Pro-Permeability Factors After Dexamethasone Implant in Retinal Vein Occlusion; the Ozurdex for Retinal Vein Occlusion (ORVO) Study

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• PURPOSE: To correlate aqueous vasoactive protein changes with macular edema after dexamethasone implant in retinal vein occlusion (RVO).
• DESIGN: Prospective, interventional case series.
• METHODS: Twenty-three central RVO (CRVO) and 17 branch RVO (BRVO) subjects with edema despite prior anti–vascular endothelial growth factor (VEGF) treatment had aqueous taps at baseline and 4 and 16 weeks after dexamethasone implant. Best-corrected visual acuity (BCVA) and center subfield thickness were measured every 4 weeks. Aqueous vasoactive protein levels were measured by protein array or enzyme-linked immunosorbent assay.
• RESULTS: Thirty-two vasoactive proteins were detected in aqueous in untreated eyes with macular edema due to RVO. Reduction in excess foveal thickness after dexamethasone implant correlated with reduction in persephin and pentraxin 3 (Pearson correlation coefficients = 0.682 and 0.638, $P = .014$ and $P = .003$). Other protein changes differed among RVO patients as edema decreased, but ≥50% of patients showed reductions in hepatocyte growth factor, endocrine gland VEGF, insulin-like growth factor binding proteins, or endostatin by ≥30%. Enzyme-linked immunosorbent assay in 18 eyes (12 CRVO, 6 BRVO) showed baseline levels of hepatocyte growth factor and VEGF of 168.2 ± 20.1 pg/mL and 78.7 ± 10.0 pg/mL, and each was reduced in 12 eyes after dexamethasone implant.
• CONCLUSIONS: Dexamethasone implants reduce several pro-permeability proteins providing a multitargeted approach in RVO. No single protein in addition to VEGF can be implicated as a contributor in all patients. Candidates for contribution to chronic edema in subgroups of patients that deserve further study include persephin, hepatocyte growth factor, and endocrine gland VEGF. (Am J Ophthalmol 2015;160(2):313–321. © 2015 by Elsevier Inc. All rights reserved.)

AJO.com Supplemental Material available at AJO.com.

Accepted for publication Apr 15, 2015.

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CENTRAL RETINAL VEIN OCCLUSION (CRVO) IS initiated by thrombotic occlusion of the main outflow vessel of the retina resulting in retinal hemorrhages, variable amounts of retinal nonperfusion, and macular edema. Branch vein occlusion (BRVO) is initiated from thrombotic occlusion of a proximal branch of the central retinal vein that drains ≤50% of the retina. Retinal hemorrhages, variable amounts of retinal nonperfusion, and macular edema also occur after BRVO but on average tend to be less severe, because less of the retina is involved by the occlusion compared to CRVO. While ischemic damage to the macula may contribute, the major cause of reduced visual acuity is macular edema. In patients with relatively recent-onset CRVO or BRVO, intraocular injections of a specific antagonist of vascular endothelial growth factor (VEGF) results in dramatic reductions in macular edema and improvements in visual acuity.[1] This indicates that VEGF is a major cause of macular edema in patients with RVO. This was confirmed by large multicenter trials, and intraocular injections of a VEGF antagonist has become first-line therapy for patients with CRVO or BRVO.[2–6] Frequent injections of a VEGF antagonist are able to completely eliminate edema in some patients, suggesting that VEGF is necessary for edema in those patients; however, it is difficult to maintain a dry retina, because recurrences often occur when the duration between injections is increased. In other patients, it is not possible to achieve complete elimination of the edema despite monthly injections of a VEGF antagonist, suggesting an inability to neutralize all VEGF or contributions from other pro-permeability factors.

It was initially thought that the goal of treatment in RVO would be to control edema and maintain vision until recanalization of the occluded vessel allowed for normalization of the underlying disease process and elimination of the need for injections. However, it appears that the occlusion is merely the initiator of a dynamic disease process that is driven by retinal ischemia and high levels of VEGF, which promote leukostasis, progression of capillary closure, and increased ischemia.[7] This progression of disease makes continued injections necessary to control edema and some patients experience permanent loss of vision from ischemic damage to the macula or damage from chronic/recurrent

0002-9394/$36.00
http://dx.doi.org/10.1016/j.ajo.2015.04.025

edema.[8] In the RETAIN study, with a mean follow-up of 49 months after the initiation of anti-VEGF treatment, only 50% of BRVO patients and 44% of CRVO patients no longer required injections to control edema.[9] In many patients, injections of a VEGF antagonist seemed less effective over time, suggesting evolution or change in the disease process such that other pro-permeability factors may play a more important role.

Corticosteroids bind to cytoplasmic receptors that translocate to the nucleus and cause transcriptional repression of a large number of genes whose products participate in inflammation, vascular leakage, and angiogenesis.[10–12] The dexamethasone implant reduces edema and improves vision in patients with RVO and has a longer duration of effect than intraocular injection of currently available VEGF antagonists.[13] It is an appealing alternative in patients who have residual edema despite anti-VEGF injections or who require frequent injections to control edema. While it is assumed that it reduces many factors that might contribute to edema, there are little data regarding this point. In this study, a vasoactive protein array was used to measure levels of aqueous proteins known to influence vascular cells prior to and after injection of a dexamethasone implant, and changes in protein levels were correlated with changes in edema.

METHODS

• **STUDY PROCEDURES:** The Ozurdex for Retinal Vein Occlusion (ORVO) Study was an investigator-initiated study funded by Allergan, Inc (Irvine, California, USA). The protocol was approved by the Institutional Review Board of the Johns Hopkins Medical Institutions and was conducted in compliance with the Declaration of Helsinki, US Code 21 of Federal Regulations, and the Harmonized "Tripartite Guidelines for Good Clinical Practice (1996). The study was registered on February 8, 2013 at www.clinicaltrials.gov (NCT01790685). All patients provided informed consent. Forty subjects with RVO (17 with BRVO and 23 with CRVO) were enrolled. Disease duration for each patient was calculated from when the patient first developed macular edema until the baseline visit. Because patient reporting is often unreliable, only injections documented in records were used to determine the number of prior anti-VEGF injections. Response to prior anti-VEGF therapy was graded as good, moderate, or poor depending on whether all intraretinal fluid could be eliminated by monthly injections or how frequently anti-VEGF injections had to be given to maintain a dry macula. Qualitative measurement of nonperfusion at baseline were made using ultra-widefield fluorescein angiography (Optos 200Tx, Optos, Dunfermline, Scotland, UK) done at or prior to the baseline visit. At baseline and at all subsequent visits, subjects had measurement of best-corrected visual acuity (BCVA) using the Early Treatment Diabetic Retinopathy Study (ETDRS) protocol, ophthalmologic examination including measurement of intraocular pressure, spectral-domain optical coherence tomography (SD OCT) using the Spectralis machine (Heidelberg Engineering, Inc, Carlsbad, California, USA), and an anterior chamber tap. Aqueous samples were stored at −80 C. Patients were given an intraocular injection of a dexamethasone implant in the study eye at baseline. Povidone-iodine was used to clean the conjunctiva and 2% lidocaine was injected under the conjunctiva. The 22 gauge needle of the injector was inserted through the pars plana and the dexamethasone implant was injected into the vitreous cavity.

• **FUNCTIONAL AND ANATOMIC OUTCOMES:** The major functional outcome measure was change from baseline BCVA in ETDRS letter score at weeks 4, 8, 12, and 16. One anatomic outcome was the change from baseline center subfield thickness at weeks 4, 8, 12, and 16. Excess foveal thickness (EFT) was calculated for each subject by subtracting the minimum foveal thickness during the course of the disease (edema-free thickness, which in each case was <320 μm) from the foveal thickness at baseline and at weeks 4, 8, 12, and 16. Percent change in EFT at each time point was calculated using the following formula: % change in EFT at a time point = (EFT at baseline–EFT at time point)/EFT at baseline. In addition, qualitative changes in intraretinal fluid were graded by side-to-side comparisons of baseline, week 4, and week 16 SD OCT scans.

• **VASOACTIVE PROTEIN ARRAYS:** The levels of vasoactive proteins in aqueous at baseline and at week 4 were measured in 11 eyes with BRVO and 11 eyes with CRVO at IMGENEX Corporation (San Diego, California, USA) using the Human Angiogenesis Antibody Array Kit (catalog number ARY007; R&D Systems, Inc, Minneapolis, Minnesota, USA). Samples obtained at week 16 were also included for 3 eyes with BRVO and 1 with CRVO. Aqueous samples (115 μL) were blotted on the membrane, which was stored for 16 hours at 4 C and further processed according to the manufacturer's instructions. Each array membrane was exposed to x-ray film for short-term and long-term exposures. The positive signals detected on the developed x-ray film were quantitated using TotalLab Quant software (Gentel Biosciences, Inc, Madison, Wisconsin, USA). Results are reported as percent change from baseline.

• **ENZYME-LINKED IMMUNOSORBENT ASSAYS:** Protein arrays indicated a reduction in aqueous levels of hepatocyte growth factor in several patients between baseline and week 4 and therefore enzyme-linked immunosorbent assay (ELISA) was used to measure aqueous levels of hepatocyte growth factor and VEGF in another cohort of RVO patients at baseline, week 4, and week 16 (when week 16

samples were available). Levels of hepatocyte growth factor and VEGF were measured in aqueous samples using ELISA kits for each (Abcam, Cambridge, Massachusetts, USA) using the manufacturer's instructions. Briefly, 100 μL of each aqueous sample diluted 1:1 in dilution buffer or 100 μL of hepatocyte growth factor or VEGF protein standard was added to a well of a 96-well plate and incubated at 4 C overnight. After wells were washed 4 times, 100 μL of biotinylated anti–hepatocyte growth factor or anti-VEGF antibody was added to each well and incubated for 1 hour at room temperature. After 4 washes, 100 μL of horseradish peroxidase–streptavidin solution was added to each well and incubated for 45 minutes at room temperature. After wells were washed 4 times, 100 μL of 3,3′–5,5′ tetramethylbenzidine substrate reagent was added to each well and after 30 minutes the reaction was stopped by adding 50 μL of stop solution. Absorbance at 450 nm was measured on a plate reader. The readings from the standards were used to generate standard curves of absorbance vs hepatocyte growth factor or VEGF. The hepatocyte growth factor and VEGF concentration in each sample was calculated by plotting absorbance on the respective standard curve.

• **CORRELATION OF CHANGES IN VASOACTIVE PROTEINS WITH CHANGES IN EDEMA:** The diversity of protein levels and center subfield thickness were calculated as percentage change in their measurements at week 4 relative to baseline:

$$FC_{Protein} = (Protein_B - Protein_{W4})/Protein_B$$

$$FC_{CST} = (CST_B - CST_{W4})/CST_B$$

where $FC_{Protein}$ and FC_{CST} are the changes in protein level and center subfield thickness, and E_B, E_{W4}, CST_B, and CST_{W4} represent the protein level and center subfield thickness at baseline and week 4, respectively. In order to estimate the correlation between percent reduction in protein level and percent reduction in excess foveal thickness, the Pearson correlation coefficient was calculated using R.

