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Minireview

Beyond VEGF: Targeting Inflammation and Other Pathways for Treatment of Retinal Disease

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ABSTRACT

Neovascular eye diseases include conditions such as retinopathy of prematurity, proliferative diabetic retinopathy, and neovascular age-related macular degeneration. Together, they are a major cause of vision loss and blindness worldwide. The current therapeutic mainstay for these diseases is intravitreal injections of biologics targeting vascular endothelial growth factor (VEGF) signaling. Lack of universal response to these anti-VEGF agents coupled with the challenging delivery method underscore a need for new therapeutic targets and agents. In particular, proteins that mediate both inflammatory and proangiogenic signaling are appealing targets for new therapeutic development. Here, we review agents currently in clinical trials and highlight some promising targets in preclinical and early clinical development, focusing on the redox-regulatory transcriptional activator APE1/Ref-1, the bioactive lipid modulator soluble epoxide hydrolase, the transcription factor RUNX1, and others. Small molecules targeting each of these proteins show promise for blocking neovascularization and inflammation. The affected signaling pathways illustrate the potential of new antiangiogenic strategies for posterior ocular disease.

SIGNIFICANCE STATEMENT

Discovery and therapeutic targeting of new angiogenesis mediators is necessary to improve treatment of blinding eye diseases like retinopathy of prematurity, diabetic retinopathy, and neovascular age-related macular degeneration. Novel targets undergoing evaluation and drug discovery work include proteins important for both angiogenesis and inflammation signaling, including APE1/Ref-1, soluble epoxide hydrolase, RUNX1, and others.

Introduction: The Challenge of Neovascular Eye Diseases

Neovascularization,or aberrant blood vessel growth, is a defiiningphenotype of an array of blinding eye diseases. Major neovascular posterior eye diseases include retinopathy of prematurity (ROP), proliferative diabetic retinopathy (PDR), and the neovascular form of age-related macular degeneration (nAMD). Together, these are major contributors to blindness worldwide. ROP is among the most common causes of vision loss in children, with more than 30,000 estimated cases annually worldwide (Hong et al., 2022). Diabetic retinopathy (DR) affects 35% (Yau et al., 2012) of the growing diabetic population, which is now estimated at more than half a billion people globally (International Diabetes Federation, 2021). PDR affects about 7%, and diabetic macular edema affects 7% (Yau et al., 2012). nAMD, although only accounting for about 10% of the more than 200 million total age-related macular degeneration (AMD) patients worldwide (Wong et al., 2014), is responsible for 90% of AMD-related blindness (Ferris et al., 1984).

ROP develops in premature infants exposed to postnatal hyperoxia, which pauses retinal vasculature development (phase 1). On return to normoxia, aberrant, leaky preretinal neovascularization develops (phase 2) (Fevereiro-Martins et al., 2023) (Fig. 1). This disease is modeled quite faithfully in the oxygen-induced retinopathy (OIR) rodent model involving postnatal hyperoxia exposure

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TABLE 1
List of clinical candidate drugs primarily targeting VEGF (obtained from clinicaltrials.gov)

Drug	Mechanism of Action/Target	Company	Indication	Route of Administration	Clinical Study Phase
KSI-301 (Tarcocimab)	VEGF	Kodiak Sciences	nAMD, DME, retinal vein occlusion	Intravitreal	Phase 3
RGX-314	AAV8-VEGF	REGENXBIO	nAMD, DR	Suprachoroidal (gene therapy)	Phase 3
EYP-1901	Voloranib (TKI)	EyePoint	nAMD	Intravitreal	Phase 2
BI 764524	Anti-Sema3A	Boehringer Ingelheim	DR with DMI	Intravitreal	Phase 2
OTX-TKI	Axitinib (TKI)	Ocular Therapeutix	nAMD	Intravitreal implant	Phase 1

DMI, diabetic macular ischemia.

(Smith et al., 1994). In DR, hyperglycemia initially leads to vascular dysfunction; inflammation characterized by gliosis, microglial activation, and edema (Rübsam et al., 2018); and ischemic areas. In some cases, this is followed by aberrant compensation in the form of retinal neovascularization, a hallmark of PDR (Antonetti et al., 2021) (Fig. 1). The OIR model, as an ischemic retinopathy model, is also often used as a surrogate in PDR research, given that hyperglycemia-driven PDR animal models are not widespread (Antonetti et al., 2021). Both nonproliferative diabetic retinopathy (NPDR) and PDR in humans can be associated with diabetic macular edema (DME), fluid leakage leading to retinal thickening (Antonetti et al., 2021) (Fig. 1). Finally, nAMD develops from the "dry" form of AMD, a chronic disease of aging characterized by lipid-rich deposits (drusen), and photoreceptor and retinal pigment epithelium (RPE) degeneration (Fleckenstein et al., 2021). In some cases, dry AMD progresses to nAMD, when macular neovascularization, usually arising from the choroid [choroidal neovascularization (CNV)] invades the outer retina (Fernandes et al., 2022) (Fig. 1). A widely used model with features of nAMD is laser-induced choroidal neovascularization (L-CNV) in rodents or primates, in which a laser burn disrupts Bruch's membrane that separates the choroid from the RPE, promoting an acute neovascular response (Grossniklaus et al., 2010; Malek et al., 2018).

The insults that drive neovascularization in these diseases are varied: largely ischemia/hypoxia (in ROP and PDR) or largely oxidative stress/inflammation (in nAMD), but in most cases, the eventual proangiogenic stimulus is provided by the vascular endothelial growth factor (VEGF) signaling cascade (Ramakrishnan et al., 2014; Apte et al., 2019). Since the approval of the first anti-VEGF ocular agent, pegaptanib (an aptamer), almost 2 decades ago (Gragoudas et al., 2004), therapy of these neovascular eye diseases has been revolutionized (Furino et al., 2021). The current anti-VEGF agents approved by the Food and Drug Administration for various neovascular eye disease indications include ranibizumab (antibody) (Folk and Stone, 2010), aflibercept (fusion protein) (Do et al., 2011), brolucizumab (antibody) (Dugel et al., 2020), and, most recently, faricimab (dual-targeting antibody that also targets angiopoietin signaling) (Heier et al., 2022; Wykoff et al., 2022). Bevacizumab, the prototypical anti-VEGF antibody, is also widely used off label (Tufail et al., 2010). In addition, there are multiple new candidate drugs currently in different phases of clinical trials, primarily targeting VEGF (Table 1). These include KSI-301, an antibody-polymer conjugate from Kodiak; RGX-314, an anti-VEGF gene therapy from REGENXBIO; and two tyrosine kinase inhibitor formulations blocking vascular endothelial growth factor receptor 2 (VEGFR2) and other receptor activities (EYP-1901 from EyePoint and OTX-TKI from Ocular Therapeutix). BI 764524, from Boehringer Ingelheim, is an exception to this anti-VEGF trend: it is an antibody that targets semaphorin 3A, a vasorepulsive axon guidance cue; semaphorin 3A inhibition may ameliorate diabetic macular ischemia (Zippel et al., 2022).

Although the existing approved agents have greatly improved outcomes for patients with ROP, PDR, nAMD, and other neovascular eye diseases, they are not without their shortcomings. Anti-VEGF biologics can lead to tachyphylaxis, resistance due to compensatory mechanisms via usage of alternative angiogenic pathways, tolerance, and systemic side effects (Stewart, 2012; Yang et al., 2016; Ricci et al., 2020). Real-world responses have not lived up to the promise of the registration trials, largely due to less-frequent dosing outside the highly regimented context of clinical trials (Hsu and Regillo, 2020; Mehta et al., 2022). And delivery is a significant challenge: all the currently approved agents must be intravitreally (IVT) injected, with the exception of a recently approved refillable port delivery system for ranibizumab (Susvimo), which is surgically implanted (Adamis and de Juan, 2022; Holekamp et al., 2022). IVT injections must be done by an ophthalmologist and have a small but significant risk of serious complications, such as endophthalmitis (Day et al., 2011; Patel et al., 2022). Moreover, understandably, these injections are unpopular with patients; 80% of patients in one study stated a preference for oral or topical therapies if such were available (Jacobs et al., 2021). And this is not only a question of preference: the time, travel, and cost required for appointments for IVT injections can lead to noncompliance or outright lack of access to

ABBREVIATIONS: AMD, age-related macular degeneration; APE1, apurinic/apyrimidinic endonuclease 1; CNV, choroidal neovascularization; DHA, docosahexaenoic acid; DHDP, dihydroxydocosapentaenoic acid; DME, diabetic macular edema; DR, diabetic retinopathy; EDP, epoxydocosapentaenoic acid; EEQ, 17, 18-epoxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; EpFA, epoxygenated fatty acid; FECH, ferrochelatase; HREC, human retinal microvascular endothelial cell; IL, interleukin; IVT, intravitreal; JNK, c-Jun N-terminal kinase; L-CNV, laser-induced choroidal neovascularization; nAMD, neovascular age-related macular degeneration; NF-κB, nuclear factor κ light-chain-enhancer of activated B cells; NPDR, nonproliferative diabetic retinopathy; OIR, oxygen-induced retinopathy; P450, cytochrome P450; PDR, proliferative diabetic retinopathy; PRMT5, protein arginine methyltransferase-5; PUFA, polyunsaturated fatty acid; PVR, proliferative vitreoretinopathy; Ref-1, reduction-oxidation factor 1; ROP, retinopathy of prematurity; RPE, retinal pigment epithelium; RUNX1, runt-related transcription factor 1; sEH, soluble epoxide hydrolase; shRNA, small hairpin RNA; STAT3, signal transducer and activator of transcription 3; *t*-AUCB, *trans*-4-(4-(3-adamantan-1-yl-ureido)-cyclohexyloxy)-benzoic acid; TF, transcription factor; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

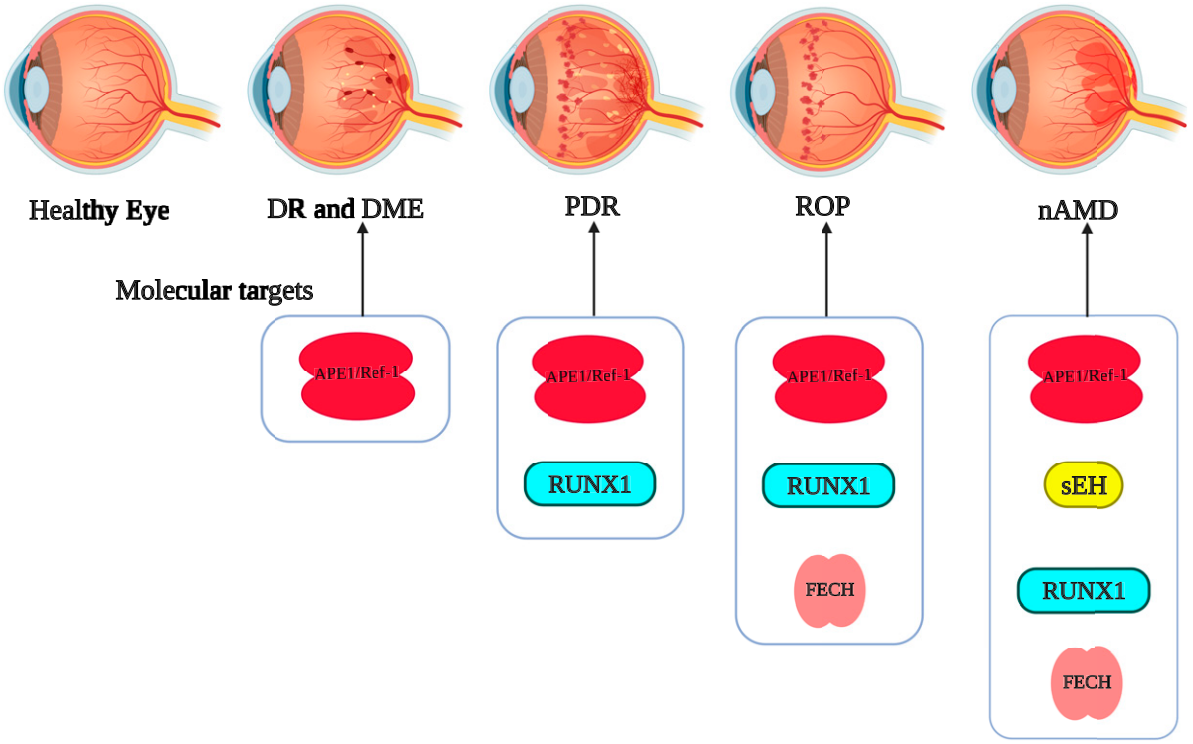

Fig. 1. Pathologies of selected posterior ocular diseases (DR and DME, PDR, ROP, and nAMD) and identified novel angiogenic targets. From left to right, healthy eye; an eye with DR and DME shows edema in the macula and vascular leakage, presence of microaneurysms, and cotton wool spots in the retina; an eye with PDR shows aberrant new blood vessel genesis and vascular tufts, vascular leakage, and hard exudates in the retina; an eye with ROP shows neovascular tufts in the retina; and an eye with nAMD shows inflammation and neovascularization in the choroid and hemorrhage/vascular leakage in the macular retina. Molecular targets discussed in the text (APE1/Ref-1, sEH, RUNX1, and FECH) are linked to the diseases in which they are implicated.

