Acta Ophthalmologica

EXTEND-I: safety and efficacy of ranibizumab in Japanese patients with subfoveal choroidal neovascularization secondary to age-related macular degeneration

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ABSTRACT.

Purpose: **To evaluate the efficacy and safety of intravitreal ranibizumab for subfoveal choroidal neovascularization (CNV) secondary to age-related macular degeneration (AMD) in Japanese patients.**

Methods: **This open-label, multicentre, Phase I/II study enroled patients into Group A (single injection of ranibizumab nonrandomized doses of 0.3 or 0.5 mg followed by 11 monthly injections of the same dose) and Group B (12 monthly injections of ranibizumab randomized to 0.3 or 0.5 mg). The primary efficacy endpoint was the mean change from baseline in best-corrected visual acuity (BCVA) score at Month 6. Safety was evaluated in all patients who received ranibizumab.**

Results: **Of 88 patients enroled, 12 entered Group A (six per dose) and 76 entered Group B (0.3 mg: $n = 35$; 0.5 mg: $n = 41$). Mean change from baseline in BCVA was significantly increased for both doses (Group B) at Month 6 (0.3 mg: +8.1 letters, $p = 0.0006$; 0.5 mg: +9.0 letters, $p < 0.0001$) and Month 12 (0.3 mg: +9.5 letters, $p = 0.0001$; 0.5 mg: +10.5 letters, $p < 0.0001$). At Month 12, one patient (0.3 mg) and 0 patients (0.5 mg) lost ≥15 letters, while 37.1% (0.3 mg) and 31.7% (0.5 mg) of patients gained ≥15 letters. Ocular serious adverse events (SAEs) of the study eye were reported in 1 and 2 patients in the 0.3- and 0.5-mg groups, respectively. Nonocular SAEs were experienced by 2 and 5 patients in the 0.3- and 0.5-mg groups, respectively. No cases of endophthalmitis were reported.**

Conclusion: **Ranibizumab was effective and well tolerated in Japanese patients with subfoveal CNV secondary to AMD.**

Key words: age-related macular degeneration – best-corrected visual acuity score – choroidal neovascularization – efficacy – Japanese patients – ranibizumab – safety – subfoveal – tolerability

Acta Ophthalmol. 2010: 88: 309–316

doi: 10.1111/j.1755-3768.2009.01843.x

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Introduction

Age-related macular degeneration (AMD) is a significant health problem. In particular, neovascular AMD is a progressive retinal disease that can cause severe and irreversible vision loss and can lead to legal blindness if left untreated (Nowak 2006). Furthermore, neovascular AMD can cause severe emotional distress and have a profound impact on patients' quality of life (Hassell et al. 2006; Augustin et al. 2007).

According to findings of the Hisayama study (prospective cohort study in Japan), the prevalence of neovascular AMD in residents aged 50 years or older ($n = 1486$) was 0.67% (1.2% in men, 0.34% in women) in 1998, which was lower than that observed in Caucasians (Oshima et al. 2001). However, another recent study (the Funagata study) of Japanese residents aged 35 years or older ($n = 1758$) between 2000 and 2002 suggested that although the prevalence of neovascular AMD was lower in Japanese women, it was similar to that seen in Caucasians for Japanese men (Kawasaki et al. 2008).

Until recently, available therapies for neovascular AMD in Japan were laser photocoagulation and photodynamic therapy (PDT) with verteporfin. Pegaptanib sodium was launched in 2008, and ranibizumab was launched

in early 2009 in Japan. Several studies have demonstrated the long-term safety and efficacy of verteporfin PDT in Japanese patients with AMD (Japanese Age-Related Macular Degeneration Trial (JAT) Study Group (2003, 2008); however, there is still little experience of pegaptanib sodium and ranibizumab in Japanese patients.

Ranibizumab (Lucentis®; Novartis Pharma AG, Basel, Switzerland and Genentech, Inc., South San Francisco, CA, USA) is a Fab fragment of a recombinant, humanized, monoclonal antibody. Ranibizumab specifically binds to and inhibits all biologically active isoforms of vascular endothelial growth factor-A (VEGF), thus blocking vascular permeability and angiogenesis in neovascular AMD (Ferrara et al. 2006; Dadgostar & Waheed 2008). Two pivotal Phase III clinical studies (ANCHOR and MARINA) have demonstrated unprecedented good efficacy and acceptable safety profiles for ranibizumab in patients with neovascular AMD (Brown et al. 2006; Rosenfeld et al. 2006), leading to ranibizumab being licensed for neovascular AMD in the United States by the Food and Drug Administration in 2006 (FDA 2006) and in the European Union in 2007.

The ANCHOR and MARINA studies were conducted in populations comprising predominantly Caucasian patients. EXTEND-I is the first clinical study to investigate the efficacy and safety of intravitreal ranibizumab specifically in Japanese patients with subfoveal choroidal neovascularization (CNV) secondary to AMD. This report describes the 12-month safety of single and multiple dosing and efficacy of multiple dosing of ranibizumab from the EXTEND-I study.

Methods

Study objectives

The primary objectives of the EXTEND-I study were to evaluate the safety of intravitreal administration of ranibizumab as single or multiple doses and to assess the efficacy of ranibizumab multiple dosing for 6 months. The secondary objectives were to compare the efficacy of multiple doses of ranibizumab (also at Month 12) in Japanese patients with that observed in previous non-Japanese studies and to characterize the pharmacokinetics of intravitreal ranibizumab in Japanese patients.

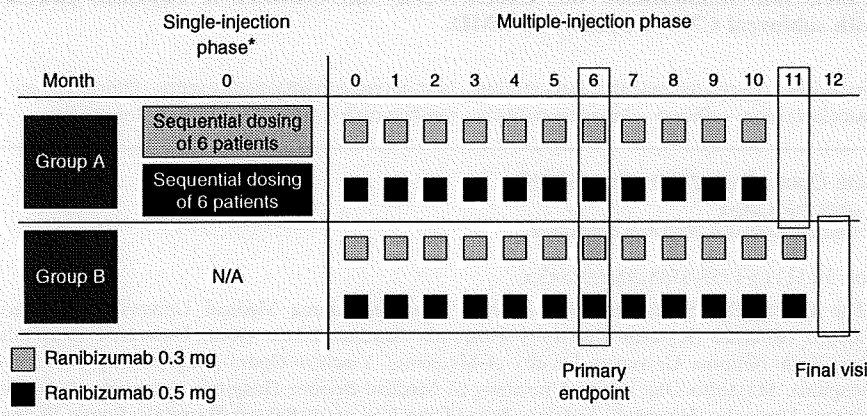
EXTEND-I study design

This was an open-label, multicentre, Phase I/II study comprising two phases (a single-injection phase and a multiple-injection phase) and two groups of patients (Groups A and B) (Fig. 1). In the single-injection phase, the safety of single intravitreal injections of ranibizumab (not randomized, doses of 0.3 or 0.5 mg) was evaluated in sequential cohorts of two patients in Group A. Those patients in Group A who successfully completed the single-injection phase [i.e. did not experience a grade-3 targeted adverse event (AE)] could enter a multiple-injection phase whereby they received monthly injections of ranibizumab for an additional 11 months at the same dose as they received in the single-injection phase. This multiple-injection phase was also initiated in a population of patients classified as Group B. In the multiple-injection phase, Group-B patients were randomized equally to receive a total of 12 monthly intravitreal injections of ranibizumab at doses of 0.3 or 0.5 mg. Both safety and efficacy were evaluated in Group B.

The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice, and was approved by the Institutional Review Board at each site. All patients provided written, informed consent before determination of their full eligibility.

Patients

Male or female patients aged ≥50 years with primary or recurrent subfoveal CNV secondary to AMD (including patients with predominantly classic lesions, minimally classic lesions or occult lesions with no classic component in a ratio of 1:1:1) were enroled from 15 study sites in Japan. Other inclusion criteria were a total area of CNV (including both classic and occult components) ≥50% of the total lesion area, total lesion size ≤5400 μm in the greatest linear dimension and a best-corrected visual acuity (BCVA) score between 73 and 24 letters in the study eye (approximate Snellen equivalent of 20/40 to 20/320), assessed with the use of Early Treatment Diabetic Retinopathy Study (ETDRS) charts. Patients were excluded if they had a BCVA score of < 34 letters in both eyes, had previously participated in a clinical study involving antiangiogenic drugs (for either eye), or had participated in a clinical study of any investigational drugs (excluding vitamins and minerals) within 1 month preceding EXTEND-I study commencement. Patients were also excluded if they had received previous treatment in the study eye with PDT with verteporfin, radiation therapy, macular laser photocoagulation, vitrectomy or transpupillary thermotherapy, or had a subretinal haemorrhage in the study eye involving the centre of the fovea with a size of either ≥50% of the total lesion area or ≥1 disc area (DA) in size.



*Upon completion of the single-injection 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

Fig. 1. EXTEND-I study design.

Assessments

Efficacy

The primary efficacy variable was the mean change from baseline in BCVA score, assessed with the use of ETDRS charts at a starting distance of 2 m, in the study eye at Month 6 for both ranibizumab doses; the mean change from baseline in BCVA score at Month 12 was evaluated as a secondary variable. Other secondary efficacy variables included the assessment at Months 3, 6, 9 and 12 of the total area of CNV, the total area of leakage from CNV plus staining of the retinal pigment epithelium (RPE), and the proportion of patients with absence of leakage from CNV. Total area of CNV and total area of leakage from CNV plus RPE staining were measured in the study eye by fluorescein angiography (FA). In addition, the proportions of patients at Months 6 and 12 with a BCVA score loss in the study eye of <15 letters, ≥ 15 letters and ≥ 30 letters, a BCVA score gain of ≥ 15 letters, and a BCVA score of <34 letters (approximate Snellen equivalent of 20/200) were evaluated. Snellen equivalents were determined with the ETDRS chart at a starting distance of 2 m. The change and per cent change from baseline in foveal retinal thickness at Months 3, 6, 9 and 12 were also investigated in study eyes using optical coherence tomography (OCT). The OCT imaging was performed using OCT 3000 (Stratus OCT™; Carl Zeiss Meditec AG, Jena, Germany) with software version A1.1 or later. OCT operators, systems and software were certified by the reading centre prior to the enrolment of study patients. Similarly for FA, photographers were also certified by the reading centre. FA and OCT images were interpreted at a designated central reading centre, the University of Wisconsin Fundus Photograph Reading Center (Madison, WI, USA).

Pharmacokinetics

Pharmacokinetic analysis was conducted in the single-injection phase of Group A. Blood samples were taken from all 12 patients in Group A at six time-points (1 hr before and 2 hr after ranibizumab single injection at baseline, and 24 hr, 3, 7 and 14 days after single injection). Serum ranibizumab concentration was assayed by Genentech, Inc. The pharmacokinetics of serum ranibizumab was analysed by noncompartmental methods (using WinNonlin Pro, Version 5: Pharsight, St Louis, MO, USA) and the pharmacokinetic parameters were summarized for each dose group. Area under the curve (AUC) was measured from time 0 to the last measurable time-point.

Safety

The primary safety variable was the incidence of grade-3 targeted AEs up to Month 6; targeted AEs were assessed in both the study and fellow eye consisted of intraocular inflammation (with grade-3 defined as any 4+ intraocular inflammation or 2–3+ intraocular inflammation that fails to decrease to $\leq 1+$ within 30 days) (Hogan et al. 1959), decreases in VA (with grade-3 defined as >29-letter decrease within 14 days after ranibizumab administration compared with before administration), retinal tear or detachment (with grade-3 defined as a new tear or detachment developing during the study and involving the macula), retinal haemorrhage (with grade-3 defined as any new haemorrhage >1 DA in size and involving the fovea, or an increase of a pre-existing haemorrhage by >1 DA and involving the fovea), vitreous haemorrhage (with grade-3 defined as any vitreous haemorrhage of $\geq 2 \pm$ severity lasting >14 days), and increases or decreases in intraocular pressure [with grade-3 defined as a persistent (>15 min) loss of light perception because of increased intraocular pressure, or a >20 mmHg increase or decrease in intraocular pressure lasting ≥ 14 days].

Serious adverse events (SAEs) were identified for special reporting requirement. Eye-related AEs were assessed by nondirective questioning and ophthalmic examinations; other AEs were detected by nondirective questioning, vital signs, laboratory values or other assessments. Pregnancy testing (urine) was performed on female patients (of child-bearing potential) at the screening visit. Serum samples for the evaluation of immunoreactivity to ranibizumab (antiranibizumab antibodies) were obtained from all patients prior to the first study drug administration, and from patients who had been treated with multiple doses of ranibizumab for ≥ 6 months at Month 6 in both Groups A and B, at Month 11 in Group A and at Month 12 in Group B.

Haematology, serum chemistry, urinalysis and vital signs were monitored regularly, and all AEs were collected and evaluated for their severity and relationship to the study drug.