RESULTS

• **DEMOGRAPHICS AND BASELINE CHARACTERISTICS:** The ORVO trial enrolled 40 subjects with macular edema, 17 with BRVO and 23 with CRVO. Most subjects had long-standing macular edema with a median duration of 40 months for patients with BRVO and 45 months for patients with CRVO, but there were also a few patients who had a relatively short duration of disease (Table 1). All subjects had previously been treated with anti-VEGF injections, with a median of 13.9 (BRVO, n = 14) or 18.9 (CRVO, n = 21); only documented injections were counted, not those based on patient history, and therefore these means are minimums and actual means may be larger. Detailed information regarding prior response to anti-VEGF injections was available for 12 patients with BRVO and 20 patients with CRVO. During periods of monthly or in some cases less frequent injections, there was minimal residual intraretinal fluid in 4 subjects with BRVO and 11 subjects with CRVO, while in 8 subjects with BRVO and 9 with CRVO there was substantial residual intraretinal fluid even during periods of monthly injections. The median BCVA at baseline in ETDRS letter score (Snellen equivalent) was 60 (20/63) in eyes with

TABLE 1. Patient Demographics and Baseline Characteristics for Patients With Retinal Vein Occlusion who Received a Dexamethasone Implant

Variable	BRVO (N = 17)	CRVO (N = 23)
Age (y), median (range)	72 (54–89)	74 (54–90)
Sex, female, n (%)	5 (29.4%)	11 (47.8%)
Median disease duration (mo)	40	45
Retinal nonperfusion based on wide-angle FA, n (%)		
Mild	9 (52.9)	12 (52.2)
Moderate	4 (23.5)	4 (17.4)
Severe	2 (11.8)	4 (17.4)
No gradable FA	2 (11.8)	3 (13.0)
Prior anti-VEGF injections,[a] mean (range)	13.9 (1–36)	18.9 (2–41)
Response to anti-VEGF injections		
Good[b]	4	11
Poor[c]	8	9
Indeterminate[d]	5	3
Prior intraocular steroids, n (%)	5 (29.4%)	1 (4.3%)
Grid laser (%)	5 (29.4)	8 (34.8)
Scatter laser photocoagulation (%)	4 (23.5)	13 (56.5)
Baseline BCVA (letter score)		
Median (range)	60 (29–71)	54 (19–76)
Baseline CST (μm)		
Median (range)	453 (225–792)	539 (251–941)
Baseline intraretinal fluid		
Mild	4	1
Moderate	4	9
Severe	9	13

BCVA = best-corrected visual acuity; BRVO = branch retinal vein occlusion; CRVO = central retinal vein occlusion; CST = central subfield thickness; FA = fluorescein angiogram; VEGF = vascular endothelial growth factor.

[a]Based on verified observed data and electronic patient records for 14 BRVO and 21 CRVO patients.

[b]Good response = elimination of all or most intraretinal fluid during periods of monthly or less frequent injections.

[c]Poor response = substantial recurrent/residual intraretinal fluid even during periods of monthly injections.

[d]Indeterminate = unable to determine from available data.

BRVO and 54 (20/80) in eyes with CRVO. The median central subfield thickness at baseline was 453 μm in eyes with BRVO and 539 μm in eyes with CRVO, but there were differences among patients with thickening and intraretinal fluid ranging from mild to severe.

• **PATIENT DISPOSITION:** Three patients exited the study early. Subject B7 fell and suffered trauma after the week 4 visit and could not continue, Subject B11 withdrew consent after the week 12 visit, and Subject C14 was lost to follow-up after the week 4 visit. This had little impact on the study because the primary outcome was based on the correlation between change in central subfield thickness and aqueous vasoactive protein levels between baseline and week 4.

• **ANATOMIC AND VISUAL OUTCOMES:** In this population of patients with chronic/recurrent macular edema due to BRVO or CRVO, there were substantial reductions from baseline mean central subfield thickness at weeks 4 and 8, with increases at week 12 (Figure 1, Top). This was accompanied by improvements in BCVA at weeks 4 and 8 after injection of a dexamethasone implant with reductions at week 12 and some reversal at week 16 owing to rescue injections (Figure 1, Bottom).

In addition to the mean changes in the subject population, it is important to examine changes in individual subjects considering the differences among patients in duration of disease, severity of edema at baseline, amount of retinal nonperfusion at baseline, and number of prior injections of a VEGF antagonist (Supplemental Tables 1 and 2, available at AJO.com). To assess differences among patients with regard to severity of edema at baseline and response to dexamethasone implant injection, we examined SD OCT horizontal cross sections through the fovea, BCVA, and center subfield thickness at each study visit (Supplemental Figures 1 and 2, available at AJO.com). However, a horizontal cross section may not provide a good indicator of edema in all patients, because in some, intraretinal fluid was slightly above or below the horizontal meridian (Supplemental Table 2, B7). While the anatomic responses were generally very good and in many cases impressive, improvements in BCVA were more variable, probably owing to macular damage from chronic/recurrent edema or ischemic damage, as suggested by macular thinness and/or irregularity after edema reduction in many patients.

• **CHANGES IN AQUEOUS LEVELS OF VASOACTIVE PROTEINS AFTER INJECTION OF DEXAMETHASONE IMPLANT:** Aqueous samples obtained at baseline and 4 weeks after injection of a dexamethasone implant for 11 subjects with BRVO and 11 subjects with CRVO were run on a vasoactive protein array. Table 2 shows the proteins on the array that were detected in at least some of the aqueous samples of some patients and those that were not detected

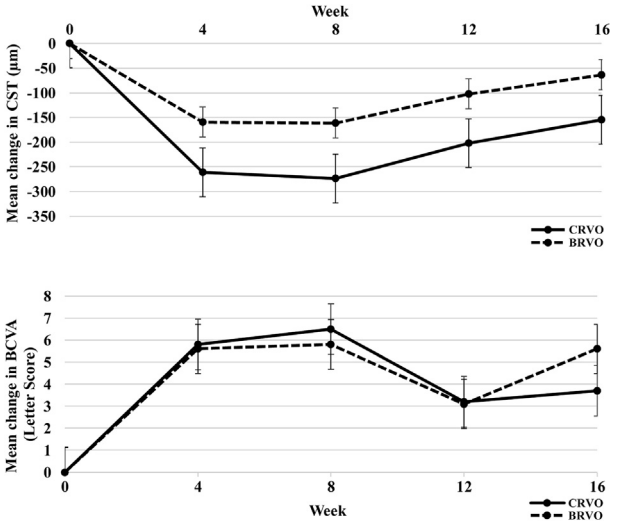

FIGURE 1. Improvement in (Top) central subfield thickness and (Bottom) best-corrected visual acuity after injection of dexamethasone implant in patients with macular edema due to retinal vein occlusion. Patients with macular edema due to central retinal vein occlusion (CRVO) or branch retinal vein occlusion (BRVO) were given an intravitreous injection of a dexamethasone implant at baseline. Mean (±standard error of the mean) change from baseline central subfield thickness (CST, Top) and mean (±standard error of the mean) change from baseline best-corrected visual acuity (BCVA, Bottom) are shown at each study visit.

in any of the samples. The major objective of this study was to determine the relative differences in aqueous vasoactive protein levels between baseline and week 4 and correlate those changes with reduction in edema. To quantitatively assess the correlation between percentage decrease in excess foveal thickness and percentage reduction in each protein between baseline and week 4, percentage decrease in excess foveal thickness was calculated as described in Methods and plotted against percentage change in protein level. The percentage decrease in pentraxin 3 (Pearson correlation coefficient= 0.685, $P = .014$) and persephin (Pearson correlation coefficient = 0.638, $P = .003$) correlated with reduction in excess foveal thickness (Figure 2), but for all of the other proteins there was not a significant correlation.

Supplemental Tables 3 and 4 (available at AJO.com) show the proteins for which there were changes between baseline and week 4 for BRVO and CRVO subjects, respectively. The proteins are divided into the following categories: (1) reduced ≥70%, (2) reduced ≥50%, (3) reduced ≥30%, (4) reduced 10%–0%, (5) no change (defined as decreased or increased by <10%), (6) increased by ≥10%, or (7) undetected in baseline sample. These data demonstrate that there are a large number of proteins that are reduced in eyes with macular edema after injection of a dexamethasone implant and there is substantial

TABLE 2. Proteins on Array Used to Test Aqueous of Patients With Macular Edema Due to Retinal Vein Occlusion who Received a Dexamethasone Implant

Proteins Detected	Proteins Consistently Undetected
Activin A	ADAMTS-1
Angiogenin	Amphiregulin
Angiopoietin-1	Coagulation factor III
Angiopoietin-2	EGF
Angiostatin/plasminogen	Endoglin
Artemin	FGF-1
CXCL4	FGF-2
CXCL16	FGF-4
DPP-IV	FGF-7
EG-VEGF	GDNF
Endostatin/collagen XVIII	GM-CSF
Endothelin-1	IL-1β
HB-EGF	IL-8
HGF	MCP-1
IGFBP-1	MIP-1α
IGFBP-2	MMP-8
IGFBP-3	NRG1-β1
Leptin	PDGF-AB/PDGF-BB
MMP-9	Serpin B5
Pentraxin 3	TGF-β1
PD-ECGF	uPA
PDGF-AA	Vasohibin
Persephin	VEGF-C
PlGF	
Prolactin	
Serpin E1	
Serpin F1	
TIMP-1	
TIMP-4	
Thrombospondin-1	
Thrombospondin-2	
VEGF-A	

ADAMTS-1 = A disintegrin and metalloproteinase with thrombospondin motif protein-1; CXCL4 = C-X-C motif ligand 4; CXCL16 = C-X-C motif ligand 16; DPP-IV = dipeptidyl peptidase-4; EGF = epidermal growth factor; EG-VEGF = endocrine gland vascular endothelial growth factor; FGF = fibroblast growth factor; GDNF = glial cell line–derived neurotrophic factor; GM-CSF = granulocyte macrophage colony-stimulating factor; HB-EGF = heparin-binding epidermal growth factor; HGF = hepatocyte growth factor; IGFBP = insulin-like growth factor binding protein; IL = interleukin; MCP-1 = monocyte chemoattractant protein-1; MIP-1α = macrophage inhibitory protein-1α; MMP = matrix metalloproteinase; NRG1 = neuroregulin 1; PD-ECGF = platelet-derived endothelial cell growth factor; PDGF = platelet-derived growth factor; PlGF = placental growth factor; TGF-β1 = transforming growth factor-β1; TIMP = tissue inhibitor of metalloproteinases; uPA = urokinase; VEGF = vascular endothelial growth factor.

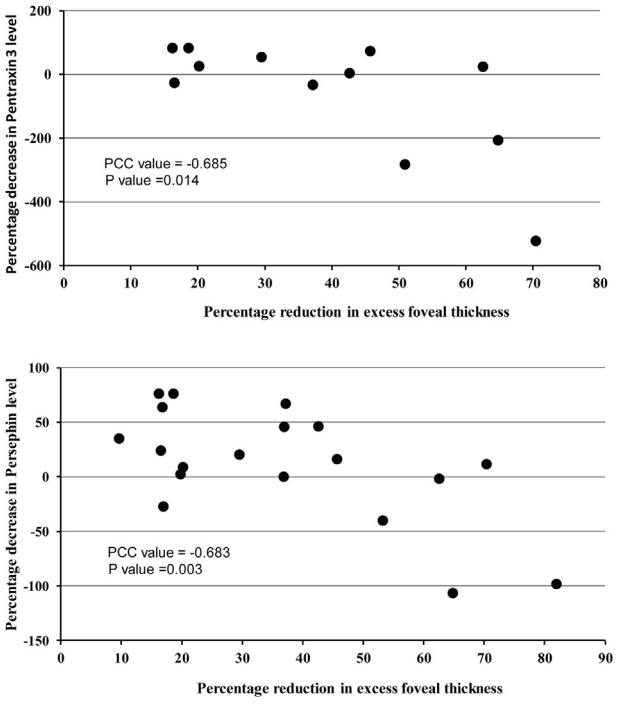

FIGURE 2. Correlation between reduction from baseline and week 4 in aqueous level of (Top) pentraxin 3 or (Bottom) persephin and reduction from baseline in excess foveal thickness after dexamethasone implant in patients with retinal vein occlusion. Patients who had detectable levels of pentraxin 3 (Top) or persephin (Bottom) at baseline and week 4 had percentage reduction in protein level plotted vs percentage reduction in excess foveal thickness. Pearson correlation coefficient was 0.685 for pentraxin 3 ($P = .014$) and 0.638 for persephin ($P = .003$).

heterogeneity among patients. We selected a 30% reduction as the threshold of a clinically meaningful reduction that potentially could contribute to reduction in edema. The median number of proteins that were decreased ≥30% between baseline and week 4 was 7 (range 0–21) in eyes with BRVO and 9 (range 3–17) in eyes with CRVO (Supplemental Tables 3 and 4, available at AJO.com).