therapy for some individuals and in low-resource settings (Weiss et al., 2018; Bascaran et al., 2021; Okada et al., 2021).

Given these shortcomings, there continues to be substantial interest in developing new approaches for treating ocular neovascularization. Especially intriguing are efforts to move beyond directly regulating VEGF signaling to impinge on the multiplex signaling pathways that drive angiogenesis, including inflammation, hypoxia, oxidative stress, and other pathways. In this minireview, we highlight some of the approaches currently in clinical trials, then focus on novel targets under investigation in our laboratories and others to illustrate the diversity of targets that may hold promise for future neovascular eye disease therapies (Fig. 1).

New Targets (Non-VEGF) in Clinical Trials: Links to Inflammation

Inflammatory signaling pathways are highly active in ocular settings, which results in a wide range of ocular inflammatory diseases, including DR and nAMD (Kim et al., 2021). Prolonged and exacerbated local inflammation in the eyes is directly or indirectly a major cause of vision impairment or blindness. For instance, inflamed eyes, in some cases, can develop CNV or retinal neovascularization, which may be successfully treated with anti-inflammatories alone. Inflammation responses such as oxidative stress, leukocyte migration, and lipid deposition lead to RPE and photoreceptor degeneration in nAMD by increasing the cell permeability and the production of angiogenic factors (Carmi et al., 2009; Xu et al., 2009; Toomey et al., 2018). Advances in the understanding of inflammatory processes have revealed new key pathways, such as VEGF and molecular factors involved in the mechanisms of inflammation.

Anti-VEGF injections do not completely meet patients' needs due to the multifactorial nature of retinal diseases, which may include an inflammatory component. Furthermore, long-term administration of IVT anti-VEGF injections could increase the risk of developing retinal scarring and could result in other complications, such as vitreous or subconjunctival hemorrhage and inflammation in the eyes (Daniel et al., 2014; Yerramothu, 2018).

Multiple signaling pathways, such as the complement pathway, stromal derived factor-1 (SDF-1)/chemokine CXC receptor-4 (CXCR4), inflammasome nucleotide-binding oligomerization domain-like receptor containing domain 3 (NLRP3), interleukin (IL)-18, programed cell death ligand-1 (PD-L1), insulin-like growth factor (IGF), and Yes-associated protein (YAP) signaling pathways, were recently implicated in the pathogenesis of DR, nAMD, and ocular inflammation (Wu et al., 2017; Yan et al., 2018; Yerramothu, 2018; Kim et al., 2021). However, some of these signaling pathways work together with upstream or downstream effectors of VEGF signaling to some extent, such as the complement pathway and transforming growth factor (TGF)-β signaling, as well as activation of crucial oncogenic transcription factors (TFs) such as Nuclear Factor kappa B (NF-κB) and signal transducer and activator of transcription 3 (STAT3) (Wang et al., 2017; Sardar Pasha et al., 2018; Dong et al., 2020). Complement signaling has a potential role in the resolution phase of inflammation. For example, both complement components C5a and C3a stimulate VEGF expression in postinjury angiogenesis and could also be involved in CNV (Nozaki et al., 2006; Grambergs et al., 2019).

TABLE 2
New drugs targeting inflammation beyond VEGF or partially involving VEGF that are undergoing clinical trials (oral or eyedrop)

Drug	Mechanism of Action/Target	Company	Indication	Route of Administration	Clinical Study Phase
APX3330	Ref-1 inhibitor (anti-inflammatory/anti-VEGF)	Ocuphire	DR, DME, nAMD	Oral	Phase 2b
BAY1101042	Guanylate cyclase activator	Bayer	NPDR	Oral	Phase 2
AKST4290	CCR3 eotaxin inhibitor	Alkahest	nAMD	Oral	Phase 2
RG7774	CB2 receptor	Roche	NPDR	Oral	Phase 2
HCB1019 (Xiflam)	Connexin 43	InflammX	DR/AMD	Oral	Phase 2
OPL-0401	ROCK 1/2 inhibitor	Valo	NPDR	Oral	Phase 2
RZ402	Plasma kallikrein	Rezolute	DME	Oral	Phase 2
OCS-01	Steroid	Oculis	DME	Eyedrop	Phase 2
OTT166	Integrin inhibitor	OcuTerra	DR	Eyedrop	Phase 1

AAV8, adeno-associated virus serotype 8; CB2, cannabinoid type 2; CCR3, C-C motif chemokine receptor 3. Source: clinicaltrials.gov.

As mentioned above, several targets are being pursued for non-VEGF ocular inflammation treatments and are at the clinical trial stage (Table 2), notably for DR, DME, and nAMD.

Runcaciguat (BAY1101042) is a soluble guanylate cyclase (aids in maintaining the retinal vasculature homeostasis) activator developed by Bayer as an oral clinical candidate in a phase 2 clinical trial for NPDR (NCT04722991) (Hahn et al., 2021). AKST4290 is an oral drug developed by Alkahest, designed to block the chemokine eotaxin from binding to its receptor, C-C motif chemokine receptor 3 (CCR3), as a novel oral therapy for inflammation-mediated nAMD (NCT04331730) (Sharma et al., 2012; Hirahara et al., 2017; Stewart et al., 2022). Vicasinabin (RG7774) is a cannabinoid 2 receptor (CB2R) agonist that could modulate pro- and anti-inflammatory signaling in cells, developed by Roche for the treatment of DR (NCT04265261).

Xiflam/tonabersat (HCB1019) is a blocker of connexin hemichannels, such as connexin 43 (a gap junction protein that facilitates secretion of inflammatory cytokines) and the NLRP3 inflammasome. It is currently in phase 2 clinical trials for treating DME (Danesh-Meyer et al., 2016; Lyon et al., 2020; Mat Nor et al., 2020) (NCT05727891). OPL-0401 is a small-molecule Rho kinase 1/2 [(ROCK) plays a key role in endothelial cell migration induced by VEGF; Arita et al., 2010] inhibitor from Valo that is a potential first-in-class oral drug for NPDR (NCT05393284). RZ402 is a selective and potent plasma kallikrein inhibitor developed by Rezolute for the treatment of DME (NCT05712720).

OCS-01 from Oculis is a dexamethasone eyedrop being tested in DME (NCT05066997), whereas OTT166/SF0166 is a novel small-molecule selective integrin ($\alpha_v\beta_3$) inhibitor designed to reach the retina via topical administration for the treatment of DME (Askew et al., 2018; Boyer et al., 2022b) (NCT02914613).

Finally, APX3330 is a novel oral agent that targets apurinic/apyrimidinic endonuclease 1 (APE1)/reduction-oxidation factor 1 (Ref-1), an attractive molecular target alleviating inflammatory burden in ocular settings. This target is discussed more in the following section (Hartman et al., 2021; Heisel et al., 2021). Most of the above agents have completed phase 1 trials and are in phase 2 (Table 2). All the candidates discussed above are given orally, except for OCS-01 and OTT166, which are eyedrops. Anti-VEGF combination therapies could provide significant improvement in overall treatment outcomes. This would include compounds targeting inflammation coupled with anti-VEGF therapy in the appropriate disease to afford an increased efficacy and potentially increased time between treatments. Additionally, use of agents that have anti-VEGF effectiveness but are delivered in a route independent of IVT injection, by eyedrops or systemically, could significantly improve treatment outcomes. This approach could have many benefits: increased efficacy, reduced trips for injections, and potential cost savings over a longer period of time, resulting in multiple benefits from the advancement of new targets involved in ocular diseases.

New Targets in Development

APE1/Ref-1. APE1/Ref-1 (gene name: *APEX1*) is one of several novel targets in development for treating retinal diseases. This multifunctional protein possesses both DNA repair activity (APE1) and a redox-transcription regulation role (Ref-1) and has been linked to pathways involved in several retinal disease states, including PDR, ROP, and nAMD (Heisel et al., 2021; Mijit et al., 2021). The DNA repair activity of APE1 regulates both short- and long-patch base excision repair by hydrolyzing the phosphodiester backbone of an abasic site, enabling DNA polymerase to integrate new nucleotides (Caston et al., 2021). The repair of oxidative DNA damage is a major focus of APE1/Ref-1 and the base excision repair pathway. The redox activity of Ref-1 regulates TFs that play a role in driving angiogenesis and inflammation, including hypoxia inducible factor 1α (HIF-1α), NF-κB, and STAT3 (Heisel et al., 2021; Mijit et al., 2021). By chemically reducing critical cysteine residues, Ref-1 allows the TFs to bind to DNA and influence gene expression and protein synthesis, thereby allowing for the activation of key pathways involved in neovascular eye diseases, such as inflammation, angiogenesis, oxidative stress response, and cell survival pathways (Kelley et al., 2012; Shah et al., 2017) (Fig. 2A). Additionally, in a recently completed study using RNA sequencing analysis following knockdown of *APEX1* in human retinal endothelial cells (HRECs), we identified multiple downregulated genes. These were involved in DNA base excision repair, other DNA repair pathways, purine or pyrimidine metabolism signaling, and histidine/one-carbon metabolism pathways. This contrasts with *APEX1* knockdown data in multiple human cancer cell lines and highlights the distinctive role of Ref-1 in the eye and possible ocular therapeutic opportunities (Mijit et al., 2023).

