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Authors
Ferrara, Napoleone
Adamis, Anthony P

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Ten years of anti-vascular endothelial growth factor therapy

Napoleone Ferrara and Anthony P. Adamis

Abstract | The targeting of vascular endothelial growth factor A (VEGFA), a crucial regulator of both normal and pathological angiogenesis, has revealed innovative therapeutic approaches in oncology and ophthalmology. The first VEGFA inhibitor, bevacizumab, was approved by the US Food and Drug Administration in 2004 for the first-line treatment of metastatic colorectal cancer, and the first VEGFA inhibitors in ophthalmology, pegaptanib and ranibizumab, were approved in 2004 and 2006, respectively. To mark this tenth anniversary of anti-VEGFA therapy, we discuss the discovery of VEGFA, the successes and challenges in the development of VEGFA inhibitors and the impact of these agents on the treatment of cancers and ophthalmic diseases.

Angiogenesis, the formation of new blood vessels from pre-existing vessels, is essential for both normal embryonic and adult development, as well as the progression of cancer and ophthalmic diseases[1].

The first description of a link between human tumours and their blood supply occurred more than 100 years ago[2], but it was only in 1939 that tumour cells themselves were hypothesized to release a blood vessel growth stimulating factor (REF. 3), that was later associated with rapid growth of transplanted tumours[4]. In 1971, Folkman proposed anti-angiogenesis as a new anticancer strategy[5]. During the next 15 years, several molecules that can induce blood vessel growth in various bioassays were identified, including fibroblast growth factor 1 (FGF1; also known as aFGF), bFGF, angiogenin and transforming growth factor-α (TGFα), but their role in the regulation of angiogenesis remained unclear[6].

In 1989, the isolation and cloning of vascular endothelial growth factor A (VEGFA, previously known as vascular permeability factor (VPF))[7,8], was a major step forward in understanding angiogenic mechanisms. This knowledge, combined with both *in vitro* and *in vivo* functional studies[9] (FIG. 1), demonstrated that VEGFA possesses both mitogenic and angiogenic properties. These milestones laid the foundations for exciting new fields of research into improved treatments not only for cancer, but also for a range of vascular-related diseases[9,10].

In 1993, Kim and colleagues identified monoclonal antibodies that can target and neutralize VEGFA and inhibit tumour growth in preclinical studies[11]. This led to the production of the recombinant humanized VEGFA-specific monoclonal antibody bevacizumab (Avastin; Genentech/Roche), which was approved in 2004 by the US Food and Drug Administration (FDA) for the first-line treatment of metastatic colorectal cancer[12]. Parallel discoveries revealed that VEGFA was associated with ocular neovascular conditions in patients[13,14] and that VEGFA inhibition could suppress ocular neovascularization in animal models[15,16]. Consequently, pegaptanib (Macugen; Pfizer/Valeant)[17] and ranibizumab (Lucentis; Genentech/Novartis)[18] received FDA approval for neovascular age-related macular degeneration (AMD) in 2004 and 2006, respectively.

These achievements have resulted in the continuing development of other VEGFA signalling pathway inhibitors. The receptor Tyr kinase inhibitors (RTKIs) sunitinib (Sutent; Pfizer)[19] and sorafenib (Nexavar; Bayer and Onyx Pharmaceuticals)[20], as well as others, are currently approved for use in various cancers. Similarly, aflibercept (zvi-aflibercept, Eylea; Regeneron Pharmaceuticals), a soluble VEGFA-neutralizing VEGF receptor 1 (VEGFR1)–VEGFR2 chimeric protein[21] is approved for use in metastatic colorectal cancer as well as wet macular degeneration, while ramucirumab (Cyramza; Eli Lilly & Co.), an anti-VEGFR2 monoclonal antibody[22], is approved for use in various solid tumours. Compounded bevacizumab is widely used off-label to treat a variety of ophthalmic diseases.

To mark the tenth anniversary of anti-VEGFA therapy, this Review provides a perspective on the status of therapeutically targeting VEGFA in cancer and ophthalmology. Despite clinical improvements, there are still unanswered questions regarding the effects of the anti-VEGFA agents and how to optimize treatment to improve patient outcomes.

VEGFA and VEGF receptors

The discovery of VEGFA. In 1983, Senger and colleagues identified VPF in culture supernatants of a guinea pig tumour cell line[23]. In 1990, the same group purified guinea pig VPF and determined its amino-terminal amino acid sequence[24]. In 1989, Ferrara and colleagues isolated and sequenced VEGFA, a diffusible mitogenic 45 kDa heparin-binding protein, from cultured bovine pituitary follicular cells[25]. In the same year, Connolly and colleagues isolated and sequenced the human VPF protein from U937 cells[26]. cDNA and protein sequence analyses confirmed that VEGFA and VPF were in fact the same molecule[7,8] (FIG. 1).

VEGFA is the prototype member of a family of proteins that includes VEGFB, VEGFC, VEGFD, VEGFE (a virally encoded protein) and placental growth factor (PlGF; also known as PGF)[2]. These proteins, which are structurally related to the platelet-derived growth factor (PDGF) family of proteins[7,8], have a range of tissue distributions and functions[9,27,28].

