AMERICAN ACADEMY™ OF OPHTHALMOLOGY

Dual Antagonism of PDGF and VEGF in Neovascular Age-Related Macular Degeneration

A Phase IIb, Multicenter, Randomized Controlled Trial

Glenn J. Jaffe, MD,[1] Thomas A. Ciulla, MD, MBA,[2] Antonio P. Ciardella, MD,[3] Francois Devin, MD,[4] Pravin U. Dugel, MD,[5] Chiara M. Eandi, MD,[6] Harvey Masonson, MD,[2] Jordi Monés, MD, PhD,[7] Joel A. Pearlman, MD, PhD,[8] Maddalena Quaranta-El Maftouhi, MD,[9] Federico Ricci, MD,[10] Keith Westby, MBA,[2] Samir C. Patel, MD[2]

Purpose: To assess the safety and efficacy of E10030 (Fovista; Ophthotech, New York, NY), a platelet-derived growth factor (PDGF) antagonist, administered in combination with the anti–vascular endothelial growth factor (VEGF) agent ranibizumab (Lucentis; Roche, Basel, Switzerland) compared with ranibizumab monotherapy in patients with neovascular age-related macular degeneration (nAMD).

Design: Phase IIb global, multicenter, randomized, prospective, double-masked, controlled superiority trial.

Participants: Four hundred forty-nine patients with treatment-naïve nAMD.

Methods: Participants were randomized in a 1:1:1 ratio to 1 of the following 3 intravitreal treatment groups: E10030 0.3 mg in combination with ranibizumab 0.5 mg, E10030 1.5 mg in combination with ranibizumab 0.5 mg, and sham in combination with ranibizumab 0.5 mg (anti-VEGF monotherapy). Drugs were administered monthly in each of the groups for a total duration of 24 weeks.

Main Outcome Measures: The prespecified primary end point was the mean change in visual acuity (VA; Early Treatment Diabetic Retinopathy [ETDRS] letters) from baseline to 24 weeks.

Results: No significant safety issues were observed in any treatment group. The E10030 (1.5 mg) combination therapy regimen met the prespecified primary end point of superiority in mean VA gain compared with anti-VEGF monotherapy (10.6 compared with 6.5 ETDRS letters at week 24; $P = 0.019$). A dose-response relationship was evident at each measured time point commencing at 4 weeks. Visual acuity outcomes favored the E10030 1.5 mg combination therapy group regardless of baseline VA, lesion size, or central subfield thickness on optical coherence tomography. All clinically relevant treatment end points of visual benefit ($\geq$15 ETDRS letter gain, final VA $\geq$20/40 or $\geq$20/25) and visual loss ($\geq$1 ETDRS line loss, $\geq$2 ETDRS line loss, final VA $\leq$20/125 or $\leq$20/200) favored the E10030 1.5 mg combination group.

Conclusions: In this phase IIb clinical trial, a 62% relative benefit from baseline was noted in the E10030 1.5 mg combination therapy group compared with the anti-VEGF monotherapy group. A favorable safety and efficacy profile of E10030 combination therapy for nAMD was evident across multiple clinically relevant end points. This highly powered study provides strong rationale for a confirmatory phase III clinical trial. *Ophthalmology 2017;124:224-234 © 2016 by the American Academy of Ophthalmology*

Supplemental material is available at www.aaojournal.org.

Currently, all commonly used anti–vascular endothelial growth factor (VEGF) agents for the treatment of neovascular age-related macular degeneration (nAMD) show similar safety and efficacy profiles.[1–5] However, research over the past decade has highlighted numerous limitations of anti-VEGF strategies. Despite continuous (i.e., monthly) dosing over 1 year, 18% to 22% of patients lose visual acuity (VA), approximately 50% do not achieve 20/40 or better VA necessary for an unrestricted driver's license in regions of the United States, and approximately 62% to 75% do not achieve a significant gain of 3 lines or more of Early Treatment Diabetic Retinopathy (ETDRS) VA.[5–7] Discontinuous (i.e., less than monthly or bimonthly) dosing results in worse visual outcomes compared with continuous doing.[1,3] Furthermore, the ceiling of anti-VEGF monotherapy has been reached with currently available agents; despite increased anti-VEGF dosage or various regimens, no additional benefit is evident.[2,4,5] Unfortunately, post-drug approval real-world analyses reveal even worse VA outcomes compared with randomized clinical trials.[8–17] During the first 4 years of treatment or sooner, VA declines beyond baseline levels in most patients.[10–12,16]

http://dx.doi.org/10.1016/j.ophtha.2016.10.010
ISSN 0161-6420/16

This experience over the past decade highlights the limitations of anti-VEGF agents and the unmet need for more effective therapies.

Many studies indicate that pericytes play an important role in the limitations of anti-VEGF therapy, in both the short and long term.[18–24] Pericytes share a common basement membrane with endothelial cells, intimately coating them.[25] Pericytes provide endothelial cells with VEGF and other growth and cell survival factors by paracrine and/or juxtacrine signaling mechanisms.[26] Consequently, the neovascular endothelial cells are protected in the setting of anti-VEGF therapy.

Pericyte recruitment, maturation, and survival are mediated by platelet-derived growth factor (PDGF).[27] E10030 (Fovista; Ophthotech, New York, NY) is a 32-mer pegylated DNA aptamer that selectively binds to PDGF-BB and PDGF-AB homodimers and heterodimers, respectively, thereby disrupting the interaction with their cognate tyrosine kinase receptors (PDGF-BB with PDGFR-αα, PDGFR-ββ, and PDGFR-αβ; PDGF-AB with PDGFR-αα and PDGFR-αβ). These receptors are commonly expressed on cells of mesenchymal origin, such as pericytes.[18,24,27–29] In a preclinical model, E10030 potently stripped neovascular pericytes from the underlying endothelial cells.[30] Pericyte stripping from a neovascular complex may leave the underlying endothelial cells in an unprotected and vulnerable state, thereby increasing their sensitivity to the effects of VEGF blockade.[18,19,21,24,28,31]

Dual targeting of PDGF and VEGF in nAMD has been assessed in a phase I clinical trial of E10030 administered in combination with ranibizumab (Lucentis); this therapy had a favorable safety profile, produced improved VA when compared with baseline, and caused biomarker changes supporting the enhanced efficacy.[32] In this article, we describe the results of a subsequent phase IIb randomized, prospective clinical trial of treatment-naïve nAMD eyes, comparing E10030 in combination with anti-VEGF therapy versus anti-VEGF monotherapy. To the best of our knowledge, this clinical trial represents the largest phase IIb pharmacologic superiority study conducted to date for a retinal disorder.

Methods

Study Design

This global phase IIb clinical trial (www.clinicaltrials.gov identifier, NCT01089517) used a parallel-group, randomized, double-masked, prospective superiority design to establish the safety and efficacy of intravitreal E10030 administered in combination with an anti-VEGF agent in patients with nAMD. The study was conducted at 69 study sites in 9 countries (in North and South America, Europe, and Israel) between April 2010 and January 2012. A list of study sites and investigators can be found in Appendix 1 (available at www.aaojournal.org). The appropriate ethics committees or institutional review boards at each study center approved the protocol. Informed consent was obtained from all participants. All data were collected in a Health Insurance Portability and Accountability Act–compliant manner.

Study Population Eligibility Criteria

Eligibility criteria included age 50 years or older, study eye with treatment-naïve subfoveal choroidal neovascularization (CNV), a classic component on fluorescein angiography (FA), and total neovascular lesion area (including blood, neovascularization, and scar or atrophy) of 5 disc areas (DAs) or less, of which at least 50% was active. Other inclusion criteria included best-corrected ETDRS VA between 20/63 and 20/200 Snellen equivalent in the study eye and the presence of subretinal fluid, intraretinal fluid, subretinal pigment epithelium (RPE) fluid, or a combination thereof on optical coherence tomography (OCT). The VA inclusion cutoff at 20/63 Snellen equivalent (instead of 20/40 Snellen equivalent) was selected to minimize the potential influence of a ceiling effect that could confound the mathematical inference(s) in a superiority trial design.

Key ocular exclusion criteria included prior treatment for nAMD in the study eye, prior intravitreal drug exposure regardless of indication (including corticosteroids), subretinal hemorrhage more than 50% of the total lesion size, and RPE tears. Patients with diabetes were excluded. Eligibility was confirmed by masked assessment of FA and OCT images by a centralized and independent image reading center (Duke Reading Center). A comprehensive list of inclusion and exclusion criteria can be found in Appendix 2 (available at www.aaojournal.org).