Ref-1 redox activity is essential for angiogenesis, and there is strong in vitro evidence that Ref-1 redox inhibitors suppress angiogenesis in several cell types. APX3330, a dimethoxy benzoquinone, is a small-molecule redox inhibitor of Ref-1 that increases oxidation of the active disulfide residues in Ref-1 to block activation of key TFs without inhibiting the DNA repair activity (Luo et al., 2008; Fishel et al., 2010; Fishel et al., 2011; Jedinak et al., 2011; Su et al., 2011; Cardoso et al., 2012; Zhang

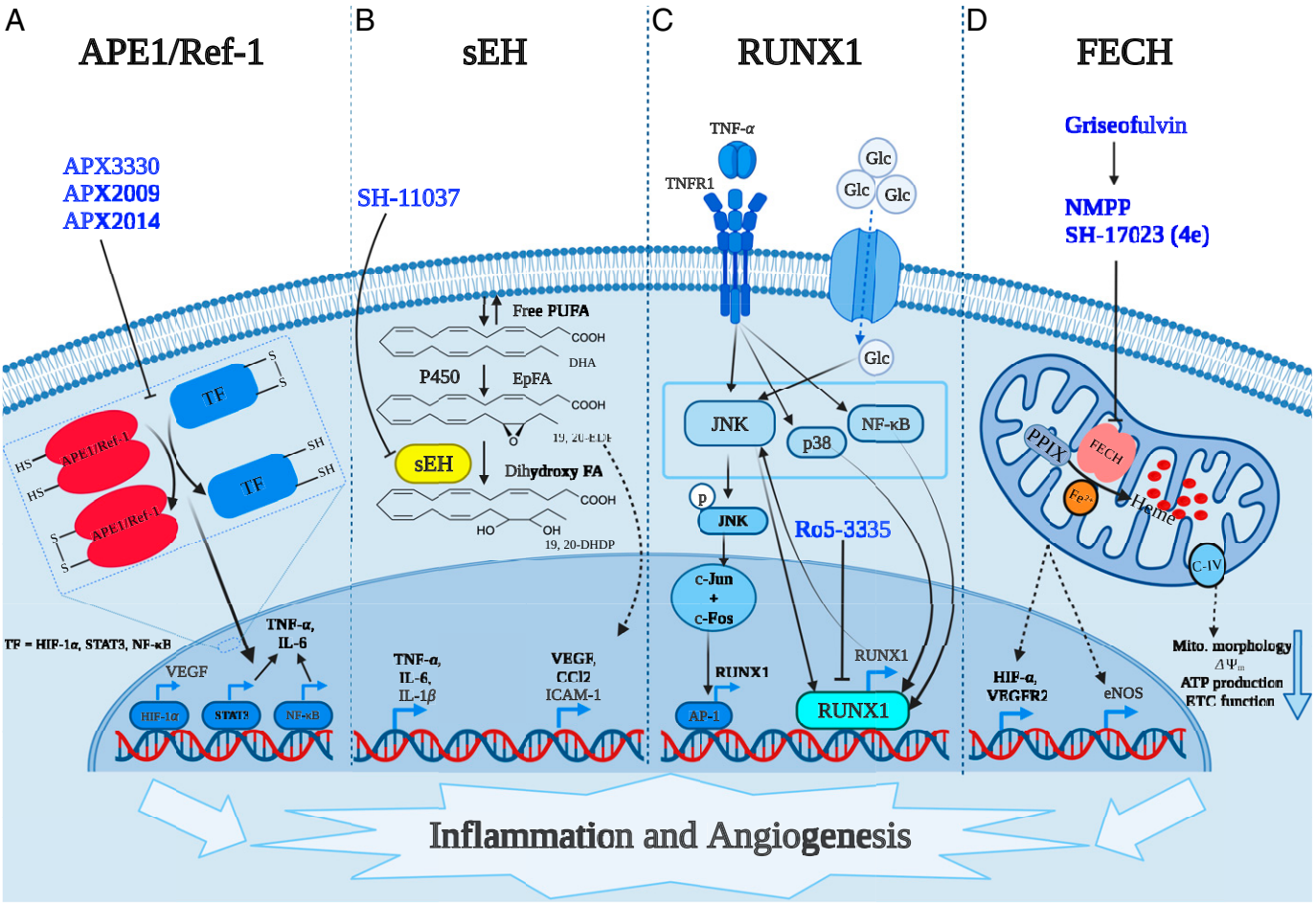

Fig. 2. Mechanisms of selected angiogenic targets that underlie inflammation and angiogenesis in ocular neovascular diseases. (A) The redox function of APE1/Ref-1 regulates TFs (possibly in the nucleus) such as hypoxia inducible factor 1α (HIF-1α), STAT3, and NF-κB via reducing their disulfide bonds and activates inflammation and angiogenesis genes. HIF-1α regulates the expression of proangiogenic VEGF whereas STAT3 and NF-κB modulate the inflammatory cytokines TNF-α and IL-6. Inhibitors APX3330, APX2009, and APX2014 inhibit the redox function of APE1/Ref-1 that alters TFs from oxidized to reduced states. (B) sEH, a metabolizing enzyme of EpFAs, mediates the hydrolysis of PUFA-derived EpFAs, which enhance angiogenesis and inflammation in the eye. Bioactive EpFAs indirectly regulate the inflammatory TNF-α, IL-6, IL-1β, chemokine (C-C motif) ligand 2 (CCL2), intercellular adhesion molecule 1 (ICAM-1), and angiogenic VEGF genes. sEH inhibition using a small-molecule inhibitor, SH-11037, stabilizes EpFAs and improves the antiangiogenic and anti-inflammatory mechanisms. (C) TF RUNX1 is regulated by TNF-α and high glucose via JNK, p38, and NF-κB signaling. Whereas p38 and NF-κB directly regulate the transcription of RUNX1, p-JNK potentiates RUNX1 transcription via activator protein 1 (AP-1) activation. Ro5-3335 blocks the transcriptional activation and expression of RUNX1. (D) FECH catalyzes the insertion of ferrous iron (Fe^{2+}) into protoporphyrin IX (PPIX) to produce heme. Heme is required for the function of endothelial nitric oxide synthase (eNOS) and complex IV of the electron transport chain (ETC), and loss of FECH depletes HIF1-α and vascular endothelial growth factor receptor 2 (VEGFR2). Griseofulvin, metabolized to *N*-methylprotoporphyrin (NMPP), and SH-17023 (4e) all inhibit ocular neovascularization by blocking FECH-directed mitochondrial dynamics with the disruption of mitochondrial morphology, $\Delta\Psi_m$ loss, decreased ATP production, and complex IV dysfunction of the ETC. $\Delta\Psi_m$, mitochondrial membrane potential.

et al., 2013b; Fishel et al., 2015; Choi et al., 2016; Shah et al., 2017). APX3330 dose-dependently decreases cell proliferation, migration, and tube formation in HRECs and a macaque choroidal endothelial cell-like cell line (Rf/6a), demonstrating the potent antiangiogenic activity of the small-molecule inhibitor (Jiang et al., 2011; Li et al., 2014b; Sardar Pasha et al., 2018). NF-κB, a TF known to activate genes involved in inflammation, proliferation, migration, and invasion, exhibits decreased activity in response to APX3330 (Hiramoto et al., 1998; Li et al., 2014a). APX3330 also reduces STAT3 DNA binding, reduces intracellular reactive oxygen species levels, and protects cells from senescence (Luo et al., 2008; Li and Wilson, 2014; Li et al., 2014a; Sardar Pasha et al., 2018). Furthermore, inhibition of Ref-1 with APX3330 produces an anti-inflammatory response and alleviates oxidative stress in a mouse model of inflammatory bowel disease, suggesting that Ref-1 mediates inflammation signaling (Sahakian et al., 2021). APX3330 does not contribute to apoptosis and even provides neuronal protection, primarily from oxidative DNA damage, by enhancing the apurinic/apyrimidinic endonuclease repair activity of APE1/Ref-1 (Fehrenbacher et al., 2017; Sahakian et al., 2021).

In vivo, APX3330 shows promising therapeutic effects in several mouse models, suggesting Ref-1 to be a suitable target for treatment of neovascular eye diseases. One IVT injection of APX3330 decreased neovascularization in the *Vldlr*$^{-/-}$ mouse model, a model of subretinal neovascularization (Jiang et al., 2011). Intraperitoneal injection of APX3330 twice daily for 2 weeks decreased L-CNV lesion volume by 25% (Sardar Pasha et al., 2018) and reduced lesion size by 50% when delivered via gavage in the L-CNV model (Hartman et al., 2021; Lachi Silva et al., 2021). This is comparable to IVT injections of anti-VEGF antibody, which decrease lesion size by 30%–50% in the L-CNV

model (Sulaiman et al., 2016). These considerable findings and the oral bioavailability of APX3330 illustrate that Ref-1 redox inhibitors may be a more effective treatment option for retinal diseases than current regimens by targeting multiple pathways involved in these diseases.

Like some current approved anti-VEGF therapeutics, APX3330 has an oncology origin and was originally developed by Eisai for multiple hepatic inflammatory indications. To date, APX3330 has completed 12 phase 1 and phase 2 clinical trials and has been extensively investigated in vitro and in vivo, yielding positive safety and efficacy findings. Efforts to develop second-generation Ref-1 redox inhibitors are already underway, including compounds APX2009 and APX2014. These second-generation Ref-1 redox inhibitors have reduced lipophilicity than APX3330, thus increasing efficacy and possibly bioavailability (Kelley et al., 2011; Sardar Pasha et al., 2018). These small molecules effectively reduce endothelial cell proliferation, migration, and tube formation, and intraperitoneal APX2009 effectively reduces lesion size in the L-CNV mouse model (Sardar Pasha et al., 2018).

APX3330's ability to regulate multiple TFs regulating proangiogenic and proinflammatory pathways makes it an attractive therapeutic for eye diseases. In addition, its ability to promote repair of oxidatively damaged DNA by APE1/Ref-1 adds a unique component. Thus, it has undergone clinical evaluation. The ZETA-1 trial was a 24-week, randomized, placebo-controlled, double-masked study in which patients received twice-daily oral tablets (total 600 mg/day) APX3330 or placebo to evaluate the safety and efficacy of APX3330 for patients with DR and DME (NCT04692688). APX3330 achieved statistical significance on a secondary endpoint of preventing clinically meaningful progression of DR, as defined by binocular three or more steps worsening on the Diabetic Retinopathy Severity Scale. Prevention of three-step worsening (binocular) is a suitable endpoint for an oral, systemic drug. Following confirmation of this as a potential registration endpoint with the Food and Drug Administration for a phase 3 trial for DR, a phase 3 meeting is planned. The safety results from the ZETA-1 trial also demonstrate that the profile of APX3330 as an oral drug remains excellent and is consistent with what was previously reported (Boyer et al., 2022a).

Because Ref-1 redox activity regulates multiple TFs that are linked to neovascular eye diseases, it may be a more promising therapeutic target than current treatments that only target the VEGF signaling pathway. The targets of APX3330 are validated retinal disease pathways, and extensive in vitro and in vivo validation demonstrate the specificity and effectiveness of APX3330 with favorable human safety data (Boyer et al., 2022a). With current treatments consisting of invasive IVT injections, an orally bioavailable drug such as APX3330 or other Ref-1 redox inhibitors offer a noninvasive route compared with standard-of-care IVT injections. Together, this evidence suggests that targeting Ref-1 for neovascular eye diseases may address several clinical problems with current approved therapeutics.

Soluble Epoxide Hydrolase. Polyunsaturated fatty acid (PUFA) metabolism, specifically targeting the function of soluble epoxide hydrolase (sEH) (gene name: *Ephx2*), is another promising area for combined antiangiogenic/anti-inflammatory therapy in neovascularization. Docosahexaenoic acid (DHA) is a PUFA and one of the major components of the retina (makes up ~60% of the fatty acids in the photoreceptors), whereas the abundance of DHA in other tissues is much lower (~5% of total fatty acids) (Stinson et al., 1991; Bush et al., 1994; Stillwell and Wassall, 2003; Querques et al., 2011). DHA can be epoxidated to epoxydocosapentaenoic acids (EDPs) by cytochrome P450 (P450). Hydrolysis via sEH generates a group of metabolites called dihydroxydocosapentaenoic acids (DHDPs), which lead to proangiogenic phenotypes (Fig. 2B) (Arnold et al., 2010a; Harris and Hammock, 2013).

