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# PATHWAY-BASED THERAPIES FOR AGE-RELATED MACULAR DEGENERATION

## An Integrated Survey of Emerging Treatment Alternatives

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**Purpose:** To review treatments under development for age-related macular degeneration (AMD) in the context of current knowledge of AMD pathogenesis.

**Methods:** Review of the scientific literature published in English.

**Results:** Steps in AMD pathogenesis that appear to be good targets for drug development include 1) oxidative damage; 2) lipofuscin accumulation; 3) chronic inflammation; 4) mutations in the complement pathway; and 5) noncomplement mutations that influence chronic inflammation and/or oxidative damage (e.g., mitochondria and extracellular matrix structure). Steps in neovascularization that can be targeted for drug development and combination therapy include 1) angiogenic factor production; 2) factor release; 3) binding of factors to extracellular receptors (and activation of intracellular signaling after receptor binding); 4) endothelial cell activation (and basement membrane degradation); 5) endothelial cell proliferation; 6) directed endothelial cell migration; 7) extracellular matrix remodeling; 8) tube formation; and 9) vascular stabilization.

**Conclusion:** The era of pathway-based therapy for the early and late stages of AMD has begun. At each step in the pathway, a new treatment could be developed, but complete inhibition of disease progression will likely require a combination of the various treatments. Combination therapy will likely supplant monotherapy as the treatment of choice because the clinical benefits (visual acuity and frequency of treatment) will likely be superior to monotherapy in preventing the late-stage complications of AMD.

**RETINA** 30:1350–1367, 2010

A large number of treatments for exudative and nonexudative age-related macular degeneration (AMD) are in preclinical development or in early-stage clinical trials (Figure 1). In this review, six observations relevant to the pathogenesis of AMD will be described. Emerging and established AMD treatments will then be reviewed within the context of these pathogenic schemes. This information should be especially useful for the rational development of combination therapies.

### Pathogenesis of Age-Related Macular Degeneration

Detailed consideration of the pathogenesis of AMD is beyond the scope of this perspective, but it has been discussed extensively elsewhere.[1,2] Six concepts will be considered briefly.

First, biochemical studies and histological studies of AMD have implicated oxidative damage as a possible cause of this disease. Eyes with geographic atrophy



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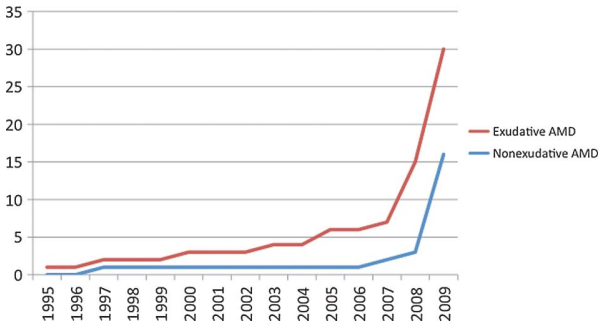


**Fig. 1.** Rate of AMD treatment growth. The number of treatments for AMD in preclinical testing, early clinical testing, or clinical practice has undergone exponential growth during the past 5 years.

(GA) exhibit DNA strand breaks and lipoperoxidation.[3] Antioxidant changes in the retinal pigment epithelium (RPE) of AMD eyes indicate that the RPE cells are under oxidative stress (e.g., increased levels of heme oxygenase-1 and heme oxygenase-2 and Cu-Zn superoxide dismutase).[4] Advanced glycation end products occur in soft drusen, basal laminar and basal linear deposits, and the cytoplasm of RPE cells associated with choroidal neovascularization (CNV).[5,6] Carboxymethyl lysine is present in drusen and CNV[5,7] as are carboxyethyl pyrrole protein adducts.[5] Additionally, $Fe^{2+}$—which is an essential element for enzymes involved in the phototransduction cascade, outer segment disk membrane synthesis, and conversion of all-trans-retinyl ester to 11-cis-retinol in RPE—also catalyzes the conversion of hydrogen peroxide to hydroxyl radicals and is known to accumulate in Bruch's membrane in AMD eyes.[8,9] Epidemiologic studies indicate that one of the main risk factors for AMD is smoking, which is known to cause oxidative damage. One interpretation of the Age-Related Eye Disease Study (AREDS) (http://clinicaltrials.gov/ct2/show/NCT00000145?term=Age-Related+Eye+Disease+Study+%28AREDS%29&rank=3) results is that antioxidant supplementation reduces the risk of visual loss associated with AMD among properly selected patients, especially for patients with the *CFHTT* genotype.[10]

Second, excessive accumulation of lipofuscin in the RPE may play an important role in the pathogenesis AMD.[11] In RPE cells, the main source of lipofuscin is probably the undegradable components of phagocytized outer segments.[12] In vertebrate photoreceptors, light causes isomerization of visual pigment chromophore, 11-*cis*-retinylidene, to all-*trans*-retinylidene, followed by release of all-*trans*-retinal from the opsin binding pocket and its reduction to all-*trans*-retinol (Figure 2).[13] ABCA4, an adenosine triphosphate–binding cassette transporter present in the outer segment of rods and cones, transports *N*-retinylidene-phosphatidylethanolamine from the outer segment disks to the photoreceptor cytoplasm.[14,15] Retinol dehydrogenase 8 (in outer segments) and retinal dehydrogenase 12 (in inner segments) reduces all-*trans*-retinal to all-*trans*-retinol.[16,17] Vitamin A (all-*trans*-retinol) diffuses to RPE where it is esterified by lecithin/retinol acyltransferase (LRAT) to all-*trans*-retinyl esters and is stored in retinosomes.[18,19] All-*trans*-retinyl esters are isomerized to 11-*cis*-retinol in a reaction involving RPE-65.[20–22] Next, 11-*cis*-retinol is oxidized to 11-*cis*-retinal,[23,24] which then diffuses across the extracellular space to photoreceptors and recombines with rod-and-cone opsin proteins to regenerate visual pigments. Within the outer segment disks, ethanolamine can combine with two retinaldehyde molecules to form *N*-retinylidene-*N*-retinylethanolamine (A2E); A2E is a major fluorophore in lipofuscin found in the RPE.[25]

Third, AMD is associated with chronic inflammation in the region of the RPE, Bruch's membrane, and choroid.[26] Several lines of evidence demonstrate this fact. Drusen, for example, contain many components of the activated complement cascade.[27–29] Anatomical studies demonstrate the presence of inflammatory cells in Bruch membrane.[30] Bioactive fragments of C3 (C3a) and C5 (C5a) are present in the drusen of AMD eyes and induce vascular endothelial growth factor (VEGF) expression in RPE cells.[31] The latter findings may explain why confluent soft drusen are a risk factor for CNVs in AMD eyes. The presence of proinflammatory molecules in drusen constitutes a stimulus for chronic inflammation in the RPE–Bruch membrane–choriocapillaris complex that may result in some features of late AMD. One interpretation of the AREDS is that zinc, one of the main therapeutic ingredients of this treatment, also affects the complement system, which in turn may slow

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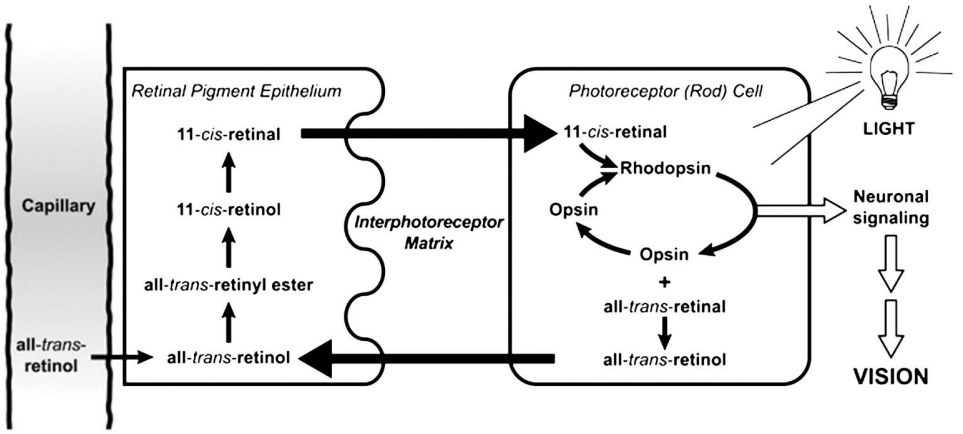


**Fig. 2.** The visual cycle. Reproduced with permission from http://lpi.oregonstate.edu/infocenter/vitamins/vitaminA/visualcycle.html. Courtesy of Jane Higdon, Linus Pauling Institute, Oregon State University, copyright 2010.

disease progression. Zinc inhibits C3 convertase activity,[32] and levels of C3a des Arg, which is a cleavage product of C3a and reflects complement activation, are higher in patients with AMD (including patients with early as well as late AMD) versus controls.[33] We are not aware of published data demonstrating that zinc supplements lower C3a des Arg levels in AMD patients.

