Table 2 Selected covariates in the increased visual function

Visual function	Selected variable	T value	*p* value
Low-contrast VA	No cardiovascular disease	2.14	0.0386
	CSC in the fellow eye	−1.78	0.0822
	Baseline low-contrast VA	−3.18	0.0029
AULCSF	Non smoking	2.49	0.0172
	Phakic eye	2.16	0.0372
	Baseline AULCSF	−3.97	0.0003
Retinal sensitivity within 0.5 degrees	Phakic eye	2.42	0.0202
	Baseline retinal sensitivity within 0.5 degrees	−2.92	0.0058
Retinal sensitivity within 6.0 degrees	CSC in the fellow eye	−2.03	0.0491

VA visual acuity; *AULCSF* area under the log contrast sensitivity function; *CSC* central serous chorioretinopathy

took supplementation containing 6 mg of lutein were followed for 1 year and the MPOD level and visual function were measured every 3 or 6 months. We followed all subjects for 1 year, which satisfied the condition of the number needed for the primary outcome measure. As affected eyes with CSC and AMD had some foveal abnormalities when observed by fundus autofluorescence spectrometry and the measured MPOD levels were unstable, we did not include any affected eyes in the current study.

At baseline, we found that the eyes with clear IOL had higher MPOD levels. This agrees with our previous report that showed the MPOD level measured by autofluorescence spectrometry with two-wavelength autofluorescence method become higher after cataract surgery [45].

After 1 year of follow-up, the MPOD levels did not increase significantly, but it was stable throughout the study period and no covariates were significant for the changes in the MPOD levels. The LUtein Nutrition effects measured by Autofluorescence (LUNA) study in Germany, in which subjects with normal eyes or AMD took a supplement containing 12 mg of lutein, 1 mg of zeaxanthin, 120 mg of vitamin C, 17.6 mg of vitamin E, 10 mg of zinc, and 40 μg of selenium for 6 months, reported a significant increase in the MPOD level in the intervention group [24, 25]. Because the LUNA study used the same autofluorescence methods to measure the MPOD as we used in the current study, the different responses in MPOD seemed to be due to the different amounts of supplemental lutein. Although this difference may also be influenced by the racial difference, number of tested subjects and the amount of other antioxidants, 6 mg of lutein supplementation may be too low to increase the MPOD level. On the other hand, it is possible that the development of cataract might affect the measurement of MPOD since the decrease of the MPOD level within 1.0 degree correlated with the phakic eye. This theory is also supported by our previous report that showed a higher nuclear color grading score was correlated with a lower MPOD level measured by autofluorescence spectrometry with two-wavelength autofluorescence method [45]. Although we did not observe the apparent decrease of visual acuity during follow-up period, cataract might develop and mask the increase of MPOD induced by lutein supplementation. In addition, the 1-year increase in the MPOD level was correlated with subjects with no cardiovascular diseases. As previous reports showed that the cardiovascular risk factors are associated with AMD, subjects with cardiovascular diseases may have less response to lutein supplementation [46, 47].

The contribution of macular pigments to visual performance has been reported. Macular pigments can improve visual performance including contrast sensitivity by reducing chromatic aberration, blue haze, and the intensity of the rod signal, for example [28–36]. To identify the minute changes in visual contrast, we measured the low-contrast VA and contrast sensitivity [40]. The low-contrast VA was correlated with aging at baseline and did not change during the follow-up period; therefore, antioxidants may not affect the low-contrast VA. However, the AULCSF, which is a representative index for evaluating the contrast sensitivity, significantly increased at 12 months (p=0.0124). Supplementation containing lutein may have maintained the MPOD level and improved the contrast sensitivity. Interestingly, the increase in AULCSF was correlated with nonsmoking status, although the AULCSF was not correlated with smoking at baseline. Uz et al. reported that smoking reduced contrast sensitivity possibly because of the decreased serum level of the trace elements manganese and zinc [35]. Because the supplement we used in the current study contained manganese and zinc, they may have caused the contrast sensitivity to improve especially in nonsmokers. Contrary to our results, Bartlett and Eperjesi reported no correlation between contrast sensitivity and supplementation with 6 mg of lutein, vitamins, and minerals [29]. The relationship between antioxidant supplement and contrast sensitivity should be investigated further.

Retinal sensitivity was measured by microperimetry within 0.5 and 6.0 degrees. The baseline retinal sensitivity within 0.5 and 6.0 degrees was correlated with aging as reported previously [48, 49]. The retinal sensitivity significantly increased during the follow-up period. To the best of our knowledge, this is the first report to show a correlation between supplementation with antioxidants containing lutein and the retinal sensitivity. Because retinal sensitivity is measured by a white target on a white background by changing the light intensity of the target, there may be some mechanisms other than macular pigment playing roles in contrast sensitivity; one hypothesis is that antioxidants may improve the light threshold of the photoreceptors. Stepwise regression analysis showed that the fellow eyes with CSC had a lower increase of retinal sensitivity within 6.0 degrees. These results might be associated with lower MPOD in the fellow eyes of those with CSC, which have a high risk of developing CSC [15]. On the other hand, stepwise regression analysis revealed that the subjects with cardiovascular diseases showed lower increase in the low-contrast VA, and the subjects with clear IOL showed lower change of AULCSF and retinal sensitivity within 0.5 degree. The reasons for these results are hard to explain at the moment, possibly because the number of the subgroup is too small. Accordingly, further studies are needed on those points.

