In the extension phase, the efficacy and safety of individualized flexible interval regimen (PRN regimen) of ranibizumab was assessed. In other words, the study consecutively investigated 12 monthly injections in the multiple-injection phase followed by the extension phase with PRN regimen guided by monthly BCVA score and by other ophthalmic examina-

Table 5. Best-corrected visual acuity (BCVA) of the study eye at the last visit in Group B (Enrolled patients).

BCVA	Ranibizumab 0.3 mg (N = 28)	Ranibizumab 0.5 mg (N = 33)
Loss of < 15 letters from baseline		
n (%)	24 (85.7)	32 (97.0)
95% CI of %*	67.3, 96.0	84.2, 99.9
Gain of ≥15 letters from baseline		
n (%)	9 (32.1)	9 (27.3)
95% CI of %*	15.9, 52.4	13.3, 45.5
Loss of ≥30 letters from baseline		
n (%)	1 (3.6)	1 (3.0)
95% CI of %*	0.09, 18.3	0.08, 15.8
Visual acuity < 34 letters		
n (%)	3 (10.7)	1 (3.0)
95% CI of %*	2.27, 28.2	0.08, 15.8
Approximate Snellen equivalent of 20/200 or worse		
n (%)	4 (14.3)	2 (6.1)
95% CI of %*	4.03, 32.7	0.74, 20.2
Approximate Snellen equivalent better than 20/200 but worse than 20/40		
n (%)	18 (64.3)	20 (60.6)
95% CI of %*	44.1, 81.4	42.1, 77.1
Approximate Snellen equivalent of 20/40 or better		
n (%)	6 (21.4)	11 (33.3)
95% CI of %*	8.30, 41.0	18.0, 51.8

* Derived from the exact confidence interval. Baseline value is defined as the last available measurement prior to the first injection in the multiple dose phase of the study; N = number of enrolled patients; n = number of patients.

tions, such as slit-lamp examination, ophthalmoscopy, fundus photography, fluorescein angiography and optical coherence tomography.

The estimated number of ranibizumab injections per year in the extension phase was approximately four injections in both the dose groups, which is equivalent to one-third of the maximally possible number of injections per year. The actual injection interval during the extension phase was not fixed and varied among patients and even in individual subject. Consequently, the PRN regimen with monthly monitoring resulted in considerably less frequent injections than a monthly regimen in this study. This seems to suggest that fixed monthly injection of ranibizumab is not necessary for all patients to maintain the improved VA gained through the initial monthly injections.

Results from the extension phase show a slight, but not significant, decrease in BCVA score when the regimen was switched from monthly injections to the PRN regimen. Thus, based on the mean change in BCVA scores in both the multiple-injection phase and the extension phase, the monthly regimen seems to be more effective in obtaining the best treatment outcome in VA than PRN regimen. However, continuous monthly injections are not feasible for many patients because of the physical and psychological burden and risk of AEs such as eye infections associated with the invasive intravitreal injection procedure.

Based on the results of the pivotal randomized Phase III studies, MARINA, ANCHOR and PIER, a drug and disease model with good agreement with study data was developed to simulate BCVA outcomes by individualized flexible VA-guided regimen following the initial three consecutive monthly injections of ranibizumab (Holz et al. 2010). Individualized flexible VA-guided regimen (administered if BCVA decreased by > 5 letters) is suggested to sustain initial BCVA gains following the initial three consecutive monthly injections of ranibizumab. According to the model prediction, it was recommended that patients should be monitored with monthly visits and further treatment should be considered if BCVA decreased by > 5 letters.

As discussed in the modelling and simulation study and as observed in the present study, slight decrease in BCVA was noted during the PRN regimen in the extension phase unlike the monthly treatment regimen. Because the concept of the PRN regimen is to treat in case of deterioration, especially a decrease in BCVA score, a corresponding decline in the BCVA curve over time is expected, i.e., the observed decline in BCVA during the extension phase is imminent to the PRN regimen concept.

