Retinal and choroidal vascular diseases are highly prevalent causes of visual disability. The most common choroidal vascular disease is neovascular AMD (nAMD). AMD was estimated to have a world-wide prevalence of 196 million in 2020 and because the world’s population is aging, 288 million is projected for 2040. Roughly 10% of patients with AMD develop neovascularization (NV) and hence the prevalence of nAMD was about 20 million in 2020. Diabetic retinopathy is the most common retinal vascular disease and in 2012 the world-wide prevalence of any diabetic retinopathy, proliferative diabetic retinopathy (PDR), and diabetic macular edema (DME) was 93 million, 17 million, and 21 million, respectively. Retinal vein occlusion (RVO) is the second most common retinal vascular disease with a world-wide prevalence of 16.4 million in 2008, and a large percentage of patients with RVO develop macular edema. These three disorders account for a large proportion of global severe and moderate vision loss.

WHAT IS nAMD AND HOW WAS IT TREATED IN THE PAST?

AMD is a complex disease for which the risk of occurrence is increased by age, multiple genetic variants, and environmental exposures. Most patients with AMD have early or intermediate stages characterized by deposits on Bruch’s membrane called drusen and pigmented changes in the macula, which is usually associated with mild vision loss that is worse in dim illumination. Roughly 10% of patients develop choroidal NV (CNV), which leaks fluid into the macula, causing a rapid loss of vision. If untreated, the new vessels recruit other cells, resulting in subretinal fibrosis that damages photoreceptors and RPE cells, causing permanent loss of central vision. Early treatments sought to ablate CNV with laser photocoagulation focusing on lesions not involving the fovea. This treatment spared central vision in some patients for a time, but recurrences were common and almost always involved the fovea, causing the loss of central vision. Because the majority of patients presented with foveal involvement or developed foveal involvement from recurrent CNV after laser ablation, subfoveal CNV was treated with laser, which sacrificed central vision, but prevented profound vision loss from continued lesion growth and subretinal fibrosis. Another approach was the surgical removal of CNV, which damaged the central retina and RPE and was found to be ineffective. Laser ablation of the fovea or surgical removal of CNV that also removed adherent RPE and photoreceptors provide insight into the desperation that was engendered by nAMD. Photodynamic therapy used the intravascular injection of a photosensitizing dye to minimize laser power and provide more selective damage to NV, but still caused substantial bystander damage to photoreceptors and the RPE, slowing but not eliminating the loss of central vision.

MOLECULAR PATHOGENESIS OF nAMD

Elucidation of the molecular pathogenesis of CNV turned the tide regarding the treatment of nAMD. The first step was the isolation and cloning of VEGF-A, a potent hypoxia-regulated angiogenic factor. Intraocular levels of VEGF-A were found to be elevated in patients with various types of ocular NV and blockade of VEGF-A suppressed ischemia-induced retinal or iris NV. Transgenic mice in which the rhodopsin promoter drives expression of VEGF165 in photoreceptors (rhop/Vegf) mice) demonstrated that increased expression of VEGF165 in the outer retina was sufficient to cause CNV. In these mice, the expression of VEGF165 begins at postnatal day (P) 7; at P14, endothelial cells begin migrating into the outer retina from the deep capillary bed, and by P21 there are perfused vessels extending from the deep retinal capillaries to the subretinal space. This type of CNV that originates from the deep retinal capillaries occurs in 30% to 40% of patients with nAMD and has been named retinal angiomaticous proliferation and more recently type 3 CNV. The tet/ on inducible expression system was used to generate double transgenic mice with doxycycline-inducible expression of VEGF165 in photoreceptors (Tet/opsin/Vegf) mice). When treated with 2 mg/mL of doxycycline in drinking water, the expression of VEGF165 in photoreceptors is so high (10-fold higher than that in rhop/Vegf mice) that there is not only type 3 CNV, but there is also severe retinal vascular leakage resulting in exudative retinal detachment. Rupture of Bruch’s membrane with laser photocoagulation in primates resulted in CNV that extends from the choroid into the subretinal space. This process is referred to as type 2 CNV and is the most common type seen in nAMD. Generation of this model in mice made it possi-
ble to use knockout and transgenic mice to investigate the effect of gene products in the pathogenesis of CNV and made screening of pharmacologic agents more feasible. At the time, fibroblast growth factor 2 was hypothesized to be a contributor to CNV, but fibroblast growth factor 2 knockout mice showed no difference in the amount of CNV at Bruch’s membrane rupture sites compared with wild-type mice, suggesting against this hypothesis. Because the targeted disruption of even one Vegfa allele is embryonic lethal, it was not possible to use a similar approach to test the contribution of VEGF-A; therefore, pharmacologic inhibitors of VEGF were used and found to strongly suppress CNV at Bruch’s membrane rupture sites. Bevacizumab is a full-length monoclonal antibody and ranibizumab is a Fab that each specifically neutralize primate VEGF-A and do not cross-react with murine VEGF-A, and therefore could not be tested in the mouse model. Ranibizumab was demonstrated to suppress CNV at Bruch’s membrane rupture sites in primates. Aflibercept is a recombinant protein containing binding domains from VEGF receptors 1 and 2, and does not exhibit species specificity with regard to the neutralization of VEGF-A; it strongly suppressed CNV at Bruch’s membrane rupture sites in mice. These studies suggested that VEGF-A is a major contributor to CNV and this finding was confirmed to be the case in patients with nAMD in clinical trials in which monthly injections of ranibizumab or aflibercept every 2 months suppressed exudation and markedly improved visual acuity. The value of an animal model is determined in part by its ability to predict outcomes in patients, and thus the mouse model of CNV following rupture of Bruch’s membrane has become widely used in academic laboratories and industry.