In conclusion, although there is a fear that the autofluorescence spectrometry with two-wavelength method may not be appropriate to detect MPOD augmentation in elderly eyes, daily supplementation with 6 mg of lutein, vitamins, and minerals did not affect the MPOD level measured by this method for 1 year. Supplementation of antioxidants containing 6 mg of lutein may be insufficient to increase the MPOD level, however, it may improve visual function such as contrast sensitivity and retinal sensitivity. So far, we cannot deny the possibility that the improved visual function was due to a learning effect, because our study had no placebo control group. Further studies may provide deeper insights into the role of antioxidant supplementation in maintaining the function of macula.

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Intravitreal bevacizumab for exudative branching vascular networks in polypoidal choroidal vasculopathy

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ABSTRACT

Aims To assess the long-term efficacy of intravitreal bevacizumab for recurrent leakage owing to the residual branching vascular networks in polypoidal choroidal vasculopathy after photodynamic therapy.

Methods Forty-five eyes with exudative branching vascular networks were treated with intravitreal bevacizumab and followed for at least 24 months. Original polypoidal lesions had been treated successfully with previous photodynamic therapy in all eyes. The best-corrected visual acuity and retinal morphological changes were assessed retrospectively.

Results Exudative branching vascular networks were characterised as occult choroidal neovascularisation (38 eyes) or classic choroidal neovascularisation (7 eyes) on fluorescein angiography. Intravitreal bevacizumab maintained or improved vision in 38 eyes (84%) over 12 months and in 36 eyes (80%) over 24 months, although the mean visual acuity at 12 and 24 months did not differ significantly compared with baseline. Complete resolution of macular fluid was achieved continuously in 26 eyes (58%) during 24 months. Sixteen eyes (36%) responded once to treatment but became unresponsive to additional injections for recurrent exudation. Three eyes (7%) were refractory to treatment throughout follow-up. Cystoid macular oedema eventually developed in 10 eyes and was a poor prognostic sign for visual outcome.

Conclusion Intravitreal bevacizumab improved the retinal morphology and maintained vision over 1 year in most eyes with recurrent fluid owing to persistent abnormal vascular networks in polypoidal choroidal vasculopathy. The therapeutic response, however, may decrease during the second year.

Polypoidal choroidal vasculopathy (PCV), characterised by a complex of branching vascular networks terminating in polypoidal lesions,[1–5] accounts for 23–54% of neovascular age-related macular degeneration (AMD) in Asian populations.[6 7] Photodynamic therapy (PDT) maintains or improves vision by resolving the polypoidal lesions and accompanying fluid beneath the neurosensory retina.[8–10] However, the branching vascular networks usually remain even after PDT,[11–13] and may enlarge further over time, resulting in persistent exudation appearing as choroidal neovascularisation (CNV) secondary to AMD in some cases.[14] Therefore, stabilisation of the branching vascular networks may be crucial for long-term management of PCV.

Recent studies have reported the efficacy and safety of bevacizumab (Avastin, Genentech, South San Francisco, California) for stabilising neovascular activity and maintaining vision in patients with neovascular AMD.[15] Those results promoted us to consider the drug as the treatment of choice for CNV-like branching vascular networks of PCV after PDT.

The purpose of this retrospective study is to assess the long-term efficacy of intravitreal bevacizumab in the management of exudative branching vascular networks in eyes with PCV.

METHODS

This study was a retrospective, consecutive, interventional case series conducted at Osaka University Hospital. Patients who received intravitreal bevacizumab for the treatment of recurrent exudation associated with branching vascular networks between December 2006 and April 2008 and followed for at least 24 months were initially enrolled. All eyes had a previous history of successful PDT for PCV with resolution of polypoidal lesions. Recurrent exudation was determined by the presence of subretinal fluid (SRF) or macular oedema on optical coherence tomography (OCT) with CNV-like fluorescein leakage caused by branching vascular networks. Patients were excluded if they had SRF or macular oedema caused by recurrent polypoidal lesions, follow-up <24 months after intravitreal bevacizumab or clinically relevant media opacity.

All patients underwent a comprehensive ocular examination, including measurement of the best-corrected visual acuity (BCVA), intraocular pressure, binocular indirect ophthalmoscopy and contact lens slit-lamp biomicroscopy, colour fundus photography, OCT, fluorescein angiography (FA) and indocyanine green angiography (ICGA). The patients were examined after a detailed explanation of the study was provided, and they provided informed consent. This study was approved by the institutional review board committee of Osaka University Hospital.