As a guidance for retreatment during the PRN regimen, in this study, BCVA decrease by > 5 letters between two consecutive scheduled visits (including the current visit) was applied, so that the decision of retreatment at the current visit was made on the basis of changes calculated between BCVA scores of the last and current scheduled visit, taking the other ophthalmic conditions into account. On the other hand, in SAILOR and SUSTAIN, although the applied retreatment criterion of BCVA was the same as adopted in this study, the starting point of calculation was any previous visit wherein the BCVA score was the highest, especially in SUSTAIN the previous visit was limited to the first three months (Mitchell et al. 2010). Therefore, the decrease of BCVA score by > 5 letters was less likely to occur in this study than in both SAILOR and SUSTAIN. From this perspective, if the retreatment criterion based on the previous highest score is applied, it is speculated that both the number of injection and the BCVA score are apt to increase in comparison with the criterion based on the two consecutive scheduled visits. In both this study and SUSTAIN, monitoring of BCVA scores and other ophthalmic examinations was performed monthly in the same manner; the decline of the BCVA score in the 0.3 mg dose group from Month 12 in this study and from Month 3 in SUSTAIN was almost the same (decrease of 2–3 letters) on an average. Furthermore, in SUSTAIN, the number of retreatments in 9 months of maintenance phase with PRN regimen after three consecutive monthly injection was 2.7 on average, which translates into approximately four times per year. This estimated number of retreatments per year in the SUSTAIN study is roughly the same as the estimated number of injections per year in the extension phase with the PRN regimen of this study. Thus, the

Table 6. Summary of ocular and nonocular adverse events during the extension phase.

Preferred term	Group A: Ranibizumab, 0.3 mg $N = 3$	Group A: Ranibizumab, 0.5 mg $N = 6$	Group B: Ranibizumab, 0.3 mg $N = 28$	Group B: Ranibizumab 0.5 mg $N = 33$
Ocular				
Total, *n* (%)	2 (66.7)	3 (50.0)	20 (71.4)	18 (54.5)
Asthenopia	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
Cataract	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)
Conjunctival haemorrhage	1 (33.3)	3 (50.0)	12 (42.9)	11 (33.3)
Conjunctival hyperaemia	0 (0.0)	0 (0.0)	2 (7.1)	1 (3.0)
Conjunctivitis	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.0)
Conjunctivitis allergic	0 (0.0)	1 (16.7)	0 (0.0)	1 (3.0)
Dry eye	1 (33.3)	0 (0.0)	0 (0.0)	1 (3.0)
Eye pain	0 (0.0)	0 (0.0)	2 (7.1)	0 (0.0)
Glaucomatocyclitic crisis	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)
Injection site discomfort	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.0)
Intraocular pressure increased*	0 (0.0)	0 (0.0)	2 (7.1)	4 (12.1)
Iritis	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)
Maculopathy	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)
Myodesopsia	0 (0.0)	1 (16.7)	0 (0.0)	2 (6.1)
Ocular hypertension	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)
Punctate keratitis	0 (0.0)	0 (0.0)	1 (3.6)	1 (3.0)
Retinal detachment#	0 (0.0)	1 (16.7)	3 (10.7)	4 (12.1)
Retinal haemorrhage†	1 (33.3)	2 (33.3)	8 (28.6)	8 (24.2)
Retinal oedema	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)
Visual acuity reduced	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)
Vitreous haemorrhage	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)
Nonocular (> 5% in any group)‡				
Total	2 (66.7)	2 (33.3)	19 (67.9)	24 (72.7)
Colonic polyp	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)
Cough	0 (0.0)	0 (0.0)	0 (0.0)	2 (6.1)
Dental caries	0 (0.0)	0 (0.0)	1 (3.6)	2 (6.1)
Diabetes mellitus	0 (0.0)	0 (0.0)	3 (10.7)	0 (0.0)
Fall	0 (0.0)	0 (0.0)	1 (3.6)	2 (6.1)
Gastroenteritis	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
Hypertension	0 (0.0)	0 (0.0)	3 (10.7)	1 (3.0)
Nasopharyngitis	1 (33.3)	1 (16.7)	5 (17.9)	8 (24.2)

