

New molecular targets for the treatment of neovascular age-related macular degeneration

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Abstract

Age-related macular degeneration (AMD) is a progressive chronic disease that currently represents the leading cause of irreversible vision loss in the western world. Experimental and clinical evidence has demonstrated that vascular endothelial growth factor A (VEGF-A) plays an important role in promoting the choroidal neovascularization that characterizes the wet form of AMD. Intravitreal injection of anti-VEGF-A agents is the current treatment of choice for neovascular AMD (nAMD). These agents have brought about dramatic changes in the treatment of nAMD, but most patients require frequently repeated injections and regular long-term follow-up, with a significant percentage of them showing resistance to anti-VEGF-A drugs. Thus, the identification of additional therapies that could improve the treatment protocols is needed. There are numerous areas of investigation into new treatments, with increasing efforts being made to study drugs that address various targets along the angiogenic signaling cascade, or other pathways related to the onset of nAMD. The aim of the present review is to summarize and discuss promising new therapies and targets that have the potential to improve outcomes and to lengthen treatment durability, especially in patients with recalcitrant or recurrent forms of nAMD.

Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in developed countries and its prevalence and incidence is likely to increase dramatically with the aging of the population.¹ The

exudative form of AMD, although less common than the atrophic form, is more threatening to vision: 90% of those with severe visual loss due to AMD suffer from the exudative type.

The main pathological feature that characterizes the exudative form of AMD is choroidal neovascularization (CNV). Although many aspects of the molecular pathogenesis of exudative AMD have not yet been elucidated, experimental and clinical evidence has demonstrated that vascular endothelial growth factor A (VEGF-A) plays a central role in promoting CNV.²⁻⁴ Intravitreal injection of anti-VEGF-A agents is the current treatment of choice for neovascular AMD (nAMD), and five molecules have been introduced into clinical practice since 2004 (bevacizumab, pegaptanib, ranibizumab, aflibercept and a molecule newly approved for the Chinese market, conbercept). This has brought about dramatic changes in the management of the pathology, but the challenges related to the treatment of nAMD remain numerous. Thus, there is a need for efforts to investigate new areas involved in the pathophysiology of neovascularization (NV) of the eye. Many fields of interest seem to be promising: among them, research regarding new molecular targets involved in the neoangiogenic pathway, together with VEGF-A, would appear to have the potential to bring significant therapeutic improvements in the near future.

Neoangiogenesis in age-related macular degeneration

Neoangiogenesis is a physiological mechanism involved in many embryonic and adult life processes, such as wound healing, blood vessels reforming during menstruation, placenta formation, *etc.* Neoangiogenesis may also occur as an unfavourable phenomenon, as is the case, for example, with tumor growth, or in physiologically avascular tissues, such as the cornea, vitreous body or the macular region of the retina. Two major types of ocular NV affect the retina: retinal NV, which is the pathogenetic basis for proliferative diabetic retinopathy, retinal vein occlusions, and retinopathy of prematurity; and subretinal or CNV.⁵ Subretinal and CNV occur in diseases of the outer retina and Bruch's membrane, the most prevalent being nAMD. There is a considerable overlap in the vasoactive molecules involved in retinal and CNV, as both result from a functional dominance of angiogenic factors over antiangiogenic/angiostatic factors, regardless of whether the primary event is an

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increase in angiogenic activity or a decrease in antiangiogenic activity. Although it has not been clearly demonstrated, hypoxia is probably the common *primum movens*: it provokes the expression of hypoxia-inducible factor-1 α (HIF-1 α), which, after binding with HIF-1 β , becomes an active molecule (HIF-1) capable of promoting the expression of several genes⁶ including VEGF-A, platelet-derived growth factor (PDGF), stromal cell-derived growth factor-1 (SDF-1), and placental growth factor (PIGF).

Challenges to current therapeutic strategies

Despite the success of anti-VEGF-A drugs, modifications and improvements in the management of nAMD are still warranted in relation to several aspects.

Treatment regimens and follow-up frequency

The treatment of nAMD requires repeated injections and long-term, frequent follow-up. Different treatment schedules have been designed in order to reduce the treatment burden. The most commonly used

ones are the *pro-re-nata* (PRN) and the *treat-and-extend* regimens.⁷⁻⁸

Analysis of the CATT and the IVAN trials shows that monthly administration has a better effect on vision than an *as needed* approach,⁹⁻¹⁰ although clinicians have not yet reached a consensus regarding the best treatment regimen. Nevertheless, there is evidence that shorter injection intervals lead to better visual outcome and steady anatomic improvements. The high costs of frequent anti-VEGF-A drug injections and of a strict regimen of follow-up visits render the treatment of nAMD financially burdensome.