P450 is responsible for converting PUFAs to bioactive epoxygenated fatty acids (EpFAs). Specifically, P450 targets the ω-6 double bond of arachidonic acid to produce epoxyeicosatrienoic acids (EETs). P450 also targets the ω-3 double bond of DHA, with a higher catalytic efficiency than arachidonic acid (Arnold et al., 2010a), and produces EDPs (Fer et al., 2008; Arnold et al., 2010b). Both metabolized EpFAs have been studied in retinopathy due to their functions of vasodilation and anti-inflammation (Ye et al., 2002; Zhang et al., 2014; Capozzi et al., 2016).

EpFAs, such as EETs and EDPs, are unstable and can be rapidly metabolized by enzymes, mainly sEH (Chacos et al., 1983). 19,20-EDP is the most abundant ω-3 EpFA isomer since it is the least efficient substrate for sEH (Zhang et al., 2014). Inhibition of sEH stabilizes and enhances bioactivities of EpFAs, and although EETs are thought to promote angiogenesis (Oltman et al., 1998; Zhang et al., 2001; Ye et al., 2002), EDPs are antiangiogenic in some studies (Zhang et al., 2013a; Capozzi et al., 2014; Hasegawa et al., 2017; Hu et al., 2017). Thus, sEH inhibitors are thought to be antiangiogenic in the eye as the result of accumulation of EDPs after sEH inhibition (Sulaiman et al., 2016; Sulaiman et al., 2018). We recently showed that IVT delivery of 19,20-EDP reduces L-CNV, whereas 19,20-DHDP has no effect (Park et al., 2022). However, conflicting results have been observed in some other studies: Tie2-driven sEH-overexpressing mice experience reduced CNV (Gong et al., 2017). And a proangiogenic role of 19,20-EDP was seen in OIR (Shao et al., 2014). As reviewed previously (Park and Corson, 2019), different experimental setups possibly contribute to these contradictory findings. Systemic versus tissue-specific inhibition of sEH (oral administration/intraperitoneal injection versus tissue specific inhibition) might have different effects considering the unique retinal lipid composition. In addition, administering 19,20-EDP without sEH inhibitors may not only increase EDP but also increase 19,20-DHDP by sEH. Thus, the observed angiogenesis with 19,20-EDP treatment could be potentially due to the increased 19,20-DHDP.

Dietary PUFAs have been extensively studied as antiangiogenic therapies in animal models, such as L-CNV. Anti-inflammatory and antiangiogenic effects were observed by providing ω-3 PUFAs but not ω-6 PUFAs in the diet of this model (Yanai et al., 2014). Providing 17,18-epoxyeicosatetraenoic acid (EEQ) and 19,20-EDP instead of ω-3 PUFAs in the diet of L-CNV mice also decreased CNV, suggesting that this protective effect was regulated by PUFAs' downstream metabolites catalyzed by P450 (Yanai et al., 2014). Exogenous ω-3 PUFAs in the diet also reduced lesion size in L-CNV mice with *Ephx2* knockout, which further confirmed the sEH-dependent metabolism of 17,18-EEQ and 19,20-EDP (Hasegawa et al., 2017). However, due to varying compositions of ω-3 PUFAs in different tissues, systemic administration of dietary PUFAs might have unexpected effects as noted above. Thus, treatment localized to the eye for neovascular eye diseases may be a promising therapeutic strategy.

In the retinas of L-CNV mice, sEH was elevated in the photoreceptors, and enzyme activity increased in adult mice after laser induction (Sulaiman et al., 2018). Significantly decreased 19,20-EDP:19,20-DHDP ratio in retinas of L-CNV mice implicated increased sEH activity (Sulaiman et al., 2018). Recently, both immunohistochemistry and RNAscope in situ hybridization data indicated that sEH was overexpressed in photoreceptors and RPE in nAMD human retinas and L-CNV mouse retinas (Park et al., 2022).

Small-molecule sEH inhibitors are effective in reducing ocular neovascularization in mouse models. We developed a synthetic homoisoflavonoid sEH inhibitor (Sulaiman et al., 2018), SH-11037, that inhibits the growth and migration of HRECs, without cytotoxicity (Basavarajappa et al., 2015), and is antiangiogenic in both the choroidal sprouting assay ex vivo and in zebrafish larvae (Sulaiman et al., 2016). In addition, IVT injection of SH-11037 reduced CNV lesions in the L-CNV model in vivo (Sulaiman et al., 2016) and in OIR (Basavarajappa et al., 2015). Similar results were seen in L-CNV with other sEH inhibitors *trans*-4-(4-(3-adamantan-1-yl-ureido)-cyclohexyloxy)-benzoic acid (*t*-AUCB) and "compound 7" (Sulaiman et al., 2018). The lipid composition of the mouse eye could be altered effectively by IVT injection of 10 μM SH-11037 or *t*-AUCB—the 19,20-EDP:19,20-DHDP ratio increased (Sulaiman et al., 2018). sEH inhibitor treatment by other administration routes has also been reported. *t*-AUCB in drinking water (2 mg/L) significantly reduced 19,20-DHDP in diabetic mouse retina and rescued vascular defects (reduced pericyte number, increased vascular permeability), which further showed a protective effect of sEH inhibitors (Hu et al., 2017). These results indicated that sEH could also be relevant to nonproliferative DR. Orally dosed 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU), an sEH inhibitor that can pass through the blood-brain barrier, coadministrated with 17,18-EEQ or 19,20-EDP (intraperitoneal) suppressed CNV (Hasegawa et al., 2017), whereas *t*-AUCB given to normal neonatal mice (i.p. injection, 2 mg/kg twice daily) reduced retinal vascularization significantly (Hu et al., 2014). Our recent work showed that adeno-associated virus serotype 8 vector expressing small hairpin RNA (shRNA) against *Ephx2* decreased L-CNV lesion volume when delivered IVT (Park et al., 2022). *Ephx2* shRNA also decreased expression of inflammatory cytokines [IL-1β, IL-6, tumor necrosis factor-α (TNF-α), etc.] (Park et al., 2022). Taken together, these results confirm that acute inflammation is tightly related with CNV progression, especially in the early stage (Choudhary and Malek, 2019), and that sEH inhibition can ameliorate this. Based on these fundamental research results about the metabolism of the P450-sEH pathway and the effects of sEH inhibition on inflammation and neovascularization, sEH could be a potential target against ocular neovascularization and DR.

Runt-Related Transcription Factor 1. A protein that controls the development of blood vessels, runt-related transcription factor 1 (RUNX1), is another novel target under exploration for varied ocular neovascular pathologies. RUNX1 (also known as AML1) is a transcription factor that was initially identified to regulate hematopoiesis in acute myeloid leukemia patients (Miyoshi et al., 1991). Core-binding factor (CBF) protein is a heterodimeric protein complex wherein RUNX1 forms the α-subunit responsible for DNA binding and activation of transcriptional targets while the CBFβ subunit stabilizes this complex, in addition to increasing binding affinity of DNA (Lam et al., 2017) (Fig. 2C). RUNX1 is upregulated in numerous cancers, including glioblastoma (Zhao et al., 2019), and this protein regulates angiogenic cell migration, proliferation, cell differentiation, invasion, tubule formation, and other phenotypes (Lam et al., 2017). As such, RUNX1 is implicated in various ocular pathologies, including PDR (Lam et al., 2017), proliferative vitreoretinopathy (PVR) (Delgado-Tirado et al., 2020), and CNV (Gonzalez-Buendia et al., 2021). In PVR, which occurs after rhegmatogenous retinal detachment, RUNX1 promotes epithelial-to-mesenchymal transition of RPE cells facilitated through TGF-β2 signaling. Plus, PVR tissue specimens have high expression of RUNX1 (Delgado-Tirado et al., 2020). In PDR, fibrovascular membrane–derived $CD31^+$ vascular endothelial cells show aberrant upregulation of RUNX1. In L-CNV, RUNX1 is highly expressed in the lesion area and in cell types contributing to neovascularization, including endothelial cells, macrophages, microglia, RPE cells, Müller cells, and vascular smooth muscle cells (Gonzalez-Buendia et al., 2021).

Further, in HRECs and human umbilical vein endothelial cells, RUNX1 protein increases in response to high glucose conditions and regulates HREC migration, proliferation, and tubule formation (Lam et al., 2017). Since high glucose can modulate RUNX1, the involvement of RUNX1 in high glucose-induced post-translational modification of *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) also promotes HREC proliferation and migration (Xing et al., 2021) in DR. Activation of p38 MAPK causes RUNX1 upregulation and RUNX1-dependent abnormal angiogenic properties in HRECs and in a mouse model of streptozotocin-induced DR (Zou et al., 2020). As added evidence, RUNX1 regulates the transcriptional activation of Trefoil factor family 1 (Tff1) peptide to repress this molecule in DR via dysregulating NF-κB signaling (Zhang et al., 2022). These studies show that RUNX1 plays a key role in ocular angiogenesis through multiple signaling pathways, which opens an avenue for the development of novel therapeutics. These links have generated increased attention in recent years to identify small-molecule inhibitors to modulate RUNX1-mediated angiogenesis in the eye.

To inhibit RUNX1's function, a specific lipophilic inhibitor of its transcriptional activation, Ro5-3335, has been widely used in vitro and in vivo. Both small interfering RNA (siRNA) knockdown of *RUNX1* and Ro5-3335 treatment in HRECs reduces angiogenic properties (Lam et al., 2017). Interestingly, Ro5-3335 dose-dependently reduced the proliferation of a primary culture established from PVR membranes. Upon inflammation, TNF-α activates c-Jun N-terminal kinase (JNK) signaling, which, in turn, accelerates *RUNX1* transcription in HRECs. Ro5-3335 blocks RUNX1-mediated transcription (since this gene self-regulates) and so downregulates the mRNA expression of TNF-α induced *RUNX1* and JNK in HRECs (Whitmore et al., 2021), suggesting the potential of Ro5-3335 for blocking TNF-α–mediated inflammatory mechanisms in ocular angiogenesis. Beyond Ro5-3335, another small-molecule inhibitor of RUNX1, Ro24-7429, as a nano-emulsion, obstructs migration and proliferation in HRECs (Arevalo-Alquichire et al., 2022).

TNF-α and high glucose stimulate RUNX1 transcription through JNK and activator protein 1 (AP-1), which eventually builds a feedback loop of JNK-AP-1-RUNX1 transduction, accountable for increased RUNX1 expression and angiogenesis progression. Intriguingly, VEGF modulates this feedback mechanism by inhibiting phosphorylation of JNK and thereby repressing the expression of RUNX1 (Whitmore et al., 2021). However,

the interaction between RUNX1 and VEGF in promoting angiogenesis needs to be validated further.

In vivo, inhibition of RUNX1 by topical application of Ro5-3335 nano-emulsion formulation impedes the progression of PVR in a rabbit model (Delgado-Tirado et al., 2020). RUNX1 inhibition through IVT injection of Ro5-3335 also ameliorates L-CNV, alone and in combination with aflibercept (Gonzalez-Buendia et al., 2021). Moreover, RUNX1 inhibition using IVT Ro5-3335 decreases neovascularization in murine OIR (Lam et al., 2017). No small-molecule RUNX1 inhibitors have been in clinical use yet, but these findings indicate that targeting RUNX1 using Ro5-3335 holds promise for further validation in treating ocular neovascular diseases.