**Current Treatment of nAMD**

Since the demonstration that monthly injections of ranibizumab provide substantial visual benefit in patients with nAMD, there have been studies demonstrating that other anti–VEGF-A agents are noninferior but there have been no major advances. Most attention has focused on reducing treatment burden and the most widely used regimen to do that is treat and extend, in which a patient is given an anti–VEGF-A injection at every visit while trying to increase the duration between visits to identify the maximum time between injections that avoids recurrent exudation. Although this approach works well for some patients, in others, missed visits and/or miscalculations result in recurrent bouts of exudation, subretinal fibrosis, and a gradual decrease in vision. Observational studies have shown that injection frequency is much less and visual outcomes are substantially worse in clinical practice than in clinical trials.

**Diabetic Retinopathy and DME: Ablative Treatments**

Hyperglycemia is a critical factor in the development of diabetic retinopathy. Prolonged hyperglycemia promotes damage to pericytes and endothelial cells, causing closure of some retinal vessels visualized on fluorescein angiograms as areas of retinal nonperfusion. Retinal ischemia from nonperfusion results in PDR, in which retinal NV often causes vitreous hemorrhage and growth of fibrovascular tissue on the surface of the retina that contracts and detaches the retina. Panretinal photocoagulation (PRP) decreases retinal ischemia, causes regression of retinal NV, and, if given in a timely manner, prevents retinal detachment and severe loss of vision. PRP is an effective treatment for PDR that continues to be used today to prevent severe vision loss and provide long-term stability. However, PRP does not prevent excessive vascular leakage from the posterior retinal vessels, which causes DME, the most common cause of moderate vision loss in diabetics. Focal laser treatments to ablate microaneurysms and macular grid laser avoiding the fovea decreased vision loss from DME, but was ineffective in many patients. Elucidation of the molecular pathogenesis of PDR and DME was critical for development of more effective treatments.