Sample Size, Treatment Groups, and Masking

Patients were randomized centrally in a 1:1:1 ratio to one of the following treatment groups: 0.3 mg E10030 in combination with 0.5 mg ranibizumab, 1.5 mg E10030 in combination with 0.5 mg ranibizumab, and sham in combination with 0.5 mg ranibizumab. The study planned for the enrollment of at least 148 patients (to account for patient dropout) in each of these groups, for a total of approximately 444 patients. Participants were treated monthly with intravitreal injection, according to their assigned dose group, at day 0 and weeks 4, 8, 12, 16, and 20 (6 doses). Patients were masked to treatments. One investigator performed the study drug or sham injection. A separate masked investigator supervised masked assessment of efficacy and assessed adverse events (AEs).

Drug Administration Procedure

Intravitreal injections were performed in accordance with standard-of-care techniques that included the use of 5% povidone iodine and a sterile lid speculum. Intraocular pressure (IOP) was measured 30 minutes after the first injection (ranibizumab, 0.5 mg/eye, 50 μl) to detect delayed normalization of IOP in any patient subgroup. The IOP was monitored after the second injection until it was less than 30 mmHg.

Schedule of Visits and Assessments

Efficacy and safety were assessed at study visits on day 0 and weeks 4, 8, 12, 16, 20, and 24; there was a ±3-day visit window centered on the week 4 time point and a ±7-day visit window centered on the subsequent time points. Certified masked examiners performed protocol refraction and ETDRS VA testing at each study visit to assess best-corrected VA at 4 m. At each study visit, participants underwent assessment of vital signs, IOP testing, and examination of the anterior and posterior segments. In addition, OCT was performed at screening and weeks 4, 8, 12, and 24. Fluorescein angiography was performed at screening and weeks 4, 12, and 24. Image acquisition and assessment parameters for OCT, fundus photographs, and FA can be found in Appendix 3 (available at www.aaojournal.org). Laboratory tests included hematologic analysis, renal function analysis, hepatic function analysis, electrolyte concentrations, and urinalysis; a complete list of

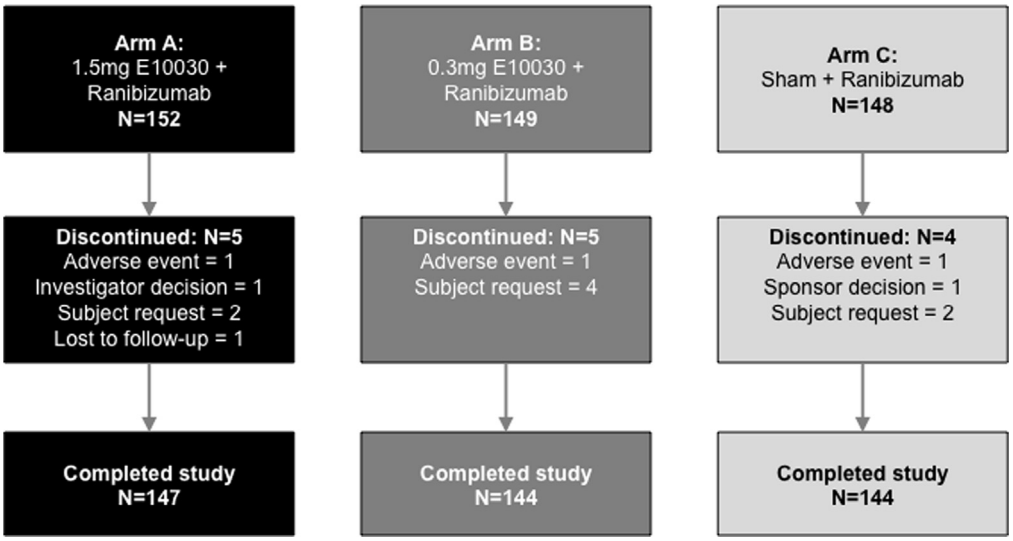

Figure 1. Flowchart showing patient disposition. A total of 449 patients were randomized into the study. This included 152 in the 1.5 mg E10030 plus ranibizumab combination arm, 149 patients in the 0.3 mg E10030 plus ranibizumab combination arm, and 148 patients in the ranibizumab monotherapy arm. There were 14 withdrawals, evenly balanced across the treatment arms.

laboratory tests can be found in Appendix 4 (available at www.aaojournal.org).

End Points and Statistical Analysis

The prespecified primary efficacy end point was the mean change in VA at week 24 when compared with baseline for participants treated with the combinations of E10030 and ranibizumab 0.5 mg versus those receiving ranibizumab 0.5 mg monotherapy. Patients were allocated centrally to 1 of 3 treatment groups. Secondary VA end points included the mean change in VA at week 12 and the proportion of participants gaining 15 ETDRS letters (3 ETDRS lines) or more from baseline at weeks 12 and 24. Secondary anatomic end points included the mean change in CNV area as determined by FA. Additional supportive VA end points included the proportion of participants who gained or lost significant VA based on a change in the number of ETDRS lines read.

The Duke Reading Center independently analyzed and graded FA and OCT images in a masked fashion. The OCT anatomic parameters identified before the study onset for analysis, with respect to their presence or absence at baseline and at 24 weeks, included RPE atrophy, intraretinal and subretinal fluid relative to the foveal location, as well as subretinal hyperreflective material (SHRM). On OCT, RPE atrophy was defined by loss of RPE, loss of overlying outer retinal neurosensory retinal layer, and signal penetration into the choroid. Subretinal hyperreflective material was identified on OCT scans and was defined as hyperreflective material external to the photoreceptors and internal to the RPE.[33]

A retrospective masked analysis also was conducted to assess the development and progression of subretinal fibrosis. Color fundus photographic standards depicting 5 progressive grades of subretinal fibrosis in nAMD from 0 to 4 (absent, barely visible, mild, moderate, or severe) were developed by Usha Chakravarthy at the Belfast Reading Center. This reading center evaluated the amount of subretinal fibrosis by masked analysis of color fundus photographs for all participants experiencing visual loss. Fibrosis development or progression was defined as a 2-step progression on this scale.

Safety end points included AEs, vital signs, laboratory variables, and ophthalmic variables including VA, IOP, ophthalmic examination findings, and FA and OCT findings. According to the statistical analysis plan, the Hochberg procedure was used to address multiplicity. In addition, intention-to-treat last observation carried forward methodology was prespecified to account for missing values, and descriptive statistics were used for secondary and supportive analysis. The safety analysis included all patients who had at least 1 administration of the trial drug.

Results

Patient Disposition and Baseline Characteristics

A total of 449 patients with treatment-naïve nAMD were randomized to each of the treatment groups as follows: 1.5 mg E10030 combination therapy (n = 152), 0.3 mg E10030 combination therapy (n = 149), and anti-VEGF monotherapy (n = 148). Of these 449 patients, 14 withdrew before completion. There were 4 withdrawals in the anti-VEGF monotherapy group (2.7%) and 5 withdrawals in each of the E10030 combination therapy groups (3.3% overall). As summarized in Figure 1, the most common reason for withdrawal was participant request (8 participants), followed by AEs (3 participants), investigator decision (1 participant), sponsor decision (1 participant), and being lost to follow-up (1 participant). Baseline demographic features were balanced between treatment groups (Table 1). Most patients were women and were white. The mean participant age was 78 years, and the mean baseline VA was 50 ETDRS letters.

Primary End Point Analysis

The 1.5 mg E10030 combination therapy group met the prespecified superiority primary end point of mean change in VA from baseline to 24 weeks compared with anti-VEGF monotherapy (Fig 2). At 24 weeks, participants receiving 1.5 mg E10030 combination therapy had a statistically significant improvement in mean VA (10.6 ETDRS letters) compared with those participants receiving anti-VEGF monotherapy (6.5 ETDRS letters; $P = 0.019$). Participants receiving 0.3 mg E10030 combination therapy gained a mean of 8.8 ETDRS letters at week 24, consistent with a trend in VA improvement ($P = 0.17$).

