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Anti-angiogenic Therapy for Retinal Disease

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Abstract

Recent breakthroughs in our understanding of the molecular pathophysiology of retinal vascular disease have allowed us to specifically target pathological angiogenesis while minimizing damage to the neurosensory retina. This is perhaps best exemplified by the development of therapies targeting the potent angiogenic growth factor and vascular permeability mediator, vascular endothelial growth factor (VEGF). Anti-VEGF therapies, initially introduced for the treatment of choroidal neovascularization in patients with age-related macular degeneration, have also had a dramatic impact on the management of retinal vascular disease and are currently an indispensable component for the treatment of macular edema in patients with diabetic eye disease and retinal vein occlusions. Emerging evidence supports expanding the use of therapies targeting VEGF for the treatment of retinal neovascularization in patients with diabetic retinopathy and retinopathy of prematurity. However, VEGF is among a growing list of angiogenic and vascular hyperpermeability factors that promote retinal vascular disease. Many of these mediators are expressed in response to stabilization of a single family of transcription factors, the hypoxia-inducible factors (HIFs), that regulate the expression of these angiogenic stimulators. Here we review the basic principles driving pathological angiogenesis and discuss the current state of retinal anti-angiogenic pharmacotherapy and as well as future directions.

Keywords

angiogenesis; neovascularization; vascular permeability; macular edema; ischemia; oxidative stress; hypoxia-inducible factor; vascular endothelial growth factor

Introduction

The development of the vascular system (i.e., vasculogenesis) occurs early during development and is an important foundational stage in organogenesis. In adults, the growth of new blood vessels (i.e., physiologic angiogenesis) is limited to wound healing and ovarian and cervical cycling (DiPietro 2016). Conversely, pathological angiogenesis is an essential component in disease, most notably in cancer, rheumatologic conditions including psoriasis, and ischemic disease (e.g., myocardial and cerebral infarction). The impact of pathological

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Conflict of Interest: Johns Hopkins has filed a patent application on the use of technology to modulate the levels of ANGPTL4 for the treatment of ocular neovascular disease (US patent 14/394, 152).

angiogenesis in disease is particularly evident for diseases of the retinal, where it can lead to dramatic and permanent loss of vision. Pathological angiogenesis occurs a list of settings including diabetic retinopathy, retinal vein occlusions, sickle cell retinopathy, hyperviscosity syndromes, pathologic myopia, age-related macular degeneration, retinal arterial occlusion, radiation retinopathy, ocular ischemic syndrome, retinopathy of prematurity, familial exudative vitreoretinopathy (FEVR), retinal vasculitis, uveitic and inflammatory conditions, tumors, retinal vascular tumors including hemangiomas, retinal degeneration, post-traumatic changes, macular dystrophies, angioid streaks, choroidal rupture, Eales disease, sarcoidosis, chronic retinal detachment, carotid-cavernous fistula, Coats' disease, retinal artery macroaneurysms, and incontinentia pigmenti. Due to its easy accessibility and visualization, the retina is also an ideal model to study both vasculogenesis and angiogenesis: the retina is thin and transparent; retinal vascular development is stereotyped both spatially and temporally; and vascularization of the retina occurs post-natally in rodents, the most commonly used vertebrate animals to study ocular disease.

Retinal laser therapy was developed over 50 years ago with investigations by Kapany and colleagues in 1963 using the ruby laser (Kapany *et al.* 1963) and has had a profound impact on the treatment of numerous retinal neovascular disorders. Seminal early studies established laser as the first treatment for numerous eye conditions. The Diabetic Retinopathy Study (DRS) established panretinal laser photocoagulation as the first effective treatment for high risk proliferative diabetic retinopathy (The Diabetic Retinopathy Study Research Group 1981). And the Early Treatment Diabetic Retinopathy Study (ETDRS) study established laser as the first effective treatment for clinically significant diabetic macular edema (Early Treatment Diabetic Retinopathy Study Research Group 1991). The branch retinal vein occlusion study (BVOS) later demonstrated the efficacy of focal laser for the treatment of macular edema associated with branch vein occlusions (The Branch Vein Occlusion Study Group 1984). However, recent scientific discoveries have led to the development of new pharmacotherapies which have demonstrated significantly improved visual acuity outcomes with decreased side-effect profile. Indeed, the recent introduction of targeted pharmacologic anti-angiogenic therapies has transformed the care of patients with ocular neovascular diseases. This chapter will briefly describe the normal development of the retinal vasculature, the pathologic molecular events that lead to retinal and choroidal neovascularization, and currently available and possible future targets for anti-angiogenic therapy.

Embryology and Anatomy of the Retina

The development of the human eye has been described and refined over the past 90 years (Mann 1928). The outer layer of the optic cup evolves as a monolayer into the retinal pigment epithelium (RPE). The neurosensory retina develops from the inner layer of the neuroectodermal cells of the optic cup that begin to migrate in the second month of fetal development (Tripathi *et al* 1997). Vascular channels from the internal carotid artery develop in the mesenchyme around the optic vesicle late in the fourth week of gestation and the ophthalmic artery develops by the 6th week of gestation.

The most superficial retinal vascular layer first begins around 14 weeks gestation age as spindle-shaped undifferentiated mesenchymal cells arise from the hyaloid artery at the optic disc and develop into radial vessel extensions from the optic nerve head. Lumina develop behind the advancing edge of the mesenchymal cells. The vessels spread peripherally to reach the ora serrata just before term in centrifugal waves following neuronal differentiation with endothelial cells differentiating first followed by zonulae occludens and gap junctions (Penfold *et al* 1990). Deeper vascular layers arise sequentially by sprouting from the initial plexus (Michaelson 1948). The hyaloid system and tunica vasculosa lentis atrophy in the third trimester. The adult retinal vascular pattern occurs by remodeling through pruning and maturation soon after formation by 3 months after birth. The foveal pit develops in the first four years of life.

The normal human circulatory system is made of several cell types. Endothelial cells make up the inner wall of the blood vessel. Inter-endothelial cell adhesions and tight junctions comprise important blood-tissue barriers. Smooth muscle cells in arterioles and pericytes in capillaries surround the endothelial cells and provide vascular tone and regulation. Glial cells such as astrocytes and retinal Müller cells are closely apposed to vessels in neural tissue. Dendritiform cells such as microglia are antigen presenting cells associated with vessels in certain tissues.

The human retina has 4 interconnected planar vascular networks in neuronal layers in the nerve fiber layer, ganglion cell layer, and inner nuclear layer. The retinal vessels form in alignment with the radial orientation of ganglion cell axons (Figure 1). Importantly, the outer retina (the outer plexiform layer and photoreceptor layer) is avascular, and the macular pit (fovea) is also avascular at all times during human development (Figure 2). The absence of vessels in the outer retina and fovea is essential for human vision.

Vasculogenesis

There are two primary modes of blood vessel assembly: vasculogenesis and angiogenesis. Vasculogenesis is the de novo assembly of vessels from endothelial precursor cells (angioblasts) and mesenchymal precursors that form clusters (blood islands). The cells aggregate into tubes that fuse, form tube-like structures, interconnect into cords, and slowly lumenize. Vasculogenesis forms the initial embryonic vessels and begins in gastrulation but plays a more limited role in adult vessel growth (McLeod *et al.* 1987; Hughes *et al.* 2000; Chan-Ling *et al.* 2004, McLeod *et al.* 2006).

In the developing human fetal retina, mesenchymal precursors precede the ingrowth of retinal blood vessels (Ashton 1970, Chan-Ling *et al.*, 2004). Astrocytes are noted to be the majority of dividing cells in developing blood vessels in the retina (Sandercoe *et al.* 1999). Hemangioblasts serve as the precursor to angioblasts, which proliferate and migrate peripherally as a precursor to endothelial cells. Both astrocyte precursor cells and angioblasts have been described in developing fetal human retina (Chu *et al.* 2001; Chan-Ling *et al.* 2004). Once believed to happen exclusively in fetuses, new blood vessel formation due to angioblast differentiation has recently been demonstrated in pathological

angiogenesis in adults (Jiang *et al* 2005). In this setting, angioblasts stored in the bone marrow of adults are recruited to tissue to initiate vasculogenesis.

Vascular guidance and patterning during vessel growth occurs through attractant and repellent cues remarkably similar to axons in the developing nervous system. This requires a complex interplay between different cell types, including endothelial cells, pericytes, and periendothelial cells. Ganglion cells promote astrocyte spread in a placental-derived growth factor (PDGF)-dependent manner. Astrocytes appear to serve as a template for vessels by secreting vascular endothelial growth factor (VEGF) and through the expression of cadherins to promote cellular adhesion. Vessels, in turn, downregulate expression of VEGF by astrocytes and induce astrocytic maturation. Early genetic and local tissue factors determine arterial versus venous fate of a plexus (Gariano 2003).

Angiogenesis

Angiogenesis is the growth of new vessels from pre-existing vessels by a sprouting or branching process. Angiogenesis elaborates on the vascular plexus begun with vasculogenesis and accounts for most vascular growth in adults, including pathologic vessel growth in disease. In stark contrast with normal retinal vasculogenesis, pathologic retinal angiogenesis creates chaotically oriented and physiologically deficient vessels that do not conform to neuronal histology. These vessels do not respect the normally avascular outer retina and macular pit, and can contribute to profound vision loss.

There are several steps in angiogenesis, including destabilization, vasodilation, hyperpermeability, cell migration, and cell proliferation. Each of these steps is regulated by the coordinated expression and/or activation of secreted mediators, cell-surface receptors, intracellular signaling molecules, and nuclear transcription factors. Circulating progenitor cells and hematopoietic stem cells from the bone marrow can contribute to angiogenesis, providing cellular building blocks for endothelial cells and secreting angiogenic molecules or differentiating into precursor perivascular cells. Angiogenesis is regulated by numerous physiologic properties including tissue oxygen tension, extracellular matrix composition, intrinsic genetic programs of endothelial cells, and a complex milieu of pro and anti-angiogenic cells and molecules.

Vasodilation occurs in response to chemical signals (e.g., nitric oxide) as well as hyperpermeability factors (e.g., VEGF). Angiogenic growth factors (including VEGF) activate receptors on endothelial cells of pre-existing blood vessels. The vascular endothelial cells release proteases, including matrix metalloproteases (MMPs) that degrade the basement membrane, allowing vascular endothelial cells to escape from the parent vessel (Rodrigues *et al.* 2013), while plasminogen activator promotes the disruption of the endothelial cell junctions (Yao and Tsirka 2011). Migrating endothelial cells proliferate in the surrounding extracellular matrix towards the angiogenic stimulus using integrins and other adhesion molecules. Periendothelial cells and pericytes are then recruited to promote vessel maturity (Birbrair *et al.* 2014).

Additional Methods of New Vessel Development

Additional mechanisms of formation of blood vessels have also been described but play a more limited role in the retina. Intussusception involves the formation of transvascular tissue pillars and includes intussusceptive microvascular growth to expand a capillary network, intussusceptive arborization to form feeding vessels from capillaries, and intussusceptive branching remodeling through pillars arising close to a bifurcation or through pillars arising at some distance from the bifurcation point. Intussusception has been described in numerous organs including the eye, as well as lung, intestine, and kidney (Djonov *et al.* 2002; Burri *et al.* 2004). Tube formation and remodeling, vascular mimicry, and biochemical translocation and expansion have also been described. Another mechanism, hamo-vasculogenesis, has been described in which blood vessel and blood cells differentiate from a common precursor, the haemangioblast. This is thought to play a role in human choroidal vascular development from 6 until 22 weeks gestation and involves endothelial cells or angioblasts and erythroblasts in the choriocapillaris. Fenestrations, smooth muscle actin, extracellular basal lamina, and pericytes develop later (Lutty *et al.* 2010).

Hypoxia and the Retinal Vasculature

In 1948, Michaelson used dye-perfusion techniques to evaluate the embryonic and perinatal retinal vasculature. He found that capillaries grow extensively around venules and less around arteries. Michaelson posited a “Factor X”, an oxygen-sensitive molecule, which controlled retinal vascular development through a concentration gradient (Michaelson 1948). Indeed, decreased oxygenation due to relative non-perfusion or an increase in metabolic demand is thought to drive the development of blood vessels in normal development (Yu *et al.* 2001).

The important role of oxygen in retinal vascular development has been further refined since the observations of Michaelson. The superficial vascular plexus in the developing retina follows the metabolic demand of neuronal development and physiologic hypoxia (Chan-Ling 1995). Lowering the percentage of inhaled oxygen in animals reduces the rate and density of the retinal vasculature (Phelps 1990; Li *et al.* 2008). Hypoxia also plays an essential role in many retinal vascular diseases (Kaur *et al.* 2008), providing a link between physiologic and pathologic vascular processes in the eye. Indeed, the oxygen-dependent angiogenic stimulators expressed during normal vascular development are also expressed (albeit at much higher levels) in retinal vascular disease (Gariano *et al.* 2005).

Hypoxia Inducible Factor (HIF)

A group of transcriptional activators, the hypoxia-inducible factor (HIF)s, have recently emerged as the master regulators of these hypoxia-regulated angiogenic stimulators (Semenza 2012) and have helped illuminate the overlap between physiologic and pathologic vascular processes in the eye. HIFs are heterodimeric proteins composed of an exquisitely oxygen-sensitive α subunit and a ubiquitously-expressed β subunit (Semenza 2007). HIF-1 α was the first HIF α subunit isoform to be identified (Wang *et al.* 1995) and its role has been extensively studied in retinal vascular disease (Scholz *et al.* 2013). Two other isoforms,

HIF-2 α and HIF-3 α have since been reported; while HIF-2 α is closely related to HIF-1 α and activates hypoxia-inducible gene transcription, HIF-3 α is distantly related and may antagonize HIF-1 α and HIF-2 α (Ratcliffe 2007).

In normoxic conditions, HIF-1 α is hydroxylated and ubiquitinated by the VHL E3 ubiquitin ligase, labeling it for degradation in the proteasome (Maxwell *et al.* 1999). In hypoxic conditions, the VHL hydroxylase is inhibited. Thus, in normal conditions, HIF-1 α is produced but constantly degraded. Conversely, under conditions of hypoxia (e.g., in ischemic retinal disease) HIF-1 α is no longer targeted for degradation, and can promote the expression of pro-angiogenic gene products, including VEGF. Indeed, VEGF expression is temporally and spatially correlated with HIF-1 α expression and retinal vascular growth (Kurihara *et al.* 2014). HIF-1 α regulates expression of the angiogenic template by astrocytes (Nakamura-Ishizu *et al.* 2012) and is essential for retinal vascular development (Caprara *et al.* 2011).

The precise molecular regulation of HIF-1 α has been elegantly demonstrated. Under standard tissue culture conditions (20% O₂), proline residues 402 and 564 on the HIF-1 α subunit are hydroxylated by a family of HIF prolyl hydroxylases (PHDs) (Kaelin 2005). Hydroxylated HIF-1 α binds to the von Hippel-Lindau (VHL) tumor suppressor protein, which ubiquitinates HIF-1 α and targets it for degradation by the proteasome (Hubbi *et al.* 2014; Mole *et al.* 2002). An additional level of regulation is provided by an asparaginyl hydroxylase, factor inhibiting HIF-1 (FIH-1) (Lando *et al.* 2002; Mahon *et al.* 2001). FIH-1 hydroxylates asparagine residue 803 on HIF-1 α and prevents binding of the transcriptional co-activator, p300, to HIF-1 α , thereby inhibiting its transcriptional activity.