**Molecular Pathogenesis of Ischemic Retinopathies**

Oxygen-induced ischemic retinopathy (OIR) recapitulates critical features of diabetic retinopathy, including retinal nonperfusion. As noted elsewhere in this article, VEGF-A plays an important role in the stimulation of retinal NV in the mouse model of OIR. Hypoxia-inducible factor-1α (HIF-1α) is responsible for the increased expression of VEGF-A in ischemic retinopathies. Two hours after the onset of retinal hypoxia in the OIR model, there was a marked increase in HIF-1α in the inner retina, and 4 hours later there was a dramatic increase in Vegfa mRNA in the same cells. Twenty-four hours after the subretinal injection of an adenoviral vector expressing a constitutively active form of HIF-1α in adult mice, there were increased ocular levels of mRNA for HIF-1α, VEGF-A, and other angiogenic factors, and after 3 days, there was strong staining for HIF-1α in the RPE and type 3 CNV like that seen in rbo/VEGF transgenic mice. Six days after the intravitreal injection of the adenoviral vector expressing constitutively active HIF-1α, there was retinal NV on the surface of the retina and anterior segment NV and thus, depending on its location, increased levels of HIF-1α in the eye are sufficient to cause choroidal, retinal, or anterior segment NV. The Senemara laboratory screened a library of approved drugs in a high-throughput assay of HIF-1 transcriptional activity and found strong inhibition by digoxin, daunorubicin, and acriflavine. Each of these drugs blocked the upregulation of VEGF-A and other angiogenic factors in ischemic retina, strongly suppressed ischemia-induced retinal NV, and strongly suppressed CNV at Bruch’s membrane rupture sites. These data demonstrate that HIF-1 plays a central role in the development of both retinal NV and CNV.

Tyrosine kinase with immunoglobulin and epidermal growth factor homology domains-2 (Tie2) is a tyrosine kinase receptor that is expressed on endothelial cells and some bone marrow–derived cells that is critical for vascular development. Angiopoietin 1 is an agonist for Tie2 that is also required for vascular development. Angiopoietin 2 binds Tie2, but does not stimulate its phosphorylation and thus acts as an endogenous Tie2 antagonist. The expression of angiopoietin 2 is increased in ischemic retina in the OIR model and is localized around retinal NV. Like VEGF-A, angiopoietin 2 is upregulated by HIF-1. Double transgenic mice with doxycycline-inducible expression of angiopoietin 2 in photoreceptors (Tet/opsin/ang2 mice) have provided a useful tool to investigate the role of angiopoietin 2 in ocular NV. In the OIR model, the induced expression of angiopoietin 2 during the ischemic period between P12 and P17 when VEGF-A levels are high, resulted...
in a marked increase in retinal NV at P17, but the induced expression of angiopoietin 2 between P20 and P23, when VEGF-A levels are low, caused a rapid regression of retinal NV.57 In adult mice, induced expression of angiopoietin 2 caused no identifiable change to retinal vessels, but when it was combined with an intravitreal injection of an adenoviral vector expressing VEGF-A, it resulted in a marked increase in retinal NV compared with mice in which the vector was injected without the induced expression of angiopoietin 2.56 These data indicated that angiopoietin 2 increases the sensitivity of endothelial cells in retinal vessels to VEGF-A, and, in the absence of VEGF-A, it promotes the regression of new vessels, but not mature vessels. Vascular endothelial protein tyrosine phosphatase (VE-PTP) dephosphorylates Tie2 and is a second Tie 2 inhibitor that, like angiopoietin 2, is increased by hypoxia; it is not detectable in normal retinal vessels, but is highly expressed in endothelial cells participating in NV in mice with OIR.58 A small molecule inhibitor of VE-PTP, AKB-9778, stimulated phosphorylation of Tie2 in endothelial cells in vitro or in vivo and suppressed hypoxia-induced retinal NV, CNV at Bruch’s membrane rupture sites, and VEGF-A–induce vascular leak- age, and had an additive effect with aflibercept.58 Thus, blocking either of two Tie2 inhibitors provides added bene- fit to VEGF-A suppression in models of retinal and choroidal vascular disease. An alternative strategy to stabilize retinal and choroidal vessels and decrease their sensitivity to patho- logic effects of VEGF-A is overexpression of angiopoietin 1.59,60

Figures 1 and 2 are schematics summarizing the molec- ular pathogenesis of ischemic retinopathies and nAMD. In ischemic retinopathies, progressive closure of some retinal vessels causes retinal hypoxia and the stabilization of HIF-1, which stimulates the transcription of several vasoactive proteins and their receptors. VEGF-A plays a central role stimulating retinal NV, but other VEGF family members, angiopoietin 2, and VE-PTP also contribute. HIF-1 also plays a central role stimulating CNV and hence nAMD. Because HIF-1 is involved in both disease processes, the same vasoactive factors are involved in each.