Table 1. Baseline Demographics

Characteristic	Sham plus Ranibizumab (n = 148)	0.3 mg E10030 plus Ranibizumab (n = 149)	1.5 mg E10030 plus Ranibizumab (n = 152)	Total E10030 (0.3 mg + 1.5 mg; n = 301)
Gender				
Male	55 (37.2)	59 (39.6)	60 (39.5)	119 (39.5)
Female	93 (62.8)	90 (60.4)	92 (60.5)	182 (60.5)
Ethnicity				
Hispanic/Latino	10 (6.8)	5 (3.4)	9 (5.9)	14 (4.7)
Not Hispanic/Latino	138 (93.2)	144 (96.6)	143 (94.1)	287 (95.3)
Race				
White	144 (97.3)	145 (97.3)	149 (98.0)	294 (97.7)
Other	4 (2.7)	4 (2.7)	3 (2.0)	7 (2.3)
Iris color				
Light	56 (38.1)	56 (37.6)	46 (30.3)	102 (33.9)
Medium	68 (46.3)	70 (47.0)	79 (52.0)	149 (49.5)
Dark	23 (15.6)	23 (15.4)	27 (17.8)	50 (16.6)
Age (yrs)*				
Mean	78.0	77.6	77.8	77.7
Standard deviation	7.98	8.19	8.36	8.27
Median	79.4	78.3	78.8	78.4
Range	48.5–94.4	53.9–98.8	55.4–94.1	53.9–98.8
Current smoking status				
Not active	135 (91.2)	128 (85.9)	134 (88.7)	262 (87.3)
Active	13 (8.8)	21 (14.1)	17 (11.3)	38 (12.7)
Study eye				
Right	71 (48.0)	70 (47.0)	77 (50.7)	147 (48.8)
Left	77 (52.0)	79 (53.0)	75 (49.3)	154 (51.2)
Total lesion size (disc areas)	1.8	1.9	1.5	1.7

Data are number of patients (%) unless otherwise indicated.
*Age at randomization, intent-to-treat population.

Other Key Visual Acuity End Points

At week 12, participants receiving 1.5 mg E10030 combination therapy had an increase in mean VA of 8.7 ETDRS letters, whereas participants treated with anti-VEGF monotherapy had a mean increase of 5.1 ETDRS letters ($P = 0.016$). A dose-response relationship was evident at each measured time point commencing at 4 weeks. Relative to the anti-VEGF monotherapy group, the VA benefit favoring the 1.5 mg E10030 combination therapy patients increased in magnitude over time (Fig 3). In addition, a larger percentage of participants receiving 1.5 mg E10030 combination therapy had 3 lines or more of improvement at weeks 12 and 24 (32% and 39%, respectively) compared with those receiving anti-VEGF monotherapy (22% and 34%, respectively). Visual acuity outcomes favored the 1.5 mg E10030 combination therapy group regardless of baseline VA, lesion size, or central subfield thickness on OCT (Fig 4).

Multiple clinically relevant treatment end points of visual gain and loss favored the 1.5 mg E10030 combination therapy group compared with anti-VEGF monotherapy group (Fig 5). At 24 weeks, the proportion of participants treated with 1.5 mg E10030 combination therapy were both more likely to experience a marked amount of VA gain (>4 and >5 ETDRS lines) and to achieve improved final VA (≥20/40 and ≥20/25) compared with participants receiving anti-VEGF monotherapy. At 24 weeks, the proportion of participants treated with 1.5 mg E10030 combination therapy were less likely both to lose VA (≥1 and ≥2 ETDRS lines) and to have a lower final VA score (20/125 or worse and 20/200 or worse) compared with participants treated with anti-VEGF monotherapy.

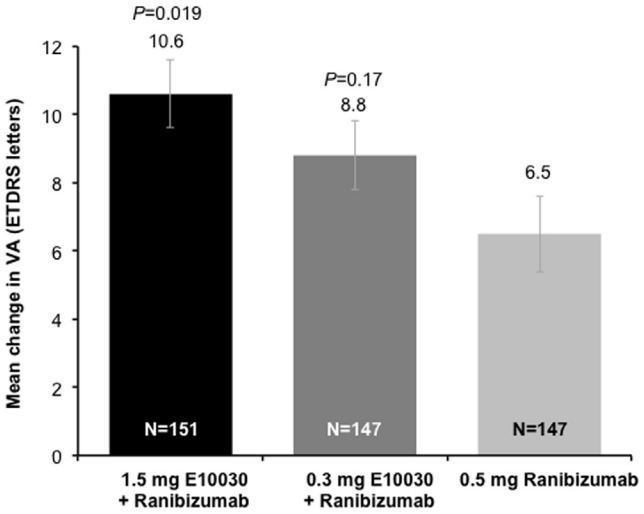

Figure 2. Bar graph showing the primary efficacy end point, mean change in visual acuity (VA) from baseline at 24 weeks. There was a statistically significant difference between the 1.5 mg E10030 plus ranibizumab arm and the sham plus ranibizumab arm (10.6 compared with 6.5 Early Treatment Diabetic Retinopathy [ETDRS] letters at week 24; $P = 0.019$), representing a 62% additional benefit from baseline. Data from the intent-to-treat analysis is depicted. The last observation carried forward method was used to handle missing data. Error bars represent standard error.

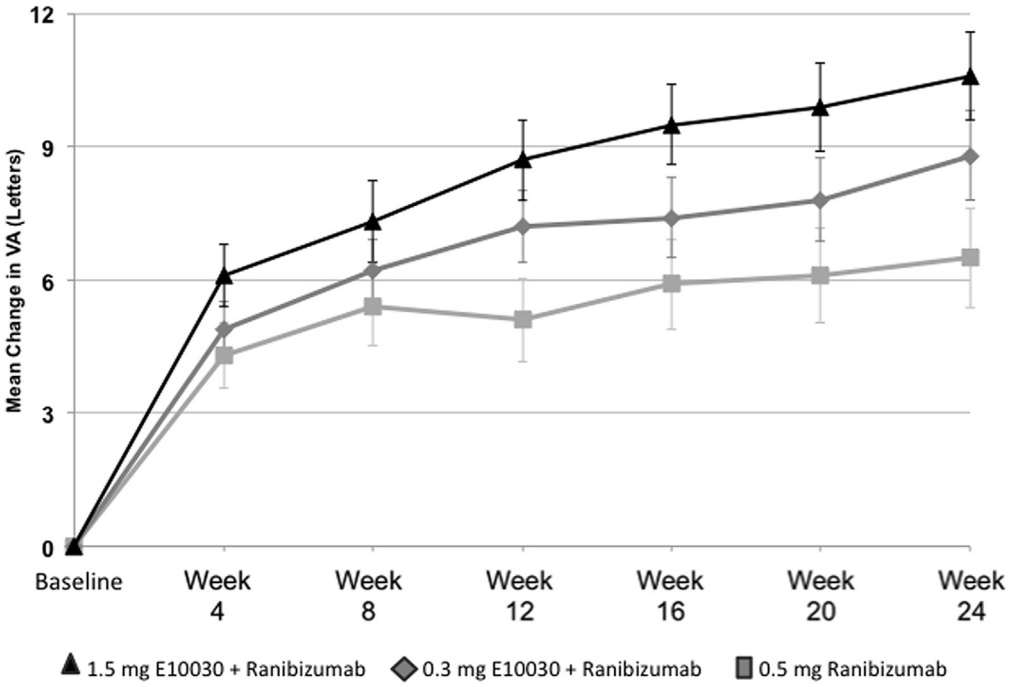

Figure 3. Graph showing the mean change in visual acuity (VA) from baseline over time. There was a benefit of E10030 plus ranibizumab treatment over sham plus ranibizumab treatment in terms of mean VA gain from week 4 onward for both dose levels. A dose-response relationship was evident at each time point for the 1.5 mg E10030 plus ranibizumab and the 0.3 mg E10030 plus ranibizumab treatment arms. The benefit expanded over time. Data from the intent-to-treat analysis are depicted. The last observation carried forward method was used to handle missing data. Error bars represent standard error.

Key Anatomic End Points

Mean CNV area decreased among all treatment groups at week 24 compared with baseline. The mean reductions in CNV area between the study arms when evaluated as a full cohort in each treatment group was −0.7 DA for anti-VEGF monotherapy, −0.7 DA for 0.3 mg E10030 combination therapy, and −0.6 DA for 1.5 mg E10030 combination therapy.