Other Developing Targets. In addition to the promising targets reviewed above, there are numerous other potential targets for ocular angiogenesis under exploration that are (at least partially) independent of the VEGF pathway, functioning through specific signaling pathways and targeted in preclinical work by small-molecule inhibitors.

Protein Arginine Methyltransferase-5. Protein arginine methyltransferase-5 (PRMT5), a type II arginine methyltransferase and novel activator of NF-κB (Wei et al., 2014; Prabhu et al., 2017), has been identified as a novel target in ocular neovascularization (Muniyandi et al., 2023). PRMT5 methylates both histone and nonhistone proteins, thereby regulating their activities. In HRECs and cancer cells, PRMT5 can dimethylate the p65 subunit of NF-κB, promoting NF-κB activation and inflammatory cell signaling (Wei et al., 2013). A variety of cancers show overexpression of PRMT5 (Han et al., 2014; Jiang et al., 2018; Zhang et al., 2018; Li et al., 2019; Qin et al., 2019; Yan et al., 2021), and we showed that PRMT5 is overexpressed in human nAMD and murine L-CNV and that it modulates proangiogenic and proinflammatory NF-κB signaling in HRECs. A novel, specific small-molecule inhibitor of PRMT5, PR5-LL-CM01, or shRNA knockdown of PRMT5 dampened these signaling and angiogenesis properties in both HRECs and in an induced pluripotent stem cell–derived choroidal endothelial cell line (iCEC2) (Muniyandi et al., 2023).

Ferrochelatase. Ferrochelatase (FECH) controls the terminal step of heme biosynthesis, inserting ferrous iron (Fe^{2+}) into protoporphyrin IX (PPIX) to form heme (Hamza and Dailey, 2012) (Fig. 2D). This heme acts as a cofactor for the hemoproteins in the cell (Smith et al., 2010). FECH is a target of the antiangiogenic natural product cremastranone (Shim et al., 2004; Kim et al., 2007; Kim et al., 2008; Lee et al., 2014; Basavarajappa et al., 2017). FECH is overexpressed in human nAMD and mice with L-CNV (Basavarajappa et al., 2017) and OIR (Pran Babu et al., 2020). Increased synthesis of heme in microvascular endothelial cells drives aberrant angiogenesis (Petrillo et al., 2018).

In vitro, FECH blockade in HRECs via siRNA knockdown and treatment with *N*-methylprotoporphyrin (NMPP) hampers proliferation, migration, and tube formation. In addition, genetic or chemical inhibition of FECH in ocular endothelial cells downregulates the protein levels of known activators of angiogenesis, including endothelial nitric oxide synthase (eNOS), HIF-1α, and VEGFR2 (Basavarajappa et al., 2017), plus mitochondrial complex IV, leading to disruption of mitochondrial morphogenesis, membrane potential loss, and diminished glycolysis and oxidative phosphorylation (Shetty et al., 2020). Therefore, we hypothesized that targeting heme synthesis via FECH would offer novel therapeutic options (Shetty and Corson, 2020). We developed a first-in-class, drug-like small-molecule FECH inhibitor, SH-17023 (4e). Strikingly, SH-17023 obstructs proliferation, migration and tube formation and dose-dependently decreases the expression of COX-IV (subunit I) protein in HRECs. SH-17023 also inhibits proliferation in iCEC2 choroidal endothelial cells (Sishtla et al., 2022).

In vivo, mice with a partial loss-of-function point mutation in FECH (*Fech*^{m1Pas}; functionally heme and iron deficient) have reduced L-CNV (Basavarajappa et al., 2017) and OIR (Pran Babu et al., 2020). Further, the antifungal drug griseofulvin (metabolized into a FECH inhibitor in vivo) attenuates L-CNV in mice (Basavarajappa et al., 2017). Intravitreal griseofulvin or NMPP reduced neovascular tuft formation and vasoobliteration without toxicity in OIR (Pran Babu et al., 2020). Likewise, novel FECH inhibitor SH-17023 reduced lesion volume in L-CNV (Sishtla et al., 2022). Thus, targeting FECH has significant therapeutic potential and might provide promising therapeutic options for ocular neovascular diseases.

Conclusions and Future Prospects

The outlook for new therapeutic approaches for posterior ocular neovascularization continues to be promising. We illustrate here just some of the many new approaches undergoing exploration (ElSheikh et al., 2022). Beyond the agents already in advanced clinical development, APE1/Ref-1 holds potential for targeting both angiogenesis and inflammation, taking advantage of APX3330, previously advanced as an anticancer drug with a good safety profile as an oral agent. Likewise, targeting sEH can suppress multiple proangiogenic/proinflammatory pathways, and extensive efforts for eye disease and other indications have presented an array of potential small-molecule inhibitors. RUNX1, as a transcription factor, can impinge on multiple pathways and is also a target of multiple small molecules. In addition, other candidates such as PRMT5 and FECH expand the arsenal of possible targets.

One key question for all these targets is the optimal delivery route for therapy. The safety and ocular uptake of APX3330 when delivered orally provides a strong rationale for pursuing this route for APE1/Ref-1 inhibition. The variability of sEH function in different tissues argues for local inhibition of this target, perhaps by eyedrops or sustained-release IVT formulations. Sustained-release small-molecule inhibition of FECH has shown proof of concept in our hands (Chobisa et al., 2021). Avoiding systemic toxicity for targets like RUNX1 and PRMT5 may also necessitate local delivery, taking advantage of novel delivery systems like nano-emulsions (Arevalo-Alquichire et al., 2022), microparticles (Kim et al., 2019), polymeric implants (Wang et al., 2013), or suprachoroidal injections (Wan et al., 2021).

Much remains to be done in dissecting the molecular mechanisms of these targets as well, including determining whether they work with or through VEGF, and, therefore, their potential in combination therapy with anti-VEGF agents and/or their efficacy in poor- or nonresponders to anti-VEGF drugs. The dearth of animal models for VEGF nonresponse hampers these efforts; synergy experiments may be helpful.

Finally, defining the diseases and disease subtypes most likely to benefit from inhibiting these targets also requires both preclinical and clinical work. If successful for (some) posterior neovascular eye diseases, there is appealing potential to explore these targets in anterior ocular neovascularization such as corneal neovascularization induced by injury or infection, which

lacks widely effective therapies (Barry et al., 2020). Given the anti-inflammatory potential of targeting APE1/Ref-1 and sEH, assessing these in inflammatory diseases like uveitis or dry AMD is also appealing. With further preclinical and clinical testing, formulation, and mechanistic studies, one or more of these disease targets may move beyond VEGF to offer new therapeutic options for neovascularization in the eye.

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Authorship Contributions

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References

Adamis AP and de Juan Jr E (2022) Development of the Port Delivery System with ranibizumab for neovascular age-related macular degeneration. *Curr Opin Ophthalmol* **33**:131–136.

Antonetti DA, Silva PS, and Stitt AW (2021) Current understanding of the molecular and cellular pathology of diabetic retinopathy. *Nat Rev Endocrinol* **17**:195–206.

Apte RS, Chen DS, and Ferrara N (2019) VEGF in signaling and disease: beyond discovery and development. *Cell* **176**:1248–1264.

Arevalo-Alquichire S, Arboleda-Velasquez J, and Kim LA (2022) Encapsulated RUNX1 inhibitor (Ro24-7429) reduces angiogenesis in retinal endothelial cells. *Invest Ophthalmol Vis Sci* **63**:677–F0131.

Arita R, Hata Y, and Ishibashi T (2010) ROCK as a therapeutic target of diabetic retinopathy. *J Ophthalmol* **2010**:175163.

Arnold C, Konkel A, Fischer R, and Schunck WH (2010a) Cytochrome P450-dependent metabolism of ω-6 and ω-3 long-chain polyunsaturated fatty acids. *Pharmacol Rep* **62**:536–547.

Arnold C, Markovic M, Blossey K, Wallukat G, Fischer R, Dechend R, Konkel A, von Schacky C, Luft FC, Muller DN, et al. (2010b) Arachidonic acid-metabolizing cytochrome P450 enzymes are targets of ω-3 fatty acids. *J Biol Chem* **285**:32720–32733.

Askew BC, Furuya T, and Edwards DS (2018) Ocular distribution and pharmacodynamics of SF0166, a topically administered αvβ3 integrin antagonist, for the treatment of retinal diseases. *J Pharmacol Exp Ther* **366**:244–250.

Barry Z, Park B, and Corson TW (2020) Pharmacological potential of small molecules for treating corneal neovascularization. *Molecules* **25**:3468.

Basavarajappa HD, Lee B, Lee H, Sulaiman RS, An H, Magaña C, Shadmand M, Vayl A, Rajashekhar G, Kim E-Y, et al. (2015) Synthesis and biological evaluation of novel homoisoflavonoids for retinal neovascularization. *J Med Chem* **58**:5015–5027.

Basavarajappa HD, Sulaiman RS, Qi X, Shetty T, Sheik Pran Babu S, Sishtla KL, Lee B, Quigley J, Alkhairy S, Briggs CM, et al. (2017) Ferrochelatase is a therapeutic target for ocular neovascularization. *EMBO Mol Med* **9**:786–801.

Bascaran C, Mwangi N, D'Esposito F, Cleland C, Gordon I, Ulloa JAL, Maswadi R, Mdala S, Ramke J, Evans JR, et al. (2021) Effectiveness of interventions to increase uptake and completion of treatment for diabetic retinopathy in low- and middle-income countries: a rapid review protocol. *Syst Rev* **10**:27.

Boyer DS, Brigell M, Kolli A, Rahmani K, Lazar A, Sooch M, Patel R, Lazar E, Pepose JS, and Kelley MR (2022a) The safety of APX3330, an oral drug candidate for the treatment of diabetic eye disease, in the ongoing masked 24-week ZETA-1 Phase 2 clinical trial. *Invest Ophthalmol Vis Sci* **63**:E-abstract 675.

Boyer DS, Kaiser PK, Magrath GN, Brady K, Edwards S, Tanzer DJ, and Heier JS (2022b) The safety and biological activity of OTT166, a novel topical selective integrin inhibitor for the treatment of diabetic eye disease: a Phase 1b study. *Ophthalmic Surg Lasers Imaging Retina* **53**:553–560.

Bush RA, Malnoë A, Remé CE, and Williams TP (1994) Dietary deficiency of N-3 fatty acids alters rhodopsin content and function in the rat retina. *Invest Ophthalmol Vis Sci* **35**:91–100.

Capozzi ME, Hammer SS, McCollum GW, and Penn JS (2016) Epoxygenated fatty acids inhibit retinal vascular inflammation. *Sci Rep* **6**:39211.

Capozzi ME, McCollum GW, and Penn JS (2014) The role of cytochrome P450 epoxygenases in retinal angiogenesis. *Invest Ophthalmol Vis Sci* **55**:4253–4260.

Cardoso AA, Jiang Y, Luo M, Reed AM, Shahda S, He Y, Maitra A, Kelley MR, and Fishel ML (2012) APE1/Ref-1 regulates STAT3 transcriptional activity and APE1/Ref-1-STAT3 dual-targeting effectively inhibits pancreatic cancer cell survival. *PLoS One* **7**:e47462.

Carmi Y, Voronov E, Dotan S, Lahat N, Rahat MA, Fogel M, Huszar M, White MR, Dinarello CA, and Apte RN (2009) The role of macrophage-derived IL-1 in induction and maintenance of angiogenesis. *J Immunol* **183**:4705–4714.