Complications

Although it is widespread as a technique, the practice of intravitreal injection is not completely free of complications. The intravitreal injection of anti-VEGF-A drugs is related to many adverse events, such as subconjunctival hemorrhages, retinal tears and retinal detachments, vitreous hemorrhages, retinal vascular occlusion, increase of intraocular pressure, cataract, tachyphylaxis, intraocular inflammation and endophthalmitis.¹¹⁻¹⁴

Blockage of the VEGF-A pathway itself seems to have potentially negative effects on retinal trophism, due to the role that VEGF-A may play in the maintenance of the choriocapillaris, Müller cells and photoreceptors.¹⁵ Long-term intravitreal injection of anti-VEGF-A drugs has been correlated with the possible progression of areas of geographic atrophy in patients with nAMD.¹⁶

Visual loss despite anti-vascular endothelial growth factor A therapy

Inadequate response to anti-VEGF-A drugs is, unfortunately, a common finding and much CNV remains active despite a protocol of frequent and timely injections. The SEVEN-UP study showed that, approximately 7 years after ranibizumab therapy in the ANCHOR and MARINA trials, one third of patients demonstrated good visual outcomes, whereas another third had poor visual outcomes.¹⁷ The causes for resistance to anti-VEGF-A therapy are hypothesized to be various: mechanisms of tolerance, tachyphylaxis, compensation by other angiogenic factors, changes in vascular architecture, sustained activation of the complement system, as well as inflammatory response, misdiagnosis and genetic variants.¹⁸

Future directions: a focus on new molecular targets

Considering the unresolved problems connected to the current management of patients affected by nAMD, many efforts are being made worldwide in order to design new therapeutic approaches to this multifactorial pathology. It is not the aim of this contribution to discuss new drug delivery methods, which nonetheless represent a very promising field of interest and a valuable potential source of improvement for the current therapeutic strategies. The purpose of this review is to briefly review the promising new molecular targets for neoangiogenesis (Figure 1), inflammation or fibrosis inhibition (Figure 2), that could represent a potential resource in the treatment of nAMD and the related drugs in various stages of development (Table 1).

Other antagonists to the vascular endothelial growth factor A pathway

- *Designed ankyrin repeat protein family (DARPin)*. Designed ankyrin repeat proteins are small, single-domain proteins that can selectively bind to a target protein with a higher level of affinity and specificity than an antibody.

Abicipar pegol (Allergan), previously known as MP0112, is a recombinant protein of the designed ankyrin repeat protein family. It binds to all isoforms of VEGF-A in a similar manner to the currently used anti-VEGF-A agents, but with a higher affinity. A phase II randomized double-blind clinical trial (ClinicalTrials.gov identifier: NCT02181517) evaluated abicipar pegol for AMD compared to ranibizumab. The first results from the phase II study have already been published¹⁹: patients received abicipar pegol every month for 3 months, while the control group received ranibizumab monthly for the entire duration of the study. The study was not powered enough to show statistically significant differences between the two drugs, but it showed good results in terms of the efficacy and durability of abicipar pegol. A phase III study evaluating the safety and efficacy of abicipar pegol in patients with nAMD is ongoing (ClinicalTrials.gov identifier: NCT02462486).

- *TH258*. RTH258 (formerly ESBA1008) is a humanized single chain antibody fragment that inhibits all isoforms of VEGF-A. It is a smaller anti-VEGF-A agent than ranibizumab or aflibercept and, because of its high stability and solubility, it is possible to concentrate RTH258 up to 120 mg/mL, allowing the