Smaller than mean area (≤1.62 DAs) and larger than mean area (>1.62 DAs) CNV at baseline were analyzed retrospectively to allow for a more arithmetically optimized investigation of CNV regression. This supportive analysis showed a greater

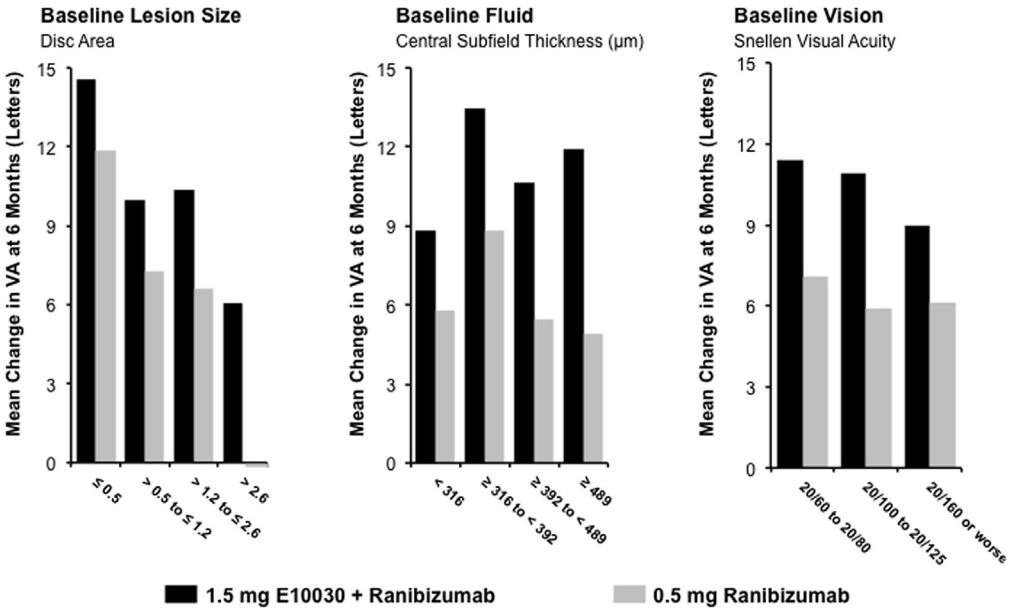

Figure 4. Bar graphs showing baseline variables and visual outcomes at 24 weeks. Visual outcomes favored the higher-dose E10030 combination therapy group regardless of baseline lesion size, baseline fluid (central subfield thickness on optical coherence tomography), or baseline vision. VA = visual acuity.

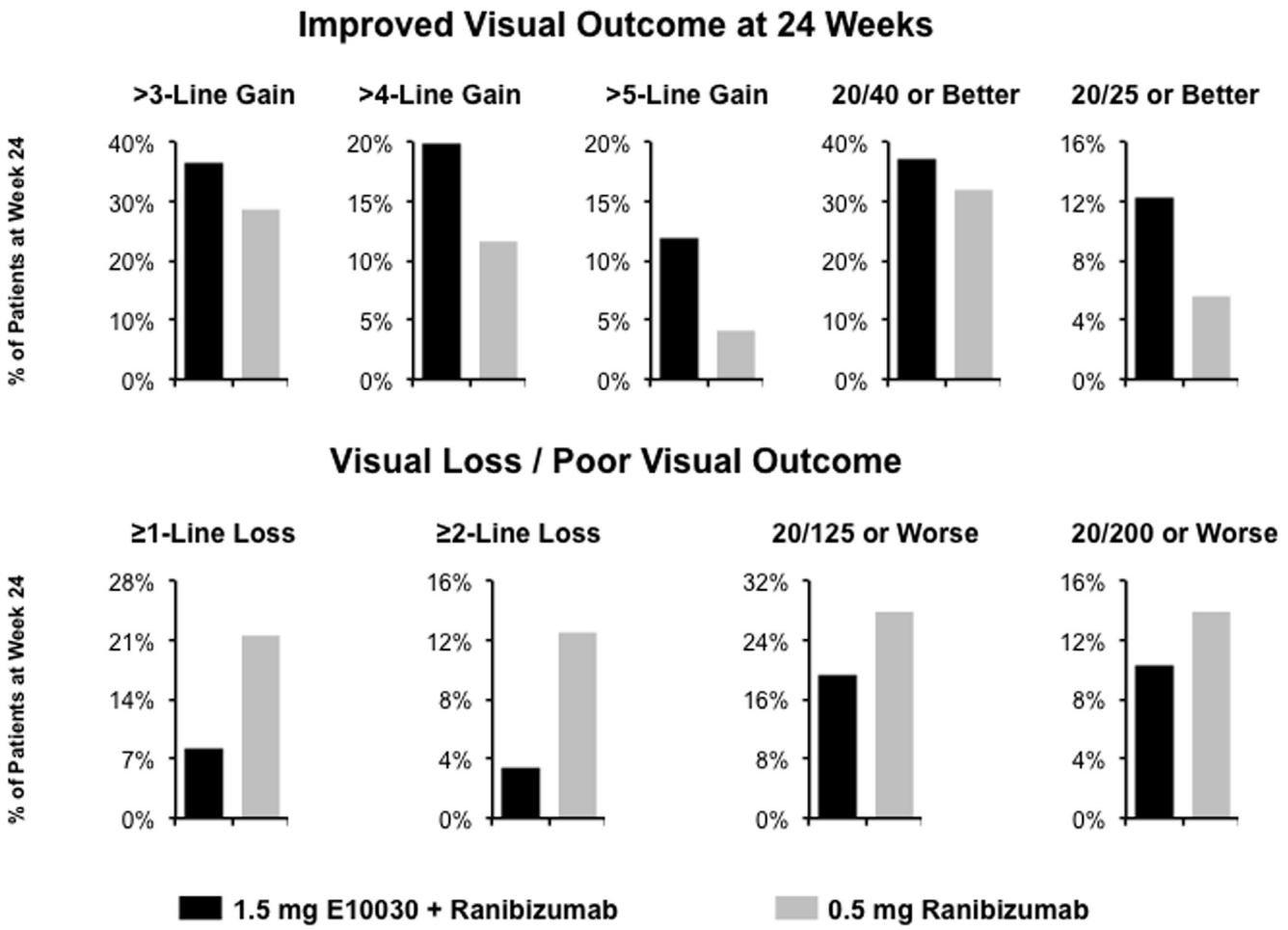

Figure 5. Bar graphs showing vision gained and lost at 24 weeks. A greater proportion of participants treated with 1.5 mg E10030 plus ranibizumab gained visual acuity (VA; >3, >4, and >5 Early Treatment Diabetic Retinopathy [ETDRS] lines) at 24 weeks compared with those treated with ranibizumab (upper left). In addition, a greater proportion of participants treated with 1.5 mg E10030 plus ranibizumab experienced better visual outcomes (20/40 or better, 20/25 or better) at 24 weeks compared with those treated with ranibizumab (upper right). A lower proportion of participants treated with 1.5 mg E10030 plus ranibizumab lost VA (≥1 and ≥2 ETDRS lines) or experienced poor visual outcomes (20/125 or worse and 20/200 or worse) at 24 weeks compared with participants treated with ranibizumab at week 24 (lower panels).

decrease in CNV area in the E10030 combination therapy arms for both small CNV at baseline (−0.15 and −0.12 DA for the 0.3 mg and 1.5 mg E10030 combination arms, respectively, compared with −0.06 DA for the anti-VEGF monotherapy arm) and for large CNV at baseline (−1.69 and −1.73 DAs for the 0.3 mg and 1.5 mg E10030 combination therapy arms, respectively, compared with −1.59 DAs for the anti-VEGF monotherapy arm).

Additional ad hoc supportive analyses were performed to assess change in CNV area for eyes gaining more than 3 lines (15 ETDRS letters) of VA at week 24. This analysis showed a greater relative decrease in CNV area for eyes in the E10030 combination therapy arms compared with the anti-VEGF monotherapy arm (Fig 6A). The decrease in CNV area was particularly evident for eyes with large CNV at baseline: −1.48 and −2.33 DAs for the 0.3 mg and 1.5 mg E10030 combination therapy arms, respectively, compared with −0.24 DA for the anti-VEGF monotherapy arm.

Changes in total macular volume, intraretinal fluid, subretinal fluid, and sub-RPE fluid were determined to evaluate alterations in vascular permeability in each treatment group. There were no significant differences in these parameters among the treatment groups (Fig 7).