Fourth, drusen, GA, and CNV are associated with mutations in components of the complement pathway, which is part of the innate immune system (Figure 3). Protective and risk-enhancing mutations in components of the complement pathways have been reported and include the following loci: complement factor H (CFH), complement component 2 (C2), factor B (CFB), complement component 3 (C3), and factor I (CFI).[27,35–47]

Fifth, oxidative damage can compromise regulation of the complement system by RPE cells. Thurman and Holers[48] noted that the alternative complement pathway is continuously activated in the fluid phase, and tissue surfaces require continuous complement inhibition to prevent spontaneous autologous cell injury. Sohn et al[49] demonstrated that the complement system is continuously activated in the eye. Thurman et al[50] showed that oxidative stress reduces the regulation of complement on the surface of ARPE-19 cells (i.e., reduces surface expression of the complement inhibitors, decay accelerating factor [CD55] and CD59) and impairs complement regulation at the cell surface by factor H. Sublytic activation of the complement cascade also causes VEGF release from the cells, which compromises RPE barrier function. Similarly, oxidative stress can reduce the ability of interferon-gamma to increase CFH expression in RPE cells.[51] In vitro evidence indicates that products of the photooxidation of A2E in RPE cells can serve as a trigger for the complement system.[52] Thus, the relative abundance of lipofuscin in the submacular RPE may predispose the macula to chronic inflammation and AMD, particularly in patients who cannot control complement activation because of inherited abnormalities in the complement system. Hollyfield et al[53] have described an animal model that links oxidative damage and complement activation to AMD.

Sixth, some AMD-risk enhancing mutations not directly involving the complement pathway are also linked to inflammation or oxidative damage.[54–59]

A proposed pathogenesis (Figure 4) of AMD suggests the possibility of therapeutic intervention at different points in the natural history of the disease with antioxidants, visual cycle inhibitors, antiinflammatory agents, antiangiogenic agents, and neuroprotective agents.

## Treatment

### *Antioxidants*

The AREDS did not show a statistically significant benefit of the AREDS formulation for either the development of new GA or the involvement of the fovea in eyes with preexisting atrophy.[60] In part, this result may be because of the paucity of GA patients in the study. AREDS II (http://clinicaltrials.gov/ct2/show/NCT00345176?term=Age-Related+Eye+Disease+Study+%28AREDS%29&rank=1) is a randomized, multicenter, clinical trial to assess 1) the role of lutein (10 mg)/zeaxanthin (2 mg) and omega-3 long-chain polyunsaturated fatty acids (docosahexaenoic acid [DHA]/eicosapentaenoic acid [EPA]) in prevention of development of GA or CNV; and 2) the possible deletion of beta-carotene and lowering the daily zinc oxide dose to 25 mg. A Phase 3 clinical trial is underway. A recently terminated Phase 2 clinical study, known as the OMEGA Study (Othera, Pharmaceuticals Inc., Conshohocken, PA) (http://clinicaltrials.gov/ct2/show/NCT00485394?term=OMEGA+Study&rank=1), investigated an eyedrop with a prodrug, known as OT 551

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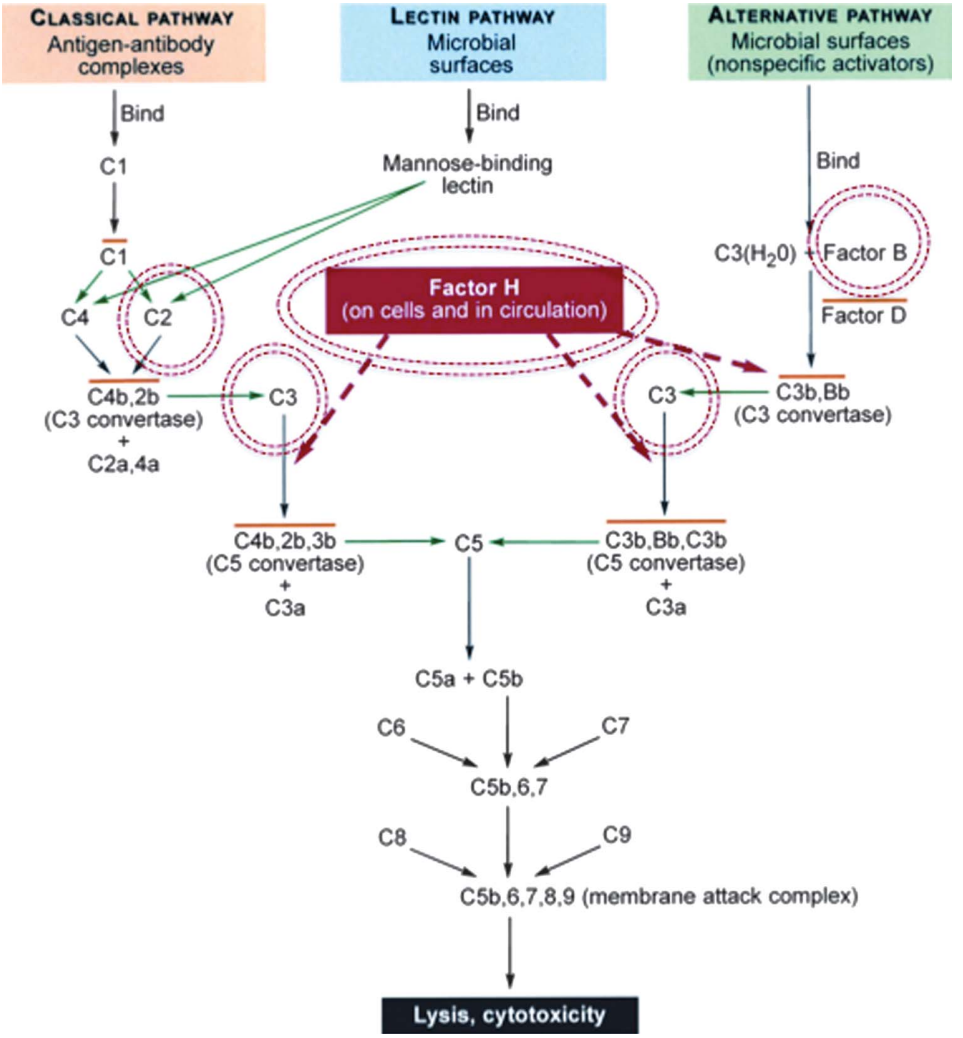


**Fig. 3.** The complement pathway. Modified with permission from Donoso et al.[2] Components of the complement system in which mutations have been associated with increased or decreased risk of drusen, GA, and CNV are circled. The coagulation system-activated intrinsic pathway is not shown.[34]

(4-cyclopropanoyloxy-1-hydroxy-2,2,6,6-tetramethypiperidine HCL), to treat GA. This prodrug penetrates the eye well and is converted to the active drug (TEMPOL-H), which has antioxidant, antiinflammatory (down regulates nuclear factor κ-B), and antiangiogenic properties in preclinical models. OT 551 failed to slow the enlargement rate of GA after 18 months. We do not know if the failure to demonstrate a treatment benefit is because of inadequate posterior segment drug delivery or because of its mechanism of action.