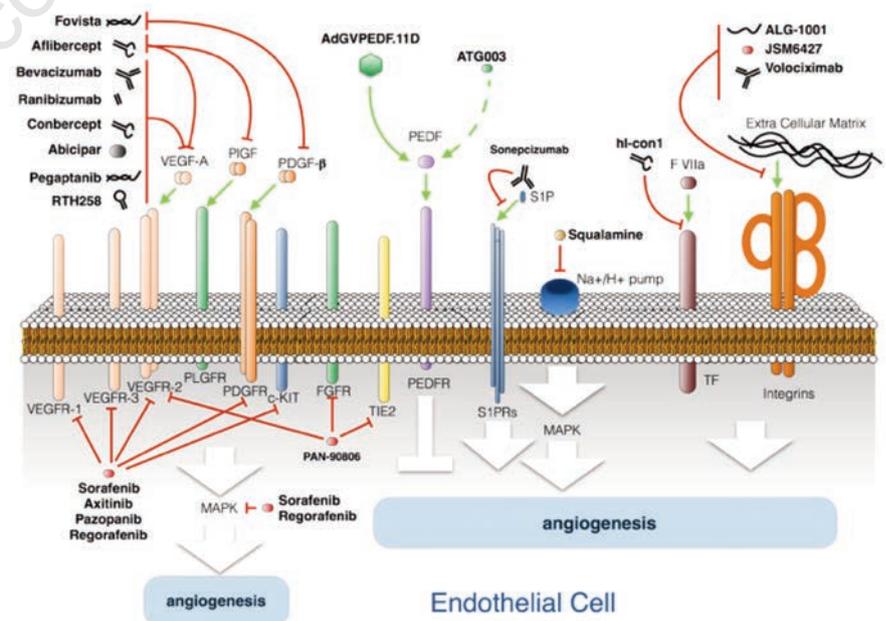


Figure 1. Schematic representation summarizing the antiangiogenic therapy drugs and their targets. See text for details.

- administration of 6 mg in a single 50- μ L intravitreal injection. This enables the delivery of a much higher molar dose in the same volume as the current VEGF-A inhibitors in clinical use, potentially supporting the early initiation and prolonged duration of the effects of treatment. Animal studies have shown that the small size of RTH258 leads to faster systemic clearance and lower systemic exposure compared to anti-VEGF-A agents such as ranibizumab and bevacizumab. The smaller size may also allow for better ocular tissue penetration.²⁰⁻²¹ A six-month phase I/II prospective multicenter double-masked and randomized clinical trial demonstrated non-inferiority in the change in central subfield thickness at 1 month for the 4.5- and 6.0-mg RTH258 doses compared to ranibizumab, and an increase of 30 days in the median time to retreatment for the 6.0-mg dose.²² A phase III clinical trial comparing the efficacy and safety of RTH258 versus aflibercept in subjects with nAMD is ongoing (ClinicalTrials.gov identifier: NCT02307682).
- **Sphingosine-1-phosphate (S1P) antibody.** S1P is a bioactive lipid mediator whose biological activities are moderated by the surface receptors found on endothelial cells. Retinal pigment epithelium (RPE) may release and store S1P in the retina and could contribute to the pathological angiogenesis, vascular permeability, fibrosis and inflammatory responses associated with nAMD.²³ Sonopimizumab, a monoclonal antibody that selectively binds to S1P, was evaluated in animal models with oxygen-induced ischemic retinopathy and was found to cause a decreased inflow of macrophages into the retina, the suppression of retinal NV, and reduced CNV after laser disruption of Bruch's membrane.²³ iSONEP (LPath, Inc, San Diego, CA, USA), the ocular formulation of sonopimizumab, was evaluated in a phase II clinical trial which did not meet its primary or key secondary endpoints. nAMD patients who had not responded adequately to existing anti-VEGF-A therapies, including Lucentis, Avastin and Eylea, did not show any statistically significant improvement in visual acuity when treated with iSONEP as an adjunctive or sole therapy (ClinicalTrials.gov identifier: NCT01414153).
 - **Squalamine lactate.** Squalamine is a small molecule derived from the cartilage of the dogfish shark (*Squalus acan-*

thias), which acts against the development of aberrant NV, by inhibiting downstream signaling pathways of multiple growth factors, including VEGF-A, PDGF and basic fibroblast growth factor (bFGF). A phase III clinical trial evaluating the efficacy and safety of ophthalmic squalamine lactate solution 0.2% (OHR-102, Ohr Pharmaceuticals, New York, NY), administered twice daily in association with intravitreal ranibizumab, is currently recruiting patients (ClinicalTrials.gov identifier: NCT02727881).

- **Small interfering RNA (siRNA).** With the aim of down-regulating the production of VEGF-A and the activity of VEGF receptors (VEGFRs), siRNA technology has been tested for the treatment of AMD. siRNAs are short RNA molecules that, once incorporated into the RNA-induced silencing complex, are able to guide the degradation of specific mRNAs. Naked siRNAs targeting VEGF-A (bevasiranib, Opko Health Inc., Miami, FL, USA) or one of its receptors, VEGFR1 (AGN 211745, previously siRNA-027; Allergan Inc., Irvine, CA, USA), were administered intravitreally and tested as drug candidates in clinical trials for CNV due to

AMD. The preliminary safety data of these two compounds were encouraging, but they failed to meet key efficacy parameters in further studies.²⁴

Platelet-derived growth factor inhibition

In vitro studies have demonstrated the role of PDGF in the process of angiogenesis.²⁵ It has been shown to promote the migration and proliferation of endothelial cells, as well as an increase in the recruitment of pericytes, which have been demonstrated to play a central role in protecting the endothelial cells against VEGF-A inhibition.²⁶

The involvement of PDGF in the ocular NV process has been demonstrated²⁷ and seems to be related to the entry of platelets and monocytes into the vitreous and sub-retinal space upon injury to the blood-retina barrier, with subsequent platelet aggregation and PDGF discharge. Interleukins such as IL-1 and TGF- β released from activated macrophages may cause further synthesis and release of PDGF.