Statistical analyses

For both Group A and Group B patients, demographic characteristics and baseline ocular characteristics were summarized for the enroled population (all enroled patients). The discrete variables were presented as the number and percentage of patients in each category, and the continuous variables were summarized using descriptive statistics (mean, median, standard deviation and range). Safety analyses, including drug exposure, were conducted in the safety population (all enroled patients who received at least one dose of the study drug and had at least one postbaseline safety assessment) in Groups A and B. Efficacy was not analysed in Group A.

For Group B, efficacy analyses were performed for three different populations: intent-to treat (ITT) population, per protocol population (PP) and patients with at least one measurement of OCT. The analysis of the primary efficacy variable was performed on the study eye in the ITT population using the last observation carried forward method to impute any missing data. In addition, to assess the robustness of the data, analysis of the primary efficacy variable was repeated on the PP population and the ITT population with observed data. Descriptive statistics for the change in BCVA score from baseline were summarized by treatment and by visit. The 95% confidence intervals (95% CI) for the change in BCVA score from baseline were based on *t*-distributions, and the p-values were based on paired *t*-tests.

Subgroup analyses were performed for the mean change from baseline in BCVA score at Months 6 and 12 by CNV lesion classification (predominantly classic, minimally classic and occult with no classic component), age (<75, ≥ 75 years), gender, baseline BCVA score in study eye (<55, ≥ 55; <45, ≥ 45 letters) and lesion size (≤ 2, 2–4 DA; >4 DA).

Analyses of the secondary efficacy variables were performed using the

same approaches as for the primary efficacy variable, with the exception of foveal retinal thickness by OCT, which was only analysed for the ITT population with observed data. These 12-month analyses of Group A and Group B data were based on data cut-offs at Month-11 and Month-12 visits for each patient, respectively, in the multiple-injection phase.

Results

Patients

Overall, 88 patients were enroled in the study: 12 in Group A (six per dose) and 76 in Group B (35 in the 0.3-mg-dose group and 41 in the 0.5-mg-dose group). Patient demographics and baseline characteristics are shown in Table 1.

In Group A, 12 patients completed the single-injection phase; of these, 11 patients subsequently entered the multiple-injection phase (one patient in the 0.3-mg-dose group chose to receive other therapy instead of entering the multiple-injection phase). Overall, 10 patients from Group A completed the multiple-injection phase; one patient in the 0.3-mg-dose group withdrew from the study because of an AE.

In Group B, eight of the 76 patients discontinued from the study prematurely; four in each of the ranibizumab 0.3-mg-dose group and 0.5-mg dose group. Discontinuations from the study were because of death (n = 2, one in each dose group), AEs (n = 2, one in each dose group), protocol violation (n = 1 in the 0.3-mg-dose group) and withdrawn consent (n = 3, one in the 0.3-mg-dose group and two in the 0.5 mg-dose group). None of the events leading to study discontinuation or death was thought to be related to the study treatment.

Efficacy (multiple-injection phase of Group B)

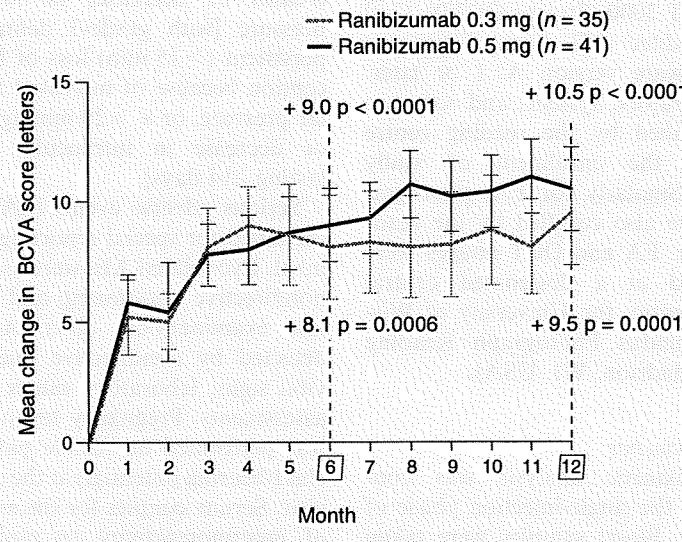
A significant increase in mean BCVA score in the study eye (standard deviation, SD), which was the primary efficacy endpoint, was observed between baseline (47.6 letters in the 0.3 mg and 48.1 letters in the 0.5 mg) and Month 6: +8.1 (12.65) letters (p = 0.0006, paired t-test) in the 0.3-mg-dose group and +9.0 (9.62) letters (p < 0.0001, paired t-test) in the 0.5-mg-dose group. Increases in mean BCVA score from baseline were seen after 1 month of ranibizumab treatment, namely increases of +5.2 (9.19) letters in the 0.3-mg-dose group and +5.8 (7.45) letters in the 0.5-mg-dose group. The improved BCVA scores at Month 6 were maintained up to Month 12, where increases of +9.5 (12.79) letters (p = 0.0001, paired t-test) in the 0.3-mg-dose group and +10.5 (11.4) letters (p < 0.0001, paired t-test) in the 0.5-mg-dose group were observed (Fig. 2).

At Month 6, the number of patients in the 0.3-mg-dose group and 0.5-mg-dose group who lost ≥15 letters in BCVA score in the study eye was 1 and 0, respectively, while the proportion of patients who gained ≥15 letters in BCVA score in the study eye was 34.3% and 24.4%, respectively. At Month 6, the proportion of patients in the 0.3-mg-dose group and 0.5-mg-dose group who had a BCVA of the approximate Snellen equivalent of 20/40 or better in the study eye was 11.4% and 29.3%, respectively, while the proportion of patients having a BCVA of the approximate Snellen equivalent of 20/200 or worse in the study eye at this time-point was 14.3% and 7.3%, respectively. Improvements in these secondary efficacy variables of

Table 1. Patient demographics and baseline characteristics.

	Group A		Group B	
	Ranibizumab 0.3 mg (n = 6)	Ranibizumab 0.5 mg (n = 6)	Ranibizumab 0.3 mg (n = 35)	Ranibizumab 0.5 mg (n = 41)
Gender (% male)	83.3	83.3	74.3	80.5
Age (mean years)	70.3	72.0	70.7	71.6
Mean BCVA letters (SD)	52.0 (16.90)	44.2 (14.95)	47.6 (11.82)	48.1 (10.75)
BCVA (median Snellen equivalent)	20/80	20/200	20/125	20/125
CNV classification (%)				
Occult with no classic	–	–	40.0	34.1
Minimally classic	–	–	34.3	41.5
Predominantly classic	–	–	25.7	24.4
Total area of lesion (DA)	–	–	2.35	2.36

BCVA = best-corrected visual acuity; CNV = choroidal neovascularization; DA = disc areas; SD = standard deviation.



BCVA = best-corrected visual acuity
p-values derived from paired t-test against baseline
Last observation carried forward method used to impute missing data
Vertical bars are (±) standard error of the mean

Fig. 2. Mean change from baseline in best-corrected visual acuity score with Early Treatment Diabetic Retinopathy Study chart over time in the study eye of multiple injection phase in Group B.

vision were also seen at Month 12 in both dose groups (Fig. 3).

The total area of CNV remained constant throughout 12 months in both dose groups. The mean change (SD) from baseline at Months 3, 6, 9 and 12 was −0.10 (0.95), −0.15 (0.97), −0.23 (0.97) and −0.16 (1.01) DA, respectively, in the 0.3-mg-dose group, and 0.13 (0.75), 0.04 (0.76), 0.21 (0.90) and 0.23 (1.08) DA, respectively, in the 0.5-mg-dose group.

Both dose groups showed a statistically significant decrease from baseline in the total area of leakage from CNV plus RPE staining and foveal retinal thickness over time. The total area of leakage from CNV plus RPE staining at Month 12 decreased by more than half of that at baseline; mean change (SD) of −1.50 (1.08) DA at Month 12 from 2.31 (1.17) DA at baseline ($p < 0.0001$, paired t-test) in the 0.3-mg-dose group, and −1.39 (1.48) DA at Month 12 from 2.49 (1.54) DA at baseline ($p < 0.0001$, paired t-test) in the 0.5-mg-dose group. At Month 12, foveal retinal thickness was significantly reduced compared with baseline in both ranibizumab-dose groups: the mean percentage change in the 0.3-mg-dose group was −41.6% (95% CI of −57.5, −25.6; $p < 0.0001$, paired t-test) and in the 0.5-mg-dose group, it was −58.9% (95% of CI −71.1, −46.7; $p < 0.0001$, paired t-test) (Fig. 4).

There was an increase in mean BCVA score at Month 12 in all analysed subgroups, with the exception of the subgroup of patients with a BCVA score of ≥55 letters at baseline in the 0.3-mg-dose group ($n = 10$, mean change −0.1 letters, SD 12.18 letters).

Pharmacokinetics

Pharmacokinetic data for each ranibizumab dose are shown in Table 2. Ranibizumab showed a slow systemic absorption with a mean T_{max} of 0.53 and 1.00 days for the 0.3-mg-dose group and 0.5-mg-dose group, respectively. Apparent mean $t_{½}$ in the 0.3-mg-dose group and 0.5-mg-dose group, respectively, was 6.56 and 7.85 days.

Safety

All 88 enroled patients received at least one dose of ranibizumab and had at least one postbaseline safety assessment: 12 in Group A; single and multiple-injection phase (six per dose group) and 76 in Group B; multiple-injection phase (35 in the 0.3-mg-dose group and 41 in the 0.5-mg-dose group). Table 3 summarizes the exposure of patients to ranibizumab treatment, including mean treatment duration. For patients in Group A, the treatment duration includes the transitional interval between the single- and multiple-injection phases. The length of this interval between the first and second injections varied for each patient. The maximum number of injections in both the groups was 12. Overall, 89% (78/88) of patients in pooled Groups A and B received >9 injections of ranibizumab.

No patients in Group A experienced a grade-3 targeted AE. However, a grade-3 targeted AE of 'retinal haemorrhage' in the study eye was

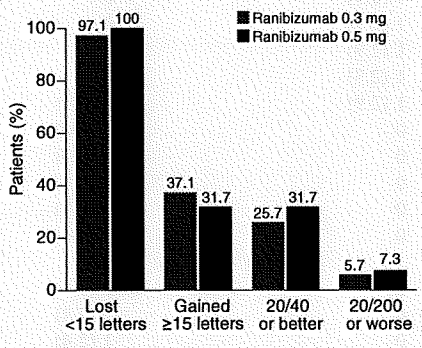
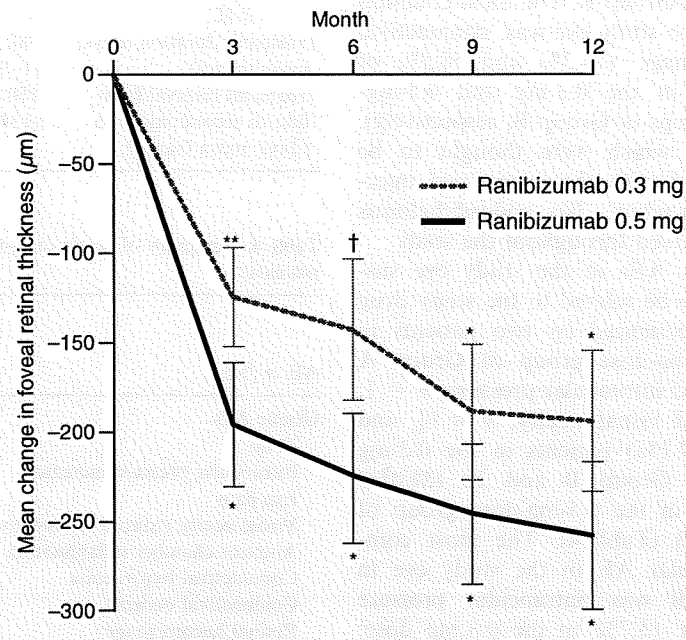


Fig. 3. Proportion of patients who lost <15 letters, gained ≥15 letters, had an approximate Snellen equivalent of 20/40 or better or had an approximate Snellen equivalent of 20/200 or worse in best-corrected visual acuity with Early Treatment Diabetic Retinopathy Study chart at Month 12 in the study eye of multiple injection phase in Group B.