Under hypoxic conditions (<5% O₂), the ability of PHDs and FIH to hydroxylate HIF-1 α is impaired (Figure 3). In the absence of hydroxylation, VHL does not bind to HIF-1 α to trigger its degradation, whereas p300 binds to HIF-1 α to enhance its transcriptional activity (Maxwell 2005; Schofield *et al.* 2004; Manalo *et al.* 2005). The resulting increased amount of active HIF-1 α protein localizes to the nucleus and binds to HIF-1 β forming a heterodimer (HIF-1) that is capable of binding to the DNA of specific (hypoxia-inducible) genes, and inducing broad changes in gene expression that mediate acclimation of cells, tissues, and the organism to conditions of low oxygen tension (Semenza 2012). Indeed, HIF-1 targets include numerous genes that play essential adaptive roles by promoting angiogenesis to increase O₂ delivery, regulating the metabolic shift from oxidative phosphorylation to glycolysis and lactic acid production to decrease O₂ demand, protecting cells from acidosis, and influencing adaptive survival mechanisms (Semenza 2012). These genes work together to collectively promote the survival of cells exposed to hypoxia.

Numerous ischemic retinopathies exist where abnormal perfusion, permeability, and vasculature creates a mismatch between tissue metabolic demand and supply. These ischemic retinopathies involving retinal neovascularization include diabetic retinopathy, central and branch retinal vein occlusions, sickle cell retinopathy, hyperviscosity syndromes, radiation retinopathy, retinal tumors including retinoblastoma and uveal melanoma, ocular ischemic syndrome, chronic retinal detachment, and retinopathy of prematurity. Proliferative diabetic retinopathy has been shown to result in an increase in numerous pro-angiogenic

factors in the vitreous, including inflammatory cytokines, chemokines, extracellular matrix adhesion molecules, complement, polyamines, vasoactive peptides, and inflammatory cells (Gariano *et al.* 2005). Retinal oxygen saturation has been shown to decrease in patients with diabetes without retinopathy (Beach *et al.* 1999) and shown to increase adjacent to panretinal photocoagulation laser scars (Stefánsson *et al.* 1992). Retinal oxygen partial pressure has been shown to be reduced in cats with diabetes of 6–8 years duration (Linsenmeier *et al.* 1988). In addition to reduced oxygen, inflammation and aberrant wound-healing play an important role in diabetic retinopathy with microglial and glial cell activation resulting in the release of cytokines and chemokines (Amin *et al.* 1997; Krady *et al.* 2005).

In age-related macular degeneration (AMD), the development of choroidal neovascularization (CNV) appears to be a consequence of the expression of the same angiogenic mediators that drive the development of retinal neovascularization in ischemic retinal disease (Figg *et al.* 2008). However, it is postulated that oxidative stress instead of – or in addition to – hypoxia may stimulate accumulation of HIF-1 α in AMD (Park *et al.* 2015). CNV occurs in conditions including age-related macular degeneration, pathologic myopia, uveitic and inflammatory conditions, choroiditis, presumed ocular histoplasmosis (POHS), punctate inner choroidopathy (PIC), multifocal choroiditis, angioid streaks, ocular trauma, vitelliform dystrophies, optic disc drusen, and idiopathic CNV. CNV occurs when the RPE or Bruch's membrane is compromised. Bruch's membrane is a 5-layered extracellular matrix that separates the RPE from the choriocapillaris and is composed of the basement membrane of the RPE, inner collagenous zone, central band of elastic fibers, outer collagenous zone, and basement membrane of the choriocapillaris. AMD can develop CNV of the choroid in addition to retinal angiomatous proliferation (RAP) originating from the deep capillary bed of the retina.

Identifying Therapeutic Targets for the Treatment of Ocular Neovascular Disease

There are numerous considerations when identifying an ideal target for treatment of ocular neovascular disease. One would consider a target whose expression is found only in the disease state (or markedly elevated in the diseased state). Low levels of the target in normal tissue would minimize potential side-effects of medications that inhibit that target. In addition, one would prefer a secreted factor or transmembrane receptor that would be readily accessible to a binding molecule given the difficulty in achieving adequate intracellular or intranuclear concentrations of some medications. An ideal therapeutic agent would be administered locally to the eye in an easily accessible form, such as topical instillation, subconjunctival depot, or intravitreal. Administration would preferably be non-invasive, so that administration would involve minimal risk to patients. And the duration of action would ideally be long, so as to minimize the number of re-treatments patients would need for these chronic diseases.

Vascular Endothelial Growth Factor (VEGF)

No single molecule has captured the attention of the retinal community as much as the angiogenic and vascular permeability stimulator, VEGF. The VEGF family consists of

VEGF-A, -B, -C, and -D, and placental-induced growth factor (PlGF) 1 and 2. VEGF is a hypoxia-inducible cytokine that is necessary for retinal vascularization and is spatially and temporally expressed in developing retinal blood vessels (Stone *et al.* 1995; Provis *et al.* 1997; Ozaki *et al.* 2000). VEGF plays numerous roles in the retina, including angiogenesis promotion, increasing vascular permeability, stimulating endothelial cell migration and proliferation, and increasing endothelial expression of plasminogen activator and interstitial collagenase. Mice expressing a single VEGF isoform demonstrate unique retinal vascular appearance, suggesting that each isoform acts within a limited spatial location to create the vasculature. Mice expressing VEGF₁₆₄ appear normal, whereas mice expressing VEGF₁₈₈ have aborted arterioles and normal veins (Stalmans *et al.* 2002). VEGF₁₂₀, a more diffusible version, results in reduced vessel branching. VEGF also plays an important role in vascular pruning in the developing retina. Relative hyperoxia surrounding arteries reduces VEGF production and its ability to maintain endothelial cell survival (Alon *et al.* 1995; Claxton *et al.* 2003). VEGF has been shown to be elevated in the eye in patients with proliferative diabetic retinopathy and retinal vein occlusions (Adamis *et al.* 1994; Aiello *et al.* 1994).

The VEGF receptors, VEGFR-1 or Flt-1, VEGFR-2 or Flk-1, VEGFR-3 or Flt-4, and neuropilin 1 and 2, are expressed in a temporally and spatially distinct pattern that also allows for vascular patterning (Shih *et al.* 2003; Favier *et al.* 2006; Gariano *et al.* 2006). Signals by a given receptor can elicit different responses at different sites. VEGFR-2 activation causes migration and filopodia in endothelial tip cells and proliferation in more proximal stalk cells (Gerhardt *et al.* 2003). Several inducing agents exist for VEGF, including TGF- α , TGF- β , PDGF, and hypoxia. VEGF also further refines vascular development through its interactions with other angiogenic factors, including placental growth factors, angiotensin, angiopoietin, and pigment epithelium-derived factor (Dawson *et al.* 1999; Feeney *et al.* 2003; Sarlos *et al.* 2003). Another potential use of anti-VEGF treatment is in the “normalization” of vasculature, reducing hypoxia and creating a window for chemotherapy (Datta *et al.* 2015).

Several therapies currently exist utilizing VEGF (table 1). The first approved therapy for age-related macular degeneration (AMD) in December 2004 was pegaptanib sodium (Macugen, EyeTech Pharmaceuticals/Pfizer), an RNA aptamer directed against VEGF-165. Pegaptanib was the first aptamer approved for use in humans (Ng *et al.* 2006). Aptamers are oligonucleotide ligands with high-affinity binding to molecular targets. Pegaptanib was demonstrated to be safe and effective in several prospective, randomized, multicenter, double-masked, sham-controlled studies (Gragoudas *et al.* 2004; Chakravarthy *et al.* 2006; D’Amico *et al.* 2006).

Bevacizumab (Avastin, Genentech/Roche) was approved by the United States Food and Drug Administration (FDA) in 2004 as treatment for metastatic colon cancer and is a whole humanized from mouse antibody to VEGF-A. It was described for use initially systemically as an intravenous treatment (Michels *et al.* 2005) and subsequently via off-label intravitreal administration for AMD (Avery *et al.* 2006; Rich *et al.* 2006). Prospective randomized controlled trials have proven the efficacy of bevacizumab in treating AMD (Sacu *et al.* 2009; Tufail *et al.* 2010), macular edema from CRVO (Epstein *et al.* 2012), and diabetic macular edema (DME) in the BOLT trial (Rajendram *et al.* 2012).

Shortly after bevacizumab was approved, the same company Genentech developed another monoclonal antibody to VEGF-A, ranibizumab or Lucentis (Ferrara *et al.* 2006). Ranibizumab is a humanized mouse Fab fragment to an epitope of VEGF (AS82-91) from within the receptor binding domain (AS8-109) of VEGF165 and was approved by the FDA in June 2006 for treatment of AMD, June 2010 for treatment of macular edema from retinal vein occlusions (RVO), August 2012 for treatment of diabetic macular edema (DME), and February 2015 for the treatment of diabetic retinopathy. Like bevacizumab, ranibizumab has neutralizing activity on all VEGF isoforms. Prospective randomized controlled trials have proven the efficacy of ranibizumab in treating classic CNV in the ANCHOR trial (Brown *et al.* 2009), occult CNV in the MARINA trial (Rosenfeld *et al.* 2006), DME in RISE, RIDE, and RESTORE (Mitchell *et al.* 2011; Nguyen *et al.* 2012), BRVO in BRAVO (Campochiaro *et al.* 2010), and CRVO in CRUISE (Brown *et al.* 2010).

Several important differences exist between ranibizumab and bevacizumab: (1) ranibizumab (48 kDa) contains only the Fab fragment of the antibody, whereas bevacizumab (149 kDa) contains both the Fab and Fc fragments; (2) ranibizumab has six corresponding amino acids which differ from bevacizumab; (3) ranibizumab has one binding site for VEGF whereas bevacizumab has two; (4) ranibizumab is produced in prokaryotic *Escherichia coli* and therefore is not glycosylated, whereas bevacizumab is produced in a eukaryotic cell line (CHO cells) and is N-glycosylated in its Fc region; and (5) ranibizumab costs over \$2000 per injection whereas bevacizumab costs approximately \$60 per injection (Krispel *et al.* 2013).

However, it is unclear how much these differences between ranibizumab and bevacizumab translate into a clinically meaningful difference. The smaller size of ranibizumab was deliberate to enhance diffusion from the vitreous cavity into the retina and choroid (Ferrara *et al.* 2006). However, subsequent studies suggest that the predicted enhanced diffusion may not translate into a clinically meaningful therapeutic advantage (Martin *et al.* 2012). Affinity maturation of ranibizumab was also deliberate to increase the binding affinity to VEGF and increase the biologic activity compared to bevacizumab (Ferrara *et al.* 2006). While initial studies using a monovalent Fab-12 were significantly lower than what was later demonstrated for ranibizumab (Chen *et al.* 1999), later evaluation demonstrated that ranibizumab and bevacizumab had a similar affinity for VEGF-A165 given bevacizumab's bivalent nature and dissociation constant (Papadopoulos *et al.* 2012). A possible disadvantage of the Fc fragment is that bevacizumab may be more stable systemically than is ranibizumab and thus have higher systemic levels (Miki *et al.* 2009). However, it is unclear whether this results in any clinically significant systemic effects.

Aflibercept (Eylea, Regeneron Pharmaceuticals Inc) is a recombinant fusion protein with the extracellular binding portions of VEGFR-1 and VEGFR-2 fused to the Fc portion of the human IgG1. Aflibercept received FDA approval in November 2011 for AMD, September 2012 for RVO, July 2014 for DME, and March 2015 for diabetic retinopathy. Aflibercept has been proven in large, randomized clinical trials for treating AMD in VIEW1 and 2 (Heier *et al.* 2012), CRVO in GALILEO (Korobelnik *et al.* 2014), BRVO in VIBRANT (Campochiaro *et al.* 2015), and DME in DA VINCI (Do *et al.* 2012).

Several studies have evaluated the relative efficacies of these bevacizumab and ranibizumab for treatment of AMD and found no difference in visual acuity or complications (Subramanian *et al.* 2010; Biswas *et al.* 2011; Chakravarthy *et al.* 2013; Kodjikian *et al.* 2013; Krebs *et al.* 2013). The CATT study found monthly treatments of bevacizumab not inferior to monthly ranibizumab, although ranibizumab had a greater mean decrease in central retinal thickness and bevacizumab had more serious systemic adverse events (CATT 2011). A Cochrane Review including 12 randomized controlled trials including 5496 patients for AMD found no difference in mean visual acuity outcomes between ranibizumab and bevacizumab, no difference in adverse events, and a statistically significant but clinically insignificant increase in reduction in central retinal thickness ($-14\ \mu\text{m}$) with ranibizumab compared to bevacizumab (Solomon *et al.* 2014). Some disagreement also exists whether a difference in safety of bevacizumab and ranibizumab exists (Martin *et al.* 2012). Some retina specialists state that ranibizumab has been well studied in many randomized clinical trials with more long-term findings when compared with bevacizumab, although the superiority of ranibizumab over bevacizumab has not been proven. This remains a hot topic of debate among retina specialists given the significant difference in price between the medications, and both drugs are actively used to treat VEGF driven retinopathies currently.

A comparison of intravitreal bevacizumab, ranibizumab, and aflibercept for DME found that for visual acuity of 20/40 or better there was on average no difference in the improvement in visual acuity between the three treatments. For patients with visual acuity of 20/50 or worse, aflibercept resulted in more mean improvement in visual acuity (Diabetic Retinopathy Clinical Research Network 2015).

Further modifications in VEGF therapy have occurred through the development of new anti-VEGF molecules. Conbercept (KH902; Chengdu Kanghong Biotech Co., Ltd., Sichuan, China) consists of the VEGF binding domains of the human VEGFR-1 and VEGFR-2 combined with the Fc portion of the human immunoglobulin G1. It binds the VEGF-A along with VEGF-B and placental growth factor (Wang *et al.* 2013). A randomized, double-masked, multicenter, phase 2 clinical trial, of conbercept, AURORA, demonstrated the drug to be safe and efficacious with as needed dosing (Li X *et al.* 2014).

Recent concerns have been raised regarding possible increase in geographic atrophy with anti-VEGF administration, particularly ranibizumab (Grunwald *et al.* 2014 & 2015). In addition, repeat administration of intravitreal anti-VEGF results in sustained elevation of intraocular in some patients (Bakri *et al.* 2008; Pershing *et al.* 2013; Bakri *et al.* 2014; Dedania & Bakri 2015), suggesting the importance of VEGF to the trabecular meshwork.

Other Secreted Growth Factors Currently Under Study

In addition to VEGF, numerous other secreted growth factors that contribute to the angiogenesis cycle exist and are currently under investigation as potential therapeutic targets. Current anti-angiogenic targets are summarized in table 2. An important proangiogenic secreted molecule implicated in retinal neovascular disease is fibroblast derived growth factor (FGF) (Presta *et al.* 2005). FGF are a family that play an important role in angiogenesis, wound healing, and embryonic development. FGF plays a role in the

migration and proliferation of endothelial cells along with the proliferation of smooth muscle cells and fibroblasts. Basic FGF (bFGF), a secreted cytokine, regulates angiogenesis through induction of VEGF expression via the FGFR1/c-Src/p38/NF- κ B (nuclear factor κ B) signaling pathway, triggering angiogenesis of endothelial progenitor cells (Tzeng *et al.* 2015).

Platelet derived growth factor (PDGF) is critical for pericyte recruitment and maturation of neovascular vessels (Alvarez *et al.* 2006). Therapies targeting PDGF may destabilize neovascular tissue thereby enhancing current anti-VEGF approaches. This interplay among angiogenic factors is also observed between FGF and PDGF. FGF-2 works with PDGF-BB to upregulate expression of PDGFR- α and - β in newly formed blood vessels (Cao *et al.* 2003). Thus therapies targeting PDGF and FGF may prove to complement one another as well as current therapies targeting VEGF.