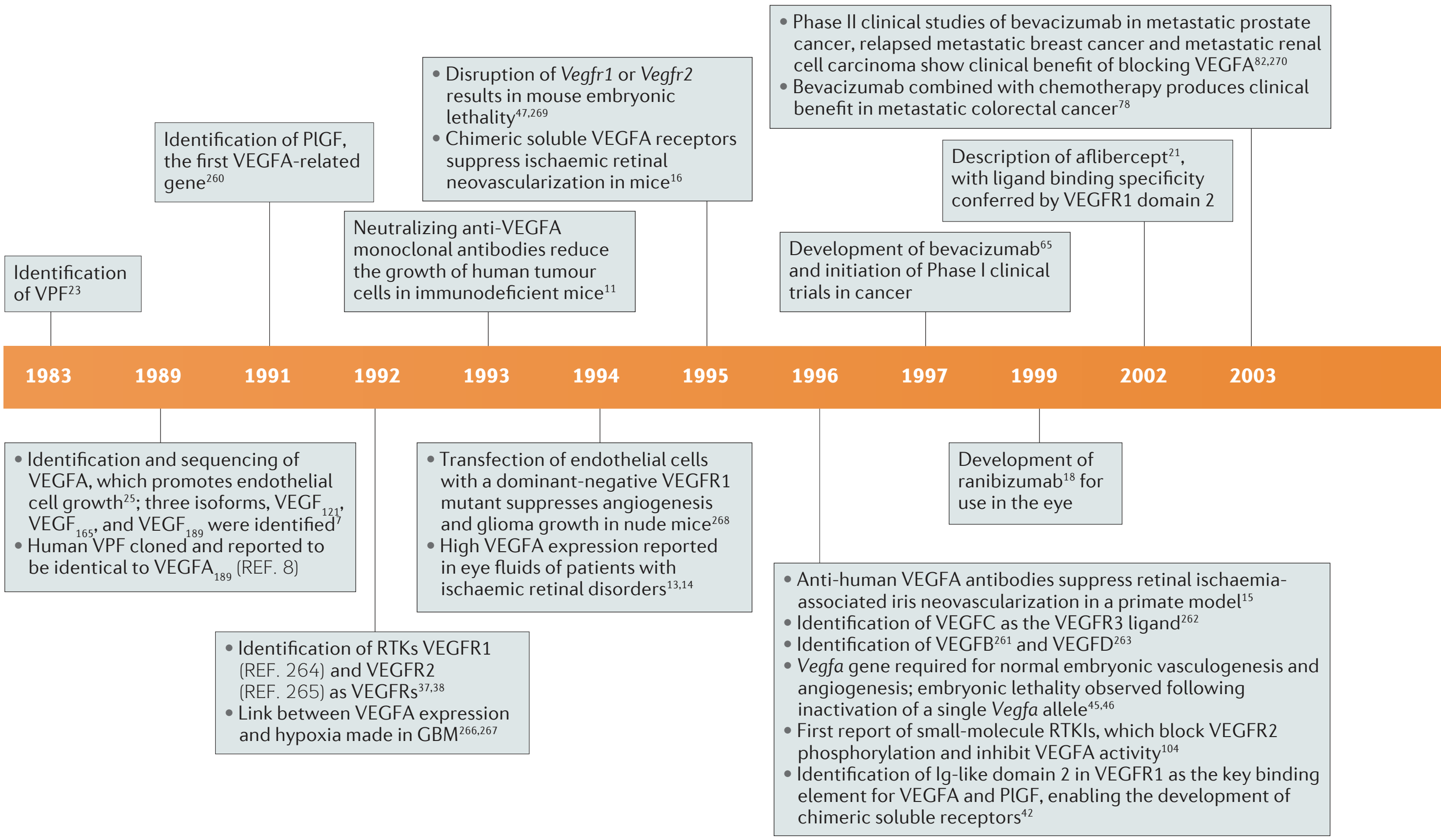


Figure 1 | **Discovery of VEGFA and VEGFA-targeted therapies.** The timeline shows progress in the field following the initial identification of vascular permeability factor (VPF) in 1983, and the more definitive biochemical and molecular studies done in 1989, to the present day. AMD, age-related macular degeneration; DME, diabetic macular oedema; DR, diabetic retinopathy; DRCR.net, Diabetic Retinopathy Clinical Research Network; EMA, European Medicines Agency; FDA, US Food and Drug Administration; GBM, glioblastoma multiforme; IFN, interferon; Ig, immunoglobulin; NSCLC, non-small cell lung cancer; PFS, progression-free survival; PlGF, placental growth factor; RTKI, receptor Tyr kinase inhibitors; RVO, retinal vein occlusion; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

VEGFA gene, isoforms and encoded proteins. There are multiple isoforms of VEGFA, derived from alternative splicing of exons 6 and 7, which gives rise to VEGFA$_{121}$, VEGFA$_{165}$, VEGFA$_{189}$ and VEGFA$_{206}$ (reviewed in REF. 29). VEGFA$_{165}$ is the most frequently expressed isoform in normal tissues and in tumours, although less common isoforms, such as VEGFA$_{145}$ and VEGFA$_{183}$, have also been identified[30]. VEGFA$_{165}$ has an intermediary behaviour between the highly diffusible VEGFA$_{121}$ and the extracellular matrix (ECM)-bound VEGFA$_{189}$, and is thought to be the most physiologically relevant VEGFA isoform (reviewed in REF. 31).

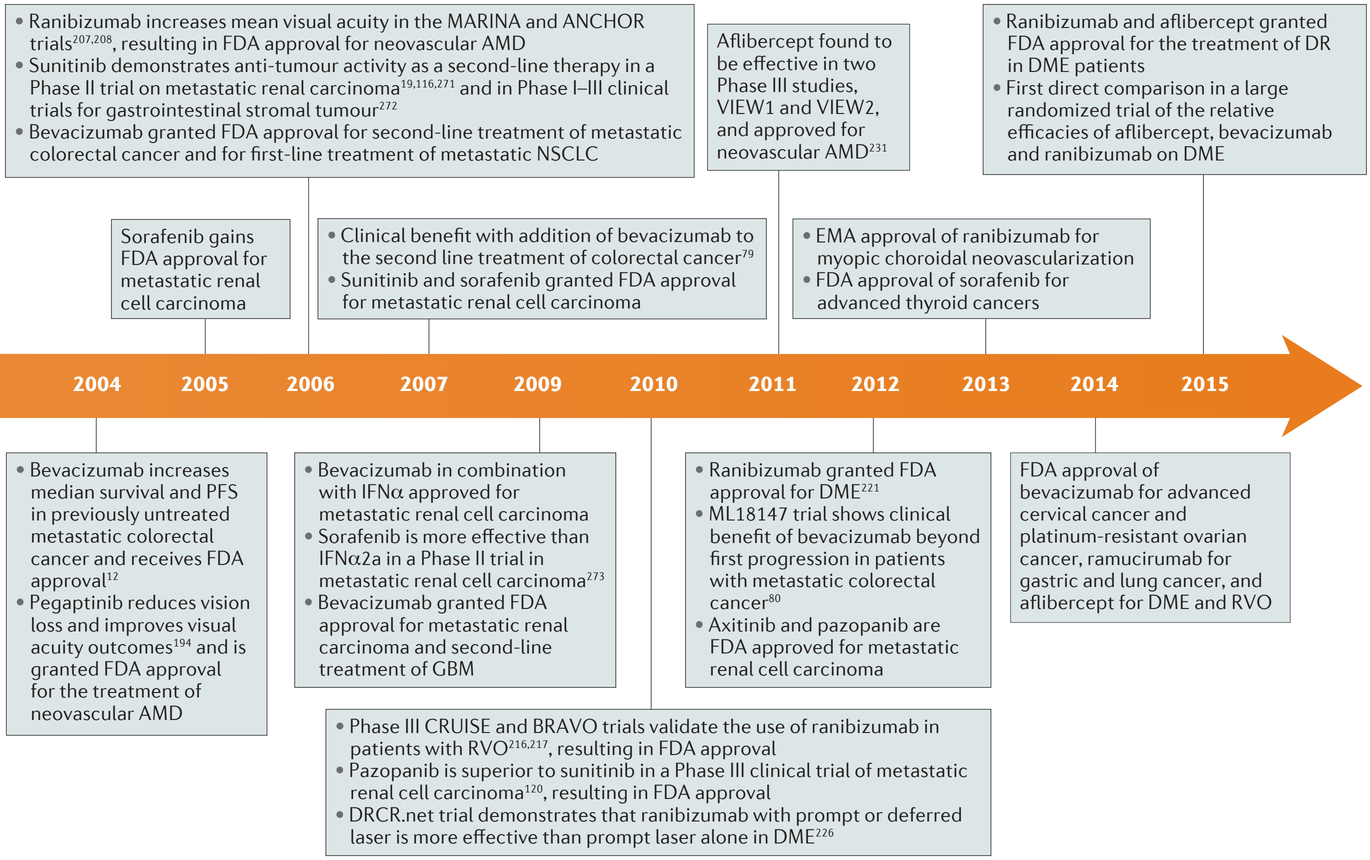
Proteolysis plays an important part in regulating the biological activity of VEGFA proteins. The proteolytic cleavage of VEGFA$_{165}$ at the carboxyl terminus, for example, gives rise to biologically active VEGFA$_{110}$ or VEGFA$_{113}$ (REF. 31). Inhibitory isoforms such as VEGFA$_{165b}$ (REF. 32) and VEGFA-Ax[33] have been described, but their significance remains to be further elucidated.

Regulation of* VEGFA *gene expression. The expression of *VEGFA* is primarily stimulated by hypoxia, mediated by the hypoxia-inducible factor (HIF), which also triggers the expression of other hypoxia-regulated genes[34]. Under normoxic conditions, HIF is hydroxylated by a class of oxygen- and iron-dependent enzymes known as HIF prolyl hydroxylases, leading to HIF recognition by the von-Hippel Lindau (VHL) tumour suppressor protein. As a result, HIF becomes a target for polyubiquitylation and proteosomal degradation[35]. Inactivating mutations in *VHL*, such as those occurring in the VHL hereditary cancer syndrome or in renal cell carcinomas, result in inefficient degradation of HIF and in VEGFA upregulation in normoxic conditions[35].

VEGFA expression is also regulated by other factors, such as epidermal growth factor (EGF) and PDGF[9], and by oncogenic mutations. The latter include *VHL* mutations, as well as mutations affecting the RAS pathway and the WNT–KRAS signalling pathway[9].

VEGFA signalling. Binding sites for VEGFA on endothelial cells *in vivo* were first described in 1992 (REF. 36), and two VEGFA RTKs, VEGFR1 (also known as FLT1)[37] and VEGFR2 (also known as KDR and FLK1)[38] have been reported since. A highly homologous RTK, VEGFR3 (also known as FLT4)[39] was also described and later shown to bind VEGFC and VEGFD[9,27,28] (which promote both angiogenesis and the development of lymphatic vessels). With the exception of VEGFA$_{121}$, VEGFA isoforms also interact with the neuropilin co-receptors (NRP1 and NRP2)[27], which can signal independently of VEGFRs and further influence VEGFR2 signalling[40]. The interactions of VEGFA family members with different VEGFRs are outlined in FIG. 2.

