SHORT-TERM SAFETY PROFILE OF INTRAVITREAL ZIV-AFLIBERCEPT

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Aim: To evaluate the safety of intravitreal ziv-aflibercept (Zaltrap) in the treatment choroidal neovascularization secondary to age-related macular degeneration.

Methods: Eligible eyes with choroidal neovascularization secondary to age-related macular degeneration each received a single intravitreal injection of ziv-aflibercept. Comprehensive ophthalmic examinations and detailed systemic evaluations were performed at baseline and Days 1, 7, and 30 after injection, and International Society for Clinical Electrophysiology of Vision standard electroretinography was performed at baseline and Day 30. Primary outcome measures were safety parameters that included signs of clinical and electroretinographic toxicity. Secondary outcome measures included changes in best-corrected visual acuity and central subfield thickness.

Results: Twelve eyes of 12 patients were treated. None of the patients complained of blurred vision, ocular pain, or bulbar injection at any of the follow-up visits, nor was intraocular inflammation noted. There were no significant differences in implicit times, "a" and "b" wave amplitudes, or b/a ratios at 1 month when compared with baseline ($P = 0.4$). None of the patients experienced serious ocular or systemic adverse events. Mean best-corrected visual acuity improved only slightly at 30 days (LogMAR 0.45 ± 0.31 [Snellen equivalent: 20/60]) compared with baseline (LogMAR 0.37 ± 0.24 [Snellen equivalent: 20/50]; $P = 0.51$).

Conclusion: Single intravitreal injections of ziv-aflibercept into eyes with neovascular age-related macular degeneration appear to be safe through 1 month. Ziv-aflibercept could become a safe, low-cost therapy for macular diseases in developing countries and in those where intravitreal aflibercept (Eylea) is not available.

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Drugs that inhibit the actions of vascular endothelial growth factor (VEGF) have become the standard-of-care for several chorioretinal vascular conditions including choroidal neovascularization due to age-related macular degeneration (AMD), diabetic macular edema, and macular edema due to retinal vein occlusions.[1–3] The US Food and Drug Administration has approved three anti-VEGF drugs (pegaptanib [Macugen; Eyetech, New York, NY], ranibizumab [Lucentis; Genentech, S. San Francisco, CA/Roche, Basel, Switzerland], and aflibercept [Eylea; Regeneron, Tarrytown, NY]) for many of these conditions,[4–6] but a less expensive alternative (off-label bevacizumab [Avastin; Genentech/Roche]) is the most commonly used anti-VEGF drug throughout the world.[7] According to the 2014 annual "Preferences and Trends Survey" by the American Society of Retina Specialists, 64.5% of US retina specialists and 41.7% of international retina specialists choose compounded bevacizumab as their primary therapy for neovascular AMD.[8] Several factors influence the surgeons' choice of drugs, but low cost is important in many cases.

The Comparison of AMD Treatments Trials (CATT) reported similar outcomes when patients with neovascular AMD were treated with intravitreal bevacizumab or ranibizumab.[7] Visual improvements

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ranged from +5 to +9 letters at 2 years, but 56% to 82% of eyes had persistent intraretinal, subretinal or subretinal pigment epithelial fluid.[9,10] Intravitreal aflibercept has emerged as an effective alternative for the primary treatment of neovascular AMD[11] or as "salvage" therapy for eyes that inadequately respond to bevacizumab or ranibizumab.[12,13] Unfortunately, ranibizumab ($1,950/dose) and aflibercept ($1,850/dose) are quite expensive, and the latter is not yet available in many countries such as India.

Ziv-aflibercept (Zaltrap; Regeneron) is a recombinant fusion protein composed of the extracellular VEGF-binding domains from human VEGF receptors 1 and 2 fused to the Fc portion of a human IgG1.[14] It was approved by the Food and Drug Administration in August 2012 for the treatment of metastatic colorectal carcinoma that is resistant to or has progressed following an oxiplatin-containing chemotherapeutic regimen.[14]

Although structurally identical to intravitreal aflibercept, ziv-aflibercept has been formulated with buffers that result in a much higher osmolarity. Some investigators contend that intravitreal injections of ziv-aflibercept solution may be toxic to the retina or uveal tract, but others believe that it might be safe and, therefore, useful against chorioretinal vascular conditions.[15]

We believe that intravitreal ziv-aflibercept may be safe and effective when used in human eyes with neovascular AMD, and herein, we present a detailed safety evaluation using sequential clinical evaluations and electroretinographic (ERG) testing after single intravitreal injections.

