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Authors

Cao, Yihai
Langer, Robert
Ferrara, Napoleone

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Review article

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Targeting angiogenesis in oncology, ophthalmology and beyond

Yihai Cao [1] ✉, Robert Langer [2,3] & Napoleone Ferrara [4,5,6] ✉

Abstract

Angiogenesis is an essential process in normal development and in adult physiology, but can be disrupted in numerous diseases. The concept of targeting angiogenesis for treating diseases was proposed more than 50 years ago, and the first two drugs targeting vascular endothelial growth factor (VEGF), bevacizumab and pegaptanib, were approved in 2004 for the treatment of cancer and neovascular ophthalmic diseases, respectively. Since then, nearly 20 years of clinical experience with anti-angiogenic drugs (AADs) have demonstrated the importance of this therapeutic modality for these disorders. However, there is a need to improve clinical outcomes by enhancing therapeutic efficacy, overcoming drug resistance, defining surrogate markers, combining with other drugs and developing the next generation of therapeutics. In this Review, we examine emerging new targets, the development of new drugs and challenging issues such as the mode of action of AADs and elucidating mechanisms underlying clinical benefits; we also discuss possible future directions of the field.

Sections

[1]Department of Microbiology, Tumour and Cell Biology, Karolinska Institute, Stockholm, Sweden. [2]David H Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, USA. [3]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA. [4]Department of Pathology, University of California San Diego, La Jolla, CA, USA. [5]Department of Ophthalmology, University of California San Diego, La Jolla, CA, USA. [6]Moores Cancer Center, University of California San Diego, La Jolla, CA, USA. ✉e-mail: yihai.cao@ki.se; nferrara@health.ucsd.edu

Introduction

The blood vessels probably constitute the largest tissue mass in the body and have central roles in the maintenance of homeostasis, metabolism and blood–tissue exchanges. Blood vessel formation, or angiogenesis, entails the sprouting of new capillaries from pre-existing vessels. Angiogenesis is regulated by a multitude of pro-angiogenic and angio-inhibitory signals. Given the ubiquitous roles of blood vessels, targeting angiogenesis is likely to be an approach suitable for treating numerous diseases.

Multiple complex and coordinated processes[1] are involved in angiogenesis, including the proliferation and migration of endothelial cells, vascular lumen formation and the construction of vascular networks. Each of these processes is regulated by specific, but often overlapping, signalling molecules. However, imbalanced expression of angiogenic factors can result in the formation of abnormal vascular networks that, eventually, cause tissue and organ dysfunction[2,3] and lead to disease states.

For example, solid tumours are highly vascularized relative to their adjacent healthy tissues. In addition, neovascularization and inappropriate vascular remodelling are common causes of visual loss in several disorders, including age-related macular degeneration (AMD). The existence of high numbers of microvessels that are often leaky alters tissue architectures and, eventually, causes malfunctions in multiple tissues and organs. In cancer, aberrant angiogenesis promotes tumour growth and metastasis and can affect the response to anticancer drugs.

Tumour blood vessels exhibit distinct features[1,4], including an immature and leaky endothelial lining that generally lacks perivascular cell coverage, loss of surrounding basement membrane, a lack of clear distinction between arterioles and venules, and chaotic and sluggish blood flow. The formation of these disorganized vascular networks is related to the unique microenvironment within tumours, which is usually inflammatory, hypoxic and acidotic[5]. However, these aberrant features provide unique opportunities for drug development and therapeutic interventions. The concept of anti-angiogenic therapy was first proposed by Folkman in 1971 (ref. 6) (Fig. 1).

Vascular endothelial growth factor (VEGF; also known as vascular permeability factor (VPF) and VEGFA) was isolated and cloned in 1989 (refs. 7–9). It is thought to be the key factor that initiates development of the embryonic haemangioblasts that subsequently differentiate into haematopoietic cells and endothelial cells[10]. Genetic deletion of a single allele of the mouse *Vegf* gene leads to embryonic lethality owing to a loss of haematopoietic cells and blood vessels[2,3]. Therefore, an optimal VEGF level is required for normal embryonic development.

VEGF is the key angiogenic factor that contributes to the formation of disorganized and primitive vasculature in various tumour tissues[7,8] (Fig. 1). It stimulates diverse biological processes, many of which are relevant to cancer[11–14]. Consequently, numerous drugs that block the VEGF signalling pathway have been developed, and they have received US Food and Drug Administration (FDA) approval for the treatment of various cancers (Table 1) as well as for neovascular eye disorders.

Today, nearly all clinically approved anti-angiogenic drugs (AADs) for cancer therapy and ophthalmic disorders target the VEGF pathway (Table 1). In 2004, the humanized anti-VEGF monoclonal antibody bevacizumab (Avastin)[15] was approved by the FDA for previously untreated metastatic colorectal cancer (CRC). This approval represented an important milestone for the concept of anti-angiogenic therapy in patients with cancer[16] (Fig. 1). Bevacizumab is still one of the most widely used cancer therapeutics, with 12 FDA approvals for multiple indications[17]. It is also widely used off-label to treat ophthalmic neovascular disorders. Owing to the diverse biological functions of VEGF, drugs that block the VEGF pathway are likely to act through complex mechanisms, including anti-angiogenesis, normalization of tumour vasculature, regression of existing tumour vasculatures, reducing vascular leakage, improving delivery of other anticancer drugs and the alteration of immune functions[18]. Although these various mechanisms could underlie clinical benefits, we define these drugs as AADs in this Review.

Despite the widespread use of anti-VEGF drugs, their therapeutic benefits in improving survival of patients with cancer are relatively limited and some cancer types are intrinsically resistant[19–21]. Challenges for the clinical use of AADs include improving clinical benefits, overcoming drug resistance, identifying reliable biomarkers, prolonging duration of clinical responses and optimizing combinations with other therapeutic modalities. Vascular targeting agents or vascular disrupting agents represent another class of anticancer drugs that can occlude pre-existing blood vessels within tumours[22], but they have not been

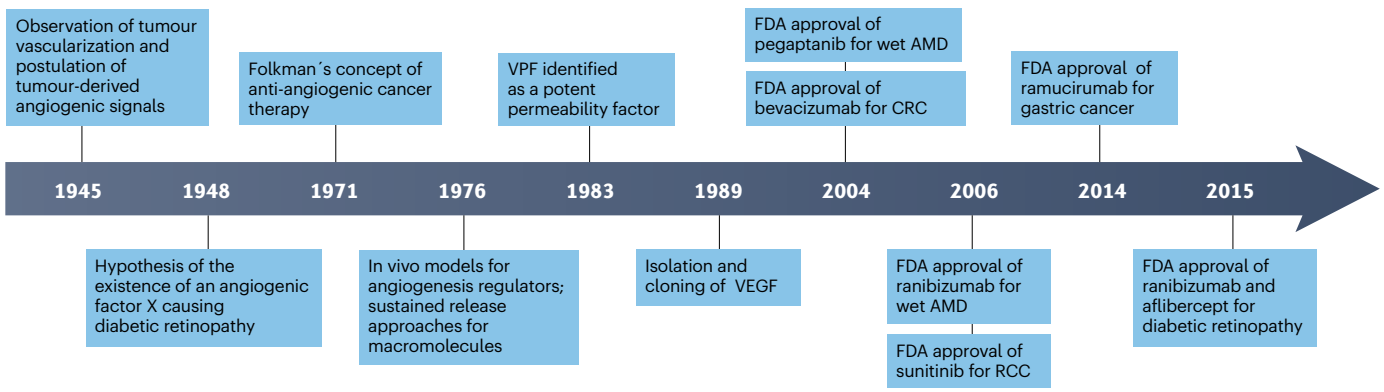

Fig. 1 | Key milestones in angiogenesis research and drug discovery. Early observations of tumour vascularization and the existence of a potential angiogenic factor were described in the 1940s[278,279]. The initial hypothesis of anti-angiogenic cancer therapy was proposed by Folkman in 1971 (ref. 6). Langer and Folkman developed in vivo models to discover angiogenesis regulators[280] and also developed the first approaches for sustained release of proteins and other macromolecules[281]. The first anti-angiogenic drugs (AADs) for treating cancer and wet age-related macular disease (AMD) were approved by the US Food and Drug Administration (FDA) in 2004 (refs. 16,162). CRC, colorectal cancer; RCC, renal cell carcinoma; VEGF, vascular endothelial growth factor; VPF, vascular permeability factor.

Table 1 | Examples of different classes of anti-VEGF drugs for clinical oncology

Drug	Target	Feature	Indication	Refs.
Biologics				
Bevacizumab	VEGF	Monospecific antibody	1st line: metastatic CRC, NSCLC, recurrent GBM, metastatic RCC, metastatic ovarian cancer	16,236–238
Aflibercept	VEGF, VEGFB, PlGF	Chimeric sVEGFR1/2	2nd line: metastatic CRC	239
Ramucirumab	VEGFR2	Monospecific antibody	1st line: gastric cancer, GEJ adenocarcinoma, NSCLC, metastatic CRC, HCC	64,65, 240,241
TKIs[a]				
Apatinib (Rivoceranib)	VEGFR2, KIT, SRC, RET	Orally active small molecule	1st line: HCC	242
Axitinib (Inlyta)	VEGFR1,2,3, PDGFRs, KIT	Orally active small molecule	1st line: metastatic RCC	243
Cabozantinib (Cabometyx)	VEGFR1,2,3, TIE2, MET	Orally active small molecule	1st line: metastatic RCC, MTC 2nd line: HCC	243–245
Lenvatinib (Lenvima)	VEGFR1,2,3, PDGFRα, FGFRs	Orally active small molecule	1st line: HCC	246
Pazopanib (Votrient)	VEGFR1,2,3, PDGFRs, KIT	Orally active small molecule	1st line: metastatic RCC 2nd line: STS	247,248
Ragorafenib (Stivarga)	VEGFR1,2,3, PDGFRs, TIE2	Orally active small molecule	1st line: metastatic CRC, GIST 2nd line: HCC	249–251
Sorafenib (Nexavar)	VEGFR1,2,3, PDGFRs, RET	Orally active small molecule	1st line: metastatic RCC, HCC, thyroid cancer	252–254
Sunitinib (Sutent)	VEGFR1,2,3, PDGFRs, KIT	Orally active small molecule	1st line: metastatic RCC, PNT 2nd line: GIST	255–257
Vandetanib (Caprelsa)	VEGFR1,2,3, EGFR, RET	Orally active small molecule	1st line: MTC	258
Other AADs[b]				
Welireg (Belzutifan)	HIF2α inhibitor	Orally active small molecule	1st line: VHL disease-associated RCC	145

AAD, anti-angiogenic drug; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; GBM, glioblastoma multiforme; GEJ, gastro-oesophageal junction; GIST, gastrointestinal stromal tumour; HCC, hepatocellular carcinoma; HIF2α, hypoxia-inducible factor 2α; MET, mesenchymal–epithelial transition factor; MTC, medullary thyroid cancer; NSCLC, non-small-cell lung cancer; PDGFR, platelet-derived growth factor receptor; PlGF, placental growth factor; PNT, pancreatic neuroendocrine tumour; RCC, renal cell carcinoma; RET, proto-oncogene rearranged during transfection; STS, soft tissue sarcoma; sVEGFR, soluble VEGFR; TIE2, TIE receptor tyrosine kinase 2; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; VHL, von Hippel–Lindau protein. [a]Only a few examples of anti-angiogenic TKIs are listed. [b]Other non-specific inhibitors that target downstream signalling components are not listed.

approved for clinical use. However, a surgical procedure of embolization by blocking arterial perfusion in hepatocellular carcinoma (HCC) is probably the oldest inventive approach of anti-angiogenic cancer therapy[23].

Anti-VEGF-based drugs are widely used for treating neovascular eye disorders[24] (Fig. 2). Anti-VEGF monotherapy provides remarkable improvement of vision and quality of life in patients with neovascular AMD and diabetic macular oedema (DME) and represents the standard of care for these disorders[25–27]. However, frequent injections are required to sustain clinical benefits, so new technologies for longer-lasting treatment are needed.