### *Visual Cycle Modulators*

Visual cycle modulators are intended to reduce the accumulation of toxic fluorophores (e.g., A2E) and lipofuscin in RPE cells. Retinol binding protein (RBP) possesses a high-affinity binding site for all-*trans*-retinol. The binding of retinol to RBP, in turn, creates a high-affinity binding site for transthyretin (TTR). Binding of TTR to the RBP–retinol complex creates a large molecular complex that resists filtration in the kidney and permits a high steady-state concentration of retinol in the circulation, which facilitates delivery of retinol to extrahepatic target tissues such as the eye. Unlike other extrahepatic tissues, the eye demonstrates a unique preference for uptake of retinol when it is presented in the RBP–TTR complex. *N*-(4-hydroxyphenyl) retinamide (Fenretinide; Sirion Therapeutics, Inc, Tampa, FL) displaces all-*trans*-retinol from RBP in blood. Fenretinide possesses a bulky side chain on its terminal end that prevents interaction of the complex with TTR. In the absence of TTR binding, the RBP–fenretinide complex is eliminated through glomerular filtration (excreted in urine) because of its relatively small size. Thus, fenretinide treatment causes a dose-dependent reversible reduction in circulating RBP and retinol. The unique requirement of the eye for retinol delivered by RBP renders the eye more susceptible to

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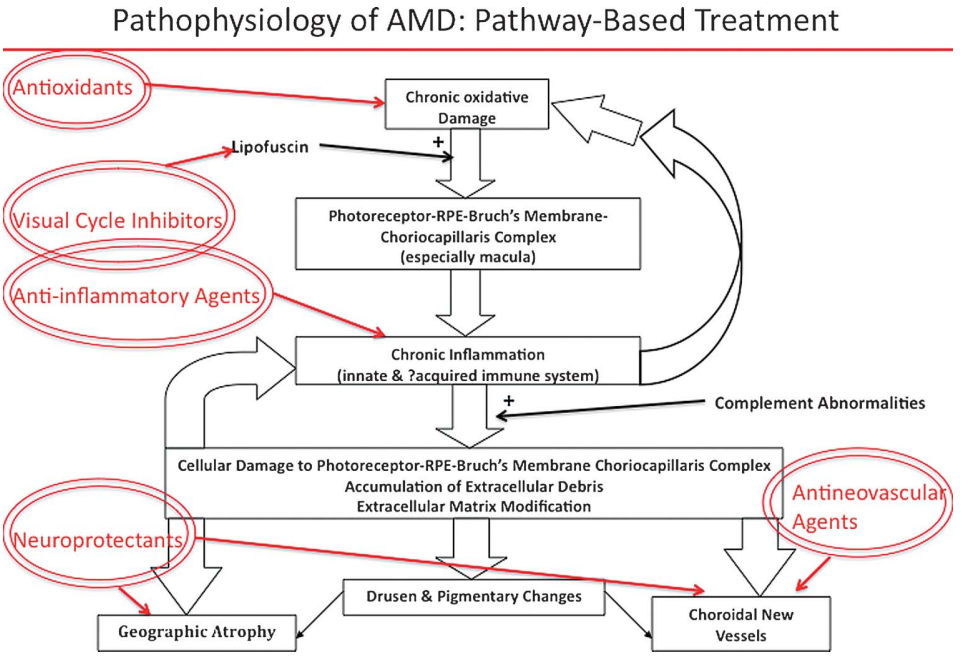


**Fig. 4.** Proposed pathophysiology of AMD and locations in the pathway in which different therapeutic interventions might be effective. Modified from Zarbin, M, Sunness JS. Dry age-related macular degeneration and age-related macular degeneration pathogenesis. In: Levin LA, Albert DM, eds. Ocular Disease: Mechanisms and Management. China: Saunders (Elsevier); 2010:527–535.

reductions in serum RBP retinol compared with other tissues. Consequently, during chronic fenretinide administration, levels of retinol within the eye will be reduced dramatically while other extrahepatic tissues will obtain retinol from alternate sources. Fenretinide reduces lipofuscin and A2E accumulation in the RPE of ABCA4$^{-/-}$ mice and causes modest delays in dark adaptation (Figure 5).[61] A Phase 2 clinical trial of this oral agent is underway. Patients receive placebo, 100-mg, or a 300-mg dose. Interim analyses reported at scientific meetings suggest a possible therapeutic effect from the drug, but the results are preliminary. Regarding possible side effects from fenretinide, we note that fenretinide has been used in clinical trials for cancer therapy and prevention of malignant neoplasms.[62–68] In these studies, which involve patients ranging in age from approximately 30 years to 60 years, the incidence of acquired night blindness ranges from 2% to 20%, with a median of approximately 15%.[65,67–72] The incidence of dry eye ranges from 3% to 53%, with a median of approximately 5%.[65,67,69,70,72] Typically, only a minority (approximately <5%) of patients had to discontinue therapy because of these side effects. Reversible dark-adaptation changes and electroretinogram abnormalities were associated with fenretinide chemotherapy (800 mg/day) for basal cell carcinoma[73] and for breast cancer (200 mg/day),[74] which is associated with a decline in plasma retinol concentration.[75] Among breast cancer patients (mean age, 48 years) using 200 mg/day, the changes in night vision were rarely symptomatic.[76] Predictive factors with a significant effect on the electroretinogram at a dose of 200 mg/day in women with breast cancer were 1) qualitative interaction between age and treatment duration and 2) plasma retinol.[64] A trend toward mild inhibition of retinal photoreceptor function after prolonged duration of intervention was observed in the older women.[64]

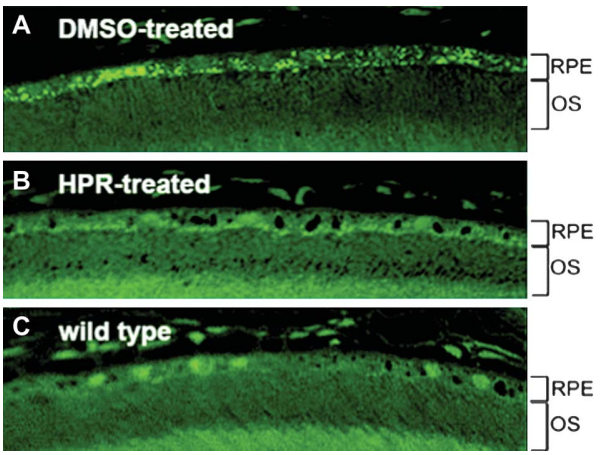


**Fig. 5.** Microscopic analysis of lipofuscin autofluorescence and cytostructure of the retina. Tissue sections were prepared from the eyes of *ABCA4*$^{-/-}$ albino and pigmented mice that had been treated with either DMSO or fenretinide (HPR) (10 mg/kg) for 42 days. Sections from albino mice were analyzed by fluorescence microscopy, while sections from pigmented mice were used for light microscopy. **A.** Analysis of lipofuscin autofluorescence revealed considerable accumulation within the RPE of DMSO-treated mice. **B.** In contrast, HPR-treated mice showed significantly reduced levels of lipofuscin fluorophores. **C.** Tissue sections prepared from an age-matched and strain-matched wild-type mouse are shown for comparison. Analysis of RPE and retina cytostructure by light microscopy revealed no aberrant morphology associated with either DMSO or HPR treatment (not shown). OS, outer segment. Reproduced with permission from Radu et al.[61]

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Aging and obesity are risk factors for diminished night vision because of a strong association with lower plasma retinol concentrations.[75] We emphasize, however, that the clinical trial in progress will determine whether observed benefits outweigh these potential side effects.