Methods

This prospective study adhered to the tenets of the Declaration of Helsinki and was approved by the L.V. Prasad Eye Institute Institutional Review Board. All study participants gave written informed consent before enrollment. Patients were recruited in April 2015 at the L.V. Prasad Eye Institute, Hyderabad, India.

Eligible patients had active, treatment-naïve or previously treated (with a VEGF inhibitory drug) neovascular AMD. Signs of neovascular activity included leakage on fundus fluorescein angiography and/or intraretinal and/or subretinal fluid on spectral-domain optical coherence tomography (OCT). Exclusion criteria included signs of ocular infection, prior periocular or intraocular corticosteroid use, previous vitrectomy, and a history of cerebrovascular accident or myocardial infarction.

Comprehensive ocular examinations performed by masked investigators included measurement of best-corrected visual acuity (BCVA) using standardized Snellen visual acuity charts, applanation tonometry, slit lamp biomicroscopy of the anterior and posterior segments, and indirect ophthalmoscopy. Comprehensive ocular examinations were performed at baseline and Days 1, 7, and 30. All patients underwent fundus photography (Zeiss Visupac FF450-plus; Carl Zeiss, Dublin, CA) and spectral-domain OCT (Carl Zeiss Meditec Cirrus HD-OCT; Carl Zeiss) at baseline and Days 7 and 30 after the intravitreal injection. Fundus autofluorescence, fundus fluorescein angiography, and indocyanine green angiography (Zeiss Visupac FF450-plus; Carl Zeiss) were performed at baseline and repeated at 30 days at the treating physician's discretion.

Full-field ERG (Metrovision, Paris, France) studies were performed at baseline and at 1 month according to the International Society for Clinical Electrophysiology of Vision guidelines.[16]

Systemic evaluations at baseline and at 30 days included a detailed medical history, during which patients were asked about current medications and systemic adverse events (AEs), including anti-platelets trialists collaboration (APTC) adverse events, and measurement of arterial blood pressure and random blood glucose.

Intravitreal Injection

Ziv-aflibercept is packaged in vials of 100 mg per 4 mL (concentration of 1.25 mg/0.05 mL). Aliquots of 0.05 mL were prepared by compounding pharmacists under a sterile, laminar flow hood using 1 mL Tuberculin BD syringes (BD, Sparks, MD) and filter needles. Sterile vials were punctured only once, and the aliquoted drug was stored at 4°C and used within 2 weeks.

Preinjection topical antibiotics were not administered, but all patients received topical ciprofloxacin HCl 0.3 mg/mL four times per day for 4 days after the injections. Intravitreal injections were performed according to the physicians' standard protocol using a strict aseptic technique under topical anesthesia in an outpatient procedure room. Intravitreal injections were performed with 29-gauge needles inserted through the inferotemporal pars plana, 4 mm posterior to the limbus in phakic eyes and 3.5 mm in pseudophakic eyes.

Outcome Measures

Primary outcome measures evaluated drug safety. These included any signs of toxicity noted on clinical examination, such as increased conjunctival congestion, progression of cataract, retinal hemorrhages, retinal vasculitis, retinal necrosis, or detachment, or on ERG testing. ERG changes were considered significant if the 30-day a- and b-wave amplitudes or

implicit times changed by more than 30% from the baseline values. Systemic evaluations included survey questions regarding systemic AEs and severe AEs. Table 1 contains a complete list of screened-for AEs and serious AEs. Secondary outcome measures included changes in BCVA, central subfield thickness and fundus autofluorescence.

Statistical Analysis

Continuous variables were analyzed with descriptive statistics that included mean and standard deviation. Pre- and postinjection changes in BCVA, ERG parameters, and central subfield thickness were compared with paired *t*-test. Statistical analyses were performed using commercial software (Stata data analysis and statistical software, version 12.1; StataCorp, College Station, TX). A P value of <0.05 was considered statistically significant.

Results

Twelve eyes of 12 patients with choroidal neovascularization due to AMD were enrolled in this study. Four patients were male and eight were female; four eyes were phakic and eight were pseudophakic. One patient had diabetes mellitus and five had systemic arterial hypertension. None of the patients had previously suffered from cerebrovascular events or myocardial infarctions.