N = number of enrolled patients; n = number of patients.
* Five incidences in Group B (2 from 0.3 mg; 3 from 0.5 mg) are suspected to be study-drug related.
# Serous retinal detachment in all cases.
† One incident in 0.5 mg (Group B) is suspected to be study-drug related.
‡ Full list provided in Table S1.

influence of the starting point to calculate the decrease in BCVA score for retreatment criterion on the stabilization of BCVA score may not be so large. Apart from the difference in the starting point for the PRN regimen, the duration of consecutive monthly injection before start of PRN regimen in this study and SUSTAIN was different, i.e., 12 and 3 months, respectively, and the duration of the extension phase in this study (about one and half year) and that of maintenance phase in SUSTAIN (9 months) were also different, so that it seems to be difficult to simply compare the mean change in BCVA score between these two studies. Although the starting point to calculate the decrease of BCVA for retreatment criterion still remains to be investigated, it can be argued that a more stringent retreatment criterion may lead to better results, taking into consideration the best treatment outcome obtained by monthly injection.

Recently, based on the evidence available from prospective, multicentre studies evaluating different ranibizumab treatment schedules (ANCHOR, MARINA, PIER, PrONTO, SUSTAIN and EXCITE), it was summarized that the treatment initiation with three consecutive monthly injections of ranibizumab, followed by continued monthly injections, has provided the best VA outcomes in pivotal clinical studies (Mitchell et al. 2010). Furthermore, Mitchell et al. (2010) recommended that if continued monthly injections are not feasible after initiation, a flexible regimen may be adopted with monthly monitoring of lesion activity. The results from the extension phase with PRN regimen in EXTEND-I study are consistent with these clinical recommendations on ranibizumab treatment.

Regarding safety, the comparison between the multiple-injection phase and the extension phase is difficult as there were substantial differences between these two phases with regard to the duration, the number of patients and the number of injections. Although the mean duration of observation in the extension phase was longer than 12 months (1.45 and 1.36 years in the 0.3 and 0.5 mg dose groups, respectively), the incidence rate of ocular AEs appears to be lower than those during the 12-month multiple-injection phase (Tano & Ohji 2010). As the incidence

Table 7. Serious adverse events (SAEs) observed during the extension phase.

	Group A Ranibizumab 0.3 mg $N = 3$	Group A Ranibizumab 0.5 mg $N = 6$	Group B Ranibizumab 0.3 mg $N = 28$	Group B Ranibizumab 0.5 mg $N = 33$
Total, *n* (%)	1 (33.3)	1 (16.7)	4 (14.3)	7 (21.2)
Ocular SAE of study eye	1 (33.3)	0 (0.0)	2 (7.1)	0 (0.0)
Glaucomatocylitic crisis	0 (0.0)	0 (0.0)	1 (3.6)*	0 (0.0)
Macular degeneration	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
Retinal detachment	0 (0.0)	0 (0.0)	1 (3.6)†	0 (0.0)
Vitreous haemorrhage	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)
Ocular SAE of fellow eye	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.0)
Visual acuity reduced	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.0)
Nonocular SAE	0 (0.0)	1 (16.7)	2 (7.1)	6 (18.2)
Abscess neck	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.0) *
Cerebral infarction	0 (0.0)	0 (0.0)	1 (3.6)*	1 (3.0)*
Colon cancer	0 (0.0)	0 (0.0)	1 (3.6)†	0 (0.0)
Colon polyp	0 (0.0)	1 (16.7)*	0 (0.0)	0 (0.0)
Depression	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.0)†
Emphysema	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.0)
Enterocele	0 (0.0)	0 (0.0)	1 (3.6)*	0 (0.0)
Gastric cancer	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.0)†
Gastric polyps	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.0)*
Small cell lung cancer stage unspecified	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.0)†
Spondylitic myelopathy	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.0)*
SAEs causing discontinuation from study drug/study	0 (0.0)	0 (0.0)	2 (7.1)	2 (6.1)
Ocular SAE of study eye	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)
Ocular SAE of fellow eye	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Nonocular SAE	0 (0.0)	0 (0.0)	1 (3.6)	2 (6.1)