*p < 0.0001; ** p = 0.0001; †p = 0.0012

Error bars are ±1 standard error of the mean

p -values derived from paired t-test against baseline

The numbers of patients in the 0.3 and 0.5 mg dose groups, respectively, were as follows:

Baseline; $n = 28$, $n = 30$

Month 3; $n = 27$, $n = 29$

Month 6; $n = 26$, $n = 28$

Month 9; $n = 26$, $n = 27$

Month 12; $n = 26$, $n = 26$

Fig. 4. Mean change from baseline in foveal retinal thickness in the study eye of multiple injection phase in Group B.

experienced by one patient (2.4%) in the 0.5-mg-dose group of Group B during the first 6 months of the multiple-injection phase. The overall incidence of grade-3 targeted AEs at Month 6 (the primary safety endpoint) was 1.3% (n = 1) of 76 patients in Group B. Additionally, grade-3-targeted AEs of 'visual acuity reduced transiently' in the study eye were experienced by two patients (4.9%) in the 0.5-mg-dose group of Group B immediately after an accidental overdose of ranibizumab (approximately 0.2–0.3 ml). The overall incidence of grade-3-targeted AEs at Month 12 was 3.9% (n = 3) in Group B. Approximately 90% of patients experienced at least one ocular AE in the study eye during the 12-month study period; namely, 94.3% (n = 33 of 35) of patients in the 0.3-mg-dose group and 82.9% (n = 34 of 41) of patients in the 0.5-mg-dose group of Group B. The most common AE of the study eye was 'conjunctival haemorrhage' (74.3% and 58.5% of patients in the 0.3 mg and 0.5-mg-dose groups of Group B, respectively), most of which were thought to be associated with the intravitreal injection procedure. No endophthalmitis was observed throughout the study.

Ocular AEs in the study eye suspected to be related to the study drug were experienced by two patients in the 0.5-mg-dose group of Group A (increased intraocular pressure, n = 1; decreased visual acuity, n = 1), and by 6 (17.1%) patients in the 0.3 mg dose of Group B and 10 (24.4%) patients in the 0.5-mg-dose group of Group B (Table 4). The most common ocular AE in the study eye in Group B was 'intraocular pressure increased' (5.7% in the 0.3 mg dose; 12.2% in the 0.5 mg dose). Furthermore, two patients in the 0.5-mg-dose group of Group B who received an accidental overdose of ranibizumab experienced the following ocular AEs: 'intraocular pressure increased' (study eye, both patients), 'visual acuity reduced transiently' (study eye, both patients), 'eye pain' (study eye, one patient), 'corneal oedema' (study eye, one patient) and 'asthenopia' (study eye, one patient).

Adverse events of intraocular inflammation were not observed in Group A. 'Anterior chamber inflammation' and 'iritis' of the study eye were observed in one patient each in the 0.3-mg-dose group of Group B. 'Anterior chamber inflammation' of the fellow eye was experienced by one patient in the 0.3-mg-dose group and 'iritis' of the fellow eye was experienced by one patient in each of the 0.3-mg-dose group and 0.5-mg-dose group of Group B.

Table 2. Pharmacokinetic parameters for ranibizumab after single administration.

Pharmacokinetic parameter	Ranibizumab 0.3 mg (n = 6)	Ranibizumab 0.5 mg (n = 6)
T_{max}, mean (range), days	0.53 (0.08–3.02)	1.00 (0.97–2.97)
C_{max}, mean (SD), ng/ml	1.96 (1.65)	1.86 (0.61)
$AUC_{0\text{-}T}$, mean (SD), ng* day/ml	7.47 (3.98)	14.90 (2.86)
$T_{½}$, mean (SD), days	6.56 (3.85)	7.85 (3.38)

$AUC_{0\text{-}T}$ = area under the curve (time 0 to last measurable time-point); C_{max} = highest systemic drug level; SD = standard deviation; $T_{½}$ = serum elimination half-life; T_{max} = time to achieve the highest systemic drug level.

Table 3. Summary of patient exposure to ranibizumab.

	Group A		Group B	
	Ranibizumab 0.3 mg (n = 6)	Ranibizumab 0.5 mg (n = 6)	Ranibizumab 0.3 mg (n = 35)	Ranibizumab 0.5 mg (n = 41)
No. of injections, mean (range)	9.3 (1–12)	12.0 (12–12)	11.2 (3–12)	11.1 (3–12)
<3	1	0	0	0
3–6	0	0	3	4
>6–9	1	0	1	0
>9–12	4	6	31	37
Treatment duration (days) mean (range)	382.2 (1–578)	417.8 (345–463)	306.6 (66–337)	305.3 (57–337)
Treatment interval from first to second injection (days) mean (range)	180.2 (134–281)	118.7 (51–166)	–	–

Table 4. Summary of study drug-related ocular and nonocular adverse events (AEs) (Group B patients).

AE, n (%)	Ranibizumab 0.3 mg (n = 35)	Ranibizumab 0.5 mg (n = 41)
Ocular AEs		
Total	6 (17.1)	10 (24.4)
Intraocular pressure increased	2 (5.7)	5 (12.2)
Eye pain	0	3 (7.3)
Visual acuity reduced transiently	0	2 (4.9)
Anterior chamber inflammation	1 (2.9)	0
Conjunctival hyperaemia	1 (2.9)	0
Conjunctival oedema	1 (2.9)	0
Retinal haemorrhage	1 (2.9)	0
Visual acuity reduced	1 (2.9)	1 (2.4)
Asthenopia	0	1 (2.4)
Corneal oedema	0	1 (2.4)
Lymphangiectasia	0	1 (2.4)
Posterior capsule opacification	0	1 (2.4)
Vitreous floaters	0	1 (2.4)
Nonocular AEs		
Total	0	3 (7.3)
Angina pectoris	0	1 (2.4)
Eczema	0	1 (2.4)
Hypertension	0	1 (2.4)

Table 5. Deaths and serious adverse events (SAEs) during the study period.

	Group A Ranibizumab 0.3 mg $n = 6$	Group A Ranibizumab 0.5 mg $n = 6$	Group B Ranibizumab 0.3 mg $n = 35$	Group B Ranibizumab 0.5 mg $n = 41$
Death	0 (0.0)	0 (0.0)	1 (2.9)	1 (2.4)
Total SAEs	1 (16.7)	0 (0.0)	4 (11.4)	8 (19.5)
Ocular SAE of study eye	0 (0.0)	0 (0.0)	1 (2.9)	2 (4.9)
Corneal oedema	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)
Eye pain	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)
Intraocular pressure increased	0 (0.0)	0 (0.0)	0 (0.0)	2 (4.9)
Visual acuity reduced	0 (0.0)	0 (0.0)	1 (2.9)	0 (0.0)
Visual acuity reduced transiently	0 (0.0)	0 (0.0)	0 (0.0)	2 (4.9)
Ocular SAE of fellow eye	0 (0.0)	0 (0.0)	2 (5.7)	1 (2.4)
Cataract	0 (0.0)	0 (0.0)	1 (2.9)	1 (2.4)
Macular degeneration	0 (0.0)	0 (0.0)	1 (2.9)	0 (0.0)
Nonocular SAE	1 (16.7)	0 (0.0)	1 (2.9)	5 (12.2)
Angina pectoris	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)
Anorexia	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)
Ataxia	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)
Bladder neoplasm	0 (0.0)	0 (0.0)	1 (2.9)	0 (0.0)
Cerebral haemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)
Diabetes mellitus	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)
Gastric cancer	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)
Hypoesthesia	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)
Lung carcinoma cell type unspecified recurrent	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)

Nonocular AEs suspected to be related to ranibizumab were reported by one patient in the 0.3-mg-dose group of Group A (intestinal diverticulum), and three patients in the 0.5-mg-dose group of Group B (Table 4).

There were 13 SAEs and two deaths in this study (Table 5). In Group A, one nonocular SAE of 'diabetes mellitus' was experienced by one patient (0.3-mg-dose group) during the multiple-injection phase. In Group B, ocular (study and fellow eye) or nonocular SAEs were experienced by four patients in the 0.3-mg-dose group and eight patients in the 0.5-mg-dose group. The SAEs in the Group B were 'corneal oedema', 'eye pain', 'intraocular pressure increased', 'visual acuity reduced' 'visual acuity reduced transiently' (accidental overdose of ranibizumab),'cataract', 'macular degeneration', 'angina pectoris', 'bladder neoplasm', 'cerebral haemorrhage', 'gastric cancer' and 'lung carcinoma cell type unspecified recurrent'. One patient experienced 'anorexia', 'ataxia' and 'hypoesthesia'. 'Bladder neoplasm' (0.3 mg Group B) resulted in the death of a patient during the first 6 months of the multiple-injection phase. There was another death (0.5-mg Group B) that resulted from a nonocular SAE of 'lung carcinoma cell type unspecified recurrent'. Neither of the deaths was suspected to be related to the study drug.

The study was discontinued by five patients because of nonocular AEs. This includes the two patients who died, two patients who reported an SAE and one patient in Group A (0.3-mg-dose group; 'vomiting' because of Fluorescein injection at Month 6).

The analyses of AEs potentially related to systemic VEGF inhibition focused on the incidence of hypertension, arterial thromboembolic events and nonocular haemorrhage. Overall, no such AEs were observed in the Group-A patients. In Group-B patients, they were observed in three of 35 patients (8.6%) in the 0.3-mg-dose group and in three of 41 patients (7.3%) in the 0.5-mg-dose group. 'Angina pectoris' and 'cerebral haemorrhage' were experienced by one patient (2.4%) each in the 0.5-mg-dose group. 'Hypertension' was experienced by three patients (8.6%) in the 0.3-mg-dose group and one patient (2.4%) in the 0.5-mg-dose group. Stroke ('cerebral haemorrhage') was observed in one patient (2.4%) in the 0.5-mg-dose group. Of these events, 'angina pectoris' and 'hypertension' in the 0.5-mg-dose group were suspected to be related to the study drug.

At Month 11 in the multiple-injection phase of Group A, immunoreactivity to ranibizumab (antiranibizumab antibodies) was not detected in any patient. At Month 12 in Group B, immunoreactivity to ranibizumab was detected in one of 32 evaluated patients (3.1%) in the 0.3-mg-dose group and three of 37 evaluated patients (8.1%) in the 0.5-mg-dose group. However, none of these patients had any AEs suspected to be related to the study drug.

Discussion

The results reported in this study demonstrate that monthly intravitreal ranibizumab significantly improves VA, FA and OCT outcomes in Japanese patients with subfoveal CNV secondary to AMD. Ranibizumab significantly increased the mean BCVA score from baseline to Month 6 with both 0.3 mg (+8.1 letters) and 0.5 mg (+9.0 letters) doses. The improved BCVA scores persisted to Month 12 in both the 0.3-mg-dose group (+9.5 letters) and 0.5-mg-dose group (+10.5 letters).

In addition, compared with baseline, ranibizumab significantly reduced the total area of leakage from CNV plus RPE staining and foveal retinal thickness.

The results in this study are consistent with those previously reported in the pivotal Phase III studies (MARINA and ANCHOR) conducted in a predominantly Caucasian population of patients with neovascular AMD (Brown et al. 2006; Rosenfeld et al. 2006). These studies showed an increase from baseline in mean BCVA score at 12 months of 6.5–8.5 letters with ranibizumab 0.3 mg and 7.2–11.3 letters with ranibizumab 0.5 mg, compared with decreases in mean BCVA score of 10.4 letters observed with sham treatment in the MARINA study and 9.5 letters with verteporfin PDT in the ANCHOR study. In addition, ≥97% of patients in this study lost fewer than 15 letters after 12 months of ranibizumab treatment, which is also similar to that seen in the MARINA and ANCHOR studies (approximately 95%). The proportions of patients who gained ≥15 letters were also similar among these three studies (approximately 30–40%).

The improvement in BCVA score observed with ranibizumab in this study and large-scale randomized double-masked clinical studies may reflect the ability of ranibizumab to inhibit all diffusible isoforms of VEGF that are biologically active, specifically $VEGF_{165}$, $VEGF_{121}$ and $VEGF_{110}$ (Lowe et al. 2007).

The calculated $t_{½}$ corresponded with the absorption rate of ranibizumab from the eye into the systemic circulation as a result of flip-flop pharmacokinetics associated with sustained release, and suggested a low elimination rate of ranibizumab from the eye. These pharmacokinetic findings in Japanese patients are consistent with those in non-Japanese patients described in the prescribing information of Lucentis (FDA, 2006).

Intravitreal ranibizumab treatment was associated with an acceptable safety and tolerability profile in this Japanese patient population. The most common AE was conjunctival haemorrhage of mild severity, most of which was thought to be because of the intravitreal injection procedure. Notably, there were no incidences of endophthalmitis in this study, which has also been mainly attributed to an intravitreal injection procedure. Among the observed ocular and nonocular SAEs, with the exception of overdose-related SAEs, only one SAE of angina pectoris in the 0.5-mg-dose group of Group B was suspected to be related to the study drug. The incidence of grade-3 targeted AEs in Group B was 1.3% (n = 1 of 35) at Month 6 and 3.9% (n = 3 of 41) at Month 12 in Group B. In addition, the incidences of nonocular AEs suspected to be related to ranibizumab and AEs potentially related to systemic VEGF inhibition were also within the acceptable range and consistent with the earlier studies (Brown et al. 2006; Rosenfeld et al. 2006).

In conclusion, the results of this study are comparable with previous randomized double-masked Phase III studies in predominantly Caucasian patients and indicate that monthly intravitreal ranibizumab therapy with 0.3 and 0.5 mg doses has an acceptable safe profile and is highly effective in Japanese patients with subfoveal CNV secondary to neovascular AMD.