**Molecular Pathogenesis of DME**

As noted elsewhere in this article, DME often occurs inde- pendent of PDR, and although it was clear that retinal hypoxia played a critical role in PDR, it was not known whether hypoxia played a role in DME or whether it was solely due to structural damage to blood vessels resulting in leaking microaneurysms. There are no animal models of DME to investigate this question and therefore a small clinical trial was designed to address it. Patients with chronic DME despite focal laser treatment were given oxygen by nasal cannula around the clock for 3 months and all eyes showed improvement in central subfield thickness measured by optical coherence tomography.55 Oxygen was withdrawn and over the next 3 months the edema worsened. This study suggests that retinal hypoxia contributes to DME.

VEGF-A was a strong candidate for a hypoxia-regulated gene product that contributes to DME, because the sustained release of VEGF from the eyes of nonhuman primates caused severe leakage from retinal vessels.62 A clinical trial testing oral PKC412, a VEGF receptor tyrosine kinase inhibitor, for 3 months in patients with DME showed a dose-dependent decrease in macular edema measured by optical coherence tomography that recurred after the drug was stopped.63 This work provided proof of concept for targeting VEGF-A for the treatment of DME, but systemic suppression of VEGF is not the best approach because of the risk of hyperten- sion and thromboembolism. A clinical trial testing intravit- reous injections of 0.5 mg ranibizumab in patients with DME demonstrated a mean improvement of 12 Early Treat- ment Diabetic Retinopathy Study (ETDRS) letters and an 85% decrease in excess foveal thickness at the month 7 primary end point.64 The READ2 multicenter randomized trial demonstrated that compared with focal/grid laser ther- apy, which was the standard of care at the time, intravit- reous injections of ranibizumab caused significantly greater improvement in best corrected visual acuity and a decrease in DME.65,66 This finding was subsequently confirmed by the Diabetic Retinopathy Clinical Research Network.67 The RIDE and RISE trials showed prolonged benefit from ranibizumab injections and led to its approval for DME.68

**Role of VEGF-A in RVO**

In diabetic retinopathy, the onset of retinal nonperfusion is gradual, but in central RVO (CRVO) or branch RVO (BRVO), the onset of retinal nonperfusion and hence retinal ischemia is abrupt. The onset of retinal vascular leakage is also acute in RVO, and edema is often severe. A clinical trial test- ing the effect of ranibizumab injections showed even more dramatic effects in CRVO and BRVO than in DME, with

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**Figure 1.** Molecular pathogenesis of ischemic retinopathies. Damage to retinal vessels results in decreased perfusion and reti- nal hypoxia causing stabilization of HIF-1. Increased levels of HIF-1 in the inner retina cause increased expression of multiple vasoactive factors and their receptors. The most important stimulator of reti- nal NV and excessive vascular leakage leading to macular edema is VEGF-A, but other VEGF family members, including VEGF-C and -D, which can stimulate VEGF receptor 2 (VEGFR2), and placen- tal growth factor and VEGF-B, which stimulate VEGF receptor-1 (VEGFR1) may also contribute. Angiopoietin-2 (Angpt2) and vascu- lar endothelial–protein tyrosine phosphatase (VE-PTP) inhibit Tie2, which decreases its stabilizing effect on endothelial cells, making them more responsive to VEGF family members. Recruitment of bone marrow–derived cells provides additional vasoactive stimula- tors and promotes leukostasis, which can further compromise perfu- sion. This recruitment occurs because leukocytes contain CXCR4, the receptor for stromal-derived factor-1 (SDF-1), and VEGFR1, which is activated by VEGF-A, VEGF-B, and placental growth factor. Activated endothelial cells release platelet-derived growth factor- 1 (PDGF-B) which recruits pericytes, glial cells, and RPE, which promote scarring.
FIGURE 2. Molecular pathogenesis of nAMD. HIF-1 is stabilized by oxidative stress and hypoxia, and both have been implicated in nAMD. Complement activation on choriocapillaris (CC) has been shown to cause CC drop out and hypoxia in the RPE and outer retina. Bruch's membrane is compromised by focal and diffuse deposits. Increased levels of HIF-1 in the outer retina and RPE cause increased expression of the same vasoactive factors and their receptors as seen in ischemic retinopathies. The most important stimulator of growth and leakiness of types 1, 2, and 3 CNV is vascular endothelial growth factor-A (VEGF-A), but other VEGF family members including VEGF-C and -D which can stimulate VEGF receptor 2 (VEGFR2), and placental growth factor and VEGF-B which stimulate VEGF receptor-1 (VEGFR1) may also contribute. Angiopoietin-2 (Angpt2) and vascular endothelial–protein tyrosine phosphatase (VE-PTP) inhibit Tie2, which decreases its stabilizing effect on endothelial cells, making them more responsive to VEGF family members. The recruitment of bone marrow–derived cells provides additional vasoactive stimulators. This recruitment occurs because leukocytes contain CXCR4, the receptor for stromal-derived factor-1 (SDF-1), and VEGFR1, which is activated by VEGF-A, VEGF-B, and placental growth factor. Activated endothelial cells release platelet-derived growth factor-B (PDGF-B), which recruits pericytes, glial cells, and RPE, which promotes subretinal fibrosis.