The clinical benefits of AADs are determined by functional changes in specific cell types for various diseases. For example, anticancer effects are determined by suppression of tumour growth, whereas visual improvement in ophthalmic diseases is executed by specialized cells in the neural retina, and adipose metabolic effects are determined by adipocytes. Owing to the marked heterogeneities within the same disease and among various disorders, the clinical benefits of AADs for treating various diseases are, not surprisingly, different.

In this Review, we discuss the successes and challenges in using AADs in oncology and ophthalmology, possible solutions for improving clinical outcomes, potential next-generation anti-angiogenic agents and the possibility of expanding AADs beyond the treatment of cancer and eye diseases.

Angiogenesis signalling and regulation

VEGF is the prototype member of a growth factor family consisting of five structurally related molecules: VEGF, placental growth factor (PlGF), VEGFB, VEGFC and VEGFD[11,28,29]. Biological functions of these angiogenic factors are primarily mediated by two tyrosine kinase receptors (TKRs), vascular endothelial growth factor receptor 1 (VEGFR1) and VEGFR2, which are mainly expressed in endothelial cells, although the non-TKR family of neuropilins (NRPs) also transduce some VEGF functions[29]. Members of the VEGF family exhibit specific binding to VEGFRs: VEGF binds to VEGFR1 and VEGFR2; PlGF and VEGFB specifically interact with VEGFR1; and VEGFC and VEGFD are natural VEGFR3 ligands but, following proteolysis, can also activate VEGFR2 (ref. 29). VEGFR2 is the major mediator of the vascular functions of the VEGF family, whereas VEGFR1 seems to serve as a decoy receptor, at least in some circumstances, owing to its weak intrinsic signalling and tight VEGF binding properties, which prevent VEGF from binding to VEGFR2 (refs. 28,29). Also, an alternatively spliced VEGFR1 variant (sFLT), consisting of the first six immunoglobulin-like loops in the extracellular domain, can function as an endogenous VEGF inhibitor[30]. sFLT released by the ischaemic placenta has been implicated in the pathogenesis of pre-eclampsia, a condition characterized by hypertension, proteinuria and, frequently, fetal distress[31]. VEGFR3 is primarily expressed in lymphatic endothelial cells and transduces lymphangiogenic signals for VEGFC and VEGFD, although VEGFR3 is also transiently

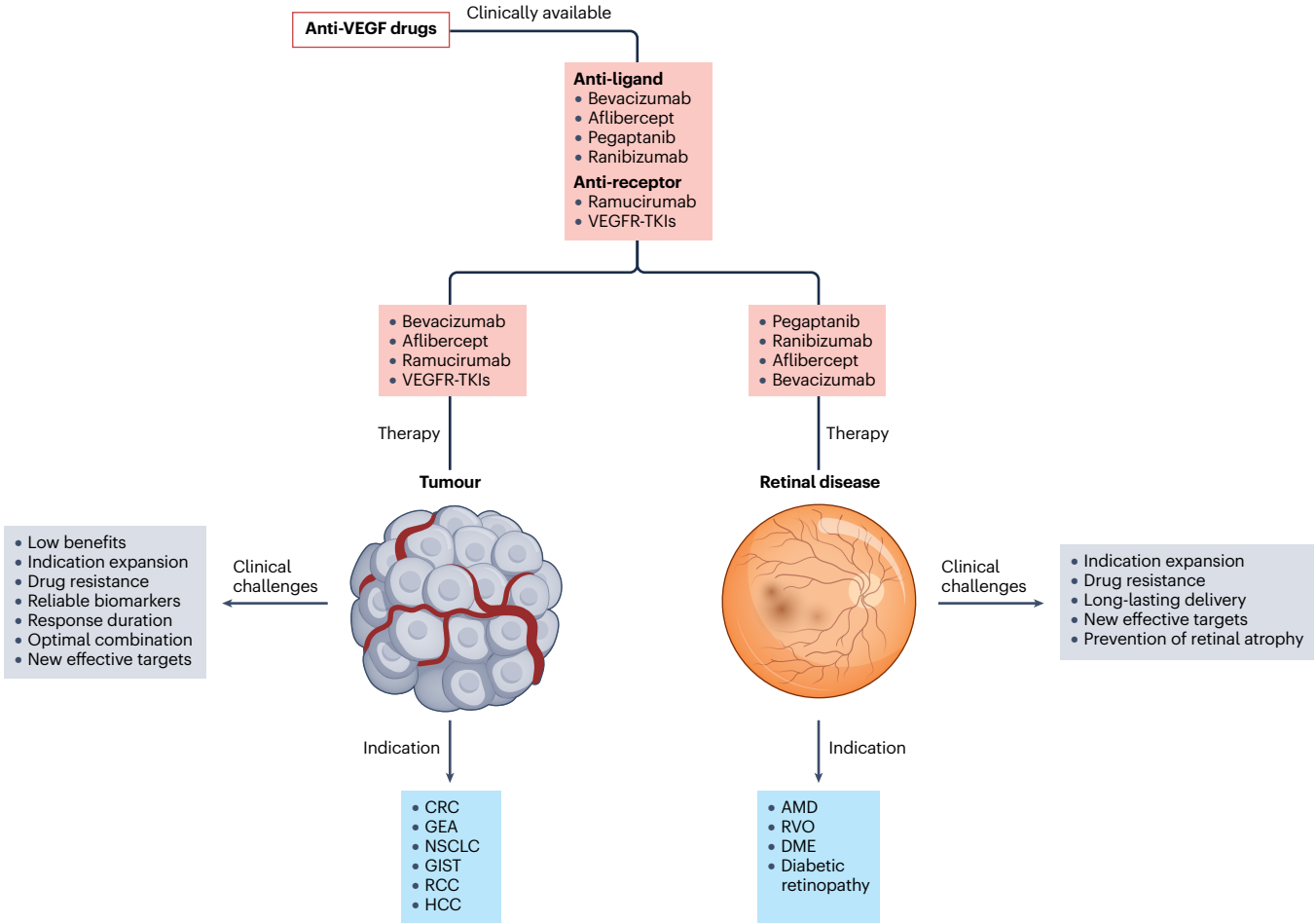

Fig. 2 | Clinically approved anti-VEGF drugs for treating cancer and ophthalmic diseases. Anti-vascular endothelial growth factor (VEGF) drugs can be divided into ligand or receptor inhibitors. Numerous clinically challenging issues remain for both oncology and ophthalmic indications. AMD, age-related macular degeneration; CRC, colorectal cancer; DME, diabetic macular oedema; GEA, gastroesophageal adenocarcinoma; GIST, gastrointestinal stromal tumour; HCC, hepatocellular carcinoma; NSCLC, non-small-cell lung carcinoma; RCC, renal cell carcinoma; RVO, retinal vein occlusion; TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor.

expressed in angiogenic endothelial cells during formation of sprouting tips[32,33].

VEGF can elicit multiple biological effects[11–14,34] (Fig. 3), including the stimulation of endothelial cell proliferation, migration, survival and remodelling; induction of vascular permeability; guidance of vascular sprouting; induction of inflammation; regulation of metabolism and endocrine functions; neurotrophic functions; stimulation of haematopoiesis; and regulation of immune functions. However, the significance of some of these effects is not always clear and the expression of VEGF receptors in vivo is largely restricted to vascular endothelial cells.

In addition to the VEGF–VEGFR2 pathway, members of the fibroblast growth factor (FGF) family, particularly FGF2, also have angiogenic properties under physiological and pathological settings[35]. FGF2 binds to its specific fibroblast growth factor receptors (FGFRs), which are TKRs expressed on endothelial cells, to execute angiogenic functions. Unlike VEGFRs, FGFRs including FGFR1, FGFR2, FGFR3 and FGFR4 are ubiquitously expressed in various cell types[35]. FGF2 potently promotes angiogenesis by stimulating endothelial cell proliferation, migration and tube formation[36]. Despite the fact that FGFRs are expressed on the cell surface, FGF2 lacks a classical signal peptide for secretion and its release into the extracellular space still remains an enigma[37]. Both *fgf1* and *fgf2* null mice have a mild phenotype and even the double knockouts are viable and do not exhibit defective angiogenesis[38]. Also, *fgf2* inactivation did not prevent retinal or choroidal angiogenesis in mouse models, casting doubt on the significance of FGF2 as a therapeutic target in these settings[39,40]. Therefore, in spite of the potent pro-angiogenic properties of these molecules, development of FGF pathway inhibitors to treat pathological angiogenesis has a limited rationale.

Angiopoietins (ANGs), including ANG1 and ANG2, interact with TIE receptor tyrosine kinase 2 (TIE2), an RTK which is mostly expressed in endothelial cells, and have overlapping yet distinctive functions from VEGF in regulating angiogenesis. Whereas ANG1 acts as a vasculoprotective factor by stabilizing vascular networks and preventing vascular permeability[41,42], ANG2 operates as a context-dependent agonist and antagonist of TIE2 (refs. 41,42). Endothelial cell-derived

ANG2 is associated with regression of co-opted tumour vasculatures, allowing neoangiogenesis by a hypoxia–VEGF-dependent mechanism[43]. ANG2 is highly expressed in tip endothelial cells at the growth cone of the leading edge of developing vessels[44]. Here, it overcomes the protective effect of ANG1 and is thought to stimulate angiogenesis through an integrin-mediated pathway[45]. ANG1 and ANG2 also exert opposing effects on pericyte coverage in the microvessels[43]. Whereas ANG1 promotes pericyte coverage, vessel stabilization and blood flow, ANG2 ablates pericytes from microvessels, allowing vascular sprouting and increased permeability (Fig. 3).

In addition to ANG–TIE2 signalling, delta-like ligand 4 (DLL4) and Jagged1 positively and negatively regulate angiogenesis by competing for binding to the NOTCH1 receptor[46]. DLL4 acts as a negative downstream regulator of VEGF-induced angiogenesis by preventing excessive sprouting[46]. Thus, NOTCH1 signalling participates in positive and negative regulation of angiogenesis through context-dependent mechanisms depending on the presence of other angiogenic signals[47]. Together with the NOTCH1 and VEGF signalling pathways, another ligand–receptor system, EphB4–ephrin B2, defines arterial–venous specification and segregation[48] (Fig. 3).

As well as vertically transducing angiogenic signals through their specific receptors, these angiogenic factors and receptors often produce synergistic effects via horizontal crosstalk. For example, combinations of VEGF plus FGF2, VEGF plus ANG2 or FGF2 plus platelet-derived growth factor B (PDGFB) synergistically promote angiogenesis when present in the same tissue environment[48–50]. An important aspect of angiogenic synergism is that although the expression level of each individual angiogenic factor might not be high, the overall angiogenic effects can be profound[51].

Altered angiogenic pathways in disease

In tumours, genetic alteration, epigenetic regulation, infiltration of stromal cells, metabolites and tissue hypoxia collectively contribute to high expression of VEGF[52,53]. Systematic analysis of various human tumour tissues shows that VEGF expression levels are almost always higher in solid tumours than in their corresponding surrounding healthy tissues[54].