Table 1. The Table Lists the Ocular and Systemic AEs and Serious AEs Screened for in the Trial

Ocular AEs and Serious AEs	
Adverse Events	
Blurred vision	Posterior vitreous detachment
Pain	Vitreous hemorrhage
Conjunctival injection	Conjunctival hemorrhage
Mild intraocular inflammation	Increased intraocular pressure
Serious AEs	
Conjunctival or scleral necrosis	Cataracts
Severe intraocular inflammation	Retinal detachment
Retinal hemorrhages	Corneal edema
Endophthalmitis	Optic atrophy
Electroretinographic abnormalities	Moderate or severe loss of vision
Systemic AEs and Serious AEs	
Systemic AEs	
Hypertension	Fevers
Surgical or medical procedure	Gastrointestinal disorders
Infections	Neurologic disorder
Serious AEs	
Deaths	Hospitalization
APTC events	Wound healing complications
Congestive heart failure	

APTC, antiplatelet trialists collaboration.

Two eyes had treatment-naive choroidal neovascular membranes and 10 had been previously treated with anti-VEGF agents (Table 2). The mean duration between the last anti-VEGF injections and enrollment into this study was 3.4 ± 2.9 months. None of the eyes had previously received photodynamic therapy for choroidal neovascularization, and none had reacted adversely to previous anti-VEGF injections.

The mean BCVA improved only slightly from baseline (LogMAR 0.45 ± 0.31; Snellen equivalent: 20/60) to Day 30 (LogMAR 0.37 ± 0.24 [Snellen equivalent: 20/50]; $P = 0.51$). At 1 month, the BCVA improved in six eyes, remained the same in five eyes, and decreased by one line (Δ LogMAR of 0.1) in one eye. The mean intraocular pressures at baseline and at Day 30 were 12.3 ± 4.0 mmHg and 13.2 ± 3.6 mmHg, respectively.

All of the patients were examined at post-injection days 1, 7, and 30. None of the patients complained of blurred vision, ocular pain, or bulbar injection at any of the follow-up visits. None of the eyes developed inflammation in the anterior or posterior segments, and none had corneal edema, cataract, vitreous hemorrhage, retinal detachment, or optic atrophy.

One of the eyes developed conjunctival thinning (suspicious for conjunctival necrosis) at the 7-day examination without significant hyperemia, ocular pain, or blurred vision at the injection site. The conjunctival swab was cultured for microorganisms, and the patient was treated with topical gatifloxacin 0.3 mg/mL four times per day for 7 days. The cultures failed to grow pathogenic organisms, and the appearance of the injection site normalized by 5 days.

None of the patients developed ocular serious AEs such as severe intraocular inflammation, endophthalmitis, or moderate or severe loss of vision (>0.3 LogMAR). No systemic AEs or serious AEs were reported.

Imaging Findings

At baseline and 30 days, the mean central subfield thicknesses were 321.5 ± 127.0 μm and 265.9 ± 104.3 μm ($P = 0.28$). Eleven eyes had subretinal fluid at baseline and 3 of these had no subretinal fluid at Day 30. The findings on fundus autofluorescence were unchanged at day 30. Spectral-domain OCT showed no change in the vitreoretinal interface at 1 month compared with baseline (Figure 1).

Table 2. Clinical Characterstics of Study Patients

Patient	Eye	Systemic Diseases	Prior Therapies	Duration From Last Anti-VEGF Injections, Months	BCVA at Baseline	Lens Status at Baseline	CST at Baseline, μm	BCVA at Day 30	CST at Day 30, μm
1	OS	NIL	5B	2	20/40	Pseudophakia	349	20/40	232
2	OD	NIL	NIL	NA	20/40	Clear	335	20/30	312
3	OD	NIL	3B	2	20/40	Pseudophakia	166	20/50	162
4	OD	NIL	3B	2	20/32	Pseudophakia	188	20/32	190
5	OD	HTN	2B	4	20/25	Pseudophakia	139	20/20	139
6	OD	NIL	4B + 3R	8	20/40	Pseudophakia	439	20/40	260
7	OD	HTN, DM	4B + 4R	1	20/100	Pseudophakia	183	20/63	163
8	OS	HTN	8B	1	20/200	Pseudophakia	503	20/63	522
9	OD	HTN	3B + 4R	10	20/50	NS2+PSC	418	20/50	220
10	OS	HTN	3B	3	20/25	Clear	346	20/20	330
11	OD	NIL	1B	1	20/252	Pseudophakia	273	20/160	239
12	OD	NIL	NIL	NA	20/60P	Clear	519	20/60	346

OD, right eye; OS, left eye; HTN, hypertension; DM, diabetes mellitus; B, bevacizumab; R, ranibizumab; CST, central subfield thickness; NA, not applicable; NS, nuclear sclerosis; PSC, posterior subcapsular.