- **Fovista.** Ophthotech (New York, U.S.A.) has developed a high affinity

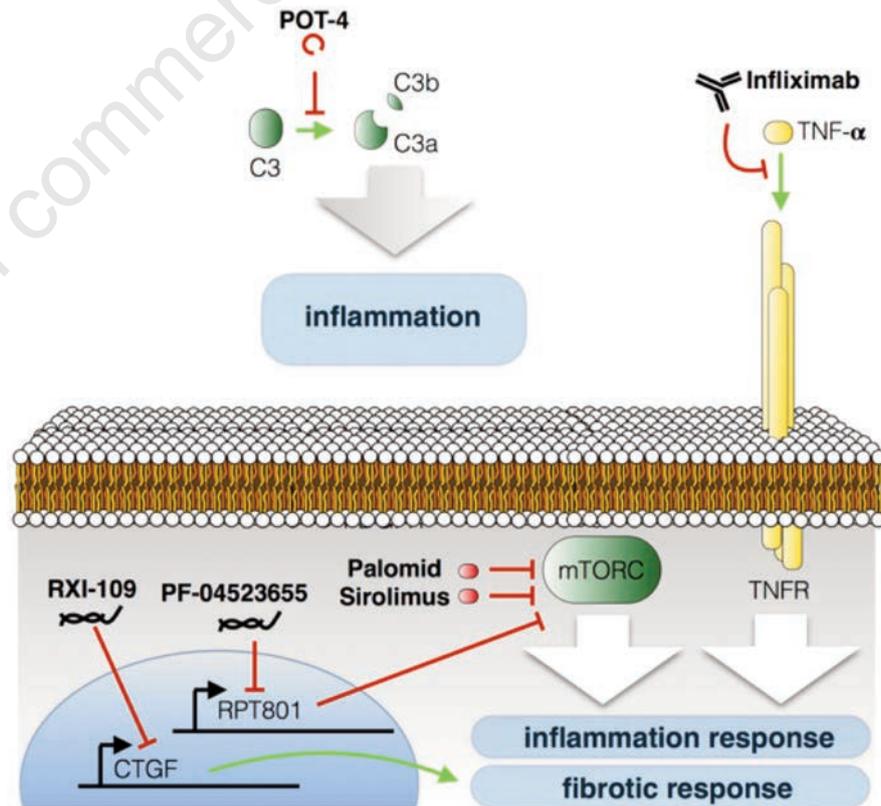


Figure 2. Schematic representation summarizing the anti-inflammatory and anti-fibrotic therapy drugs and their targets. See text for details.

PDGF antagonist called E10030 (Fovista). This molecule binds to PDGF and prevents it from binding to its receptor PDGFR- β . In vitro/in vivo studies using this compound have shown its effectiveness, in combination with an anti-VEGF-A agent, in preventing the formation of CNV, as well as in stripping pericytes from vessels that have already formed, leading to their regression. Phase-I and phase-II clinical trials investigating the safety and tolerability, and safety and efficacy, of intravitreal injection of Fovista in humans, respectively in combination with anti-VEGF-A agents or as a monotherapy, have been completed, showing Fovista as a possible future therapeutic agent when used in combination with anti-VEGF-A drugs (Clinical Trials.gov identifier: NCT00569140) (ClinicalTrials.gov identifier: NCT01089517). Two phase-III, randomized, double-blind clinical trials evaluating the safety and efficacy of the intravitreal administration of Fovista in combination with ranibizumab, compared to ranibizumab monotherapy, have recently been terminated (ClinicalTrials.gov identifier: NCT01944839) (ClinicalTrials.gov identifier: NCT01940900). Unfortunately, Ophthotech Corporation announced that the addition of Fovista to a monthly Lucentis regimen did not result in benefit as measured by the mean change in visual acuity at the 12 month time point.