In some tumours, such as clear cell renal cell carcinoma (RCC), VEGF expression is markedly upregulated owing to the functional inactivation of von Hippel–Lindau protein (VHL), a crucial substrate recognition component of an E3 ubiquitin ligase that directs hypoxia-inducible factor 1α (HIF1α) for degradation[54]. HIF1α is a transcription factor that targets the hypoxia response element (HRE) in the VEGF promoter to transcriptionally upregulate VEGF expression[55,56]. The high

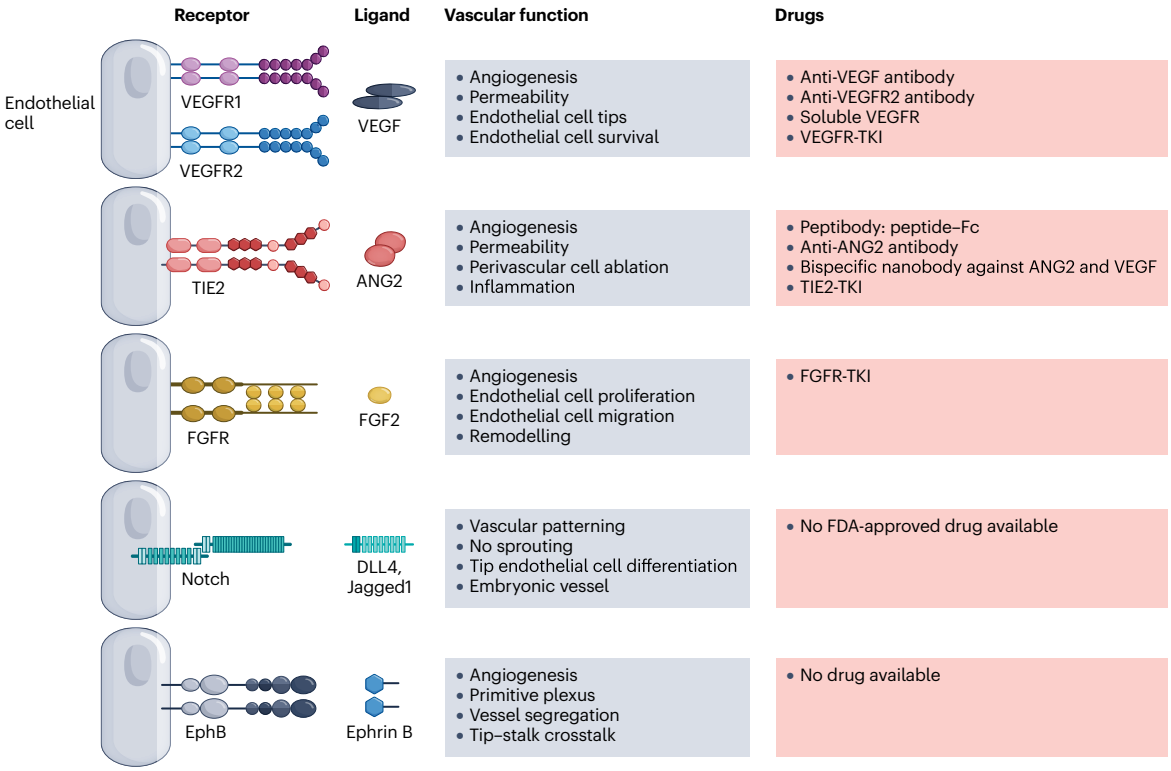

Fig. 3 | Angiogenic signalling molecules and their vascular functions. Vascular endothelial growth factor (VEGF) binds to vascular endothelial growth factor receptor 1 (VEGFR1) and VEGFR2 tyrosine kinase receptors (TKRs) that mediate various biological functions, including angiogenesis, vascular permeability, endothelial cell survival, endothelial cell tip formation, endothelial cell proliferation and migration, and vascular remodelling. Angiopoietin 2 (ANG2) binds to the TIE receptor tyrosine kinase 2 (TIE2) TKR to induce angiogenesis, vascular permeability, inflammation and perivascular cell disassociation. Fibroblast growth factor 2 (FGF2) stimulates angiogenesis and regulates vascular remodelling. Delta-like ligand 4 (DLL4)/Jagged1–Notch signalling regulates vascular development, sprouting, patterning and maturation. The ephrin B–EphB signalling pathway modulates crosstalk between endothelial cell tip and stalk, angiogenesis, vessel segregation and formation of the primitive plexus. Drugs targeting VEGF, ANG and FGF signalling pathways are listed. FDA, US Food and Drug Administration; FGFR, fibroblast growth factor receptor; TKI, tyrosine kinase inhibitor.

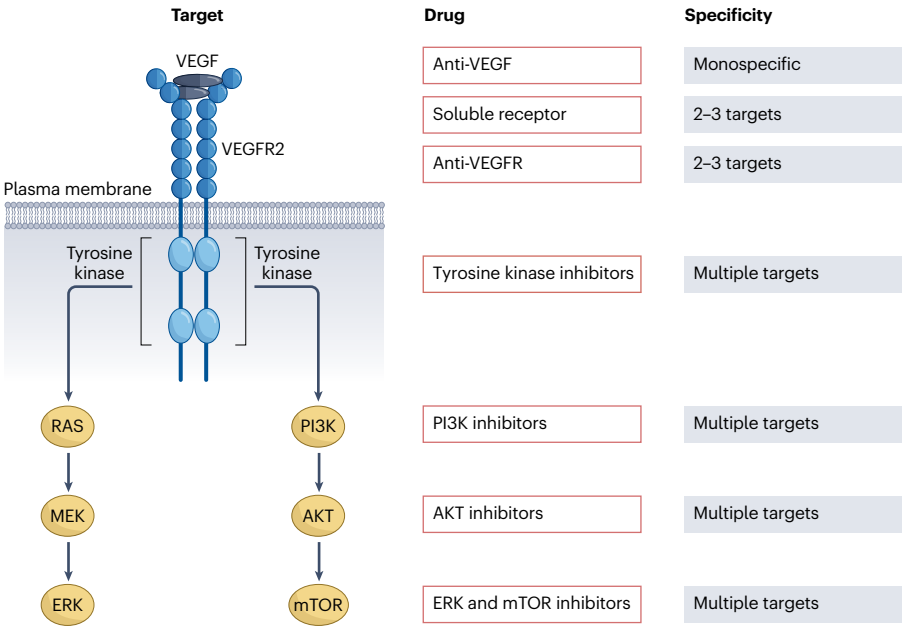

Fig. 4 | Anti-angiogenic drugs target different VEGF signalling events. Monospecific drugs such as bevacizumab specifically neutralize angiogenic factors. Soluble receptors such as aflibercept consisting of the extracellular domains but lacking kinase activities can neutralize two or three angiogenic ligands. Similarly, an anti-receptor neutralizing antibody such as ramucirumab can block angiogenic functions of two or three factors. Inhibitors targeting intracellular downstream signalling components such as tyrosine kinase, phosphoinositide 3-kinase (PI3K), AKT and mammalian target of rapamycin (mTOR) have multiple targets. ERK, extracellular-signal-regulated kinase; MEK, MAPK/ERK kinase; VEGF, vascular endothelial growth factor; VEGFR, VEGFR, vascular endothelial growth factor receptor.

levels of VEGF in the local tumour microenvironment resulting from increased HIF1α promote the formation of tortuous, primitive and leaky vascular networks that often lack overt distinction between arterioles and venules[57]. In mouse models, the diffusible VEGF molecules — mainly consisting of smaller non-heparin-binding isoforms generated by alternative splicing — can enter the circulation and have the potential to alter vascular homeostasis in remote healthy tissues by triggering angiogenesis and vascular leakage[58–60].

Notably, *VEGF* upregulation in human tumours can occur in the absence of HIF upregulation, and hypoxia does not always increase VEGF expression, suggesting a context dependence in the role of the hypoxic pathways[54]. Interestingly, earlier studies reported that deletion of the HRE in the mouse *Vegf* promoter did not result in embryonic lethality[61]. This indicated that HIF regulation is not required for VEGF-dependent embryonic vasculogenesis and angiogenesis. However, HRE deletion led to death of approximately half of the mice during the neonatal/perinatal period, possibly reflecting a role of HIF-regulated VEGF in the adaptation to a new environment with different oxygen levels and hypoxic stress[61]. It is also noteworthy that HIF-independent hypoxic pathways regulating VEGF expression have been described. The transcriptional co-activator PGC1α upregulates VEGF in response to hypoxia in the heart and in the skeletal muscle[62]. However, the role of this pathway in regulating VEGF expression in tumours and other pathologic conditions is less clear. Overall, multiple signals including oncogenes, hormones, growth factors and pro-inflammatory cytokines can regulate VEGF expression independent from hypoxic pathways (reviewed elsewhere[34]).

Similarly to tumours, high levels of VEGF are present in the retina in various eye diseases such as AMD, DME, retinopathy of prematurity, diabetic retinopathy and retinal vein occlusion (RVO) and serve as the driving force for retinal angiogenesis[24]. The predominant role of VEGF in retinal neovascularization defines a crucial therapeutic target for treating the most common eye diseases that cause blindness[24]. In addition to VEGF-based therapeutic targets, several other pro-angiogenic factors, including PlGF, VEGFC, VEGFD, PDGF, erythropoietin, ANG2 and stromal-derived factor 1 (SDF1), might also augment retinal angiogenesis[63] at least in some animal models.

Anti-angiogenic cancer therapy

Clinically available AADs can be classified as either biological agents (biologics) or small molecules[11,19,20,24,53] (Table 1). Biologics, including neutralizing antibodies and extracellular domains of receptors, block specific angiogenic factors or their receptors. As the clinically available drugs mainly block the VEGF–VEGFR2 axis, we will use this signalling pathway as an example for defining therapeutic targets and drug development. Bevacizumab is a monoclonal antibody that specifically inhibits VEGF[16] (Fig. 4). Ramucirumab (Cyramza) is a neutralizing antibody that blocks VEGFR2, a receptor mainly expressed in vascular endothelial cells[64,65]. Aflibercept (ziv-aflibercept, VEGF-Trap, Eylea, Zaltrap) is a genetically engineered soluble receptor consisting of the immunoglobulin-like domain 2 of VEGFR1 and the immunoglobulin-like domain 3 of VEGFR2, fused to Fc IgG[66–68]. Whereas bevacizumab only neutralizes VEGF, ramucirumab neutralizes three angiogenic factors because VEGFR2 binds to VEGF, VEGFC and VEGFD[69–72]. Similarly, aflibercept neutralizes the VEGFR1 ligands VEGF, PlGF and VEGFB[67]. Nevertheless, as noted in other sections of this Review, the benefit of neutralizing these additional factors often remains unclear. In addition to VEGF neutralizing agents, other anti-angiogenic biologics such as the ANG1/2 neutralizing peptibody trebananib are under development[44].

Unlike biologics, anti-angiogenic tyrosine kinase inhibitors (TKIs) are small molecules that, in addition to the intended target, inhibit a broad spectrum of tyrosine and serine-threonine kinases[73]. Almost all anti-angiogenic TKIs inhibit VEGFR signalling (Table 1). Owing to their wide-ranging targets, TKIs often exhibit serious toxicity profiles, which restrain long-term clinical use and usually prevent combinations with cytotoxic agents[11,19]. Additionally, treatment of tumour-bearing mice with AADs has been reported to promote metastasis[74,75]. However, these findings in mouse models are controversial[76–78], and increases

in metastasis have not been observed in patients with cancer treated with anti-angiogenic agents[79,80].

Key clinical challenges

Despite the clinical successes of AADs for the treatment of various human cancers, the overall survival benefits to patients with cancer are incremental. However, improving overall survival remains a major challenge for all classes of drugs and therapeutic strategies, including immunotherapy, hence the need to identify novel combinations. Among multiple challenges, overcoming drug resistance, defining reliable biomarkers for responders and development of more effective drugs are needed (Box 1).

Although the notion of angiogenesis dependence of tumour growth remains a guiding concept, the fact that not all patients with cancer benefit from AAD treatment raises questions regarding mechanisms underlying clinical benefits. It is plausible that some cancers, owing to metabolic adaptions, are less dependent on angiogenesis compared with others. Also, other possible mechanisms have been invoked to explain the limited clinical benefits, including tumour vessel normalization by AADs[81], compensatory angiogenesis by non-VEGF factors[82], co-option of pre-existing vasculatures surrounding tumour tissues[83], alternative mechanisms for tumour neovascularization by intussusception and vasculogenesis[84,85], and insufficient penetration of AADs into tumour tissues[86]. Although these hypothetical mechanisms are attractive and partly supported by preclinical evidence, none of them has been clinically validated.