Electroretinographic Findings

The mean (±standard deviation) ERG findings at baseline and Day 30 are listed in Table 3. There were no significant difference in implicit times, "a" and "b" wave amplitudes, and b/a ratios at Day 30 when compared with baseline.

Discussion

Our study failed to uncover clinical or electroretinographic evidence of toxicity following single intravitreal injections of ziv-aflibercept in 12 eyes with neovascular AMD. This was only a small pilot study, so we cannot endorse routine use of intravitreal ziv-aflibercept for the treatment of chorioretinal vascular conditions, but these encouraging results support the call for further investigations.

For several years, the differences in osmolarity between intraocular aflibercept and ziv-aflibercept were believed to constitute a barrier to the off-label intraocular use of ziv-aflibercept. Aflibercept (Eylea) is an isoosmotic solution (300 mOsm/kg), whereas the buffers in ziv-aflibercept make it far more concentrated (1,000 mOsm/kg). Marmor et al[17] showed that solutions of less than 500 mOsm cause

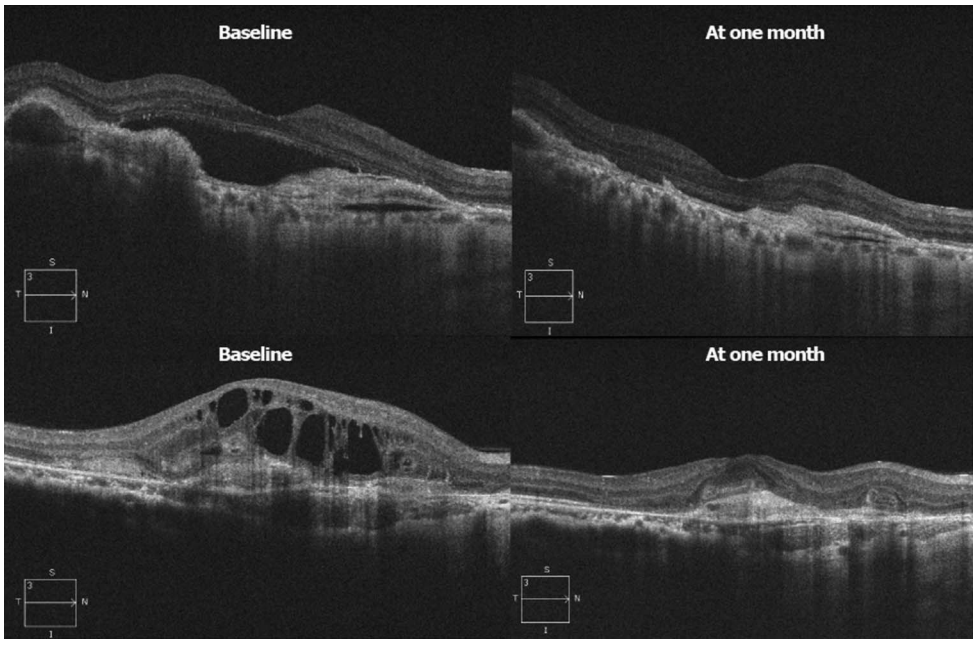

Fig. 1. Spectral-domain optical coherence tomography scans of 2 eyes (Cases 6 and 12), which underwent intravitreal ziv-aflibercept, at baseline (left) and at 1-month follow-up (right) showing resolution of subretinal fluid and intraretinal fluid, with no change in vitreoretinal interface.