Proteins that were reduced by ≥30% in the majority of the 22 dexamethasone implant–injected eyes with RVO were hepatocyte growth factor; endocrine gland VEGF; insulin-like growth factor binding proteins 1, 2, and 3; and endostatin (Table 3). Considering BRVO (Table 3, Top) and CRVO (Table 3, Bottom) separately, at least 4 of each showed ≥30% reductions in hepatocyte growth factor; endocrine gland VEGF; insulin-like growth factor binding proteins 1, 2, and 3; activin-A; and endostatin.

The levels of hepatocyte growth factor and VEGF at baseline, week 4, and (when available) week 16 were measured in aqueous samples from the 6 remaining eyes with BRVO and the 12 remaining eyes with CRVO by ELISA. Levels of hepatocyte growth factor were greater than those of VEGF in all eyes, but they were in the same range, with a mean of 145.9 pg/mL in eyes with CRVO and 212.8 pg/mL in eyes with BRVO for hepatocyte

TABLE 3. Aqueous Proteins Reduced by ≥30% Between Baseline and Week 4 in At Least 30% of Patients With Retinal Vein Occlusion Treated With Dexamethasone Implant

Number of Patients With ≥30% Reduction (N = 11)	Proteins
BRVO	
8	Persephin
7	IGFBP-2
	IGFBP-3
6	Activin-A
	Endostatin
5	HGF
4	EG-VEGF
	IGFBP-1
CRVO	
7	HGF
	EG-VEGF
	IGFBP-1
	Endostatin
6	IGFBP-2
	IGFBP-3
	CXCL16
	MMP-9
5	HB-EGF
	Thrombospondin-1
4	Activin-A
	DPP-IV
	Angiopoietin-1
	PDGF-AA

BRVO = branch retinal vein occlusion; CRVO = central retinal vein occlusion; CXCL16 = C-X-C motif ligand 16; DPP-IV = dipeptidyl peptidase-4; EG-VEGF = endocrine gland vascular endothelial growth factor; HB-EGF = heparin-binding epidermal growth factor; HGF = hepatocyte growth factor; IGFBP = insulin-like growth factor binding protein; MMP = matrix metalloproteinase; PDGF = platelet-derived growth factor.

growth factor vs 86.6 pg/mL and 63.0 pg/mL for VEGF. There were 20%–64% reductions in hepatocyte growth factor in association with reduced macular edema in 5 of 6 patients with BRVO, and in 2 patients for whom samples were available at week 16 there was an increase in hepatocyte growth factor between weeks 4 and 16 associated with recurrent edema (Supplemental Table 5, available at AJO.com). Of the 5 eyes with BRVO that showed a reduction in hepatocyte growth factor, 4 showed 18%–74% concomitant decreases in VEGF. The 1 BRVO eye that showed an 187% increase in hepatocyte growth factor between baseline and week 4 showed a 37% reduction in VEGF. Of the 12 eyes with CRVO, 9 showed complete or nearly complete resolution of severe or moderate macular edema and 3 showed modest reductions in edema between baseline and week 4. Hepatocyte growth factor was reduced in 7, unchanged in 2, and increased in 3, while VEGF was reduced in 5, unchanged in 2, increased in 4, and not tested in 1 owing to insufficient sample (Supplemental Table 6, available at AJO.com). All of the eyes showed a reduction in hepatocyte growth factor or VEGF except 1 eye in which both were increased; this eye had minimal reduction in central subfield thickness, which was 539 μm at baseline and 435μm at week 4.

• **EFFECTS OF DEXAMETHASONE IMPLANTS ON INTRAOCULAR PRESSURE:** Three of the 40 patients (7.5%) had an IOP above 30 during the trial and 2 of those patients had the increase in IOP after a second rescue injection of a dexamethasone implant. Seven patients (17.5%) required an IOP-lowering drop and in 2 it was administered after a second dexamethasone implant injection.

DISCUSSION

MOST PATIENTS WITH BRVO OR CRVO RECEIVE SUBSTANTIAL benefits from injections of a VEGF-neutralizing protein, but prolonged treatment is often needed. In some patients, monthly injections of an anti-VEGF neutralizing protein do not eliminate edema, suggesting that in these patients VEGF is not completely neutralized or other pro-permeability factors are contributing to edema. Only 50% of patients with BRVO and 44% of patients with CRVO have resolution of edema with no need for further injections after 4 years of anti-VEGF injections.[9] In many of these patients, anti-VEGF injections become less effective at reducing intraretinal fluid over time, suggesting that while VEGF was the predominant cause of leakage initially, other vasoactive factors may contribute to chronic/recurrent edema. Many studies have reported elevated levels of a single or a few vasoactive proteins in eyes with macular edema due to RVO, but the mere presence of a protein does not prove that it contributes to edema, particularly in an eye in which injection of a specific VEGF antagonist is able to eliminate all edema.

In this study, we used a different strategy. First, we focused on patients with chronic/recurrent macular edema secondary to RVO. Some of these patients had elimination of most intraretinal fluid during periods of monthly injections (or in some patients, less frequent injections) of a VEGF antagonist; however, some patients chose to reduce visit frequency and tolerate bouts of recurrent edema. Eight patients with BRVO and 9 with CRVO had substantial residual intraretinal or subretinal fluid even during periods of monthly injections. The second aspect of the strategy was to measure levels of 55 vasoactive proteins before and after injection of a dexamethasone implant and correlate improvements in edema with reductions in aqueous proteins.

The first important observation is that injection of a dexamethasone implant resulted in a marked reduction in intraretinal fluid in most patients with chronic/recurrent edema due to RVO, but there were some differences among

patients, with most having no or minimal residual intraretinal fluid at week 4, while others had substantial residual fluid. In general, benefits were maintained for 8 weeks in almost all patients, 12 weeks in some patients, and 16 weeks in only a few patients. A second important observation is that there is substantial heterogeneity with regard to measureable levels of vasoactive proteins in the aqueous of patients with chronic/recurrent macular edema due to RVO and the manner in which those proteins change in association with reduction in edema. The median number of proteins reduced by ≥30% between baseline and week 4 in 11 eyes with BRVO was 7, but there was substantial variability, with a range of 0–21. In 2 eyes with BRVO in which severe edema was substantially reduced after injection of a dexamethasone implant, none of the 55 proteins on the array were reduced by ≥30%, while in another eye in which edema was reduced from moderate at baseline to mild at week 4 there was a reduction ≥30% in 21 proteins. In 11 eyes with CRVO the number of proteins reduced by ≥30% in association with reduced edema ranged from 3 to 17 with a median of 9. These data indicate that there is not one single factor in addition to VEGF that is a major contributor to macular edema in all patients with RVO, but instead there are likely to be different contributors in different patients. Despite the high degree of heterogeneity, there were 2 factors, persephin and pentraxin-3, for which there was a modest (0.64, 0.68) statistically significant correlation between percentage reduction in protein level and percentage reduction in edema. Persephin belongs to the glial cell line–derived neurotrophic factor family of ligands, a subgroup of the transforming growth factor β superfamily.[14] Binding of persephin to its receptor results in Ret kinase activation.[15] Ret kinase mutations promote papillary thyroid carcinoma and multiple endocrine neoplasia types 2A and 2B.[16] Persephin knockout mice are hypersensitive to cerebral ischemia and have a 3-fold increase in infarct volume compared to wild-type mice after occlusion of the middle cerebral artery.[17] The role of persephin in the retina is uncertain and therefore it is not known if the dexamethasone implant–induced reduction in persephin that correlates with reduction in edema has any physiologic or pathologic effect. Pentraxin-3 is a member of the pentraxin family of fluid phase pattern recognition molecules that is induced in many cell types, including endothelial cells and leukocytes, by the inflammatory cytokines interleukin-1β and tumor necrosis factor-α.[18,19] Serum levels of pentraxin 3 provide a biomarker of inflammation particularly associated with vascular injury.[20,21] Several studies have suggested that an increase in serum pentraxin 3 levels in patients with coronary artery disease suggests vulnerable plaque and is a negative prognostic sign in patients with acute myocardial infarction.[22,23] Previous studies have measured increased levels of pentraxin 3 in the vitreous of patients with BRVO[24] or CRVO.[25] Steroids have been shown to reduce leukocyte-derived pentraxin 3 and increase fibroblast-derived pentraxin 3,[26] and thus the reduction of pentraxin 3 after injection of a dexamethasone implant in eyes with RVO may indicate reduced inflammatory cells and/or their activity.

Understanding that RVO patient heterogeneity regarding ocular levels of vasoactive proteins and their correlation with macular edema prevents definitive conclusions as to which other proteins contribute to edema, it is still useful to examine which proteins with known pro-permeability activity most consistently showed reductions of ≥30% to provide candidates for future studies. Those pro-permeability factors that correlate with changes in macular edema in the largest percentage of patients with RVO are hepatocyte growth factor, endocrine gland VEGF, and activin-A. The protein array findings for hepatocyte growth factor were confirmed by ELISA, increasing confidence in their validity. Hepatocyte growth factor is proangiogenic,[27] increases permeability through endothelial cell monolayers,[28] and causes vascular leakage in the eye.[29] Patients with proliferative diabetic retinopathy have increased levels of HGF in the vitreous.[30] Under normal conditions, endocrine gland VEGF is expressed only in endocrine glands,[31,32] so its presence in eyes with macular edema due to RVO is a bit surprising, but perhaps it should not be, because aberrant expression is a common feature of diseased tissue. Endocrine gland VEGF is not structurally related to VEGF and binds to G protein–coupled receptors rather than tyrosine kinase receptors. Under normal circumstances expression of the receptor may be limited to endothelial cells of endocrine glands contributing to tissue specificity, because injection of adenoviral vectors that increase expression of endocrine gland VEGF has no effect in skin or skeletal muscle but induces angiogenesis in the ovary[31]; therefore, in order for the increased levels of endocrine gland VEGF in some eyes with RVO to have biologic significance, there must also be aberrant expression of 1 of the 2 endocrine gland VEGF receptors on diseased retinal endothelial cells. If there is, then steroid-induced reduction of endocrine gland VEGF could contribute to the anti-permeability effects of dexamethasone implants, because stimulation of endocrine gland VEGF receptors promotes fenestrae and leakage. Activin-A is a glycoprotein that is a member of the transforming growth factor-β superfamily.[33] It forms dimers that bind to activin receptors on endothelial cells and stimulates tube formation in vitro.[34] Activin A stimulates expression of VEGF and promotes corneal neovascularization.[35] Thus it is reasonable to postulate that its reduction after dexamethasone implant injection may contribute to edema reduction.

Our discussion has been limited to proteins that showed changes that correlated with edema in a substantial number of patients, but there were many proteins that showed correlations in a few patients. It is expected that many of these are due to chance, but we cannot rule out the possibility that some of these proteins should also receive

consideration and further testing. Protein changes detected in all patients are shown in Supplemental Tables 5 and 6 (available at AJO.com) so that other investigators can give this matter consideration. It should also be noted that there are vasoactive proteins that were not included on the protein array and it is possible that some of those deserve consideration.

In summary, our data suggest that patients with chronic/recurrent edema due to RVO differ with respect to the number and identity of vasoactive proteins detectable in the aqueous. This suggests that there may be changes in the disease process over time that could influence therapeutic response. Despite this heterogeneity, intraocular injections of dexamethasone implants are generally quite effective in most patients with chronic/recurrent edema due to RVO, causing substantial reduction in edema for about 2–3 months. The reduction in edema is associated with decreases in multiple factors, many of which increase as edema recurs. While we cannot say for certain which of these factors contribute to edema, it is likely that multiple proteins contribute and the contributors differ among patients. This indicates that dexamethasone implants provide a useful multitargeted approach. Regardless of the benefit provided by dexamethasone implants in the majority of patients, knowing which of these factors contribute to edema would be useful to design more specific combination treatments, since not all patients can tolerate prolonged use of intraocular steroids because of increased intraocular pressure. The most compelling candidates are hepatocyte growth factor, endocrine gland VEGF, and activin-A. To test the hypothesis that these proteins contribute to edema, it will be necessary to determine the effect of specific antagonists for these proteins on edema in a similar patient population.