Drug resistance. Preclinical studies show that blocking VEGF–VEGFR signalling often triggers overproduction of other pro-angiogenic factors such as FGFs, ANGs, hepatocyte growth factor (HGF), integrin and PDGFs via a possible mechanism of hypoxia[87]. These additional pro-angiogenic factors are not targets of anti-VEGF-based AADs and thus could provide mechanisms of drug resistance. Further, infiltration of inflammatory cells, including myeloid-derived suppressor cells (MDSCs; also known as $Gr1^+$ $CD11b^+$ myeloid cells), neutrophils, macrophages and tumour-infiltrating T helper 17 cells, significantly contributes to AAD resistance by producing various cytokines, including granulocyte colony-stimulating factor (G-CSF), interleukins, chemokines, bombina variegate peptide 8 (Bv8) and transforming growth factor-β (TGFβ)[88–93]. Other studies demonstrate that alteration of metabolic pathways in cancer cells and adipocytes confers AAD resistance[94,95]. AAD-induced hypoxia triggers lipolysis in cancer-associated adipocytes, which provides free fatty acids for lipid metabolism and evades glycolysis-dependent metabolism to enable cancer cell proliferation[94]. Given the complex mechanisms underlying AAD resistance, combination therapies that simultaneous target non-VEGF signalling pathways and even metabolic pathways could, in principle, improve therapeutic outcomes.

Timing of administration. Optimization of therapeutic scheduling is potentially key for improving clinical benefits. In principle, a long-lasting therapeutic regimen is needed to maximize therapeutic benefits, because withdrawal of AADs allows tumour vessels to regrow[96–98]. Studies in animal tumour models demonstrated that AAD withdrawal engenders revascularization in tumours, which occurs within a few days after cessation of anti-angiogenic therapy[96–98]. In particular, removal of anti-angiogenic TKIs triggers an almost immediate angiogenic response, most likely due to their relatively short half-life in the body[96,97]. It should be noted, however, that clinical trials do not generally support the concept of 'rebound', that is, acceleration of tumour growth after anti-angiogenic therapy. A retrospective analysis of five placebo-controlled clinical trials with bevacizumab did not document a decreased time to disease progression, increased mortality or altered disease progression pattern after cessation of bevacizumab therapy[79]. However, discontinuation of long half-life anti-VEGF antibodies in tumour-bearing mice results in tumour revascularization within a week[97,98]. In addition to tumours, revascularization triggered by drug cessation also occurs in several healthy tissues such as endocrine organs and the liver, which under physiological conditions are dependent on VEGF to maintain vascular homeostasis, survival, fenestration and sinusoidal architectures[60,98,99]. In preclinical models, liver revascularization after AAD withdrawal promotes metastatic spreading by a mechanism of extravasation[98]. Indeed, clinical studies show increased benefits after long-term bevacizumab treatment in solid tumours[100]. For long-term maintenance therapy, oral administration of a low and non-toxic dose of capecitabine that suppresses tumour angiogenesis prevents further growth of solid tumours[97], although these findings warrant clinical validation. The goals of future efforts are the development of more effective AADs, with fewer side effects, longer half-life and more convenient routes of administration.

Biomarkers. Identifying reliable biomarkers for the selection of AAD responders, and surrogate markers to monitor therapeutic effects, remains an unmet clinical need[19]. In principle, VEGF itself as a specific target for drugs such as bevacizumab should fulfil the criteria to serve as a reliable biomarker. Unfortunately, although plasma VEGF levels have prognostic value in multiple tumour types, they do not have predictive value for clinical benefit, at least for bevacizumab[17,101].

Box 1

Clinical challenges of anti-angiogenic cancer therapy

- Incremental survival benefits: the lack of validated biomarkers does not allow selection of patients who are most likely to be responsive to treatment
- Drug resistance: intrinsic and acquired resistance seen in patients with various cancers; multiple and complex mechanisms likely contribute
- Timing of therapy: interrupted versus non-stop therapy; short-term versus long-lasting therapy; development of drug-release polymers for long-term therapy
- Delivery of anti-angiogenic drugs (AADs) to the tumour local environment versus systemic therapy
- Impacts of systemic AADs on healthy tissues and organs
- Impacts of systemic AADs on metastasis and systemic cancer
- Identification of reliable surrogate markers to monitor therapeutic benefits and predictive biomarkers for patient selection
- Optimizing combination therapy with other conventional, targeted therapeutics and immunotherapy to improve clinical benefits
- Minimizing adverse effects

It has been hypothesized that the relationship between VEGF and its related VEGF family members could determine antitumour effects of AADs. For example, PlGF and VEGF can form heterodimers and high expression of PlGF remodels tumour vessels towards a 'normalized' phenotype, which constitutes large-diameter and branchless tumour vessels[102,103]. Surprisingly, in some animal models the PlGF-normalized tumour vessels are highly sensitive to anti-VEGF drugs, raising the possibility of PlGF as a potential biomarker[104]. Additionally, VEGFR1/2 can be synthesized or proteolytically processed as ligand-binding extracellular domains, named natural soluble VEGFRs (sVEGFRs), that neutralize VEGF ligands[105,106]. The levels of sVEGFRs also affect AAD responses[107]. For example, plasma sVEGFR1 levels in patients with CRC are inversely correlated with survival advantages in response to bevacizumab[108]. Recent studies suggest that biomarkers for clinical benefit to bevacizumab might be tumour type-dependent. For example, in non-small-cell lung carcinoma (NSCLC) the pretreatment plasma concentrations of VEGF had a prognostic value[94]. Whereas in ovarian cancer, tumour microvessel density was a potential predictive biomarker for bevacizumab benefit[109]. Most recently, *TP53* sequencing and p53 immunohistochemistry predicted outcomes of bevacizumab treatment in an endometrial cancer trial, with p53 overexpression having a particularly strong association with bevacizumab benefit[110].

Other phase III clinical studies suggest that plasma levels of several growth factors and cytokines, either alone or in combination, serve as predictive biomarkers for AAD benefits or lack thereof. For example, HGF and IL-6 are potential predictive biomarkers for selecting patients with metastatic RCC who are likely to benefit from AADs[111]. Similarly, IL-6 was a positive predictive marker for bevacizumab in patients with epithelial ovarian cancer[112]. Conversely, high plasma levels of PlGF and VEGFD might predict the lack of benefit in progression-free survival in patients with metastatic CRC who receive bevacizumab plus chemotherapy[113]. Another study shows that levels of osteopontin (OPN), TIMP1, thrombospondin 2 (TSP2), HGF and VCAM1 correlate with poor overall survival in patients with non-clear cell RCC[114]. In contrast, SDF1 was associated with improved survival. However, all of these encouraging findings remain to be prospectively validated.

Impact on non-tumour targets. AADs are systemically administered to patients with cancer and so all tissues and organs are exposed to drugs. Given the small volume of a tumour mass relative to other heathy tissues and organs, most drug molecules will be distributed to non-tumour tissues[58]. It is unclear whether non-tumour targets of AAD are beneficial or harmful for patients with cancer. In mice, the vasculature in endocrine organs, the liver, bone marrow and the gastrointestinal wall is dependent on VEGF to maintain fenestrations, sinusoidal architecture and homeostasis[11]. Systemic administration of anti-VEGF-based AADs leads to vascular regression and alters vascular structures in these tissues and organs[60]. In addition, systemic administration of TKIs in non-tumour-bearing mice results in regression of microvessels in the thyroid by nearly 80%, leading to defective production of thyroid hormones[60,99,115]. In fact, hypothyroidism is one of the common adverse effects seen in patients with TKI-treated cancer[116]. Other AAD-associated common adverse effects include hypertension, proteinuria, haemorrhages and gastrointestinal perforation. However, these effects are considerably less pronounced using more selective agents such as antibodies[117].

Microvessels can also be highly sensitive to VEGF stimulation. In mice, high circulating levels of tumour-derived VEGF cause a paraneoplastic syndrome by dilating and destroying vessels and manifesting in defective haematopoiesis, hepatomegaly and endocrine dysfunction[59,118]. Additionally, autopsy analysis revealed that approximately 20% of patients with RCC had hepatomegaly due to vascular dilation, most likely due to high circulating VEGF caused by VHL loss or dysfunctional mutations[119]. Indeed, blocking tumour-derived VEGF by AADs results in survival benefits in patients with RCC[58].

Combining AADs with immunotherapy

Improving the clinical benefits of AADs by combining them with other anticancer modalities such as immunotherapy is achieving success in the clinic, although challenges remain. Cancer immunotherapy aims to stimulate the immune system to attack cancer cells, for example by inhibiting immune checkpoint molecules such as programmed cell death 1 (PD1), programmed cell death 1 ligand 1 (PDL1) and cytotoxic T lymphocyte antigen 4 (CTLA4)[120]. Antibodies against these molecules are used to treat a wide range of cancer types[121,122]. Another approach is adoptive cell therapy, also known as cellular immunotherapy. This involves isolation of cancer-recognizing immune cells from patients with cancer and expanding them in vitro, or genetically engineering T cells to express chimeric antigen receptors (CAR T cells). These immune cells are then transferred to patients with cancer[123].

Several anticancer immunotherapies have been investigated as combination regimens with anti-angiogenic therapy in preclinical studies[18]. Additionally, the therapeutic effects of immune checkpoint inhibitors (ICIs) in combination with AADs have been evaluated in clinical trials and produced encouraging results[124].

Mechanisms of immunosuppression by angiogenic factors. The infiltration of immune cells, especially T cells, is the key determinant for clinical responses to immunotherapy. Immune-inflamed tumours, also known as 'hot tumours', are characterized by elevated infiltration of T cells, accumulation of pro-inflammatory cytokines, increased expression of PDL1, high tumour mutational burden and better responses to ICIs[125] (Fig. 5). In contrast, immune-excluded and immune-desert tumours that lack tumour-infiltrating lymphocytes (TILs) and have impaired T cell priming are intrinsically resistant to immunotherapy. The infiltration of TILs into the tumour microenvironment is dependent on several factors: a tumour vasculature that is adequately perfused; chemoattractive signals such as chemokines to recruit immune cells; and the trafficking of immune cells to tumours which involves intravasation, adhesion to vascular endothelial cells and extravasation across the vessel wall. Poorly perfused, disorganized and leaky tumour vessels impair TIL infiltration in tumours[18]. Thus, the tumour vasculature has crucial roles in controlling immune cell infiltration in tumours.

Considerable evidence supports a role for VEGF in eliciting immunosuppressive effects and various mechanisms have been proposed[18] (Fig. 5). These mechanisms include the induction of Fas ligand (FasL) by VEGF in tumour endothelial cells, leading to the loss of $CD8^+$ T cells by apoptosis; the recruitment of $VEGFR2^+$ regulatory T (T_{reg}) cells, which have strong immunosuppressive effects in the tumour microenvironment; VEGF serving as a chemoattractant for MDSCs; and the functional impairment of dendritic cells by VEGF.

ANG2 is also proposed to induce immunosuppression in tumours via multiple mechanisms. For example, by recruiting T_{reg} cells and M2-like macrophages, or by suppressing monocyte-mediated antitumour activity[126]. FGF2 also can suppress cytotoxic T lymphocyte recruitment in tumours by polarizing macrophages towards a M2 phenotype[127]. Thus, multiple pro-angiogenic factors might potentially exert immunosuppressive functions by targeting a range of immune cell types in the tumour microenvironment.