Placental-induced growth factor (PIGF) is a member of the VEGF family and plays an important role in several steps of vasculogenesis and angiogenesis through its receptor VEGFR-1 (Flt-1). An anti-Flt-1 antibody suppressed neovascularization in tumors and ischemic retina, and angiogenesis and inflammatory joint destruction in arthritis (Luttun *et al.* 2002). PIGF plays a role in endothelial cell migration and survival, recruitment of smooth muscle cells, and differentiation and activation of monocytes. PIGF mRNA expression was reduced by hypoxia in mice and elevated with anti-VEGF therapy (Zhou *et al.* 2014).

Hepatocyte growth factor (HGF) is a mesenchyme-derived pleiotropic factor and acts upon vascular endothelial cells to regulate cell growth, cell motility, and morphogenesis through epithelial mesenchymal interactions (Morishita *et al.* 2004). NK4, the N-terminal hairpin and subsequent four kringle domains of HGF, acts as the competitive antagonist for HGF and has been demonstrated to inhibit HGF-induced ERK1/2 (p44/42 mitogen-activated protein kinase) activation to prevent angiogenesis and tumor growth in mice (Kuba *et al.* 2000). HGF has also been implicated in papillary thyroid cancer angiogenesis (Scarpino *et al.* 2003).

Pigment epithelium-derived factor (PEDF) plays an important role in endothelial cell migration and proliferation, apoptosis through the p38 MAPK pathway or FAS/FASL, and mediates angiogenesis through effects on VEGFR-1 and -2 (Longeras *et al.* 2012). An N-terminal 34-amino acid peptide (PEDF-34) has been demonstrated to have antiangiogenic properties along with anti-vasculogenic properties.

Pro-angiogenic Signaling Molecules as Therapeutic Targets

The VEGFR is a receptor tyrosine kinase and one of the earliest and most studied kinases targeted by synthetic small molecules for oncologic applications and may prove to be effective for the treatment of ocular neovascular disease. There are currently 7 approved small molecules inhibitors targeting the VEGFR: sorafenib (Nexavar, Bayer), sunitinib (Sutent, Pfizer), pazopanib (Votrient, GlaxoSmithKline), axitinib (Inlyta, Pfizer), regorafenib (Stivarga, Bayer), nintedanib (Ofev, Boehringer Ingelheim), and lenvatinib (Lenvima, Eisai Inc.). Both sorafenib and regorafenib are multitarget protein kinase inhibitors. Besides

inhibition of VEGFR and B-raf, sorafenib has also been shown to be a potent low-nanomolar inhibitor of p38a (Simard *et al.* 2009). In addition, vandetanib (Caprelsa, AstraZeneca) is a multiple kinase inhibitor against EGFR, VEGFR, and RET (Knowles *et al.* 2006). Cabozantinib (Cometriq, Exelixis) is a dual MET and VEGFR2 inhibitor.

Numerous drugs targeting other signaling pathways that promote pathological angiogenesis have been developed or are being investigated for oncologic applications. Extension of these therapies for the treatment of ocular neovascular disease may follow. Trebananib (AMG 386) is a peptide-Fc fusion protein, or peptibody, reported to neutralize the interaction between angiopoietins (Ang1/2) and their Tie2 receptors, which has been shown to be promising in ovarian cancer (Marchetti *et al.* 2015). Nintedanib has activity against platelet derived growth factor receptor (PDGFR) and fibroblast growth factor receptor (FGFR) in addition to VEGFR, thus offering a broader spectrum of anti-angiogenic activity than simply VEGFR. Nintedanib has been shown to be effective in lung cancer (Hilberg *et al.* 2008; Reck *et al.* 2014). Linifanib (ABT-869) is a tyrosine-kinase inhibitor that selectively targets VEGFR and PDGFR and has low off-target inhibitory activity and anti-angiogenic activity (Aversa *et al.* 2015).

The Wnt signaling pathways are a group of signal transduction pathways that pass extracellular signals through cell surface receptors to intracellular. The canonical Wnt signaling pathway involves β -catenin. Wnt signaling pathways are activated by the binding of a Wnt-protein to a Frizzled receptor, which activates Dishevelled. Wnt controls cell fate specification, cell proliferation, and cell migration. A glial-derived extracellular ligand, Norrin, can stimulate Wnt signaling and acts on the transmembrane receptor, Frizzled4, a coreceptor, Lrp5, and an auxiliary membrane protein, Tspan12, on the surface of developing endothelial cells. The resulting signal controls a transcriptional program that regulates endothelial growth and maturation (Ye *et al.* 2010). Therapies targeting Wnt signaling are also under development for the treatment of cancer; these, too, may prove effective as an anti-angiogenic approach for the treatment of ocular neovascular disease.

Nestin is an important molecule expressed in the cell soma of diving neural progenitor cells and their leading processes. After this, it follows vascular branches, suggesting vasculogenesis along microglia migrating routes sustains its angiogenic potential (Lee *et al.* 2012). Endothelial nitric oxide synthases occurs in the nuclei of endothelial vascular cells in vasoformative cells, suggesting it may also play a role in vasculogenesis (McLeod *et al.* 2012). Neuropilins are transmembrane glycoproteins that are receptors for VEGF and are essential for blood vessel development and assists with the separation of veins and arteries (Fantin *et al.* 2011). In addition to VEGF, neuropilins bind to semaphorins, molecules critical for axon guidance which have recently been implicated also in retinal neovascular disease (Cerani *et al.* 2013).

Akt-mediated phosphorylation of Girdin, an actin-binding protein, promotes VEGF-dependent migration of endothelial cells and tube formation. Exogenously delivered adenovirus harbouring Girdin short interfering RNA markedly inhibited VEGF-mediated angiogenesis (Kitamura *et al.* 2008). Mammalian target of rapamycin (mTOR), a key mediator of PI3K/Akt/mTOR signaling pathway, has recently emerged as a compelling

molecular target in glioblastoma. The mTOR is a member of serine/threonine protein kinase family that functions as a central controller of growth, proliferation, metabolism and angiogenesis, but its signaling is dysregulated in various human diseases especially in certain solid tumors including the glioblastoma (Cui *et al.* 2015). mTOR has been implicated in the regulation of HIF-1 translation and may play a critical role in regulating HIF-directed angiogenesis in retinal neovascular disease.

The small GTPase RhoA and its downstream effectors, ROCK1 and ROCK2, are important mediators in a number of angiogenic processes, including EC migration, survival, and cell permeability, and suggest that Rho/ROCK inhibition may prove useful for the treatment of angiogenesis-related disorders (Bryan *et al.* 2010).

$\alpha_v\beta_3$ integrin has been reported as a promising therapeutic target for angiogenesis. GOPPP, a novel antagonist of $\alpha_v\beta_3$ integrin, has been shown to inhibit the pro-angiogenic effects of vitronectin on HUVECs, including adhesion, proliferation, and migration, and inhibit ERK1/2 and Akt phosphorylation. HIF-1 α and VEGF were also inhibited by GOPPP in mice (Li YJ *et al.* 2014). Proliferative diabetic retinopathy has been shown to recruit bone-marrow-derived CD133+ endothelial progenitor cells and CD14+ monocytes to assist (Abu El-Asrar *et al.* 2011). Other integrins under active investigation include $\alpha_v\beta_5$ and $\alpha_5\beta_1$ in current clinical trials for AMD and diabetic macular edema.

MicroRNAs (miRNA) are small non-coding RNA molecules (containing approximately 22 nucleotides) that functions in RNA silencing and post-transcriptional regulation of gene expression. A single miRNA can regulate the expression of hundreds of gene products and can have broad effects on cell function. MiRNA-132 as a highly upregulated miRNA in a human embryonic stem cell model of vasculogenesis and was found to be highly expressed in the endothelium of human tumors. Anti-miRNA-132 has been shown to effectively inhibit neovascularization (Anand *et al.* 2010). However, whether therapies targeting microRNAs will be specific enough for the treatment of human disease remains unknown.

Emerging Therapeutic Targets

Future anti-angiogenic approaches may include therapies upstream of the angiogenic secreted factors [e.g., HIF-1 (Subhani *et al.* 2016)] or novel angiogenic secreted molecules [e.g., angiopoietin-like 4, or ANGPTL4 (Xin *et al.* 2013; Sodhi *et al.* 2015)] or signaling pathways regulated by these molecules. However, recent focus has been directed at the molecules that regulate other steps in pathological angiogenesis (table 3). Appreciation for the role of circulating progenitor cells and hematopoietic stem cells from the bone marrow to pathological angiogenesis (Liekens *et al.* 2010) has exposed the SDF1/CXCR4 axis as a novel therapeutic target for ocular neovascular disease. The extracellular matrix plays an essential role in retinal neovascularization. MMPs are necessary to degrade the basement membrane to allow migrating vascular endothelial cells to escape from the parent vessel (Rodrigues *et al.* 2013). Although therapies targeting MMPs have not proven successful, other molecules that regulate extracellular matrix protein production have emerged as potential targets including transforming growth factor (TGF)- α and - β , endoglin, epidermal growth factor (EGF), inflammatory cytokines including IL-6 and 8, TNF-alpha and beta

inhibitors, $\alpha_5\beta_1$ integrin receptor inhibitor, and $\alpha_v\beta_3$ and $\alpha_v\beta_5$. Another critical step in pathological angiogenesis is the promotion of endothelial cell survival through both intrinsic (e.g., VEGF) and extrinsic mechanisms. The latter is ensured through stabilization of endothelial cells through recruitment of pericytes, smooth muscle cells, and deposition of extracellular matrix proteins in addition to focal adhesion kinase (FAK) (Roy-Luzarraga *et al.* 2016). In addition, endothelial cell transdifferentiation has exposed additional targets including ID1 and ID3 (Ruzinova *et al.* 2003).

Vascular Permeability Targets

There is significant overlap between pathological angiogenesis (and retinal neovascularization) and vascular hyper-permeability (and macular edema). Indeed, one of the first steps in pathological angiogenesis is the induction of vascular permeability. It is therefore not surprising that many of the therapeutic targets for retinal neovascularization have proven (or may prove) effective for the treatment of macular edema. This is most notable for therapies targeting VEGF. Nonetheless, macular edema is a major cause of vision loss in numerous disorders, including uveitis, pars planitis, post-operative, and retinal degenerations such as retinitis pigmentosa, in which retinal neovascularization is less common, and there are several mediators that promote vascular permeability independent of their ability to promote angiogenesis.

Macular edema results from a breakdown of the blood-retinal barrier (BRB), which is composed of an inner and outer component (Cunha-Vaz 1976). The inner component is formed by tight junctions and adherens junction between vascular endothelial cells. Pericytes and perivascular astrocytes mediate the inner component also and the paucity of intraendothelial cell vesicles. The outer component is established by tight junctions between RPE cells that prevents fluid from choroidal vessels entering the retina (Vinores 1995; Rizzolo 1997; Vinores *et al.* 1999).

In diabetes, an upregulation of trans-endothelial vesicular transport and increased membrane permeability correlates with BRB breakdown. The players in vascular permeability are identical to those in pathological angiogenesis, including retinal glial cells, vascular endothelial cells, and neutrophils, which together promote breakdown of adherens and tight junctions and increase vesicular transport. And the secreted factors observed in neovascularization, including VEGF, TNF- α , and IL-1 β , are elevated also in macular edema (Cuff *et al.* 1996; Luna *et al.* 1997). Similarly, ANGPTL4, an emerging target in pathological angiogenesis and retinal neovascularization (Babapoor-Farrokhran *et al.* 2015) may also play an important role in vascular permeability and macular edema (Xin *et al.* 2013). The downstream signaling molecules are also similar between retinal neovascularization and diabetic macular edema. As such, src kinase inhibitors (Doukas *et al.* 2008) and tyrosine kinase inhibitors such as pazopanib have been shown to reduce diabetic macular edema in animal models (Thakur *et al.* 2011). Protein kinase C (PKC) inhibitors such as ruboxistaurin showed initial promise in reducing diabetic macular edema (Gálvez 2011; Aiello *et al.* 2011), although some phase 3 studies have shown mixed results (Sheetz *et al.* 2013).

Molecular Imaging and Theranostics

Currently available ophthalmic imaging modalities are capable of imaging retinal pathologic anatomic changes, including hemorrhages, exudates, and edema, with unprecedented resolution. However, anatomical abnormalities are the end-product of complex molecular processes. Subclinical molecular changes occur before retinal disease can be detected by current anatomy-driven imaging instrumentations. Thus, the field of molecular imaging is emerging as an important factor to assist with early disease detection, improved treatment monitoring, and improved understanding of retinal pathophysiology (Xie *et al.* 2012; Capozzi *et al.* 2013; Evans *et al.* 2014). Several recent studies have reported feasibility of molecular imaging in detecting retinal ganglion cells (RGCs), RPE cells, vascular endothelial cells, VEGFR, and leukocytes.

Detection of apoptosing retinal cells (DARC) has been described for the single-cell detection of RGC apoptosis (Cordeiro *et al.* 2004; Coxon *et al.* 2011). In DARC, Annexin V was intravitreally injected to specifically bind the apoptosis biomarker phosphatidylserine (PS), and then it was detected by ophthalmic fluorescence imaging instrumentation. A peptide-based fluorescent probe (TcapQ) sensitive to active caspases such as caspase 3 involved in RGC apoptosis has also been described *in vivo* to quantify apoptotic RGCs (Barnett *et al.* 2009). Another promising opportunity for molecular imaging of RGCs lies in imaging RGC dysfunction before cell death. Several imaging probe such as reactive oxygen (ROS) (Dickinson *et al.* 2011), mitochondria permeability transition pore (Vrabec *et al.* 2003), and E glutamate (Okubo *et al.* 2010) hold great promise in improving the molecular imaging of RGCs. RPE-related molecular targets that have been evaluated include ROS (King *et al.* 2004), β -amyloid, esterified cholesterol and carbohydrate moieties in drusen (Hageman *et al.* 2001).

Vascular endothelial cells have emerged as critical surface biomarkers of neovascularization and as potential candidates for development of targeted contrast agents for ophthalmic imaging of retinal and choroidal neovascularization. The C-C chemokine receptor 3 (CCR3) is a promising biomarker of choroidal neovascularization (CNV), as demonstrated by CCR3 expression on choroidal neovascular endothelial cells in human CNV specimens (Takeda *et al.* 2009). Other promising biomarkers include targeting proliferating endothelial, endoglin (Grisanti *et al.* 2004), and integrin $\alpha_v\beta_3$ (Li *et al.* 2010; Peiris *et al.* 2012). VEGFR-2 expression has been demonstrated with molecular imaging to be elevated in retinal capillaries in diabetes (Sun *et al.* 2014). OCT has been used with anti-mouse CD45 coated gold nanorods to visualize leukocytes at sites of laser-induced retinal injury (Sen *et al.* 2016). Photoacoustic imaging can also be used to perform molecular imaging (de la Zerda *et al.* 2010; Hu *et al.* 2015). Molecular imaging is now further evolving into the development of theranostics, agents that combine diagnostic imaging with targeted therapy (Ding *et al.* 2013; Yan *et al.* 2016). While currently in its infancy, molecular imaging of the retina is rapidly developing and will play a critical role in early disease diagnosis and treatment monitoring in the near future.

Drug Delivery Pathways

Treatment with anti-VEGF is often transient and must be repeated with rapid regrowth of neovascularization on cessation of treatment. In addition to intravitreal injections which are the most common routes of administration, numerous additional pathways of delivery are being investigated. Recent developments in drug delivery have led to investigations on longer-lasting agents through biodegradable implants, gene delivery, biodegradable polymers, suprachoroidal delivery, microspheres, contact lenses with sustained delivery, punctal plugs with sustained delivery, nanoparticles, liposomes, and gels (Querques *et al.* 2015). ForSight Vision has developed a refillable, nonbiodegradable port delivery system that is surgically implanted in the pars plana beneath the conjunctiva through a 3.2 mm scleral incision. A phase 1 study was conducted in Latvia included 20 treatment-naïve patients with neovascular AMD (Rubio 2014). The primary endpoint of the study was at 1 year. There were 4 serious adverse effects, including 1 case of endophthalmitis, 2 cases of persistent vitreous hemorrhage, and 1 case of traumatic cataract. The visual acuity gain for all patients at month 12 was 10 letters, and the average number of refills for the full 20-patient cohort was 4.8. A phase 2 study is being planned.