Caston RA, Gampala S, Armstrong L, Messmann RA, Fishel ML, and Kelley MR (2021) The multifunctional APE1 DNA repair-redox signaling protein as a drug target in human disease. *Drug Discov Today* **26**:218–228.

Chacos N, Capdevila J, Falck JR, Manna S, Martin-Wixtrom C, Gill SS, Hammock BD, and Estabrook RW (1983) The reaction of arachidonic acid epoxides (epoxyeicosatrienoic acids) with a cytosolic epoxide hydrolase. *Arch Biochem Biophys* **223**:639–648.

Chobisa D, Sishtla KL, Corson TW, and Yoon Y (2021) Sustained delivery of griseofulvin by polymeric microparticles for neovascular eye disease treatment. *Invest Ophthalmol Vis Sci* **62**:E-abstract 199.

Choi S, Joo HK, and Jeon BH (2016) Dynamic regulation of APE1/Ref-1 as a therapeutic target protein. *Chonnam Med J* **52**:75–80.

Choudhary M and Malek G (2019) A review of pathogenic drivers of age-related macular degeneration, beyond complement, with a focus on potential endpoints for testing therapeutic interventions in preclinical studies, in *Retinal Degenerative Diseases* (Bowes Rickman C, Grimm C, Anderson RE, Ash JD, LaVail MM, and Hollyfield JG, eds) pp 9–13, Springer International Publishing, New York.

Danesh-Meyer HV, Zhang J, Acosta ML, Rupenthal ID, and Green CR (2016) Connexin43 in retinal injury and disease. *Prog Retin Eye Res* **51**:41–68.

Daniel E, Toth CA, Grunwald JE, Jaffe GJ, Martin DF, Fine SL, Huang J, Ying GS, Hagstrom SA, Winter K, et al.; Comparison of Age-related Macular Degeneration Treatments Trials Research Group (2014) Risk of scar in the comparison of age-related macular degeneration treatments trials. *Ophthalmology* **121**:656–666.

Day S, Acquah K, Mruthyunjaya P, Grossman DS, Lee PP, and Sloan FA (2011) Ocular complications after anti-vascular endothelial growth factor therapy in Medicare patients with age-related macular degeneration. *Am J Ophthalmol* **152**:266–272.

Delgado-Tirado S, Amarnani D, Zhao G, Rossin EJ, Eliott D, Miller JB, Greene WA, Ramos L, Arevalo-Alquichire S, Leyton-Cifuentes D, et al. (2020) Topical delivery of a small molecule RUNX1 transcription factor inhibitor for the treatment of proliferative vitreoretinopathy. *Sci Rep* **10**:20554.

Do DV, Schmidt-Erfurth U, Gonzalez VH, Gordon CM, Tolentino M, Berliner AJ, Vitti R, Rückert R, Sandbrink R, Stein D, et al. (2011) The DA VINCI Study: phase 2 primary results of VEGF Trap-Eye in patients with diabetic macular edema. *Ophthalmology* **118**:1819–1826.

Dong S, Wu X, Xu Y, Yang G, and Yan M (2020) Immunohistochemical study of STAT3, HIF-1α and VEGF in pterygium and normal conjunctiva: Experimental research and literature review. *Mol Vis* **26**:510–516.

Dugel PU, Koh A, Ogura Y, Jaffe GJ, Schmidt-Erfurth U, Brown DM, Gomes AV, Warburton J, Weichselberger A, and Holz FG; HAWK and HARRIER Study Investigators (2020) HAWK and HARRIER: Phase 3, multicenter, randomized, double-masked trials of brolucizumab for neovascular age-related macular degeneration. *Ophthalmology* **127**:72–84.

ElSheikh RH, Chauhan MZ, and Sallam AB (2022) Current and novel therapeutic approaches for treatment of neovascular age-related macular degeneration. *Biomolecules* **12**:1629.

Fehrenbacher JC, Guo C, Kelley MR, and Vasko MR (2017) DNA damage mediates changes in neuronal sensitivity induced by the inflammatory mediators, MCP-1 and LPS, and can be reversed by enhancing the DNA repair function of APE1. *Neuroscience* **366**:23–35.

Fer M, Dréano Y, Lucas D, Corcos L, Salaün JP, Berthou F, and Amet Y (2008) Metabolism of eicosapentaenoic and docosahexaenoic acids by recombinant human cytochromes P450. *Arch Biochem Biophys* **471**:116–125.

Fernandes AR, Zielińska A, Sanchez-Lopez E, Dos Santos T, Garcia ML, Silva AM, Karczewski J, and Souto EB (2022) Exudative versus nonexudative age-related macular degeneration: physiopathology and treatment options. *Int J Mol Sci* **23**:2592.

Ferris 3rd FL, Fine SL, and Hyman L (1984) Age-related macular degeneration and blindness due to neovascular maculopathy. *Arch Ophthalmol* **102**:1640–1642.

Fevereiro-Martins MDR, Marques-Neves CAM, Areias M, and Bicho MDP (2023) Retinopathy of prematurity: A review of pathophysiology and signaling pathways. *Surv Ophthalmol* **68**:175–210.

Fishel ML, Jiang Y, Rajeshkumar NV, Scandura G, Sinn AL, He Y, Shen C, Jones DR, Pollok KE, Ivan M, et al. (2011) Impact of APE1/Ref-1 redox inhibition on pancreatic tumor growth. *Mol Cancer Ther* **10**:1698–1708.

Fishel ML, Cheng H, Shahda S, and Kelley MR(2015) APX3330 drug development for clinical trials targeting APE1/Ref-1 in pancreatic cancer, in *AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics, Mol Cancer Ther* December 2015; B167, Boston, MA.

Fishel ML, Colvin ES, Luo M, Kelley MR, and Robertson KA (2010) Inhibition of the redox function of APE1/Ref-1 in myeloid leukemia cell lines results in a hypersensitive response to retinoic acid-induced differentiation and apoptosis. *Exp Hematol* **38**:1178–1188.

Fleckenstein M, Keenan TDL, Guymer RH, Chakravarthy U, Schmitz-Valckenberg S, Klaver CC, Wong WT, and Chew EY (2021) Age-related macular degeneration. *Nat Rev Dis Primers* **7**:31.

Folk JC and Stone EM (2010) Ranibizumab therapy for neovascular age-related macular degeneration [published correction appears in *N Engl J Med* (2010) 363:2474]. *N Engl J Med* **363**:1648–1655.

Furino C, Boscia F, Reibaldi M, and Alessio G (2021) Intravitreal therapy for diabetic macular edema: an update. *J Ophthalmol* **2021**:6654168.

Gong Y, Fu Z, Liegl R, Chen J, Hellström A, and Smith LEH (2017) ω-3 and ω-6 long-chain PUFAs and their enzymatic metabolites in neovascular eye diseases. *Am J Clin Nutr* **106**:16–26.

Gonzalez-Buendia L, Delgado-Tirado S, An M, O'Hare M, Amarnani D, A B Whitmore H, Zhao G, Ruiz-Moreno JM, Arboleda-Velasquez JF, and Kim LA (2021) Treatment of experimental choroidal neovascularization via RUNX1 inhibition. *Am J Pathol* **191**:418–424.

Gragoudas ES, Adamis AP, Cunningham Jr ET, Feinsod M, and Guyer DR; VEGF Inhibition Study in Ocular Neovascularization Clinical Trial Group (2004) Pegaptanib for neovascular age-related macular degeneration. *N Engl J Med* **351**:2805–2816.

Grambergs R, Mondal K, and Mandal N (2019) Inflammatory ocular diseases and sphingolipid signaling. *Adv Exp Med Biol* **1159**:139–152.

Grossniklaus HE, Kang SJ, and Berglin L (2010) Animal models of choroidal and retinal neovascularization. *Prog Retin Eye Res* **29**:500–519.

Hamza I and Dailey HA (2012) One ring to rule them all: trafficking of heme and heme synthesis intermediates in the metazoans. *Biochim Biophys Acta* **1823**:1617–1632.

Hahn MG, Lampe T, El Sheikh S, Griebenow N, Woltering E, Schlemmer KH, Dietz L, Gerisch M, Wunder F, Becker-Pelster EM, et al. (2021) Discovery of the soluble guanylate cyclase activator runcaciguat (BAY 1101042). *J Med Chem* **64**:5323–5344.

Han X, Li R, Zhang W, Yang X, Wheeler CG, Friedman GK, Province P, Ding Q, You Z, Fathallah-Shaykh HM, et al. (2014) Expression of PRMT5 correlates with malignant grade in gliomas and plays a pivotal role in tumor growth in vitro. *J Neurooncol* **118**:61–72.

Harris TR and Hammock BD (2013) Soluble epoxide hydrolase: gene structure, expression and deletion. *Gene* **526**:61–74.

Hartman GD, Lambert-Cheatham NA, Kelley MR, and Corson TW (2021) Inhibition of APE1/Ref-1 for neovascular eye diseases: from biology to therapy. *Int J Mol Sci* **22**:10279.

Hasegawa E, Inafuku S, Mulki L, Okunuki Y, Yanai R, Smith KE, Kim CB, Klokman G, Bielenberg DR, Puli N, et al. (2017) Cytochrome P450 monooxygenase lipid metabolites are significant second messengers in the resolution of choroidal neovascularization. *Proc Natl Acad Sci USA* **114**:E7545–E7553.

Heier JS, Khanani AM, Quezada Ruiz C, Basu K, Ferrone PJ, Brittain C, Figueroa MS, Lin H, Holz FG, Patel V, et al.; TENAYA and LUCERNE Investigators (2022) Efficacy, durability, and safety of intravitreal faricimab up to every 16 weeks for neovascular age-related macular degeneration (TENAYA and LUCERandomizedandomised, double-masked, phase 3, non-inferiority trials. *Lancet* **399**:729–740.

Heisel C, Yousif J, Mijiti M, Charizanis K, Brigell M, Corson TW, and Kelley MR (2021) APE1/Ref-1 as a novel target for retinal diseases. *J Cell Signal* **2**:133–138.

Hirahara S, Nozaki M, Ohbayashi M, Hasegawa N, Ozone D, and Ogura Y (2017) Suppression of retinal neovascularization by anti-ccr3 treatment in an oxygen-induced retinopathy model in mice. *Ophthalmic Res* **58**:56–66.

Hiramoto M, Shimizu N, Sugimoto K, Tang J, Kawakami Y, Ito M, Aizawa S, Tanaka H, Makino I, and Handa H (1998) Nuclear targeted suppression of NF-κ B activity by the novel quinone derivative E3330. *J Immunol* **160**:810–819.

Holekamp NM, Campochiaro PA, Chang MA, Miller D, Pieramici D, Adamis AP, Brittain C, Evans E, Kaufman D, Maass KF, et al.; all Archway Investigators (2022) Archway randomized phase 3 trial of the port delivery system with ranibizumab for neovascular age-related macular degeneration. *Ophthalmology* **129**:295–307.

Hong EH, Shin YU, and Cho H (2022) Retinopathy of prematurity: a review of epidemiology and current treatment strategies. *Clin Exp Pediatr* **65**:115–126.

Hsu J and Regillo CD (2020) Poorer outcomes in real-world studies of anti-vascular endothelial growth factor therapy for neovascular age-related macular degeneration. *Ophthalmology* **127**:1189–1190.

Hu J, Dziumbla S, Lin J, Bibli S-I, Zukunft S, de Mos J, Awwad K, Frömel T, Jungmann A, Devraj K, et al. (2017) Inhibition of soluble epoxide hydrolase prevents diabetic retinopathy. *Nature* **552**:248–252.