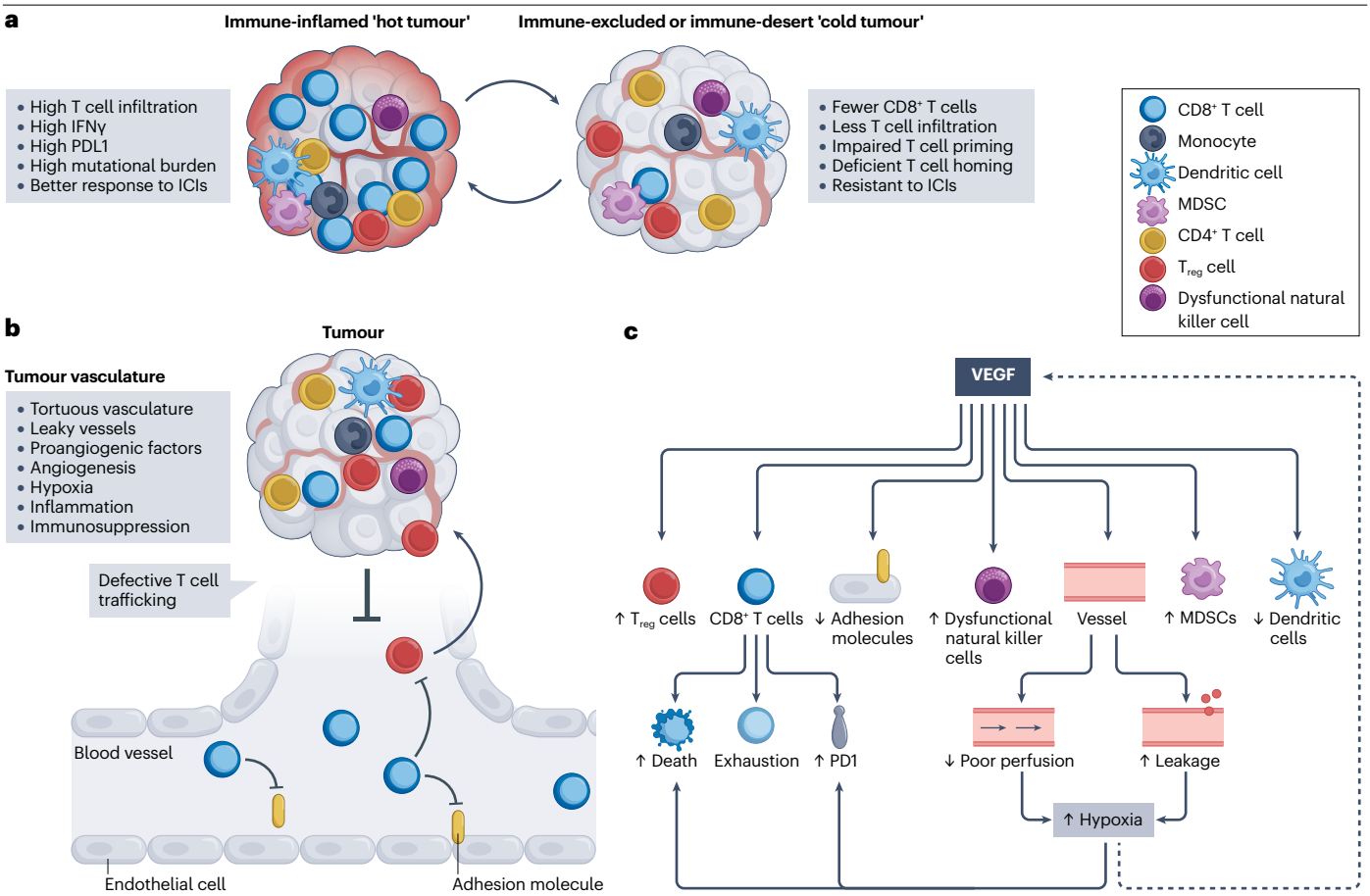

Fig. 5 | Immunosuppressive functions of the tumour vasculature and VEGF. **a**, Immune-inflamed 'hot tumours' contain high numbers of T cells and express high levels of interferon-γ (IFNγ) and inflammatory cytokines. Malignant cells in 'hot tumours' carry high mutational burden and express high levels of programmed cell death 1 ligand 1 (PDL1). These inflamed tumours usually respond better to immune checkpoint inhibitors (ICIs). By contrast, immune-excluded or immune-desert 'cold tumours' are often intrinsically resistant to ICIs as they lack effective T cells for killing tumour cells. **b**, Dysfunction of the tumour vasculature, including poor blood perfusion and lack of appropriate adhesion molecules in endothelial cells, confers ICI resistance due to defective trafficking of T cells to tumour tissues. **c**, Immunosuppressive functions of vascular endothelial growth factor (VEGF) on various immune cell types. VEGF directly suppresses $CD8^+$ T cell functions and upregulates regulatory T (T_{reg}) cells and myeloid-derived suppressor cells (MDSCs). Vascular leakiness and poor perfusion significantly contribute to tumour hypoxia. Hypoxia limits recruitment of cytolytic T cells to tumours and increases $CD8^+$ T cell death. PD1, programmed cell death 1.

Preclinical cancer models. Numerous preclinical studies have shown that combining AADs with ICIs leads to enhanced antitumour effects in various tumour types[18]. Most data were obtained by combining a mouse anti-VEGFR2 neutralizing antibody (VEGFR2 blockade) or anti-angiogenic TKIs with anti-PD1/anti-PDL1 antibodies (PD1/PDL1 blockades)[18,124]. In a mouse HCC model, a combination of VEGFR2 blockade with PD1 blockade had a synergistic anticancer effect by increasing tumour infiltration of $CD8^+$ T cells and endothelial cell interferon-γ (IFNγ)-mediated upregulation of PDL1 expression. The overall survival of the HCC-bearing mice was also improved[124]. The same combination regimen also led to enhanced anticancer activity and improved survival in a mouse CRC model by suppressing angiogenesis and increasing TILs[128]. Additionally, an anti-VEGF and anti-PD1 combination led to tumour suppression and increased survival in lung cancer models through mechanisms of rescuing exhausted cytotoxic T lymphocytes and inhibiting angiogenesis[129]. In other cancer types, including pancreatic cancer, breast cancer, RCC and glioblastoma multiforma (GBM), AADs plus PD1/PDL1 blockade also have synergistic anticancer activity through similar mechanisms[18,124]. Together, these preclinical data demonstrate that combinations of AADs and immunotherapeutic agents lead to enhanced anticancer effects.

The tumour vasculature is an emerging target for CAR T cell therapy, and vascular endothelial cells are accessible to circulating CAR T cells. Numerous endothelial cell surface molecules, including VEGFR1, VEGFR2 and integrins, have been engineered as CARs for cancer therapy[130]. However, only limited anticancer effects have been observed in animal tumour models, which might involve competitive binding between anti-VEGFR2 CAR T cells and VEGF to VEGFR2, which is a functional receptor for tumour angiogenesis[131].

Clinical studies. On the basis of positive results from phase III studies, combinations of AADs with ICIs have recently received FDA approval for the treatment of various cancers, including RCC, HCC, NSCLC and endometrial carcinoma[18,132]. Owing to extensive discussion of these

clinical studies elsewhere[18,132], we choose a few cancer types as examples (Table 2) to provide mechanistic insights into the effectiveness of these combinations. The most extensive clinical data of AAD plus ICI combination therapy were generated in patients with RCC (Table 2). In general, safety profiles of these combination regimens are tolerable and clinically manageable, with the exception of more severe toxicities associated with the VEGFR-TKIs[132]. Based on the phase III studies, axitinib plus pembrolizumab (Keytruda) or avelumab (Bavencio) has become standard care in the front-line management of RCC[133] and has improved overall response rates by 55% in patients with advanced RCC (Table 2).

Studies aiming to demonstrate the efficacy of AADs plus ICIs in metastatic CRC have been inconclusive so far. Phase III studies are ongoing to assess the clinical benefits of bevacizumab and FOLFOX (a combination of chemotherapy drugs consisting of folinic acid, fluorouracil and oxaliplatin), with or without atezolizumab (Tecentriq), and bevacizumab plus nivolumab (Opdivo) in metastatic CRC[132]. However, a phase II study of bevacizumab plus nivolumab for treating metastatic CRC in maintenance settings has not produced positive data[134]. The bevacizumab plus atezolizumab regimen is most extensively studied in patients with advanced HCC[135]. This combination has produced promising clinical benefits, with overall response rates from 11 to 50% and a manageable safety profile. Several phase III studies are ongoing to assess combinations of VEGFR-TKIs with ICIs, and clinical studies in endometrial, ovarian and cervical cancer support benefits of anti-VEGF plus ICIs[136]. In summary, the notion that combining AADs with ICIs improves clinical benefits in cancer treatment has been extensively validated.

Clinical development of non-anti-VEGF AADs

Non-VEGF factors might provide compensatory mechanisms to circumvent the effects of anti-VEGF AADs by enabling a switch to VEGF-independent angiogenesis. However, as discussed in the next sections, there is unfortunately no conclusive evidence that targeting non-VEGF factors together with VEGF provides any additional benefit[52].

ANG2 inhibitors. ANG2 is upregulated in various cancer types and promotes the formation of primitive and leaky vascular beds, which are associated with cancer invasion and poor survival[44]. Importantly, patients with CRC responding poorly to bevacizumab had high levels of ANG2, suggesting its involvement in AAD resistance[137]. Several ANG2 inhibitors have been developed or are under development[138]. In particular, the approach of simultaneous targeting ANG2 and VEGF has drawn considerable attention. However, there is no clear evidence that such dual targeting provides clinical advantages. For example, a phase II study in patients with metastatic CRC receiving vanecizumab (a bispecific monoclonal antibody that targets both VEGF and ANG2) plus FOLFOX failed to show an improvement in progression-free survival compared with patients treated with bevacizumab plus FOLFOX, arguing that, at least in this setting, ANG2 is not a relevant therapeutic

Table 2 | Examples of randomized phase II/III trials of anti-VEGF combined with ICI drugs

Drug	Target	Clinical trial (phase)	Outcome[a]			Ref.
			Overall survival	Progression-free survival	Overall response rate	
Colorectal cancer (CRC)						
Bev+Ate	VEGF, PDL1	NCT02873195 (II)	10.3 vs 10.2	4.4 vs 3.3	9.0% vs 4%	259
Bev+Niv	VEGF, PD1	NCT04072198 (II)	ND	ND	ND	134
Non-small-cell lung carcinoma (NSCLC)						
Bev+Ate	VEGF, PDL1	NCT02366143 (III)	19.2 vs 14.7	8.3 vs 6.8	64.0% vs 48.0%	260
Renal cell carcinoma (RCC)						
Bev+Ate	VEGF, PDL1	NCT02420821 (III)	34.0 vs 32.7	11.2 vs 7.7	3.0% vs 35.0%	261
Bev+Ate	VEGF, PDL1	NCT01984242 (II)	ND	11.7 vs 8.4	46.0% vs 27.0%	262
Axi+Ave	VEGFRs, PDL1	NCT02684006 (III)	11.6 vs 10.7	13.8 vs 8.4	55.2% vs. 25.5%	11
Tiv+Niv	VEGFRs, PD1	NCT03136627 (I/II)	ND	18.9%	56.0%	263
Axi+Pem	VEGFRs, PD1	NCT02853331 (III)	ND	15.1 vs 11.1	59.3.% vs. 35.7%	264
Len+Pem	VEGFRs, PD1	NCT02811861 (III)	33.6 vs 24.0	23.9 vs. 9.2	ND	265
Hepatocellular carcinoma (HCC)						
Bev+Ate	VEGF, PDL1	NCT03434379 (III)	67.2% vs 54.6% (1 year)	6.8 vs 4.3	27.3% vs 11.9%	135
Len+Pem	VEGFRs, PD1	NCT03418922 (II)	ND	ND	76.7% vs 66.7	266
Cab+Niv+Ipi	VEGFRs, PD1, CTLA4	NCT01658878 (III)	ND	6.8 vs. 5.5	26% vs 17%	266
Endometrial cancer						
Len+Pem	VEGFRs, PD1	NCT02501096 (II)	17.4 vs 12.0	6.6 vs. 3.8	31.9% vs 14.7.%	136
Len+Pem	VEGFRs, PD1	NCT03517449 (III)	18.3 vs. 11.4	6.6 vs. 3.8	ND	136

Ate, atezolizumab; Ave, avelumab; Axi, axitinib; Bev, bevacizumab; Cab, cabozantinib; CTLA4, cytotoxic T lymphocyte antigen 4; ICI, immune checkpoint inhibitor; Ipi, ipilimumab (Yervoy); Len, lenvatinib; ND, not determined; Niv, nivolumab; Pem, pembrolizumab; Tiv, tivozanib (Fotivda); VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.
[a]For outcome, numbers in the overall survival column represent overall survival of months in the investigational groups versus the placebo groups; numbers in the progression-free survival column represent progression-free survival of months in the investigational groups versus the placebo groups; and numbers in the overall response rate column represent overall response rates of percentages in the investigational groups versus the placebo groups.

target[139]. Further studies are required to fully assess the benefits of such strategies. An alternative approach consists of directly activating TIE2 by using, for example, agonistic antibodies. This approach theoretically bypasses the challenges of the different ANG1 to ANG2 ratios in various contexts, which might limit the effectiveness of ANG2 inhibitors[138].