Tyrosine kinase inhibition

Since, as suggested by recent research, the activity of VEGF-A and other angiogenic factors is regulated by tyrosine kinases, kinase inhibitors may be used to target VEGFRs. The high homology between VEGFRs and PDGFRs allows many kinase inhibitors to simultaneously block both.²⁸

- *Axitinib*. Axitinib is a multi-receptor tyrosine kinase inhibitor that has marked inhibitory effects on VEGFR2, PDGFR- β and c-KIT receptors. Axitinib is currently approved for use in advanced human renal cell carcinoma. Because of its anti-VEGFR and anti-PDGFR- β action, its use in nAMD treatment has been suggested. It recently showed good efficacy at a low dose in a laser-induced CNV model in rats.²⁹
- *Pazopanib*. Pazopanib (GlaxoSmithKline, Brentford, UK) (GW786034) is a small molecule that has been investigated as an

eye drop in CNV, due to its ability to act as an inhibitor of VEGFRs 1, 2, and 3; PDGF- α and β ; and the stem cell factor receptor c-KIT.³⁰⁻³⁴ A multi-country, randomized, double-masked, dose-ranging study phase IIb clinical trial was completed in 2015. It demonstrated the inferiority of daily pazopanib eye drops compared to intravitreal injection of ranibizumab in the treatment of nAMD.³⁵

- *Regorafenib*. Regorafenib (Bayer Healthcare, Leverkusen, Germany) is an eye drop multikinase inhibitor (MKI) which targets several angiogenic kinases, including VEGFR-2, VEGFR-1, VEGFR-3, fibroblast growth factor receptor 1 (FGFR1), PDGFR- β and the mutant oncogenic kinases c-KIT, RET and B-RAF. It showed positive results in a phase I clinical trial in healthy volunteers and a phase II clinical trial is

currently in progress.³⁶

- *Pan 90806*. Pan 90806 (PanOptica) is a MKI of VEGFR-2, FGFR1-3, TIE2, and of the autophosphorylation of other pro-angiogenic tyrosine kinase receptors. This molecule was initially investigated as an oral antineoplastic agent but showed poor results. Subsequently it was adopted as an eye drop for AMD and diabetic retinopathy, since it is a small molecule with high permeability and has the ability to concentrate high doses in the active form at the posterior pole. Phase I/II studies are currently ongoing (ClinicalTrials.gov identifier: NCT02022540).
- *Sorafenib*. Sorafenib is an oral MKI whose antiangiogenic action inhibits VEGFR-1, -2, and -3, as well as PDGFR- β , RAF kinase and c-KIT.³⁷ In a study using a mouse model of CNV,

Table 1. Approved and not-yet-approved drugs for age-related macular degeneration treatment.

Drug	Type of molecule	Target	Clinical trial
Drug	Type of molecule	Target	Clinical trial
Bevacizumab	Monoclonal antibody	VEGF-A	Approved
Aflibercept	Chimeric soluble receptor	VEGF-A/PIGF	Approved
Conbercept	Chimeric soluble receptor	VEGF-A	Approved
Ranibizumab	Fab fragment of antibody	VEGF-A	Approved
Pegaptanib	Pegylated aptamer	VEGF-A	Approved
Abicipar	Single-domain protein	VEGF-A	Phase III
RTH258	Single-Chain Antibody Fragment	VEGF-A (all isoforms)	Phase III
Axitinib	Small molecule	VEGFR-2/PDGFR- β /c-KIT	Phase I/II
Pazopanib	Small molecule	VEGFR-1,2,3/PDGFR α,β , c-KIT	Phase II
Regorafenib	Small molecule	VEGFR-1,2,3/c-KIT/PDGFR β /FGFR1/MAPK	Phase II
Pan 90806	Small molecule	VEGFR-1,2,3/TIE2/FGFR1,2,3	Phase I/II
Sorafenib	Small molecule	VEGFR-1,2,3/c-KIT/PDGFR β /RAF	Phase II
Sonepizumab	Monoclonal antibody	SIP	Phase II
Squalamine	Small molecule	proton pumps	Phase III
Fovista	Pegylated Aptamer	PDGF	Phase III
AdGVPEDF.11D	adenoviral vector	PEDF expression	Phase I
ATG003	Small molecule	nAChR/PEDF expression	Phase II
JSM6427	Small molecule	$\alpha\beta$ 1 integrins	Phase I
Volociximab	Chimeric monoclonal antibody	α 5 β 1 integrin	Phase I
ALG-1001	Oligopeptide	α 5 β 1, α v β 3 and α v β 5 integrins	Phase I
Infliximab	Monoclonal antibody	TNF- α	Phase II
POT-4	Oligopeptide	Complement C3	Phase I
Sirolimus	Small molecule	mTOR	Phase II
Palomid 529	Small molecule	mTORC1	Phase I
PF-04523655	siRNA	RTP801 expression	Phase II
RXI-109	sd-rxRNA	CTGF expression	Phase I
hI-con1	Chimeric protein	Tissue factor	Phase II