Topical therapy has also been developed, pazopanib (GlaxoSmithKline, Brentford, UK), a multi-tyrosine kinase inhibitor having an effect on VEGFR-1, VEGFR-2, VEGFR-3, PDGFR- α , PDGFR- β . A study evaluating 70 patients with AMD treated for 28 days found a decrease in central retinal thickness and an increase in visual acuity in only a subset of patients (Danis *et al.* 2014). Bevacizumab therapy was initially investigated in AMD as a systemic treatment given intravenously, and oral therapies are also being investigated.

Another strategy of sustained delivery is through gene delivery through adeno-associated viral vectors (AAV). Gene therapy trials have been completed for Leber's congenital amaurosis and have shown safety and promising results (Maguire *et al.* 2008). AAV2-sFLT01 (Genzyme, Cambridge, MA, USA) allows delivery of VEGFR1 (sFlt1) and has demonstrated sustained expression in animals (Pechan *et al.* 2009; Lukason *et al.* 2011). Similarly, AVA-101 (rAAV.sFLT-1 recombinant adeno-associated virus) is currently undergoing a phase I study ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01494805) identifier: NCT01494805).

Additional routes of administration which can be considered include transcleral, subretinal, and high-velocity (needleless) delivery (Todorich *et al.* 2014), siRNA, and cell-based therapy. Single chain antibody fragments are being developed which allow for sustained delivery due to their small size. A phase I study of the safety and tolerability of a single chain antibody fragment ESBA1008 (Alcon, Fort Worth, Tex., USA) versus ranibizumab has been completed in 194 patients in a prospective, randomized, multicenter trial. Encapsulated cell technology involves implants which can continuously produce recombinant therapy without needing to be refilled. NT-503 (Neurotech Pharmaceuticals, RI, USA), an intraocular implant delivering anti-VEGF, is being tested in a phase I prospective, multicenter study (Querques *et al.* 2015). In addition to pharmacologic therapies, laser photocoagulation can be used along with photodynamic therapy, transpupillary thermotherapy, and radiation therapy as a possible treatment by itself or in conjunction with pharmacotherapy.

Conclusion

Anti-angiogenic pharmacologic therapy has become an indispensable component in the management of diseases of the vitreous and retina. Anti-angiogenic pharmacotherapy has revolutionized the treatment of many ocular conditions, including diabetic retinopathy, retinal vein occlusions, pathologic myopia, choroidal neovascularization, and age-related macular degeneration. Our improving understanding of the pathophysiology of embryology, angiogenesis, and vasculogenesis has allowed us to increasingly target a select tissue while minimizing the side effects of therapy. Selective therapeutic approaches will achieve desirable biochemical, cellular, and tissue effects while excluding unwanted damage, and will improve our understanding of the function and pathology of posterior segment diseases. Continuous innovations in pharmacotherapy and progress in understanding of retinal pathophysiology make us believe that improvements in the treatment of retinal diseases using anti-angiogenic therapy will continue for many years to come.

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References

- Abu El-Asrar AM, Struyf S, Verbeke H, Van Damme J, Geboes K. Circulating bone-marrow-derived endothelial precursor cells contribute to neovascularization in diabetic epiretinal membranes. *Acta Ophthalmol.* 2011 May; 89(3):222–8. [PubMed: 19764917]
- Adamis AP, Miller JW, Bernal MT, D'Amico DJ, Folkman J, Yeo TK, Yeo KT. Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. *Am J Ophthalmol.* 1994 Oct 15; 118(4):445–50. [PubMed: 7943121]
- Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, Pasquale LR, Thieme H, Iwamoto MA, Park JE, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med.* 1994 Dec 1; 331(22):1480–7. [PubMed: 7526212]
- Aiello LP, Vignati L, Sheetz MJ, Zhi X, Girach A, Davis MD, Wolka AM, Shahri N, Milton RC, PKC-DRS and PKC-DRS2 Study Groups. Oral protein kinase c β inhibition using ruboxistaurin: efficacy, safety, and causes of vision loss among 813 patients (1,392 eyes) with diabetic retinopathy in the Protein Kinase C β Inhibitor-Diabetic Retinopathy Study and the Protein Kinase C β Inhibitor-Diabetic Retinopathy Study 2. *Retina.* 2011 Nov; 31(10):2084–94. [PubMed: 21862954]
- Alon T, Hemo I, Itin A, Pe'er J, Stone J, Keshet E. Vascular endothelial growth factor acts as a survival factor for new formed retinal vessels and has implications for retinopathy of prematurity. *Nature Med.* 1995; 1:1024–1028. [PubMed: 7489357]
- Alvarez RH, Kantarjian HM, Cortes JE. Biology of platelet-derived growth factor and its involvement in disease. *Mayo Clin Proc.* 2006; 81:1241–57. [PubMed: 16970222]
- Amin RH, Frank RN, Kennedy A, Elliott D, Puklin JE, Abrams GW. Vascular endothelial growth factor is present in glial cells of the retina and optic nerve of human subjects with nonproliferative diabetic retinopathy. *Invest Ophthalmol Vis Sci.* 1997 Jan; 38(1):36–47. [PubMed: 9008628]
- Anand S, Majeti BK, Acevedo LM, Murphy EA, Mukthavaram R, Schepke L, Huang M, Shields DJ, Lindquist JN, Lapinski PE, King PD, Weis SM, Cheres DA. MicroRNA-132-mediated loss of p120RasGAP activates the endothelium to facilitate pathological angiogenesis. *Nat Med.* 2010 Aug; 16(8):909–14. [PubMed: 20676106]

- Ashton N. Retinal angiogenesis in the human embryo. *Brit Med Bull.* 1970; 26:103–106. [PubMed: 4911762]
- Aversa C, Leone F, Zucchini G, Serini G, Geuna E, Milani A, Valdembri D, Martinello R, Montemurro F. Linifanib: current status and future potential in cancer therapy. *Expert Rev Anticancer Ther.* 2015 Jun; 15(6):677–87. [PubMed: 25936222]
- Avery RL, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA, Giust MJ. Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. *Ophthalmology.* 2006 Mar; 113(3): 363–372. [PubMed: 16458968]
- Babapoor-Farrokhran S, Jee K, Puchner B, Hassan SJ, Xin X, Rodrigues M, Kashiwabuchi F, Ma T, Hu K, Deshpande M, Daoud Y, Solomon S, Wenick A, Luty GA, Semenza GL, Montaner S, Sodhi A. Angiopoietin-like 4 is a potent angiogenic factor and a novel therapeutic target for patients with proliferative diabetic retinopathy. *Proc Natl Acad Sci U S A.* 2015 Jun 9; 112(23):E3030–9. [PubMed: 26039997]
- Bakri SJ, McCannel CA, Edwards AO, Moshfeghi DM. Persistent ocular hypertension following intravitreal ranibizumab. *Graefes Arch Clin Exp Ophthalmol.* 2008; 246:955–958. [PubMed: 18425523]
- Bakri SJ, Moshfeghi DM, Francom S, Rundle AC, Reshef DS, Lee PP, Schaeffer C, Rubio RG, Lai P. Intraocular pressure in eyes receiving monthly ranibizumab in 2 pivotal age-related macular degeneration clinical trials. *Ophthalmology.* 2014; 121:1102–1108. [PubMed: 24393349]
- Barnett EM, Zhang X, Maxwell D, Chang Q, Piwnica-Worms D. Single-cell imaging of retinal ganglion cell apoptosis with a cell-penetrating, activatable peptide probe in an in vivo glaucoma model. *Proceedings of the National Academy of Sciences of the United States of America.* 2009; 106:9391–9396. [PubMed: 19458250]
- Beach JM, Schwenzer KJ, Srinivas S, Kim D, Tiedeman JS. Oximetry of retinal vessels by dual-wavelength imaging: calibration and influence of pigmentation. *J Appl Physiol.* 1999 Feb; 86(2): 748–58. [PubMed: 9931217]
- Birbrair A, Zhang T, Wang ZM, Messi ML, Olson JD, Mintz A, Delbono O. Type-2 pericytes participate in normal and tumoral angiogenesis. *Am J Physiol Cell Physiol.* 2014 Jul 1; 307(1):C25–38. [PubMed: 24788248]
- Biswas P, Sengupta S, Choudhary R, Home S, Paul A, Sinha S. Comparative role of intravitreal ranibizumab versus bevacizumab in choroidal neovascular membrane in age-related macular degeneration. *Indian J Ophthalmol.* 2011 May-Jun; 59(3):191–6. [PubMed: 21586838]
- The Branch Vein Occlusion Study Group. Argon laser photocoagulation for macular edema in branch vein occlusion. *Am J Ophthalmol.* 1984; 98(3):271–82. [PubMed: 6383055]
- Brown DM, Michels M, Kaiser PK, Heier JS, Sy JP, Ianchulev T, ANCHOR Study Group. Ranibizumab versus verteporfin photodynamic therapy for neovascular age-related macular degeneration: Two-year results of the ANCHOR study. *Ophthalmology.* 2009 Jan; 116(1):57–65. [PubMed: 19118696]
- Brown DM, Campochiaro PA, Singh RP, Li Z, Gray S, Saroj N, Rundle AC, Rubio RG, Murahashi WY, CRUISE Investigators. Ranibizumab for macular edema after central retinal vein occlusion: six-month primary end point results of a phase III study. *Ophthalmology.* 2010; 117:1124–1133. [PubMed: 20381871]
- Bryan BA, Dennstedt E, Mitchell DC, Walshe TE, Noma K, Loureiro R, Saint-Geniez M, Campaigniac JP, Liao JK, D'Amore PA. RhoA/ROCK signaling is essential for multiple aspects of VEGF-mediated angiogenesis. *FASEB J.* 2010 Sep; 24(9):3186–95. [PubMed: 20400538]
- Burri PH, Hlushchuk R, Djonov V. Intussusceptive angiogenesis: its emergence, its characteristics, and its significance. *Dev Dyn.* 2004 Nov; 231(3):474–88. [PubMed: 15376313]
- Campochiaro PA, Sen HA. Adenosine and its agonists cause retinal vasodilation and hemorrhages: Implications for ischemic retinopathies. *Arch Ophthalmol.* 1989; 107:412–6. [PubMed: 2493781]
- Campochiaro PA, Heier JS, Feiner L, Gray S, Saroj N, Rundle AC, Murahashi WY, Rubio RG, BRAVO Investigators. Ranibizumab for macular edema after branch retinal vein occlusion: six-month primary end point results of a phase III study. *Ophthalmology.* 2010; 117:1102–1112. [PubMed: 20398941]

- Campochiaro PA, Clark WL, Boyer DS, Heier JS, Brown DM, Vitti R, Kazmi H, Berliner AJ, Erickson K, Chu KW, Soo Y, Cheng Y, Haller JA. Intravitreal aflibercept for macular edema following branch retinal vein occlusion: the 24-week results of the VIBRANT study. *Ophthalmology*. 2015 Mar; 122(3):538–44. [PubMed: 25315663]
- Cao R, Brakenhielm E, Pawliuk R, Wariaro D, Post MJ, Wahlberg E, Leboulch P, Cao Y. Angiogenic synergism, vascular stability and improvement of hind-limb ischemia by a combination of PDGF-BB and FGF-2. *Nature Medicine*. 2003; 9(5):604–13.
- Capozzi ME, Gordon AY, Penn JS, Jayagopal A. Molecular imaging of retinal disease. *J Ocul Pharmacol Ther*. 2013 Mar; 29(2):275–86. [PubMed: 23421501]
- Caprara C, Thiersch M, Lange C, Joly S, Samardzija M, Grimm C. HIF1A is essential for the development of the intermediate plexus of the retinal vasculature. *Invest Ophthalmol Vis Sci*. 2011; 52:2109–2117. [PubMed: 21212189]
- CATT Research Group. Martin DF, Maguire MG, Ying GS, Grunwald JE, Fine SL, Jaffe GJ. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. *N Engl J Med*. 2011 May 19; 364(20):1897–908. [PubMed: 21526923]
- Cerani A, Tetreault N, Menard C, Lapalme E, Patel C, Sitaras N, Beaudoin F, Leboeuf D, De Guire V, Binet F, Dejda A, Rezende FA, Miloudi K, Sapienza P. Neuron-derived semaphorin 3A is an early inducer of vascular permeability in diabetic retinopathy via neuropilin-1. *Cell Metab*. 2013 Oct 1; 18(4):505–18. [PubMed: 24093675]
- Chakravarthy U, Adamis AP, Cunningham ET Jr, Goldbaum M, Guyer DR, Katz B, Patel M, VEGF Inhibition Study in Ocular Neovascularization (V.I.S.I.O.N.) Clinical Trial Group. Year 2 efficacy results of 2 randomized controlled clinical trials of pegaptanib for neovascular age-related macular degeneration. *Ophthalmology*. 2006 Sep; 113(9):1508.e1–25. [PubMed: 16828500]
- Chakravarthy U, Harding SP, Rogers CA, Downes SM, Lotery AJ, Culliford LA, Reeves BC, IVAN study investigators. Alternative treatments to inhibit VEGF in age-related choroidal neovascularisation: 2-year findings of the IVAN randomised controlled trial. *Lancet*. 2013 Oct 12; 382(9900):1258–67. [PubMed: 23870813]
- Chan-Ling T, Gock B, Stone J. The effect of oxygen on vasoformative cell division. Evidence that 'physiologic hypoxia' is the stimulus for normal retinal vasculogenesis. *Invest Ophthalmol Vis Sci*. 1995; 36:1201–1214. [PubMed: 7775098]
- Chan-Ling T, Mcleod DS, Hughes S, Bax-ter L, Chu Y, Hasegawa T, Luttly GA. Astrocyte-endothelial cell relationships during human retinal vascular development. *Invest Ophthalmol Vis Sci*. 2004; 45:2020–2032. [PubMed: 15161871]
- Chang JH, et al. Matrix metalloproteinase 14 modulates signal transduction and angiogenesis in the cornea. *Surv Ophthalmol*. 2016 Jul-Aug; 61(4):478–97. [PubMed: 26647161]
- Che X, Fan XQ, Wang ZL. Mechanism of blood-retinal barrier breakdown induced by HIV-1 (Review). *Exp Ther Med*. 2014 Apr; 7(4):768–772. [PubMed: 24660027]
- Chen Y, Wiesmann C, Fuh G, Li B, Christinger HW, McKay P, de Vos AM, Lowman HB. Selection and analysis of an optimized anti-VEGF antibody: crystal structure of an affinity matured Fab in complex with antigen. *J Mol Biol*. 1999; 293:865–881. [PubMed: 10543973]
- Chu Y, Hughes S, Chan-Ling T. Differentiation and migration of astrocyte precursor cells and astrocytes in human fetal retina: relevance to optic nerve coloboma. *FASEB J*. 2001; 15:2013–2015. [PubMed: 11511521]
- Claxton S, Fruttiger M. Role of arteries in oxygen induced vaso-obliteration. *Exp Eye Res*. 2003; 77:305–311. [PubMed: 12907163]
- Cohen MH, Gootenberg J, Keegan P, Pazdur R. FDA drug approval summary: bevacizumab plus FOLFOX4 as second-line treatment of colorectal cancer. *Oncologist*. 2007 Mar; 12(3):356–61. [PubMed: 17405901]
- Cordeiro MF, Guo L, Luong V, Harding G, Wang W, Jones HE, Moss SE, Sillito AM, Fitzke FW. Real-time imaging of single nerve cell apoptosis in retinal neurodegeneration. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:13352–13356. [PubMed: 15340151]
- Coxon KM, Duggan J, Cordeiro MF, Moss SE. Purification of annexin V and its use in the detection of apoptotic cells. *Methods in molecular biology* (Clifton, NJ). 2011; 731:293–308.