almost complete elimination of excess foveal thickness and a mean improvement in the best corrected visual acuity of about 15 ETDRS letters in both CRVO and BRVO.69,70 The phase III BRAVO and CRUISE trials showed prolonged benefit from ranibizumab injections in BRVO and CRVO and led to its approval.71–74

EFFECT OF VEGF-A SUPPRESSION ON RETINAL NONPERFUSION IN ISCHEMIC RETINOPATHIES

When the results from the initial study testing ranibizumab in patients with RVO were presented,69 some clinicians and investigators raised the concern that, because VEGF-A is a survival factor for endothelial cells and retinal vascular endothelial cells are so distressed by widespread ischemia in RVO, the suppression of VEGF-A might further compromise the endothelial cells and worsen retinal nonperfusion. In fact, several reports were published suggesting that the intraocular injection of an anti-VEGF-A agent had caused worsening of retinal nonperfusion in patients with RVO.75–78 Therefore in BRAVO and CRUISE, fluorescein angiograms were obtained at baseline and every 3 months, and retinal nonperfusion was measured by an independent reading center within a template centered on the fovea and consisting of the center, inner, and outer ETDRS subfields.
Diabetic Retinopathy

The percentage of patients with no posterior retinal nonperfusion decreased in the sham groups, indicating worsening of nonperfusion, whereas it was relatively stable in the ranibizumab groups resulting in a significant difference at the 6-month primary end point. After 6 months, all treatment groups received ranibizumab as needed and the differences were eliminated. Similar measurements of retinal nonperfusion were done in patients with DME who participated in the RISE and RIDE trials. During the first 24 months, when the ranibizumab groups received monthly injections and the sham group had no treatment, there was little change in nonperfusion in the ranibizumab groups, but there was a steady decrease in the percentage of patients with no posterior nonperfusion in the sham group, indicating worsening of nonperfusion. After month 24, all treatment groups received ranibizumab as needed and the difference was eliminated. These data indicate that high levels of VEGF-A promote the progressive worsening of retinal nonperfusion in patients with ischemic retinopathy and that VEGF-A suppression does not worsen nonperfusion, but instead slows it and even causes improvement in some patients. The beneficial effect of VEGF-A suppression on retinal nonperfusion in RVO was confirmed in the COPERNICUS and GALILEO phase III trials testing aflibercept in CRVO. In those trials, ultrawide angle fluorescein angiograms were used to measure the total area of retinal nonperfusion per retina. Monthly injections of aflibercept caused an improvement in the percentage of patients with 10 or more disc areas of nonperfusion from 23.5% at baseline to 11.6% at week 24, which was significantly different from the sham group, in which there was no improvement. To investigate the mechanism by which VEGF promotes retinal nonperfusion, intravascular leukocytes were visualized by perfusion with concanavalin A after the intravitreal injection of 1 μg of VEGF in adult mice. There was a striking increase in leukostasis 24 hours after VEGF injection, which returned to baseline by 48 hours. A sustained, increased expression of VEGF165 in the retinas of Tet/opsin/Vegf double transgenic mice by the administration of doxycycline resulted in sustained leukostasis, leukocytic plugging, and retinal nonperfusion. Mice in which nonperfusion was visualized by fluorescein angiography after 3 days of doxycycline administration showed reperfusion of the closed vessels 14 days after stopping doxycycline. Thus, in both diabetic retinopathy and RVO, there are initial insults that cause retinal nonperfusion, retinal ischemia, and increased levels of VEGF-A. Once VEGF-A is increased, it becomes an important driver of disease progression by promoting leukostasis and the plugging of blood vessels. This process results in a positive feedback loop, causing the progressive worsening of nonperfusion and other outcomes (Figures 3 and 4).