In Japanese patients, clinically and statistically significant improvements in mean BCVA score of approximately two lines on the ETDRS chart have been achieved during 12 months of monthly treatment with ranibizumab.

Acknowledgements

The authors acknowledge former principal investigator, Dr Shinobu Takeuchi (Toho University Ohashi Medical Center), and Dr Takashi Tokoro (Tokyo Medical and Dental University) for their contribution to the study protocol and clinical study report.

The authors acknowledge medical writing assistance from Vanessa Cobb from Complete Medical Communications. This study was funded unconditionally by Novartis. The views and opinions expressed herein are those of the authors.

The EXTEND-I study was presented at the ARVO 2008 annual meeting as a poster presentation.

References

Augustin A, Sahel JA, Bandello F, Dardennes R, Maurel F, Negrini C, Hieke K & Berdeaux G (2007): Anxiety and depression prevalence rates in age-related macular degeneration. Invest Ophthalmol Vis Sci **48**: 1498–1503.

Brown DM, Kaiser PK, Michels M, Soubrane G, Heier JS, Kim RY, Sy JP & Schneider S (2006): Ranibizumab versus verteporfin for neovascular age-related macular degeneration. N Engl J Med **355**: 1432–1444.

Dadgostar H & Waheed N (2008): The evolving role of vascular endothelial growth factor inhibitors in the treatment of neovascular age-related macular degeneration. Eye **22**: 761–767.

FDA (2006). Highlights of Lucentis prescribing information. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2006/125156lbl.pdf.

Ferrara N, Damico L, Shams N, Lowman H & Kim R (2006): Development of ranibizumab, an antivascular endothelial growth factor antigen binding fragment, as therapy for neovascular age-related macular degeneration. Retina **26**: 859–870.

Hassell JB, Lamoureux EL & Keeffe JE (2006): Impact of age related macular degeneration on quality of life. Br J Ophthalmol **90**: 593–596.

Hogan M, Kimura S & Thygeson P (1959): Signs and symptoms of uveitis. I. Anterior uveitis. Am J Ophthalmol **47**: 155–170.

Japanese Age-Related Macular Degeneration Trial (JAT) Study Group (2003): Japanese age-related macular degeneration trial: 1-year results of photodynamic therapy with verteporfin in Japanese patients with subfoveal choroidal neovascularization secondary to age-related macular degeneration. Am J Ophthalmol **136**: 1049–1061.

Japanese Age-Related Macular Degeneration Trial (JAT) Study Group (2008): Photodynamic therapy with verteporfin in Japanese patients with subfoveal choroidal neovascularization secondary to age-related macular degeneration (AMD): results of the Japanese AMD Trial (JAT) extension. Jpn J Ophthalmol **52**: 99–107.

Kawasaki R, Wang JJ, Ji GJ et al. (2008): Prevalence and risk factors for age-related macular degeneration in an adult Japanese population: the Funagata study. Ophthalmology **115**: 1376–1381.

Lowe J, Araujo J, Yang J et al. (2007): Ranibizumab inhibits multiple forms of biologically active vascular endothelial growth factor in vitro and in vivo. Exp Eye Res **85**: 425–430.

Nowak JZ (2006): Age-related macular degeneration (AMD): pathogenesis and therapy. Pharmacol Rep **58**: 353–363.

Oshima Y, Ishibashi T, Murata T, Tahara Y, Kiyohara Y & Kubota T (2001): Prevalence of age related maculopathy in a representative Japanese population: the Hisayama study. Br J Ophthalmol **85**: 1153–1157.

Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY & Kim RY (2006): Ranibizumab for neovascular age-related macular degeneration. N Engl J Med **355**: 1419–1431.

Received on May 22nd, 2009.
Accepted on November 27th, 2009.

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Pharmacokinetics of Bevacizumab and Its Effect on Vascular Endothelial Growth Factor after Intravitreal Injection of Bevacizumab in Macaque Eyes

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Purpose. To evaluate the pharmacokinetics of intravitreally injected bevacizumab in the systemic circulation and the aqueous humor and its effect on vascular endothelial growth factor (VEGF) in the aqueous humor.

Methods. Bevacizumab (1.25 mg/50 μL) was injected into the vitreous cavity of the right eyes of three cynomolgus macaques. Aqueous humor and serum were obtained from the macaques just before injection and on days 1, 3, and 7 and weeks 2, 4, 6, and 8 after injection. The bevacizumab and VEGF concentrations were measured using enzyme-linked immunosorbent assay.

Results. Aqueous VEGF concentrations ranged from 63.2 to 106 pg/mL (mean, 80.0 ± 22.6 pg/mL) before injection; decreased to <31.2 pg/mL, the lower limit of detection, in all eyes between 1 and 28 days after injection; and returned to the preinjection concentration at 42 days. Aqueous VEGF concentrations in the fellow eyes did not change throughout the experiment. Aqueous bevacizumab concentrations in the treated eyes reached a mean peak concentration of 49,500 ± 10,900 ng/mL the day after injection and gradually declined, whereas those in the untreated eyes peaked at 3 days, with a mean concentration of 18.5 ± 25.5 ng/mL, and declined to below 0.156 ng/mL, the limit of detection at 2 weeks. A maximum mean bevacizumab concentration of 1430 ± 186 ng/mL was achieved in the serum 1 week after injection.

Conclusions. Intravitreal injection of bevacizumab decreased the VEGF concentration in the treated eyes for at least 4 weeks and had no or a minimal effect on the untreated fellow eyes. (*Invest Ophthalmol Vis Sci.* 2010;51:1606–1608) DOI:10.1167/iovs.09-4140

Bevacizumab (Avastin; Genentech, South San Francisco, CA) is a full-length humanized monoclonal antibody to all isoforms of vascular endothelial growth factor (VEGF) and has been approved by the Food and Drug Administration for intravenous treatment of metastatic colorectal cancer. Recently, intravenous injection of bevacizumab was reported to be effective for treating age-related macular degeneration (AMD),[1,2] whereas intravitreal injection of bevacizumab has been used widely to treat various ocular diseases including AMD and proliferative diabetic retinopathy.[1–5] Although numerous reports about the efficacy of intravitreal injection of bevacizumab have been published, few studies have reported on the pharmacokinetics of bevacizumab. Bakri et al.[6] reported the pharmacokinetics of intravitreal bevacizumab in a rabbit model and clearly showed that the vitreous half-life of 1.25 mg intravitreal bevacizumab is 4.32 days in rabbit eyes, with minute amounts of bevacizumab detected in the serum and the fellow untreated eye. However, the study had some limitations because of differences in vitreous volume and anatomy of human eyes. Therefore, we used a primate model, which has several advantages in that the ocular volume and anatomy are similar to those of humans. We measured the VEGF and bevacizumab concentrations over time in the aqueous humor of the treated and the untreated eyes after intravitreal injection of bevacizumab in cynomolgus macaques and the pharmacokinetics of bevacizumab in the aqueous humor of the treated and untreated eyes and in the serum.

Methods

All treatments were conducted in agreement with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and the animal research was approved by the Animal Experimentation Committee at Shiga University of Medical Science. Three male cynomolgus macaques, aged 8 to 9 years and weighing 3.9 to 5.5 kg, were anesthetized with 5 mg/kg intramuscular ketamine hydrochloride and 1 mg/kg intramuscular xylazine hydrochloride. Povidone iodine was placed on the conjunctiva of each eye. With the use of a 29-gauge needle, bevacizumab (1.25 mg/50 μL) was injected into the vitreous cavity of the right eye of each macaque. The left eyes received no intravitreal injections and served as controls. Both aqueous humor samples (200 μL) and venous blood samples (2 mL) were obtained from the macaque just before injection and 1, 3, and 7 days and 2, 4, 6, and 8 weeks after injection. Aqueous humor samples were obtained with a 29-gauge syringe. Anterior chamber depth recovered at all times when the samples were obtained. Serum was obtained by allowing the blood sample to clot overnight at 4°C followed by centrifugation. Samples were stored in a freezer at −80°C until analysis. The eyes were monitored before injection and 1, 3, and 7 days and then weekly after injection for signs of inflammation.

Measurement of VEGF

VEGF concentrations in the aqueous humor and the serum were measured with a commercial immunoassay (Quantikine Human VEGF Immunoassay; R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. The limit of the detectable VEGF concentration was 31.2 pg/mL. We measured serum VEGF concentrations twice. However, we measured VEGF concentrations in the aqueous humor once because the sample volumes were small.

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Supported in part by Grant 21592255 from the Ministry of Education, Culture, Sports, Science and Technology of Japan and a grant from the Ministry of Health, Labour and Welfare.

Submitted for publication June 13, 2009; revised August 14, 2009; accepted September 27, 2009.

Disclosure: **T. Miyake**, None; **O. Sawada**, None; **M. Kakinoki**, None; **T. Sawada**, None; **H. Kawamura**, None; **K. Ogasawara**, None; **M. Ohji**, None

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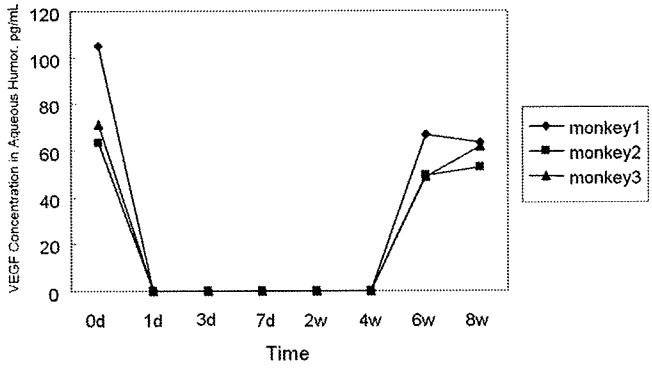

FIGURE 1. VEGF concentrations in the aqueous humor of the monkey eyes treated with bevacizumab. The 0 pg/mL VEGF concentration indicates concentrations below the lower limit of detection (31.2 pg/mL).

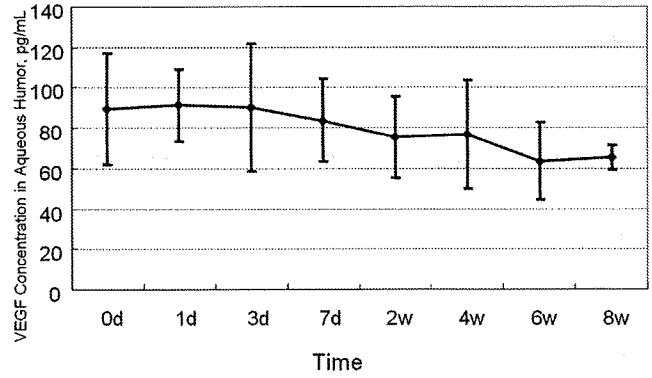

FIGURE 2. Mean VEGF concentrations in the aqueous humor of the fellow untreated eyes.

Bevacizumab Immunoassay

The concentration of bevacizumab was measured using an enzyme-linked immunosorbent assay, as previously described with slight modification.[7] Ninety-six-well plates were coated with recombinant human $VEGF_{165}$ (R&D Systems) at a concentration of 1 μg/mL overnight at 4°C (100 μL/well). After washing three times with phosphate-buffered saline (PBS) containing 0.05% Tween-20, the wells were blocked with 3% bovine serum albumin/PBS overnight at 4°C (200 μL/well). The wells then were washed five times with PBS containing 0.05% Tween-20 and stored dry at 4°C for later use. Aqueous humor or serum diluted in 0.1% bovine serum albumin/PBS was added to the plates overnight at 4°C (50 μL/well). Bevacizumab was detected by horseradish peroxidase-goat anti-human IgG (H+L) conjugate (Invitrogen Corporation, Carlsbad, CA) with a concentration of 1 μg/mL after a 3-hour incubation at room temperature. After five washes, color development was performed with 100-μL tetramethyl benzidine substrates (3,3′,5,5″-tetramethyl benzidine substrate), and the reaction was stopped by the addition of 1 M hydrogen chloride (100 μL). Optical density was measured at 450 nm with correction at 570 nm. A standard curve was prepared, with bevacizumab ranging from 15.6 to 1000 pg/mL. Because the sample volumes were small, we diluted them to 10 times and performed the measurement. Therefore, the limit of the detectable bevacizumab concentration was 0.156 ng/mL. We measured the bevacizumab concentration in each sample twice.

Statistical Analysis

All statistical analyses were carried out with a statistical analysis program (SAS 9.1.3; SAS Institute Japan, Tokyo, Japan).

RESULTS

VEGF concentrations in the aqueous humor of the right eyes ranged from 63.2 to 106 pg/mL (mean ± SD, 80.0 ± 22.6 pg/mL) before intravitreal injection of bevacizumab. One day after injection of bevacizumab, the VEGF concentrations in the aqueous humor decreased to <31.2 pg/mL, the lower limit of detection, in all treated eyes. The concentration below the lower limit was maintained until 4 weeks in all eyes (Fig. 1). VEGF concentrations in the aqueous humor of the fellow untreated eyes (left eyes) ranged from 57.9 to 108 pg/mL (mean, 89.4 ± 27.5 pg/mL) before intravitreal injection. There were no significant differences between the treated and the untreated eyes before intravitreal injection. VEGF concentrations in the aqueous humor of the fellow eyes did not change (Fig. 2). VEGF concentrations in the serum were <31.2 pg/mL, the limit of detection, before intravitreal injection of bevacizumab throughout the experiment.