- Cuff CA, Martiney JA, Berman JW, Brosnan CF. Differential effects of transforming growth factor- β -1 on interleukin-1-induced cellular inflammation and vascular permeability in the rabbit retina. *J Neuroimmunol.* 1996; 70:21–8. [PubMed: 8862131]
- Cui YH, Chen J, Xu T, Tian HL. Structure-based grafting and identification of kinase-inhibitors to target mTOR signaling pathway as potential therapeutics for glioblastoma. *Comput Biol Chem.* 2015 Feb;54:57–65. [PubMed: 25625417]
- Cunha-Vaz JG. The blood-retinal barriers. *Doc Ophthalmol.* 1976; 41:287–327. [PubMed: 1009819]
- D'Amico DJ, Masonson HN, Patel M, Adamis AP, Cunningham ET Jr, Guyer DR, Katz B, VEGF Inhibition Study in Ocular Neovascularization (V.I.S.I.O.N.) Clinical Trial Group. Pegaptanib sodium for neovascular age-related macular degeneration: two-year safety results of the two prospective, multicenter, controlled clinical trials. *Ophthalmology.* 2006 Jun; 113(6):992–1001. [PubMed: 16647134]
- Danis R, McLaughlin MM, Tolentino M, Staurengi G, Ye L, Xu CF, Kim RY, Johnson MW, Pazopanib Eye Drops Study Group. Pazopanib eye drops: a randomised trial in neovascular age-related macular degeneration. *Br J Ophthalmol.* 2014; 98:172–178. [PubMed: 24227801]
- Datta M, Via LE, Kamoun WS, Liu C, Chen W, Seano G, Weiner DM, Schimel D, England K, Martin JD, Gao X, Xu L, Barry CE 3rd, Jain RK. Anti-vascular endothelial growth factor treatment normalizes tuberculosis granuloma vasculature and improves small molecule delivery. *Proc Natl Acad Sci U S A.* 2015 Feb 10; 112(6):1827–32. [PubMed: 25624495]
- Dawson DW, Volpert OV, Gillis P, Crawford SE, Xu H, Benedict W, Bouck NP. Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. *Science.* 1999 Jul 9; 285(5425):245–8. [PubMed: 10398599]
- de la Zerda A, Paulus YM, Teed R, Bodapati S, Dollberg Y, Khuri-Yakub BT, Blumenkranz MS, Moshfeghi DM, Gambhir SS. Photoacoustic Ocular Imaging. *Optics Letters.* 2010; 35(3):270–272. [PubMed: 20125691]
- de Oliveira Dias JR, Rodrigues EB, Maia M, Magalhães O Jr, Penha FM, Farah ME. Cytokines in neovascular age-related macular degeneration: fundamentals of targeted combination therapy. *Br J Ophthalmol.* 2011 Dec; 95(12):1631–7. [PubMed: 21546514]
- Dedania VS, Bakri SJ. Sustained elevation of intraocular pressure after intravitreal anti-VEGF agents: What is the evidence? *Retina.* 2015 May; 35(5):841–58. [PubMed: 25905784]
- Diabetic Retinopathy Clinical Research Network. Wells JA, Glassman AR, Ayala AR, Jampol LM, Aiello LP, Antoszyk AN, Arnold-Bush B, Baker CW, Bressler NM, Browning DJ, Elman MJ, Ferris FL, Friedman SM, Melia M, Pieramici DJ, Sun JK, Beck RW. Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema. *N Engl J Med.* 2015 Mar 26; 372(13):1193–203. [PubMed: 25692915]
- The Diabetic Retinopathy Study Research Group. Photocoagulation treatment of proliferative diabetic retinopathy. Clinical application of Diabetic Retinopathy Study (DRS) findings. DRS report number 8. *Ophthalmology.* 1981; 88(7):583–600. [PubMed: 7196564]
- Dickinson BC, Tang Y, Chang Z, Chang CJ. A nuclear-localized fluorescent hydrogen peroxide probe for monitoring sirtuin-mediated oxidative stress responses in vivo. *Chemistry & biology.* 2011; 18:943–948. [PubMed: 21867909]
- Ding Y, Li S, Nie G. Nanotechnological strategies for therapeutic targeting of tumor vasculature. *Nanomedicine (Lond).* 2013 Jul; 8(7):1209–22. [PubMed: 23837858]
- DiPietro LA. Angiogenesis and wound repair: when enough is enough. *J Leukoc Biol.* 2016 Jul 12. pii: jlb.4MR0316-102R. [Epub ahead of print].
- Djonov VG, Kurz H, Burri PH. Optimality in the developing vascular system: branching remodeling by means of intussusception as an efficient adaptation mechanism. *Dev Dyn.* 2002 Aug; 224(4): 391–402. [PubMed: 12203731]
- Do DV, Nguyen QD, Boyer D, Schmidt-Erfurth U, Brown DM, Vitti R, Berliner AJ, Gao B, Zeitz O, Ruckert R, Schmelter T, Sandbrink R, Heier JS, da Vinci Study Group. One-year outcomes of the da Vinci Study of VEGF Trap-Eye in eyes with diabetic macular edema. *Ophthalmology.* 2012 Aug; 119(8):1658–65. [PubMed: 22537617]
- Doukas J, Mahesh S, Umeda N, Kachi S, Akiyama H, Yokoi K, Cao J, Chen Z, Dellamary L, Tam B, Racanelli-Layton A, Hood J, Martin M, Noronha G, Soll R, Campochiaro PA. Topical

- administration of a multi-targeted kinase inhibitor suppresses choroidal neovascularization and retinal edema. *J Cell Physiol.* 2008 Jul; 216(1):29–37. [PubMed: 18330892]
- Early Treatment Diabetic Retinopathy Study Research Group. Results from the Early Treatment Diabetic Retinopathy Study. *Ophthalmology.* 1991; 98(Suppl 5):739–840. [PubMed: 2062509]
- Epstein DL, Algvere PV, von Wendt G, Seregard S, Kvanta A. Bevacizumab for macular edema in central retinal vein occlusion: a prospective, randomized, double-masked clinical study. *Ophthalmology.* 2012 Jun; 119(6):1184–9. [PubMed: 22424833]
- Evans SM, Kim K, Moore CE, Uddin MI, Capozzi ME, Craft JR, Sulikowski GA, Jayagopal A. Molecular probes for imaging of hypoxia in the retina. *Bioconjug Chem.* 2014 Nov 19; 25(11):2030–7. [PubMed: 25250692]
- Fantin A, Schwarz Q, Davidson K, Normando EM, Denti L, Ruhrberg C. The cytoplasmic domain of neuropilin 1 is dispensable for angiogenesis, but promotes the spatial separation of retinal arteries and veins. *Development.* 2011 Oct; 138(19):4185–91. [PubMed: 21852397]
- Favier B, Alam A, Barron P, Bonnin J, Laboudie P, Fons P, Mandron M, Herault JP, Neufeld G, Savi P, Herbert JM, Bono F. Neuropilin-2 interacts with VEGFR-2 and VEGFR-3 and promotes human endothelial cell survival and migration. *Blood.* 2006 Aug 15; 108(4):1243–50. [PubMed: 16621967]
- Feeney SA, Simpson DA, Gardiner TA, Boyle C, Jamison P, Stitt AW. Role of vascular endothelial growth factor and placental growth factors during retinal vascular development and hyaloid regression. *Invest Ophthalmol Vis Sci.* 2003 Feb; 44(2):839–47. [PubMed: 12556420]
- Fernando NH, Hurwitz HI. Inhibition of vascular endothelial growth factor in the treatment of colorectal cancer. *Semin Oncol.* 2003 Jun; 30(3 Suppl 6):39–50.
- Ferrara N. VEGF as a therapeutic target in cancer. *Oncology.* 2005; 69(Suppl 3):11–6. [PubMed: 16301831]
- Ferrara N, Damico L, Shams N, Lowman H, Kim R. Development of ranibizumab, an anti-vascular endothelial growth factor antigen binding fragment, as therapy for neovascular age-related macular degeneration. *Retina.* 2006; 26:859–870. [PubMed: 17031284]
- Figg, W., Folkman, J., editors. *Angiogenesis: An Integrative Approach from Science to Medicine.* New York, NY: Springer; 2008.
- Gálvez MI. Protein kinase C inhibitors in the treatment of diabetic retinopathy. *Review Curr Pharm Biotechnol.* 2011 Mar 1; 12(3):386–91. [PubMed: 20939796]
- Gariano RF. Cellular mechanisms in retinal vascular development. *Prof Retinal Eye Res.* 2003; 22:295–306.
- Gariano RF, Gardner TW. Retinal angiogenesis in development and disease. *Nature.* 2005; 438:960–6. [PubMed: 16355161]
- Gariano RF, Hu D, Helms J. Expression of angiogenesis-related genes during retinal development. *Gene Expr Patterns.* 2006 Jan; 6(2):187–92. [PubMed: 16330258]
- Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, Jeltsch M, Mitchell C, Alitalo K, Shima D, Betsholtz C. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *J Cell Biol.* 2003 Jun 23; 161(6):1163–77. [PubMed: 12810700]
- Ghanem I, Riveiro ME, Paradis V, Faivre S, de Parga PM, Raymond E. Insights on the CXCL12-CXCR4 axis in hepatocellular carcinoma carcinogenesis. *Am J Transl Res.* 2014 Jul 18; 6(4):340–52. [PubMed: 25075251]
- Giantonio BJ, Catalano PJ, Meropol NJ, O'Dwyer PJ, Mitchell EP, Alberts SR, Schwartz MA, Benson AB III, Eastern Cooperative Oncology Group Study E3200. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. *J Clin Oncol.* 2007 Apr; 25(12):20. 1539–44.
- Gragoudas ES, Adamis AP, Cunningham ET Jr, Feinsod M, Guyer DR, VEGF Inhibition Study in Ocular Neovascularization Clinical Trial Group. Pegaptanib for neovascular age-related macular degeneration. *N Engl J Med.* 2004 Dec; 351(27):30. 2805–16.
- Grisanti S, Canbek S, Kaiserling E, Adam A, Lafaut B, Gelisken F, Szurman P, Henke-Fahle S, Oficjalska-Mlynczak J, Bartz-Schmidt KU. Expression of endoglin in choroidal neovascularization. *Exp Eye Res.* 2004; 78:207–213. [PubMed: 14729353]

- Grunwald JE, Daniel E, Huang J, Ying GS, Maguire MG, Toth CA, Jaffe GJ, Fine SL, Blodi B, Klein ML, Martin AA, Hagstrom SA, Martin DF, CATT Research Group. Risk of geographic atrophy in the comparison of age-related macular degeneration treatments trials. *Ophthalmology*. 2014 Jan; 121(1):150–61. [PubMed: 24084496]
- Grunwald JE, Pistilli M, Ying GS, Maguire MG, Daniel E, Martin DF, Comparison of Age-related Macular Degeneration Treatments Trials Research Group. Growth of geographic atrophy in the comparison of age-related macular degeneration treatments trials. *Ophthalmology*. 2015 Apr; 122(4):809–16. [PubMed: 25542520]
- Hageman GS, Luthert PJ, Victor Chong NH, Johnson LV, Anderson DH, Mullins RF. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. *Prog Retin Eye Res*. 2001; 20:705–732. [PubMed: 11587915]
- Heier JS, Brown DM, Chong V, Korobelnik JF, Kaiser PK, Nguyen QD, Kirchhof B, Ho A, Ogura Y, Yancopoulos GD, Stahl N, Vitti R, Berliner AJ, Soo Y, Anderesi M, Groetzbach G, Sommerauer B, Sandbrink R, Simader C, Schmidt-Erfurth U, VIEW 1 and VIEW 2 Study Groups. Intravitreal aflibercept (VEGF trap-eye) in wet age-related macular degeneration. *Ophthalmology*. 2012 Dec; 119(12):2537–48. [PubMed: 23084240]
- Hilberg F, Roth GJ, Krssak M, Kautschitsch S, Sommergruber W, Tontsch-Grunt U, Garin-Chesa P, Bader G, Zoephel A, Quant J, Heckel A, Rettig WJ. BIBF 1120: triple angiokinase inhibitor with sustained receptor blockade and good antitumor efficacy. *Cancer Res*. 2008; 68:4774–82. [PubMed: 18559524]
- Hu Z, Wang X, Liu Q, Paulus YM. Photoacoustic Imaging in Ophthalmology. *International Journal of Ophthalmology & Eye Science*. 2015; 3(8):126–132.
- Hubbi ME, Gilkes DM, Hu H, Kshitiz, Ahmed I, Semenza GL. Cyclin-dependent kinases regulate lysosomal degradation of hypoxia-inducible factor 1 α to promote cell-cycle progression. *Proc Natl Acad Sci U S A*. 2014 Aug 12; 111(32):E3325–34. [PubMed: 25071185]
- Hughes S, Yang H, Chan-Ling T. Vascularization of the human fetal retina: roles of vasculogenesis and angiogenesis. *Invest Ophthalmol Vis Sci*. 2000; 41:1217–1228. [PubMed: 10752963]
- Jiang A, Zhang M, Liu Z. Angioblasts in adult and its role in ocular disorders due to neovascularization. *Yan Ke Xue Bao*. 2005 Sep; 21(3):158–62, 178. [PubMed: 17162854]
- Kaelin WG. Proline hydroxylation and gene expression. *Annu Rev Biochem*. 2005; 74:115–28. [PubMed: 15952883]
- Kapany NS, Peppers NA, Zweng HC, Flocks M. Retinal Photocoagulation by Lasers. *Nature*. 1963; 199:146–9. [PubMed: 14043179]
- Kaur C, Foulds WS, Ling EA. Blood-retinal barrier in hypoxic ischaemic conditions: basic concepts, clinical features and management. *Prog Retin Eye Res*. 2008 Nov; 27(6):622–47. [PubMed: 18940262]
- King A, Gottlieb E, Brooks DG, Murphy MP, Dunaief JL. Mitochondria-derived reactive oxygen species mediate blue light-induced death of retinal pigment epithelial cells. *Photochemistry and photobiology*. 2004; 79:470–475. [PubMed: 15191057]
- Kitamura T, Asai N, Enomoto A, Maeda K, Kato T, Ishida M, Jiang P, Watanabe T, Usukura J, Kondo T, Costantini F, Murohara T, Takahashi M. Regulation of VEGF-mediated angiogenesis by the Akt/PKB substrate Girdin. *Nat Cell Biol*. 2008 Mar; 10(3):329–37. [PubMed: 18264090]
- Knowles PP, Murray-Rust J, Kjaer S, Scott RP, Hanrahan S, Santoro M, Ibáñez CF, McDonald NQ. Structure and chemical inhibition of the RET tyrosine kinase domain. *J Biol Chem*. 2006; 281:33577–33587. [PubMed: 16928683]
- Kodjikian L, Souied EH, Mimoun G, Maugé-Faÿsse M, Behar-Cohen F, Decullier E, Huot L, Aulagner G, GEFAL Study Group. Ranibizumab versus Bevacizumab for Neovascular Age-related Macular Degeneration: Results from the GEFAL Noninferiority Randomized Trial. *Ophthalmology*. 2013 Nov; 120(11):2300–9. [PubMed: 23916488]
- Korobelnik JF, Holz FG, Roeder J, Ogura Y, Simader C, Schmidt-Erfurth U, Lorenz K, Honda M, Vitti R, Berliner AJ, Hiemeyer F, Stemper B, Zeitz O, Sandbrink R, GALILEO Study Group. Intravitreal Aflibercept Injection for Macular Edema Resulting from Central Retinal Vein