HIF inhibitors. As noted, hypoxia is one of the hallmarks of solid tumours and HIF often becomes activated[140]. HIF is a heterodimeric transcription factor consisting of HIF1α and HIF1β subunits[141]. HIF1α is induced by hypoxia, and HIF1β is constitutively expressed independent of oxygen levels. In addition to hypoxia, functional inactivation of VHL by genetic mutations — such as in a subset of RCCs and in VHL syndrome (a genetic disorder characterized by abnormal vascular proliferation and tumours in various organs) — also markedly increases HIF expression through stabilization[140]. HIF also has multiple roles in promoting tumorigenesis and drug resistance[140,141], Given these diverse functions, therapeutic targeting of HIF became an attractive approach for cancer therapy and numerous molecules were reported to suppress tumour angiogenesis and growth through HIF inhibition[140]. However, the agents initially tested lacked specificity, and thus it is difficult to determine whether any of the effects reported were truly due to HIF inhibition. Notably, blocking the mammalian target of rapamycin (mTOR) can lead to suppression of the HIF1α–VEGF-mediated angiogenic pathway[142], suggesting enhanced clinical benefits by combining mTOR inhibitors and anti-VEGF therapy. Indeed, the mTOR inhibitor everolimus improved progression-free survival in patients with metastatic RCC who had disease progression when treated with the first-line VEGFR-targeted TKI lenvatinib[143].

More recently, impressive clinical results were reported in patients with VHL-mutant RCC using belzutifan, a highly specific small-molecule inhibitor of HIF2α (ref. 144). Oncogenic activation of the HIF pathway in VHL-mutant RCC results in enhanced tumour growth and angiogenesis, which is blocked by belzutifan. The drug was approved by the FDA in 2021 for treatment of patients with mutant VHL[145]. Additional HIF inhibitors are being developed.

Notch inhibitors. The NOTCH1 receptor and its ligand DLL4 are important regulators of angiogenesis. High expression of DLL4 in tumours occurs in vascular endothelial cells and is associated with reduced survival[146,147]. DLL4–NOTCH1 and VEGF–VEGFR2 signalling pathways reciprocally regulate each other in the formation of microvascular networks in tumours[46]. Paradoxically, blocking DLL4 inhibits tumour growth by increasing, but not decreasing, vascular density through the formation of vessels that lack blood perfusion and are non-functional[148]. Numerous DLL4–NOTCH1 inhibitors have been tested in preclinical models, including anti-DLL4 antibodies, anti-NOTCH1 antibodies, soluble DLL4-Fc, NOTCH1-Fc decoy, γ-secretase inhibitors and DNA vaccines[149]. However, despite potent antitumour activity, DLL4 inhibitors exhibit broad toxicities in the liver, heart, lung and skin[150]. A humanized anti-DLL4 antibody, demcizumab, was tested in a phase I cancer study[151], but phase II studies in pancreatic and lung cancer were discontinued due to toxicity and lack of efficacy.

Targeting PlGF. PlGF binds to VEGFR1 but not VEGFR2, and is often upregulated in tumours[28]. However, its role in promoting tumour growth, angiogenesis and metastasis remains unclear[28]. Some preclinical studies showed that PlGF shares redundant functions with VEGF, and inhibition of PlGF with an anti-PlGF antibody inhibited primary tumour growth and metastasis[152]. Other studies yielded conflicting findings, showing, for example, that PlGF blockade in multiple cancer models lacked anti-angiogenic effects[153]. Furthermore, clinical trials with aflibercept, which neutralizes VEGF, PlGF and VEGFB, provide additional evidence for the limited role of PlGF as a cancer target. Contrary to some expectations, the impact of aflibercept in cancer therapy has been considerably less pronounced than that of bevacizumab; it gained FDA approval only for treatment of second-line CRC, despite clinical studies in multiple tumours[154].

Clinical studies with a humanized anti-PlGF monoclonal antibody (TB-403) in combination with bevacizumab in solid tumours were discontinued. Clinical trials with TB-403 were additionally performed in ophthalmic disorders such as neovascular AMD and DME, in combination with ranibizumab (Lucentis)[155], but it appears that these studies were also discontinued.

Interestingly, it has been shown that, at least in some circumstances, PlGF expression reduces rather than promotes tumour growth due to the formation of PlGF–VEGF heterodimers, which are less effective at stimulating angiogenesis than VEGF[103,156]. High expression of PlGF in in mouse models correlates with 'normalized' vessels, which are reported to be highly sensitive to anti-VEGF therapy[102].

Anti-angiogenic therapy in ophthalmology

Excessive neovascularization and inappropriate vascular remodelling are common causes of visual loss in several intraocular disorders, including AMD, diabetic retinopathy and RVO[24]. AMD is a progressive chronic disease and a worldwide leading cause of visual loss[157]. Neovascular AMD, accounting for about 10% of total AMD cases and responsible for 80–90% of AMD-associated legal blindness, is characterized by robust choroidal neovascularization (CNV) that often penetrates through Bruch's membrane into the subretinal space, resulting in exudation, retinal oedema, haemorrhage, pigment epithelial detachment and fibrous scarring[158]. These CNV-associated pathological changes cause serious and often irreversible visual impairment. Additionally, ophthalmic inflammation is the next most frequently implicated pathological process accompanying CNV[159].

Preclinical and clinical evidence demonstrates that VEGF is the key angiogenic driver of CNV[24]. Hypoxia and inflammation are the main triggers for switching on VEGF expression in neovascular AMD[160]. In animal models and human patients, VEGF levels positively correlate with retinal ischaemia[24]. Intravitreal administration of VEGF into non-human primate eyes stimulated retinal neovascularization similarly to human ophthalmic diseases[161]. On the basis of these findings, various angiogenic factors became attractive targets for treating neovascular AMD, DME and RVO[24] (Fig. 6). Nearly all anti-VEGF biologics developed for oncological indications have been tested in ophthalmic diseases (Table 3). Additionally, emerging new therapeutics that target non-VEGF signalling pathways are under preclinical and clinical investigation (Table 3).

Anti-VEGF drugs in clinical use

Pegaptanib (Macugen), an anti-VEGF aptamer, was the first milestone for treatment of neovascular AMD with AADs, and received FDA approval in the same year that bevacizumab was approved for oncology use[162] (Fig. 1). However, pegaptanib was followed by more effective inhibitors such as ranibizumab and aflibercept, and today pegaptanib is rarely used. Ranibizumab is an affinity-matured Fab derived from bevacizumab[163]. Mechanistic challenges that were overcome during the development of ranibizumab included[24] increasing the ability to penetrate the retinal layer, reducing the half-life of an intact antibody in the circulation to avoid adverse events and eliminating the antibody Fc to

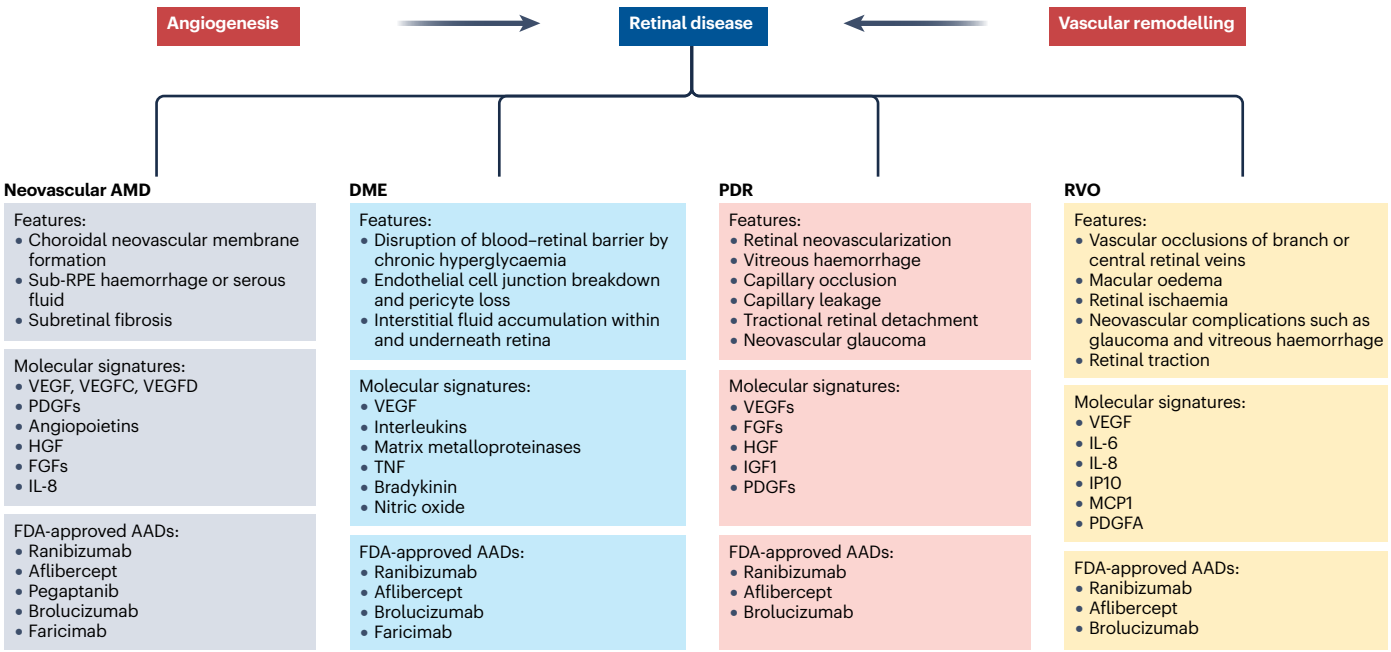

Fig. 6 | Targeting angiogenesis for treatment of ophthalmic diseases. Neovascularization and vascular dysfunctions are common mechanisms underlying various eye diseases, including neovascular age-related macular disease (AMD), diabetic macular oedema (DME), proliferative diabetic retinopathy (PDR) and retinal vein occlusion (RVO), which are major causes of blindness. Multiple angiogenic factors and cytokines contribute to retinal neovascularization and pathological processes. Targeting these pro-angiogenic factors has provided effective therapeutic approaches for treating ophthalmic disorders. A few examples of US Food and Drug Administration (FDA)-approved anti-angiogenic drugs (AADs) are given. FGF, fibroblast growth factor; HGF, hepatocyte growth factor; IGF1, insulin-like growth factor 1; IP10, interferon-γ-induced protein 10; MCP1, monocyte chemoattractant protein 1; PDGF, platelet-derived growth factor; RPE, retinal pigment epithelial; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

avoid pro-inflammatory effects. On the basis of two successful phase III trials[164,165], the 0.5 mg monthly dose of ranibizumab was approved for treatment of AMD in 2006 (ref. 166). Owing to its considerably lower cost, bevacizumab has been widely used off-label for treating neovascular AMD since 2005, when the clinical results of ranibizumab were disclosed[167].