Both of gastric cancer and small cell lung cancer stage unspecified occurred in same patient of the 0.5 mg dose group.
N = number of enrolled patients; n = number of patients.
* SAE resolved by the last visit of the study.
† SAE led to discontinuation.

rate of conjunctival haemorrhage, conjunctival hyperaemia and eye pain in the study eye appears to be lower in the extension phase than those in the multiple-injection phase, these AEs are likely to be related to the intravitreal injection of ranibizumab and subconjunctival anaesthesia. Because the estimated number of ranibizumab injections per year was reduced by about one-third because of the PRN regimen in comparison with monthly regimen, there appears to be a relationship between the lower incidence of ocular AEs and reduction of number of injections. On the other hand, the incidence rate of nonocular AEs appears to be similar to those in the multiple-injection phase.

In conclusion, given the efficacy and safety profile observed in the extension phase, an individualized flexible interval regimen (PRN regimen) of ranibizumab, guided by monthly monitoring of BCVA score and other ophthalmic examinations, appears sufficiently effective and feasible in sustaining BCVA gained by consecutive monthly treatment and helps reducing the number of injections and treatment burden. Ranibizumab administered over the extension phase in Japanese patients with subfoveal CNV secondary to AMD was safe and well tolerated.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Number (%) of patients with nonocular adverse events by preferred term in Part B (Enrolled patients).

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Success Rates of Trabeculotomy for Steroid-Induced Glaucoma: A Comparative, Multicenter, Retrospective Cohort Study

KEIICHIRO IWAO, MASARU INATANI, AND HIDENOBU TANIHARA, ON BEHALF OF THE JAPANESE STEROID-INDUCED GLAUCOMA MULTICENTER STUDY GROUP

• PURPOSE: To evaluate the surgical outcomes of trabeculotomy for steroid-induced glaucoma.

• DESIGN: Multicenter, retrospective cohort study.

• METHODS: At 17 Japanese clinical centers, 121 steroid-induced glaucoma patients who underwent trabeculotomy between 1997 and 2006 were reviewed. Surgical failure was defined by the need for additional glaucoma surgery, deterioration of visual acuity to no light perception, or intraocular pressure ≥21 mm Hg (criterion A) and ≥18 mm Hg (criterion B). Surgical outcomes were compared with those of 108 primary open-angle glaucoma (POAG) patients who underwent trabeculotomy and 42 steroid-induced glaucoma patients who underwent trabeculectomy. Prognostic factors for failure were evaluated using the Cox proportional hazards model.

• RESULTS: The probabilities of success at 3 years for trabeculotomy for steroid-induced glaucoma vs trabeculotomy for POAG was 78.1% vs 55.8% for criterion A ($P = .0008$) and 56.4% vs 30.6% for criterion B ($P < .0001$), respectively. At 3 years, the success of trabeculotomy for steroid-induced glaucoma was comparable to trabeculectomy for steroid-induced glaucoma for criterion A (83.8%; $P = .3636$), but lower for criterion B (71.6%; $P = .0352$). Prognostic factors for failure of trabeculotomy for steroid-induced glaucoma were previous vitrectomy (relative risk [RR] = 5.340; $P = .0452$ on criterion A, RR = 3.898; $P = .0360$ for criterion B) and corticosteroid administration other than ocular instillation (RR = 2.752; $P = .0352$ for criterion B).

• CONCLUSIONS: Trabeculotomy is effective for controlling intraocular pressure <21 mm Hg in steroid-induced glaucoma eyes. (Am J Ophthalmol 2011;151: 1047–1056. © 2011 by Elsevier Inc. All rights reserved.)