- Occlusion: One-Year Results of the Phase 3 GALILEO Study. *Ophthalmology*. 2014 Jan; 121(1): 202–8. [PubMed: 24084497]
- Koyama R, Nakanishi T, Ikeda T, Shimizu A. Catalogue of soluble proteins in human vitreous humor by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrospray ionization mass spectrometry including seven angiogenesis-regulating factors. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2003 Jul 15; 792(1):5–21.
- Krady JK, Basu A, Allen CM, Xu Y, LaNoue KF, Gardner TW, Levison SW. Minocycline reduces proinflammatory cytokine expression, microglial activation, and caspase-3 activation in a rodent model of diabetic retinopathy. *Diabetes*. 2005 May; 54(5):1559–65. [PubMed: 15855346]
- Krebs I, Schmetterer L, Boltz A, Told R, Vécsei-Marlovits V, Egger S, Schönherr U, Haas A, Ansari-Shahrezaei S, Binder S, MANTA Research Group. A randomised double-masked trial comparing the visual outcome after treatment with ranibizumab or bevacizumab in patients with neovascular age-related macular degeneration. *Br J Ophthalmol*. 2013 Mar; 97(3):266–71. [PubMed: 23292928]
- Krispel C, Rodrigues M, Xin X, Sodhi A. Ranibizumab in diabetic macular edema. *World J Diabetes*. 2013 Dec 15; 4(6):310–8. [PubMed: 24379922]
- Kuba K, Matsumoto K, Date K, Shimura H, Tanaka M, Nakamura T. HGF/NK4, a four-kringle antagonist of hepatocyte growth factor, is an angiogenesis inhibitor that suppresses tumor growth and metastasis in mice. *Cancer Res*. 2000 Dec 1; 60(23):6737–43. [PubMed: 11118060]
- Kurihara T, Westenskow PD, Friedlander M. Hypoxia-inducible factor (HIF)/vascular endothelial growth factor (VEGF) signaling in the retina. *Adv Exp Med Biol*. 2014; 801:275–81. [PubMed: 24664708]
- Kwon SH, Shin JP, Kim IT, Park DH. Aqueous Levels of Angiopoietin-like 4 and Semaphorin 3E Correlate with Nonperfusion Area and Macular Volume in Diabetic Retinopathy. *Ophthalmology*. 2015 May; 122(5):968–75. [PubMed: 25687026]
- Lad EM, Cheshier SH, Kalani MY. Wnt-signaling in retinal development and disease. *Stem Cells Dev*. 2009 Jan-Feb; 18(1):7–16. [PubMed: 18690791]
- Lando D, Peet DJ, Gorman JJ, Whelan DA, Whitelaw ML, Bruick RK. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. *Genes Dev*. 2002 Jun 15; 16(12):1466–71. [PubMed: 12080085]
- Lee JH, Park HS, Shin JM, Chun MH, Oh SJ. Nestin expressing progenitor cells during establishment of the neural retina and its vasculature. *Anat Cell Biol*. 2012 Mar; 45(1):38–46. [PubMed: 22536550]
- Li F, Liu J, Jas GS, Zhang J, Qin G, Xing J, Cotes C, Zhao H, Wang X, Diaz LA, Shi ZZ, Lee DY, Li KC, Li Z. Synthesis and evaluation of a near-infrared fluorescent non-peptidic bivalent integrin $\alpha(v)\beta(3)$ antagonist for cancer imaging. *Bioconjugate chemistry*. 2010; 21:270–278. [PubMed: 20102181]
- Li Y, Cheng H, Duong TQ. Blood-flow magnetic resonance imaging of the retina. *Neuroimage*. 2008; 39(4):1744–51. [PubMed: 18063388]
- Li X, Xu G, Wang Y, Xu X, Liu X, Tang S, Zhang F, Zhang J, Tang L, Wu Q, Luo D, Ke X, AURORA Study Group. Safety and efficacy of conbercept in neovascular age-related macular degeneration: results from a 12-month randomized phase 2 study: AURORA study. *Ophthalmology*. 2014; 121:1740. [PubMed: 24793528]
- Li YJ, Li XH, Wang LF, Kuang X, Hang ZX, Deng Y, Du JR. Therapeutic efficacy of a novel non-peptide $\alpha v \beta 3$ integrin antagonist for pathological retinal angiogenesis in mice. *Exp Eye Res*. 2014 Dec; 129:119–26. [PubMed: 25446322]
- Liekens S, Schols D, Hatse S. CXCL12-CXCR4 axis in angiogenesis, metastasis and stem cell mobilization. *Curr Pharm Des*. 2010; 16(35):3903–20. [PubMed: 21158728]
- Lin M, Hu Y, Chen Y, Zhou KK, Jin J, Zhu M, Le YZ, Ge J, Ma JX. Impacts of hypoxia-inducible factor-1 knockout in the retinal pigment epithelium on choroidal neovascularization. *Invest Ophthalmol Vis Sci*. 2012 Sep 14; 53(10):6197–206. [PubMed: 22915031]
- Linsenmeier RA, Braun RD, McRipley MA, Padnick LB, Ahmed J, Hatchell DL, McLeod DS, Lutty GA. Retinal hypoxia in long-term diabetic cats. *Invest Ophthalmol Vis Sci*. 1998 Aug; 39(9): 1647–57. [PubMed: 9699554]

- Longeras R, Farjo K, Ihnat M, Ma JX. A PEDF-derived peptide inhibits retinal neovascularization and blocks mobilization of bone marrow-derived endothelial progenitor cells. *Exp Diabetes Res*. 2012; 2012:518426. [PubMed: 21754923]
- Lukason M, DuFresne E, Rubin H, Pechan P, Li Q, Kim I, Kiss S, Flaxel C, Collins M, Miller J, Hauswirth W, MacLachlan T, Wadsworth S, Scaria A. Inhibition of choroidal neovascularization in a nonhuman primate model by intravitreal administration of an AAV2 vector expressing a novel anti-VEGF molecule. *Mol Ther*. 2011 Feb; 19(2):260–5. [PubMed: 20978476]
- Luna JD, Chan C-C, Derevjani N, Mahlow J, Chiu C, Peng B, Tobe T, Campochiaro PA, Viores SA. Blood-retinal barrier (BRB) breakdown in experimental autoimmune uveoretinitis: Comparison with vascular endothelial growth factor, tumor necrosis factor, and interleukin-1 β -mediated breakdown. *J Neurosci Res*. 1997; 49:268–80. [PubMed: 9260738]
- Luttun A, Tjwa M, Carmeliet P. Placental growth factor (PlGF) and its receptor Flt-1 (VEGFR-1): novel therapeutic targets for angiogenic disorders. *Ann N Y Acad Sci*. 2002 Dec; 979:80–93. [PubMed: 12543719]
- Lutty GA, Hasegawa T, Baba T, Grebe R, Bhutto I, McLeod DS. Development of the human choriocapillaris. *Eye (Lond)*. 2010 Mar; 24(3):408–15. [PubMed: 20075975]
- Lutty GA, McLeod DS, Bhutto I, Wiegand SJ. Effect of VEGF trap on normal retinal vascular development and oxygen-induced retinopathy in the dog. *Invest Ophthalmol Vis Sci*. 2011 Jun 8; 52(7):4039–47. [PubMed: 21357392]
- Ma AC, Fung TK, Lin RH, Chung MI, Yang D, Ekker SC, Leung AY. Methionine aminopeptidase 2 is required for HSC initiation and proliferation. *Blood*. 2011 Nov 17; 118(20):5448–57. [PubMed: 21937698]
- Maguire AM, Simonelli F, Pierce EA, Pugh EN Jr, Mingozzi F, Bennicelli J, Banfi S, Marshall KA, Testa F, Surace EM, Rossi S, Lyubarsky A, Arruda VR, Konkle B, Stone E, Sun J, Jacobs J, Dell'Osso L, Hertle R, Ma JX, Redmond TM, Zhu X, Hauck B, Zelenia O, Shindler KS, Maguire MG, Wright JF, Volpe NJ, McDonnell JW, Auricchio A, High KA, Bennett J. Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N Engl J Med*. 2008 May 22; 358(21):2240–8. [PubMed: 18441370]
- Mahon PC, Hirota K, Semenza GL. FIH-1: a novel protein that interacts with HIF-1 α and VHL to mediate repression of HIF-1 transcriptional activity. *Genes Dev*. 2001 Oct 15; 15(20):2675–86. [PubMed: 11641274]
- Maiti D, Xu Z, Duh EJ. Vascular endothelial growth factor induces MEF2C and MEF2-dependent activity in endothelial cells. *Invest Ophthalmol Vis Sci*. 2008 Aug; 49(8):3640–8. [PubMed: 18450586]
- Mann, IC. The development of the human eye. Cambridge: University Press; 1928.
- Manalo DJ, Rowan A, Lavoie T, Natarajan L, Kelly BD, Ye SQ, Garcia JG, Semenza GL. Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood*. 2005 Jan 15; 105(2):659–69. [PubMed: 15374877]
- Marchetti C, Gasparri ML, Ruscito I, Palaia I, Perniola G, Carrone A, Farooqi AA, Pecorini F, Muzii L, Panici PB. Advances in anti-angiogenic agents for ovarian cancer treatment: The role of trebananib (AMG 386). *Crit Rev Oncol Hematol*. 2015 Jun; 94(3):302–310. [PubMed: 25783620]
- Martin DF, Maguire MG, Fine SL, Ying GS, Jaffe GJ, Grunwald JE, Toth C, Redford M, Ferris FL. Ranibizumab and bevacizumab for treatment of neovascular age-related macular degeneration: two-year results. *Ophthalmology*. 2012; 119:1388–1398. [PubMed: 22555112]
- Mauriz JL, Martín-Renedo J, García-Palomo A, Tuñón MJ, González-Gallego J. Methionine aminopeptidases as potential targets for treatment of gastrointestinal cancers and other tumours. *Curr Drug Targets*. 2010 Nov; 11(11):1439–57. [PubMed: 20583970]
- Maxwell PH. Hypoxia-inducible factor as a physiological regulator. *Exp Physiol*. 2005 Nov; 90(6):791–7. [PubMed: 16157658]
- Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, Ratcliffe PJ. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature*. 1999; 399(6733):271–5. [PubMed: 10353251]

- McLeod DS, Luty GA, Wajer SD, Flower RW. Visualization of a developing vasculature. *Microvasc Res*. 1987; 33:257–269. [PubMed: 2438539]
- McLeod DS, Hasegawa T, Prow T, Merges C, Luty G. The initial fetal human retinal vasculature develops by vasculogenesis. *Dev Dyn*. 2006 Dec; 235(12):3336–47. [PubMed: 17061263]
- McLeod DS, Baba T, Bhutto IA, Luty GA. Co-expression of endothelial and neuronal nitric oxide synthases in the developing vasculatures of the human fetal eye. *Graefes Arch Clin Exp Ophthalmol*. 2012 Jun; 250(6):839–48. [PubMed: 22411126]
- Michaelson IC. The mode of development of the vascular system of the retina, with some observations on its significance for certain retinal diseases. *Trans Ophthalmol Soc UK*. 1948; 68:137–181.
- Michels S, Rosenfeld PJ, Puliafito CA, Marcus EN, Venkatraman AS. Systemic bevacizumab (Avastin) therapy for neovascular age-related macular degeneration twelve-week results of an uncontrolled open-label clinical study. *Ophthalmology*. 2005 Jun; 112(6):1035–47. [PubMed: 15936441]
- Miki K, Miki A, Matsuoka M, Muramatsu D, Hackett SF, Campochiaro PA. Effects of intraocular ranibizumab and bevacizumab in transgenic mice expressing human vascular endothelial growth factor. *Ophthalmology*. 2009; 116:1748–1754. [PubMed: 19643496]
- Mitchell P, Bandello F, Schmidt-Erfurth U, Lang GE, Massin P, Schlingemann RO, Sutter F, Simader C, Burian G, Gerstner O, Weichselberger A, RESTORE study group. The RESTORE study: Ranibizumab monotherapy or combined with laser versus laser monotherapy for DME. *Ophthalmology*. 2011 Apr; 118(4):615–25. [PubMed: 21459215]
- Mole DR, Pugh CW, Ratcliffe PJ, Maxwell PH. Regulation of the HIF pathway: enzymatic hydroxylation of a conserved prolyl residue in hypoxia-inducible factor alpha subunits governs capture by the pVHL E3 ubiquitin ligase complex. *Adv Enzyme Regul*. 2002; 42:333–47. [PubMed: 12123724]
- Morishita R, Aoki M, Hashiya N, Yamasaki K, Kurinami H, Shimizu S, Makino H, Takesya Y, Azuma J, Ogihara T. Therapeutic angiogenesis using hepatocyte growth factor (HGF). *Curr Gene Ther*. 2004 Jun; 4(2):199–206. [PubMed: 15180586]
- Nakamura-Ishizu A, Kurihara T, Okuno Y, Ozawa Y, Kishi K, Goda N. The formation of an angiogenic astrocyte template is regulated by the neuroretina in a HIF-1-dependent manner. *Dev Biol*. 2012; 363:106–114. [PubMed: 2226979]
- Nakanishi T, Koyama R, Ikeda T, Shimizu A. Catalogue of soluble proteins in the human vitreous humor: comparison between diabetic retinopathy and macular hole. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2002 Aug 25; 776(1):89–100.
- Nazari H, Zhang L, Zhu D, et al. Stem cell based therapies for age-related macular degeneration: The promises and the challenges. *Prog Retin Eye Res*. 2015 Sep; 48:1–39. [PubMed: 26113213]
- Ng EW, Shima DT, Calias P, Cunningham ET Jr, Guyer DR, Adamis AP. Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. *Nat Rev Drug Discov*. 2006 Feb; 5(2):123–32. [PubMed: 16518379]
- Nguyen QD, Brown DM, Marcus DM, Boyer DS, Patel S, Feiner L, Gibson A, Sy J, Rundle AC, Hopkins JJ, Rubio RG, Ehrlich JS, RISE and RIDE Research Group. Ranibizumab for DME: Results from 2 phase III randomized trials: RISE and RIDE. *Ophthalmology*. 2012 Apr; 119(4):789–801. [PubMed: 22330964]
- Okubo Y, Sekiya H, Namiki S, Sakamoto H, Iinuma S, Yamasaki M, Watanabe M, Hirose K, Iino M. Imaging extrasynaptic glutamate dynamics in the brain. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:6526–6531. [PubMed: 20308566]
- Ozaki H, Seo MS, Ozaki K, Yamada H, Yamada E, Okamoto N, Hofmann F, Wood JM, Campochiaro PA. Blockage of vascular endothelial cell growth factor receptor signaling is sufficient to completely prevent retinal neovascularization. *Am J Pathol*. 2000; 156:697–707. [PubMed: 10666398]
- Papadopoulos N, Martin J, Ruan Q, Rafique A, Rosconi MP, Shi E, Pyles EA, Yancopoulos GD, Stahl N, Wiegand SJ. Binding and neutralization of vascular endothelial growth factor (VEGF) and related ligands by VEGF Trap, ranibizumab and bevacizumab. *Angiogenesis*. 2012; 15:171–185. [PubMed: 22302382]
- Park H, Lee DS, Yim MJ, Choi YH, Park S, Seo SK, Choi JS, Jang WH, Yea SS, Park WS, Lee CM, Jung WK, Choi IW. 3,3'-Diindolylmethane inhibits VEGF expression through the HIF-1 α and

NF- κ B pathways in human retinal pigment epithelial cells under chemical hypoxic conditions. *Int J Mol Med*. 2015 Jul; 36(1):301–8. [PubMed: 25955241]

Pechan P, Rubin H, Lukason M, Ardinger J, DuFresne E, Hauswirth WW, Wadsworth SC, Scaria A. Novel anti-VEGF chimeric molecules delivered by AAV vectors for inhibition of retinal neovascularization. *Gene Ther*. 2009 Jan; 16(1):10–6. [PubMed: 18633446]