Aflibercept was another milestone for treating neovascular AMD due to the equivalent efficacy of injections every other month of a higher dose (2 mg) with the monthly ranibizumab administration[168]. Since then, various studies have emphasized the importance of testing higher doses in order to increase the response duration, as discussed later in this Review. Recently, clinical trials testing 8 mg aflibercept in AMD and DME have been initiated. Although its high-affinity VEGF binding and neutralization of PlGF and VEGFB were thought to provide aflibercept with a major therapeutic advantage relative to agents that only block VEGF, some clinical trials in patients with cancer and ophthalmology have not supported this hypothesis. As noted, aflibercept was tested in multiple cancer types, but in contrast to bevacizumab gained FDA approval only for treatment of second-line CRC[154].

The original monthly frequency of intravitreal injections poses treatment burdens and logistical challenges to patients and healthcare providers. Alteration of dosing schedules 'as-needed' (pro re nata) has significantly reduced injection frequencies and produced similar gains in vision to monthly dosing[169]. A commonly adopted clinical approach is 'treat and extend', which employs the initial treatment to induce stabilization of the disease, followed by extended injection intervals if disease remains[170]. Clinical studies show that the treat and extend regimen is non-inferior to monthly injections.

Diabetic retinopathy is a common cause of visual loss and has several pathological features, including DME, macular ischaemia, vitreous haemorrhage and tractional retinal detachment. DME has the major impact on vision loss[171], and vascular leakiness is the main mechanism behind its development. Anti-VEGF drugs have considerably improved the DME treatment paradigm and ranibizumab was the first anti-VEGF agent[67]. An analysis of two phase III studies of ranibizumab (RISE and RIDE) in 759 patients showed that 57–69% of patients had visual acuity gains of >10 letters and 37–50% had gains of >15 letters, which were maintained throughout the 36-month study[172]. Therefore, a substantial proportion of patients had clinically meaningful visual acuity benefits. Another important conclusion of the RISE and RIDE trials was that approximately one third of patients with DME no longer needed treatment, suggesting that anti-VEGF had a disease-modifying effect. Comparable results were reported with aflibercept[173].

The efficacy and safety of aflibercept in patients with diabetes with DME were demonstrated in two phase III studies[24,174] and it was approved for DME in 2014. Aflibercept treatment led to anatomical and vision improvements in patients with persistent DME or unresponsive to bevacizumab and ranibizumab[175], although the higher dose of aflibercept used might confound the conclusion that it has intrinsically greater efficacy.

RVO is the second common cause of vascular disorders of the retina[176]. After occlusion, the retinal tissue undergoes ischaemia that results in macular oedema via VEGF-mediated vascular permeability. After long-term follow-up, ranibizumab demonstrated clinical benefits

in patients with RVO and was approved in 2010 (refs. 177,178). Aflibercept has similar efficacy and safety and was approved for the treatment of RVO-associated macular oedema in 2012 (ref. 179).

Despite the clinical success of anti-VEGF agents in the treatment of neovascular AMD, DME and RVO, there are challenges that need to be addressed. For example, there are important differences in therapeutic outcomes between real-world data and clinical trials[180]. Real-world data demonstrate that, depending on the country and the robustness of the health system, patients with neovascular AMD receive fewer anti-VEGF injections and experience less visual improvement relative to patients recruited into clinical trials. A retrospective analysis of more than 2,000 patients with neovascular AMD treated with ranibizumab for 2 years in different European countries showed substantial differences in outcomes, which were related to injection frequency between countries[181]. More frequent visits and injections were associated with greater improvements in visual acuity[181]. Similar findings of under-treatment linked to reduced effectiveness were reported in patients with DME treated with ranibizumab[182]. Also, several clinical trials show that available anti-VEGF drugs achieve comparable improvement in visual acuity, therefore switching to a different agent rarely results in greater efficacy. Another issue is inherent drug resistance[183]. A subpopulation of approximately 15–40% of patients lack significant responses to anti-VEGF therapy. The reasons are not clear and might reflect existing fibrosis or atrophy, conditions which are not improved by anti-VEGF agents. It has been also suggested that prolonged anti-VEGF treatment in some patients with neovascular AMD might be associated with progression of geographic atrophy, a late stage of dry AMD. However, it is well established that geographic atrophy expands over time in patients with AMD in the absence of anti-VEGF treatment[184], and thus it is unclear whether the increases in geographic atrophy seen in trials with anti-VEGF are influenced by the drug treatment or merely reflect the natural progression of AMD.

Notwithstanding these issues, anti-VEGF agents represent the best available treatment for intraocular neovascular disorders. Long-term studies have documented visual acuity outcomes that vastly exceed those before anti-VEGF agents were available[185].

Table 3 | Examples of approved and investigative AADs for ophthalmic disease

Drug	Target	Feature	Indication	Phase	Refs.
Approved drugs					
Aflibercept	VEGF, VEGFB, PlGF	Chimeric sVEGFR1/2	AMD, diabetic retinopathy, DME, RVO	Approved	168,172, 267,268
Pegaptanib	VEGF165	Aptamer, a single-strand nucleic acid	AMD	Approved	162
Ranibizumab	VEGF	Monospecific antibody Fab fragment	AMD, DME, diabetic retinopathy, RVO	Approved	165,178, 269
Bevacizumab	VEGF	Monospecific antibody	Off-label: AMD, DME, RVO		167
Conbercept (Lumitin)	VEGF, VEGFB, PlGF	Chimeric sVEGFR1/2	AMD, DME	CFDA approved	270
Brolucizumab (Beovu)	VEGF	Monospecific single-chain antibody	AMD, DME, diabetic retinopathy, RVO	Approved	186
Investigative drugs					
Abicipar Pegol (Allergan)	VEGF	Antibody with DARPins, long half-life	AMD	II/III	192
KSI-301 (Tarcocimab tedromer)	VEGF	Antibody biopolymer, high bioavailability	AMD, DME, ROV	I/II/III	193
Nesvacumab (REGN910)	ANG2	Monospecific antibody	DME, AMD	I/II	271
GB-102 (Sunitinib)	VEGFR	Sunitinib-TKI	AMD	II	195
PAN-90806 (CP-547632)	VEGFR, PDGFR	Topical TKI	AMD	II	196
Faricimab (RG7716)	VEGF, ANG2	Bispecific antibody	AMD, DME, RVO	III, approved	188,272
OPT-302 (Opthea)	VEGFC, VEGFD	sVEGFR3	AMD, DME	III	194
X-82 (Vorolanib, CM082)	VEGFR, PDGFR	Oral TKI	AMD	II, halted	273
Razuprotafib (AKB-9778)	VE-PTP agonist	Endothelial cell tyrosine phosphatase agonist	DME	II	274
Risuteganib (ALG 1001)	Integrin inhibitor	Small molecule	DME	II/III	275
Carotuximab (De-122)	Endoglin	Antibody	AMD	I/II	276
Rinucumab (REGN2176)	PDGFRβ	Antibody	AMD	II/III	197
Pegpleranib	PDGF-BB/AB	DNA aptamer	AMD	II/III	277
RGX-314	VEGF	AAV8-anti-VEGF antibody fragment	AMD, diabetic retinopathy	I/II	195
ADVM-022 (AAV.7m8-aflibercept)	VEGF, VEGFB, PlGF	AAV-based aflibercept for gene therapy	AMD, diabetic retinopathy	I/II	203

AAD, anti-angiogenic drug; AAV, adeno-associated virus; AMD, age-related macular degeneration; ANG2, angiopoietin 2; CFDA, China Food and Drug Administration; DARPin, designed ankyrin repeat protein; DME, diabetic macular oedema; PDGFR, platelet-derived growth factor receptor; PlGF, placental growth factor; RVO, retinal vein occlusion; sVEGFR, soluble VEGFR; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; VE-PTP, vascular endothelial tyrosine phosphatase.

New anti-VEGF drugs

Brolucizumab, a humanized single-chain variable fragment (scFv) that neutralizes all VEGF isoforms, received approval for treatment of neovascular AMD in 2019 (ref. 186). However, in spite of remarkable clinical efficacy including non-inferiority to aflibercept, the occurrence of adverse events such as intraocular inflammation and, rarely, retinal artery occlusion, which can lead to blindness, has considerably limited the use of this drug[187].

Together with VEGF, ANG2 has been implicated in the pathogenesis of vascular abnormalities in AMD and DME in human patients. As already noted, ANG1 has opposing effects to ANG2 by stabilizing blood vessels. In principle, blocking ANG2 should make ANG1 available to activate TIE2 for vascular stabilization and restoration of retinal vascular homeostasis. Faricimab, a bispecific antibody that neutralizes VEGF and ANG2, was recently approved for treatment of neovascular AMD and DME[188,189]; administration of faricimab at up to 16-week intervals demonstrated visual benefits, thus reducing treatment burden. However, it remains unclear to what extent ANG2 blockade contributed to the therapeutic efficacy of faricimab, considering that there was no improvement in visual acuity compared with aflibercept or other anti-VEGF agents tested. The high dose of faricimab tested (6 mg) might be a major reason for the durability of the therapeutic effects. In this context, combining an anti-ANG2 antibody (nesvacumab) with aflibercept failed to result in additional visual acuity improvement in patients with DME[190]. However, administration of AKB-9778, a small-molecule inhibitor of vascular endothelial tyrosine phosphatase (VE-PTP), which inactivates TIE2, enhanced the ability of ranibizumab to reduce DME in a phase II study[191], although subsequent studies did not fully validate these findings. As mentioned, an alternative approach is being pursued to activate TIE2 through agonistic antibodies[138].

Abicipar is a pegylated 'designed ankyrin repeats protein' (DARPin) that binds to all isoforms of VEGF[192]. DARPins are small proteins derived from naturally occurring ankyrin repeat proteins. Two phase III studies demonstrated non-inferiority of abicipar relative to ranibizumab, with less frequent injections[192]. However, a high incidence of intraocular inflammation so far has precluded further clinical development of this agent.

KSI-301 is a humanized anti-VEGF neutralizing antibody conjugated to a biopolymer in order to increase durability[193]. In a recent phase IIb/III clinical trial, KSI-301 failed to meet the primary end point of non-inferior visual acuity gains compared with aflibercept[193].

OPT-302, a soluble receptor consisting of the immunoglobulin-like domains 1–3 of VEGFR3 fused to Fc IgG, neutralizes VEGFC/VEGFD[194]. Early-stage clinical trials in patients with neovascular AMD suggest that OPT-302 in combination with ranibizumab results in improved visual acuity relative to ranibizumab monotherapy[194], and similar results were seen in patients with diabetic retinopathy[194,195]. These preliminary clinical findings suggest that OPT-302 might potentially offer improved clinical benefits. In conclusion, documenting therapeutic advantages of these new anti-VEGF agents relative to the existing drugs will require considerable additional clinical research.

Developing longer-acting VEGF inhibitors

Despite considerable efforts to engineer newer anti-VEGF drugs with prolonged half-life and bioavailability, long-term treatment burdens and injection-related risks still pose challenges for ophthalmic clinical practice. Development of sustained drug delivery systems offers new opportunities to achieve long-lasting effects. The hydrogel-based drug delivery platform is under development by formulating biodegradable polyethylene glycol (PEG) networks embedded with drug particles, which slowly release drugs by hydrolysis. AAD-TKIs, including axitinib (OTX-TKI) and sunitinib malate (GB-102), have been formulated with PEG-based microparticles for intravitreal injections[195,196]. Both OTX-TKI and GB-102 have entered early phase clinical trials. These TKIs also target PDGF receptors, which might participate in retinal diseases and, especially, in the development of fibrosis. However, targeting of PDGF signalling to treat ophthalmic neovascular diseases remains controversial after the anti-PDGFB aptamer pegpleranib (Fovista) failed to improve outcomes when combined with anti-VEGF agents in neovascular AMD[197].