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STEROID-INDUCED GLAUCOMA IS A FORM OF OPEN-angle glaucoma associated with various modalities of corticosteroid administration such as oral, intravenous, inhaled, ocular instilled, intravitreal, and periocular.[1–6] Some histologic studies have reported the accumulation of extracellular matrices including basement membrane–like material,[7–9] fine fibrillar-like material,[8] or proteoglycans[9] in the trabecular meshwork of steroid-induced glaucoma patients. These observations suggest that such accumulation could lead to an increased resistance to aqueous outflow in the trabecular meshwork of steroid-induced glaucoma patients.

Surgical procedures for intraocular pressure (IOP) reduction in eyes with steroid-induced glaucoma include trabeculectomy,[2,10,11] trabeculotomy,[4,12] viscocanalostomy,[13] and laser trabeculoplasty.[14–18] Although several case series have shown that these surgeries are effective for IOP reduction, surgical outcomes for steroid-induced glaucoma are not fully understood due to lack of large case-control studies aiming to investigate the success rates of trabeculotomy in steroid-induced glaucoma eyes. It has previously been reported that trabeculotomy more effectively reduces IOP in adult Japanese patients with exfoliative glaucoma than primary open-angle glaucoma (POAG).[19] This IOP-lowering effect in eyes with exfoliative glaucoma is thought to be attributable to the relief of abnormally increased outflow resistance that was induced by the accumulation of exfoliative material within the trabecular meshwork.

For the same reason, trabeculotomy has been the surgical procedure of choice for adult patients with steroid-induced glaucoma among Japanese surgeons.[20] We previously showed that trabeculotomy helped to reduce IOPs to 21 mm Hg or less in 14 Japanese patients with steroid-induced glaucoma.[12] However, large-scale, comparative clinical data remain elusive on, for example, whether trabeculotomy is more effective for steroid-induced glaucoma than POAG, whether trabeculotomy for steroid-induced glaucoma offers better IOP management than other surgeries such as trabeculectomy with mitomycin C (MMC), or which characteristics of patients with steroid-induced glaucoma exhibit better prognosis after trabeculotomy. To evaluate the surgical outcomes of trabeculotomy for steroid-induced

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TABLE 1. Patients With Steroid-Induced Glaucoma and Primary Open-Angle Glaucoma who Underwent Trabeculotomy

	SIG-LOT, n (%) (n = 121)	SIG-LET, n (%) (n = 42)	*P* Value	POAG-LOT, n (%) (n = 108)	*P* Value
Female	62 (51.2)	26 (61.9)	.232[a]	38 (35.2)	.014[a]
Right eye	62 (51.2)	22 (52.4)	.899[a]	50 (46.3)	.455[a]
Age (years), mean ± SD	38.4 ± 17.6	42.3 ± 17.9	.153[b]	45.2 ± 15.0	.001[b]
Preoperative IOP (mm Hg), mean ± SD	38.1 ± 10.0	35.6 ± 8.3	.169[b]	28.9 ± 8.4	<.001[b]
Combined sinusotomy	20 (16.5)	—	—	33 (30.6)	.012[a]
Previous cataract surgery	17 (14.0)	4 (9.5)	.626[c]	5 (4.6)	.029[c]
Previous vitrectomy	6 (5.0)	0 (0.0)	.320[c]	0 (0.0)	.054[c]
Diabetic mellitus	13 (10.7)	6 (14.3)	.736[c]	10 (9.3)	.709[a]
Hypertension	18 (14.9)	8 (19.0)	.695[c]	15 (13.9)	.832[a]
Cause of corticosteroid use					
Atopic dermatitis	21 (17.4)	4 (9.5)	.335[c]		
Uveitis	25 (20.7)	11 (26.2)	.457[a]		
Collagen disease	37 (30.6)	17 (40.5)	.240[a]		
Route of administration					
Ocular instillation only	17 (14.0)	12 (28.6)	.591[a]		
Posterior sub-Tenon's injection of TA	13 (10.7)	1 (2.4)	.178[c]		
Intravitreal injection of TA	10 (8.3)	0 (0.0)	.121[c]		
Oral administration	72 (59.5)	26 (61.9)	.784[a]		
Intravenous administration	3 (2.5)	2 (4.8)	.826[c]		
Corticosteroid administration for more than 3 months after surgery	68 (56.2)	25 (59.5)	.708[a]		

