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.

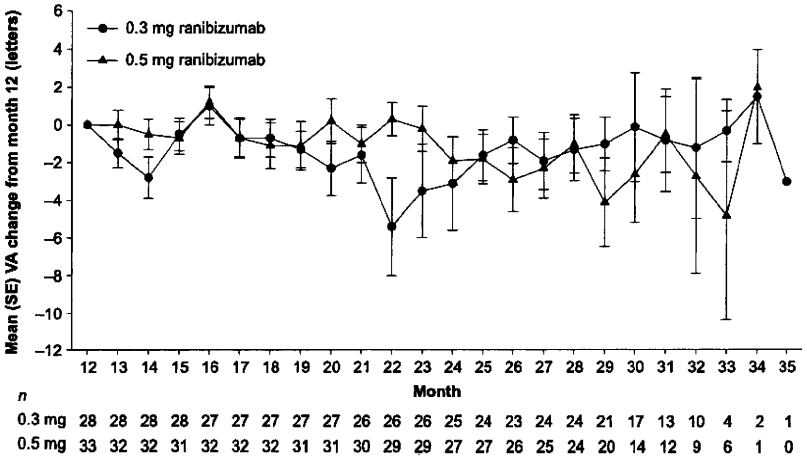

Fig. 2. Mean change from Month 12 (the start of Extension phase) in best-corrected visual acuity score (±SE) of study eye by visit during extension phase (Group B patients).

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).

Nonocular AEs were observed in four patients (44.4%) in Group A (two

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.

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 examinations, such as slit-lamp examination, ophthalmoscopy, fundus photography,

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.

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 influence of the starting point to calculate the decrease in BCVA score for

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.

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 rate of conjunctival haemorrhage, conjunctival hyperaemia and eye pain in

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.

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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Negative Correlation Between Aqueous Vascular Endothelial Growth Factor Levels and Axial Length

Aqueous VEGF levels and axial length

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Abstract

Purpose: The aim of this study was to evaluate the relationship between concentration of vascular endothelial growth factor (VEGF) in the aqueous humor and axial length.

Methods: Aqueous humor samples were obtained from 60 eyes of 60 patients without ocular diseases other than cataract. No patient with diabetes mellitus was included. The VEGF concentration in the aqueous humor was measured using an enzyme-linked immunosorbent assay.

Results: The VEGF concentrations in the aqueous humor samples ranged from 25 to 241 pg/mL (mean ± standard deviation [SD], 116.6 ± 46.7 pg/mL). The axial lengths ranged from 20.98 to 31.95 mm (mean ± SD, 24.09 ± 2.06 mm). The VEGF concentrations in the aqueous humor samples were correlated with axial length (Pearson product moment correlation test, $\rho = -0.373$; $P = 0.003$).

Conclusions: Concentration of VEGF in the aqueous humor is negatively correlated with axial length.

Keywords: vascular endothelial growth factor, aqueous humor, cataract, axial length

Introduction

Vascular endothelial growth factor (VEGF) is a pathogenic factor that affects the clinical condition in vitreoretinal diseases. The intraocular VEGF level is elevated in diabetic retinopathy, retinal vein occlusion, and retinopathy of prematurity [1-7]. Anti-VEGF drugs are widely used to treat retinal diseases such as age-related macular degeneration (AMD), proliferative diabetic retinopathy (PDR), and macular edema secondary to retinal vein occlusion [8-19]. Some phenomena concerning VEGF remain puzzling, one of which is the lesser severity of diabetic retinopathy in patients with myopia than in patients with emmetropia or hypermetropia [20-22]. Another is the significantly lower VEGF concentration in the aqueous humor of eyes with myopic choroidal neovascularization (mCNV) [23, 24], although intravitreal injection of bevacizumab, an anti-VEGF antibody, is effective for treating mCNV [25-27]. The above-described phenomena seem to be ~~related~~ correlated with myopia or axial length.

Despite the attention that VEGF has been attracting, to the best of our knowledge, no reports have been published on the relationship between the aqueous VEGF level and the axial length of "normal" eyes. Therefore, we measured the VEGF concentration in the aqueous humor of patients without ocular diseases other than cataract and without diabetes mellitus and evaluated the relationship between the VEGF concentration and the axial

length.

Methods

In this prospective study, we measured the VEGF concentration in the aqueous humor of 60 eyes of 60 patients (20 men, 40 women) without ocular diseases other than cataract. We excluded patients with myopic changes such as staphyloma and myopic atrophy and patients with diabetes mellitus. The mean patient age was 72.1 years (range, 44-89). No ocular treatments including steroids and ocular surgery were administered before the cataract surgery.

Undiluted aqueous humor samples (0.2 mL) were obtained from the eyes of the patients immediately before the cataract surgery. All samples were collected using standard aseptic techniques that included the use of topical povidone-iodine and levofloxacin drops. The samples were stored in a freezer at −80°C until analysis.