Of the two RTKs, VEGFR2 is the main mediator of the roles of VEGFA in cell proliferation, angiogenesis and vessel permeabilization[27]. Binding of VEGFA to VEGFR2 on endothelial cells leads to receptor dimerization and autophosphorylation,



which activates multiple downstream signalling cascades involved in proliferation, filopodial extension, chemotaxis and ECM degradation (reviewed in REF. 27). The higher binding affinity of VEGFA to VEGFR1 compared with VEGFR2, combined with the lack of consistent mitogenic effects following VEGFR1 activation, suggest that VEGFR1 may act at least in some circumstances as a decoy receptor, sequestering VEGFA and thus regulating VEGFR2 activity[41]. Structure–function studies demonstrated that VEGFA and PlGF bind to domain 2 of the seven immunoglobulin (Ig)-like domains in the extracellular portion of VEGFR1 (REF. 42), a finding which was instrumental to the design of chimeric soluble receptors such as aflibercept[21]. An alternatively spliced, soluble form of VEGFR1 that is expressed in a variety of other tissues has been implicated as a negative regulator of angiogenesis in the eye[43]. VEGFR1 is also expressed by monocytes and macrophages as well as in tumour cells[27], and VEGFR3 is mainly present in lymphatic endothelial cells, where it regulates lymphangiogenesis[28].

VEGFA and VEGFRs in angiogenesis. VEGFA is the master regulator of angiogenesis (FIG. 2), binding to VEGFR2 to stimulate the proliferation of endothelial cells via the RAS–RAF–MAPK (mitogen-activated protein kinase)–ERK (extracellular signal-regulated protein kinase) signalling pathway[44]. VEGFA triggers endothelial cell migration, which is an integral component of angiogenesis. Indeed, *Vegfa*$^{+/-}$ (REFS 45,46) and *Vegfr2*$^{-/-}$ mouse embryos[47] have severe defects in angiogenesis and die *in utero* at embryonic days 8.5–10.5.

More recent studies have shown that phosphorylation of VEGFR2 Tyr1175 (in humans; Tyr1173 in mice) has a crucial role in regulating VEGFA-dependent angiogenesis. This amino acid residue is required to activate the MAPK and possibly also the phosphoinositide 3-kinase (PI3K) signalling pathways[27]. Mice homozygous for the single substitution Tyr to Phe at position 1173 (*Vegfr2*$^{1173Phe/1173Phe}$) show defective vasculogenesis and angiogenesis and die *in utero* around embryonic day 8.5–9.5, similar to *Vegfr2*-null mice[48].

Role of VEGFA in regulation of vascular permeability. Senger and colleagues initially characterized VPF as a protein that rapidly and transiently enhances vascular permeability of an intact endothelium[23]. Although VEGFA-mediated production of endothelial nitric oxide synthetase (eNOS)[49], activation of SRC and YES signalling to regulate cell-to-cell contacts[50] and activation of VE-cadherin contribute to regulation of vascular permeability[51], more recent studies have emphasized the role of phosphorylation of Tyr949 (Tyr951 in humans) in VEGFR2. This phosphorylated residue interacts with an adaptor protein (TsAd), which in turn activates SRC, resulting in the formation of complexes with VE-cadherin[52]. Inactivating mutations in this pathway largely abolished the direct permeability-enhancing effects of VEGFA in mice, without causing any developmental abnormality or deficits in adult physiological parameters, including blood flow and pressure[50,52].

Importantly, the chronic vascular hyper-permeability associated with tumours and intraocular neovascular diseases primarily reflects the growth of structurally abnormal and immature vessels that, among other defects, are deficient in pericytes (the cells that surround endothelial cells on the vascular wall), have a thin endothelium and develop microaneurisms, which frequently result in bleeding and leakage[10,53,54]. Interestingly, injections of recombinant VEGFA into the vitreous humor of the eye reproduce virtually all of the aforementioned abnormalities[55].