Changes in the concentration of bevacizumab over time in the aqueous humor of the treated and the untreated eyes and in the serum after intravitreal injection are shown in Figure 3. Bevacizumab concentrations in the aqueous humor of the treated eyes peaked at 49,500 ± 10,900 ng/mL the day after injection and gradually declined. Bevacizumab also was detected in the untreated eyes; however, the levels were very low. Concentrations of bevacizumab in the aqueous humor of the untreated eyes peaked 3 days after injection, with a mean concentration 18.5 ± 25.5 ng/mL, and declined to <0.156 ng/mL, the lower limit of detection, at 2 weeks in all macaques. Bevacizumab was detected in the serum after intravitreal injection, though the concentrations were much lower than in the aqueous humor for 1 to 2 weeks after intravitreal injection. A maximum concentration of 1430 ± 186 ng/mL was achieved 1 week after injection and then gradually declined. However, the reduction rate was lower than that in the aqueous humor of the treated eyes, and the bevacizumab concentration in the serum was higher than that in the aqueous humor in the treated eyes at 4 weeks and thereafter. The bevacizumab concentration in the serum 8 weeks after injection was 67.1 ± 24.3 ng/mL, which was approximately 187 times higher than that in the aqueous humor of the treated eyes. The half-life of 1.25 mg intravitreally injected bevacizumab was 2.8 ± 0.6 days (n = 3; range, 2.3–3.5 days) in the aqueous humor and 12.3 ± 2.6 days (n = 3; range, 9.2–14.1 days) in the serum. The area under curve was 5680 ± 2336 (μg/mL × h) in the aqueous humor and 526.2 ± 17.1 (μg/mL × h) in the serum. No complications, such as uveitis or endophthalmitis, developed after the bevacizumab injections.

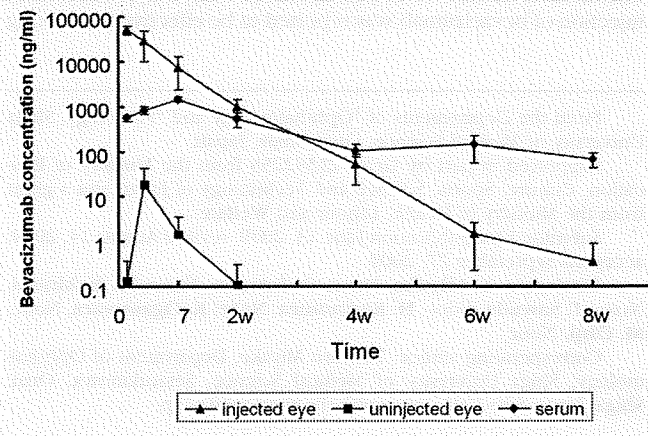

FIGURE 3. Concentrations of bevacizumab in the aqueous humor of the treated eye and in the serum after intravitreal injection.

Discussion

Because we used a macaque model and obtained aqueous humor samples repeatedly over time, we observed the VEGF levels at different time points in the same macaque eyes. To our best knowledge, this is the first study to report the time course of the VEGF level in the same macaques. Although macaque eyes are not the same as human eyes, VEGF levels in the aqueous humor of the macaques before injection were similar to those in human eyes.[5,7]

Concentrations of bevacizumab in macaques also were similar to those in humans.[7,8] Therefore, the current results could be applicable to human eyes. The only difference in the bevacizumab concentrations between macaques and humans was that the drug decreased in concentration in a shorter time in macaques than in humans. Krohne et al.[8] reported that the half-life of an intravitreal injection of 1.5 mg bevacizumab in humans was 9.82 days in the aqueous humor. However, in the present study, the half-life of 1.25 mg bevacizumab was 3.1 days in the aqueous humor. There are several explanations for this difference. First, we observed the bevacizumab concentrations at different time points in the same macaque eyes, whereas the same patients were not observed in the clinical study. Second, we used naive macaques in the present study, whereas the patients in the clinical study had some diseases. Measuring the VEGF and bevacizumab concentrations in the vitreous cavity rather than in the aqueous humor seems to be better for evaluating the intraocular concentration or the pharmacokinetics; however, it would be almost impossible to obtain vitreous samples from the same eyes repeatedly. Therefore, we measured VEGF and bevacizumab concentrations in the aqueous humor. The concentration in the aqueous humor can be useful because the VEGF level in the aqueous humor was reported to be significantly correlated with the VEGF level in the vitreous.[9] Funatsu et al.[10] measured VEGF and interleukin (IL)-6 levels in the aqueous humor, vitreous fluid, and plasma and reported a significant relationship between VEGF and IL-6 levels in the aqueous humor and vitreous fluid. The VEGF level in the vitreous fluid was about five to six times higher than in the aqueous humor. Because we clearly showed that the VEGF concentration in the aqueous humor decreased substantially after intravitreal injection of bevacizumab, the VEGF concentration in the vitreous also should decrease substantially after intravitreal injection of bevacizumab. In the present study, the VEGF level in the aqueous humor fell below the lower limit of detection after bevacizumab injection, similar to results reported in humans.[5] The decreased concentration was maintained for approximately 4 weeks and returned to a level similar to that before injection at 6 weeks after injection. Therefore, the effect of intravitreal injection of bevacizumab is expected to continue for approximately 1 month in macaques; although we do not know the exact length of time, the intravitreal injection of bevacizumab continued to be effective for at least 1 month in humans.[11]

Aqueous humor concentrations of bevacizumab gradually declined; however, low bevacizumab concentrations were detected over 8 weeks after the intravitreal injection, and the time course of the decreasing concentration in humans is longer than in macaques, indicating that the effect might continue longer in humans.[7,8] We previously reported that intravitreal injection of bevacizumab did not decrease the VEGF level in the aqueous humor of the fellow eyes and did not have as great a beneficial effect as a direct intravitreal injection of bevacizumab.[12] However, because that was a clinical study, we could not measure the VEGF concentration in the aqueous humor of the untreated fellow eyes before intravitreal injection of bevacizumab in the treated eye. Therefore, we could not measure the exact decrease in those eyes.

In the present study, the VEGF concentrations in the aqueous humor of the fellow eyes did not change throughout the experiments, although a minute amount of bevacizumab was detected in the fellow untreated eyes and peaked at 3 days with a concentration of 18.5 ng/mL. Avery et al.[3] reported that intravitreal injection of 6200 ng bevacizumab decreased fluorescein leakage in some cases. Because the vitreous volume is approximately 4 mL, an intravitreal injection of 6200 ng bevacizumab results in approximately 1500 ng/mL in the vitreous fluid. According to a previous study, the VEGF level in the vitreous fluid was approximately five to six times higher than in the aqueous humor.[10] Therefore, 1500 ng/mL in vitreous is at least >250 ng/mL in the aqueous humor. However, in the present study, only 18.5 ng/mL was detected, and it might have been too small to have an effect. Bevacizumab was detected in the serum after intravitreal injection, though the concentrations were much lower than in the aqueous humor until 2 weeks after injection. Intravenous injection of bevacizumab 2 mg/kg once weekly in macaques was not toxic after 26 weeks, and bevacizumab concentrations in the serum 1 week after one intravenous injection of 2 mg/mL bevacizumab were higher than 10,000 ng/mL (interview form for bevacizumab, Chugai Oncology, Tokyo, Japan; available only in Japanese). The maximum concentration in serum was 1430 ± 186 ng/mL, which is much lower than 10,000 ng/mL. Therefore 1.25 mg intravitreal injections of bevacizumab are not toxic systemically.

In conclusion, intravitreal injection of bevacizumab decreased the VEGF concentration in the treated eyes for approximately 4 weeks but had no or a minimal effect on the untreated fellow eyes in macaques.

References

1. Michels S, Rosenfeld PJ, Puliafito CA, Marcus EN, Vemlatraman AS. Systemic bevacizumab (Avastin) therapy for neovascular age-related macular degeneration: twelve-week results of an uncontrolled open-label clinical study. *Ophthalmology.* 2005;112:1035-1047.
2. Moshfeghi AA, Rosenfeld PJ, Puliafito CA, et al. Systemic bevacizumab (Avastin) therapy for neovascular age-related macular degeneration: twenty-four-week results of an uncontrolled open-label clinical study. *Ophthalmology.* 2006;113:2002-2011.
3. Avery RL, Pearlman J, Pieramici D, et al. Intravitreal bevacizumab (Avastin) in the treatment of proliferative diabetic retinopathy. *Ophthalmology.* 2006;113:1695-1705.
4. Spaide RF, Fisher YL. Intravitreal bevacizumab (Avastin) treatment of proliferative diabetic retinopathy complicated by vitreous hemorrhage. *Retina.* 2006;26:275-278.
5. Sawada O, Kawamura H, Kakinoki M, Ohji M. Vascular endothelial growth factor in aqueous humor before and after intravitreal injection of bevacizumab in eyes with diabetic retinopathy. *Arch Ophthalmol.* 2007;125:1363-1366.
6. Bakri SJ, Snyder MR, Reid JM, Pulido JS, Ezzat MK, Singh RJ. Pharmacokinetics of intravitreal bevacizumab (Avastin). *Ophthalmology.* 2007;114:855-859.
7. Zhu Q, Ziemssen F, Henke-Fahle S, et al. Vitreous levels of bevacizumab and vascular endothelial growth factor-α in patients with choroidal neovascularization. *Ophthalmology.* 2008;115:1750-1755.
8. Krohne TU, Eter N, Holz FG, Meyer CH. Intraocular pharmacokinetics of bevacizumab after a single intravitreal injection in humans. *Am J Ophthalmol.* 2008;146:508-512.
9. Aiello LP, Avery RL, Arrigg PG, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med.* 1994;331:1480-1487.
10. Funatsu H, Yamashita H, Noma H, et al. Aqueous humor levels of cytokines are related to vitreous levels and progression of diabetic retinopathy in diabetic patients. *Graefes Arch Clin Exp Ophthalmol.* 2005;243:3-8.
11. Funk M, Kriechbaum K, Prager F, et al. Intraocular concentrations of growth factors and cytokines in retinal vein occlusion and the effect of therapy with bevacizumab. *Invest Ophthalmol Vis Sci.* 2009;50:1025-1032.
12. Sawada O, Kawamura H, Kakinoki M, Ohji M. Vascular endothelial growth factor in fellow eyes of eyes injected with intravitreal bevacizumab. *Graefes Arch Clin Exp Ophthalmol.* 2008;246:1379-1381.

ラニビズマブ(遺伝子組換え)の維持期における再投与ガイドライン

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I 緒 言

中心窩下脈絡膜新生血管を伴う加齢黄斑変性症の治療薬であるラニビズマブ(遺伝子組換え)が「ルセンティス®硝子体内注射液 2.3 mg/0.23 ml(ノバルティスファーマ株式会社)」として平成21年1月に承認された．その用法および用量は，表1のとおり，導入期として1か月ごとに連続3か月間(本剤の投与は連続3回)硝子体内投与し，その後の維持期においては，症状により投与間隔を調節する，いわゆるフレキシブル用法(pro re nata：prn，as needed)となっている〔ルセンティス®硝子体内注射液 2.3 mg/0.23 ml 添付文書，2009年1月作成(新様式第1版)〕．さらに，用法および用量に関連する使用上の注意として，維持期には1か月に1回視力などを測定し，その結果および患者の状態を考慮し，本剤投与の要否を判断する旨規定されている．

このフレキシブル用法には，導入期の投与により平均として得られる視力改善を維持期においては最少限の投与回数で維持することが期待できるという有効性上のメリットがある．それに加えて，毎月投与する必要のある患者も想定されるものの，大多数の患者では硝子体内注射という高侵襲な投与の回数を減らせるという安全性上のメリットもあり，合理的な用法と考えられる．実際に投与回数が減れば，注射による身体的・精神的負担や，注射後の感染リスクが低減するだけでなく，結膜出血などの注射手順に関連する有害事象を含む有害事象全体の発現も低減すると考えられる．

この用法は，本剤の国内外の臨床試験でEarly Treatment Diabetic Retinopathy Study(ETDRS)視力検査表による最高矯正視力スコアの平均値が，本剤を1か月ごとに連続硝子体内投与することにより投与開始後から急速に改善し，3か月後までにはプラトーに達するとの結果に基づいて設定された．すなわち，最初の3か月間に本剤を1か月ごとに連続3回投与すれば，平均として視力改善が得られることから，まず3か月間の導入期が設定された．さらに，その後は患者ごとに症状，特に視力などが悪化した場合に投与すれば，導入期に得られた視力改善をその後も維持できるとの国内臨床試験結果に基づいて維持期の用法が設定された．この国内臨床試験では，最初の12か月間は本剤が月1回硝子体内注射されたが，その後の継続投与期では，連続する2回の来院時にETDRS視力検査表による最高矯正視力スコアとして5文字を超える悪化が認められた場合，光干渉断層計(optical coherence tomography：OCT)やフルオレセイン蛍光眼底造影(fluorescein angiography：FA)などの所見も考慮して再投与することを基準として実施された．継続投与期での投与間隔は同一患者内でも一定ではないものの，全例の年間平均投与回数は3.98回と推定され，月1回投与の1/3程度に減少したが，投与12か月後までに得られていた最高矯正視力スコアの平均値は

表1 ルセンティス®硝子体内注射液 2.3 mg/0.23 ml の承認された用法および用量

用法および用量	ラニビズマブ(遺伝子組換え)として0.5 mg(0.05 ml)を1か月ごとに連続3か月間(導入期[a])硝子体内投与する．その後の維持期においては，症状により投与間隔を適宜調節するが，1か月以上の間隔をあけること．
用法および用量に関連する使用上の注意(抜粋)	1．維持期においては，1か月に1回視力などを測定し，その結果および患者の状態を考慮し，本剤投与の要否を判断すること．また，定期的に有効性を評価し，有効性が認められない場合には漫然と投与しないこと．

a)：導入期における本剤の投与回数は合計3回である．

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(Received April 27, 2009 and accepted in revised form June 11, 2009)

その後もほぼ維持された(中間集計).