Another visual cycle modulator is 13-*cis*-retinoic acid (Accutane, Hoffmann-La Roche, Inc., Nutley, NJ), which inhibits the conversion of all-*trans*-retinyl esters (in retinosomes) to 11-*cis*-retinol and the conversion of 11-*cis*-retinol to 11-*cis*-retinal by retinol dehydrogenase and also reduces lipofuscin accumulation in ABCA4$^{-/-}$ mice.[77] This oral agent may be associated with a high incidence of nyctalopia.[78] All-*trans*-retinylamine (ACU-4429; Acucela, Seattle, WA) is an orally administered compound that inhibits conversion of all-*trans*-retinyl ester to 11-*cis*-retinol via blockade of RPE65 or another protein needed for isomerization of all-*trans*-retinol. ACU-4429 also reduces lipofuscin and A2E accumulation in the RPE of ABCA4$^{-/-}$ mice. Because this molecule works as an enzyme inhibitor (rather than by reducing availability of precursor, thus reducing rhodopsin formation via mass action kinetics), its effects should last longer than fenretinide, thus permitting less frequent dosing. However, there may be greater risk of side effects, such as nyctalopia. Retinoids and farnesyl-containing isoprenoids (TDT and TDH) also block RPE65.

Although the use of beta-carotene in the AREDS formulation and attempts to block the visual cycle as a treatment for AMD may seem contradictory, it is not clear that these treatment approaches are antagonistic. Normally, beta-carotene is metabolized to retinaldehyde. Relatively high-dose beta-carotene supplementation (not vitamin A) was used in the AREDS. In low doses, beta-carotene can act as an antioxidant.[79] High doses of beta-carotene can reduce retinoic acid levels, possibly via stimulation of cytochrome P450 activity because of the formation of eccentric cleavage products (vs. the central cleavage of beta-carotene, which forms 2 retinal molecules).[80] In addition, a free radical–rich environment also favors the formation of eccentric cleavage products, cytochrome P450 stimulation, and local retinoic acid deficiency. Thus, it is possible that in the local environment of the outer retina–RPE–Bruch membrane–choroid, fenretinide and beta-carotene may not have completely antagonistic effects.

### *Antiinflammatory Agents*

Corticosteroids have a number of antiangiogenic effects (Table 1). They have been used previously as sole treatment and as part of combination treatment for CNV.[81] Iluvien (Alimera Sciences, Alpharetta, GA) is a nonbioerodible polyimide tube containing 180 μg of the corticosteroid fluocinolone acetonide. It is inserted via a 25-gauge intravitreal injector, which creates a self-sealing wound. A Phase 2 study is underway involving 40 patients with bilateral GA, and the primary outcome is a difference in the enlargement rate of GA in treated versus untreated eyes. The study eye is randomized to high (0.5 μg/day) or low (0.2 μg/day) dose Iluvien. The fellow eye serves as a control.

Table 1. Some Antiangiogenic Effects of Corticosteroids

| |
|---|
| Induce capillary basement membrane dissolution (in growing capillaries). |
| Alter the behavior of inflammatory cells that stimulate angiogenesis. |
| Inhibit bFGF-stimulated choroidal endothelial cell migration and tube formation. |
| Inhibit bFGF-induced activation of matrix metalloproteinase-2. |
| Reduce oxidative stress–induced VEGF messenger RNA expression in ARPE-19 cells. |
| Alter intercellular adhesion molecule expression of nonendothelial cells. |
| Reduce blood–retinal barrier breakdown in rabbit eyes. |
| Inhibit platelet-derived growth factor–induced VEGF expression. |
| Reduce numbers of microglia in AMD-associated choroidal new vessels. |

Modified from Zarbin.[81]

A number of agents that modulate different parts of the complement system are in Phase 1 and Phase 2 clinical trials (Figure 6). In general, these agents work either by replacing a defective complement component (e.g., providing normal factor H to patients with Y402H mutations) so that complement activation can be modulated properly or by blocking the complement pathway (e.g., POT-4, which inhibits C3). Several examples will be discussed because they illustrate some of the challenges associated with manipulation of this pathway.

The classical, lectin, and alternative pathways generate bioactive fragments C3a and C5a and the membrane attack complex (C5b,6,7,8,9) via C3 cleavage. As a result, C3 inhibition should be very effective at blocking complement activation that arises from many different mutations involving the complement system (thus, targeting a relatively large population of AMD patients), which should be a therapeutic advantage. However, this degree of complement inhibition may create risks such as an increased risk of injection-associated endophthalmitis. In a murine model, it seems that C3 deficiency does not increase the risk of *Staphylococcus aureus* endophthalmitis.[82] Conversely, in a guinea pig model, complement depletion with cobra venom factor does seem to increase the risk of *S. aureus*, *Staphylococcus epidermidis*, and

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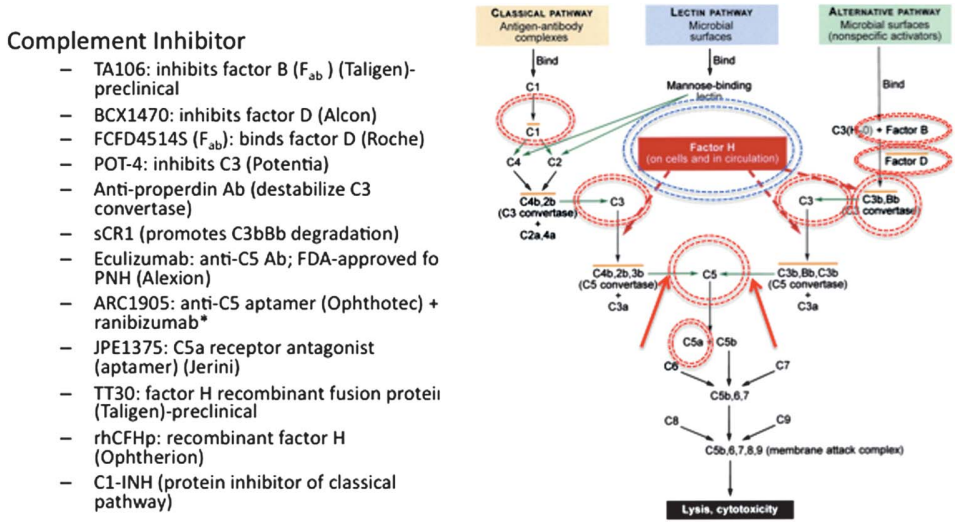


**Fig. 6.** Treatment of AMD through management of complement abnormalities. Depiction of complement pathways (on the right) is modified with permission from Donoso et al.[2] Circles and the two red arrows indicate parts of the pathway that are targets of current therapy (listed on the left).

*Pseudomonas aeruginosa* endophthalmitis.[83,84] POT-4 (Potentia Pharmaceuticals, Louisville, KY), a cyclic peptide of 13 amino acids that is a derivative of Compstatin, is a C3 inhibitor and is administered by intravitreal injection. An attractive feature of this preparation is that gel-like deposits will form in the vitreous when POT-4 is injected at high concentrations. These deposits last at least 6 months, thus providing a sustained-release delivery system. It is not known whether the doses administered intravitreally will have systemic effects, but a Phase 1 study of POT-4 in AMD eyes with CNV was completed successfully without any safety concerns (http://clinicaltrials.gov/ct2/show/NCT00473928?term=POT-4&rank=1).

Inhibition of C5 is attractive because terminal complement activity is blocked, but proximal complement functions remain intact, for example, C3a anaphylatoxin production, C3b opsonization, and immune complex and apoptotic body clearance. ARC1905 (Ophthotech Corp., Princeton, NJ) is an anti-C5 aptamer delivered by intravitreal injection. It is in Phase 1 trials for nonexudative (http://clinicaltrials.gov/ct2/show/NCT00950638?term=ARC-1905&rank=1) and exudative complications of AMD (http://clinicaltrials.gov/ct2/show/NCT00709527?term=ARC-1905&rank=2). Eculizumab (SOLIRIS, Alexion Pharmaceuticals, Cheshire, CT) is a humanized monoclonal antibody that blocks C5 and is administered intravenously. Eculizamab is already Food and Drug Administration–approved for the treatment of paroxysmal nocturnal hemoglobinuria and is in Phase 2 trials for treatment of nonexudative complications of AMD (http://clinicaltrials.gov/ct2/show/NCT00935883?term=eculizumab&rank=2). C5a receptor blockade, for example, JPE1375 (Jerini AG, Berlin, Germany); PMX025 (Arana Therapeutics, Sydney, Australia); Neutrazimab (G2 Therapies, Darlinghurst, New South Wales, Australia), might have an advantage or a disadvantage over direct C5a inhibition. C5a receptor blockade might inhibit some important inflammatory pathways[31] without preventing membrane attack complex formation.