Arguably, currently the most effective approach to reduce frequency of intravitreal injections is the Port Delivery System (PDS), a device containing highly concentrated ranibizumab that is gradually released for up to 6 months and can be refilled with a custom syringe[198]. The PDS is implanted into the pars plana through a scleral incision. In a phase III clinical study, PDS Q24W approaches the efficacy of anti-VEGF, and was equivalent to monthly ranibizumab[198]. However, the implant was associated with a significant increase in the incidence of the inflammatory condition endophthalmitis relative to monthly ranibizumab injections. Nonetheless, there are other materials and drug delivery designs that could be developed to address such issues and, potentially, also increase drug duration[199,200].

Anti-angiogenic gene therapy

Owing to its small size, immune privilege and compartmentalization, the eye is an excellent site to achieve high therapeutic efficacy of gene therapy. In addition, the blood–retinal barrier prevents systemic diffusion, and non-dividing retinal cells are suitable for the delivery of non-integrating vectors to reduce risks of mutagenesis[201]. Several viral vector-based gene expression vectors have been developed for ophthalmic uses. Adeno-associated viruses (AAVs) expressing anti-VEGF proteins are commonly used and are able to transduce multiple cell types in the retina[195]. RGX-314 and ADVM-022 are two examples of AAV-based anti-VEGF gene therapeutics for treating neovascular AMD and diabetic retinopathy[202,203]. RGX-314 is an AAV serotype 8 vector expressing an anti-VEGF antibody fragment and is under investigation for treatment of neovascular AMD and diabetic retinopathy by one-time intravitreal injection. ADVM-022 (AAV2.7m8-aflibercept) coding for aflibercept protein exhibits high transduction efficiency and a single intravitreal injection validates the anti-VEGF response in patients with neovascular AMD[203]. Together, gene therapy holds promises to reduce treatment burdens for eye disorders. However, issues of potential high costs, transduction efficiency, long-term effects and safety warrant future investigation. Also, it is unclear whether frequent injections are needed to achieve long-term effects.

Emerging new targets

As noted, considerable efforts have been devoted to develop new generations of AADs for eye diseases, but various studies indicate that a ceiling effect is reached by targeting VEGF alone. Therefore, it is conceivable that further advances will result from targeting non-angiogenic pathways, such as those associated with fibrosis or atrophy. Also, development of digitalized deep-learning algorithms is likely to be key to assessing therapeutic responses to novel agents.

Genome-wide association studies have identified numerous genetic variants associated with AMD[204]. In particular, polymorphisms in the complement factor H (*CFH*) gene have been identified as a major risk factor and the complement pathway is a target for treatment of

geographic atrophy. Although most of the variants identified in genome-wide association studies are associated with both neovascular and dry AMD, at least some have been especially implicated in angiogenesis or vascular assembly, namely *HTRA1*, *CETP*, *MMP9* and *SYN3/TIMP3* polymorphisms[204]. Among these, the serine protease HTRA1 is particularly intriguing. Its transgenic overexpression in retinal pigment epithelial (RPE) cells led to the development of CNV[205]. Anti-HTRA1 antibodies are currently being tested in patients with AMD[206]. It is conceivable that additional promising targets will emerge from genomic analysis.

Several studies indicate that the blood vessels have a crucial role in the development of geographic atrophy, but in an almost opposite way to neovascular AMD. Loss of choriocapillaris, a layer of capillaries closely adjacent to Bruch's membrane in the choroid, is an early event in AMD, and precedes RPE degeneration[207]. Recent studies provided evidence for deposition of membrane attack complexes in the choroid of patients with a high-risk *CFH* genotype[208]. Therefore, loss of choriocapillaris could be a key event in the pathogenesis of geographic atrophy, raising the possibility that strategies aiming at protecting and/or regenerating the choriocapillaris would be effective. VEGF would not be suitable in this setting, given its well-established effects in enhancing vascular permeability.

The organ-specific structural characteristics of blood vessels have been long recognized[209,210]. Interestingly, earlier studies described a mitogen specific for particular endothelial cells, raising the possibility that other vascular bed-specific endothelial cell mitogens might exist[211]. Indeed, leukaemia inhibitory factor (LIF), a member of the IL-6 family, was recently identified as a mitogen for choroidal endothelial cells that also protects the choroidal vasculature from oxidative damage without inducing vascular leakage[212]. Single-cell transcriptomic data show high expression of *LIFR* in human choroidal endothelial cells, comparable with *VEGFR2* or *TIE2*. In early studies, LIF was characterized as a growth inhibitor for aortic endothelial cells[213], indicating that the same signalling pathway might have opposite effects depending on the endothelial cell type. These findings suggest that LIF administration might prevent choriocapillary loss and geographic atrophy.

Elimination of senescent cells to prevent ageing and various age-related disorders[214] using 'senolytic' drugs[215] is currently the object of considerable investigation, although there is no definitive evidence that this approach is effective in humans. Interestingly, the use of senolytics has been reported to inhibit neovascularization in mouse models of retinopathy[216]. Clinical trials in patients with DME and AMD are ongoing.

Targeting angiogenesis to treat other diseases

In addition to cancer and ophthalmic diseases, targeting angiogenesis also has implications for treatment of other disorders, including cardiovascular diseases, metabolic disorders, inflammation and infection.

Therapeutic angiogenesis in cardiovascular disease

The rationale of 'therapeutic angiogenesis' for treating cardiovascular disease is to stimulate the development of new vessels that improve blood perfusion in the ischaemic myocardium or limbs and enable functional recovery. Delivery of pro-angiogenic and arteriogenic factors to the ischaemic region of the infarcted myocardium might provide an important approach for these patients[217] and several angiogenic factors were shown to increase vascularity in animal models. However, in clinical trials, the functional recovery induced by FGF, VEGF and HGF was no greater than that of placebo[218]. Potential impediments to clinical benefits could be inefficient delivery, inadequate expression of the factors or suboptimal selection of end points or patients[219]. Other unresolved issues include whether angiogenic vessels can be remodelled to become functional conduits as well as whether they can be stabilized by appropriate coverage of perivascular cells[50]. Simultaneous delivery of dual pro-angiogenic and perivascular factors has produced encouraging functional outcomes in improving myocardial function in large animal models, although this combination approach requires clinical validation[50,220].

An additional pro-angiogenic strategy consists of activating stress pathways in endothelial cells[221]. The hexosamine D-mannosamine (ManN) is an endothelial cell mitogen and survival factor that acts additively with VEGF. ManN inhibits glycosylation in endothelial cells, leading to activation of the unfolded protein response and stimulation of pro-angiogenic signalling pathways. ManN administration enhanced angiogenesis in mouse ischaemia models, accelerating recovery of blood flow[221]. Thus, despite the disappointing clinical data in the past, there is reason to hope that a better understanding of the molecular and biological basis of therapeutic angiogenesis will lead to better clinical outcomes.

Targeting adipose vasculature in metabolic disease

The adipose tissue is one of the most vascularized tissues in the adult body[222] and undergoes constant changes in size and function. The vasculature has important roles in maintaining an optimal microenvironment for adipocytes by transporting nutrients, oxygen and metabolites[223]. Furthermore, in addition to releasing signalling molecules, cells in the vessel wall such as endothelial and perivascular cells have stem cell-like features and can differentiate into adipocytes[224,225]. Thus, vessel numbers and vascular structures are key determinants for adipose tissue mass and metabolic functions[225]. The expansion of white adipose tissue (WAT) was hypothesized to be dependent on angiogenesis, similar to a growing tumour[226,227]. If so, blocking angiogenesis would provide a therapeutic option for treating obesity and metabolic disorders. In support of this view, preclinical studies have revealed a role for angiogenic vessels in expanding WAT and shown that blocking adipose angiogenesis leads to potent anti-obesity effects[226,227]. Importantly, anti-angiogenic therapy improves insulin sensitivity in obese animals, implying that it might be useful to treat type 2 diabetes[226].

Paradoxically, metabolic activation of brown adipose tissue and browning WAT is accompanied by robust angiogenesis[228], which accelerates thermogenic metabolism[229–232]. Stimulation, but not inhibition, of angiogenesis dissipates energy in brown adipose tissue and browning WAT, and ultimately improves metabolic dysfunction in obese and diabetic animals[229,232,233]. Thus, both stimulation and inhibition of angiogenesis might be harnessed for treating obesity and type 2 diabetes, depending on the metabolic status of the adipose tissue[225]. In metabolically inert WAT, inhibition of angiogenesis would suppress lipid transport and deposition in expanding adipose tissue. By contrast, enhancing angiogenesis in metabolically active brown adipose tissue and browning WAT instigates energy expenditure by thermogenesis.

Similar to tumours and the eye, VEGF–VEGFR signalling is a key angiogenic pathway in adipose tissues and sustains vascular homeostasis under physiological conditions[228,229,232,234]. Suppression of VEGFR1 alone augments angiogenic and browning phenotypes in WAT and triggers non-shivering thermogenesis[229]. Pharmacological inhibition or genetic deletion of VEGFR2 in vascular endothelial cells prevents WAT browning and thermogenesis[232]. It appears that VEGF-stimulated endothelial cells produce paracrine factors that either induce differentiation of preadipocytes into browning mature adipocytes or convert

existing mature adipocytes into browning adipocytes. Thus, defining these endothelial cell-derived paracrine factors provides a strategy for developing novel therapeutics. For example, members of the PDGF family have important paracrine functions[232].

Although the concept of targeting adipose angiogenesis for treating obesity and metabolic disease is in its infancy, it might shift the treatment paradigm in future. Because AADs are routinely used for treating patients with cancer and eye disease, the impacts of these drugs on adipose tissue and global metabolism warrant further investigation.

Concluding remarks and perspectives

Almost a century of angiogenesis research, starting from the observation of vascularization in various pathological tissues to the discovery of key angiogenic pathways, has led to the development of unprecedented therapeutic modalities for treating malignant and non-malignant diseases. The clinical success of AADs has provided one of most remarkable examples of translational research, from initial hypotheses and discoveries to successful treatment of human patients. Conceivably, the use of drugs targeting angiogenesis will expand beyond cancer and ophthalmic disease. Indeed, drugs targeting angiogenesis will likely have a role in the treatment of numerous human diseases, including cardiovascular and metabolic diseases.

In cancer treatment, AADs in combination with immunotherapy have demonstrated superior clinical benefits over monotherapies and are standards of care in conditions such as HCC and RCC. Anti-angiogenic therapy is also an important component of combinations with other anticancer drugs besides immunotherapy. In ophthalmic disease, anti-angiogenic therapy has emerged as the most effective treatment for AMD, diabetic retinopathy, DMO and RVO.

Despite the success of anti-angiogenic therapy, several issues need to be addressed. For cancer, issues include the clinical relevance of animal models, mechanisms of drug resistance, the timing and delivery of therapy, the optimization of therapeutic regimens and the definition of reliable biomarkers. For ophthalmic disease, long-lasting drug delivery and more convenient drug delivery systems need to be developed. Formulas for eye-drop administration would be ideal for clinical use. Also, combination therapy needs to be investigated; similarly to RCC treatment, maximizing clinical benefit might require combinations of drugs with different modes of action.

Unfortunately, in spite of extensive preclinical and clinical efforts, there is no compelling evidence that targeting angiogenic pathways other than VEGF provides a therapeutic benefit, in cancer or ophthalmic disease. Although HIF and mTOR inhibitors have been approved for treating RCC, their action is mediated, at least in part, by VEGF inhibition. Hopefully, this picture will change if some of the approaches or combinations described in this Review prove successful. It should also be considered that drugs targeting VEGF itself might engage pathways other than angiogenesis, because VEGF has multiple biological functions in the tumour microenvironment and body. Another area that is receiving considerable attention is exploiting endothelial cell metabolism, in parallel to targeting well-established growth factors, to achieve greater anti-angiogenic effects[235]. Furthermore, it is conceivable that genome-wide association studies and other genomic analyses will yield novel targets. In the future, we expect that drugs modulating angiogenesis and vascular functions will be further expanded to treat metabolic diseases, inflammatory diseases such as rheumatoid arthritis and various autoimmune diseases.

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