ALL AUTHORS HAVE COMPLETED AND SUBMITTED THE ICMJE FORM FOR DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST. Financial Disclosures: Peter A. Campochiaro: Consultant, Genentech, South San Francisco, California; Regeneron, Tarrytown, New York; Aerpio, Cincinnati, Ohio; Alimera, Atlanta, Georgia; Allegro, San Diego, California; Eleven, Cambridge, Massachusetts; Kala, Cambridge, Massachusetts; Applied Genetic Technologies Corporation, Gainesville, Florida; Ocata Therapeutics, Marlborough, Massachusetts; and AsclepiX, Baltimore, Maryland; Institutional Grants: Allergan, Irvine, California; Genentech, South San Francisco, California; Regeneron, Tarrytown, New York; Aerpio, Cincinnati, Ohio, Abbvie, North Chicago, Illinois; Genzyme, Cambridge, Massachusetts; Oxford Biomedica, Oxford, United Kingdom; Roche, Basel, Switzerland; GlaxoSmithKline, Middlesex, United Kingdom; AscepiX, Baltimore, Maryland, Graybug, Baltimore, Maryland. Howard S. Ying: Institutional Grant, Regeneron, Tarrytown, New York. Adam Wenick: Consultant, Bristol-Myers Squibb, New York, New York; Institutional Grant, Novo Nordisk, Bagsvaerd, Denmark. This study was supported by a grant from Allergan (Irvine, California, USA). The authors would like to acknowledge the Wilmer Biostatistics Core Grant EY01765. All authors attest that they meet the current ICMJE requirements to qualify as authors.

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Biosketch

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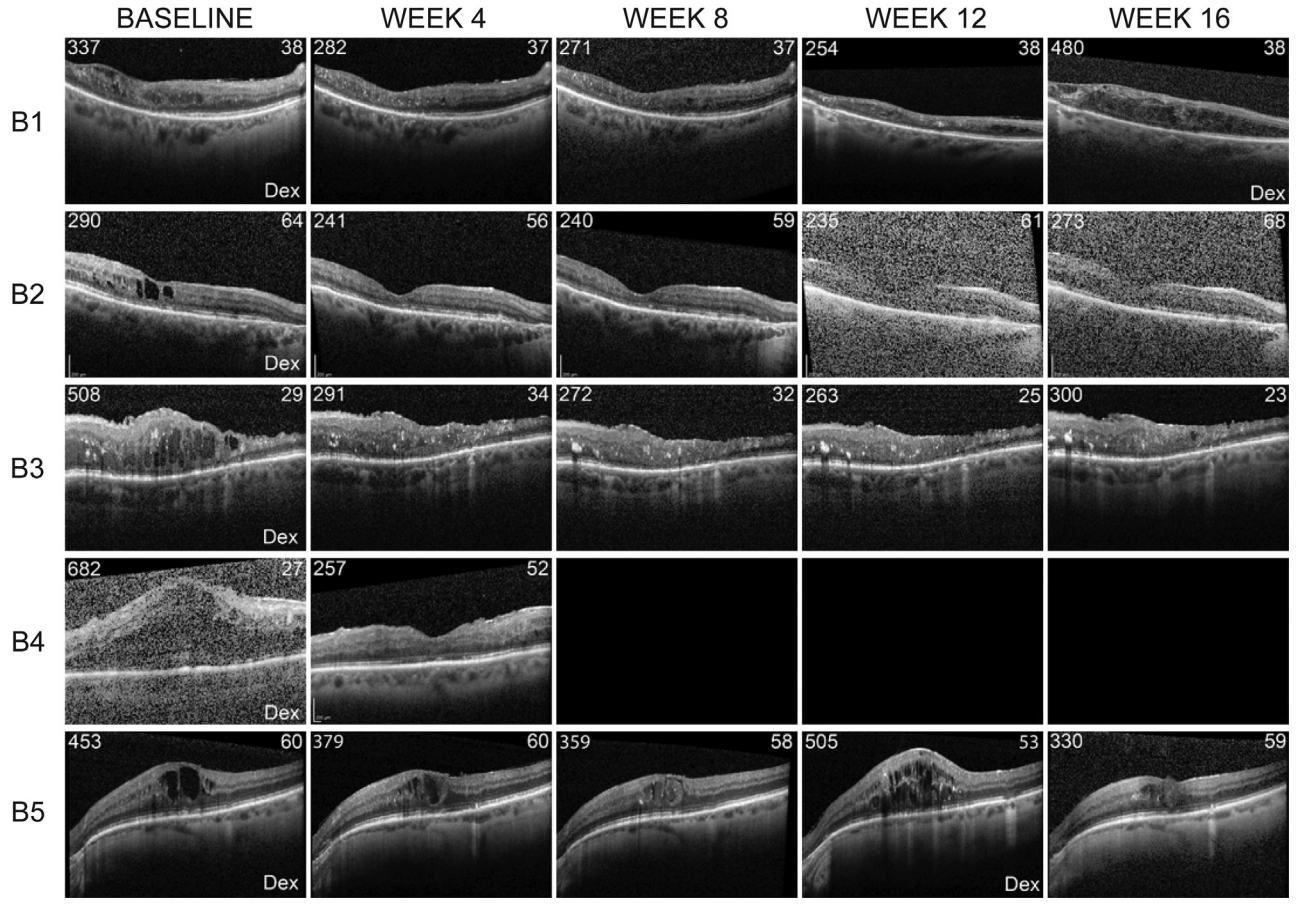
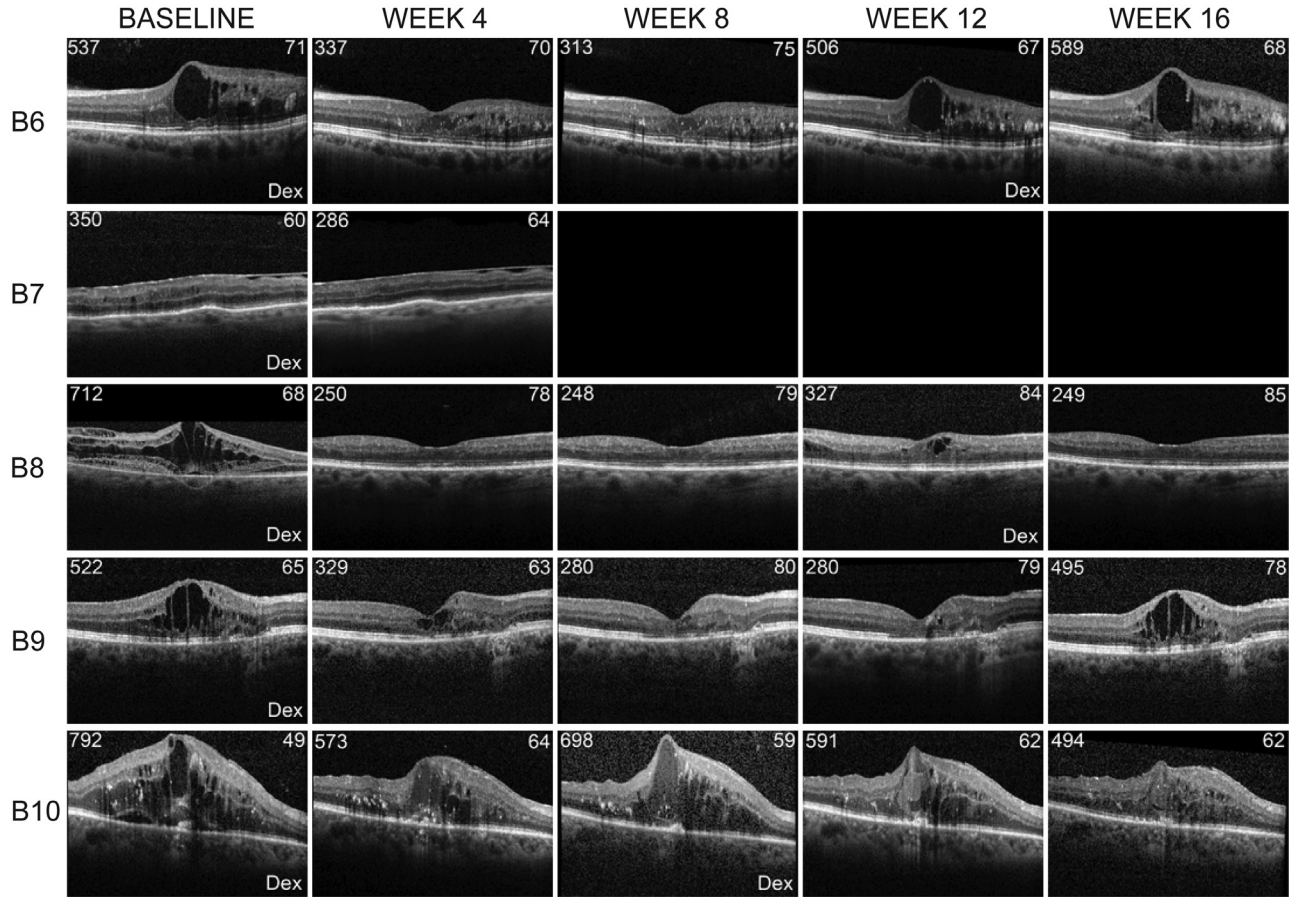
Biosketch

Gulnar Hafiz, MD, MPH, is a research faculty member at the Wilmer Eye Institute of Johns Hopkins School of Medicine, who has expertise in Public Health and interventional clinical trials in retinal diseases. She supervises a large clinical trial team and has participated in development of several new treatments for retinal diseases.



SUPPLEMENTAL FIGURE 1. Foveal horizontal spectral-domain optical coherence tomography scans at each study visit for patients with branch retinal vein occlusion who received a dexamethasone implant. Horizontal scans through the fovea at each study visit are shown for 17 patients with branch vein occlusion (B1-B17), divided into 4 images: (1) B1-B5, (2) B6-B10, (3) B11-B15, and (4) B16-B17. Visits at which a dexamethasone implant was injected are indicated by Dex in the lower right of the box and visits at which an anti–vascular endothelial growth factor (anti-VEGF) injection was given are indicated by anti-VEGF in the lower right of the box. The central subfield thickness is shown in the upper left and the best-corrected visual acuity in Early Treatment Diabetic Retinopathy Study letter score is shown in the upper right of each box. Blank boxes indicate a missed visit.