Does VEGF-A Suppression Cause Retinal Damage?

Like ischemic retinopathies, nAMD is chronic, and the chronicity of these diseases results in a high treatment burden, which often leads to undertreatment and poor outcomes. A potential solution is the sustained suppression of VEGF-A, but some investigators and clinicians have cautioned against this strategy because they contend that VEGF-A is a survival factor for photoreceptors and sustained suppression of VEGF-A could kill photoreceptors and cause geographic atrophy. Another concern is that the knock-out of VEGF-A in RPE cells causes loss of the choriocapillaris and retinal degeneration, and it has been suggested that treatments that block VEGF-A could mimic the effect of VEGF-A knockout. However, there is ample evidence suggesting that the strong, sustained suppression of VEGF-A does not cause retinal damage. Transgenic mice with induced high-level expression for up to 7 months of a potent VEGF-binding protein, sVEGFR1-Fc, have marked suppression of NV or retinal vascular leakage, but have no functional or ultrastructural evidence of retinal damage. The prolonged pharmacologic blockade of VEGF receptors also failed to decrease ERG function or cause any thinning of the outer nuclear layer. Photoreceptor survival factors such as fibroblast growth factor 2 or brain-derived neurotrophic factors promote photoreceptor survival and function in rats or mice with inherited photoreceptor degeneration, but VEGF does not. Injection of VEGF165 or prolonged blockade of VEGF receptors in rd10 mice had no effect on photoreceptor ERG function or photoreceptor survival. These data suggest against VEGF-A being a survival factor for photoreceptors, but then why do photoreceptors have type 2 VEGFR? This question was answered by Okabe et al., who showed that the conditional knockout
FIGURE 4. Role of VEGF-A in progression of retinopathy due to RVO. RVO causes increased resistance in the retinal circulation. If there is little or no preexistent arterial disease, the increased resistance causes decreased blood flow and some retinal hypoxia, but little retinal nonperfusion (RNP) and modest increase in VEGF-A expression. The increase in VEGF results in macular edema, but it is easily controlled with anti–VEGF injections and after a moderate number of VEGF injections, they may no longer be needed. This course is common in young patients who on average have less retinal arterial disease than older patients. If there is substantial preexistent retinal arterial disease, the increased resistance from RVO results in closure of many capillaries and small arterioles which causes substantial increase in VEGF expression. The high levels of VEGF become the driver of the disease by stimulating leukostasis causing progression of RNP and further increasing VEGF expression (↑↑VEGF), resulting in a positive feedback loop for disease progression. If untreated or insufficiently treated, there is a poor outcome. Frequent anti-VEGF injections suppress leukostasis which decreases the progression of RNP and often causes it to improve. Some patients may eventually stabilize and no longer require injections, but the majority require long-term anti–VEGF injections to prevent the worsening of RNP and to suppress macular edema, which can result in a good visual outcome. Reprinted with permission from Campochiaro PA, Akhlaq A. Sustained suppression of VEGF for treatment of retinal/choroidal vascular diseases. Prog Retin Eye Res. 2021;83:1-10.921. Copyright © 2020 Elsevier Ltd.

of VEGFR2 in photoreceptors had no deleterious effects on photoreceptor structure or function, but decreased VEGF internalization and degradation, thereby disrupting the reverse VEGF gradient surrounding retinal neurons and causing vessels to grow closer. Thus, the function of VEGFR2 on retinal neurons is to make blood vessels socially distance from photoreceptors and other retinal neurons, not to promote photoreceptor survival.

Warnings that anti–VEGF-A injection frequency should be moderated to decrease the risk of macular atrophy in patients with nAMD have been controversial, because of a lack of any evidence of a causal link, the occurrence of macular atrophy as part of the natural history of AMD, and the potential for poor outcomes from undertreatment. Patients with type 3 CNV have a particularly high incidence of macular atrophy. Rho/Vegf transgenic mice develop type 3 CNV and, as new vessels grow from deep retinal capillaries through the outer nuclear layer to the subretinal space, photoreceptors adjacent to the vessels stain for a specific marker of oxidative damage, after which the outer nuclear layer becomes thin and irregular indicating outer retinal atrophy visualized as hypopigmented spots on fundus photographs. The atrophy is prevented by treatment with the potent antioxidant N-acetylcysteine, confirming that it is caused by oxidative damage. Aflibercept did not decrease or exacerbate the retinal atrophy. These data indicate that type 3 CNV, in which there are perfused vessels carrying oxygen through the photoreceptor layer, causes oxidative damage to photoreceptors resulting in retinal atrophy and that VEGF-A suppression plays no role.