VEGF-A, vascular endothelial growth factor A; PIGF, placental growth factor; VEGFR, vascular endothelial growth factor receptor; PDGF, platelet derived growth factor; PDGFR, platelet derived growth factor receptor; FGFR, fibroblast growth factor receptor; KIT, v-kit feline sarcoma viral oncogene homolog; RAF, v-raf-1 murine leukemia viral oncogene homolog 1; TIE, TEK tyrosine kinase endothelial; MAPK, mitogen-activated protein kinase; PEDF, Pigment epithelium-derived factor; mTOR, mammalian target of rapamycin; mTORC, mTOR complex; TNF- α , Tumor necrosis factor; SIP, Sphingosine-1-phosphate; nAChR, Nicotinic acetylcholine receptor; C3, Component 3.

sorafenib showed the ability to decrease the extent of CNV in a dose-dependent manner.³⁸ In two case reports, sorafenib was administered orally in combination with intravitreal anti-VEGF-A treatment and all patients appeared to gain some benefit.³⁹⁻⁴⁰

Pigment epithelium-derived factor

PEDF is a cell survival factor secreted by the RPE, widely expressed in the central and peripheral nervous system,^{41,42} and apparently an endogenous inhibitor of angiogenesis in the eye.⁴³ A critical balance appears to exist between PEDF and VEGF-A, with PEDF counteracting the angiogenic potential of VEGF-A. A physiological decrease in PEDF occurring in the elderly may disrupt this balance and create a permissive environment for the formation of CNV in AMD.⁴⁴ PEDF gene polymorphisms may also contribute to AMD.⁴⁵

- *ATG003*. PEDF protein expression has been found to be decreased in RPE from smoker patients with AMD compared to controls.⁴⁶ It has also been reported that nicotine, a potent angiogenic agent, increased the VEGF-A/PEDF ratio in the RPE through nicotinic acetylcholine receptor (nAChR).⁴⁷ *ATG003* (CoMentis, formerly Athenagen, South San Francisco, CA, USA) is a topical mecamylamine that antagonizes the nAChR pathway which mediates angiogenesis. It was designed as an eye drop therapy for AMD, and phase II clinical trials testing its safety and efficacy were completed in 2010 (ClinicalTrials.gov identifier: NCT00414206) (ClinicalTrials.gov identifier: NCT00607750), although no results have been published yet.
- *AdGVPEDF.11D*. An adenoviral vector containing complementary DNA encoding human PEDF, known as AdGVPEDF.11D (GenVec, Inc., Gaithersburg, MD, USA), seems to allow the expression of large amounts of PEDF in the target tissue and inhibit ocular NV in murine AMD models.⁴⁸ Intravitreal injection of AdGVPEDF.11D results in the local production of PEDF. A phase I single-dose trial enrolled 28 patients with severe nAMD.⁴⁹ This clinical study suggested that antiangiogenic activity may last for up to 6 months after a single intravitreal injection, since half of the lesions treated did not change in size from baseline. However, no further studies have corroborated this hypothesis.

Integrin receptor antagonists

Integrins are transmembrane proteins that mediate the attachment between cells and the surrounding extracellular matrix. Their ligands are fibronectin, vitronectin, collagen and laminin.⁵⁰ Integrins localized at the apical surface of RPE bind to ligands in the interphotoreceptor matrix⁵¹ and participate in the interactions between photoreceptors and the RPE.⁵² During aging, the dissociation between the interphotoreceptor matrix and retinal cells seems to contribute to decreasing the supply of oxygen, nutrients and growth factors from the choroidal or retinal vessels, as well as to inhibit the phagocytosis of photoreceptor outer segments.⁵³ It has been shown that $\alpha 5\beta 1$, $\alpha \beta 3$ and $\alpha \nu \beta 5$ integrins are expressed in CNV tissue,⁵⁴ and many studies have demonstrated a possible role for their antagonists in preventing ocular NV.⁵⁵⁻⁵⁹