ICGA analysis

ICGA was performed at baseline before intravitreal bevacizumab and 3–6 months and 12–24 months after intravitreal bevacizumab. A confocal scanning laser ophthalmoscope (Heidelberg Retina Angiograph 2 (HRA2), Heidelberg Engineering GmbH, Dossenheim, Germany) was used as previously described.[14] The planimetric size of the branching vascular networks was measured from the early-phase ICGA at baseline to the final visits using software included in the HRA2.[14] The individual readings of two investigators were averaged.

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A change in lesion size was recorded when the lesion increased or decreased more than 50% in the corresponding area.

OCT analysis

OCT images were obtained by Stratus OCT (Carl Zeiss Meditec, Dublin, California) or the Cirrus HD-OCT (Carl Zeiss Meditec). The central retinal thickness (CRT), defined as the distance between the internal limiting membrane and the inner surface of the retinal pigment epithelium (RPE), was measured manually at the fovea.[16] The SRF and intraretinal fluid were included in the CRT measurements, whereas sub-RPE fluid was not included. The fluid in the macula was identified as intraretinal fluid (macular oedema) and SRF, and a fluid-free macula was defined by the absence of macular oedema and SRF as determined by OCT.

Follow-up and reinjection protocols

Intravitreal bevacizumab (1.25 mg) was injected during an outpatient procedure under strict aseptic conditions. All patients were followed monthly for more than 24 months. The VA and OCT were examined at every visit. Re-treatment with intravitreal bevacizumab was considered if there was OCT evidence of macular fluid with at least one-line loss of VA, new macular haemorrhage or newly developed fibrinous changes. A treatment response on OCT was defined as complete if there was no macular fluid, partial if there was no macular fluid initially but the lesions become refractory to treatment after recurrence of exudation, or no response if there was not an absence of macular fluid. When recurrence was suspected, FA and ICGA were performed at the discretion of the physician.

Data collection and statistical analysis

The main outcome measures were the changes in exudative fluid and the BCVA during 24 months after the initial injection. The changes in the CRT and the size of the branching vascular networks before and after injection were also evaluated. Statistical analyses were performed with SAS software, version 9.1 (SAS Institute). P-values <0.05 were considered significant.

RESULTS

Forty-five eyes of 45 patients met the criteria for data analysis. The patient demographics are shown in table 1. Original polypoidal lesions had resolved in all eyes on ICGA with complete absence of fluid after previous PDT, but the branching vascular network remained in all eyes. The development of subsequent exudative changes associated with residual branching vascular networks without recurrent polypoidal lesions was seen 9.3±8.4 months (range 1–36) after the previous PDT (figure 1).

Table 1 Patient baseline characteristics

No eyes	45
No patients	45
Age (years) (mean±SD; range)	70.4±6.9 (53–83)
Gender (no/%)	
Men	35 (78)
Women	10 (22)
Eye (no/%)	
Right	20 (44)
Left	25 (56)
Interval between intravitreal bevacizumab and previous photodynamic therapy (mean±SD; range)	9.3±8.4 (1–36)
Optical coherence tomography characteristics	
Subretinal fluid (no/%)	43 (96)
Pigment epithelial detachment (no/%)	26 (58)
Macular oedema (no/%)	10 (22)
Leakage pattern on fluorescein angiography	
Classic choroidal neovascularisation	7 (16)
Occult choroidal neovascularisation	38 (84)
Baseline best-corrected visual acuity	
Landolt C acuity chart (mean; range)	0.35 (0.09–1.2)
Logarithm of the minimum angle of resolution (mean±SD)	0.45±0.30

Angiographic and OCT characteristics of exudative branching vascular networks

FA showed leakage mimicking occult CNV in 38 eyes (84%) (figure 1C,G) and classic CNV in seven eyes (16%) (figure 1K). Of the 38 eyes with occult CNV-like lesions, ICGA showed thin branching choroidal vessels that depicted relatively well-delineated plaque in the late phase. Of the seven eyes with classic CNV-like lesions, subretinal fibrinous exudation was seen in all eyes. The exudation associated with the branching vascular networks was characterised by OCT as SRF in 43 eyes (96%) and macular oedema in 10 eyes (22%). Apparent PED was seen in 24 eyes (53%), and the limited RPE elevation was detected in 14 eyes (31%).

Visual acuity, ICG angiography and OCT outcomes

The mean number of injections in the 45 eyes during the first 12 months was 2.9±1.8 (range 1–8) and 1.9±1.8 (range 0–6) during the second year.

The mean baseline BCVA was 0.45±0.30 (table 1). The mean BCVA values were 0.43±0.33, 0.43±0.30, 0.48±0.32 and 0.51±0.38 at 3, 6, 12 and 24 months, respectively ($p=0.118$, $p=0.428$, $p=0.523$ and $p=0.206$, respectively) (figure 2A). The BCVA at the 12-month follow-up visit improved by three or more lines in four eyes (9%), was unchanged within three lines in 34 eyes (76%) and worsened in seven eyes (16%). At 24 months, BCVA improved by three or more lines in six eyes (13%), was unchanged in 30 eyes (67%) and worsened in nine eyes (20%).