このように，用法上の維持期における本剤の再投与は，有効性指標である自覚的視力の毎月の検査結果を主とし，他覚的な眼科学的検査結果も考慮して総合的に判断することが基本と考えられる.

一方，臨床試験で用いられたETDRS視力検査表は本邦のみならず，世界的にも一般診療にはほとんど用いられておらず，臨床試験での再投与基準をそのまま一般診療に応用することはできない．しかしながら，上記のフレキシブル用法を実際の診療で活用するには，ETDRS視力検査表で測定可能な微小な視力変化を同程度に検出することが可能で，かつ実用的な視力検査方法の確立が必須となる.

諸外国での本剤の用法をみると，米国では月1回投与が推奨されているが，月1回投与ができない場合は，効果は減弱するものの，最初に月1回で4回連続投与した後は3か月に1回投与に投与頻度を減らせる旨のみを記載し，維持期の再投与の基準は規定されていない．一方，米国と異なり，欧州の用法では，月1回，連続3か月間投与する導入期(本剤の投与は連続3回)から開始し，その後の維持期では視力を月1回検査し，5文字(ETDRS視力検査表，またはSnellen視力検査表の1行に相当)を超える視力低下が認められた場合に再投与するが，投与間隔は1か月を下回らないと規定されている．欧州では5文字を超える視力低下を再投与の基準として規定しているものの，実際の診療での視力検査方法や，他の検査所見を考慮するか否かなど，具体的な再投与基準としては，欧州の5文字超以外，治療ガイドラインを含めて特に触れられていない.

一方，本邦では，視力は一般に万国式試視力表(以下，小数視力検査表)を用いて検査され，小数視力で表示される．この小数視力は最小可視角(分)の逆数で表されるため，各視標間の視力差は等間隔ではない．このため，本邦で一般的な小数視力に基づいて，本剤投与の要否をどのように判断するのか，さらには他の他覚的な眼科学的検査結果をどのように考慮するのかは，添付文書の用法および用量の記載だけでは明確ではない.

そこで，本邦における実際の診療状況を踏まえて，より具体的に本剤の維持期においてフレキシブル用法により適切に再投与を行うためのガイドラインを作成した．以下では，まず小数視力検査表を用いて微小な視力変化を検出するための視力測定方法と視力悪化の基準を提示し，次いで視力検査以外の眼科学的検査による網膜病態の判断基準を提示した上で，これらを総合的に考慮し，視力維持を目的として考案した本剤の維持期における再投与アルゴリズムを供覧する.

Ⅱ　ガイドライン

1．本剤の維持期におけるフレキシブル用法に適した小数視力の測定方法

本邦で視力検査に通常使われる小数視力検査表では，視標列間の最小可視角(分)の差が高視力領域に比べて低視力領域で大きくなるため，通常の測定方法では特に低視力領域における微小な視力変化を検出することはできない．一方，ETDRS視力検査表では，視標が1列5文字ずつ最小可視角(分)の対数(logarithmic Minimum Angle of Resolution：logMAR)として等間隔で配列されているため，小数視力0.02から2.0までを0～100文字のスコアとして1文字単位(0.02 logMAR相当)で測定可能であり，1列5文字ごと(0.1 logMAR相当)の小数視力としても測定可能である．例えば，小数視力0.2から0.1への1段階の悪化はETDRS視力検査表の文字スコアとして15文字の悪化に相当するが，小数視力検査表では0.2と0.1の間には視標列がないため，この視標間の視力悪化を検出できない．これに対して，ETDRS視力検査表には0.1と0.2の視標間を5文字間隔の3段階に分割し，0.125と0.16の視標列も設定されているため，この領域の視力悪化も1列5文字ごとに相当する小数視力として，より詳細に測定できる．このため，ETDRS視力検査表を用いた前述の国内臨床試験では，5文字超の視力悪化が検出された場合に本剤を投与するフレキシブル用法が可能であった．これに対して，通常の小数視力測定方法では5文字超の視力悪化を検出できないため，実際の診療でフレキシブル用法を臨床試験と同水準で行うことはできない．そこで，通常の小数視力検査表を用いて，ETDRS視力検査表で5文字超に相当する視力悪化を検出することが可能で，かつ実用的な測定方法を考案した．以下に基本的な考え方と具体的な測定方法を記述する.

本邦では，小数視力0.1未満の視力は，通常使われる測定距離5m用の小数視力検査表の0.1の視標が判別できる距離まで被検者に近づいてもらうか，検査者が0.1の視標を持って視標を判別できる距離まで被検者に近づく方法により測定される．例えば，測定距離3mまで近づいたときに0.1の視標を判別できた場合の視力は，0.1×3m/5m=0.06と比例計算される．このような視力の比例計算を容易にするため，被検者と視力検査表との距離は通常5m，4m，3m，2.5m，2m，1.5m，1mが推奨される．このように0.1の視標を用いて測定距離を調節して0.1未満の小数視力を測定する方法を，視標間の視力差が大きい0.1と0.2の間(15文字の差に相当)，ならびに0.2と0.3の間(8文字の差に相当)についても応用することにより，表2のとおり，小数視力検査表を用いてETDRS視力検査表による0～100文字の範囲をほぼ5文字間隔で検査することが可能

表2 小数視力検査表(5m用)を用いて測定距離を調節して視力を測定する方法と各小数視力間の文字数の差

使用視標	測定距離	小数視力(括弧内は計算値)[a)b)]	視力計算方法	各小数視力(計算値を含む)に相当するETDRS視力検査表による文字数の理論値[c)]	1段階高い小数視力(計算値を含む)との差(ETDRS視力検査表の文字数として換算)[d)]
0.1	1m	(0.02)	0.1×1m/5m=0.02	0	−8
	1.5m	(0.03)	0.1×1.5m/5m=0.03	8	−7
	2m	(0.04)	0.1×2m/5m=0.04	15	−5
	2.5m	(0.05)	0.1×2.5m/5m=0.05	20	−4
	3m	(0.06)	0.1×3m/5m=0.06	24	−6
	4m	(0.08)	0.1×4m/5m=0.08	30	−5
	5m	0.1	—	35	−4
0.2	3m	(0.12)	0.2×3m/5m=0.12	39	−6
	4m	(0.16)	0.2×4m/5m=0.16	45	−5
	5m	0.2	—	50	−4
0.3	4m	(0.24)	0.3×4m/5m=0.24	54	−4
	5m	0.3	—	58	−7
0.4	5m	0.4	—	65	−5
0.5	5m	0.5	—	70	−4
0.6	5m	0.6	—	74	−3
0.7	5m	0.7	—	77	−3
0.8	5m	0.8	—	80	−2
0.9	5m	0.9	—	82	−3
1.0	5m	1.0	—	85	−4
1.2	5m	1.2	—	89	−5
1.5	5m	1.5	—	94	−6
2.0	5m	2.0	—	100	—

a)：括弧のない小数視力は，小数視力検査表に対応する視標が予め設定されているものを示す(測定距離5m)．

b)：括弧内の小数視力は，小数視力検査表に対応する視標が設定されていないものを示す(測定距離を調節して測定する視力)．

c)：ETDRS(Early Treatment Diabetic Retinopathy Study)視力検査表による文字数について，例えば小数視力0.1と判定される文字数の範囲は34～38文字に相当するが，小数視力0.1に対応する理論値35文字を示す．

d)：ある小数視力に相当するETDRS視力検査表による文字数から，その小数視力より1段階高い小数視力に相当するETDRS視力検査表による文字数を減じた値．例：0.02と0.03では0−8=−8

—：該当せず．

となる．

本剤による治療を受ける患者は，毎月の来院時に上記の測定距離を調節する方法を応用して，最高矯正視力を小数視力(計算値を含む)として毎回測定し，ほぼ5文字超に相当する視力低下の有無を確認する必要がある．例えば，前回来院時に0.2の視標が5mの距離で判別できた患者(小数視力0.2)の場合，その視力はETDRS視力検査表による文字数では50文字に相当する．1か月後の来院時に5mで0.1の視標は判別できたが，0.2の視標は判別できなかった場合，4mで0.2の視標を判別できたら，その小数視力は0.16，すなわちETDRS視力検査表による文字数で45文字に相当する．この場合，前回に比べて理論上は視力が5文字低下したことを意味する．もし，このとき，4mでも0.2の視標を判別できない場合は5文字を超える視力低下と判断できる．

このように，ETDRS視力検査表による文字数でほぼ5文字超に相当する最高矯正視力の変化を，測定距離調節すれば小数視力検査表によっても測定できること，以下の視力悪化の基準を設定した．さらにこの基に該当する小数視力の変化を具体的に表3に示した．

視力悪化の基準：
前回の視力を基準として，ETDRS視力検査表の文数に換算してほぼ5文字超の悪化に相当する小数視の視標が判別できない場合

2．その他の眼科学的検査

前項に示した，測定距離を調節して行う小数視力検表による視力検査によって，ETDRS視力検査表に視力検査とほぼ同等に微小な視力変化を検出すること可能となったことから，この方法による視力検査だけも本剤の再投与を判断することは十分可能と考える．かしながら，視標間の視力差が視力悪化の基準であ

表3　ETDRS視力検査表でほぼ5文字超に相当する小数視力検査表における小数視力の変化

小数視力検査表(5m用)					ETDRS視力検査表
使用視標	測定距離	前回の視力(括弧内は計算値)[a)b)]	→	1か月後の来院時に，下記視力の視標が判別できない場合は，視力悪化の基準に該当する(括弧内は計算値)[a)b)]	理論上推定される減少文字数
0.1	1m	(0.02)	→	—	—
	1.5m	(0.03)	→	(0.02)	>8
	2m	(0.04)	→	(0.03)	>7
	2.5m	(0.05)	→	(0.04)	>5
	3m	(0.06)	→	(0.05)	>4
	4m	(0.08)	→	(0.06)	>6
	5m	0.1	→	(0.08)	>5
0.2	3m	(0.12)	→	0.1	>4
	4m	(0.16)	→	(0.12)	>6
	5m	0.2	→	(0.16)	>5
0.3	4m	(0.24)	→	0.2	>4
	5m	0.3	→	(0.24)	>4
0.4	5m	0.4	→	0.3	>7
0.5	5m	0.5	→	0.4	>5
0.6	5m	0.6	→	0.5	>4
0.7	5m	0.7	→	0.5[c)]	>7
0.8	5m	0.8	→	0.6[c)]	>6
0.9	5m	0.9	→	0.7[c)]	>5
1.0	5m	1.0	→	0.8[c)]	>5
1.2	5m	1.2	→	1.0	>4
1.5	5m	1.5	→	1.2	>5
2.0	5m	2.0	→	1.5	>6