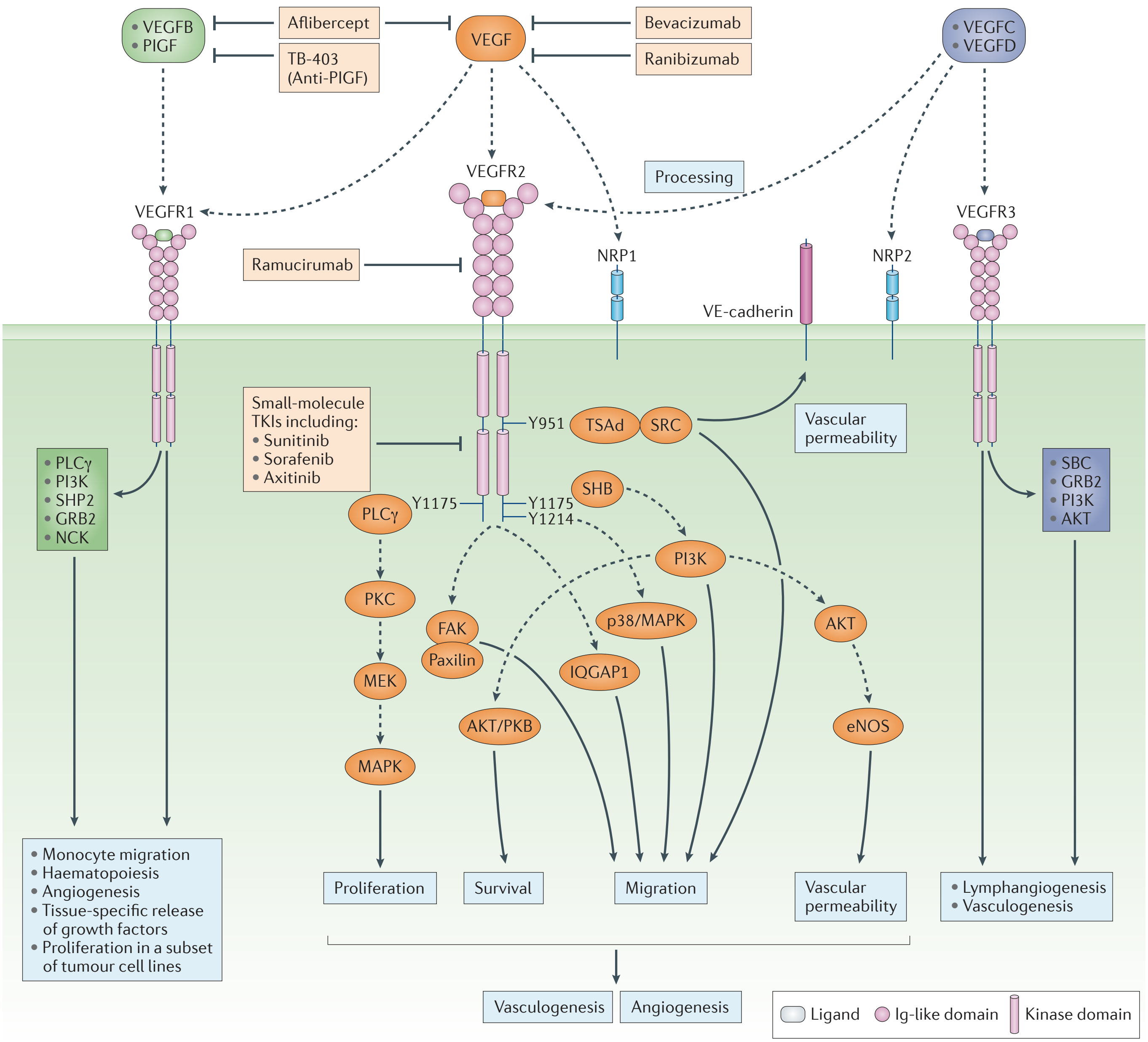


Figure 2 | **VEGF signalling pathways and inhibitors.** The vascular endothelial growth factor (VEGF) Tyr kinase receptors are primarily expressed by endothelial cells. Placenta growth factor (PlGF) and VEGFB bind selectively to VEGF receptor 1 (VEGFR1), whereas VEGFA binds both VEGFR1 and VEGFR2, although VEGFR2 is the major signalling receptor for VEGFA. VEGFC and VEGFD bind to VEGFR3, a key regulator of lymphangiogenesis; however, following proteolytic processing they can also bind to and activate VEGFR2 (REF. 25). Heparin-binding VEGFA isoforms and PlGF also bind the co-receptor neuropilin 1 (NRP1)[40] (PlGF binding not shown). This interaction between VEGFA and NRP1 increases the binding affinity of VEGFA for VEGFR2 (REF. 27). VEGFA or PlGF may directly act on NRP1, independently of VEGF receptor activation[27]. NRP2 regulates lymphangiogenesis, primarily through its interaction with VEGFR3 (REF. 274). VEGFR1 may function as a decoy receptor, sequestering VEGFA and preventing it from binding to VEGFR2 (REF. 41). It can, however, regulate the expression of a variety of genes in the endothelium, including matrix metalloproteinase 9 (MMP9) and certain growth factors such as hepatocyte growth factor and connective tissue growth factor, which play an important part in tissue homeostasis and regeneration[275]. VEGFR1 is also expressed by monocytes and macrophages and, in some cases, also by tumour cells, in which it can mediate tumour cell proliferation in response to VEGFA or PlGF[276]. VEGFR2 mediates endothelial cell mitogenesis and vascular permeability. Multiple inhibitors block VEGFA-induced signalling. Bevacizumab and ranibizumab bind VEGFA. The soluble chimeric receptor aflibercept binds VEGFA, PlGF and VEGFB. TB403, a PlGF-specific antibody, is being tested for the treatment medulloblastoma. The VEGFR2-specific monoclonal antibody ramucirumab prevents VEGFR2-dependent signalling. Numerous small molecule Tyr kinase inhibitors block VEGFR signalling. Phosphorylated Tyr in position 1175 (in humans) in VEGFR2 is required for the activation of the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways and is essential for embryonic vasculogenesis and angiogenesis[48]. Phosphorylated Tyr in position 951 (in humans) interacts with the adaptor protein TsAd, which in turn activates SRC and enhances vascular permeability via formation of complexes with vascular endothelial (VE)-cadherin[52]. Signalling molecules that have been implicated in VEGFA-induced migration through VEGFR2 include the focal adhesion kinase (FAK) and its substrate paxillin (reviewed in REF. 27). Upregulation of endothelial nitric oxide synthetase (eNOS) and local production of arachidonic acid metabolites has also been implicated in VEGFA-induced vascular permeability. Figure is modified from REF. 57. GRB2, growth factor receptor-bound protein 2; ig, immunoglobulin; MEK, MAPK/ERK kinase; PLCγ, phospholipase Cγ; SBC, sodium bicarbonate cotransporter; SHB, SH2 domain-containing adapter protein B.

VEGFA inhibitors in oncology
Angiogenesis has a key role in maintaining the continued expansion of tumours. VEGFA secreted by tumour cells and surrounding stroma stimulates the proliferation and survival of endothelial cells, leading to the formation of new blood vessels, which may be structurally abnormal and leaky[10,53,54]. *VEGFA* mRNA is overexpressed in most human tumours, where its expression correlates with invasiveness, increased vascular density, metastasis, tumour recurrence and poor prognosis[56]. Accordingly, several strategies to inhibit the VEGFA–VEGFR signalling pathway for the treatment of cancer have been explored[57,58].

Development of bevacizumab. Neutralizing monoclonal antibodies to VEGFA were produced to further investigate the function of this growth factor[59]. In 1993, the mouse antibody A.4.6.1, which specifically recognizes and neutralizes all bioactive isoforms of human, but not mouse, VEGFA, was reported to inhibit the growth of human tumour xenografts in mice in a dose-dependent manner[11]. Further studies confirmed these findings and extended them to additional tumour models[60,61]. These studies produced the first direct evidence that tumour growth depends on angiogenesis and confirmed the importance of VEGFA in this process. Subsequent research revealed that the contribution of VEGFA to tumour angiogenesis in human xenografts in mice was underestimated in the studies using the A.4.6.1 antibody, as VEGFA can also be produced by host stromal cells, which in this case would not be blocked by this antibody[62]. Accordingly, soluble VEGFA receptors[21,62] or cross-species VEGFA-blocking antibodies[63] result in more complete VEGFA inhibition and greater suppression of tumour growth in these hybrid models.

The same antibody was also tested in the ischaemic retinas of adult cynomolgus monkeys, which have been shown to express transcripts encoding $VEGFA_{121}$ and $VEGF_{165}$ (REF. 64). Intravitreal injections of antibody A.4.6.1 into the eyes of cynomolgus monkeys with retinal ischaemia specifically inhibited capillary cell proliferation and vascular leakage, thereby providing proof-of-concept for the role of VEGFA in intraocular neovascularization in primates[15].

Antibody A.4.6.1 was subsequently humanized[65] by transfer of its six complementarity-determining regions into a normal human Ig framework[66]. The resulting recombinant antibody, bevacizumab, retained the same binding characteristics and inhibited the *in vivo* growth of human tumour cell lines with similar potency and efficacy to the original monoclonal antibody[65] and was assessed for use in human clinical trials[67].

FDA approval of bevacizumab in metastatic colorectal cancer. In Phase I clinical trials, bevacizumab monotherapy was generally well tolerated, with no severe (grade III or grade IV) adverse events[68]; typical side effects of bevacizumab were hypertension and mild proteinuria[69]. Preliminary studies also suggested that the addition of bevacizumab to most conventional chemotherapy regimes resulted in clinical improvements in a number of tumour types[70]. Importantly, bevacizumab did not markedly increase toxicity when used in combination with a range of chemotherapeutic agents[70], although subsequent studies revealed infrequent adverse events including gastrointestinal perforations, nephrotic syndrome and arterial thromboembolic complications such as myocardial infarction and stroke, especially in patients with a prior thromboembolic event or of age 65 or older[69,71,72].

The rationale behind combination therapy was to simultaneously target the endothelial cells and the tumour cells, and, indeed, preclinical studies confirmed a synergistic effect between bevacizumab and cytotoxic therapies, in part because VEGFA blockade seems to sensitize the endothelium to the effects of the cytotoxic agents[73–75]. It was also postulated that VEGFA inhibition results in the apoptosis of endothelial cells that are not covered by pericytes and reduces the abnormal tortuosity and hyperpermeability of the tumour vasculature (‘normalization’), thus reducing tumour interstitial pressure and enhancing the delivery of cytotoxic agents[76,77].

In Phase II randomized clinical studies in metastatic colorectal cancer, a combination of bevacizumab with standard first-line treatment 5-fluorouracil (5′-FU) and leucovorin improved treatment response rates compared with using 5′-FU–leucovorin alone and increased progression-free survival (PFS)[78]. Moreover, in a pivotal Phase III clinical trial in 2004 (AVF2107), bevacizumab in combination with irinitecan and a 5′-FU–leucovorin chemotherapy regimen significantly increased treatment response rates, PFS and overall survival (OS) in previously untreated patients with metastatic colorectal cancer, compared with the irinitecan, 5′-FU–leucovorin chemotherapy alone[12] (TABLE 1). Consequently, in February 2004, the FDA approved the use of bevacizumab for the first-line treatment of metastatic colorectal cancer. This was followed by approvals from the European Medicines Agency (EMA) and many other regulatory authorities.

Bevacizumab was also efficacious in second-line metastatic colorectal cancer. In the ECOG E3200 study, the addition of bevacizumab to second-line chemotherapy with FOLFOX4 (5′-FU–leucovorin–oxaliplatin) after tumour progression, improved response rates, PFS and OS[79], a result that led to the approval of bevacizumab for second-line treatment of metastatic colon cancer in June 2006. Additionally, a randomized Phase III study (ML18147) showed that the continued use of bevacizumab with either oxaliplatin- or irinotecan-based therapy beyond the first progression significantly increased PFS and OS, compared with chemotherapy alone. This led to an additional FDA approval in 2013 for the use of bevacizumab in combination with either oxaliplatin- or irinotecan-based chemotherapy for the treatment of patients with metastatic colorectal cancer whose disease has progressed on a first-line bevacizumab-containing regimen[80].