ICGA images showed persistent branching vascular networks in all 45 eyes during follow-up. The size of the branching vascular network increased in 20 eyes (44%), remained unchanged in 21 eyes (47%) and decreased in four eyes (9%) at the final ICGA examination compared with baseline. In six eyes, polypoidal lesions reappeared at the site connected to the branching vascular networks. The period between the initial intravitreal bevacizumab and detection of newly developed polypoidal lesions was 3 months in one eye, 6 months in one eye and 24 months in four eyes.

The mean baseline CRT measured on OCT was 222±71 μm (range 87–434). The CRT decreased an average of 31.0 μm from baseline by 3 months ($p<0.001$) and an average of 40.0 μm by 6 months ($p<0.001$) (figure 2B). Those initial decreases lessened over time after initial intravitreal bevacizumab, with an average reduction of 25.8 μm by 12 months ($p=0.050$) and 1.8 μm by 24 months ($p=0.296$). There tended to be a correlation between the CRT at 24 months and the BCVA at 24 months ($r=0.292$, $p=0.051$).

A fluid-free macula was achieved during the first 12 months in 42 eyes (93%). The mean number of injections required to achieve a fluid-free macula was 1.9±1.6 (range 1–9). Subsequently, 36 eyes developed recurrent macular fluid and received additional injections. Overall, 26 eyes (58%) were regarded on OCT as complete responders because intravitreal bevacizumab was effective throughout the 24 months of follow-up in completely resolving the exudation. One initial injection was effective to keep the macula dry for 24 months in two eyes.

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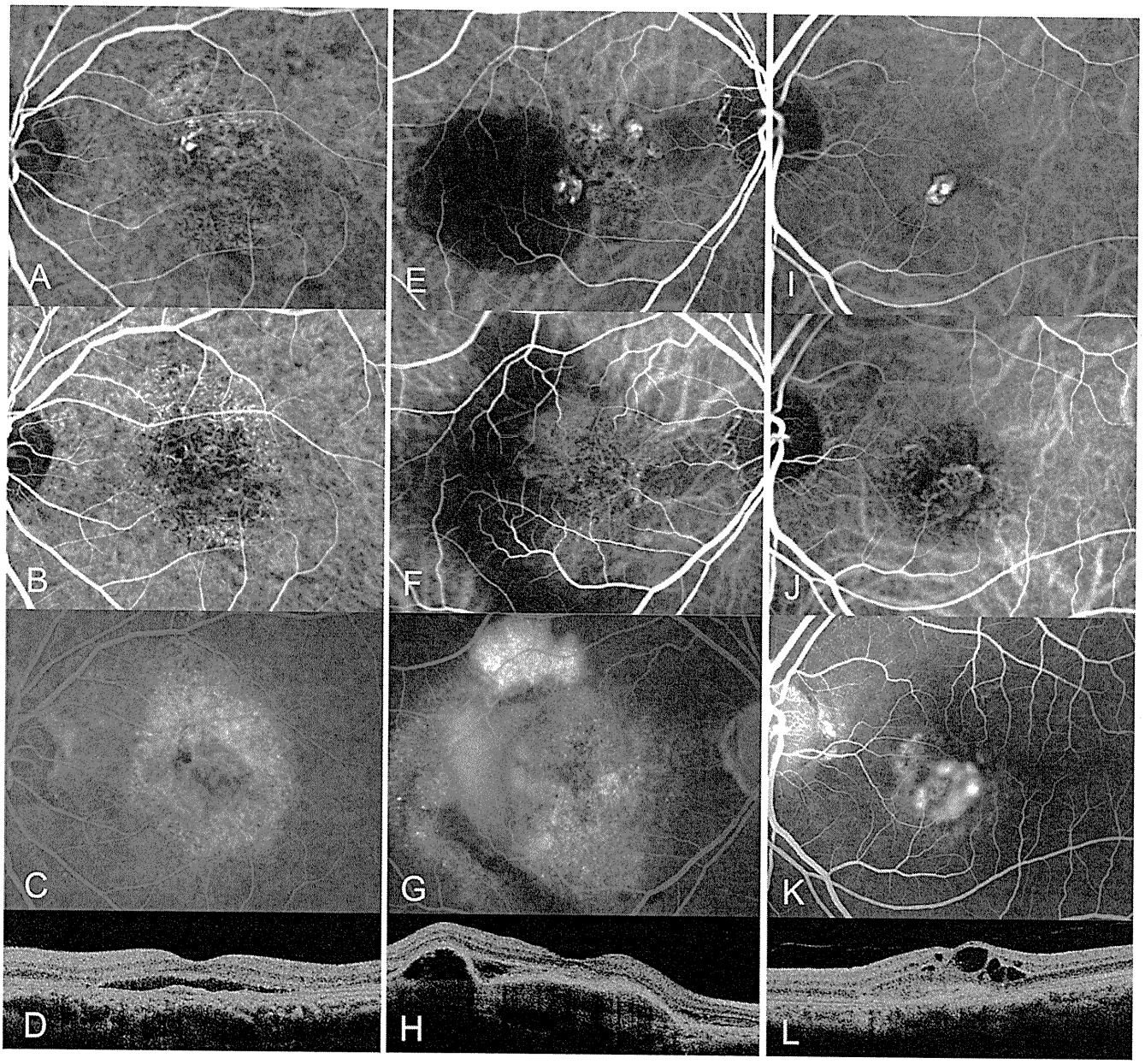