Replacement of CFH should inhibit inflammation in AMD patients with risk-enhancing mutations in *CFH*. It is not clear whether patients with other mutations will benefit from this therapy. An attractive feature of this approach, which might require genetic screening before treatment, is that there is no increased risk of infection because we have innate systems that permit CFH to modulate C3 activation locally. The recombinant human form of the full-length CFH protein in its ''protective'' form is known as rhCFHp (Ophtherion, Inc, New Haven, CT). This protein can be administered intravenously or intravitreally. In preclinical models, intravitreal adenoviral vector delivery of the *CFH* gene has been effective and offers the promise of a sustained delivery system. (Our understanding is that Ophtherion, Inc., is not going to continue with its rhCFHp program.) Replacement of defective CFH is also being developed by Taligen (TT30, a recombinant fusion protein, Taligen Therapeutics, Cambridge, MA) (http://www.taligentherapeutics.com/pipeline/index.html). Taligen is also exploring factor B inhibition using a humanized antibody fragment (TA106).

Gene therapy to silence genes by preventing messenger RNA expression might be useful for treatment of AMD because deletion of genes closely related to *CFH* (i.e., *CFHR1* and *CFHR3*) seems to be strongly protective against AMD.[38] However, short-interfering RNA therapies in the eye may be toxic,[85]

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and it seems that the deletion of *CFHR1* and *CFHR3* protects against development of AMD at least in part because the deletion tags a protective haplotype and does not occur in association with the Y402H single-nucleotide polymorphism.[86]

Sirolimus (rapamycin; Macusight/Santen, Union City, CA) is a macrolide fungicide that targets mTOR (mammalian target of rapamycin) and is antiinflammatory, antiangiogenic, and antifibrotic; mTOR is a protein kinase that regulates proliferation, motility, survival, and protein synthesis. Rapamycin can be administered subconjunctivally and was in Phase 1/2 studies in patients with GA (http://clinicaltrials.gov/ct2/show/NCT00766649?term=rapamycin&cond=Macular+Degeneration&rank=3) as well as in monotherapy (http://clinicaltrials.gov/ct2/show/NCT00712491?term=rapamycin&cond=Macular+Degeneration&rank=1) and combination therapy trials with ranibizumab for exudative complications of AMD (http://clinicaltrials.gov/ct2/show/NCT00766337?term=rapamycin&cond=Macular+Degeneration&rank=2). Glatiramer acetate (Copaxone; TEVA, Petach Tikva, Israel) induces glatiramer acetate–specific suppressor T cells and downregulates inflammatory cytokines. It can be administered subcutaneously and is in Phase 2 and Phase 3 studies in patients with drusen (http://clinicaltrials.gov/ct2/show/NCT00466076?term=copaxone&rank=10). A small, randomized, controlled study demonstrated efficacy after 12 weeks of subcutaneous injections.[87] It remains to be shown whether drusen disappearance, the end point of this study, represents an appropriate surrogate end point for long-term visual acuity preservation in AMD eyes. The Complications of Age-Related Macular Degeneration Prevention (CAPT) trial demonstrated no long-term visual benefit to laser photocoagulation-induced drusen resorption.[88] The mechanism of action of the two treatment modalities, however, is fundamentally different. Laser treatment induces inflammation, and glatiramer acetate is antiinflammatory.

Amyloid-β oligomers are toxic to cells (soluble monomers are not). Amyloid diseases typically exhibit abundant fibrils of various lengths. These fibrils are an end product of stepwise protein/peptide misfolding, and they accumulate as long-lived extracellular deposits. Drusen vesicles probably contain fibrillar amyloid composed in part of amyloid-β, which may damage RPE cells and/or incite inflammation that contributes to AMD progression.[89,90] RN6 G (PF-4382923; Pfizer, New York, NY) is a humanized monoclonal antibody that targets the C-termini of amyloid β-40 and amyloid β-42. Both these peptides have been implicated in neurodegenerative diseases. Treatment with intravenous RN6 G is intended to prevent the accumulation of amyloid β-40 and amyloid β-42 and to prevent their cytotoxic effects. A Phase 1 clinical trial has been completed successfully (http://clinicaltrials.gov/ct2/show/NCT00877032?term=RN6G&rank=1), and a Phase 2 trial is underway for treatment of subjects with advanced nonexudative AMD.

### *Neurotrophic Agents*

Neuroprotectants can rescue photoreceptors in preclinical models of retinal degeneration such as light damage and in animal models of glaucoma and retinitis pigmentosa (RP). The pathogenesis of these conditions is not completely understood. The mechanism(s) by which neurotrophic factors promote retinal survival in these models is not established fully and may vary depending on the disease setting.[91,92]

Molecules that seem to provide the broadest degree of protection against photoreceptor degeneration include basic fibroblast growth factor (bFGF), ciliary neurotrophic factor (CNTF), brain-derived neurotrophic factor, pigment epithelium–derived factor, and interleukin-1.[93–97] Some of these molecules can be produced by RPE cells.[98–101] The RPE and retina, for example, seem to produce FGF, and the retina produces CNTF.[102] Constitutive production of bFGF is probably important for photoreceptor survival normally.[103,104] In addition, rod cells produce cone survival factors, which may explain why mutations specific for rods can cause early rod and later cone death in patients with typical RP.[105,106] Heat shock proteins may also promote retinal survival in some paradigms of retinal degeneration, including light damage, ischemia, and RP.[107–110] Administration of alpha-2 adrenergic agonists protects photoreceptors against light damage, probably because of specific induction of bFGF expression in photoreceptors.[111] Sustained delivery of CNTF can slow photoreceptor degeneration in animal models of RP, although it can be associated with side effects such as decreased electroretinogram amplitude.[104,112] It is important to note that neurotrophins do not prevent photoreceptor death in animal models of RP, they merely delay it. In addition, the duration and amplitude of pathway activation by a given neurotrophin receptor modulate the biologic response one observes.[91]

Many of the neurotrophic factors that seem to provide the broadest degree of protection are ligands for two major families of membrane-bound receptor tyrosine kinases: FGF receptors and Trk neurotrophin receptors.[93,94] Ligand binding to FGF and CNTF receptors activates various enzymes including the phosphatidyl inositol 3-kinase (PI3-K), mitogen-activated protein kinases (MEK and ERK), and Akt,

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which in turn can inhibit apoptosis (see Chaum[91] for references). In addition, phosphorylation of cAMP response element binding protein 1 (CREB1) and activating transcription factor 1 (ATF1) is an intrinsic response to photoreceptor injury arising from photoreceptor gene mutations and is found in AMD eyes.[113] Ciliary neurotrophic factor induces CREB1/ATF1 phosphorylation in normal retinas and induces increased phosphorylated CREB1/ATF1 in canine retina with the *rcd* 1 mutation.[113] At least in some cases, the photoreceptor rescue effect of neurotrophic factors (e.g., brain-derived neurotrophic factor, CNTF, and bFGF) may be mediated via Muller cells,[98–101,114–116] although a direct effect on photoreceptors is possible.[117] Nerve growth factor and brain-derived neurotrophic factor may inhibit cell death through multiple pathways, for example, PI3-K activation and *c*-jun protein inhibition (see Chaum[91] for references). Gene therapy to modulate expression of components of the signaling pathways stimulated by neurotrophins (e.g., Akt, antiapoptotic genes, or heat shock protein) might be superior to treatment with neurotrophic factors themselves.[91]