Bevacicumab in other tumour types. The addition of bevacizumab to conventional chemotherapies, either as first-line therapy to treatment-naive patients or second-line treatment to refractory patients, has resulted in significant clinical benefits in various advanced cancers beyond metastatic colorectal cancer (TABLE 1).

In non-squamous non-small cell lung cancer (NSCLC), the ECOG E4599 study reported increased response rates on incorporating bevacizumab with paclitaxel and carboplatin, accompanied by significantly improved PFS and OS[81] (TABLE 1), resulting in FDA regulatory approval in October 2006.

In renal cell carcinomas, inactivating mutations in the *VHL* gene are frequent and lead to *VEGFA* upregulation[35], providing a rationale to target this protein for treatment. Accordingly, bevacizumab monotherapy increased PFS in an early placebo-controlled Phase II study of advanced metastatic renal cell carcinoma[82]. Two Phase III studies, CALGB 90206 and AVOREN, found that the addition of bevacizumab to interferon-α2a (IFNα2a) significantly improved PFS[83,84] (TABLE 1), supporting this combination as first-line treatment in patients with metastatic renal cell carcinoma. The combination treatment was subsequently approved for the treatment of this disease by the FDA in July 2009.

Table 1 | **Selected Phase III clinical trial data for therapies targeting the VEGFA pathway in advanced cancer**

Clinical trial (patient population)	Treatment	Response rate (%)	Median PFS (months)	Median OS (months)	Refs
Metastatic colorectal cancer					
AVF2107	1st line bevacizumab + IFL	44.8	10.6	20.3	12
	1st line IFL only	34.8	6.2	15.6	
	Comparison	Difference = 10.0; $p = 0.004$	HR = 0.54; $p < 0.001$	HR = 0.62; $p < 0.001$	
ECOG E3200	2nd line bevacizumab + FOLFOX4	22.6	7.3	12.9	79
	FOLFOX4 only	8.6	4.7	10.8	
	2nd line bevacizumab monotherapy	3.3	2.7	10.2	
	Comparison*	Difference = 14.0; $p < 0.001$	HR = 0.61; $p < 0.0001$	HR = 0.75; $p = 0.0011$	
ML18147	1st line bevacizumab + FOLFIRI	57.9	5.7	11.2	80
	1st line bolus IFL + bevacizumab	53.3	4.1	9.8	
	Comparison	Difference = 4.6; NS	HR = 0.68; $p < 0.0001$	HR = 0.81; $p = 0.0062$	
Horizon III	1st line cediranib + FOLFOX6	46.3	9.9	22.8	129
	1st line bevacizumab + FOLFOX6	47.3	10.3	21.3	
	Comparison	Difference = 1.0; NS	HR = 1.10; $p = 0.119$	HR = 0.95; $p = 0.541$	
VELOUR	2nd line aflibercept + FOLFIRI	19.8	6.90	13.50	135
	2nd line FOLFIRI	11.1	4.67	12.06	
	Comparison	Difference = 8.7; $p = 0.0001$	HR = 0.758; $p < 0.0001$	HR = 0.817; $p = 0.0032$	
CORRECT	2nd line regorafenib	1.0	1.9	6.4	125
	Placebo	0.4	1.7	5.0	
	Comparison	Difference = 0.6; $p = 0.19$	HR = 0.49; $p < 0.0001$	HR = 0.77; $p = 0.0052$	
PRAISE	2nd line ramucirumab + FOLFIRI	13.4	5.7	13.3	145
	2nd line placebo + FOLFIRI	12.5	4.5	11.7	
	Comparison	Difference = 0.9; $p = 0.63$	HR = 0.79; $p = 0.0005$	HR = 0.84; $p = 0.0219$	
Gastroesophageal cancer					
REGARD	2nd line ramucirumab	3.4	2.1	5.2	142
	Placebo	2.6	1.3	3.8	
	Comparison	Difference = 0.8 (ORR)	HR = 0.483; $p < 0.0001$	HR = 0.776; $p = 0.047$	
RAINBOW	2nd line ramucirumab + paclitaxel	27.9	4.4	9.6	143
	Placebo + paclitaxel	16.1	2.9	7.4	
	Comparison	Difference = 11.8; $p = 0.0001$ (ORR)	HR = 0.635; $p < 0.0001$	HR = 0.807; $p = 0.017$	
Non-small-cell lung cancer					
ECOG E4599	1st line bevacizumab + paclitaxel and carboplatin	35	6.2	12.3	81
	1st line paclitaxel and carboplatin	15	4.5	10.3	
	Comparison	Difference = 20; $p < 0.001$	HR = 0.66; $p < 0.001$	HR = 0.79; $p = 0.003$	
AVAIL	1st line bevacizumab (7.5 mg per kg or 15 mg per kg) + cisplatin + gemcitabine	37.8 (7.5 mg per kg)	14.1	13.6 (7.5 mg per kg)	277
	1st line cisplatin + gemcitabine + placebo	21.6	12.3–16.9	13.1	
	Comparison	Difference = 16.2; $p < 0.0001$	HR = 0.94; $p = 0.553$	HR = 0.93; $p = 0.420$	

Table 1 (cont.) | **Selected Phase III clinical trial data for therapies targeting the VEGFA pathway in advanced cancer**

Clinical trial (patient population)	Treatment	Response rate (%)	Median PFS (months)	Median OS (months)	Refs
Non-small-cell lung cancer (cont.)					
VITAL	2nd line aflibercept + doxacetal	23.3	5.2	10.1	138
	2nd line placebo + doxacetal	8.9	4.4	10.4	
	Comparison	Difference = 14.4; $p<0.01$	HR = 0.82; $p=0.0035$	HR = 1.01; $p=0.90$	
REVEL	2nd line ramucirumab + docetaxel	22.9	4.5	10.5	144
	2nd line placebo + docetaxel	13.6	3.0	9.1	
	Comparison	Difference = 9.3 (ORR)	HR = 0.76; $p<0.0001$	HR = 0.86; $p=0.023$	
LUME Lung 1	2nd line nindedanib + doxacetal	NR	3.4	10.9	133
	2nd line placebo + doxacetal	NR	2.7	7.9	
	Comparison	NR	HR = 0.79; $p=0.0019$	HR = 0.75; $p=0.0073$	
Metastatic renal cell carcinoma					
CALGB90206	1st line bevacizumab + IFNα2a	25.5	8.5	NR	83
	1st line IFNα2a + placebo	13.1	5.2	NR	
	Comparison	Difference = 12.4; $p<0.0001$	HR = 0.71; $p<0.0001$	NR	
NCT00083889	1st line sunitinib	47	11	26.4	117, 278
	1st line IFNα2a only	12	5	21.8	
	Comparison	Difference = 35; $p<0.001$	HR = 0.42; $p<0.001$	HR = 0.821; $p=0.051$	
TARGET	2nd line placebo, crossover to sorafenib	NA	NA	17.8	279
	Placebo	NA	NA	14.3	
	Comparison	NA	NA	HR = 0.78; $p=0.0287$	
AVOREN	1st line bevacizumab + IFNα2a	31	10.2	23.3	84
	1st line IFNα2a + placebo	13	5.4	21.3	
	Comparison	Difference = 18; $p<0.001$	HR = 0.63; $p<0.001$	HR = 0.86; $p=0.1291$	
Glioblastoma multiforme					
AVAglio	1st line bevacizumab + radiotherapy and tomozolomide	NR	10.6	16.8	87
	1st line radiotherapy and tomozolomide	NR	6.2	16.7	
	Comparison	NR	HR = 0.64; $p<0.01$	HR = 1.02; $p=0.10$	
RTOG0825	1st line bevacizumab + radiotherapy and tomozolomide	NR	10.7	15.7	88
	1st line radiotherapy and tomozolomide	NR	7.3	16.1	
	Comparison	NR	HR = 0.79; $p=0.007$	HR = 1.13; $p=0.21$	
Persistent, recurrent or metastatic cervical cancer					
GOG240	1st line bevacizumab + paclitaxel and cisplatin or paclitaxel and topotecan	48	8.2	17.0	89
	Paclitaxel and cisplatin or paclitaxel and topotecan	36	5.9	13.3	
	Comparison	Difference = 12; $p=0.008$	HR = 0.67; $p=0.002$	HR = 0.71; $p=0.004$	
Ovarian cancer					
AURELIA (platinum resistant)	2nd line chemotherapy + bevacizumab	27.3	6.7	16.16	90
	Chemotherapy only	11.8	3.4	13.3	
	Comparison	Difference = 15.5; $p=0.01$	HR = 0.48; $p<0.01$	HR = 0.85; $p<0.174$	