- *SM6427*. JSM6427 is a small molecule antagonist of the $\alpha 5\beta 1$ integrin, which has been shown to inhibit retinal and CNV in preclinical models.⁶⁰ A phase I clinical trial of JSM6427 for nAMD has been completed (ClinicalTrials.gov identifier: NCT00536016). Although encouraging results were obtained, no further studies have evaluated JSM6427.
- *Volociximab*. Volociximab (Ophthotech Corporation, Princeton, NY, USA) is a chimeric monoclonal antibody specifically blocking the binding of fibronectin to the $\alpha 5\beta 1$ integrin. A phase I study evaluating the safety of intravitreal volociximab combined with ranibizumab for nAMD (ClinicalTrials.gov identifier: NCT00782093) revealed good improvements in visual acuity at nine weeks, although the results did not distinguish the independent contributions of ranibizumab and volociximab.
- *ALG-1001*. ALG-1001, also known as Luminite (Allegro Ophthalmics, San Juan Capistrano, CA, USA), is a synthetic oligopeptide that targets $\alpha 5\beta 1$, $\alpha \nu \beta 3$ and $\alpha \nu \beta 5$ integrins. It has been investigated for 3 indications: nAMD, diabetic macular edema and vitreomacular traction. In a phase Ib clinical trial ALG-1001 used in combination with ranibizumab for the treatment of nAMD the drug was found to be safe.⁶¹

Anti-inflammatory and anti-immune therapies

Over the last few years many authors

have identified inflammatory mechanisms and immune responses as central features in all AMD phenotypes, consolidating the hypothesis that AMD is a singular disease with multiple outcomes.⁶²⁻⁶⁴

Investigating the role of immune response in the pathogenesis of ocular NV may therefore represent a promising field in the search for new targets for AMD treatments (Figure 2).

- *Infliximab*. Tumor necrosis factor alpha (TNF- α) plays a central role in inflammation, apoptosis and immune reactions. The anti-TNF- α monoclonal antibody infliximab (Remicade, Centocor, Inc., Horsham, PA, USA) is commonly used to treat various inflammatory diseases. Intravitreal infliximab has been reported to inhibit laser-induced CNV in rats and has been safely administered up to a dose of 2 mg in the rabbit eye.⁶⁵⁻⁶⁶ Intravitreal infliximab was also administered to three patients with nAMD, resulting in improved visual acuity and central foveal thickness on Optical Coherence Tomography (OCT).⁶⁷
- *POT-4*. The alternative complement pathway has also been implicated in the development of AMD.⁶⁸ POT-4 is a potent inhibitor of complement factor C3 activation. A phase I clinical trial was designed to evaluate the safety and tolerability of POT-4 administered via intravitreal injections (ClinicalTrials.gov identifier: NCT00473928). Preliminary results indicated that intravitreal POT-4 is safe and well-tolerated and supported its administration in larger randomized clinical trials in order to further define its efficacy profile. Even though no significant improvement in visual acuity was observed, there was no significant visual loss either.⁶⁹
- *Sirolimus*. Mammalian target of rapamycin (mTOR) is an evolutionarily conserved serine/threonine kinase which appears to function as a central node in a signaling cascade directing the integration of diverse environmental inputs into the immune microenvironment.⁷⁰ Sirolimus (previously known as rapamycin, Santen Pharmaceutical, Inc., Osaka, Kapan, and MacuSight, Inc., Union City, CA) was discovered in the 1970s as a soil bacterium metabolite. It was collected on Easter Island (Rapa Nui) and was originally developed as a macrolide antifungal agent. Subsequently, it was found to possess potent immunosuppressive and antiproliferative properties due to its ability to bind to the protein FKBP12. The resulting sirolimus-FKBP12 complex binds

to and inhibits mTOR.⁷¹ It is used as an anticancer drug against several solid tumors and an immunosuppressive agent in the transplantation of various organs, especially kidneys.⁷² Preclinical studies in experimental models have shown promising results regarding the use of this pharmacological agent to inhibit ocular neovascularization.⁷³⁻⁷⁴ Early phase I/II studies have subsequently provided encouraging safety and efficacy data (ClinicalTrials.gov identifier: NCT00712491). A phase II study (EMERALD) of an ocular sirolimus (rapamycin) formulation in combination with ranibizumab in patients with AMD has been terminated: the last update was in June 2013 (ClinicalTrials.gov identifier: NCT00766337).

- *Palomid 529*. Unlike Sirolimus, which binds to mTOR Complex 1 (mTORC1), Palomid 529 is capable of blocking both mTORC1 and mTORC2. A phase I clinical trial enrolled five participants with nAMD who were refractory to intravitreal anti-VEGF-A, and received three consecutive monthly subconjunctival doses of 1.9 mg Palomid 529. Unfortunately, compared to baseline no clinically important changes were observed in best-corrected visual acuity, fluorescein leakage pattern, CNV size at indocyanine green angiography, or autofluorescence patterns at fundus autofluorescence.⁷⁵
- *PF-04523655*. PF-04523655 is a 19-ribonucleotide double-stranded siRNA which inhibits the expression of the hypoxia-inducible gene, RTP801, an inhibitor mTOR signaling.⁷⁶ A single intravitreal injection of PF-0523655 ≤3000 mg seemed to be safe and well-tolerated in a phase I study involving 27 patients with AMD which had been unresponsive to prior treatment.⁷⁷ A phase II clinical trial, the multicenter and randomized MONET study, sought to prove the efficacy of PF-04523655 in combination with Lucentis. It demonstrated the superiority of PF-04523655+ranibizumab over ranibizumab monotherapy in terms of best corrected visual acuity improvement.⁷⁸