IOP = intraocular pressure; POAG-LOT = primary open-angle glaucoma patients who underwent trabeculotomy; SD = standard deviation; SIG-LET = steroid-induced glaucoma patients who underwent trabeculectomy with mitomycin C; SIG-LOT = steroid-induced glaucoma patients who underwent trabeculotomy; TA = triamcinolone acetonide.

[a] *P* values are based on the χ^2 for independence test.

[b] *P* values are based on Mann-Whitney *U* test.

[c] *P* values are based on the χ^2 for independence test with Yates' correction.

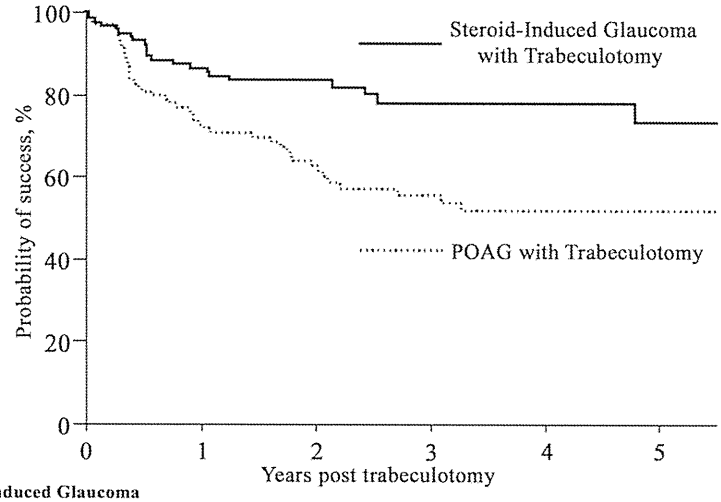
glaucoma, we retrospectively reviewed clinical charts at 17 clinical centers in Japan.

METHODS

• PATIENT SELECTION AND SURGICAL PROCEDURES: We retrospectively reviewed the medical records of patients with steroid-induced glaucoma who underwent trabeculotomy or trabeculectomy with MMC and those with POAG who underwent trabeculotomy between January 1, 1997, and December 31, 2006, at the following 17 clinical centers in Japan: Kumamoto University Hospital (Kumamoto), Niigata University Medical and Dental Hospital (Niigata), University of Tokyo Hospital (Tokyo), Kanazawa University Hospital (Kanazawa), Gifu University Hospital (Gifu), Kagawa University Hospital (Miki), University of Yamanashi Hospital (Chuo), Tohoku University Hospital (Sendai), Ryukyu University Hospital (Nishihara), Kyoto Prefectural University Hospital (Kyoto), Kagoshima University Medical and Dental Hospital (Kagoshima), Kyoto University Hospital (Kyoto), Nagoya City University Hospital (Nagoya), Saga University Hospital (Saga), Kobe University Hospital (Kobe), Hiroshima University Hospital (Hiroshima), and NTT West Kyushu Hospital (Kumamoto).

Eyes that presented with an IOP ≥22 mm Hg while on ocular hypotensive medications before surgery were included in this study. Steroid-induced glaucoma eyes were defined as open-angle eyes with an IOP elevation ≥22 mm Hg after the administration of corticosteroid. If both eyes underwent glaucoma surgeries, the eye that was treated first was investigated. Exclusion criteria were as follows: eyes with a history of previous glaucoma surgery, eyes that had undergone intraocular surgery up to 3 months before trabeculotomy or trabeculectomy, steroid-induced glaucoma eyes in the active phase of uveitis, eyes associated with IOP ≥22 mm Hg before corticosteroid administration in the medical records, and eyes that underwent combined glaucoma and cataract surgeries.