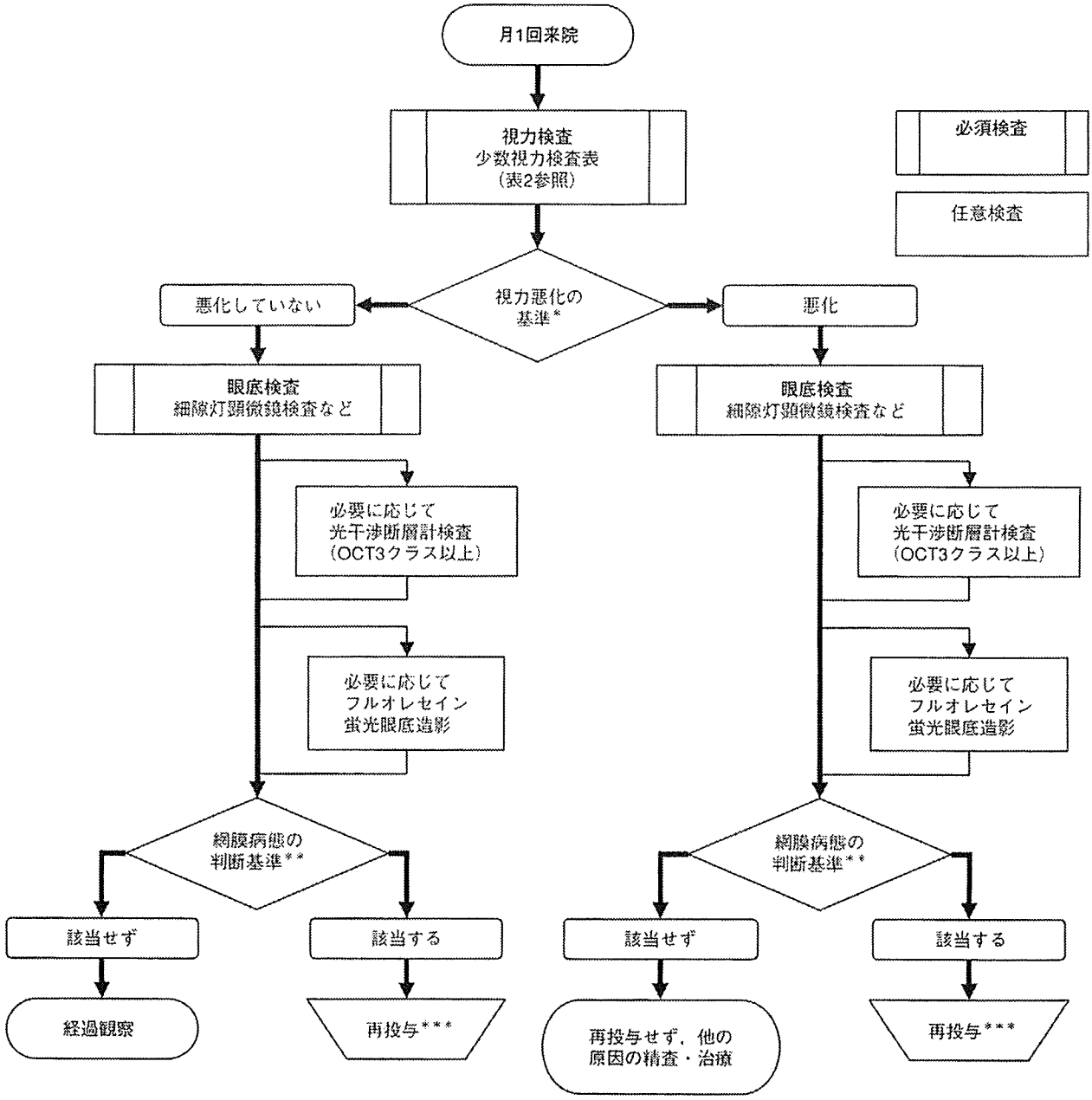
a)：括弧のない小数視力は，小数視力検査表に対応する視標が予め設定されているものを示す(測定距離5m)．
b)：括弧内の小数視力は，小数視力検査表に対応する視標が設定されていないものを示す(測定距離を調節して測定する)．
c)：小数視力検査表で2段階視力が低い視標が判別できない場合に該当し，その他は1段階視力が低い視標が判別できない場合に該当する．
—：該当せず．

文字超より大きい箇所(視力差として6文字超～8文字超)があることに加え，加齢黄斑変性症(age-related macular degeneration：AMD)の病態の進行を考慮すると，視力の悪化に先行して生じると考えられる網膜病態の変化などを各種眼科学的検査によって客観的に把握し，その結果も考慮した上で，本剤の再投与を判断することは重要と考えられる．

眼科診療で通常実施される眼科学的検査のうち，眼底検査(細隙灯顕微鏡検査など)では滲出型AMDによる網膜出血などの有無を観察することが重要である．また，OCTおよびFAは滲出型AMDの診断や，網膜浮腫などの滲出性変化の経過の観察には不可欠な検査となっている．しかしながら，FAは網膜，脈絡膜の血管の形態学的変化や，循環動態を把握する上では重要な検査ではあるものの，フルオレセインに対する過敏症などの安全性上の懸念を伴う侵襲的検査であることから，毎月来院時に実施することは事実上不可能である．一方，OCTは，FAと異なり，非接触，非侵襲，非破壊で，操作も容易かつ簡便で，網膜の断面の画像が得られ，網膜厚や網膜の各層での形態学的な変化などを捉えることが可能で，滲出型AMDの網膜浮腫などを容易に検出できる検査としてきわめて有用である．解像度(横方向および深さ方向)や，網膜厚の繰り返し性および再現性の点で，OCT 3000など，通称OCT 3クラス以上のOCTであれば，OCT画像の視覚的・定性的判断によって網膜浮腫などの網膜病態の把握が可能となっている．

以上のことを踏まえて，小数視力検査に基づく視力悪化の有無の判断に加えて，本剤の再投与の要否を総合的に判断する上で，視力悪化に先行して生じると考えられる網膜病態の変化やその持続を考慮する目的で，網膜病態の判断基準を以下のとおり設定した．

網膜病態の判断基準：
出血あるいは滲出性変化(網膜浮腫など)がある場合



*：前回の視力を基準として，Early Treatment Diabetic Retinopathy Study(ETDRS)視力検査表の文字数に換算してほぼ5文字超の悪化に相当する少数視力の視標が判別できない場合(表3参照)
**：出血あるいは滲出性変化(網膜浮腫など)がある場合
***：投与に影響する有害事象がない限り，原則として再投与とするが，再投与するか否かは最終的に眼科医が総合的に判断する．

図1 維持期においてラニビズマブ(遺伝子組換え)の再投与を判断するためのアルゴリズム．

図1は，ラニビズマブ(遺伝子組換え)の維持期(表1参照)において，ラニビズマブ(遺伝子組換え)を再投与するか否かを判断するためのアルゴリズムである．すなわち，月1回来院から始まる矢印に従って進み，必須検査の視力検査で視力悪化の基準(表3参照)に照らして悪化している場合と悪化していない場合に分かれる．それぞれの場合について，必須検査の眼底検査，必要に応じて実施する任意検査の光干渉断層計検査およびフルオレセイン蛍光眼底造影の結果に基づいて，網膜病態の判断基準〔出血あるいは滲出性変化(網膜浮腫など)がある場合〕に該当するか否かに従って，ラニビズマブ(遺伝子組換え)を再投与するか否かを判断する．

3．維持期において本剤の再投与を判断するためのアルゴリズム

以上に基づいて，本剤を導入期として月1回，連続3か月間(本剤の投与は連続3回)硝子体内に投与した後の維持期において，毎月の来院時に本剤を再投与するか否かを判断するための再投与の判断アルゴリズムを図1に示した．

今回考案したアルゴリズムでは，連続する2回の来院でほぼ5文字超に相当する最高矯正視力の悪化が認められた場合，その原因が滲出型AMD以外の場合を除いて，原則として本剤を再投与することが推奨される．一方，連続する2回の来院で視力が5文字以下の低下に留まる場合は，再投与せず経過観察が原則であるが，出血や，必要に応じて実施するOCT検査やFA検査などの結果で網膜浮腫などの滲出性変化が存在していれば，それを考慮して本剤を再投与することが推奨される．この点で，欧州の視力のみを判断指標として，5文字(ETDRS視力検査表，またはSnellen視力検査表の1行に相当)を超える視力低下が認められた場合に再投与するフレキシブル用法に比べると，本アルゴリズムは視力維持を図るという目的として，より優れていると考えられる．

以上のとおり，このガイドラインに規定した測定距離を調節して測定する小数視力測定法と，その他の眼科学的検査結果も考慮する再投与アルゴリズムは，本剤の適正使用に資するものと考える．なお，今後，本剤とともに本アルゴリズムも実際の診療に供されることとなるが，その内容は市販後の使用経験の集積に応じて継続的に見直していく必要性がある．特に，OCT検査は滲出型AMDの診断や経過観察には不可欠で重要な検査の1つであるが，前述のとおり，本剤の国内臨床試験の限られた結果ではあるものの，視力維持のためには必ずしも不可欠とはいえなかったことから，本アルゴリズムでは必須検査には位置付けなかった．しかしながら，OCTは平成20年4月に眼底三次元画像解析として保険収載され，その実施率は今後飛躍的に高くなることが容易に予想される．これに伴って網膜病態がより確実に把握できるようになることから，本剤の維持期における再投与を判断する上でOCTの位置付けを見直すことは必至と考えられ，今後の検討が待たれる．

医療は本来医師の裁量に基づいて行われるものであり，医師は個々の症例に最も適した診断と治療を行うべきである．ラニビズマブ治療指針策定委員会は，本ガイドラインを用いて行われた医療行為により生じた法律上のいかなる問題に対して，その責任義務を負うものではない．

Comparison of Intravitreal Triamcinolone Acetonide With Photodynamic Therapy and Intravitreal Bevacizumab with Photodynamic Therapy for Retinal Angiomatous Proliferation

MASAAKI SAITO, CHIEKO SHIRAGAMI, FUMIO SHIRAGA, MARIKO KANO, AND TOMOHIRO IIDA

• PURPOSE: To compare the efficacy of combined therapy with intravitreal triamcinolone (IVTA) and photodynamic therapy (PDT; IVTA plus PDT) with intravitreal bevacizumab (IVB) and PDT (IVB plus PDT) for patients with retinal angiomatous proliferation (RAP).

• DESIGN: Retrospective, observational case series.

• METHODS: We retrospectively reviewed 25 treatment-naïve eyes of 22 Japanese patients (11 men, 11 women) with retinal angiomatous proliferation. Twelve eyes of 11 patients were treated with combined therapy of IVTA plus PDT from September 1, 2004, through July 31, 2006. Thirteen eyes of 11 patients were treated with combined therapy of IVB plus PDT from February 1, 2007, through January 31, 2008.

• RESULTS: In 12 eyes treated with IVTA plus PDT, the mean best-corrected visual acuity (BCVA) levels at baseline and 12 months were 0.29 and 0.13, respectively. A significant ($P < .05$) decline in the mean BCVA from baseline was observed at 12 months. In 13 eyes treated with IVB plus PDT, the mean BCVA levels at baseline and 12 months were 0.25 and 0.37. A significant ($P < .05$) improvement in the mean BCVA from baseline was observed. At 12 months, the difference in BCVA between the 2 groups was significant ($P < .05$). The mean numbers of treatments at 12 months in the IVTA plus PDT group and the IVB plus PDT group were 2.7 and 1.6, respectively. The difference between the 2 treatments reached significance ($P < .05$). No complications developed.

• CONCLUSIONS: Compared with IVTA plus PDT, IVB plus PDT was significantly more effective in maintaining and improving visual acuity and in reducing the number of treatment for patients with retinal angiomatous proliferation. (Am J Ophthalmol 2010;149: 472–481. © 2010 by Elsevier Inc. All rights reserved.)

Accepted for publication Sep 21, 2009.

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RETINAL ANGIOMATOUS PROLIFERATION (RAP) HAS been described as a variant of exudative age-related macular degeneration (AMD).[1] The term RAP was first coined by Yannuzzi and associates in 2001.[1] RAP is differentiated into 3 stages based on clinical and angiographic observations: stage 1, proliferation of intraretinal capillaries originating from the deep retinal complex (intraretinal neovascularization); stage 2, growth of the retinal vessels into the subretinal space (subretinal neovascularization); and stage 3, clinically or angiographically observed choroidal neovascularization (CNV).[1] RAP sometimes is referred to as type 3 neovascularization to distinguish it from the type 1 and 2 CNV anatomic classifications described by Freund and associates.[2]

RAP represents 15% of all neovascular AMD in white patients and 4.5% of all neovascular AMD in Japanese patients.[3,4] The natural course of RAP differs from typical exudative AMD and has poor visual outcomes.[5–7] Furthermore, various treatments for RAP such as conventional laser photocoagulation,[6,8] transpupillary thermotherapy,[6,9] surgical ablation,[10,11] and monotherapy of photodynamic therapy (PDT) with verteporfin (Visudyne; Novartis Pharma AG, Basel, Switzerland)[12,13] have not been efficacious.