Peiris PM, Toy R, Doolittle E, Pansky J, Abramowski A, Tam M, Vicente P, Tran E, Hayden E, Camann A, Mayer A, Erokwu BO, Berman Z, Wilson D, Baskaran H, Flask CA, Keri RA, Karathanasis E. Imaging metastasis using an integrin-targeting chain-shaped nanoparticle. *ACS nano*. 2012; 6:8783–8795. [PubMed: 23005348]

Penfold PL, Provis JM, Madigan MC, van Driel D, Billson FA. Angiogenesis in normal human retinal development: the involvement of astrocytes and macrophages. *Graefes Arch Clin Exp Ophthalmol*. 1990; 228(3):255–263. [PubMed: 2361598]

Pershing S, Bakri SJ, Moshfeghi DM. Ocular hypertension and intraocular pressure asymmetry after intravitreal injection of anti-vascular endothelial growth factor agents. *Ophthalmic Surg Lasers Imaging Retina*. 2013; 44:460–464. [PubMed: 24044708]

Phelps DL. Oxygen and developmental retinal capillary remodeling in the kitten. *Invest Ophthalmol Vis Sci*. 1990; 31:2194–2200. [PubMed: 2211017]

Presta M, Dell'Era P, Mitola S, Moroni E, Ronca R, Rusnati M. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. *Cytokine Growth Factor Rev*. 2005; 16:159–78. [PubMed: 15863032]

Provis JM, Leech J, Diaz CM, Penfold PL, Stone J, Keshet E. Development of the human retinal vasculature: cellular relations and VEGF expression. *Exp Eye Res*. 1997; 65:555–568. [PubMed: 9464188]

Querques G, Capuano V, Frascio P, Bandello F, Souied EH. Emerging therapeutic options in age-related macular degeneration. *Ophthalmic Res*. 2015; 53(4):194–9. [PubMed: 25871486]

Rajendram R, Fraser-Bell S, Kaines A, Michaelides M, Hamilton RD, Esposti SD, Peto T, Egan C, Bunce C, Leslie RD, Hykin PG. A 2-year prospective randomized controlled trial of intravitreal bevacizumab or laser therapy (BOLT) in the management of diabetic macular edema: 24-month data: report 3. *Arch Ophthalmol*. 2012 Aug; 130(8):972–9. [PubMed: 22491395]

Rakoczy EP, Lai CM, Magno AL, Wikstrom ME, French MA, Pierce CM, Schwartz SD, Blumenkranz MS, Chalberg TW, Degli-Esposti MA, Constable IJ. Gene therapy with recombinant adeno-associated vectors for neovascular age-related macular degeneration: 1 year follow-up of a phase 1 randomised clinical trial. *Lancet*. 2015 Dec 12; 386(10011):2395–403. [PubMed: 26431823]

Ratcliffe PJ. HIF-1 and HIF-2: working alone or together in hypoxia? *J Clin Invest*. 2007 Apr; 117(4):862–5. [PubMed: 17404612]

Reck M, Kaiser R, Mellemgaard A, Douillard JY, Orlov S, Krzakowski M, von Pawel J, Gottfried M, Bondarenko I, Liao M, Gann CN, Barrueco J, Gaschler-Markefski B, Novello S, LUME-Lung 1 Study Group. Docetaxel plus nintedanib versus docetaxel plus placebo in patients with previously treated non-small-cell lung cancer (LUME-Lung 1): a phase 3, double-blind, randomised controlled trial. *Lancet Oncol*. 2014; 15:143–55. [PubMed: 24411639]

Rich RM, Rosenfeld PJ, Puliafito CA, Dubovy SR, Davis JL, Flynn HW Jr, Gonzalez S, Feuer WJ, Lin RC, Lalwani GA, Nguyen JK, Kumar G. Short-term safety and efficacy of intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. *Retina*. 2006 May-Jun; 26(5):495–511. [PubMed: 16770255]

Rizzolo LJ. Polarity and the development of the outer blood-retinal barrier. *Histol Histopathol*. 1997; 12:1057–67. [PubMed: 9302567]

Rodrigues M, Xin X, Jee K, Babapoor-Farrokhran S, Kashiwabuchi F, Ma T, Bhutto I, Hassan SJ, Daoud Y, Baranano D, Solomon S, Luttly G, Semenza GL, Montaner S, Sodhi A. VEGF secreted by hypoxic Müller cells induces MMP-2 expression and activity in endothelial cells to promote retinal neovascularization in proliferative diabetic retinopathy. *Diabetes*. 2013 Nov; 62(11):3863–73. [PubMed: 23884892]

Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY, Kim RY, MARINA Study Group. Ranibizumab for neovascular age-related macular degeneration. *N Engl J Med*. 2006 Oct 5; 355(14):1419–31. [PubMed: 17021318]

- Roy-Luzarraga M1, Hodivala-Dilke K. Molecular Pathways: Endothelial Cell FAK-A Target for Cancer Treatment. *Clin Cancer Res*. 2016 Aug 1; 22(15):3718–24. [PubMed: 27262114]
- Rubio R. Long-Acting Anti-VEGF Delivery. *Retina Today*. 2014 Jul-Aug;:78–80.
- Rudkin AK, Lee AW, Chen CS. Ocular neovascularization following central retinal artery occlusion: prevalence and timing of onset. *Eur J Ophthalmol*. 2010 Nov-Dec;20(6):1042–6. [PubMed: 20544682]
- Ruzinova MB, Schoer RA, Gerald W, Egan JE, Pandolfi PP, Rafii S, Manova K, Mittal V, Benezra R. Effect of angiogenesis inhibition by Id loss and the contribution of bone-marrow-derived endothelial cells in spontaneous murine tumors. *Cancer Cell*. 2003 Oct; 4(4):277–89. [PubMed: 14585355]
- Sacu S, Michels S, Prager F, Weigert G, Dunavoelgyi R, Geitzenauer W, Prunte C, Schmidt-Erfurth U. Randomised clinical trial of intravitreal Avastin vs photodynamic therapy and intravitreal triamcinolone: long-term results. *Eye (Lond)*. 2009 Dec; 23(12):2223–7. [PubMed: 19169239]
- Salehi-Had H, Roh MI, Giani A, Hisatomi T, Nakao S, Kim IK, Gragoudas ES, Vavvas D, Guccione S, Miller JW. Utilizing targeted gene therapy with nanoparticles binding alpha v beta 3 for imaging and treating choroidal neovascularization. *PLoS One*. 2011 Apr 29;6(4):e18864. [PubMed: 21559527]
- Sampieri CL, León-Córdoba K, Remes-Troche JM. Matrix metalloproteinases and their tissue inhibitors in gastric cancer as molecular markers. *J Cancer Res Ther*. 2013 Jul-Sep;9(3):356–63. [PubMed: 24125966]
- Sandercoe TM, Madigan MC, Billson FA, Penfold PL, Provis JM. Astrocyte proliferation during development of the human retinal vasculature. *Exp Eye Res*. 1999; 69:511–523. [PubMed: 10548471]
- Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilienbaum R, Johnson DH. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med*. 2006; 355:2542–50. [PubMed: 17167137]
- Sarlos S, Rizkalla B, Moravski CJ, Cao Z, Cooper ME, Wilkinson-Berka JL. Retinal angiogenesis is mediated by an interaction between the angiotensin type 2 receptor, VEGF, and angiopoietin. *Am J Pathol*. 2003 Sep; 163(3):879–87. [PubMed: 12937129]
- Scarpino S, D'Alena FC, Di Napoli A, Ballarini F, Prat M, Ruco LP. Papillary carcinoma of the thyroid: evidence for a role for hepatocyte growth factor (HGF) in promoting tumour angiogenesis. *J Pathol*. 2003 Feb; 199(2):243–50. [PubMed: 12533838]
- Schofield CJ, Ratcliffe PJ. Oxygen sensing by HIF hydroxylases. *Nat Rev Mol Cell Biol*. 2004 May; 5(5):343–54. [PubMed: 15122348]
- Scholz CC, Taylor CT. Targeting the HIF pathway in inflammation and immunity. *Curr Opin Pharmacol*. 2013 Aug; 13(4):646–53. [PubMed: 23660374]
- Schor AM, Schor SL. Angiogenesis and tumour progression: migration-stimulating factor as a novel target for clinical intervention. *Eye (Lond)*. 2010 Mar; 24(3):450–8. [PubMed: 20019766]
- Sears ML. Aphakic cystoid macular edema: The pharmacology of ocular trauma. *Surv Ophthalmol*. 1984; 28:525–34. [PubMed: 6379950]
- Semenza GL. Vasculogenesis, angiogenesis, and arteriogenesis: mechanisms of blood vessel formation and remodeling. *J Cell Biochem*. 2007 Nov 1; 102(4):840–7. [PubMed: 17891779]
- Semenza GL. Hypoxia-inducible factors in physiology and medicine. *Cell*. 2012 Feb 3; 148(3):399–408. [PubMed: 22304911]
- Sen D, SoRelle ED, Liba O, Dalal R, Paulus YM, Kim T-W, Moshfeghi DM, de la Zerda A. High resolution contrast-enhanced Optical Coherence Tomography in mice retinæ. *Journal of Biomedical Optics*. 2016; 21(6):066002.
- Sheetz MJ, Aiello LP, Davis MD, Danis R, Bek T, Cunha-Vaz J, Shahri N, Berg PH, MBDL and MBCU Study Groups. The effect of the oral PKC β inhibitor ruboxistaurin on vision loss in two phase 3 studies. *Invest Ophthalmol Vis Sci*. 2013 Mar 11; 54(3):1750–7. [PubMed: 23404115]
- Shih SC, Ju M, Liu N, Smith LE. Selective stimulation of VEGFR-1 prevents oxygen-induced retinal vascular degeneration in retinopathy of prematurity. *J Clin Invest*. 2003 Jul; 112(1):50–7. [PubMed: 12840058]

- Shojaei F, Wu X, Qu X, Kowanetz M, Yu L, Tan M. G-CSF-initiated myeloid cell mobilization and angiogenesis mediate tumor refractoriness to anti-VEGF therapy in mouse models. *Proc Natl Acad Sci U S A*. 2009; 106:6742–47. [PubMed: 19346489]
- Simard JR, Getlik M, Grütter C, Pawar V, Wulfert S, Rabiller M, Rauh D. Development of a fluorescent-tagged kinase assay system for the detection and characterization of allosteric kinase inhibitors. *J Am Chem Soc*. 2009; 131:13286–13296. [PubMed: 19572644]
- Smith LE, Wesolowski E, McLellan A, Kostyk SK, D'Amato R, Sullivan R, D'Amore PA. Oxygen-induced retinopathy in the mouse. *Invest Ophthalmol Vis Sci*. 1994 Jan; 35(1):101–11. [PubMed: 7507904]
- Sodhi A, Montaner S, Patel V, Zohar M, Bais C, Mesri E, Gutkind JS. The Kaposi's Sarcoma Associated Herpesvirus G Protein-Coupled Receptor Upregulates VEGF Expression and Secretion through Multiple Signaling Pathways Acting on Hypoxia Inducible Factor-1alpha. *Cancer Research*. 2000; 60:4873–80. [PubMed: 10987301]
- Sodhi A, Montaner S. Angiopoietin-like 4 as an Emerging Therapeutic Target for Diabetic Eye Disease. *JAMA Ophthalmol*. 2015 Dec; 133(12):1375–6. [PubMed: 26469243]
- Solomon SD, Lindsley K, Vedula SS, Krzystolik MG, Hawkins BS. Anti-vascular endothelial growth factor for neovascular age-related macular degeneration. *Cochrane Database Syst Rev*. 2014 Aug. 29;8, CD005139.
- Stalmans I, Ng YS, Rohan R, Fruttiger M, Bouché A, Yuce A, Fujisawa H, Hermans B, Shani M, Jansen S, Hicklin D, Anderson DJ, Gardiner T, Hammes HP, Moons L, Dewerchin M, Collen D, Carmeliet P, D'Amore PA. Arteriolar and venular patterning in retinas of mice selectively expressing VEGF isoforms. *J Clin Invest*. 2002; 109:327–336. [PubMed: 11827992]
- Stefánsson E, Machemer R, de Juan E Jr, McCuen BW II, Peterson J. Retinal oxygenation and laser treatment in patients with diabetic retinopathy. *Am J Ophthalmol*. 1992 Jan 15; 113(1):36–8. [PubMed: 1728144]
- Stone J, Itin A, Alon T, Pe'er J, Gnessin H, Chan-Ling T, Keshet E. Development of retinal vasculature is mediated by hypoxia-induced vascular endothelial growth factor (VEGF) expression in neuroglia. *J Neurosci*. 1995; 15:4738–4747. [PubMed: 7623107]
- Subhani S, Vavilala DT, Mukherji M. HIF inhibitors for ischemic retinopathies and cancers: options beyond anti-VEGF therapies. *Angiogenesis*. 2016 Jul; 19(3):257–73. [PubMed: 27146677]
- Subramanian ML, Abedi G, Ness S, Ahmed E, Fenberg M, Daly MK, Houranieh A, Feinberg EB. Bevacizumab vs ranibizumab for age-related macular degeneration: 1-year outcomes of a prospective, double-masked randomised clinical trial. *Eye (Lond)*. 2010 Nov; 24(11):1708–15. [PubMed: 20885427]
- Sun D, Nakao S, Xie F, Zandi S, Bagheri A, Kanavi MR, Samiei S, Soheili ZS, Frimmel S, Zhang Z, Ablonczy Z, Ahmadi H, Hafezi-Moghadam A. Molecular imaging reveals elevated VEGFR-2 expression in retinal capillaries in diabetes: a novel biomarker for early diagnosis. *FASEB J*. 2014 Sep; 28(9):3942–51. [PubMed: 24903276]
- Takeda A, Baffi JZ, Kleinman ME, Cho WG, Nozaki M, Yamada K, Kaneko H, Albuquerque RJ, Dridi S, Saito K, Raisler BJ, Budd SJ, Geisen P, Munitz A, Ambati BK, Green MG, Ishibashi T, Wright JD, Humbles AA, Gerard CJ, Ogura Y, Pan Y, Smith JR, Grisanti S, Hartnett ME, Rothenberg ME, Ambati J. CCR3 is a target for age-related macular degeneration diagnosis and therapy. *Nature*. 2009; 460:225–230. [PubMed: 19525930]
- Tawfik A, Gnana-Prakasam JP, Smith SB, Ganapathy V. Deletion of hemojuvelin, an iron-regulatory protein, in mice results in abnormal angiogenesis and vasculogenesis in retina along with reactive gliosis. *Invest Ophthalmol Vis Sci*. 2014 May 8; 55(6):3616–25. [PubMed: 24812553]
- Thakur A, Scheinman RI, Rao VR, Kompella UB. Pazopanib, a multitargeted tyrosine kinase inhibitor, reduces diabetic retinal vascular leukostasis and leakage. *Microvasc Res*. 2011 Nov; 82(3):346–50. [PubMed: 21945644]
- Todorich B, Yiu G, Hahn P. Current and investigational pharmacotherapeutic approaches for modulating retinal angiogenesis. *Expert Rev Clin Pharmacol*. 2014 May; 7(3):375–91. [PubMed: 24580084]
- Tolentino MJ. Current molecular understanding and future treatment strategies for pathologic ocular neovascularization. *Curr Mol Med*. 2009 Nov; 9(8):973–81. [PubMed: 19925410]