Figure 1 Exudation associated with branching vascular networks in eyes with polypoidal choroidal vasculopathy. The exudation patterns are characterised as occult choroidal neovascularisation (CNV) without apparent pigment epithelial detachment (PED) (A—D), occult CNV with fibrovascular PED (E—H) or classic CNV (I—L). (A) Indocyanine green angiography (ICGA) before previous photodynamic therapy (PDT) showing branching vascular networks terminating in small polypoidal lesions in a 64-year-old man. The polypoidal lesions resolved completely after PDT. However, the branching vascular networks remained (B) and subsequently developed exudative changes resembling occult CNV on fluorescein angiography (FA) 6 months after the previous PDT (C). (D) Optical coherence tomography (OCT) showing subretinal fluid (SRF) with limited retinal pigment epithelium elevation. (E) ICGA before previous PDT showing branching vascular networks terminating in polypoidal lesions with a serous PED in a 77-year-old man. The polypoidal lesions have resolved completely after PDT. However, the branching vascular networks have enlarged (F) and subsequently developed exudative changes resembling occult CNV with a fibrovascular PED on FA 3 months after previous PDT (G). (H) OCT showing a large PED with SRF. (I) ICGA before previous PDT showing small branching vascular networks terminating in polypoidal lesions in a 57-year-old woman. The polypoidal lesions have resolved completely after PDT. Without recurrence of the polypoidal lesion, the branching vascular networks show exudative changes at the macula (J) resembling classic CNV on FA 6 months after previous PDT (K). (L) The OCT shows fibrin accumulation and macular oedema.

Sixteen eyes (36%) were considered to be partial responders. Those eyes once had no macular fluid after injection during the first 12 months; however, they became unresponsive to treatment despite repeated injections for recurrent exudation (figure 3). Three eyes (7%) were considered non-responders because the macular fluid persisted during 24 months despite repeated injections. Eight of 16 eyes with partial responses and two of three eyes with no response eventually developed cystoid macular oedema (CMO), and six of these lost three lines or more at 24 months compared with baseline.

The differences between complete responders and partial or non-responders with respect to BCVA and OCT findings are shown in table 2. Although the baseline BCVA, the leakage pattern on FA, CRT and the lesion size did not differ significantly

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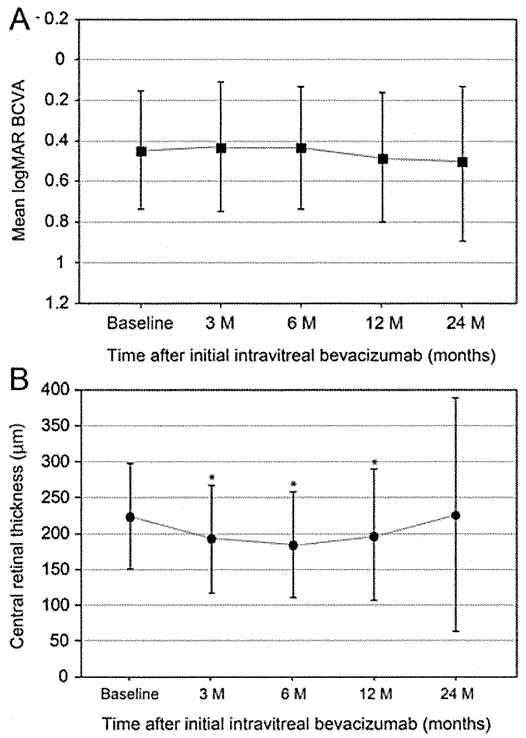

Figure 2 (A) Mean change in the best-corrected visual acuity (BCVA) (mean±SD) after intravitreal bevacizumab for exudative branching vascular networks through 24 months. The visual acuity is expressed as the logarithm of the minimum angle of resolution (logMAR). There are no statistical differences between the baseline and post-treatment BCVA values at any time point. (B) Mean change in the central retinal thickness (CRT) (mean±SD) after intravitreal bevacizumab for exudative branching vascular networks through 24 months. The mean CRT has decreased significantly at 3, 6 and 12 months after initial intravitreal bevacizumab (*$p<0.05$ compared with baseline). However, there are no statistical differences in CRT between baseline and 24 months. M, months.

between the groups, the mean BCVA in eyes with a complete response was significantly better than in eyes with a partial or no response at 24 months ($p=0.041$). However, there was no significant difference in the BCVA between eyes with a complete response and those with partial responses or no response at 24 months when the 10 eyes with CMO were excluded from the analysis (0.41±0.34 vs 0.34±0.30, $p=0.675$). The CRT also differed significantly between the groups at 24 months ($p=0.001$). The branching vascular networks were significantly larger in eyes with a partial response or no response compared with those who were complete responders at 24 months ($p=0.049$).