The pathophysiology of photoreceptor death associated with light damage, mechanical injury, and inherited retinal degeneration not only shares similarities but also differs in some important ways.[91,92] For example, endogenous bFGF, FGF receptor (FGFR-1), and CNTF are upregulated in response to retinal light damage and mechanical puncture, which would seem to indicate that the retina responds to injury via single mechanism.[102,118,119] However, insulin-like growth factor-1 and its receptor are upregulated in response to light but not mechanical injury,[120] which indicates that there are distinct differences in the pathophysiology of these two paradigms of retinal injury. Although L-NAME (*N*(G)-nitro-L-arginine methyl ester), an inhibitor of nitric oxide synthase, can partially protect rats from light damage, it is not effective in transgenic rat models of RP, for example, P23H and S334ter transgenic rats.[121,122] Transgenic overexpression of erythropoietin can protect against light damage but not retinal degeneration in the rd1 mouse.[123] Toxic accumulation of all-*trans*-retinal may be more important in light damage pathogenesis than is accumulation of A2E, which may be important in Stargardt disease.[124,125] Adding to the complexity of assessing neurotrophic agents in preclinical models, there seem to be differences in the pathophysiology of light damage in mice and rats. For example, RPE65 is an important determinant of susceptibility to light damage in mice but not in rats (see Wenzel et al[92] for references). The relevance of light damage models and animal models of RP to AMD is not clear, although mutations in ABCA4 have been associated with AMD in some, but not all, studies.[126–128] Furthermore, some strategies that are protective in a given retinal degeneration model, for example, Bcl-2 overexpression or oxidative stress reduction, may not be effective in another.[129–132] Finally, the therapeutic effect of an intervention may not only depend on the disease but also on the way the therapy is delivered. The route of delivery (e.g., intravitreal injection vs. viral vector–mediated transfection vs. cell-based delivery system) and the steady-state level seem to be important in determining the effectiveness of bFGF-mediated photoreceptor rescue in RP models.[133–136] It is with these reservations in mind that one should contemplate strategies for neuroprotection in AMD based on the results of light damage experiments and animal models of RP. Currently, a brimonidine sustained release implant (brimonidine [alpha-2 adrenergic receptor agonist] formulated in the Allergan Novadur (Allergan, Irvine, CA) sustained release delivery system) and topical tandospirone (AL-8309B; Alcon Inc., Hünenberg Switzerland, serotonin 1A receptor agonist) are in clinical trials for AMD based on their effectiveness in preventing retinal degeneration in preclinical light damage models (http://clinicaltrials.gov/ct2/show/NCT00658619?term=brimonidine&rank=6; http://clinicaltrials.gov/ct2/show/NCT00890097?term=AL-8309B&rank=1). Serotonin 1A agonists are neuroprotective in animal models of excitotoxic neuronal damage.[137] Neuroprotection may arise from their hyperpolarizing effects on cells, mediated via G protein–coupled $K^+$ channels, and/or stimulation of nerve growth factor release by neurons.[138]

The CNTF Study (http://clinicaltrials.gov/ct2/show/NCT00063765?term=CNTF&rank=1) used intravitreal implants of genetically modified RPE that overexpress CNTF and are contained within a semipermeable capsule with small pores that permit CNTF to escape into the vitreous cavity and protect the allogeneic RPE cells from immune rejection. A Phase 1 study in patients with RP was completed successfully, and the data suggested that visual acuity was improved in some patients.[139] Neurotech (Lincoln, RI) is testing the ability of these implants to arrest/retard the progression of GA in a multicenter, randomized, double-masked, sham-controlled Phase 2 study of 51 patients with GA (http://clinicaltrials.gov/ct2/show/NCT00447954?term=CNTF&rank=2). Patients receive either high-dose or low-dose implant or sham treatment in one eye only. At 12-month follow-up, 96.3% of patients in the high-dose NT-501-treated cohort lost <15 letters (Early Treatment Diabetic Retinopathy Study chart) versus 75% patients in the sham group ($P$ = 0.078). No increase in vision occurred, and no serious adverse events were reported.

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The trend in visual stabilization at 12 months was preceded (at four months) by a dose-dependent statistically significant increase ($P < 0.001$ and $P = 0.013$ for the high-dose and low-dose cohorts, respectively) in retinal thickness by optical coherence tomography. Ciliary neurotrophic factor–induced increased retinal thickness has been observed in laboratory animals with RP-like conditions.[112,140] In mice, this thickness change reflects, in part, increased photoreceptor nuclear size and increased amounts of euchromatin; in rcd-1 dogs, it reflects increased photoreceptor nuclear size and swelling of photoreceptors and/or Muller cell processes with expansion of the outer limiting membrane toward the RPE.

### *Antiangiogenic Agents*

Angiogenesis is a multistep process (Figure 7), and as a result, many different substances can stimulate or inhibit CNV in preclinical models (see Zarbin[1] for references). Choroidal neovascularization inhibitors can be classified according to these pathophysiological steps.

### *Intracellular Angiogenic Factor Production*

As noted above, activation of mTOR, a tyrosine kinase that regulates cell growth and proliferation, leads to production of hypoxia-inducible factors (e.g., HIF1-alpha) that induces gene activation, including VEGF. Rapamycin (Sirolimus; Macusight/Santen), RAD001 (Everolimus; Novartis International AG, Basel, Switzerland), and P529 (Palomid 529; Paloma Pharmaceuticals, Jamaica Plain, MA) are mTOR inhibitors. Sirolimus is in Phase 1 and Phase 2 clinical trials of AMD patients with CNV (www.clinicaltrials.gov/ct2/show/NCT00712491?term=sirolimus&rank=11), and oral Everolimus is in Phase 2 trials in combination with ranibizumab (http://clinicaltrials.gov/ct2/show/NCT00857259?term=Novartis&cond=Macular+Degeneration&rank=1). Palomid 529 is a nonsteroidal small molecule that inhibits both the TORC1 (transducer of regulated cyclic adenosine monophosphate response–element binding protein) and TORC2 complexes of mTOR and thus inhibits the PI3-K/Akt/mTOR transduction pathway (rapamycin is a TORC1 antagonist). Palomid 529 is in Phase 1 clinical trials (http://clinicaltrials.gov/ct2/results?term=Palomid+529). REDD1 acts through mTOR/HIF1-alpha to produce VEGF. RTP801i-14 (PF04523655, Quark/Pfizer, Freemont, CA), a small inhibitory RNA (siRNA) that targets REDD1 to suppress VEGF production, is in Phase 2 clinical trials for treatment of CNV (http://clinicaltrials.gov/ct2/show/NCT00713518). Bevasiranib (Opko Health, Inc., Miami, FL), an siRNA that inhibits VEGF, was in a Phase 3 clinical trial that was terminated (http://www.clinicaltrials.gov/ct2/show/NCT00499590?term=bevasiranib&rank=1). We note that the effect of siRNAs on CNV may not be through suppression of RNA-specific gene expression but through activation of toll-like receptor 3 via a class effect (i.e., any siRNA 21 nucleotides or longer seems to inhibit CNV).[141]

### *Extracellular Angiogenic Factors*

Pigment epithelium–derived factor is neuroprotective, inhibits the effects of VEGF, inhibits endothelial migration, and is decreased in AMD.[101,142–144] A Phase 1 clinical trial (http://clinicaltrials.gov/ct2/show?term=PEDF&rank=2) was initiated using an adenoviral vector to deliver pigment epithelium–derived factor via

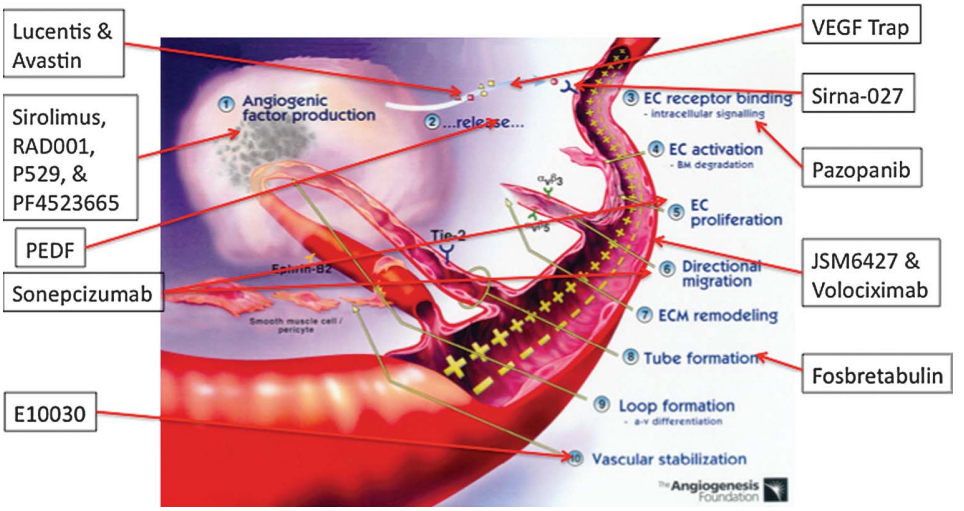


**Fig. 7.** Angiogenesis: A multistep process. The angiogenesis pathway figure is modified with permission from The Angiogenesis Foundation (www.aniog.org).