CURRENT TREATMENT FOR nAMD, DME, AND RVO

The current treatment for nAMD, DME, and RVO is intravitreal anti–VEGF-A injections. The acute effects are dramatic in that there is a rapid decrease in macular fluid and improvement in visual acuity in most patients. These benefits are maintained through the 1- and 2-year end points in clinical
trials by mandated repeated injections.\textsuperscript{27–29,68,71–74} However, these diseases are chronic and there is sustained increased expression of VEGF-A in most patients. After each injection, VEGF-A is neutralized for a finite period of time that varies depending on the level of expression and local factors that influence the responsiveness of retinal/choroidal vessels and new vessels to VEGF-A. Because these factors vary among patients, the time between an anti–VEGF-A injection and recurrent exudation varies among patients. Our current treatment regimens depend on identifying that interval in each patient and leaving patients uncovered, hoping that it will remain constant over time. Unfortunately, the interval does not remain constant over time, resulting in intermittent episodes of recurrent exudation (and progression of nonperfusion in ischemic retinopathies), which are increased in frequency by missed visits. Although one or a few recurrences may have little effect on vision, damaging effects from multiple recurrences mount over time, resulting in the gradual loss of peak vision.\textsuperscript{93,94}

\section*{Future Treatments for nAMD, DME, and RVO}

Because retinal and choroidal vascular diseases are chronic and persistent high expression of VEGF-A drives disease progression and causes gradual loss of vision in many patients, there is strong rationale for sustained suppression of VEGF-A or all VEGF family members. Several strategies are being tested. The approach that is furthest along in development is the port delivery system (PDS), a surgically implanted refillable reservoir that constantly releases ranibizumab into the vitreous cavity. The phase II Ladder trial compared the PDS filled with 10, 40, or 100 mg/mL ranibizumab with monthly injections of ranibizumab in patients with previously treated nAMD. The primary end point was the median time to the recurrence of macular fluid requiring a refill, and in the 100 mg/mL PDS group it was 15 months.\textsuperscript{95} The mean change from baseline best corrected visual acuity at 9 months was +5.0 ETDRS letters in the 100 mg/mL PDS group, which was comparable with the +3.9 letters in the monthly injection group. The phase III Archway trial met its primary outcome by demonstrating that, compared with eyes of patients with nAMD that received monthly injections of ranibizumab, visual outcomes were equivalent in eyes implanted with the PDS containing 100 mg/mL ranibizumab and refilled every 6 months.\textsuperscript{96} The PDS was approved by the FDA in October 2021 and will be available for clinical use in 2022.

Another approach is to incorporate a VEGF receptor tyrosine kinase inhibitor into a biodegradable polymer and generate microparticles that can provide sustained delivery of the inhibitor to the retina and RPE after intravitreous injection. This approach is appealing because it provides a means of blocking all VEGF family members, which may have greater efficacy than specific VEGF-A antagonists. Microparticles composed of blends of poly-lactic-co-glycolic acid and poly-lactic-co-glycolic acid conjugated to polyethylene glycol when injected into the vitreous of mice or minipigs self-aggregated to form a depot that slowly eroded to provide therapeutic levels in the retinas of minipigs for 6 months and the suppression of CNV in mice for at least 6 months.\textsuperscript{97} Clinical trials showed evidence of prolonged suppression of exudation in patients with nAMD, but the microparticle depot was not as firm in human vitreous as it had been in minipigs and mice, allowing the dispersion of clumps of microparticles that clouded the media in some patients. Modifications to this formulation or new sustained delivery platforms will be needed to take advantage of the potential of VEGF receptor tyrosine kinase inhibitors.