A greater proportion of eyes treated with 1.5 mg E10030 combination therapy had complete resolution of SHRM from baseline when compared with eyes treated with anti-VEGF monotherapy (SHRM was absent in 32% vs. 22%, respectively). In participants who achieved significant visual gain (at week 24), an even greater proportion of eyes treated with 1.5 mg E10030 combination therapy had resolution of SHRM from baseline when compared with eyes treated with anti-VEGF monotherapy (SHRM was absent in 54% vs. 38%, respectively; Fig 6B).

A retrospective masked analysis also was conducted with respect to the development and progression of subretinal fibrosis. In those eyes with VA loss at 24 weeks, the mean change in subretinal fibrosis severity from baseline to 24 weeks was less in the 1.5 mg E10030 combination therapy group compared with the anti-VEGF monotherapy group (0.97 vs. 2.0; $P = 0.003$). In this subgroup at 24 weeks, a greater percentage of eyes receiving anti-VEGF monotherapy demonstrated subretinal fibrosis and had subretinal fibrosis progression (51% and 54%, respectively) compared with those

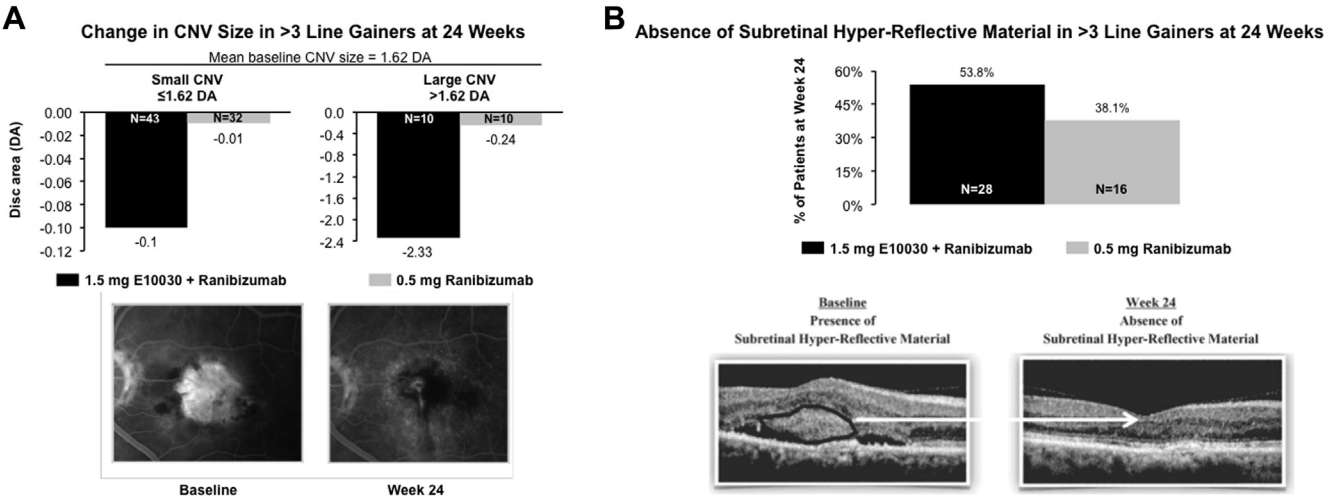

Figure 6. Anatomic changes associated with more than 3-line gains at 24 weeks. **A**, Change in choroidal neovascularization (CNV) size. A retrospective supportive analysis was performed to assess change in CNV size for patients with smaller than mean area (≤1.62 disc areas [DAs]) or larger than mean area (>1.62 DAs) CNV at baseline. In those patients gaining more than 3 lines (15 Early Treatment Diabetic Retinopathy [ETDRS] letters) of visual acuity (VA) at week 24, the decrease in area of CNV was −0.1 DA for the 1.5 mg E10030 plus ranibizumab arm compared with −0.01 DA for the sham plus ranibizumab arm in the small CNV (upper left panel). The decrease in area of CNV was −2.33 DAs for the 1.5 mg E10030 plus ranibizumab arm compared with −0.24 DA for the sham plus ranibizumab arm in the large CNV (upper right panel). A representative 1.5 mg E10030 plus ranibizumab case is shown in the bottom panels. In the baseline image, note the 1.5-DA classic subfoveal CNV with some blockage from mild subretinal hemorrhage nasally (lower left panel), which decreased in size at 24 weeks (lower right panel). **B**, Anatomic changes: absence of subretinal hyperreflective material (SHRM). Retrospective analysis of optical coherence tomography findings was performed in those patients gaining more than 3 lines (15 ETDRS letters) of VA at week 24. Subretinal hyperreflective material was absent in 54% of those patients in the 1.5 mg E10030 plus ranibizumab arm compared with 38% in the sham plus ranibizumab arm (upper panels). A representative 1.5 mg E10030 plus ranibizumab case is shown in the bottom panels. In the baseline image, note the prominent SHRM subfoveally (lower left panel), which resolved at 24 weeks (lower right panel).

receiving 1.5 mg E10030 combination therapy (10% and 27%, respectively).

Retinal pigment epithelium atrophy (RPE loss or disruption with overlying photoreceptor atrophy and signal penetration into the choroid) also was assessed by OCT in a prespecified masked fashion by the Duke Reading Center. At 24 weeks, RPE atrophy was evident in 21% of eyes in the anti-VEGF monotherapy group (n = 30/144), 17% in the 0.3 mg E10030 combination therapy group (n = 24/143), and 16% in the 1.5 mg E10030 combination therapy group (n = 23/145), respectively (Fig 8).

Safety

E10030 plus ranibizumab combination therapy was well tolerated. There were no significant differences in injection procedure AEs, study drug AEs, AEs leading to study discontinuation, or serious AEs among the treatment arms. Very few patients experienced study drug AEs, and most AEs were mild or moderate in intensity.

The most frequently reported AEs were ophthalmic AEs in the study eye related to the injection itself, such as surface irritation and subconjunctival hemorrhage. Transient elevation of mean IOP, consistent with a volume effect, was observed, and mean IOP returned to the preinjection IOP level in all arms at the next visit and at the end of the study. There were no AEs of glaucoma.

Nonophthalmic AEs were reported less frequently than ophthalmic AEs. The most frequently occurring systemic AEs were conditions that commonly occur among the general population, including hypertension, headache, nasopharyngitis, and urinary tract infection. There were no clinically meaningful laboratory abnormality trends and no significant vital sign changes.

The incidence of severe AEs in the combination and monotherapy groups was similar and is summarized in Table 2. There was a low incidence of Antiplatelet Trialists' Collaboration events and no imbalance among groups. There were no events of endophthalmitis, retinal detachment, retinal tear, or iatrogenic traumatic cataract after a total of 4431 intravitreal injections (1776 injections of E10030 and 2655 injections of ranibizumab).

Discussion

This clinical trial confirmed the initial findings of the phase I clinical trial, which suggested a favorable safety profile, improved visual outcomes, and biomarker changes consistent with the mechanism of action of E10030 combination therapy in nAMD.[32] This phase IIb study demonstrated a statistically significant VA benefit when E10030 (1.5 mg) was added to a monthly anti-VEGF regimen (E10030 combination therapy) over 6 months for nAMD, reflected by the 62% additive improvement in mean change in VA from baseline to 24 weeks. A dose-dependent benefit of E10030 combination therapy over anti-VEGF monotherapy was evident early and was sustained to the last measured time point at 24 weeks. Moreover, there was a suggestion of increasing benefit of E10030 combination therapy compared with anti-VEGF monotherapy over time, with no drug-related safety imbalances between the groups. The relative treatment benefit in the E10030 combination therapy arm was evident regardless of baseline VA, lesion size, or central