Hu J, Popp R, Frömel T, Ehling M, Awwad K, Adams RH, Hammes H-P, and Fleming I (2014) Müller glia cells regulate Notch signaling and retinal angiogenesis via the generation of 19,20-dihydroxydocosapentaenoic acid. *J Exp Med* **211**:281–295.

International Diabetes Federation (2021) *IDF Diabetes Atlas*, 10th ed, IDF, Brussels.

Jacobs B, Palmer N, Shetty T, Dimaras H, Hajrasouliha A, Jusufbegovic D, and Corson TW (2021) Patient preferences in retinal drug delivery. *Sci Rep* **11**:18996.

Jedinak A, Dudhgaonkar S, Kelley MR, and Sliva D (2011) Apurinic/Apyrimidinic endonuclease 1 regulates inflammatory response in macrophages. *Anticancer Res* **31**:379–385.

Jiang A, Gao H, Kelley MR, and Qiao X (2011) Inhibition of APE1/Ref-1 redox activity with APX3330 blocks retinal angiogenesis in vitro and in vivo. *Vision Res* **51**:93–100.

Jiang H, Zhu Y, Zhou Z, Xu J, Jin S, Xu K, Zhang H, Sun Q, Wang J, and Xu J (2018) PRMT5 promotes cell proliferation by inhibiting BTG2 expression via the ERK signaling pathway in hepatocellular carcinoma. *Cancer Med* **7**:869–882.

Kelley MR, Georgiadis MM, and Fishel ML (2012) APE1/Ref-1 role in redox signaling: translational applications of targeting the redox function of the DNA repair/redox protein APE1/Ref-1. *Curr Mol Pharmacol* **5**:36–53.

Kelley MR, Luo M, Reed A, Su D, Delaplane S, Borch RF, Nyland 2nd RL, Gross ML, and Georgiadis MM (2011) Functional analysis of novel analogues of E3330 that block the redox signaling activity of the multifunctional AP endonuclease/redox signaling enzyme APE1/Ref-1. *Antioxid Redox Signal* **14**:1387–1401.

Kim J, Lima E Silva R, Shmueli RB, Mirando AC, Tzeng SY, Pandey NB, Ben-Akiva E, Popel AS, Campochiaro PA, and Green JJ (2019) Anisotropic poly(lactic-co-glycolic acid) microparticles enable sustained release of a peptide for long-term inhibition of ocular neovascularization. *Acta Biomater* **97**:451–460.

Kim JH, Kim KH, Kim JH, Yu YS, Kim YM, Kim KW, and Kwon HJ (2007) Homoisoflavanone inhibits retinal neovascularization through cell cycle arrest with decrease of cdc2 expression. *Biochem Biophys Res Commun* **362**:848–852.

Kim JH, Kim JH, Yu YS, Jun HO, Kwon HJ, Park KH, and Kim KW (2008) Inhibition of choroidal neovascularization by homoisoflavanone, a new angiogenesis inhibitor. *Mol Vis* **14**:556–561.

Kim S-Y, Kim Y, and Oh Y (2021) Inflammatory pathways in pathological neovascularization in retina and choroid: a narrative review on the inflammatory drug target molecules in retinal and choroidal neovascularization. *Ann Eye Sci* **6**:24.

Lachi Silva L, Lambert-Cheatham NA, Stratford RE, Quinney SK, Corson TW, and Kelley MR (2021) Oral APX3330 treatment reduces L-CNV lesions in preclinical mouse model and confirms Phase 2 DR/DME clinical dose with sufficient distribution to human retina using PBPK modeling. *Invest Ophthalmol Vis Sci* **62**:E-abstract 1073.

Lam JD, Oh DJ, Wong LL, Amarnani D, Park-Windhol C, Sanchez AV, Cardona-Velez J, McGuone D, Stemmer-Rachamimov AO, Eliott D, et al. (2017) Identification of RUNX1 as a mediator of aberrant retinal angiogenesis. *Diabetes* **66**:1950–1956.

Lee B, Basavarajappa HD, Sulaiman RS, Fei X, Seo SY, and Corson TW (2014) The first synthesis of the antiangiogenic homoisoflavanone, cremastranone. *Org Biomol Chem* **12**:7673–7677.

Li M and Wilson 3rd DM (2014) Human apurinic/apyrimidinic endonuclease 1. *Antioxid Redox Signal* **20**:678–707.

Li Y, Liu X, Zhou T, Kelley MR, Edwards P, Gao H, and Qiao X (2014a) Inhibition of APE1/Ref-1 redox activity rescues human retinal pigment epithelial cells from oxidative stress and reduces choroidal neovascularization. *Redox Biol* **2**:485–494.

Li Y, Liu X, Zhou T, Kelley MR, Edwards PA, Gao H, and Qiao X (2014b) Suppression of choroidal neovascularization through inhibition of APE1/Ref-1 redox activity. *Invest Ophthalmol Vis Sci* **55**:4461–4469.

Li Y, Yang Y, Liu X, Long Y, and Zheng Y (2019) PRMT5 promotes human lung cancer cell apoptosis via Akt/Gsk3β signaling induced by resveratrol. *Cell Transplant* **28**:1664–1673.

Luo M, Delaplane S, Jiang A, Reed A, He Y, Fishel M, Nyland 2nd RL, Borch RF, Qiao X, Georgiadis MM, et al. (2008) Role of the multifunctional DNA repair and redox signaling protein Ape1/Ref-1 in cancer and endothelial cells: small-molecule inhibition of the redox function of Ape1. *Antioxid Redox Signal* **10**:1853–1867.

Lyon H, Shome A, Rupenthal ID, Green CR, and Mugisho OO (2020) Tonabersat inhibits connexin43 hemichannel opening and inflammasome activation in an in vitro retinal epithelial cell model of diabetic retinopathy. *Int J Mol Sci* **22**:298.

Malek G, Busik J, Grant MB, and Choudhary M (2018) Models of retinal diseases and their applicability in drug discovery. *Expert Opin Drug Discov* **13**:359–377.

Mat Nor MN, Rupenthal ID, Green CR, and Acosta ML (2020) Connexin hemichannel block using orally delivered tonabersat improves outcomes in animal models of retinal disease. *Neurotherapeutics* **17**:371–387.

Mehta H, Nguyen V, Barthelmes D, Pershing S, Chi GC, Dopart P, and Gillies MC (2022) Outcomes of over 40,000 eyes treated for diabetic macula edema in routine clinical practice: a systematic review and meta-analysis. *Adv Ther* **39**:5376–5390.

Mijit M, Caston R, Gampala S, Fishel ML, Fehrenbacher J, and Kelley MR (2021) APE1/Ref-1 - one target with multiple indications: emerging aspects and new directions. *J Cell Signal* **2**:151–161.

Mijit M, Liu S, Sishtla K, Hartman GD, Wan J, Corson TW, and Kelley MR (2023) Identification of novel pathways regulated by APE1/Ref-1 in human retinal endothelial cells. *Int J Mol Sci* **24**:1101.

Miyoshi H, Shimizu K, Kozu T, Maseki N, Kaneko Y, and Ohki M (1991) t(8;21) breakpoints on chromosome 21 in acute myeloid leukemia are clustered within a limited region of a single gene, AML1. *Proc Natl Acad Sci USA* **88**:10431–10434.

Muniyandi A, Martin M, Sishtla K, Motolani A, Sun M, Jensen NR, Qi X, Boulton ME, Prabhu L, Lu T, et al. (2023) PRMT5 is a therapeutic target in choroidal neovascularization. *Sci Rep* **13**:1747.

Nozaki M, Raisler BJ, Sakurai E, Sarma JV, Barnum SR, Lambris JD, Chen Y, Zhang K, Ambati BK, Baffi JZ, et al. (2006) Drusen complement components C3a and C5a promote choroidal neovascularization. *Proc Natl Acad Sci USA* **103**:2328–2333.

Okada M, Mitchell P, Finger RP, Eldem B, Talks SJ, Hirst C, Paladini L, Barratt J, Wong TY, and Loewenstein A (2021) Nonadherence or nonpersistence to intravitreal injection therapy for neovascular age-related macular degeneration: a mixed-methods systematic review. *Ophthalmology* **128**:234–247.

Oltman CL, Weintraub NL, VanRollins M, and Dellsperger KC (1998) Epoxyeicosatrienoic acids and dihydroxyeicosatrienoic acids are potent vasodilators in the canine coronary microcirculation. *Circ Res* **83**:932–939.

Park B and Corson TW (2019) Soluble epoxide hydrolase inhibition for ocular diseases: vision for the future. *Front Pharmacol* **10**:95.

Park B, Sardar Pasha SPB, Sishtla KL, Hartman GD, Qi X, Boulton ME, and Corson TW (2022) Decreased expression of soluble epoxide hydrolase suppresses murine choroidal neovascularization. *Int J Mol Sci* **23**:15595.

Patel D, Patel SN, Chaudhary V, and Garg SJ (2022) Complications of intravitreal injections: 2022. *Curr Opin Ophthalmol* **33**:137–146.

Petrillo S, Chiabrando D, Genova T, Fiorito V, Ingoglia G, Vinchi F, Mussano F, Carossa S, Silengo L, Altruda F, et al. (2018) Heme accumulation in endothelial cells impairs angiogenesis by triggering paraptosis. *Cell Death Differ* **25**:573–588.

Prabhu L, Chen L, Wei H, Demir O, Safa A, Zeng L, Amaro RE, O'Neil BH, Zhang ZY, and Lu T (2017) Development of an AlphaLISA high throughput technique to screen for small molecule inhibitors targeting protein arginine methyltransferases. *Mol Biosyst* **13**:2509–2520.

Pran Babu SPS, White D, and Corson TW (2020) Ferrochelatase regulates retinal neovascularization. *FASEB J* **34**:12419–12435.

Qin Y, Hu Q, Xu J, Ji S, Dai W, Liu W, Xu W, Sun Q, Zhang Z, Ni Q, et al. (2019) PRMT5 enhances tumorigenicity and glycolysis in pancreatic cancer via the FBW7/cMyc axis. *Cell Commun Signal* **17**:30.

Querques G, Forte R, and Souied EH (2011) Retina and omega-3. *J Nutr Metab* **2011**:748361.

Ramakrishnan S, Anand V, and Roy S (2014) Vascular endothelial growth factor signaling in hypoxia and inflammation. *J Neuroimmune Pharmacol* **9**:142–160.

Ricci F, Bandello F, Navarra P, Staurenghi G, Stumpp M, and Zarbin M (2020) Neovascular age-related macular degeneration: therapeutic management and new-upcoming approaches. *Int J Mol Sci* **21**:8242.

Rübsam A, Parikh S, and Fort PE (2018) Role of inflammation in diabetic retinopathy. *Int J Mol Sci* **19**:942.

Sahakian L, Filippone RT, Stavely R, Robinson AM, Yan XS, Abalo R, Eri R, Bornstein JC, Kelley MR, and Nurgali K (2021) Inhibition of APE1/Ref-1 redox signaling alleviates intestinal dysfunction and damage to myenteric neurons in a mouse model of spontaneous chronic colitis. *Inflamm Bowel Dis* **27**:388–406.

Sardar Pasha SPB, Sishtla K, Sulaiman RS, Park B, Shetty T, Shah F, Fishel ML, Wikel JH, Kelley MR, and Corson TW (2018) Ref-1/APE1 inhibition with novel small molecules blocks ocular neovascularization. *J Pharmacol Exp Ther* **367**:108–118.

Shah F, Logsdon D, Messmann RA, Fehrenbacher JC, Fishel ML, and Kelley MR (2017) Exploiting the Ref-1-APE1 node in cancer signaling and other diseases: from bench to clinic. *NPJ Precis Oncol* **1**:19.