The VEGF concentration in the aqueous humor was measured by an enzyme-linked immunosorbent assay for human VEGF (R&D Systems, Minneapolis, MN, USA). The primary antibody against VEGF detected 2 ($VEGF_{121}$ and $VEGF_{165}$) of the 4 VEGF isoforms [27]. The assay was performed according to the manufacturer's instructions. A standard curve was plotted from the measurements made with the standard

solution from 20 to 1000 pg/mL for VEGF, and the concentration of VEGF in the sample was determined.

The axial length was measured using the IOLMaster (Carl Zeiss Meditec, Jena, Germany).

The data were analyzed using SigmaStat software (version 3.1; Systat Software, Richmond, CA, USA) and expressed as the mean ± standard deviation (SD). An unpaired *t* test was used to evaluate the difference in the VEGF concentration of the aqueous humor samples between men and women. The Mann-Whitney test was used to evaluate the difference between men and women in axial lengths. The Pearson product moment correlation test was used to evaluate the correlation between the VEGF concentrations in the aqueous humor and age or axial length. A probability value less than 0.05 was considered statistically significant.

This study was approved by the institutional review board of Shiga University of Medical Science Hospital. All patients provided written informed consent, including consent to obtaining aqueous samples for measurement of the aqueous VEGF concentration.

Results

The VEGF concentrations in the aqueous humor of patients with cataract ranged from 25 to 241 pg/mL (mean ± SD, 116.6 ± 46.7 pg/mL). The axial lengths of the eyes with cataract ranged from 20.98 to 31.95 mm (mean ± SD, 24.09 ± 2.06 mm).

The correlation between the VEGF concentration in the aqueous humor and age or axial length was evaluated. The VEGF concentration in the aqueous humor was negatively correlated with axial length in eyes with cataract (Pearson product moment correlation test, $\rho = -0.373$; $P = 0.003$) (Figure 1). The regression line using the VEGF concentration as an outcome variable (y) and the axial length as a predictor variable (x) was $y = -9.156x + 337.226$. The VEGF concentration in the aqueous humor was not significantly correlated with age (Pearson product moment correlation test, $\rho = 0.173$; $P = 0.185$) (Figure 2). The VEGF concentrations in the aqueous humor samples from men ranged from 25 to 241 pg/mL (mean ± SD, 108.5 ± 54.4 pg/mL) and in women from 31 to 228 pg/mL (mean ± SD, 120.7 ± 47.2 pg/mL). No significant difference was found between men and women in the VEGF concentrations in the aqueous humor samples (unpaired *t* test, $P = 0.381$) (Figure 3), nor in the axial lengths (Mann-Whitney test, $P = 0.185$).

Discussion

We measured the VEGF concentrations in the aqueous humor samples from patients

without ocular diseases other than cataract and without diabetes mellitus and found that the VEGF concentration was negatively correlated with axial length.

Several explanations for the negative correlation between VEGF concentration in the aqueous humor and axial length are possible, one of which is that the VEGF in the anterior chamber and vitreous cavity might be diluted as a result of longer axial length and therefore, greater intraocular volume.

To evaluate this explanation, regression analysis of the VEGF concentrations in eyes with cataract in relation to axial length was performed, and we compared the value according to the regression line with the value calculated by the dilution ratio. It may have been better to evaluate the relationship between the VEGF concentration in aqueous humor and intraocular volume. But it is difficult to measure the intraocular volume of each patient correctly, whereas the methods to measure axial length are well established and widespread. Therefore, we employed the axial length as the index of eyeball size. A significant negative correlation was found between VEGF concentration and axial length. According to the top regression line, ([VEGF concentration] = -9.156 [axial length] + 337.226), the adjusted VEGF concentration was 154.1 pg/mL after substitution of 20 mm for the axial length and 62.5 pg/mL after substitution of 30 mm for the axial length. Because the circumferential length of eyes is similar despite differences in the axial length between myopic eyes and

122 nonmyopic eyes except for the anterior segment, the intraocular volume might be assumed
123 to be linear to the axial length. Assuming the intraocular volume was linear to the axial
124 length, the dilution ratio of the VEGF concentration at 30 mm to that at 20 mm was 20 to
125 30. The VEGF concentration at 30 mm calculated by the dilution effect was 102.7 pg/mL.
126 This result is still higher than 62.5 pg/mL, the value obtained from the regression line. The
127 lower VEGF level in the aqueous humor samples from eyes with longer axial length is not
128 explained completely by the dilution effect resulting from longer axial length.
129 Another possible explanation is that VEGF production might decrease because the
130 retina is thinner with axial elongation [29] and retinal thinning might cause relatively
131 increased choroidal perfusion and decreased retinal hypoxia resulting in decreased VEGF
132 production derived from the retinal pigment epithelium [30].
133 There was no significant difference between men and women in the VEGF
134 concentrations in the aqueous humor samples and axial lengths in this study.
135 VEGF plays a key role in the progression of PDR [1]. The current study showed
136 that VEGF concentration was negatively correlated with axial length. The lower VEGF
137 concentration in aqueous humor samples of eyes with axial elongation might explain why
138 the severity of diabetic retinopathy in patients with myopia is less than that in patients with
139 emmetropia or hypermetropia.

140 This finding might contribute to an understanding of the pathogenesis of
141 vitreoretinal disease concerning VEGF.
142
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