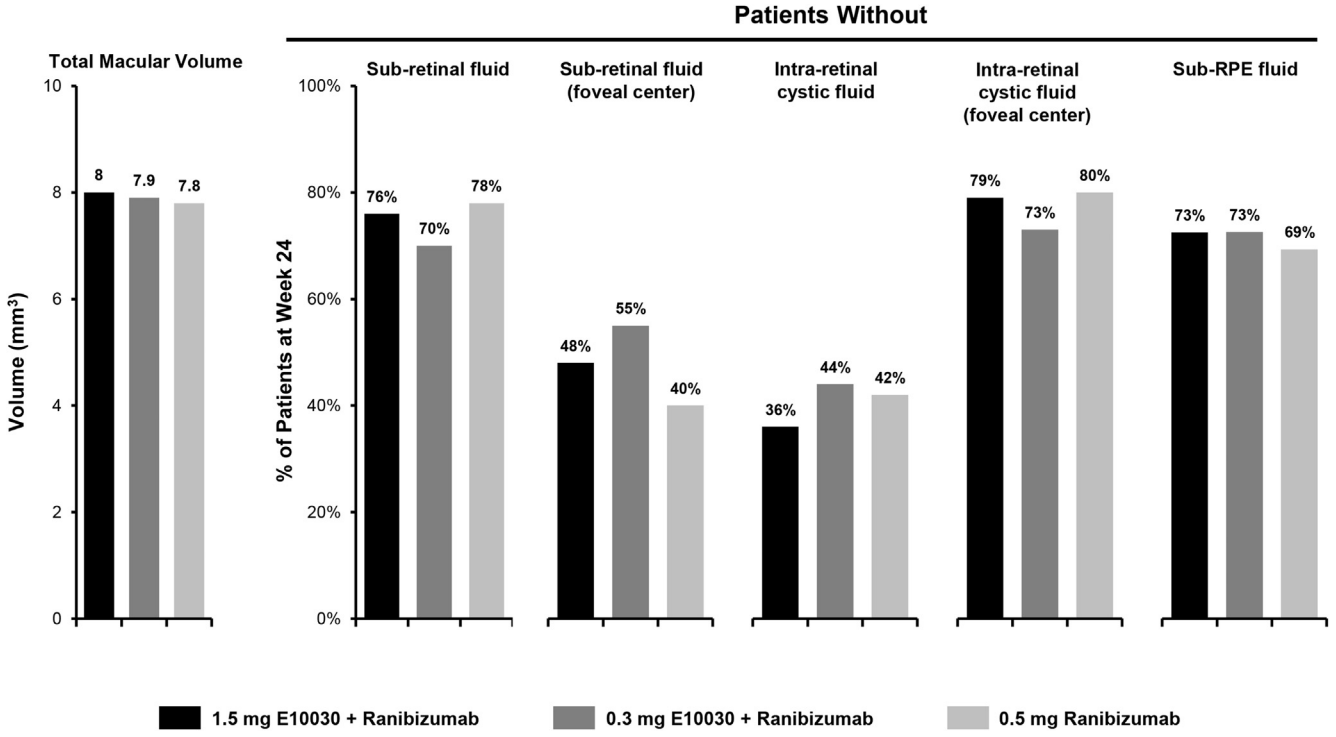

Figure 7. Bar graphs showing optical coherence tomography (OCT) analysis of permeability alterations at 24 weeks. Analysis of permeability alterations on OCT included evaluation of total macular volume or the absence of subretinal fluid, intraretinal cystic fluid, or sub—retinal pigment epithelium (RPE) fluid. No meaningful difference was noted between the groups.

subfield thickness on OCT and was evident across multiple clinically relevant treatment end points measuring VA gain and reduction of VA loss.

Multiple mechanisms involving dual antagonism of VEGF and PDGF signaling pathways may result in a variety of disease-modifying tissue responses (i.e., neovascular complex regression; reduction of fibrovascular scar and/or fibrous scar). First, in preclinical pathologic angiogenesis, when PDGF signaling is disrupted, pericytes are stripped from neovascular endothelial cells. The resulting endothelial-lined neovascular tubes are highly vulnerable to the effects of anti-VEGF therapy, thereby inducing neovascular regression.[18,21,24] Second, immunolabeling experiment studies of spatiotemporal cellular events in laser CNV models suggest that pericytes play a key role during the initial formation and growth of CNV.[34] Third, recent findings show that pericytes play an important role in local inflammatory response by orchestrating the navigation of leukocytes within the interstitial space to sites of inflammation.[35] Pericytes coordinate this interstitial leukocyte trafficking through expression of the cell-surface intercellular adhesion molecule-1 (allowing pericytes physically to engage neutrophils and monocytes or macrophages) and by releasing the chemoattractant macrophage migration-inhibitory factor.[35] Fourth, PDGF itself is chemotactic for pericytes, RPE cells, and glial cells,[25,36,37] all of which are known components of surgically extracted fibrovascular and fibrous CNV.[38] Finally, there is strong supporting evidence that pericytes are a major source of myofibroblasts, which deposit pathologic matrix.[27,39] In other organ systems, pericytes drive renal and hepatic fibrosis.[27,39] Platelet-derived growth factor also is central to wound healing and fibrosis systemically, as manifested by the US Food and Drug Administration approval of a recombinant PDGF dermatologic gel to promote the healing of diabetic ulcers, as well as the recent US Food and Drug Administration approval of nintedanib for idiopathic pulmonary fibrosis, which is known to involve PDGF signaling. In

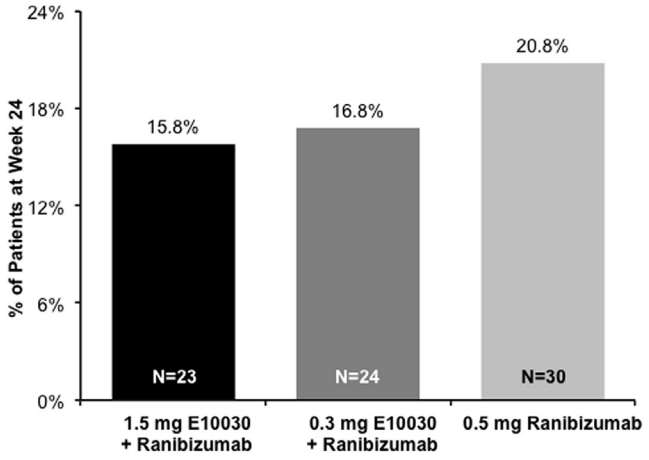

Figure 8. Bar graph showing patients with retinal pigment epithelium (RPE) atrophy at 24 weeks. Retinal pigment epithelium atrophy was assessed on optical coherence tomography at 24 weeks. At 24 weeks, the presence of RPE atrophy was evident in 16% in the 1.5 mg E10030 plus ranibizumab arm, 17% in the 0.3 mg E10030 plus ranibizumab arm, and 21% in the sham plus ranibizumab group.

Table 2. Summary of Serious Adverse Events

	Monotherapy Ranibizumab (n = 148)	0.3 mg E10030 + Ranibizumab (n = 149)	1.5 mg E10030 + Ranibizumab (n = 152)
Eye disorders	1 (0.7)	1 (0.7)	1 (0.7)
Corneal erosion	0 (0.0)	0 (0.0)	1 (0.7)
Uveitis	0 (0.0)	1 (0.7)	0 (0.0)
Visual acuity reduced	1 (0.7)	0 (0.0)	0 (0.0)
Patients with ≥1 systemic SAE	11 (7.4)	13 (8.7)	9 (5.9)
MedDRA system organ class			
Cardiac disorders	2 (1.4)	2 (1.3)	2 (1.3)
Gastrointestinal disorders	1 (0.7)	2 (1.3)	3 (2.0)
Infections	1 (0.7)	2 (1.3)	0 (0.0)
Musculoskeletal disorders	1 (0.7)	0 (0.0)	2 (1.3)
Neoplasms	3 (2.0)	3 (2.0)	1 (0.7)
Nervous system disorders	3 (2.0)	1 (0.7)	0 (0.0)
Respiratory disorders	0 (0.0)	3 (2.0)	2 (1.3)
Any APTC event	3 (2.0)	1 (0.7)	0 (0.0)
Nonfatal myocardial infarction	0 (0.0)	0 (0.0)	0 (0.0)
Nonfatal stroke	2 (1.4)	1 (0.7)	0 (0.0)
Vascular death	1 (0.7)	0 (0.0)	0 (0.0)

APTC = Antiplatelet Trialists' Collaboration; MedDRA = Medical Dictionary for Regulatory Activities; SAE = serious adverse event.
Data are number of patients (%).

the eye, E10030-mediated PDGF inhibition significantly reduced epiretinal fibrosis in an animal model of retinal scarring.[40] In the present study, we targeted these multiple mechanisms with E10030 plus anti-VEGF combination therapy. We propose that dual PDGF and VEGF inhibition induced neovascular regression after stripping of pericytes. We further believe that PDGF inhibited nonneovascular components (myofibroblasts and inflammatory, RPE, and glial cells)[41] to limit the amount of fibrovascular and fibrous tissues in these eyes with nAMD. Based on these data taken together, we hypothesize that these beneficial effects on tissue responses accounted for the improved VA observed in this study with E10030 plus anti-VEGF combination therapy.