CNV complexes are comprised of inflammatory cells and vascular endothelial growth factor (VEGF).[14–16] Corticosteroids such as triamcinolone acetonide (TA) have antiangiogenic, antiinflammatory, and anti-VEGF effects.[17,18] Recent studies have reported that combined therapy of intravitreal TA (IVTA) and PDT for RAP effectively resolves angiographic leakage and maintains or improves visual acuity (VA).[19,20]

Anti-VEGF therapy prevents formation of CNV and decreases leakage from existing CNV in animal models.[21] VEGF monoclonal antibodies and aptamers such as ranibizumab (Lucentis; Genentech, Inc, South San Francisco, California, USA), bevacizumab (Avastin; Genentech), and pegaptanib (Macugen; EyeTech Pharmaceuticals, Lexington, Massachusetts, USA) reduce vascular leakage and improve visual outcomes in patients with CNV secondary to AMD.[22–25] Moreover, combined therapy of intravitreal bevacizumab (IVB) injections and PDT administered to treat CNV reduced the retreatment rates in patients with AMD.[26–28] We reported recently that combined therapy of IVB and PDT was effective for treating RAP after 6

0002-9394/10/$36.00
doi:10.1016/j.ajo.2009.09.016

TABLE 1. Intravitreal Triamcinolone Acetonide and Photodynamic Therapy for Retinal Angiomatous Proliferation

						Baseline					12 Months after Treatment					
Case No.	Age (yrs)	Gender	Eye	RAP Stage	Lens Status	VA	Central Retinal Thickness (μm)	RRA	GLD (μm)	IOP (mmHg)	VA	Central Retinal Thickness (μm)	RRA	GLD (μm)	IOP (mmHg)	No. Treatments
1	74	F	Right	2+PED	Phakic eye	0.8	279	Yes	1590	13	0.8	34	No	0	12	1
2	85	F	Right	2	Pseudophakia	0.2	385	Yes	1100	13	0.1	115	Yes	0	11	2
3	86	M	Right	2	Phakic eye	0.4	293	Yes	2570	10	0.1	550	Yes	2197	10	4
4	86	M	Left	2+PED	Phakic eye	0.3	350	Yes	1080	11	0.2	289	Yes	957	11	4
5	74	F	Left	2	Pseudophakia	0.8	542	Yes	3600	13	0.5	232	No	0	12	3
6	63	M	Right	2+PED	Phakic eye	0.8	267	Yes	5610	17	0.06	481	Yes	4784	18	3
7	84	F	Right	2+PED	Phakic eye	0.6	355	Yes	2690	10	0.08	407	Yes	3296	9	5
8	90	F	Right	2	Pseudophakia	0.3	314	No	4516	13	0.03	83	—	0	16	1
9	70	F	Left	2	Pseudophakia	0.09	367	Yes	2450	16	0.2	324	Yes	0	19	2
10	69	F	Right	2	Phakic eye	0.15	625	Yes	3334	14	0.1	135	Yes	0	10	3
11	68	M	Right	2	Pseudophakia	0.1	521	Yes	3430	20	0.3	237	Yes	0	16	2
12	90	F	Right	2+PED	Pseudophakia	0.1	575	Yes	2648	14	0.05	423	Yes	0	15	2
Mean	78	—	—	—	—	0.29	406	—	2885	13.7	0.13	276	—	936	13.3	2.7
SD	9.5	—	—	—	—	—	125	—	1335	2.9	—	166	—	1623	3.4	1.2

F = female; GLD = greatest linear dimension; IOP = intraocular pressure; M = male; PED = pigment epithelial detachment; RAP = retinal angiomatous proliferation; RRA = retinal–retinal anastomosis; SD = standard deviation; VA = decimal visual acuity; yrs = years.

TABLE 2. Intravitreal Bevacizumab and Photodynamic Therapy for Retinal Angiomatous Proliferation

						Baseline					12 Months after Treatment					
Case No.	Age (yrs)	Gender	Eye	RAP Stage	Lens Status	VA	Central Retinal Thickness (μm)	RRA	GLD (μm)	IOP (mmHg)	VA	Central Retinal Thickness (μm)	RRA	GLD (μm)	IOP (mmHg)	No. Treatments
13	64	F	Right	2	Phakic eye	1.0	234	No	602	16	1.0	117	—	0	12	2
14	63	M	Right	2+PED	Phakic eye	0.07	601	Yes	4331	12	0.2	126	No	0	10	2
15	81	M	Right	2	Phakic eye	0.4	393	No	1900	15	1.0	124	—	0	13	1
16	78	M	Left	2	Phakic eye	0.6	406	No	1998	11	1.2	125	—	0	11	1
17	89	M	Left	2+PED	Pseudophakia	0.3	379	Yes	3531	16	0.6	182	No	0	13	1
18	87	F	Right	3	Pseudophakia	0.06	394	Yes	2368	10	0.07	140	Yes	0	10	1
19	87	F	Left	2+PED	Pseudophakia	0.7	396	Yes	5532	10	0.7	112	Yes	0	10	2
20	78	M	Left	2	Phakic eye	0.9	430	Yes	975	15	0.8	127	No	0	14	2
21	83	F	Right	2	Phakic eye	0.05	514	Yes	3489	19	0.05	88	No	0	14	2
22	83	F	Left	2+PED	Pseudophakia	0.3	479	Yes	4568	17	0.6	44	No	0	16	3
23	72	M	Right	2+PED	Phakic eye	0.3	360	Yes	3300	16	0.2	74	No	0	13	2
24	74	M	Right	3	Phakic eye	0.06	950	Yes	4494	15	0.09	426	No	0	11	1
25	79	M	Left	2+PED	Pseudophakia	0.3	389	Yes	3321	11	0.9	66	No	0	10	1
Mean	78	—	—	—	—	0.25	456	—	3108	14.1	0.37	135	—	0	12.1	1.6
SD	8.2	—	—	—	—	—	171	—	1469	2.9	—	94	—	—	1.9	0.7

F = female; GLD = greatest linear dimension; M = male; PED = pigment epithelial detachment; RAP = retinal angiomatous proliferation; RRA = retinal–retinal anastomosis; SD = standard deviation; VA = decimal visual acuity; yrs = years.

months of follow-up.[29] The purpose of the current study was to clarify the efficiency of combined therapy of IVB plus PDT compared with combined therapy of IVTA plus PDT for treating patients with RAP over 12 months.

METHODS

WE RETROSPECTIVELY REVIEWED 25 EYES OF 22 JAPANESE patients (11 men, 11 women; age range, 63 to 90 years; mean ± standard deviation, 78.3 ± 8.8 years) with RAP. Twelve eyes of 11 patients (3 men, 8 women; age range, 63 to 90 years; mean age, 78.3 years) were treated with combined therapy of IVTA plus PDT from September 1, 2004, through July 31, 2006. Thirteen eyes of 11 patients (8 men, 3 women; age range, 63 to 89 years; mean age, 78.3 years) were treated with combined therapy of IVB and PDT from February 1, 2007, through January 31, 2008. The 6-month results for 8 of the 13 eyes treated with IVB plus PDT were reported previously.[29] The patients were followed up for at least 12

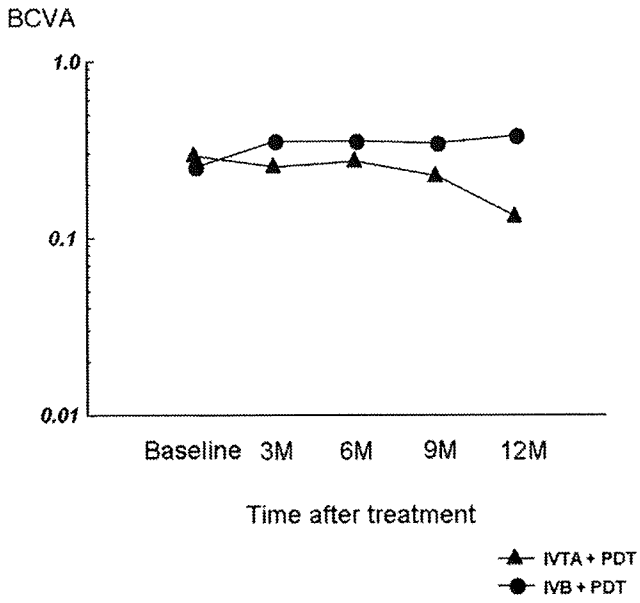

FIGURE 1. Graph showing results of intravitreal triamcinolone acetonide with photodynamic therapy (IVTA plus PDT) and intravitreal bevacizumab with photodynamic therapy (IVB plus PDT) for retinal angiomatous proliferation (RAP). In eyes treated with IVTA plus PDT, there is a significant ($P < .05$, paired t test) decline in the mean best-corrected visual acuity (BCVA) between baseline and 12 months. In eyes treated with IVB plus PDT, there is a significant improvement in the mean BCVA between baseline and 3, 6, and 12 months ($P < .01$, $P < .05$, $P < .05$, respectively, paired t test). There is no significant ($P = .74$) difference in the mean BCVA between groups at baseline; nevertheless, there is a significant difference in the mean BCVA at 12 months ($P < .05$, nonpaired t test) between the IVB plus PDT group and the IVTA plus PDT group. M = month(s).

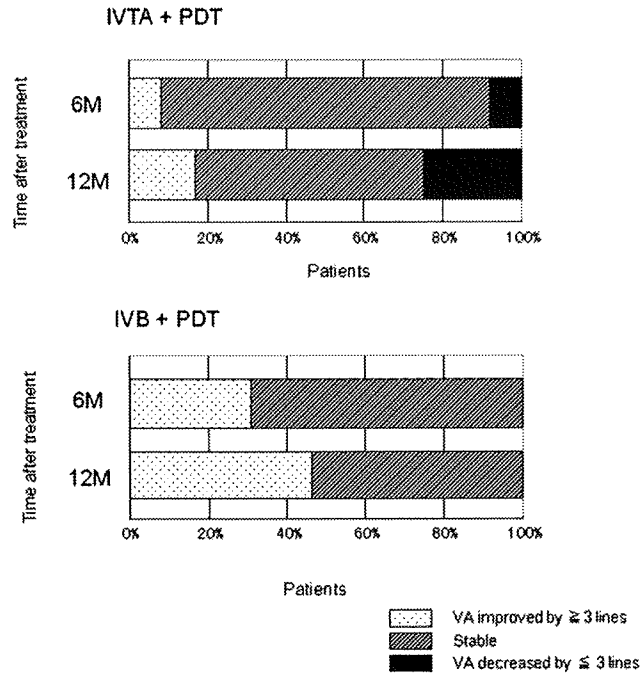

FIGURE 2. The distribution of the mean best-corrected visual acuity (BCVA) changes from baseline after treatment with combined intravitreal triamcinolone acetonide with photodynamic therapy (IVTA plus PDT) and intravitreal bevacizumab with photodynamic therapy (IVB plus PDT). (Top) One and 3 eyes treated with IVTA plus PDT had decreased BCVA at 6 and 12 months, respectively. (Bottom) No eyes treated with IVB plus PDT had decreased BCVA of 3 lines or more after treatment over 12 months. M = month(s).

months at Fukushima Medical University Hospital or Kagawa University Hospital. No patient had undergone a previous treatment. The treatment was approved by the Institutional Review Boards/Ethics Committees at Fukushima Medical University and Kagawa University. After the potential risks and benefits were explained in detail, all patients provided written informed consent. The exclusion criteria were previous treatment for RAP such as laser photocoagulation, submacular surgery, transpupillary thermotherapy, and PDT; glaucoma; tears in the retinal pigment epithelium; and maculopathies such as diabetic maculopathy, retinal vascular occlusion, or idiopathic juxtafoveal retinal telangiectasis.

We recorded the best-corrected visual acuity (BCVA) measured with a Japanese standard decimal VA chart and calculated the mean BCVA using the logarithm of the minimal angle of resolution (logMAR) scale. All patients underwent a standardized examination including slit-lamp biomicroscopy with a contact lens, fundus color photography, fluorescein angiography (FA), and indocyanine green angiography (ICGA) with a fundus camera (TRC-50 FA/IA/IMAGEnet H1024 system; Topcon, Tokyo, Japan), with a confocal scanning laser ophthalmoscope (Heidelberg Retina Angiograph 2; Heidelberg Engineering, Heidelberg, Germany), or both. All examinations were performed using time-domain optical coherence tomography (OCT; OCT 3000; Carl Zeiss, Meditec, Dublin, California, USA; or OCT-Ophthalmoscope; Nidek-OTI, Gamagori, Japan) in eyes treated with IVTA plus PDT and spectral-domain OCT (3D-OCT; Topcon; or Cirrus OCT, Carl Zeiss) in eyes treated with IVB plus PDT. All patients were examined using the same OCT machine during the follow-up. FA was performed to determine the lesion type, the location, and the activity of the RAP lesions. ICGA was performed to diagnose RAP and to identify retinal–retinal anastomosis. The central retinal thickness, defined as the distance from the retinal pigment epithelium to the inner limiting membrane, was measured at baseline and at 3, 6, 9, and 12 months after treatment using internal caliper software.

All patients had documented visual loss before treatment. IVTA (4 mg/0.1 mL) or IVB (1.25 mg/0.05 mL) was injected 3.5 to 4.0 mm posterior to the corneal limbus into the vitreous cavity using a 27-gauge needle after topical anesthesia was applied. In the patients treated with IVTA plus PDT, PDT was performed 7 days after IVTA was injected. In the patients treated with IVB plus PDT, PDT was administered 1