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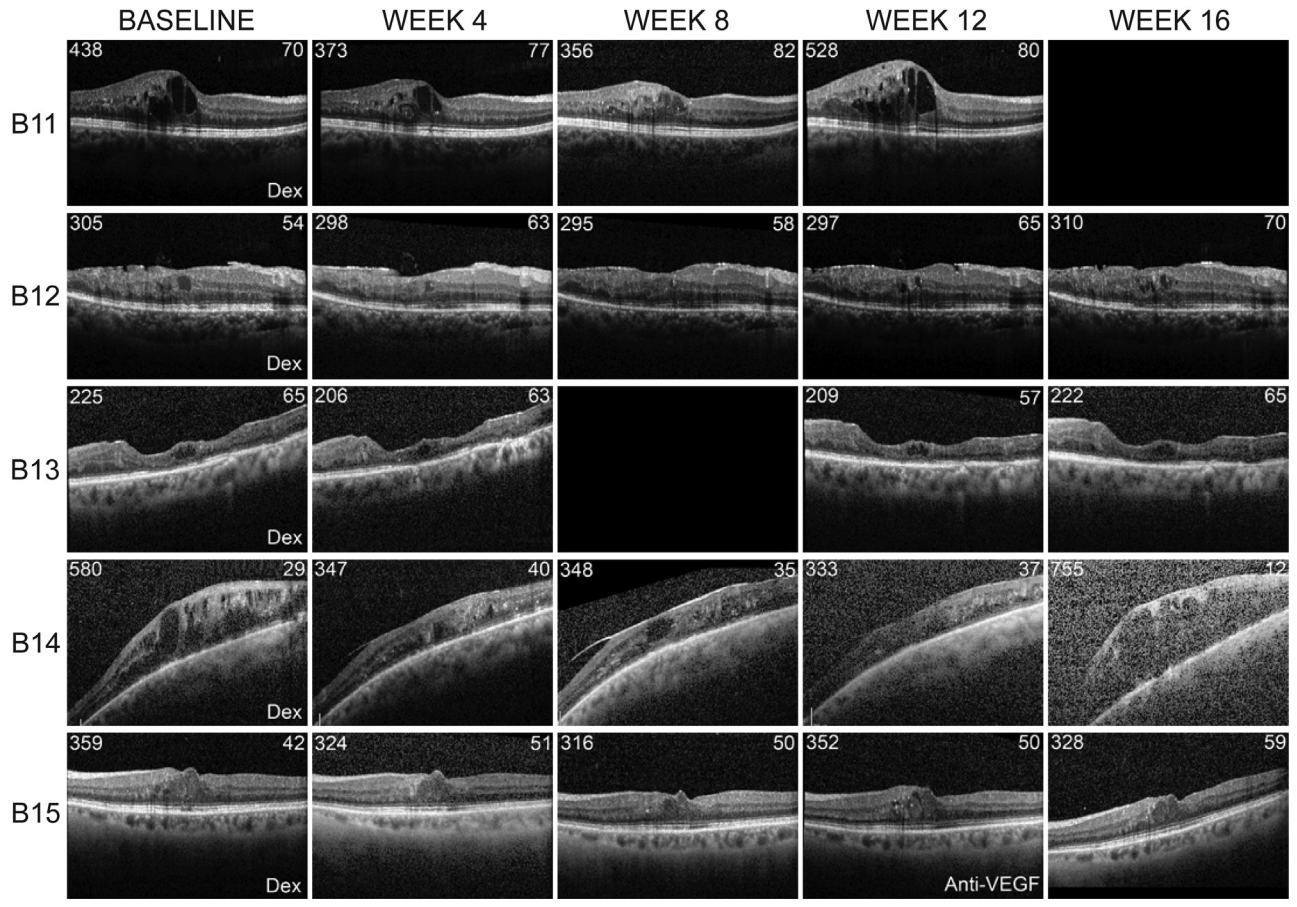

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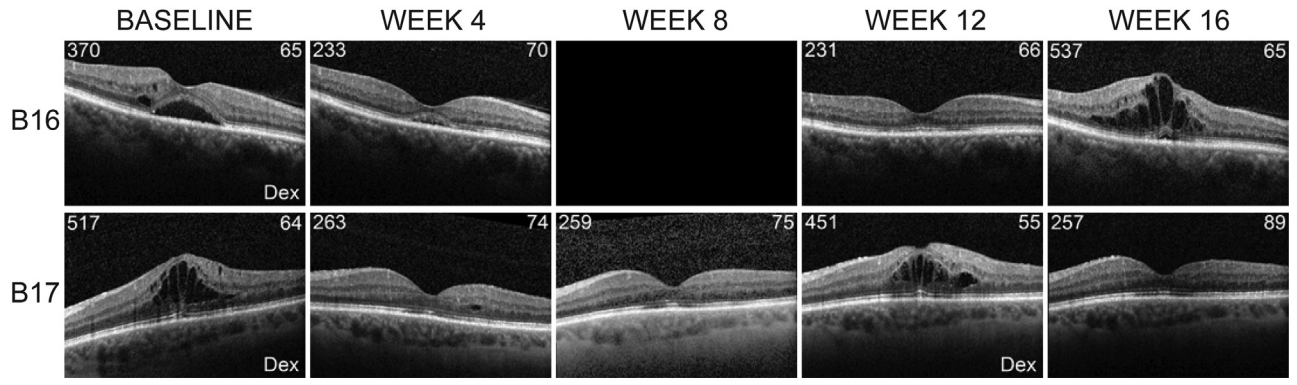

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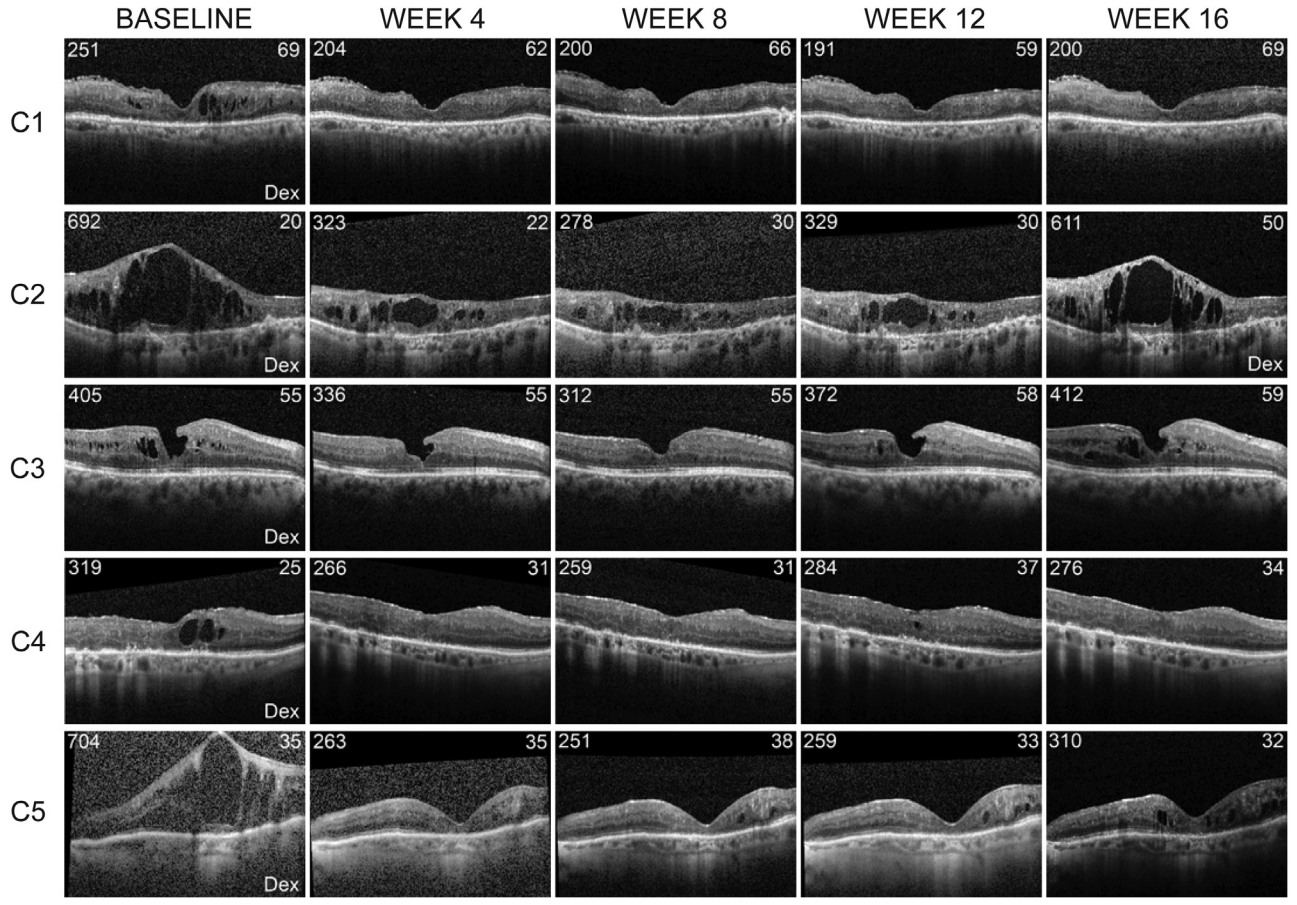
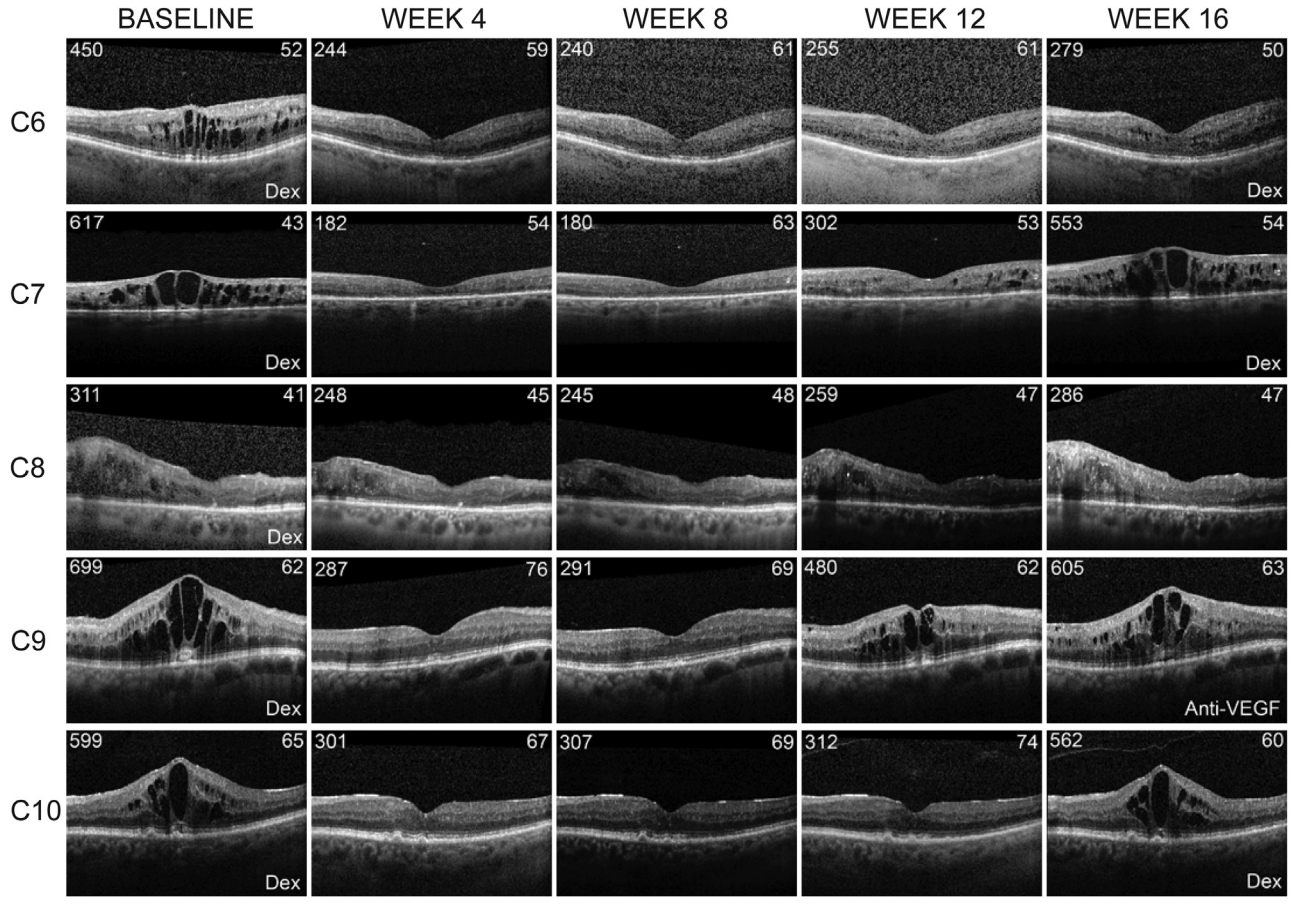


SUPPLEMENTAL FIGURE 2. Foveal horizontal spectral-domain optical coherence tomography scans at each study visit for patients with central retinal vein occlusion who received a dexamethasone implant. Horizontal scans through the fovea at each study visit are shown for 23 patients with central vein occlusion (C1-C23), divided into 5 images: (1) C1-C5, (2) C6-C10, (3) C11-C15, (4) C16-C20, and (5) C21-C23. Visits at which a dexamethasone implant was injected are indicated by Dex in the lower right of the box and visits at which an anti–vascular endothelial growth factor (anti-VEGF) injection was given are indicated by anti-VEGF in the lower right of the box. The central subfield thickness is shown in the upper left and the best-corrected visual acuity in Early Treatment Diabetic Retinopathy Study letter score is shown in the upper right of each box. Blank boxes indicate a missed visit.