The imaging studies supported the aforementioned proposed mechanisms of action for E10030 plus anti-VEGF combination therapy on tissue responses and VA. Neovascular complex regression after treatment with E10030 combination therapy was evident on OCT based on the enhanced resolution of SHRM, which also correlated with improved VA. On FA, a similar CNV regression effect was suggested by the separate evaluation of small and large CNV lesions at baseline. This division was used to address the confounding effect resulting from the disproportional numerical influence on regression imparted by the larger baseline CNV area group.

Consistent with the mechanisms highlighted in preclinical studies mentioned above, retrospective masked analysis revealed that E10030 combination therapy was more effective than anti-VEGF monotherapy in limiting the development and progression of fibrosis. The implication related to this finding is relevant because nAMD-associated fibrosis is a key cause of decreased VA in anti-VEGF-treated eyes.[33,42] In this trial, approximately half of the eyes that did not gain VA while receiving anti-VEGF monotherapy demonstrated subretinal fibrosis. In the Comparison of Age-Related Macular Degeneration Treatments Trials, approximately 25% of eyes demonstrated fibrosis by 2 years despite treatment with anti-VEGF monotherapy.[42] One Comparison of Age-Related Macular Degeneration Treatments Trials publication showed SHRM as a significant risk factor for scar formation.[42] The authors of that publication postulated that E10030-mediated PDGF inhibition and associated SHRM resolution may be one explanation for the resulting visual benefit noted in this study, which requires confirmation in future clinical trials.[42]

In summary, E10030 combination therapy yielded robust visual outcomes across multiple meaningful parameters consistent with the mechanism of action. The overall trend in imaging biomarker responses were consistent with visual benefit associated with E10030 combination therapy. To the best of our knowledge, in the current anti-VEGF monotherapy era, no biomarker has been correlated with improvement in visual outcome after the initial resolution of exudation in the induction phase. Large, confirmatory, phase III clinical trials in nAMD are underway, comparing 1.5 mg E10030 combined with each of the 3 commonly used anti-VEGF agents (ranibizumab, aflibercept, and off-label bevacizumab) with the respective anti-VEGF agent administered as monotherapy.

Acknowledgments. The authors thank Forbes Huang, Ophthotech Corporation, for his assistance in preparing the figures for publication.

References

1. Comparison of Age-related Macular Degeneration Treatments Trials Research Group, Martin DF, Maguire MG, et al. Ranibizumab and bevacizumab for treatment of neovascular age-related macular degeneration: two-year results. *Ophthalmology*. 2012;119:1388-1398.
2. Busbee BG, Ho AC, Brown DM, et al. Twelve-month efficacy and safety of 0.5 mg or 2.0 mg ranibizumab in patients with subfoveal neovascular age-related macular degeneration. *Ophthalmology*. 2013;120:1046-1056.
3. Chakravarthy U, Harding SP, Rogers CA, et al. Alternative treatments to inhibit VEGF in age-related choroidal neovascularisation: 2-year findings of the IVAN randomised controlled trial. *Lancet*. 2013;382:1258-1267.
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. Heier JS, Brown DM, Chong V, et al. Intravitreal aflibercept (VEGF trap-eye) in wet age-related macular degeneration. *Ophthalmology*. 2012;119:2537-2548.
6. Brown DM, Michels M, Kaiser PK, et al. Ranibizumab versus verteporfin photodynamic therapy for neovascular age-related macular degeneration: two-year results of the ANCHOR study. *Ophthalmology*. 2009;116:57-65.e5.

7. Rosenfeld PJ, Brown DM, Heier JS, et al. Ranibizumab for neovascular age-related macular degeneration. *N Engl J Med*. 2006;355:1419-1431.
8. Arevalo JF, Lasave AF, Wu L, et al. Intravitreal bevacizumab for choroidal neovascularization in age-related macular degeneration: 5-year results of the Pan-American Collaborative Retina Study Group (PACORES). *Retina*. 2016;36:859-867.
9. Cohen SY, Mimoun G, Oubraham H, et al. Changes in visual acuity in patients with wet age-related macular degeneration treated with intravitreal ranibizumab in daily clinical practice: the LUMIERE study. *Retina*. 2013;33:474-481.
10. Holz FG, Tadayoni R, Beatty S, et al. Multi-country real-life experience of anti-vascular endothelial growth factor therapy for wet age-related macular degeneration. *Br J Ophthalmol*. 2015;99:220-226.
11. Rakic JM, Leys A, Brie H, et al. Real-world variability in ranibizumab treatment and associated clinical, quality of life, and safety outcomes over 24 months in patients with neovascular age-related macular degeneration: the HELIOS study. *Clin Ophthalmol*. 2013;7:1849-1858.
12. Rasmussen A, Bloch SB, Fuchs J, et al. A 4-year longitudinal study of 555 patients treated with ranibizumab for neovascular age-related macular degeneration. *Ophthalmology*. 2013;120:2630-2636.
13. Ross AH, Donachie PH, Sallam A, et al. Which visual acuity measurements define high-quality care for patients with neovascular age-related macular degeneration treated with ranibizumab? *Eye (Lond)*. 2013;27:56-64.
14. Talks JS, Lotery AJ, Ghanchi F, et al. First-year visual acuity outcomes in the United Kingdom of providing aflibercept according to the VIEW study protocol for age-related macular degeneration. *Ophthalmology*. 2015;123:337-343.
15. van Asten F, Evers-Birkenkamp KU, van Lith-Verhoeven JJ, et al. A prospective, observational, open-label, multicentre study to investigate the daily treatment practice of ranibizumab in patients with neovascular age-related macular degeneration. *Acta Ophthalmol*. 2015;93:126-133.
16. Writing Committee for the U. K. Age-Related Macular Degeneration E. M. R. Users Group. The neovascular age-related macular degeneration database: multicenter study of 92 976 ranibizumab injections: report 1: visual acuity. *Ophthalmology*. 2014;121:1092-1101.
17. Souied EH, Oubraham H, Mimoun G, et al. Changes in visual acuity in patients with wet age-related macular degeneration treated with intravitreal ranibizumab in daily clinical practice: The TWIN study. *Retina*. 2015;35:1743-1749.
18. Benjamin LE, Hemo I, Keshet E. A plasticity window for blood vessel remodelling is defined by pericyte coverage of the preformed endothelial network and is regulated by PDGF-B and VEGF. *Development*. 1998;125:1591-1598.
19. Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. *Nat Rev Cancer*. 2008;8:592-603.
20. Carmeliet P, Jain RK. Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. *Nat Rev Drug Discov*. 2011;10:417-427.
21. Erber R, Thurnher A, Katsen AD, et al. Combined inhibition of VEGF and PDGF signaling enforces tumor vessel regression by interfering with pericyte-mediated endothelial cell survival mechanisms. *FASEB J*. 2004;18:338-340.
22. Helfrich I, Schadendorf D. Blood vessel maturation, vascular phenotype and angiogenic potential in malignant melanoma: one step forward for overcoming anti-angiogenic drug resistance? *Mol Oncol*. 2011;5:137-149.
23. Helfrich I, Scheffrahn I, Bartling S, et al. Resistance to antiangiogenic therapy is directed by vascular phenotype, vessel stabilization, and maturation in malignant melanoma. *J Exp Med*. 2010;207:491-503.
24. Jo N, Mailhos C, Ju M, et al. Inhibition of platelet-derived growth factor B signaling enhances the efficacy of anti-vascular endothelial growth factor therapy in multiple models of ocular neovascularization. *Am J Pathol*. 2006;168:2036-2053.
25. Hellstrom M, Kalen M, Lindahl P, et al. Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. *Development*. 1999;126:3047-3055.
26. Armulik A, Abramsson A, Betsholtz C. Endothelial/pericyte interactions. *Circ Res*. 2005;97:512-523.
27. Andrae J, Gallini R, Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. *Genes Dev*. 2008;22:1276-1312.
28. Hall AP. Review of the pericyte during angiogenesis and its role in cancer and diabetic retinopathy. *Toxicol Pathol*. 2006;34:763-775.
29. Fredriksson L, Li H, Eriksson U. The PDGF family: four gene products form five dimeric isoforms. *Cytokine Growth Factor Rev*. 2004;15:197-204.
30. Mitchell TS, Bradley J, Robinson GS, et al. RGS5 expression is a quantitative measure of pericyte coverage of blood vessels. *Angiogenesis*. 2008;11:141-151.
31. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. *Nature*. 2011;473:298-307.
32. Jaffe GJ, Eliott D, Wells JA, et al. A phase 1 study of intravitreous E10030 in combination with ranibizumab in neovascular age-related macular degeneration. *Ophthalmology*. 2015;123:78-85.
33. Willoughby AS, Ying GS, Toth CA, et al. Subretinal hyperreflective material in the Comparison of Age-Related Macular Degeneration Treatments Trials. *Ophthalmology*. 2015;122:1846-1853.e5.
34. Strittmatter K, Pomeroy H, Marneros AG. Targeting PDGFRBeta+ scaffold formation inhibits choroidal neovascularization. *Am J Pathol*. 2016;186:1890-1899.
35. Stark K, Eckart A, Haidari S, et al. Capillary and arteriolar pericytes attract innate leukocytes exiting through venules and 'instruct' them with pattern-recognition and motility programs. *Nat Immunol*. 2013;14:41-51.
36. Campochiaro PA, Glaser BM. Platelet-derived growth factor is chemotactic for human retinal pigment epithelial cells. *Arch Ophthalmol*. 1985;103:576-579.
37. Uchihori Y, Puro DG. Mitogenic and chemotactic effects of platelet-derived growth factor on human retinal glial cells. *Invest Ophthalmol Vis Sci*. 1991;32:2689-2695.
38. Grossniklaus HE, Miskala PH, Green WR, et al. Histopathologic and ultrastructural features of surgically excised subfoveal choroidal neovascular lesions: submacular surgery trials report no. 7. *Arch Ophthalmol*. 2005;123:914-921.
39. Greenhalgh SN, Iredale JP, Henderson NC. Origins of fibrosis: pericytes take centre stage. *F1000Prime Rep*. 2013;5:37-43.
40. Akiyama H, Kachi S, Silva RL, et al. Intraocular injection of an aptamer that binds PDGF-B: a potential treatment for proliferative retinopathies. *J Cell Physiol*. 2006;207:407-412.
41. Spaide RF. Rationale for combination therapies for choroidal neovascularization. *Am J Ophthalmol*. 2006;141:149-156.
42. Daniel E, Toth CA, Grunwald JE, et al. Risk of scar in the comparison of age-related macular degeneration treatments trials. *Ophthalmology*. 2014;121:656-666.