Table 1 (cont.) | **Selected Phase III clinical trial data for therapies targeting the VEGFA pathway in advanced cancer**

Clinical trial (patient population)	Treatment	Response rate (%)	Median PFS (months)	Median OS (months)	Refs
Ovarian cancer (cont.)					
GOG0218 (stage III or IV)	1st line chemotherapy only	NR	10.3	39.3	92
	1st line chemotherapy + bevacizumab initiation	NR	11.1	38.7	
	1st line chemotherapy + bevacizumab maintenance	NR	14.1	39.7	
	Comparison‡	NR	HR = 0.9; $p < 0.16$	HR = 1.078; NS	
	Comparison§	NR	HR = 0.717; $p < 0.01$	HR = 0.885; NS	
Hepatocellular carcinoma					
SHARP (naive to systemic therapy)	1st line sorafenib	NR	4.1	10.7	112
	Placebo	NR	4.9	7.9	
	Comparison	NR	HR = 1.08; $p = 0.77$	HR = 0.69; $p < 0.001$	

FOLFIRI, irinotecan, fluorouracil (5′-FU) and folinic acid; FOLFOX4, 5′-FU–leucovorin–oxaliplatin; IFL, irinotecan, 5′-FU and leucovorin; iFN, interferon; NA, not applicable; NR, not reported; NS, not significant; PFS, progession-free survival; ORR, objective response rates; OS, overall survival. *Bevacizumab and FOLFOX4 versus FOLFOX only. ‡1st line chemotherapy only versus 1st line chemotherapy + bevacizumab initiation. §1st line chemotherapy only versus 1st line chemotherapy + bevacizumab maintenance.

Bevacizumab monotherapy was also efficacious in recurrent glioblastoma multiforme (GBM), with response rates in 20% to 25% of patients[85,86], resulting in FDA approval in 2009. In two Phase III studies, AVAglio[79] and RTOG0825 (REF. 80), involving newly diagnosed GBM patients, PFS, but not OS, were improved with the combination of bevacizumab with radiotherapy and temozolomide compared with radiotherapy and temozolomide alone[87,88].

Bevacizumab is also efficacious in some difficult to treat gynaecological malignancies. A Phase III study (GOG240) in patients with advanced cervical cancer found PFS and OS improvements when bevacizumab was combined with two different chemotherapy regimens[89], leading to FDA approval in August 2014. Significant increases in PFS and a trend to improved OS in the Phase III study AURELIA[90] led to FDA approval of bevacizumab, in combination with chemotherapy, for platinum-resistant ovarian cancer in November 2014. In addition, in a placebo-controlled, randomized Phase III study (OCEANS), bevacizumab plus chemotherapy significantly increased response rates and PFS in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal or fallopian tube cancer, compared with chemotherapy alone[91]. OS was not increased, but similar to other trials[92], patient crossover to subsequent therapies (bevacizumab or other agents) complicates the assessment of the survival effects of bevacizumab[91].

Bevacizumab also significantly increased PFS in a large randomized study in patients with stage III or stage IV ovarian cancer who had undergone debulking surgery (GOG0218)[92]. Patients were randomized into three groups: chemotherapy alone, chemotherapy plus bevacizumab, chemotherapy plus bevacizumab plus maintenance and bevacizumab monotherapy for up to 15 months. The greatest PFS benefit was observed in the group that received maintenance bevacizumab, emphasizing the need for long-term inhibition of angiogenesis[92].

In addition, in vestibular schwannomas associated with neurofibromatosis type 2 (benign tumours that result in profound hearing loss), bevacizumab administration significantly reduced tumour size, associated with hearing improvement or stabilization[93].

However, not all tumour types or settings have received significant benefit from bevacizumab, or other anti-VEGFA approaches. For example, the FDA approved the use of bevacizumab in combination with paclitaxel in February 2008 for the treatment of metastatic HER2-negative breast cancer, and, although response rates and PFS were improved compared with paclitaxel alone[94], subsequent studies, using first- or second-line bevacizumab, reported smaller improvements in PFS[95–97]. This resulted in the FDA revoking the accelerated approval for bevacizumab in metastatic breast cancer in 2011.

In addition, little or no benefit from adding bevacizumab (or other anti-VEGF agents) to standard of care was observed in both prostatic and pancreatic cancer[98].

Furthermore, in two large randomized Phase III studies (NSABP C-08 (REFS 99,100) and AVANT[101]), the administration of bevacizumab for 1 year (the initial 6 months in conjunction with adjuvant chemotherapy) after colon cancer resection did not improve disease-free survival at 3 years compared with chemotherapy alone, which is in contrast to the metastatic setting[102]. However, in both C-08 (REF. 99) and AVANT[101] studies, a significant benefit was observed during bevacizumab exposure, raising the possibility that a longer treatment duration may achieve a better outcome. However, in the AVANT study OS data suggested a potential detrimental effect in the bevacizumab groups, especially in combination with oxaliplatin-based chemotherapy[101]. Although no detrimental effects were seen in the NSABP C-08 study[100], there has been reluctance in pursuing further adjuvant studies with bevacizumab.

Small molecule RTKIs. In addition to using monoclonal antibodies[11], alternative approaches of inhibiting the VEGFA–VEGFR pathway for the treatment of cancer have been explored[103]. Small molecule inhibitors of VEGFR2 were first reported in 1996 (REF. 104). These early generation molecules, which belonged to the tyrphostin family[105], inhibited VEGFA-dependent VEGFR2 autophosphorylation and several biological activities of VEGFA[104,105]. The elucidation of the crystal structure of the VEGFR2 kinase domain[106] enabled the development of other families of small-molecule VEGFR RTKIs, including the 4-anilinoquinazolines and the 3-substituted indonilones (reviewed in REF. 105). In addition to VEGFRs, these molecules inhibit other structurally related RTKs, typically PDGF receptors, cKIT, FLT3 and macrophage colony-stimulating factor 1 receptor (CSF1R), with various degrees of selectivity[107]. Some of these small molecules can also inhibit structurally unrelated RTKs, including EGFR, TIE2, cMET, RET and fibroblast growth factor receptors[107]. Therefore, the antitumour activity of these molecules potentially reflects the contribution of inhibition of multiple targets in the microenvironment and, in some cases, also direct effects on tumour cell growth[107]. In addition to the aforementioned effects of VEGFA inhibition (hypertension and proteinuria), adverse effects include fatigue, diarrhoea, thrombocytopenia, skin and hair discoloration, and hand and foot syndrome. Numerous VEGFR RTKIs entered clinical trials in the early 2000s, with semaxanib (SU5416; SUGEN) and vatalanib (PTK/787; Bayer, Novartis) representing some of the first to be clinically developed[108]. However, Phase III trials in patients with previously untreated colorectal cancer, in combination with chemotherapy, failed to show a survival benefit, leading to eventual discontinuation of both molecules. Other molecules had greater success, including sorafenib, sunitinib, pazopanib (Votrient; GlaxoSmithKline) and axitinib (Inlyta; Pfizer) (TABLE 1 and below).

Initial studies of sorafenib, which was initially characterized as a RAF kinase inhibitor and then shown to inhibit VEGFR2 autophosphorylation[109], demonstrated its limited toxicity and promising efficacy in metastatic renal cell carcinoma[110]. The Phase III TARGET study in patients with metastatic renal cell carcinoma reported that sorafenib increased the median PFS[111] and OS[20]. As a result, patients previously treated with placebo were crossed over to receive sorafenib during the trial[20], and the drug obtained FDA approval in 2005 for in the treatment of cytokine-refractory metastatic renal cell carcinoma. Sorafenib was also approved for the treatment of advanced hepatocellular carcinoma[112] in November 2007 and thyroid cancer[113] in November 2013. Three more VEGFR RTKIs, cabozantinib (Cometriq; Exelixis), vandetanib (Caprelsa; Astra Zeneca) and lenvantinib (E7080; Eisai), have been approved for thyroid cancer, based in part on their ability to inhibit the RTK RET[114].