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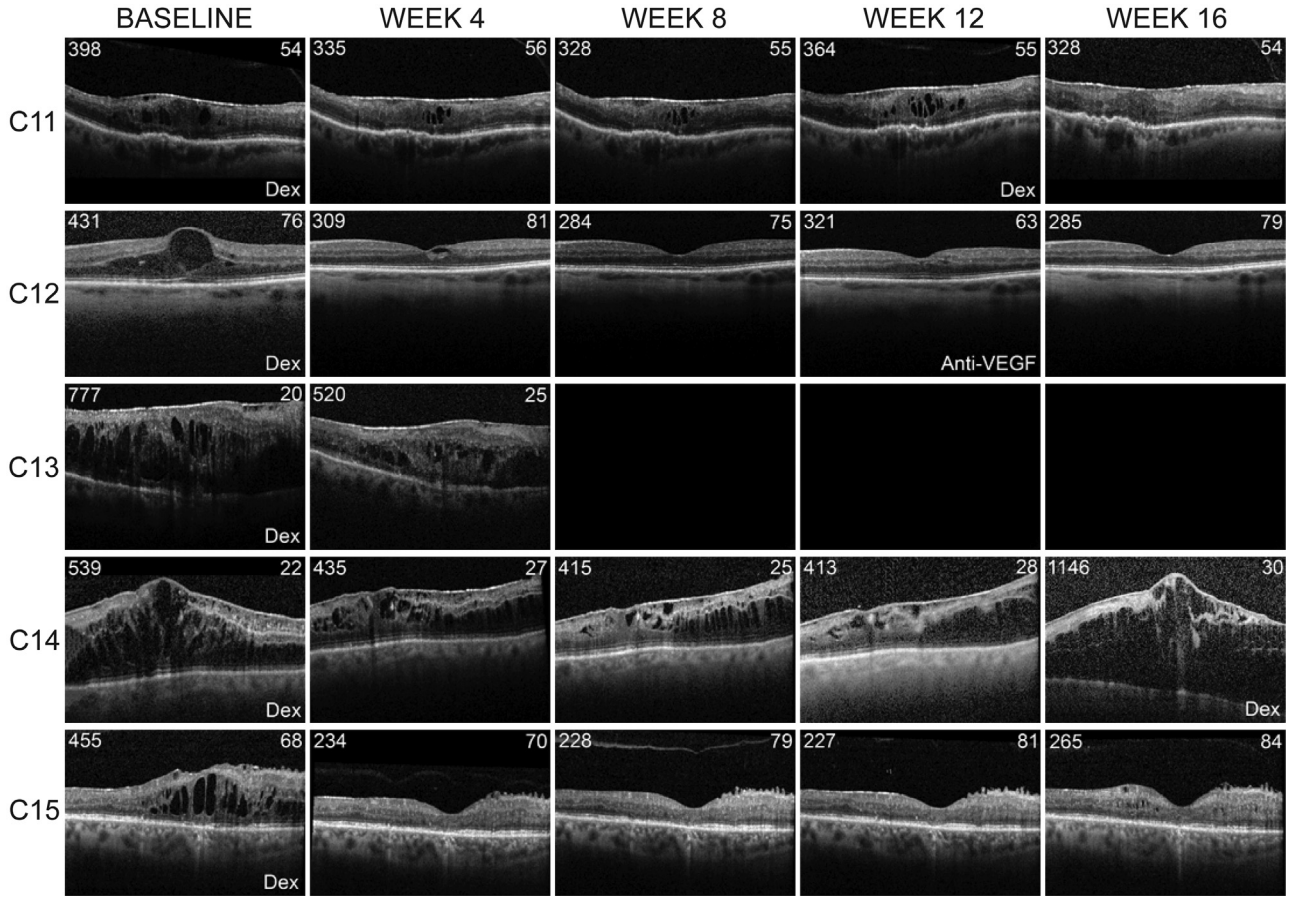

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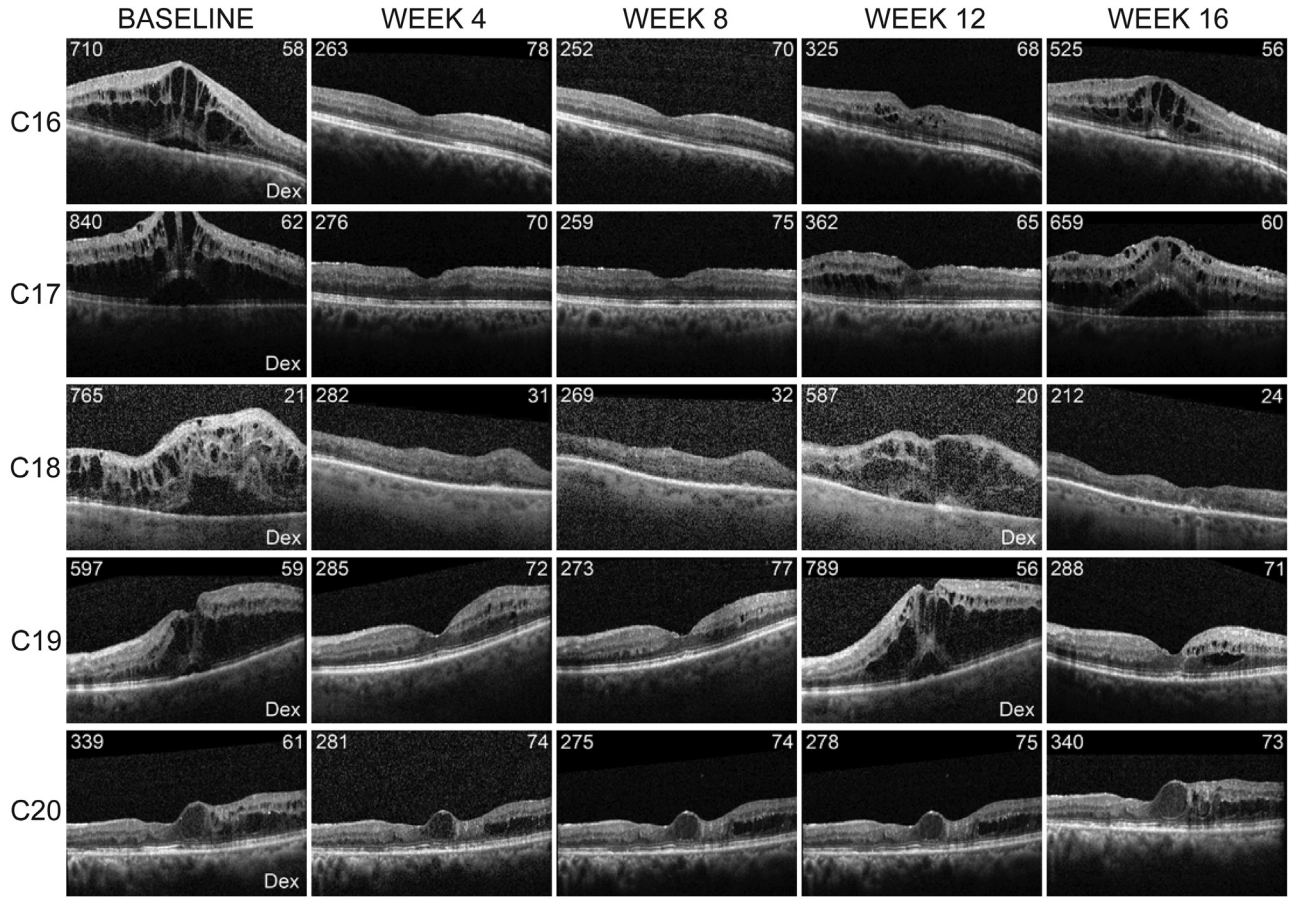

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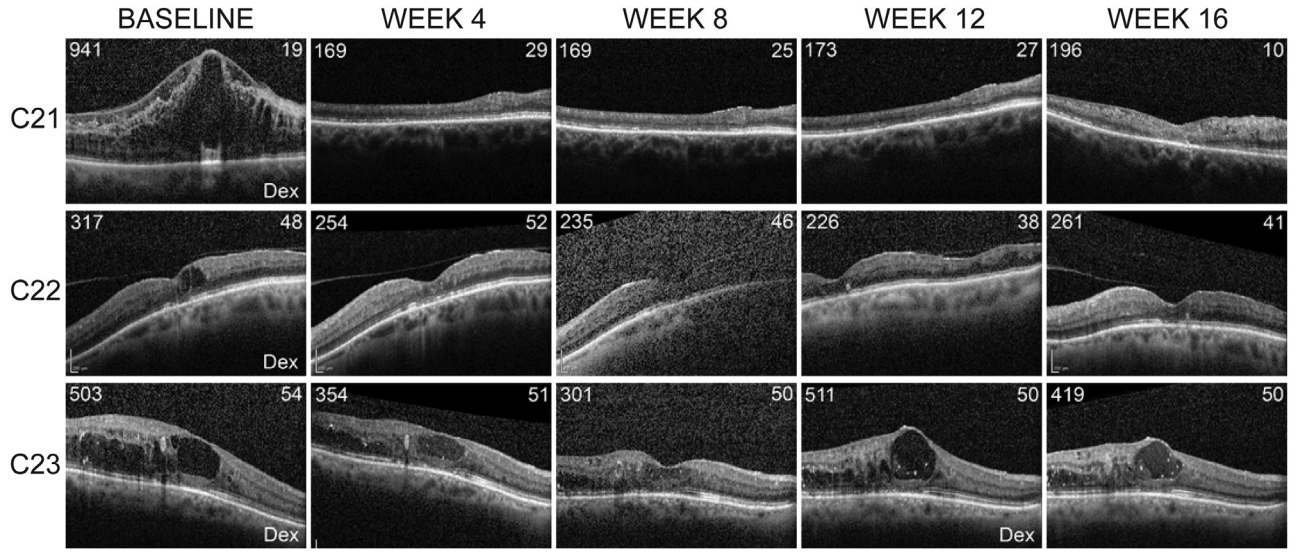

SUPPLEMENTAL FIGURE 2. (*continued*).

SUPPLEMENTAL TABLE 1. Anatomic Outcomes in Eyes With Branch Vein Occlusion After Injection of a Dexamethasone Implant

Patient ID	Age (y)	CST at Baseline (μm)	CST at Week 4 (μm)	Intraretinal Fluid at Baseline	Intraretinal Fluid at Week 4	RNP at Baseline	Edema Duration (mo)	Anti-VEGF Injections	Months Since Last Injection
B1	87	337	282	Moderate	Mild	Mild	87	3[a]	2
B2	89	290	241	Mild	Minimal	Mild	89	5	5
B3	76	508	291	Severe	Mild	Moderate	76	7	9
B4	85	682	257	Severe	Mild	Severe	85	2[a]	6
B5	80	453	379	Moderate	Mild	Mild	80	-[a]	-[a]
B6	54	537	337	Severe	Mild	Mild	54	1	9
B7	72	350	286	Mild	None	Mild	72	28	1
B8	68	712	250	Severe	None	-[b]	68	3	1
B9	59	522	329	Severe	Mild	Mild	59	36	3
B10	66	792	573	Severe	Moderate	Moderate	66	30	3
B11	58	438	373	Severe	Moderate	-[b]	58	26	1
B12	62	305	298	Mild	Minimal	Moderate	62	13	1
B13	73	225	206	Mild	Minimal	Moderate	73	2	8
B14	73	580	347	Severe	Mild	Severe	72	11	1
B15	70	359	324	Moderate	Minimal	Mild	70	25	11
B16	61	370	233	Moderate	Minimal	Mild	61	2	2
B17	74	517	263	Severe	Minimal	Mild	74	6	2

CST = central subfield thickness; ID = identifier; RNP = retinal nonperfusion; VEGF = vascular endothelial growth factor.

[a]Patient received treatment outside Johns Hopkins Hospital, the medical record for which is not available.

[b]No gradable fluorescein angiogram.