The technique of trabeculotomy performed in this study has been described previously.[19] In brief, after conjunctival incision, a 4 × 4-mm square or triangular scleral flap at four-fifths thickness was created at the corneal limbus. After identification of the Schlemm's canal, its outer wall was cut with a razor blade and excised with fine scissors. U-shaped probes were then inserted into both ends of the opened canal and rotated 90 degrees against the trabecular



Steroid-Induced Glaucoma											
No. at risk	121	121	100	89	77	63	42	32	26	22	20
Failure	0	8	7	3	0	2	1	0	0	0	1
Censored	0	13	4	9	14	19	9	6	4	2	5
POAG											
No. at risk	108	108	84	72	67	47	38	32	26	25	18
Failure	0	20	8	3	6	4	1	2	0	0	0
Censored	0	4	4	2	14	5	5	4	1	7	3

FIGURE 1. Criterion A–based Kaplan-Meier survival curves of surgical outcomes in patients with steroid-induced glaucoma (solid line) vs primary open-angle glaucoma (POAG; dotted line) that underwent trabeculotomy. The steroid-induced glaucoma eyes had a significantly higher cumulative probability of success than the POAG eyes ($P = .0008$).

meshwork. Rotation of these probes achieved 120-degree opening of the trabecular meshwork. The scleral flap was closed with 1 to 7 10-0 nylon sutures until the wound became watertight.

During trabeculotomy, some cases were combined with a sinusotomy, based upon the procedure of Mizoguchi and associates,[21] which made 1 or 2 sites of 1-mm-diameter sclerotomy with a punch through the scleral flap before closure with 10-0 nylon sutures. Trabeculectomy was performed according to a modification of the technique developed by Cairns.[22] Conjunctiva incisions included limbal-based and fornix-based procedures. After the creation of a scleral flap, sponges soaked with MMC (0.4 mg/mL) were applied to the posterior surface of the conjunctiva, Tenon's capsule, the adjacent episcleral tissue, and the scleral flap for 2 to 5 minutes, followed by irrigation with balanced salt solution. A trabecular block was excised to create a fistula in the anterior chamber, and peripheral iridectomy was then performed. The scleral flap was closed with 10-0 nylon sutures while the conjunctival flap was sutured with 10-0 nylon or 7-0 silk. All patients were required to sign informed consent forms before surgery.

• MAIN OUTCOME MEASURE: The main outcome measure was the probability of success in the Kaplan-Meier survival-curve analysis. Before data analysis, surgical failure was defined by the following IOP levels, with or without ocular hypotensive medications, which were verified at the next visit: criterion A, IOP ≥21 mm Hg; criterion B, IOP ≥18 mm Hg. IOP data that were examined using a Goldmann applanation tonometer were collected from patients' medical records. IOPs that corresponded to criteria A and B up to 3 months after surgery were not considered a surgical failure because of the occurrence of postoperative IOP fluctuations after trabeculotomy.[19] If additional glaucoma surgery was performed, or visual acuity deteriorated to an absence of light perception, the eye was regarded as a surgical failure for both criteria.

We compared the surgical outcomes between the steroid-induced glaucoma with trabeculotomy group and the POAG with trabeculotomy group, and between the steroid-induced glaucoma with trabeculotomy group and the steroid-induced glaucoma with trabeculectomy group. To determine potential risk factors for surgical failure of steroid-induced glaucoma after trabeculotomy, the following variables were assessed: gender, age, pseudophakia, previous vitrectomy, route of corticosteroid administration (ocular instillation, intravitreal injection, posterior sub-Tenon's injection, or systemic administration), duration of corticosteroid administration after glaucoma surgery, reason for corticosteroid use (collagen disease, atopic dermatitis, or uveitis), sinusotomy, previous cataract surgery, and baseline IOP. These factors were analyzed statistically in the steroid-induced glaucoma with trabeculotomy group with criteria A and B. Data on postoperative complications were also collected from the medical records.

• STATISTICAL ANALYSIS: Data analysis was performed using the JMP version 8 statistical package program (SAS