No adverse systemic and local complications related to intravitreal bevacizumab were observed during the study period. One eye developed an RPE tear.

DISCUSSION

In the current study, we focused on identifying the characteristics of the exudative features associated with branching vascular networks and assessed the potential efficacy of bevacizumab to treat those lesions. We found two angiographic patterns of exudative branching vascular networks at an average of 9.3±8.4 months (range 1–20) after previous PDT, that is, occult CNV-like lesions with or without fibrovascular PED (84%) and classic CNV-like leaky vascular lesions (16%).

In eyes with occult CNV-like leakage, the complex of abnormal vessels was clearly seen on early-phase ICGA, with hyperfluorescent plaques on late-stage ICGA. On OCT, the lesion was also identifiable as elevated RPE, indicating invasion of the networks beneath the RPE. Thus, PCV in this stage was clinically and angiographically indistinguishable from type 1 neovascularisation. Imamura and associates also indicated that it would be difficult to differenciate between PCV and CNV unless typical polypoidal lesion exists in the network vessels.[17] Serous and haemorrhagic PEDs associated with PCV before PDT seemed to be potentially predictive of the development and progression of occult CNV-like exudative vessels, because in those eyes, the rate of PED at the time of PDT was 63% and was higher than that reported previously in PCV (19–44%).[7 8 18] In eyes with classic CNV-like lesions, OCT showed subretinal fibrin formation, indicating extensive exudation. Both the occult and classic CNV-like lesions in our study did not seem to have the same characteristics as the original branching vascular networks of PCV, which had previously been considered as long-term stable abnormal choroidal vessels.[18 19] We speculate that pre-existing branching vascular networks acquired hyperpermeable properties mimicking CNV or the CNV newly emerged from residual branching vascular networks over time after PDT.

Intravitreal bevacizumab for exudative branching vascular networks maintained or improved vision in 38 eyes (84%) over 12 months, although the mean VA at 12 months did not differ significantly compared with baseline. The significant decrease in retinal thickness was achieved during the first year due to complete resolution of macular fluid in 42 (93%) eyes, indicating the potent effect of bevacizumab on antipermeability in most eyes over 12 months.

Improved or stabilised VA was continuously achieved in 36 eyes (80%) over 24 months. According to a previous study of patients with PCV treated only by PDT for 3 years, VA deteriorated three lines or more in 37%, owing to the enlargement of abnormal networks with neovascular changes and recurrent polyps.[20] In contrast to these reports, the current results using bevacizumab are encouraging because VA deterioration was seen in 20%, despite a mean follow-up period of 33 months after initial PDT.

Complete resolution of macular fluid was maintained throughout the study in 58% of eyes (complete responders). However, 36% of eyes that responded to treatment during the first 12 months showed less treatment effect, despite repeated injections for recurrent exudation (partial responders). Three eyes were considered non-responders on OCT, and the macular fluid increased further. As a result, the decrease in the mean retinal thickness during the first year lessened during the second year and became non-significant ($p=0.296$) at 24 months compared with baseline.

The loss of the therapeutic response to bevacizumab during 2 years may be explained by a potential change in the sensitivity to bevacizumab. In the current study, the vascular networks persisted on ICGA in all eyes after intravitreal bevacizumab and even enlarged in 20 eyes (44%) despite repeated injections. Continuous growth of the CNV despite repeated anti-vascular endothelial growth factor therapy also had been reported in eyes with AMD.[21] The persistence and expansion of the networks may lead to more mature and less vascular-endothelial-growth-factor-dependent vessels with increased treatment resistance. In addition, a tachyphylactic response, that is, a progressive decrease in the bioefficacy of bevacizumab, after repeated injections has been reported in AMD.[22] Those responses also