SUPPLEMENTAL TABLE 2. Anatomic Outcomes in Eyes With Central Vein Occlusion After Injection of Dexamethasone Implant

Patient ID	Age (y)	CST at Baseline (μm)	CST at Week 4 (μm)	Intraretinal Fluid at Baseline	Intraretinal Fluid at Week 4	RNP at Baseline	Edema Duration (mo)	Anti-VEGF Injections	Months Since Last Injection
C1	72	251	204	Moderate	None	Moderate	140	26	6
C2	84	692	323	Severe	Mild	Severe	82	39	8
C3	59	405	336	Moderate	None	Mild	39	32	5
C4	90	319	266	Mild	None	Mild	94	29	11
C5	81	704	263	Severe	None	Mild	70	14	2
C6	81	450	244	Moderate	None	Mild	78	2	2
C7	54	617	182	Severe	None	Moderate	46	19	3
C8	66	311	248	Moderate	Minimal	Severe	63	41	2
C9	55	699	287	Severe	None	Mild	2	2	1
C10	78	599	301	Severe	None	Mild	90	37	6
C11	68	398	335	Moderate	Mild	Severe	38	30	1
C12	69	431	309	Moderate	None	Mild	37	18	5
C13	83	777	520	Severe	Moderate	Mild	79	11	24
C14	83	539	435	Severe	Moderate	Moderate	10	4	3
C15	47	455	234	Severe	None	Mild	45	35	2
C16	58	710	263	Severe	None	Mild	43	26	2
C17	59	840	276	Severe	None	Mild	10	9	1
C18	87	765	282	Severe	None	-[b]	11	-[a]	4
C19	74	597	285	Moderate	None	Mild	14	9	-[a]
C20	74	339	281	Moderate	Mild	-[b]	54	-[a]	-[a]
C21	85	941	169	Severe	None	Severe	6	2	2
C22	77	317	254	Moderate	None	Moderate	17	10	4
C23	86	503	354	Severe	Mild	-[b]	57	2	2

CST = central subfield thickness; ID = identifier; RNP = retinal nonperfusion; VEGF = vascular endothelial growth factor.

[a]Based on history, received several injections elsewhere but not documented.

[b]No gradable fluorescein angiogram.

SUPPLEMENTAL TABLE 3. Changes in Vasoactive Protein Levels 4 Weeks After Injection of Dexamethasone Implant in 11 Patients With Macular Edema Due to Branch Retinal Vein Occlusion

Subject	Protein Level Change	Protein
Severe Edema, Complete Response		
B3	>70% decrease	Angiopoietin-1, IGFBP-3, PD-ECGF, PDGF-AA
	50%–70% decrease	Endostatin, CXCL-4, thrombospondin-2
	30%–50% decrease	Activin-A, artemin, EG-VEGF, IGFBP-2, persephin
	10%–30% decrease	DPP-IV, HB-EGF, HGF, IGFBP-1, thrombospondin-1, VEGF-A, angiogenin
	No change	Angiostatin, endothelin-1, pentraxin 3, CXCL-16, TIMP-1
	Undetected	Leptin, prolactin
	Increase	Angiopoietin-2, MMP-9, PIGF, serpin E1, serpin F1, TIMP-4
B4	>70% decrease	Activin-A, HB-EGF, HGF, leptin, MMP-9, CXCL-4
	50%–70% decrease	IGFBP-1, VEGF-A
	30%–50% decrease	Endostatin, IGFBP-3, persephin, TIMP-4, IGFBP-2
	10%–30% decrease	Angiogenin, CXCL-16, serpin F1
	No change	DPP-IV, endothelin-1, TIMP-1
	Undetected	Angiostatin, pentraxin 3, PD-ECGF, prolactin, PDGF-AA, thrombospondin-1, thrombospondin-2, angiopoietin-1, PIGF
	Increase	Angiopoietin-2, artemin, EG-VEGF, serpin E1
B8	10%–30% decrease	IGFBP-3, CXCL-16, TIMP-1
	No change	Endostatin, IGFBP-2, VEGF-A, angiogenin
	Undetected	Angiopoietin-1, angiostatin, artemin, DPP-IV, HGF, leptin, PD-ECGF, PIGF, prolactin
	Increase	Activin-A, endothelin-1, IGFBP-1, MMP-9, CXCL-4, serpin E1, angiopoietin-2, EG-VEGF, HB-ECGF, pentraxin 3, PDGF-AA, thrombospondin-1, thrombospondin-2, persephin, serpin F1, TIMP-4
B17	50%–70% decrease	IGFBP-2
	30%–50% decrease	HGF, IGFBP-1, IGFBP-3, persephin, serpin F1
	10%–30% decrease	VEGF-A, CXCL-16
	No change	Endostatin, angiogenin, TIMP-1
	Undetected	Angiostatin, artemin, endothelin-1, HB-EGF, leptin, MMP-9, pentraxin 3, PD-ECGF, PDGF-AA, CXCL-4, PIGF, prolactin, serpin E1, TIMP-4, thrombospondin-1, thrombospondin-2
	Increase	Angiopoietin-1, angiopoietin-2, DPP-IV, EG-VEGF, activin-A
Severe Edema, Partial Response		
B6	50%–70% decrease	IGFBP-2, persephin, CXCL-16
	30%–50% decrease	Activin-A, endostatin, endothelin-1, IGFBP-3
	10%–30% decrease	Artemin, EG-VEGF, IGFBP-1, PDGF-AA, angiogenin, TIMP-1
	No change	Angiostatin, VEGF-A, serpin F1
	Undetected	DPP-IV, HB-EGF, HGF, leptin, MMP-9, PD-ECGF, PIGF, CXCL-4, prolactin, serpin E1, TIMP-4, thrombospondin-1
	Increase	Angiopoietin-1, angiopoietin-2, thrombospondin-2, pentraxin 3
B9	>70% decrease	VEGF-A
	50%–70% decrease	EG-VEGF
	30%–50% decrease	TIMP-1
	10%–30% decrease	Endostatin, endothelin-1, IGFBP-2, IGFBP-3
	No change	Activin-A, persephin, angiogenin, CXCL-16, serpin F1
	Undetected	HB-EGF, HGF, leptin, MMP-9, pentraxin 3, PD-ECGF, PDGF-AA, PIGF, prolactin, serpin E1, TIMP-4, thrombospondin-2
	Increase	Angiopoietin-1, angiopoietin-2, angiostatin, artemin, CXCL-4, DPP-IV, thrombospondin-1

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SUPPLEMENTAL TABLE 3. Changes in Vasoactive Protein Levels 4 Weeks After Injection of Dexamethasone Implant in 11 Patients With Macular Edema Due to Branch Retinal Vein Occlusion (*Continued*)

Subject	Protein Level Change	Protein
B14	Undetected	Angiopoietin-1, angiopoietin-2, angiostatin, artemin, DPP-IV, endothelin-1, HB-EGF, HGF, IGFBP-1, leptin, pentraxin 3, PD-ECGF, PDGF-AA, persephin, CXCL-4, PlGF, prolactin, serpin F1
	Increase	Angiogenin, TIMP-1, TIMP-4, activin-A, EG-VEGF, endostatin, IGFBP-2, IGFBP-3, MMP-9, serpin E1, VEGF-A, CXCL-16, thrombospondin-1, thrombospondin-2
Moderate Edema, Partial Response		
B1	>70% decrease	Angiostatin, EG-VEGF, MMP-9, pentraxin 3, PDGF-AA, persephin
	50%–70% decrease	Activin-A, angiopoietin-2, artemin, endostatin, IGFBP-3, VEGF-A
	30%–50% decrease	Endothelin-1, HGF, IGFBP-1, IGFBP-2, TIMP-4, PD-ECGF, CXCL-16, thrombospondin-1, thrombospondin-2
	10%–30% decrease	DPP-IV, serpin F1
	No change	Angiogenin, TIMP-1
	Undetected	Angiopoietin-1, HB-EGF, leptin, CXCL-4, PlGF, prolactin
	Increase	Serpin E1
B15	>70% decrease	Artemin
	30%–50% decrease	Persephin
	10%–30% decrease	Endothelin-1, IGFBP-1, serpin F1
	Undetected	Angiopoietin-1, angiostatin, DPP-IV, endostatin, HGF, IGFBP-2, IGFBP-3, leptin, MMP-9, pentraxin 3, PD-ECGF, PDGF-AA, CXCL-4, PlGF, prolactin, thrombospondin-1, thrombospondin-2, CXCL-16
	Increase	Activin A, angiopoietin-2, EG-VEGF, angiogenin, TIMP-1, serpin E1, TIMP-4, VEGF-A
B16	>70% decrease	EG-VEGF, HB-EGF
	50%–70% decrease	Angiopoietin-1, endostatin, IGFBP-2, CXCL-16
	30%–50% decrease	Activin-A, HGF, IGFBP-1, IGFBP-3, persephin
	10%–30% decrease	DPP-IV, endothelin-1, VEGF-A, angiogenin, TIMP-1
	No change	Artemin
	Undetected	Angiostatin, leptin, MMP-9, pentraxin 3, PD-ECGF, PDGF-AA, CXCL-4, PlGF, prolactin, serpin E1, TIMP-4, thrombospondin-1, thrombospondin-2
	Increase	Angiopoietin-2, serpin F1
Mild Edema, Complete Response		
B2	>70% decrease	Angiostatin, HGF, IGFBP-3
	50%–70% decrease	Activin-A, endostatin, persephin
	30%–50% decrease	IGFBP-2, TIMP-4, VEGF-A
	10%–30% decrease	EG-VEGF, endothelin, IGFBP-1, angiogenin, CXCL-16, serpin F1
	No change	TIMP-1
	Undetected	Angiopoietin-1, HB-EGF, MMP-9, pentraxin 3, PD-ECGF, PDGF-AA, CXCL-4, PlGF, prolactin, thrombospondin-1, thrombospondin-2
	Increase	Artemin, DPP-IV, serpin E1, angiopoietin-2, leptin

CXCL4 = C-X-C motif ligand 4; CXCL16 = C-X-C motif ligand 16; DPP-IV = dipeptidyl peptidase-4; EG-VEGF = endocrine gland vascular endothelial growth factor; HB-EGF = heparin binding-epidermal growth factor; HGF = hepatocyte growth factor; IGFBP = insulin-like growth factor binding protein; MMP = matrix metalloproteinase; PD-ECGF = platelet-derived endothelial cell growth factor; PDGF = platelet-derived growth factor; PlGF = placental growth factor; TIMP = tissue inhibitor of metalloproteinases; VEGF = vascular endothelial growth factor.