Sunitinib, a broad-spectrum multi-targeted oral RTKI, prevented endothelial cell proliferation and neovascularization in a variety of human tumour lines in xenograft models[115]. A Phase I study showed significant but manageable toxicity and some clinical benefit in a range of different tumours[116]. In a Phase III study in previously untreated patients with metastatic renal cell carcinoma, first-line sunitinib treatment more than doubled PFS and increased response rates, compared with IFNα2a[117]. Consequently, the FDA and EMA approved sunitinib in February 2007 and January 2007, respectively, for the treatment of metastatic renal cell carcinoma.

The rare pancreatic neuroendocrine tumours (PNET) — highly vascularized malignancies that develop in pancreatic endocrine cells — could potentially benefit from anti-angiogenic therapy[118]. Indeed, in a Phase III study, sunitinib monotherapy significantly increased PFS in patients with PNET compared with best supportive care, resulting in FDA approval in May 2011 (REF. 119).

Monotherapy with the VEGFR RTKI pazopanib has proved efficacious in locally advanced or metastatic renal cell carcinoma[120] (TABLE 1), and it exhibits an improved safety profile compared with sunitinib[121]. It was approved by the FDA in October 2009 and by the EMA in June 2010 for first- and second-line treatment of advanced renal cell carcinoma.

In addition, axitinib, which has been reported to be more selective for VEGFRs than sunitinib[122], significantly improved PFS compared with sorafenib in second-line treatment of metastatic renal cell cancinoma[123]. This AXIS study led to the FDA approval of axitinib in January 2012 for the treatment of metastatic renal cell carcinoma that is refractory to sunitinib treatment.

The broad-spectrum RTKI and RAF kinase inhibitor regorafenib (Stivarga; Onyx, Bayer)[124] is the only kinase inhibitor to be approved by the FDA as a monotherapy for previously treated metastatic colorectal cancer (February 2013) following improved OS in the CORRECT placebo-controlled Phase III study[125].

In contrast to their overall success as monotherapies, VEGFR RTKIs in combination with cytotoxic agents have proved disappointing in breast, lung and colorectal cancer. For example, in metastatic breast cancer patients, the primary endpoint of improved PFS was not met in the SUN1064 and SUN1099 Phase III trials studying sunitinib in combination with docetaxol or as second-line therapy with capecitabine[126,127]. Similarly, in a Phase III study comparing sunitinib plus FOLFIRI (irinotecan, 5′-FU and folinic acid) to placebo plus FOLFIRI in previously untreated metastatic colorectal cancer, PFS in the sunitinib arm was not superior to the control arm and had a considerably higher incidence of adverse events[128]. In addition, in the HORIZON III study[129], in which cediranib (Recentin, AstraZeneca) was combined with FOLFOX6 and compared with bevacizumab plus FOLFOX6 in previously untreated metastatic colorectal cancer, PFS and OS were similar in the two arms, but the pre-specified boundary for PFS non-inferiority was not met and the safety profile with cediranib also appeared less favourable[129].

These results underscore the difficulty in combining cytotoxic chemotherapy with VEGFR RTKIs. It is conceivable that the toxicity of the RTKIs is additive to that of cytotoxic agents, limiting patient compliance and resulting in under-treatment. Also, preclinical studies testing high doses of VEGFR RTKIs have reported increased tumour aggressiveness and metastasis[130]. However, a recent study found no evidence of accelerated tumour growth in renal cell carcinoma patients treated with sunitinib[131].

An apparent exception to this is nintedanib (Ofev; Boehringer Ingelheim Pharmaceuticals), a VEGFR–PDGFR–FGFR RTKI[132], which recently demonstrated an OS benefit in patients with NSCLC in combination with doxacetal, compared with doxacetal alone (LUME Lung 1 study) in second-line therapy[133], leading to its approval by the EMA in November 2014. Nindedanib also resulted in clinical improvement in patients with idiopathic pulmonary fibrosis, a fatal non-neoplastic lung disease in which VEGFRs, PDGFRs and FGFRs have been implicated[134], gaining FDA and EMA approval for this indication.

Protein inhibitors. In addition to bevacizumab and small molecule RTKIs, two protein inhibitors of the VEGFA pathway have been approved for cancer therapy: aflibercept, a recombinant VEGFR fusion protein that binds to, and inhibits, VEGFA, VEGFB and PlGF[21]; and ramucirumab, a fully human monoclonal antibody that inhibits VEGFR2 (REF. 22).

Aflibercept was as effective as bevacizumab in the Phase III VELOUR trial on second-line metastatic colorectal cancer, although a greater incidence of adverse events was reported[135]. When used in combination with FOLFIRI, aflibercept improved median PFS and OS times compared with FOLFIRI and placebo treatments. Aflibercept received FDA approval for previously treated metastatic colorectal cancer in August 2012. However, in a large randomized Phase II study in patients with previously untreated metastatic colorectal cancer (AFFIRM trial), aflibercept in combination with FOLFOX6 did not improve PFS relative to FOLFOX6 alone[136]. Also, aflibercept monotherapy did not meet a 6-month PFS endpoint in a Phase II study in recurrent malignant glioma patients, in part because of patient attrition due to toxicity[137]. In addition, aflibercept in combination with doxacetal did not improve OS compared with doxacetal alone in a Phase III study in patients with advanced NSCLC (VITAL trial)[138]. These findings suggest that targeting PlGF and VEGFB as well as VEGFA may not confer a significant clinical advantage[139]. Indeed, the role of PlGF in tumour angiogenesis and its significance as a therapeutic target remain controversial[140,141].

Over the past few years, ramucirumab has shown efficacy in multiple tumour types, resulting in three FDA approvals. Ramucirumab significantly increased OS in patients with advanced gastric or gastro-oesophageal junction adenocarcinoma in two international multicentric Phase III studies, REGARD[142] and RAINBOW[143], and was approved by the FDA for this indication in 2014. In the same year, ramucirumab also received approval for the treatment of advanced NSCLC, following the REVEL Phase III study, which showed increased OS and PFS when used in combination with doxacetal versus doxacetal alone[144]. Most recently (April 2015), ramucirumab received FDA approval for the treatment of patients with metastatic colorectal cancer that progressed during or after first-line treatment with bevacizumab (RAISE study), in combination with second-line FOLFIRI[145], representing the fourth VEGFA pathway inhibitor to be approved for this indication.

Targeting VEGFA in combination with other angiogenic inhibitors. Targeting VEGFA and other pathways implicated in angiogenesis, simultaneously or sequentially, should theoretically result in more effective tumour growth inhibition.

One such approach is the use of sequential treatments with VEGFA inhibitors and inhibitors of mammalian target of rapamycin (mTOR) such as everolimus (Afinitor Disperz; Novartis) and temsirolimus (Torisel; Pfizer). Indeed, the use of everolimus increased PFS in patients with metastatic renal cell carcinoma who became refractory to VEGFA-targeted therapies[146].

Recent studies have shown that inhibitors of cMET, an RTK that has been implicated in angiogenesis as well as in epithelial–mesenchymal transition (EMT) and other aspects of tumorigenesis, markedly enhance the efficacy of VEGFA inhibition in preclinical tumour models[147]. In particular, cMET has been reported to be the key mediator of invasiveness and EMT in GBM cells following VEGFA blockade[148]. However, adding onartuzumab (MetMab; Roche), a cMET-blocking antibody, did not provide any additional benefit relative to bevacizumab monotherapy in patients with GBM[149]. The reasons for these disappointing results remain unclear, but recent studies in different clinical settings have cast some doubt on the significance of cMET (and of its ligand, hepatocyte growth factor) as a broad therapeutic target in human tumours[150].