Another strategy for the sustained delivery of therapeutic proteins is gene therapy. The initial approach was to express an endogenous antiangiogenic protein and a proof-of-concept clinical trial using an adenoviral vector to express pigment epithelial-derived factor showed some evidence of therapeutic activity.\textsuperscript{98} Subretinal injection of a lentiviral vector expressing endostatin and angiostatin in patients with nAMD resulted in sustained transgene expression for at least 4.5 years, but little signs of efficacy.\textsuperscript{99} Because VEGF-A is a validated target in nAMD and retinal vascular diseases, the gene transfer of a VEGF-A antagonist is a less risky approach than expressing an unvalidated antiangiogenic protein. The subretinal injection of an adeno-associated viral vector 2 expressing soluble VEGFR1 in patients with nAMD showed some initial positive effects,\textsuperscript{100} but ultimately failed; expression data were never reported and it is not clear that sufficient levels of soluble VEGFR1 were obtained to achieve efficacy in the majority of patients with nAMD. The intravitreous injection of an adeno-associated viral vector 2 expressing modified soluble VEGFR1 suppressed exudation in some patients with nAMD, but not others, suggesting that higher expression is needed. The subretinal injection of RGX-314, an adeno-associated viral vector 8 expressing an anti–VEGF Fab similar to ranibizumab, strongly suppressed type 3 CNV in rbo/VEGF\(\text{mice}\)\textsuperscript{101} and in a phase I/IIa clinical trial testing subretinal injection of RGX-314 in patients with nAMD, has shown a dose-depend and prolonged expression of the RGX-314 protein, suppression of exudation, and stable or improved visual acuity (ClinicalTrials.gov Identifier: NCT03066258). Suprachoroidal injection is a new route of delivery for ocular gene therapy that is less invasive than a subretinal injection and can be done in an outpatient setting without surgery.\textsuperscript{102} Clinical trials are underway testing suprachoroidal gene transfer of RGX-314 in patients with nAMD (ClinicalTrials.gov Identifier: NCT04514653) or diabetic retinopathy (ClinicalTrials.gov Identifier: NCT04567550).

Despite compelling evidence in animal models that other hypoxia-regulated gene products in addition to VEGF-A contribute to retinal and choroidal vascular diseases, blocking VEGF-A has been so effective in clinical trials that it has been challenging to demonstrate any added benefit by inhibiting other targets. However, three phase II trials have suggested that the activation of Tie2 may provide additional benefit in patients with DME. The TIME2 trial showed that, compared with ranibizumab alone, ranibizumab combined with the VE-PTP inhibitor, AKB-9778, caused greater reduction in DME.\textsuperscript{103} In the RUBY Trial, compared with aflibercept alone, aflibercept plus an antiAng2 antibody caused a greater decrease in edema (ClinicalTrials.gov Identifier: NCT02712008). Neither of these trials showed greater improvement in visual acuity by the combination therapy, but they included patients with chronic edema in whom visual improvement is limited by photoreceptor damage. In the Boulevard trial, Faricimab, a bispecific anti–VEGF and anti-Ang2 antibody, showed a greater decrease in edema and a greater improvement in visual acuity than ranibizumab in patients with new-onset DME.\textsuperscript{104} Faricimab also caused greater improvement in background diabetic retinopathy. In the recently completed phase III Yosemite
and Rhine trials (ClinicalTrials.gov Identifiers: NCT03622580 and NCT03622595), compared with afiblerecept, Faricimab showed evidence of a greater decrease in edema, but not greater visual improvement at the 9-month primary end point. Over a longer treatment period, greater control of edema may translate into better visual acuity.

In summary, retinal and choroidal vascular diseases are prevalent causes of vision loss treated in the past by ablative treatments that provided minimal benefit with the exception of PRP for PDR. Presently, they are treated by repeated injections of anti–VEGF-A neutralizing proteins, which provide substantial benefit when injections are given frequently and regularly, but in clinical practice undertreatment is common and leads to poor outcomes. In the future, these diseases will be treated by one of a variety approaches that provide the sustained suppression of VEGF-A or multiple VEGF family members. This treatment strategy will be sufficient for most patients, but in those with an incomplete response, it will be supplemented by agents that activate Tie2 or target other hypoxia-regulated vasoactive proteins, not yet validated in clinical trials, such as integrins \( \alpha_\beta_3 \) and \( \alpha_\beta_1 \).\(^{105}\)

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