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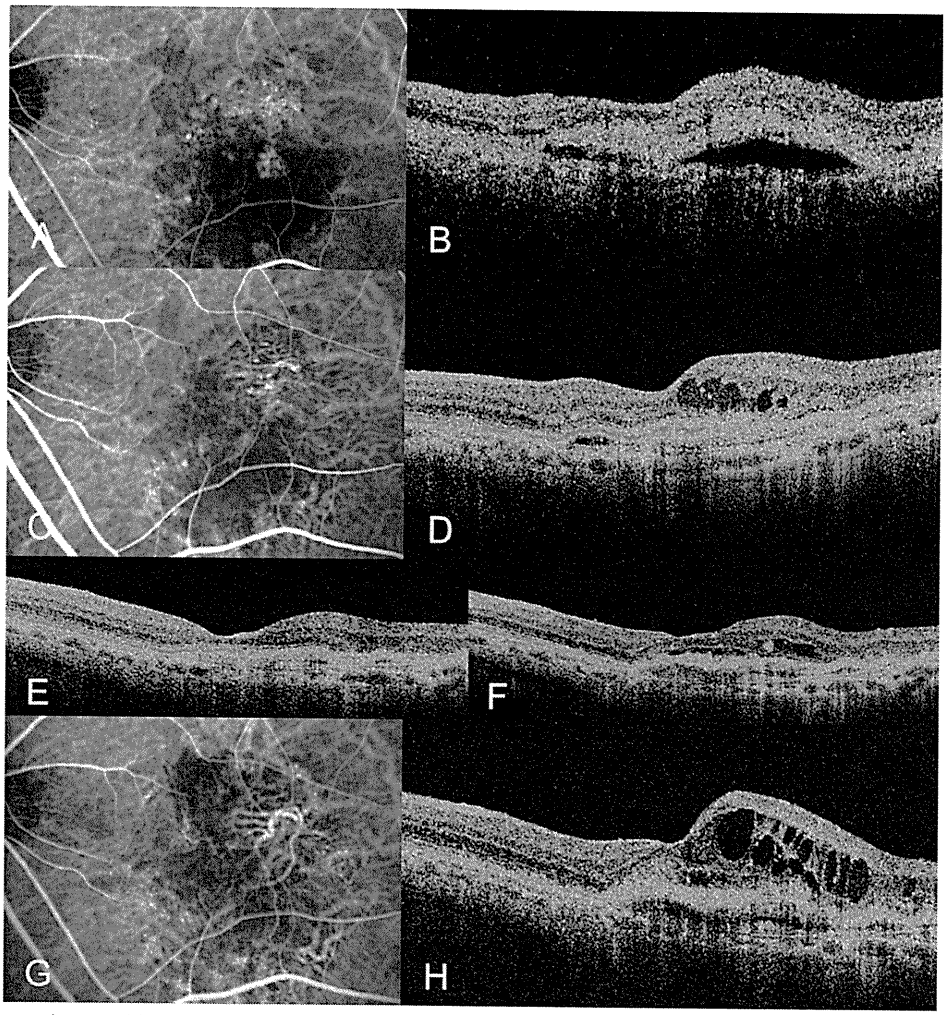

Figure 3 Indocyanine green angiography (ICGA) and optical coherence tomography (OCT) images before and after intravitreal bevacizumab in an 83-year-old man. (A) ICGA showing branching vascular networks terminating in polypoidal lesions with a serous pigment epithelial detachment (PED) before previous photodynamic therapy (PDT). (B) OCT image confirming the PED. The polypoidal lesions have resolved completely after PDT. However, the branching vascular networks remain with subsequently developing exudation 6 months after previous PDT (C). (D) OCT showing slight subretinal fluid (SRF), macular oedema and a fibrovascular PED. The visual acuity (VA) is 0.2. Intravitreal bevacizumab was administered. After intravitreal bevacizumab, the SRF and macular oedema have resolved on the OCT image (E). The VA is 0.3. However, OCT shows recurrent macular oedema 3 months after the first injection (F). The VA is 0.2. Additional intravitreal bevacizumab was administered, and the SRF and macular oedema resolved again. The macular oedema recurred once again and was successfully treated with repeated injections during the first year. Fifteen months after the initial injection, the branching vascular networks have increased in size (G). Despite repeated intravitreal bevacizumab for recurrent exudation, the SRF and macular oedema that had once responded to treatment during the first year persistently remained during the second year with deterioration of VA to 0.1, indicating loss of the treatment efficacy (H).

Table 2 Differences between complete responders and partial or non-responders in best-corrected visual acuity and optical coherence tomography findings

	Complete responders (n=26)	Partial or non-responders (n=19)	p Value
Age (years), mean±SD (range)	70.5±6.5 (57–83)	70.4±7.6 (53–83)	0.985
Patterns of leakage on fluorescein angiography at baseline			
Classic choroidal neovascularisation	5	1	
Occult choroidal neovascularisation	21	18	0.222
Baseline optical coherence tomography findings			
Pigment epithelial detachment (no/%)	15 (58)	11 (58)	0.770
Subretinal fluid (no/%)	25 (96)	18 (95)	1.000
Macular oedema (no/%)	5 (19)	5 (26)	0.720
Best-corrected visual acuity, logarithm of the minimum angle of resolution±SD			
Baseline	0.43±0.29	0.48±0.32	0.583
12 months	0.40±0.31	0.58±0.32	0.058
24 months	0.41±0.34	0.66±0.40	0.041
Logarithm of the minimum angle of resolution change between baseline and 24 months	0.02±0.37	−0.18±0.27	0.047
Central retinal thickness (μm), mean±SD			
Baseline	219±60 (87–337)	226±85 (128–434)	0.738
12 months	172±56 (80–347)	229±115 (128–664)	0.017
24 months	159±54 (76–288)	313±216 (113–834)	0.001
Change between baseline and 24 months	−61±81 (−215–118)	87±216 (−246–678)	0.003
Branching vascular network size (mm^2)			
Baseline	4.59±2.72 (1.18–9.98)	5.52±4.23 (0.88–16.86)	0.705
24 months	6.30±3.53 (1.14–15.90)	11.39±10.01 (2.85–45.23)	0.049

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may be responsible for the loss of treatment efficacy in the current study eyes.