Table 3. Electroretinographic Values at Baseline and 30 Days

ERG Parameters	Baseline	At 1 Month	*P*
"a" wave implicit time	19.8 ± 3.2	20.7 ± 3.2	0.44
"a" wave amplitude	63.0 ± 51.9	78.8 ± 67.3	0.43
"b" wave implicit time	38.1 ± 6.4	39.7 ± 7.7	0.48
"b" wave amplitude	156.5 ± 86.6	171.4 ± 120.9	0.67
"b/a" ratio	4.3 ± 4.2	3.9 ± 4.9	0.84

no retinal pigment epithelium damage in rabbits and primates. In animal experiments, Malik et al[18] reported that intravitreal ziv-aflibercept injections ranging from 1/2 to 2 times normal clinical doses produced similar changes in vitreous osmolality (range from 324 to 342 mOsm/kg) as control injections (328 mOsm/kg). Mansour et al[15] suggested that the safety of intravitreal ziv-aflibercept may be attributed to its dilution after injection into 4 mL of vitreous to a final osmolarity of 312 mOsm/kg (within the physiologic range).[15]

Viability studies failed to show cytotoxic effects when ARPE-19 cells were exposed to twice the clinical dose of ziv-aflibercept.[19] However, Malik et al[18] reported a significant reduction in mitrochondrial membrane potential with a clinical dose (2 mg in 4 mL of vitreous), although the same group showed that 2 mg of ziv-aflibercept was not toxic to Müller cells.[20] At twice the clinical dose, however, ziv-aflibercept reduced Müller cell viability, whereas aflibercept, bevacizumab and ranibizumab did not.

De Oliveira Dias et al[19] found no signs of toxicity on clinical examination, OCT, and ERG 24 hours and 7 days after a single intravitreal ziv-aflibercept injection into rabbit eyes. Histologic and electron microscopic examinations showed no toxicity.

De Oliveira Dias et al[21] actually found that a single intravitreal injection of 1.75 mg (0.07 mL) ziv-aflibercept into an eye with resistant choroidal neovascularization secondary to AMD was followed by improved ERG and microperimetric studies. Similarly, we found no ERG evidence of drug toxicity with our smaller dose of ziv-aflibercept (1.25 mg in 0.05 mL is consistent with the commonly used volume for intravitreal anti-VEGF injections), and our data suggest that a single dose of intravitreal ziv-aflibercept does not interfere with inner and outer retina function. The ziv-aflibercept dose in our study (1.25 mg/0.05 mL) was less than the approved intravitreal aflibercept dose (2 mg/0.05 mL) but still greater than one of the treatment arms in the VEGF Trap-Eye: Investigation of Efficacy and Safety in Wet AMD trials (0.5 mg/0.05 mL, which was similarly effective to 2 mg). The optimal intravitreal dose of ziv-aflibercept is not known, therefore further studies on efficacy and dosing are underway.

Intravitreal aflibercept has caused sterile inflammation,[22–24] but we found none in our small series with ziv-aflibercept. One of our eyes developed sterile conjunctival thinning that normalized after a short course of topical antibiotic, but there was no evidence of bulbar injection or intraocular inflammation. Therefore, we could not attribute the necrosis to ziv-aflibercept.

One weakness of our study is the small number of enrolled patients. Another weakness concerns the timing of the follow-up ERG (30 days) because any drug-induced toxicity may have recovered by 30 days. The promising short-term safety data from this single-dose pilot, however, suggests that longer-term studies with repeat injections and larger sample sizes are reasonable. Data regarding the injections of ziv-aflibercept into non-human primate eyes may provide reassuring data regarding systemic absorption, however, this is beyond the scope of this pilot study.

In conclusion, our study reports the safety of single intravitreal injections of ziv-aflibercept in eyes with neovascular AMD. Ziv-aflibercept for macular diseases could become a low-cost alternative in developing countries and in those where aflibercept is not available. Further investigations into its long-term safety and efficacy are warranted.

Key words: aflibercept, anti-VEGF, bevacizumab, ranibizumab, ziv-aflibercept, AMD, CNV.