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an intravitreal injection.[145] Ranibizumab (Lucentis; Genentech/Roche) and bevacizumab (Avastin; Genentech/Roche, South San Francisco, CA) block the action of released VEGF by binding all isoforms of VEGF-A, and ranibizumab is the most effective agent for CNV treatment in AMD shown in randomized, multicenter, clinical trials.[146–149] VEGF Trap (Regeneron Pharmaceuticals, Tarrytown, NY) is a fusion protein comprising segments of the extracellular domains of human VEGF receptor-1 (VEGFR-1) and VEGF receptor-2 (VEGFR-2) fused to the constant region (Fc) of human IgG1. It binds to all isoforms of VEGF-A with higher affinity than ranibizumab and bevacizumab and also binds placental growth factors-1 and 2 and was well tolerated in a Phase 1 clinical trial.[150] VEGF Trap-Eye (http://clinicaltrials.gov/ct2/show/NCT00509795?term=VEGF+Trap-Eye&rank=14) is formulated for intravitreal injection, appears to be effective in a Phase 2 trial (www.bmctoday.net/retinatoday/2009/10/article.asp?f=1009_08.php), and is now being compared with ranibizumab in a Phase 3 clinical trial. AAV2-sFLT01 (Genzyme, Cambridge, MA) is an adeno-associated virus that codes for VEGFR-1. It is delivered to retinal cells by intravitreal injection, and the goal is to bind free VEGF to interrupt VEGF signaling. A Phase 1 trial is underway (http://www.clinicaltrials.gov/ct2/show/NCT01024998?term=AAV2-sFLT01&rank=1).

### *Endothelial Cell Receptor Binding*

Vascular endothelial growth factor-A binds primarily to VEGFR-1 and VEGFR-2, both of which are tyrosine kinases.[151] AGN211745 (Sirna-027; Allergan, Irvine, CA) is an siRNA that inhibits VEGFR-1 synthesis and was tested in a Phase 2 trial that has been terminated (http://www.clinicaltrials.gov/ct2/show/NCT00395057?term=Sirna&rank=3).

### *Endothelial Cell Activation*

Pazopanib (GlaxoSmithKline-USA, Philadelphia, PA) is a tyrosine kinase inhibitor that blocks the action of VEGFR-1, VEGFR-2, and VEGFR-3 and is active against platelet-derived growth factor receptor, c-Kit, and FGFR-1. It is effective in preclinical models of CNV.[152] It is administered topically and has been tested in a Phase 2 trial (http://www.clinicaltrials.gov/ct2/show/NCT00612456term=pazopanib&rank=31). A Phase 2a study (70 patients) demonstrated a mean 4.3-letter increase in visual acuity after treatment with topical pazopanib (5 mg/ml) three times a day (*Ophthalmology Times*, March 1, 2010, p. 34). Patients with the *CFH* TT genotype (i.e., the alleles least likely to be associated with AMD) exhibited the best response, both from the standpoint of visual acuity and reduction in retinal thickness. PTK787 (Valatanib; Novartis International AG, Basel, Switzerland) is an oral protein kinase inhibitor that has been tested previously as a treatment for CNV in Phase 1 and Phase 2 clinical studies (http://www.clinicaltrials.gov/ct2/show/NCT00138632?term=Vatalanib+(PTK787,+Novartis)&rank=2). TG100801 (TargeGen, Inc., San Diego, CA), another topically administered tyrosine kinase inhibitor, was in Phase 2 clinical trial (http://clinicaltrials.gov/ct2/show/NCT00509548?term=Targegen&rank=2), but the study was terminated because of safety concerns. AL39324 (Alcon, Inc., Hünenberg, Switzerland) is an intravitreally administered tyrosine kinase inhibitor that is about to enter Phase 2 clinical trials with ranibizumab using ranibizumab alone as the active control group (www.clinicaltrials.gov/ct2/show/NCT00992563). ATG-3 (mecamylamine; CoMentis, Inc., South San Francisco, CA) antagonizes the nicotinic cholinergic receptor pathway in vasculature, inhibits endogenous and VEGF-induced angiogenesis in human endothelial cells, is administered topically, and has now completed a Phase 2 clinical trial (http://www.clinicaltrials.gov/ct2/ show/NCT00607750?term=Comentis&rank=1).

### *Endothelial Cell Proliferation*

Sonepcizumab (LT1009; Lpath, Inc., San Diego, CA) is a humanized monoclonal antibody directed against sphingosine-1 phosphate. Sphingosine-1 phosphate is the extracellular ligand for the G protein–coupled lysophospholipid receptor EDG-1 (endothelial differentiation gene-1). Sonepcizumab inhibits CNV in preclinical models,[153] is administered intravitreally, and is in a Phase 1 human trial (http://www.clinicaltrials.gov/ct2/show/NCT00767949?term=LT1009&rank=2).

### *Endothelial Cell Directional Migration*

Endothelial cell migration involves interactions between integrins (transmembrane heterodimeric proteins) and extracellular matrix ligands. Integrins are critical for endothelial cell migration.[154] JSM6427 (Jerini Ophthalmic AG, Berlin, Germany) is an apha-5 beta-1 integrin antagonist that inhibits CNV formation in preclinical models and was in a Phase 1 clinical trial (www.clinicaltrials.gov/ct2/show/NCT00536016?term=JSM6427&rank=1), although its future development is uncertain.[155] Volociximab (Ophthotec Corp., Princeton, NJ) is a monoclonal antibody that blocks integrin alpha-5 beta-1 binding to fibronectin and is in Phase 1 studies in combination with ranibizumab (http://www.clinicaltrials.gov/ct2/show/NCT00782093?term=volociximab&rank=9).

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*Extracellular Matrix Remodeling*

We are unaware of any molecules acting in this part of the pathway that are directly relevant to AMD at this time.

*Tube Formation*

Fosbretabulin (combrestatin-A4 (CA4) phosphate; OXiGENE Inc., South San Francisco, CA) is metabolized to CA4, which binds tubulin, inhibits microtubule assembly, and leads to occlusion of the vascular lumen. It is administered intravenously and is in Phase 2 clinical trials (http://clinicaltrials.gov/ct2/show/NCT01023295?term=fosbretabulin&rank=1) for treatment of CNV associated with polypoidal choroidal vasculopathy.

*Loop Formation (Arteriovenous Differentiation)*

We are unaware of any molecules acting in this part of the pathway that are directly relevant to AMD at this time.