Shao Z, Fu Z, Stahl A, Joyal J-S, Hatton C, Juan A, Hurst C, Evans L, Cui Z, Pei D, et al. (2014) Cytochrome P450 2C8 ω3-long-chain polyunsaturated fatty acid metabolites increase mouse retinal pathologic neovascularization–brief report. *Arterioscler Thromb Vasc Biol* **34**:581–586.

Sharma NK, Prabhakar S, Gupta A, Singh R, Gupta PK, Gupta PK, and Anand A (2012) New biomarker for neovascular age-related macular degeneration: eotaxin-2. *DNA Cell Biol* **31**:1618–1627.

Shetty T and Corson TW (2020) Mitochondrial heme synthesis enzymes as therapeutic targets in vascular diseases. *Front Pharmacol* **11**:1015.
Shetty T, Sishtla K, Park B, Repass MJ, and Corson TW (2020) Heme synthesis inhibition blocks angiogenesis via mitochondrial dysfunction. *iScience* **23**:101391.
Shim JS, Kim JH, Lee J, Kim SN, and Kwon HJ (2004) Anti-angiogenic activity of a homoisoflavanone from *Cremastra appendiculata*. *Planta Med* **70**:171–173.
Sishtla K, Lambert-Cheatham N, Lee B, Han DH, Park J, Sardar Pasha SPB, Lee S, Kwon S, Muniyandi A, Park B, et al. (2022) Small-molecule inhibitors of ferrochelatase are antiangiogenic agents. *Cell Chem Biol* **29**:1010–1023.e14.
Smith LE, Wesolowski E, McLellan A, Kostyk SK, D'Amato R, Sullivan R, and D'Amore PA (1994) Oxygen-induced retinopathy in the mouse. *Invest Ophthalmol Vis Sci* **35**:101–111.
Smith LJ, Kahraman A, and Thornton JM (2010) Hemeproteins–diversity in structural characteristics, function, and folding. *Proteins* **78**:2349–2368.
Stewart MW (2012) The expanding role of vascular endothelial growth factor inhibitors in ophthalmology. *Mayo Clin Proc* **87**:77–88.
Stewart MW, Garg S, Newman EM, Jeffords E, Konopińska J, Jackson S, Sikorski BL, and Rawner ES (2022) Safety and therapeutic effects of orally administered AKST4290 in newly diagnosed neovascular age-related macular degeneration. *Retina* **42**:1038–1046.
Stillwell W and Wassall SR (2003) Docosahexaenoic acid: membrane properties of a unique fatty acid. *Chem Phys Lipids* **126**:1–27.
Stinson AM, Wiegand RD, and Anderson RE (1991) Recycling of docosahexaenoic acid in rat retinas during n-3 fatty acid deficiency. *J Lipid Res* **32**:2009–2017.
Su D, Delaplane S, Luo M, Rempel DL, Vu B, Kelley MR, Gross ML, and Georgiadis MM (2011) Interactions of apurinic/apyrimidinic endonuclease with a redox inhibitor: evidence for an alternate conformation of the enzyme. *Biochemistry* **50**:82–92.
Sulaiman RS, Merrigan S, Quigley J, Qi X, Lee B, Boulton ME, Kennedy B, Seo S-Y, and Corson TW (2016) A novel small molecule ameliorates ocular neovascularisation and synergises with anti-VEGF therapy. *Sci Rep* **6**:25509.
Sulaiman RS, Park B, Sheik Pran Babu SP, Si Y, Kharwadkar R, Mitter SK, Lee B, Sun W, Qi X, Boulton ME, et al. (2018) Chemical proteomics reveals soluble epoxide hydrolase as a therapeutic target for ocular neovascularization. *ACS Chem Biol* **13**:45–52.
Toomey CB, Johnson LV, and Bowes Rickman C (2018) Complement factor H in AMD: Bridging genetic associations and pathobiology. *Prog Retin Eye Res* **62**:38–57.
Tufail A, Patel PJ, Egan C, Hykin P, da Cruz L, Gregor Z, Dowler J, Majid MA, Bailey C, Mohamed Q, et al.; ABC Trial Investigators (2010) Bevacizumab for neovascular age related macular degeneration (ABC Trial): multicenter randomized double masked study. *BMJ* **340**:c2459.
Wan CR, Muya L, Kansara V, and Ciulla TA (2021) Suprachoroidal delivery of small molecules, nanoparticles, gene and cell therapies for ocular diseases. *Pharmaceutics* **13**:288.
Wang J, Jiang A, Joshi M, and Christoforidis J (2013) Drug delivery implants in the treatment of vitreous inflammation. *Mediators Inflamm* **2013**:780634.
Wang X, Ma W, Han S, Meng Z, Zhao L, Yin Y, Wang Y, and Li J (2017) TGF-β participates choroid neovascularization through Smad2/3-VEGF/TNF-α signaling in mice with laser-induced wet age-related macular degeneration. *Sci Rep* **7**:9672.
Wei H, Mundade R, Lange KC, and Lu T (2014) Protein arginine methylation of non-histone proteins and its role in diseases. *Cell Cycle* **13**:32–41.
Wei H, Wang B, Miyagi M, She Y, Gopalan B, Huang DB, Ghosh G, Stark GR, and Lu T (2013) PRMT5 dimethylates R30 of the p65 subunit to activate NF-κB. *Proc Natl Acad Sci USA* **110**:13516–13521.
Weiss M, Sim DA, Herold T, Schumann RG, Liegl R, Kern C, Kreutzer T, Schiefelbein J, Rottmann M, Priglinger S, et al. (2018) Compliance and adherence of patients with diabetic macular edema to intravitreal anti-vascular endothelial growth factor therapy in daily practice. *Retina* **38**:2293–2300.
Whitmore HAB, Amarnani D, O'Hare M, Delgado-Tirado S, Gonzalez-Buendia L, An M, Pedron J, Bushweller JH, Arboleda-Velasquez JF, and Kim LA (2021) TNF-α signaling regulates RUNX1 function in endothelial cells. *FASEB J* **35**:e21155.
Wong WL, Su X, Li X, Cheung CM, Klein R, Cheng CY, and Wong TY (2014) Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob Health* **2**:e106–e116.
Wu H, Hwang DK, Song X, and Tao Y (2017) Association between aqueous cytokines and diabetic retinopathy stage. *J Ophthalmol* **2017**:9402198.
Wykoff CC, Abreu F, Adamis AP, Basu K, Eichenbaum DA, Haskova Z, Lin H, Loewenstein A, Mohan S, Pearce IA, et al.; YOSEMITE and RHINE Investigators (2022) Efficacy, durability, and safety of intravitreal faricimab with extended dosing up to every 16 weeks in patients with diabetic macular oedema (YOSEMITE and RHINE): two randomised, double-masked, phase 3 trials. *Lancet* **399**:741–755.
Xing X, Wang H, Niu T, Jiang Y, Shi X, and Liu K (2021) RUNX1 can mediate the glucose and O-GlcNAc-driven proliferation and migration of human retinal microvascular endothelial cells. *BMJ Open Diabetes Res Care* **9**:e001898.
Xu H, Chen M, and Forrester JV (2009) Para-inflammation in the aging retina. *Prog Retin Eye Res* **28**:348–368.
Yan Y, Zhao P, Wang Z, Liu Z, Wang Z, Zhang J, Ding Y, Hua X, and Yu L (2021) PRMT5 regulates colorectal cancer cell growth and EMT via EGFR/Akt/GSK3β signaling cascades. *Aging (Albany NY)* **13**:4468–4481.
Yan Z, Shi H, Zhu R, Li L, Qin B, Kang L, Chen H, and Guan H (2018) Inhibition of YAP ameliorates choroidal neovascularization via inhibiting endothelial cell proliferation. *Mol Vis* **24**:83–93.
Yanai R, Mulki L, Hasegawa E, Takeuchi K, Sweigard H, Suzuki J, Gaissert P, Vavvas DG, Sonoda K-H, Rothe M, et al. (2014) Cytochrome P450-generated metabolites derived from ω-3 fatty acids attenuate neovascularization. *Proc Natl Acad Sci USA* **111**:9603–9608.
Yang S, Zhao J, and Sun X (2016) Resistance to anti-VEGF therapy in neovascular age-related macular degeneration: a comprehensive review. *Drug Des Devel Ther* **10**:1857–1867.
Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, Chen SJ, Dekker JM, Fletcher A, Grauslund J, et al.; Meta-Analysis for Eye Disease (META-EYE) Study Group (2012) Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care* **35**:556–564.
Ye D, Zhang D, Oltman C, Dellsperger K, Lee H-C, and VanRollins M (2002) Cytochrome p-450 epoxygenase metabolites of docosahexaenoate potently dilate coronary arterioles by activating large-conductance calcium-activated potassium channels. *J Pharmacol Exp Ther* **303**:768–776.
Yerramothu P (2018) New therapies of neovascular AMD-beyond anti-VEGFs. *Vision (Basel)* **2**:31.
Zhang G, Kodani S, and Hammock BD (2014) Stabilized epoxygenated fatty acids regulate inflammation, pain, angiogenesis and cancer. *Prog Lipid Res* **53**:108–123.
Zhang G, Panigrahy D, Mahakian LM, Yang J, Liu J-Y, Stephen Lee KS, Wettersten HI, Ulu A, Hu X, Tam S, et al. (2013a) Epoxy metabolites of docosahexaenoic acid (DHA) inhibit angiogenesis, tumor growth, and metastasis. *Proc Natl Acad Sci USA* **110**:6530–6535.
Zhang J, Luo M, Marasco D, Logsdon D, LaFavers KA, Chen Q, Reed A, Kelley MR, Gross ML, and Georgiadis MM (2013b) Inhibition of apurinic/apyrimidinic endonuclease I's redox activity revisited. *Biochemistry* **52**:2955–2966.
Zhang W, Zhang D, Cheng Y, Liang X, and Wang J (2022) Runx1 regulates Tff1 expression to expedite viability of retinal microvascular endothelial cells in mice with diabetic retinopathy. *Exp Eye Res* **217**:108969.
Zhang X, Wu J, Wu C, Chen W, Lin R, Zhou Y, and Huang X (2018) The LINC01138 interacts with PRMT5 to promote SREBP1-mediated lipid desaturation and cell growth in clear cell renal cell carcinoma. *Biochem Biophys Res Commun* **507**:337–342.
Zhang Y, Oltman CL, Lu T, Lee H-C, Dellsperger KC, and VanRollins M (2001) EET homologs potently dilate coronary microvessels and activate BK(Ca) channels. *Am J Physiol Heart Circ Physiol* **280**:H2430–H2440.
Zhao K, Cui X, Wang Q, Fang C, Tan Y, Wang Y, Yi K, Yang C, You H, Shang R, et al. (2019) RUNX1 contributes to the mesenchymal subtype of glioblastoma in a TGFβ pathway-dependent manner. *Cell Death Dis* **10**:877.
Zippel N, Kenny CH, Wu H, Garneau M, Kroe-Barrett R, Gupta P, Low S, Bakker RA, and Thomas L (2022) Sema3A Antibody BI-X prevents cell permeability and cytoskeletal collapse in HRMECs and increases tip cell density in mouse oxygen-induced retinopathy. *Transl Vis Sci Technol* **11**:17.
Zou W, Zhang Z, Luo S, Cheng L, Huang X, Ding N, Yu J, Pan Y, and Wu Z (2020) p38 promoted retinal micro-angiogenesis through up-regulated RUNX1 expression in diabetic retinopathy. *Biosci Rep* **40**:BSR20193256.

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