References

1. Brown DM, Campochiaro PA, Singh RP, et al. Ranibizumab for macular edema following central retinal vein occlusion: six-month primary end point results of a phase III study. Ophthalmology 2010;117:1124–1133.e1121.
2. Nguyen QD, Shah SM, Khwaja AA, et al. Two-year outcomes of the ranibizumab for edema of the mAcula in diabetes (READ-2) study. Ophthalmology 2010;117:2146–2151.
3. Rofagha S, Bhisitkul RB, Boyer DS, et al. Seven-year outcomes in ranibizumab-treated patients in ANCHOR, MARINA, and HORIZON: a multicenter cohort study (SEVEN-UP). Ophthalmology 2013;120:2292–2299.
4. Schmidt-Erfurth U, Kaiser PK, Korobelnik JF, et al. Intravitreal aflibercept injection for neovascular age-related macular degeneration: ninety-six-week results of the VIEW studies. Ophthalmology 2014;121:193–201.
5. Korobelnik JF, Do DV, Schmidt-Erfurth U, et al. Intravitreal aflibercept for diabetic macular edema. Ophthalmology 2014;121:2247–2254.
6. Korobelnik JF, Holz FG, Roider J, et al. Intravitreal aflibercept injection for macular edema resulting from central retinal vein occlusion: one-year results of the phase 3 GALILEO study. Ophthalmology 2014;121:202–208.
7. Group CR, Martin DF, Maguire MG, et al. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. N Eng J Med 2011;364:1897–1908.

8. Available at: http://www.asrs.org/pat-survey/pat-survey-archive. Accessed July 6, 2015.
9. Lux A, Llacer H, Heussen FM, et al. Non-responders to bevacizumab (Avastin) therapy of choroidal neovascular lesions. Br J Ophthalmol 2007;91:1318–1322.
10. Krebs I, Glittenberg C, Ansari-Shahrezaei S, et al. Non-responders to treatment with antagonists of vascular endothelial growth factor in age-related macular degeneration. Br J Ophthalmol 2013;97:1443–1446.
11. Heier JS, Brown DM, Chong V, et al. Intravitreal aflibercept (VEGF trap-eye) in wet age-related macular degeneration. Ophthalmology 2012;119:2537–2548.
12. Pinheiro-Costa J, Costa JM, Beato JN, et al. Switch to aflibercept in the treatment of neovascular AMD: one-year results in clinical practice. Ophthalmologica 2015;233:155–161.
13. Ehlken C, Jungmann S, Bohringer D, et al. Switch of anti-VEGF agents is an option for nonresponders in the treatment of AMD. Eye (Lond) 2014;28:538–545.
14. Patel A, Sun W. Ziv-aflibercept in metastatic colorectal cancer. Biologics 2014;8:13–25.
15. Mansour AM, Al-Ghadban SI, Yunis MH, et al. Ziv-aflibercept in macular disease. Br J Ophthalmol 2015; 99:1055–1059.
16. McCulloch DL, Marmor MF, Brigell MG, et al. ISCEV standard for full-field clinical electroretinography (2015 update). Doc Ophthalmol 2015;130:1–12.
17. Marmor MF, Martin LJ, Tharpe S. Osmotically induced retinal detachment in the rabbit and primate. Electron miscoscopy of the pigment epithelium. Invest Ophthalmol Vis Sci 1980;19: 1016–1029.
18. Malik D, Tarek M, Caceres del Carpio J, et al. Safety profiles of anti-VEGF drugs: bevacizumab, ranibizumab, aflibercept and ziv-aflibercept on human retinal pigment epithelium cells in culture. Br J Ophthalmol 2014;98:i11–i16.
19. de Oliveira Dias JR, Badaro E, Novais EA, et al. Preclinical investigations of intravitreal ziv-aflibercept. Ophthalmic Surg Lasers Imaging Retina 2014;45:577–584.
20. Del Carpio JC, Moustafa MTM, Ramirez CA, et al. Effects of ranibizumab, bevacizumab, aflibercept, and ziv-aflibercept on cultured human retinal Muller cells. Poster presented at: Association for Research in Vision and Ophthalmology Annual Meeting; May 3–8, 2014; Orlando, FL.
21. de Oliveira Dias JR, Xavier CO, Maia A, et al. Intravitreal injection of ziv-aflibercept in patient with refractory age-related macular degeneration. Ophthalmic Surg Lasers Imaging Retina 2015;46:91–94.
22. Fine HF, Roth DB, Shah SP, et al. Frequency and characteristics of intraocular inflammation after aflibercept injection. Retina 2015;35:681–686.
23. Hahn P, Chung MM, Flynn HW Jr., et al. Postmarketing analysis of aflibercept-related sterile intraocular inflammation. JAMA Ophthalmol 2015;133:421–426.
24. Goldberg RA, Shah CP, Wiegand TW, et al. Noninfectious inflammation after intravitreal injection of aflibercept: clinical characteristics and visual outcomes. Am J Ophthalmol 2014; 158:733–737.e731.