Fortunately, half of the eyes that were partial and non-responders maintained the VA through 24 months despite persistent macular fluid. However, 10 eyes eventually developed significant CMO with poor visual outcomes. The CMO has been reported to be associated with all forms of neovascular AMD, including classic CNV, occult CNV, PED and disciform scars,[23 24] or with PCV.[20] We speculated that the persistent exudative vascular networks beneath the RPE may cause RPE decompensation, resulting in severe damage to the neurosensory retina, as seen in progressed AMD.

The limitations of the current study were its retrospective nature and the absence of a control group. Because the individual responses vary among the patients, we did not re-treat patients at fixed intervals but did so with the criteria based on their responses under monthly monitoring. Some patients with a lower morphological response despite consecutive injections were not always re-treated unless VA declined, as reported in the treatment of AMD.[25] To confirm whether the current strategy is optimal for long-term follow-up, a prospective, randomised, comparative study should be considered comparing different injection strategies for exudative branching vascular networks in PCV.

In summary, the intravitreal bevacizumab to treat exudative branching vascular networks in patients with PCV improves the retinal morphology and maintains vision over 1 year. However, the network vessels persist, and the therapeutic response may be lost during the second year. The visual prognosis is poor in eyes with CMO. Further studies may elucidate the appropriate use of intravitreal bevacizumab or other treatment modalities for better management of branching vascular networks in PCV.

Competing interests None.

Patient consent Obtained.

Ethics approval Ethics approval was provided by the institutional review board committee of Osaka University Hospital.

Provenance and peer review Not commissioned; externally peer reviewed.

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Intravitreal bevacizumab for exudative branching vascular networks in polypoidal choroidal vasculopathy

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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-

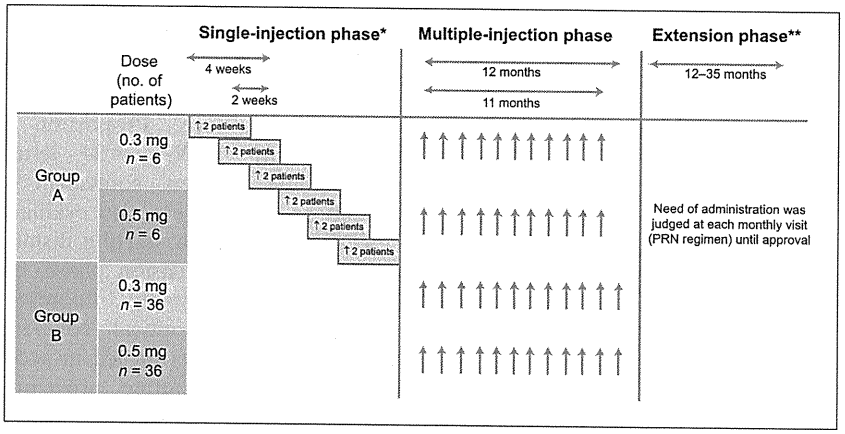

Fig. 1. EXTEND-I treatment schedule. *Upon completion of the single-dose phase, patients in Group A were eligible to enter the multiple-injection phase, which began ≥4 weeks after the final visit of the single-injection phase. Multiple injections did not begin until both doses were shown to be well tolerated 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.

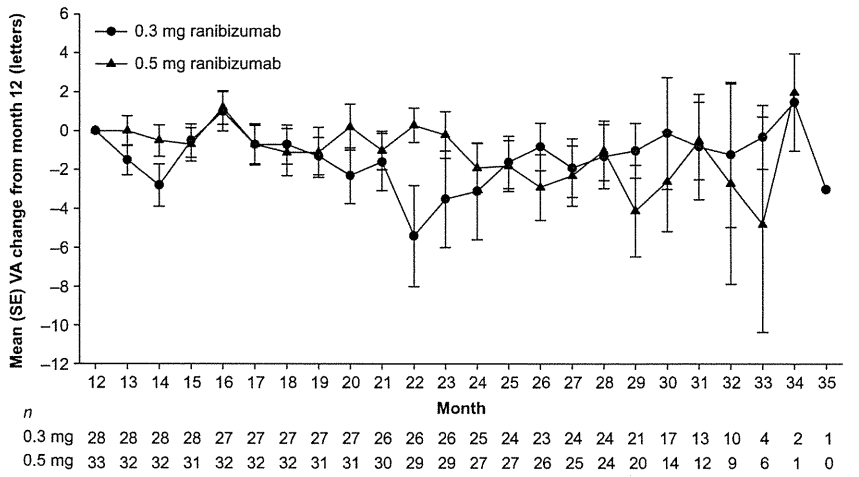

Fig. 2. Mean change from Month 12 (the start of Extension phase) in best-corrected visual acuity score (±SE) of study eye by visit during extension phase (Group B patients).

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 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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