Footnotes and Financial Disclosures

Originally received: May 19, 2016.
Final revision: October 6, 2016.
Accepted: October 8, 2016.
Available online: October 28, 2016. Manuscript no. 2016-1060.

[1] Department of Ophthalmology, Duke Reading Center, Duke University, Durham, North Carolina.

[2] Ophthotech Corporation, New York, NY.

[3] Azienda Ospedaliero-Universitaria di Bologna—Policlinico S. Orsola-Malpighi, Unità Operativa Oftalmologia—Ciardella, Bologna, Italy.

[4] Centre Paradis Monticelli, Marseilles, France.

[5] Retinal Consultants of Arizona, Phoenix, Arizona, and USC Roski Eye Institute, Keck School of Medicine, University of Southern California, Los Angeles, California.

[6] Department of Surgical Science, Eye Clinic, University of Torino, Torino, Italy.

[7] Institut de la Macula, Centro Medico Teknon, QuironSalud, and Barcelona Macula Foundation, Barcelona, Spain.

[8] Retinal Consultants, Sacramento, California.

[9] Centre Rabelais, Lyon, France.

[10] Università Tor Vergata—Fondazione PTV Policlinico Tor Vergata, Unità Operativa Semplice Dipartimentale Patologie Retiniche—Dipartimento di Chirurgia, Rome, Italy.

Financial Disclosure(s):
The author(s) have made the following disclosure(s): G.J.J.: Consultant — Alcon Laboratories, Inc./Novartis (Basel, Switzerland); Genentech/Roche (Basel, Switzerland); Neurotech (Cumberland, RI); Heidelberg Engineering (Heidelberg, Germany)

T.A.C.: Employee, Equity owner — Ophthotech Corp. (New York, NY)

P.U.D.: Consultant — Ophthotech Corp. (New York, NY); Abbott/AMO (Santa Anna, CA); Aerpio (Cincinnati, OH); Alcon/Novartis (Basel, Switzerland); Alimera Sciences (Alpharetta, GA); Allergan (Dublin, Ireland); Annidis (Grandville, MI); Acucela (Seattle, WA); ArcticDx (Toronto, Canada); Avalanche Biotechnologies; Clearside Biomedical (Alpharetta, GA); Digisight (San Francisco, CA); DOSE Medical; Genentech (South San Francisco, CA); Graybug Vision (Redwood City, CA); Lutronic (Burlington, MA); Lux BioScience (Jersey City, NJ); Neurotech (Cumberland, RI); Novartis (Basel, Switzerland); OD-OS (Teltow, Germany); Omeros (Seattle, WA); Opthea (South Yarra, Victoria, Australia); Optovue (Freemont, CA); ORA (Andover, MA); Regeneron (Tarrytown, NY); Roche (Basel, Switzerland); Pentavision (Ambler, PA); Shire Human Genetic Therapies (Lexington, MA); Stealth BioTherapeutics (Newtown, MA); Thrombogenics (Leuven, Belgium); TopCon (Tokyo, Japan); Equity owner — Ophthotech Corp. (New York, NY); Alimera (Alaphetta, GA); Aerpio (Cincinnati, OH); Annidis (Grandville, MI); Digisight (San Francisco, CA); Ophthotech (New York, NY); TrueVision (Santa Barbara, CA)

H.M.: Employee, Equity owner — Ophthotech Corp. (New York, NY); Patent — US 2016/0264969 A1

J.M.: Consultant — Ophthotech Corp. (New York, NY); Ophthotech (New York, NY); Notal Vision, Inc. (Tel Aviv, Israel); Alcon Laboratories, Inc. (Fort Worth, TX); Allergan (Dublin, Ireland); Bayer Health Pharmaceuticals (Leverkusen, Germany); Genentech/Roche (Basel, Switzerland); Novartis (Basel, Switzerland); Financial support — Novartis (Basel, Switzerland); Genentech/Roche (Basel, Switzerland); Equity owner — Ophthotech Corp. (New York, NY); Notalvision (Tel Aviv, Israel)

J.A.P.: Financial support — Ophthotech Corp. (New York, NY)

M.Q.-E.: Consultant — Novartis (Basel, Switzerland); Bayer Health Pharmaceuticals (Leverkusen, Germany)

F.R.: Consultant — Novartis (Basel, Switzerland); Alcon Laboratories, Inc. (Fort Worth, TX); Allergan (Dublin, Ireland); Bayer (Leverkusen, Germany); Regeneron (Tarrytown, NY)

K.W.: Employee, Equity owner — Ophthotech Corp. (New York, NY)

S.P.: Employee, Equity owner — Ophthotech Corp. (New York, NY)

Supported by Ophthotech Corp. (New York, NY). The sponsor participated in the study design, data collection and management, data analysis and interpretation, and manuscript preparation.

Author Contributions:

Conception and design: Masonson, Patel

Analysis and interpretation: Jaffe, Ciulla, Masonson, Westby, Patel

Data collection: Jaffe, Ciardella, Devin, Dugel, Eandi, Monés, Pearlman, Quaranta-El Maftouhi, Ricci

Obtained funding: none

Overall responsibility: Jaffe, Ciulla, Westby, Patel

Abbreviations and Acronyms:
AE = adverse event; **CNV** = choroidal neovascularization; **DA** = disc area; **ETDRS** = Early Treatment Diabetic Retinopathy Study; **FA** = fluorescein angiography; **IOP** = intraocular pressure; **nAMD** = neovascular age-related macular degeneration; **OCT** = optical coherence tomography; **PDGF** = platelet-derived growth factor; **RPE** = retinal pigment epithelium; **SHRM** = subretinal hyperreflective material; **VA** = visual acuity; **VEGF** = vascular endothelial growth factor.

Correspondence:
Glenn J. Jaffe, MD, Department of Ophthalmology, Duke Reading Center, Duke University, Box 3802, Durham, NC 27710. E-mail: jaffe001@mc.duke.edu.