SUPPLEMENTAL TABLE 4. Changes in Vasoactive Protein Levels 4 Weeks After Injection of Dexamethasone Implant in 11 Patients With Macular Edema Due to Central Retinal Vein Occlusion

Subject	Protein Level Change	Protein
Severe Edema, Complete Response		
C5	5%–70% decrease	MMP-9, PD-ECGF, PDGF-AA
	30%–50% decrease	EG-VEGF, HB-EGF, IGFBP-1, CXCL-4
	10%–30% decrease	Angiopoietin-2, angiostatin, DPP-IV, HGF, pentraxin 3, serpin F1
	No change	Activin-A, angiopoietin-1, endothelin-1, IGFBP-2, IGFBP-3, persephin, VEGF-A, thrombospondin-2, angiogenin, CXCL-16, TIMP-1
	Undetected	Leptin
	Increase	Artemin, PIGF, prolactin, serpin E1, TIMP-4, thrombospondin-1
C7	>70% decrease	DPP-IV, HGF, MMP-9
	50%–70% decrease	Angiopoietin-1, HB-EGF, IGFBP-3
	30%–50% decrease	EG-VEGF, endostatin, endothelin-1, IGFBP-1, IGFBP-2
	10%–30% decrease	Activin-A, angiopoietin-2, artemin, PDGF-AA, persephin, VEGF-A, angiogenin, CXCL-16, TIMP-1
	No change	Angiostatin, PD-ECGF, CXCL-4, serpin F1
	Undetected	Leptin, thrombospondin-1, thrombospondin-2
	Increase	PIGF, prolactin, serpin E1, TIMP-4, pentraxin 3
C21	>70% decrease	DPP-IV
	50%–70% decrease	IGFBP-1
	30%–50% decrease	IGFBP-2, IGFBP-3
	10%–30% decrease	Activin-A, endostatin, CXCL-16
	No change	Angiostatin, VEGF-A, angiogenin, TIMP-1
	Undetected	Angiopoietin-1, artemin, HB-EGF, HGF, leptin, pentraxin 3, PD-ECGF, PDGF-AA, CXCL-4, PIGF, serpin E1, TIMP-4, thrombospondin-1, thrombospondin-2
	Increase	EG-VEGF, endothelin-1, MMP-9, persephin, serpin F1, angiopoietin-2
Severe Edema, Partial Response		
C2	>70% decrease	Thrombospondin-1
	50%–70% decrease	HGF, MMP-9, CXCL-16
	10%–30% decrease	Angiopoietin-2, endostatin, IGFBP-3
	No change	EG-VEGF, IGFBP-2, leptin, angiogenin, TIMP-1
	Undetected	Angiopoietin-1, HB-EGF, pentraxin 3, PD-ECGF, PDGF-AA, CXCL-4, PIGF, prolactin, serpin E1, TIMP-4, thrombospondin-2
	Increase	DPP-IV, endothelin-1, IGFBP-1, persephin, VEGF-A, artemin, activin-A, angiostatin, serpin F1
C23	>70% decrease	EG-VEGF, endothelin-1, HG-EGF, HGF, IGFBP-1, MMP-9, thrombospondin-1
	50%–70% decrease	Activin-A, endostatin, IGFBP-2, pentraxin 3, PDGF-AA, CXCL-16
	30%–50% decrease	Thrombospondin-2
	10%–30% decrease	IGFBP-3, persephin, angiogenin
	No change	Angiostatin, VEGF-A, serpin F1, TIMP-1
	Undetected	Angiopoietin-1, artemin, DPP-IV, leptin, PD-ECGF, CXCL-4, prolactin, serpin E1, TIMP-4, PIGF
	Increase	Angiopoietin-2
Moderate Edema, Complete Response		
C1	>70% decrease	Activin-A, angiostatin, endostatin, HGF, pentraxin 3, persephin, thrombospondin-1, thrombospondin-2, serpin F1
	50%–70% decrease	CXCL-16
	30%–50% decrease	IGFBP-2, IGFBP-3
	10%–30% decrease	Angiopoietin-2, endothelin-1, IGFBP-1, leptin, VEGF-A, TIMP-1
	No change	DPP-IV, PDGF-AA, angiogenin
	Undetected	Angiopoietin-1, HB-EGF, MMP-9, PD-ECGF, CXCL-4, PIGF, prolactin, serpin E1, TIMP-4
	Increase	Artemin

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SUPPLEMENTAL TABLE 4. Changes in Vasoactive Protein Levels 4 Weeks After Injection of Dexamethasone Implant in 11 Patients With Macular Edema Due to Central Retinal Vein Occlusion (*Continued*)

Subject	Protein Level Change	Protein
C3	>70% decrease	Angiopoietin-1, IGFBP-3, leptin
	50%–70% decrease	Activin-A, angiostatin, EG-VEGF, HGF, IGFBP-1
	30%–50% decrease	DPP-IV, endostatin, CXCL-4, CXCL-16
	10%–30% decrease	IGFBP-2, MMP-9, angiogenin
	No change	PD-ECGF, thrombospondin-1, TIMP-1
	Undetected	Pentraxin 3, PDGF-AA, thrombospondin-2
	Increase	Angiopoietin-2, artemin, endothelin-1, HB-EGF, persephin, PlGF, prolactin, serpin E1, serpin F1, TIMP-4, VEGF-A
C6	>70% decrease	IGFBP-3, pentraxin 3, PDGF-AA, thrombospondin-1, thrombospondin-2
	50%–70% decrease	Angiopoietin-1, endostatin, HB-EGF, MMP-9, PD-ECGF
	30%–50% decrease	EG-VEGF, HGF, IGFBP-2, PlGF, prolactin, serpin E1, TIMP-4
	10%–30% decrease	Angiostatin, IGFBP-2, pentraxin 3, persephin, CXCL-16
	No change	Angiopoietin-1, VEGF-A, angiogenin, TIMP-1
	Undetected	Leptin
	Increase	Activin-A, serpin F1
C8	>70% decrease	HB-EGF
	50%–70% decrease	EG-VEGF, endostatin, HGF, IGFBP-3, MMP-9, PDGF-AA, thrombospondin-1
	30%–50% decrease	Activin-A, DPP-IV, IGFBP-1, PD-ECGF, CXCL-4, thrombospondin-2
	10%–30% decrease	Angiostatin, IGFBP-2, pentraxin 3, persephin, CXCL-16
	No change	Angiopoietin-1, VEGF-A, angiogenin, TIMP-1
	Undetected	Leptin
	Increase	Angiopoietin-2, artemin, endothelin-1, serpin E1, serpin F1, PlGF, prolactin, TIMP-4
C22	50%–70% decrease	CXCL-16
	30%–50% decrease	Endostatin, IGFBP-1
	10%–30% decrease	EG-VEGF, IGFBP-2, IGFBP-3
	No change	Angiostatin, angiogenin, TIMP-1
	Undetected	Angiopoietin-1, artemin, DPP-IV, endothelin-1, HB-EGF, HGF, leptin, pentraxin 3, PDGF-AA, PD-ECGF, CXCL-4, PlGF, prolactin, serpin E1, TIMP-4, thrombospondin-1, thrombospondin-2
	Increase	Activin-A, angiopoietin-2, MMP-9, VEGF-A, serpin F1
Mild Edema, Complete Response		
C4	>70% decrease	Angiopoietin-1, PD-ECGF
	30%–50% decrease	Leptin, CXCL-16
	10%–30% decrease	Angiopoietin-2, angiostatin, endostatin, HGF, IGFBP-3, PDGF-AA, persephin
	No change	Artemin, IGFBP-2, CXCL-4, TIMP-1
	Increase	DPP-IV, EG-VEGF, endothelin-1, IGFBP-1, MMP-9, pentraxin 3, PlGF, prolactin, serpin E1, serpin F1, TIMP-4, thrombospondin-1, thrombospondin-2, activin-A, HB-EGF, VEGF-A

CXCL4 = C-X-C motif ligand 4; CXCL16 = C-X-C motif ligand 16; DPP-IV = dipeptidyl peptidase-4; EG-VEGF = endocrine gland vascular endothelial growth factor; HB-EGF = heparin-binding epidermal growth factor; HGF = hepatocyte growth factor; IGFBP = insulin-like growth factor binding protein; MMP = matrix metalloproteinase; PD-ECGF = platelet-derived endothelial cell growth factor; PDGF = platelet-derived growth factor; PlGF = placental growth factor; TIMP = tissue inhibitor of metalloproteinases; VEGF = vascular endothelial growth factor.

SUPPLEMENTAL TABLE 5. Aqueous Levels of Hepatocyte Growth Factor and Vascular Endothelial Growth Factor Before and After Injection of Dexamethasone Implant in Patients With Branch Retinal Vein Occlusion

Subject ID	Disease Duration (mo)	Retinal Nonperfusion	Visit	HGF (pg/mL)	VEGF (pg/mL)	CST (μm)	Intraretinal Fluid	BCVA (Letter Score)
B5	41	Mild	Day 0	270	49.7	453	Moderate	60
			Week 4 (% change)	215 (−20.4%)	22.5 (−54.7%)	379	Moderate	60
			Week 16 (% change)	255 (+18.6%)	15.2 (−32.5%)	330	Moderate	59
B7	34	Mild	Day 0	99	71.4	350	Mild	60
			Week 4 (% change)	284 (+186.9%)	45.0 (−37.0%)	286	None	64
B10	42	Moderate	Day 0	400	40.8	792	Severe	49
			Week 4 (% change)	144 (−64.0%)	43.7 (+7.1%)	573	Moderate	64
			Week 16 (% change)	165 (+14.6%)	65.2 (49.2%)	494	Moderate	62
B11	39	-[a]	Day 0	67	20.1	438	Moderate	70
			Week 4 (% change)	53 (−20.9%)	16.2 (−19.4%)	373	Moderate	77
B12	25	Moderate	Day 0	194	140	305	Mild	54
			Week 4 (% change)	110 (−43.3%)	36.2 (−74.2%)	298	Minimal	63
			Week 16 (% change)	150 (+36.4%)	Not detected	310	Mild	70
B13	109	Moderate	Day 0	247	55.8	225	Mild	65
			Week 4 (% change)	179 (−27.5%)	45.6 (−18.3%)	206	Minimal	63

BCVA = best-corrected visual acuity; CST = center subfield thickness; HGF = hepatocyte growth factor; VEGF = vascular endothelial growth factor.

[a]No gradable fluorescein angiogram.

SUPPLEMENTAL TABLE 6. Aqueous Levels of Hepatocyte Growth Factor and Vascular Endothelial Growth Factor Before and After Injection of a Dexamethasone Implant in Patients With Central Retinal Vein Occlusion

Subject ID	Disease Duration (mo)	Retinal Nonperfusion	Visit	HGF (pg/mL)	VEGF (pg/mL)	CFT (μm)	Intraretinal Fluid	BCVA (Letter Score)
C9	2	Mild	Day 0	178	88.2	699	Severe	62
			Week 4 (% change)	170 (−4.7%)	63.2 (−28.5%)	287	None	76
			Week 16 (% change)	200 (+17.6%)	58.9 (−6.9%)	605	Moderate	63
C10	89	Mild	Day 0	140	54.0	599	Severe	65
			Week 4 (% change)	71 (−49.2%)	-[b]	301	None	67
			Week 16 (% change)	112 (+57.7%)	55.2	562	Moderate	60
C11	54	Severe	Day 0	200	170.6	398	Moderate	54
			Week 4 (% change)	173 (−13.7%)	185.7 (−7.6%)	335	Mild	56
			Week 16 (% change)	162 (−6.4%)	147.0 (−20.8%)	328	Mild	54
C12	37	Mild	Day 0	52	48.3	431	Moderate	76
			Week 4 (% change)	54 (+4.5%)	15.2 (−68.6%)	309	None	81
			Week 16 (% change)	117 (+116.7 %)	15.7 (+3.3%)	285	None	79
C13	20	Mild	Day 0	194	97.4	777	Severe	77
			Week 4 (% change)	67 (−65.3%)	51.6 (−47.0%)	520	Moderate	78
C14	10	Moderate	Day 0	203	43.0	539	Severe	22
			Week 4 (% change)	261 (+28.6%)	124.0 (+187.3%)	435	Moderate	27
			Week 16 (% change)	312 (+19.5%)	74.7 (−39.8%)	1146	Severe	30
C15	45	Mild	Day 0	85	74.7	455	Severe	68
			Week 4 (% change)	154 (+80.1%)	47.1 (−36.9%)	234	None	70
			Week 16 (% change)	161 (+4.5%)	94.6 (+100.8%)	265	Moderate	84
C16	43	Mild	Day 0	83	79.3	710	Severe	43
			Week 4 (% change)	94 (+13.2%)	47.3 (−40.4%)	263	None	44
			Week 16 (% change)	133 (+41.5%)	86.4 (+82.7%)	525	Moderate	47
C17	62	Mild	Day 0	107	88.0	840	Severe	62
			Week 4 (% change)	67 (−37.0%)	172.7 (+96.0%)	276	None	70
			Week 16 (% change)	97 (+44.8%)	129.4 (−25.1%)	659	Moderate	60
C18	10	-[a]	Day 0	176	148.8	765	Severe	21
			Week 4 (% change)	129 (−27.0%)	389.2 (+162.0%)	282	None	31
C19	13	Mild	Day 0	137	28.6	597	Moderate	59
			Week 4 (% change)	85 (−38.3%)	68.8 (+140.6%)	285	None	72
C20	53	-[a]	Day 0	196	118.0	339	Moderate	61
			Week 4 (% change)	141 (−28.1%)	116.4 (−1.4%)	281	Mild	73

BCVA = best-corrected visual acuity; CST = center subfield thickness; HGF = hepatocyte growth factor; VEGF = vascular endothelial growth factor.

[a]No gradable fluorescein angiogram.

[b]Not detectable.