Connective tissue growth factor inhibition

The 38-KDa cysteine-rich polypeptide connective tissue growth factor (CTGF), which has a pleiotropic and cell type-specific

role, was first isolated in human umbilical vein endothelial cells.⁷⁹ CTGF is considered to be involved in the pathological synthesis of peri-retinal fibrous tissue in AMD patients.⁸⁰

- *RXI-109*. RXI-109 (RXi Pharmaceuticals, Marlborough, MA) is a self-delivering RNAi compound (sd-rxRNA), designed to reduce the expression of CTGF and developed to reduce dermal scarring following planned surgery. A study designed to evaluate the safety, tolerability and clinical activity of RXI-109 administered by intravitreal injection, in order to reduce the progression of subretinal fibrosis in subjects with advanced nAMD degeneration, is currently recruiting patients (ClinicalTrials.gov identifier: NCT02599064).

Tissue factor inhibition

Tissue factor (TF) is expressed in neovascular endothelium but not in the normal vasculature of mouse and pig models.

- *hI-con1*. Factor VII-verteporfin has been tested with the aim of improving the therapeutic results of photodynamic therapy in rats, due to its ability to bind tightly and specifically to TF, which is expressed in the endothelial cells of CNV but not in the normal vasculature.⁸¹ hI-con1 (Iconic Therapeutics Inc. South San Francisco, CA, USA), a synthetic molecule composed of factor VII conjugated to an antibody Fc, binds to TF and selectively destroys pathologic blood vessels. A phase II, randomized, double-masked, multi-center study evaluating the administration of repeated intravitreal injections of hI-con1 in patients with CNV secondary to AMD has recently been completed and the results are pending (ClinicalTrials.gov identifier: NCT02358889).

CD93

Human CD93 is a 652 amino acid surface glycoprotein with a predicted molecular mass of 68 kDa; it is rich in proline and charged amino acids.⁸²⁻⁸³ CD93 expression has been found in several cell types, including the vascular endothelium.⁸⁴⁻⁸⁵ Moreover, the soluble EGF-like domain of CD93, which is the product of ectodomain cleavage or shedding, has recently been discovered to be an angiogenic factor.⁸⁶ The monoclonal antibody 4E1, binding to CD93, is capable to selectively inhibit blood vessel formation both in vitro and in vivo, without

affecting endothelial cell survival.⁸⁷

CD93 expression has recently been observed in the endothelial cells of AMD-related CNV and the soluble CD93 domain has been shown to be overexpressed in the aqueous humor of AMD patients, suggesting that CD93 may represent a potential new antiangiogenic target in the treatment of CNV.⁸⁸

HTRA1

HTRA1 is a serine protease involved in protein quality control and cell fate. HTRA1 expression has been found to be significantly higher in CNV patients compared to controls. After treatment with anti-VEGF-A, it returned to control levels.⁸⁹ The ability of HTRA1 to regulate extracellular matrix proteoglycan degradation and TGF-β activity appears to allow it to modulate AMD development.⁹⁰⁻⁹¹ HTRA1 can be considered an innovative target. This hypothesis is supported by the discovery of the antibody 94, which is an inhibitor of this protein.⁹²

Conclusions

Over the last decade, the management of AMD has changed significantly due to the advent of anti-VEGF-A therapy. However, treatment with anti-VEGF-A agents rarely brings about an improvement in visual acuity. The study of the pathogenetic mechanisms of this disease has led to the discovery of several proteins and factors, which could be used as new molecular targets in AMD therapy.

A great effort has been made to inhibit neovascularization with several drugs now in advanced clinical trials (Figure 1) targeting new antigens with complementary functions, which could offer novel opportunities to circumvent resistance mechanisms and improve the anti-VEGF-A therapy.

The unsuccessful outcomes of the anti-VEGF-A therapy have often been attributed to development of subretinal fibrosis and inflammatory response. Other interesting drugs seem to come from these fields of research (Figure 2), with at least four compounds in clinical phase II (Infliximab, Sirolimus, PF-04523655 and hI-con1).

Future directions in AMD treatment will have to focus now on applying combinations of drugs with different angiogenic and non-angiogenic targets, with the aim of achieving disease stabilization or regression, improving visual acuity, and shortening injection intervals.

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