- Treps L, Conradi LC, Harjes U, Carmeliet P. Manipulating Angiogenesis by Targeting Endothelial Metabolism: Hitting the Engine Rather than the Drivers-A New Perspective? *Pharmacol Rev.* 2016 Jul; 68(3):872–87. [PubMed: 27363442]
- Tripathi, BJ., Tripathi, RC. Development of the human eye. In: Bron, AJ.Tripathi, RC., Tripathi, BJ., editors. *Wolff's Anatomy of the Eye and Orbit*. 8th. London: Chapman & Hall; 1997.
- Tufail A, Patel PJ, Egan C, Hykin P, da Cruz L, Gregor Z, Dowler J, Majid MA, Bailey C, Mohamed Q, Johnston R, Bunce C, Xing W, ABC Trial Investigators. Bevacizumab for neovascular age related macular degeneration (ABC Trial): multicentre randomised double masked study. *BMJ.* 2010 Jun 9;340:c2459. [PubMed: 20538634]
- Tzeng HE, Chen PC, Lin KW, Lin CY, Tsai CH, Han SM, Teng CL, Hwang WL, Wang SW, Tang CH. Basic fibroblast growth factor induces VEGF expression in chondrosarcoma cells and subsequently promotes endothelial progenitor cell-primed angiogenesis. *Clin Sci (Lond).* 2015 Jul 1; 129(2):147–58. [PubMed: 25735814]
- Varner JA, Cheresch DA. Tumor angiogenesis and the role of vascular cell integrin $\alpha v \beta 3$. *Important Adv Oncol.* 1996;69–87. [PubMed: 8791129]
- Vinore SA. Assessment of blood-retinal barrier integrity. *Histol Histopath.* 1995; 10:141–54.
- Vinore SA, Derevjani NL, Ozaki H, Okamoto N, Campochiaro PA. Cellular mechanisms of blood-retinal barrier dysfunction in macular edema. *Doc Ophthalmol.* 1999; 97(3–4):217–28. [PubMed: 10896335]
- Vrabec JP, Lieven CJ, Levin LA. Cell-type-specific opening of the retinal ganglion cell mitochondrial permeability transition pore. *Invest Ophthalmol Vis Sci.* 2003; 44:2774–2782. [PubMed: 12766086]
- Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O₂ tension. *Proc Natl Acad Sci U S A.* 1995 Jun 6; 92(12):5510–4. [PubMed: 7539918]
- Wang Q, Li T, Wu Z, Wu Q, Ke X, Luo D, Wang H. Novel VEGF decoy receptor fusion protein conbercept targeting multiple VEGF isoforms provide remarkable anti-angiogenesis effect in vivo. *PLoS One.* 2013; 8:e70544. [PubMed: 23950958]
- Wilkinson-Berka JL, Rana I, Armani R, Agrotis A. Reactive oxygen species, Nox and angiotensin II in angiogenesis: implications for retinopathy. *Clin Sci (Lond).* 2013 May; 124(10):597–615. [PubMed: 23379642]
- Wu P, Nielsen TE, Clausen MH. FDA-approved small-molecule kinase inhibitors. *Trends Pharmacol Sci.* 2015 Jul; 36(7):422–39. [PubMed: 25975227]
- Xie F, Luo W, Zhang Z, Sun D. In vivo molecular imaging in retinal disease. *J Ophthalmol.* 2012; 2012:429387. [PubMed: 22363836]
- Xin X, Rodrigues M, Umapathi M, Kashiwabuchi F, Ma T, Babapoor-Farrokhran S, Wang S, Hu J, Bhutto I, Welsbie DS, Duh EJ, Handa JT, Eberhart CG, Luty G, Semenza GL, Montaner S, Sodhi A. Hypoxic retinal Muller cells promote vascular permeability by HIF-1-dependent up-regulation of angiopoietin-like 4. *Proc Natl Acad Sci U S A.* 2013 Sep 3; 110(36):E3425–34. [PubMed: 23959876]
- Yan F, Wu H, Liu H, Deng Z, Liu H, Duan W, Liu X, Zheng H. Molecular imaging-guided photothermal/photodynamic therapy against tumor by iRGD-modified indocyanine green nanoparticles. *J Control Release.* 2016 Feb 28;224:217–28. [PubMed: 26739551]
- Yao Y, Tsirka SE. Truncation of monocyte chemoattractant protein 1 by plasmin promotes blood-brain barrier disruption. *J Cell Sci.* 2011; 124(Pt 9):1486–95. [PubMed: 21486949]
- Ye X, Wang Y, Nathans J. The Norrin/Frizzled4 signaling pathway in retinal vascular development and disease. *Trends Mol Med.* 2010 Sep; 16(9):417–25. [PubMed: 20688566]
- Yu DY, Cringle SJ. Oxygen distribution and consumption within the retina in vascularised and avascular retinas and in animal models of retinal disease. *Prog Retin Eye Res.* 2001; 20:175–208. [PubMed: 11173251]
- Zhang X, Huang S, Guo J, Zhou L, You L, Zhang T, Zhao Y. Insights into the distinct roles of MMP-11 in tumor biology and future therapeutics (Review). *Int J Oncol.* 2016 May; 48(5):1783–93. [PubMed: 26892540]

Zhou AY, Bai YJ, Zhao M, Yu WZ, Huang LZ, Li XX. Placental growth factor expression is reversed by antivascular endothelial growth factor therapy under hypoxic conditions. *World J Pediatr.* 2014 Aug; 10(3):262–70. [PubMed: 25124978]

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Figure 1. Schematic of the eye and blood flow

A. Schematic of the eye demonstrating the cornea, anterior chamber (blue), vitreous cavity (red), and retina (black box). **B.** Retinal schematic diagram demonstrating juxtaposed retinal cells and the corresponding retinal and choroidal vasculature. The inner retina receives its blood supply from branches from the central retinal artery and has two distinct capillary plexi: the superficial capillary plexus within the ganglion cell layer and the deep capillary plexus within the inner nuclear layer. The outer retina, including the photoreceptors and retinal pigment epithelium, are supplied by the dense underlying vasculature of the choriocapillaris and choroid.

Figure 2. Optical Coherence Tomography Angiography (OCTA)

A. OCT (Cirrus HD-OCT, Zeiss AngioPlex, Carl Zeiss Meditec, Inc., Dublin, CA) demonstrating calculations of the retinal thickness (purple hashed lines) and location of blood flow (red). **B.** OCTA of a normal human retinal vasculature demonstrating the foveal avascular zone and surrounding vascular plexus. (Image courtesy of Gregory Hoffmeyer, Carl Zeiss Meditec, Dublin, CA)

Figure 3. Schematic of HIF-1α stabilization of the ischemic retina

A. Ultrawide field fluorescein angiogram (Optos 200Tx, Optos plc, Dunfermline, Scotland, U.K.) of a normal retina. **B.** Schematic of HIF-1α in normoxia demonstrating degradation involving von Hippel-Lindau (VHL) protein. HIF-1α is constitutively transcribed. In normoxia, HIF-1α is hydroxylated on proline and acetylated on lysine. This complex is preferentially bound by VHL, ubiquitinated, and degraded in the proteasome. **C.** Ultrawide field fluorescein angiogram demonstrating extensive peripheral capillary non-perfusion (star) and microaneurysms along with regions of neovascularization (red arrows) adjacent to regions of capillary non-perfusion (yellow arrows). **D.** Schematic of HIF-1α in hypoxia demonstrating HIF-induced transcription of hypoxia-inducible genes. In hypoxia, p300 is not inhibited by FIH (factor inhibiting HIF-1α) and interacts with the COOH-terminal transactivation domain (C-TAD). This results in HIF-1α stabilization against degradation through NH₂-terminal transactivation domain (N-TAD), resulting in nuclear localization, heterodimerization of HIF-1α and HIF-1β, and binding of hypoxia response elements (HRE). This results in the transcription of hypoxia-inducible genes, including vascular endothelial growth factor (VEGF) and angiopoietin-2 (Ang2).

Table 1
Current VEGF/KDR (kinase-insert-domain-containing receptor) anti-angiogenic targets in clinical trials

Drug	Drug target	Disease target	Clinicaltrials.gov ID	Phase	Number patients	Relevant clinical study
Pazopanib	VEGFR tyrosine kinase inhibitor	AMD	NCT00612456 NCT01134055	2, Completed 2009 2b	70 510	Danis R. <i>et al.</i> Br J Ophthalmol 2014;98:172–178
TG100801	VEGF tyrosine kinase inhibitor	AMD, PDR	NCT00414999	1, Completed	44	
TG100801	Same	AMD	NCT00509548	2, Terminated early	Terminated after 7 due to corneal deposits	
OPT-302	VEGFR-3	AMD	NCT02543229	1	Enrolling 50	
Regorafenib	Receptor tyrosine kinase VEGFR2-TIE2	AMD	NCT02222207	2	52	
rAAV.sFlt-1	Flt1	AMD	NCT01494805	1 and 2	40	
PAN-90806	Anti-VEGFR	AMD	NCT02022540	1	50	
Squalamine lactate	VEGF	AMD RVO PDR	NCT01678963 NCT02614937 NCT01769183	2 1 and 2 2	142 20 6	
Abicipar Pegol (AGN-150998)	Anti-VEGF	AMD DME	NCT01397409 NCT02181517 NCT02181504 NCT02462928 NCT02186119	2 2 2 3 2	271 25 25 900, R 151	Rakoczy EP. <i>et al.</i> Lancet. 2015;386(10011):2395–403.
PF582	Anti-VEGF	AMD	NCT02121353	1 and 2	25	
EYE001	Anti-VEGF Pegylated Aptamer	AMD	NCT00021736	2 and 3	540	
AL-39324	Inhibit VEGFR and PDGFR	AMD	NCT00992563	2	35	
LHA510	VEGFA-inhibitor	AMD	NCT02076919 NCT02355028	1 2	135	
X-82	VEGF and PDGF inhibitor	AMD	NCT02348359 NCT01674569	2, R 1 and 2	132 35	
ESBA 1008	Anti-VEGF	AMD	NCT01304693	1 and 2	376	
BCD-021	Anti-VEGF	AMD	NCT02450981	1	10	
LMG324	Anti-VEGF	AMD	NCT02398500	1 and 2	25, Terminated	
TK001	Anti-VEGF	AMD	NCT02613559	1	27, R	
ORA102		AMD	NCT00745511	1 and 2	96	
RG7716	Anti-VEGFA	AMD	NCT02484690	2	271	
AGN211745	VEGFR-1 siRNA	AMD	NCT00395057	2	138, Terminated	

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Drug	Drug target	Disease target	Clinicaltrials.gov ID	Phase	Number patients	Relevant clinical study
PTK787 (Vatalamib)	Tyrosine kinase inhibitor targets	VEGFR AMD	NCT00138632	1 and 2	50	

Abbreviations: R= recruiting; AMD = neovascular age-related macular degeneration; DME = diabetic macular edema; PDR = proliferative diabetic retinopathy; RVO = retinal vein occlusion

Table 2

Current non-VEGF targets in clinical trials

Drug	Drug target	Disease target	Clinicaltrials.gov ID	Phase	Number patients
Fractalkine (FKN)	CX3C chemokine	PDR	NCT00728598	1, completed 1998	30
OC-10X	tubulin inhibitor	PDR, AMD	NCT01869933	1	10
Fovista (E10030)	Anti-PDGF-B	AMD	NCT02591914	1	25
			NCT02214628	2	100
			NCT01089517	2	449
			NCT01944839	3,R	622
Palomid 529	TORC1/2inhibitor of mTOR	AMD	NCT01033721	1	13
			NCT01271270	1	5
AdGV/PEDF.11D	PEDF	AMD	NCT00109499	1	
Sirolimus	mTOR IL-2	AMD	NCT00766337	2	62
Everolimus (RAD001)	mTOR	AMD	NCT00857259	2	16, terminated
Pf-04523655	Block RTP801 mTOR	DME	NCT01445899	2	258
		AMD	NCT00713518	2	152
ARC1905	Inhibit complement C5 aptamer	AMD	NCT00709527	1	60
ISONEP	S IP lipid	AMD	NCT01414153	2	158
ASP8232	Vascular adhesion protein-1 inhibitor	DME	NCT02302079	2	96
hL-con1	Factor VIIa inhibitor	AMD	NCT02358889	2	88
Tocilizumab	Antibody IL-6	DME	NCT02511067	2	66
iCo-007	inhibitor C-raf	DME	NCT01565148	2	208
Pf-04634817	CCR2/5 antagonist	DME	NCT01994291	2	212
ATC003 (mecamylamine)	Tubulin depolymerize, tight junction disruption	AMD	NCT00607750	2	60
RO6867461		DME	NCT02699450	2	150, R
		AMD	NCT01941082	1	
Luminate (ALG-1001)	$\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin inhibitor	DME	NCT02348918	2	150
Apremilast (CC-10004)	Inhibit PDE4 (breaks down cAMP)	Behcet's	NCT00866359	2	111
			NCT02307513	3	204
Efalizumab	Bind CD11a	DME	NCT00676559	1	0, Withdrawn
Zimura	Anti-C5 Aptamer	Polypoidal	NCT02397954	2	5
AKB-9778	Tie-2 activator	DME	NCT02050828	2	144
AL-78898A	Complement C3 inhibitor	AMD	NCT01157065	2	99
Volociximab	$\alpha_5\beta_1$ integrin antagonist	AMD	NCT00782093	1	63

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Drug	Drug target	Disease target	Clinicaltrials.gov ID	Phase	Number patients
JSM6427	$\alpha_5\beta_1$ integrin antagonist	AMD	NCT00536016	1	36

Abbreviations: R= recruiting; AMD = neovascular age-related macular degeneration; DME = diabetic macular edema; PDR = proliferative diabetic retinopathy; RVO = retinal vein occlusion

Table 3

Future anti-angiogenic targets to consider

Drug target	Relevant literature/studies
HIF-1/2	Subhani S, <i>et al.</i> Angiogenesis. 2016 Jul;19(3):257–73.
Angiopoietin-like 4 (ANGPTL4)	Babapoor-Farrokhran S, <i>et al.</i> Proc Natl Acad Sci U S A. 2015;112(23):E3030-E3039. Xin X, <i>et al.</i> Proc Natl Acad Sci U S A. 2013 Sep 3;110(36):E3425–34. Kwon SH, <i>et al.</i> Ophthalmology. 2015 May;122(5):968–75.
Stromal-derived factor-1 (SDF-1) and its receptor CXCR4	Ghanem I, <i>et al.</i> Am J Transl Res. 2014 Jul 18;6(4):340–52.
Metabolic gene products	Treps L, <i>et al.</i> Pharmacol Rev. 2016 Jul;68(3):872–87.
Matrix metalloproteinases (MMP)	Sampieri CL, <i>et al.</i> J Cancer Res Ther. 2013 Jul-Sep;9(3):356–63. Zhang X, <i>et al.</i> Int J Oncol. 2016 May;48(5):1783–93. Chang JH, <i>et al.</i> Surv Ophthalmol. 2016 Jul-Aug;61(4):478–97.
Inflammation and inflammatory cytokines	de Oliveira Dias JR, <i>et al.</i> Br J Ophthalmol. 2011 Dec;95(12):1631–7. Schor AM, Schor SL. Eye (Lond). 2010 Mar;24(3):450–8.
Reactive oxygen species (ROS)	Wilkinson-Berka JL, <i>et al.</i> Clin Sci (Lond). 2013 May;124(10):597–615.
Stem cell and endothelial progenitor therapy	Nazari H, <i>et al.</i> Prog Retin Eye Res. 2015 Sep;48:1–39.
Integrin antagonists	Salehi-Had H, <i>et al.</i> PLoS One. 2011 Apr 29;6(4):e18864. Varner JA, <i>et al.</i> Important Adv Oncol. 1996:69–87. Tolentino MJ. Curr Mol Med. 2009 Nov;9(8):973–81.
Methionine aminopeptidase	Ma AC, <i>et al.</i> Blood. 2011 Nov 17;118(20):5448–57. Mauriz JL, <i>et al.</i> Curr Drug Targets. 2010 Nov;11(11):1439–57.