*Vascular Stabilization*

Pericyte recruitment is a critical step in vascular maturation.[156] E10030 (Ophthotec Corp., Princeton, NJ) is a pegylated aptamer that inhibits platelet-derived growth factor-beta. In preclinical studies, it strips pericytes from endothelial cells and renders more mature CNV sensitive to VEGF inhibition, thus potentially making VEGF inhibition more effective as a CNV treatment for early and late CNV.[157,158] A Phase 1 dose-escalating, multicenter, uncontrolled, single-dose and multiple-dose study (http://clinicaltrials.gov/ct2/show/NCT00569140?term=E10030&rank=1) (22 patients) evaluated the safety and tolerability of intravitreal E10030 + ranibizumab in AMD-associated CNV (www.bmctoday.net/retinatoday/2009/10/article.asp?f=1009_12.php). Moderate visual improvement occurred in 59% of the combination therapy cohort versus 36% of the ranibizumab cohort during a 3-month follow-up. In addition, 91% of cases in the combination therapy cohort demonstrated some degree of CNV regression by fluorescein angiography (in some cases, by OCT as well) versus 12% of the ranibizumab cohort. A Phase 2 study is underway (http://clinicaltrials.gov/ct2/show/NCT01089517?term=PDGF&rank=9).

## Combination Therapy

The main benefit of developing pathway-based therapies may be that they can be combined rationally to create "super" therapies. Synergistic effects of combination therapy have been demonstrated in the treatment of infectious disease and cancer. Sequential inhibition of folate metabolism by trimethoprim and sulfamethoxazole, for example, leads to a synergistic antibacterial effect. The combination of VEGF blockade (e.g., with bevacizumab) and chemotherapy or radiation therapy results in a greater antitumor effect than with either treatment alone.[159,160] Combining AMD treatments with differing mechanisms of action may have synergistic effects that might result in 1) better visual outcome; 2) reduced frequency of treatment; 3) greater patient convenience (e.g., subconjunctival vs. intravitreal injection); 4) lower risk of adverse events (e.g., endophthalmitis); and/or 5) less likelihood of "escape." (Escape refers to the phenomenon in which cells [e.g., tumor cells] develop alternative pathways that allow them to overcome the iatrogenic inhibition of a pathway essential for their survival or growth.) Some combinations have been tested already, for example, anti-VEGF therapy + verteporfin-photodynamic therapy (PDT)[161] ± dexamethasone.[162] The principal benefit of combining anti-VEGF therapy and PDT (with or without steroid) seems to be reduction in the number of treatments needed to stabilize the CNV. Large, multicenter, randomized studies (DENALI [http://www.clinicaltrials.gov/ct2/show/NCT00433017?term=Novartis&cond=Macular+Degeneration&rank=4], Mont Blanc [http://www.clinicaltrials.gov/ct2/show/NCT00433017?term=Novartis&cond=macular+degeneration&intr=verteporfin&rank=1], RADICAL [http://clinicaltrials.gov/ct2/show/NCT00492284?term=RADICAL&rank=2]) are underway, and so far, based on results presented at research meetings, the combination of anti-VEGF therapy and PDT has shown no great advantage over anti-VEGF therapy alone. For example, 12-month results of the DENALI study were reported at the World Ophthalmology Congress 2010 in Berlin (http://www.qltinc.com/newsCenter/2010/100615.htm). This Phase 3b study involved combining verteporfin PDT (Visudyne; Novartis Pharma AG and QLT, Inc., Vancouver, British Columbia, Canada) with ranibizumab (Lucentis; Genentech/Roche) with 3 ranibizumab loading doses followed by additional injections on a monthly as-needed basis in patients (n = 321) with subfoveal CNV (all lesion types) secondary to AMD. At Month 12, patients in the standard-fluence combination group gained on average 5.3 letters from baseline, and patients in the reduced-fluence combination group gained on average 4.4 letters. Patients in the ranibizumab monthly monotherapy group gained on average 8.1 letters at Month 12. DENALI did not demonstrate noninferior visual acuity gain for verteporfin PDT-ranibizumab combination therapy compared with ranibizumab monthly monotherapy. On average, patients in the combination groups required 2.2 (standard fluence) or 2.8 (reduced fluence) additional ranibizumab injections after the

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mandatory 3 loading doses as compared with an average of 7.6 additional injections in the ranibizumab monthly monotherapy cohort. The RADICAL study, a Phase 2 trial, included 162 patients randomized to 1 of 4 treatment arms: double therapy of PDT with reduced-fluence verteporfin PDT followed by ranibizumab (n = 43), reduced-fluence verteporfin PDT followed by ranibizumab-dexamethasone triple therapy (n = 39), very low-fluence verteporfin PDT followed by ranibizumab-dexamethasone triple therapy (n = 39), or ranibizumab monotherapy (n = 41). At 24-months follow-up, mean visual acuity change from baseline was not statistically different among the treatment groups. Through 24 months, patients in the triple therapy half-fluence group had a mean of 4.2 retreatment visits compared with 8.9 for patients who received ranibizumab monotherapy ($P < 0.001$). At Month 24, mean visual acuity in the triple therapy half-fluence group improved 1.8 letters fewer (95% confidence interval, 11.1 letters fewer to 7.6 letters better) compared with the ranibizumab monotherapy group ($P = 0.71$) (*Retina Today*, May/June 2010).

Anti-VEGF therapy is also being combined with macular radiation therapy. Two different radiation approaches are currently in clinical trials: the internal approach, which requires a pars plana vitrectomy and the introduction of a radioactive probe (NeoVista, Inc, Newark, CA, http://clinicaltrials.gov/ct2/show/NCT00454389?term=Neovista%2C+Inc.&rank=1), and the office-based external approach, which is delivered through the inferior sclera (Oraya Therapeutics, Inc, Newark, CA, http://clinicaltrials.gov/ct2/show/NCT01016873?term=Oraya%2C+Inc&rank=1). Combination intraocular epiretinal irradiation and ranibizumab appears to result in a reduced need for intravitreal injection (with comparable visual outcome) compared with intravitreal ranibizumab therapy alone,[163] a conclusion that is being assessed in a Phase 3 study (http://clinicaltrials.gov/ct2/show/NCT00454389?term=CABERNET&rank=1, CABERNET). The type of synergistic benefit we may hope to see by proper combination of pathway-based therapies is well illustrated by the early results of the combination E10030 + ranibizumab trial mentioned above.

## Conclusions

The era of pathway-based therapy for the early and late manifestations of AMD has begun. At each step in the pathogenesis of AMD, a new treatment (and a new company!) may be developed. No matter how many treatments are developed, they all will address some step in the pathway that leads from early to late AMD. Steps in AMD pathogenesis that appear to be good targets for drug development include 1) oxidative damage; 2) lipofuscin accumulation; 3) chronic inflammation; 4) mutations in the complement pathway; and 5) non-complement mutations that influence chronic inflammation and/or oxidative damage (e.g., mitochondria, extracellular matrix structure). Steps in neovascularization that can be targeted for drug development and combination therapy include 1) angiogenic factor production; 2) extracellular factor release; 3) binding of factors to extracellular receptors (and activation of intracellular signaling after receptor binding); 4) endothelial cell activation (and basement membrane degradation); 5) endothelial cell proliferation; 6) directed endothelial cell migration; 7) extracellular matrix remodeling; 8) tube formation; and 9) vascular stabilization. Combination therapy will likely supplant monotherapy as the treatment of choice because the clinical benefits (visual acuity and frequency of treatment) will likely be superior to monotherpay in preventing the late-stage complications of AMD. We speculate that there will be so many different possible combinations that companies demonstrating clinical efficacy for a new compound will be unlikely to identify the adjunctive agents with which that compound should be combined for maximally effective combination therapy. (Generally, clinical trials involve comparison between the test compound + standard therapy vs. an active control group receiving standard therapy. More complex trials would be quite expensive and logistically daunting.) Stochastic processes to identify the best combination are available and might provide the fastest route to the identification of ''ideal'' combinations.[164,165] Alternatively, individual physicians might be able to use knowledge of the pathways involved in AMD pathogenesis and the various treatment options to identify optimal combinations (and doses) of agents. These practicing physicians will play an important role in identifying and developing effective treatments for the blinding complications of AMD.

**Key words:** age-related macular degeneration, angiogenesis, choroidal neovascularization, complement, geographic atrophy, neuroprotection, oxidative damage, treatment.

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