Following the report that PlGF mediates angiogenic escape and resistance to anti-VEGFR2 antibody treatment in some tumour models[140], the combination of bevacizumab with the humanized anti-PIGF monoclonal antibody TB403 (RO5323441; Thrombogenics; Bioinvent; Roche) has been clinically explored in patients with multiple tumour types. However, so far only a study in patients with GBM has been published, which indicates a lack of additional benefit from the combination, relative to bevacizumab alone[151]. The clinical programmes combining TB403 with bevacizumab have been discontinued, but the same anti-PlGF antibody is now being tested in medulloblastoma patients. This is following a study showing that, in this context, PlGF promotes tumour growth by a non-angiogenic mechanism, involving direct stimulation of tumour cell growth through a NRP1-dependent pathway[152].

A potentially promising combination is the use of agents targeting the angiopoietin (ANG)–TIE2 axis — a signalling system involved in multiple physiological and pathological processes, including blood vessel sprouting and maintenance, lymphangiogenesis, recruitment of myeloid cells and metastasis[153,154], as preclinical studies have shown marked additivity with VEGFA inhibitors in various tumour models[155]. Clinical trials combining VEGFA blockers with inhibitors of one of the key TIE2 ligands, ANG2, in cancer as well as in ophthalmology are ongoing[154].

Furthermore, clinical trials combining bevacizumab with antibodies targeting NRP1 (REF. 156) or EGF-like protein 7 (EGFL7), an ECM protein that is implicated in endothelial cell survival and in vascular morphogenesis[157], have been initiated but no results have yet been published.

Challenges in the development and use of VEGFA inhibitors in oncology. The use of VEGFA inhibitors has validated VEGFA as an important clinical target and has shown considerable benefit in patients with advanced cancers with limited treatment options. However, despite the overall success of these inhibitors, it is unclear why some patients and some tumour types have a limited response. Although the responsiveness of renal cell carcinoma to VEGFA inhibitors has a well-defined molecular basis, the reasons for the greater and more consistent benefit in metastatic colon cancer compared with breast cancer, for example, remain unclear.

A key question is how to identify those patients who would receive the maximum benefit from anti-VEGFA therapies. The identification of specific predictive biomarkers therefore remains a major goal. Potential biomarkers include intratumoural and plasma VEGFA levels, as well as KRAS and BRAF status, which, while prognostic, are not predictive of response to bevacizumab treatment[158,159]. Many predictive biomarkers for VEGFA inhibitors, including hypertension[160], tumour imaging[161], pro-inflammatory cytokines[162–164], soluble VEGFA receptors[165], gene signatures[166] and polymorphisms in VEGFA pathway genes[167], have been suggested on the basis of small patient series or retrospective analyses, but none has yet been validated. This may reflect the complexity of a process such as angiogenesis that is influenced by multiple factors within the microenvironment[168], as opposed to measuring tumour-intrinsic changes such as oncogene mutations or amplifications. Therefore, biomarkers that are predictive of anti-VEGFA efficacy may be specific to different tissues and tumour subtypes. In this context, a recent retrospective analysis of the placebo-controlled Phase III study AVAglio suggested that patients with proneural GBM, but not with other subtypes, have a survival benefit from bevacizumab therapy[169].

Many patients progress despite anti-VEGFA therapy, which is indicative of drug resistance. However, the mechanisms seem to be inherently different from those typically occurring during treatment with inhibitors of well-defined oncogenic pathways that render a drug ineffective (that is, selection of pre-existing or acquired mutations in the target or in the pathway)[170]. So far, there is no convincing evidence showing that mutations in VEGFA or its receptors underlie drug resistance. The finding that continued administration of bevacizumab beyond progression still results in a small but significant OS benefit in metastatic colorectal cancer[80] suggests that the resistance is of a reversible nature and raises the possibility of re-treating with the same or an alternative VEGFA inhibitor. Indeed, it has been postulated that such plasticity may be mediated by the dynamic nature of the tumour micro-environment[171]. Preclinical studies have implicated haematopoietic growth factors (including granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage CSF (GM-CSF) and stromal cell-derived factor 1 (SDF1)) and the resulting tumour infiltration of myeloid and other pro-inflammatory cell types in the induction of VEGFA-independent angiogenic signals[147,164,172–175]. More work is clearly needed to determine whether these observations are clinically relevant.

Studies in a transgenic model of PNET have indicated that treatment with anti-VEGFR2 antibodies or other VEGFA pathway inhibitors increases tumour invasiveness and metastasis, likely mediated by hypoxia, cMET upregulation and EMT[176]. However, other studies have been unable to confirm these findings in this or other tumour models[155,177–179]. The reasons for these conflicting results remain unclear, but an analysis of multiple clinical trials with bevacizumab did not find any evidence of increased metastasis or tumour rebound after therapy discontinuation[180]. These and other findings emphasize the challenges in designing and interpreting preclinical efficacy studies and the need to develop more predictive animal models in oncology[181].

VEGFA inhibitors in ophthalmology

Retinal ischaemia is frequently associated with pathological neovascularization, with the resultant oedema and haemorrhage producing vision loss[182]. Prototypical diseases include diabetic retinopathy (DR) and retinal vein occlusion (RVO). Beginning in the 1940s, experimental and clinical data led investigators to postulate that a hypothetical diffusible substance produced in ischaemic retina, termed factor X[183–185], was causal for pathological ocular neovascularization. It was not until the 1990s that multiple lines of evidence converged on VEGFA as the sought after factor X. VEGFA is produced in human and non-human primate retina[186,187], and its levels increase when the retina becomes ischaemic[187]. VEGFA levels in ocular fluids are temporally and spatially associated with experimental neovascularization[187], and blocking VEGFA potently suppresses pathological vessel growth[15,16]. In patients with retinal ischaemia, eyes with neovascularization had increased VEGFA levels in ocular fluids[13,14,188]; and in normal non-human primate eyes, VEGFA injections recapitulated the retinal vascular pathology and ocular neovascularization seen in human disease[55,189].

Experimental data have also highlighted the critical role of VEGFA in non-ischaemic vascular disease, most importantly choroidal neovascularization[190,191], which characterizes neovascular AMD, and diabetic blood–retina barrier breakdown[192], the central pathology of diabetic macular oedema (DME).

Taken together, these data supported the testing of VEGFA inhibitors in a range of ophthalmologic conditions, including neovascular AMD, DME and RVO[193]. Pegaptanib, the first anti-VEGFA aptamer for an ocular disease, was approved in 2004 for neovascular AMD, based on the VISION trials, which found that it was associated with reduced vision loss compared with sham injection[194]. However, although the product is still marketed, it has been largely supplanted by newer, more effective agents.

Rationale for the development of ranibizumab. Targeting VEGFA in ophthalmology has presented several challenges, including the optimal route of administration and the ocular and systemic safety of the treatment. Despite a lack of evidence of major systemic toxicity from the preclinical use of intravenous bevacizumab[195], there were theoretical concerns given its long half-life in the circulation. It was also unclear whether repeated intravitreal injections are safe for patients, although there were some data from patients receiving anti-viral drugs[196], and intravitreal anti-VEGFA injections in the non-human primate model were found to be both safe and efficacious[15].

Another concern was the presence of a size-dependent barrier that could potentially limit the ability of bevacizumab to enter and cross the retina[197]. Indeed, bevacizumab Fab fragments (which are derived by digesting the antibody with enzymes) diffused more rapidly through the retina in rhesus monkeys than whole antibodies[197,198]. A Fab fragment also has the theoretical advantages of minimizing the potential toxicity of Fc antibody fragments[199], as well as exhibiting a significantly shorter systemic half-life[200], which is desirable as locally administered drugs eventually enter the systemic circulation[201]. These factors, including the necessity to potently neutralize VEGFA using a small injected volume, led to the development of ranibizumab, a high-affinity Fab fragment that could be highly concentrated for injection[202,203]. Five to 30 times more potent than bevacizumab, ranibizumab neutralized the biological activities of all VEGFA isoforms[18], and possessed a favourable pharmacokinetic profile with effective biological concentrations being present in the eye for up to 1 month or more, but with 1,000-fold to 2,000-fold lower levels being present in the systemic circulation[204]. A key reason for the latter was the removal of the Fc region, which prevents recycling of the antibody through the circulation via FcRn[200].

Ranibizumab clinical trials and FDA approval. Ranibizumab and other anti-VEGFA inhibitors have had a substantial impact in ophthalmology (BOX 1). A Phase I study demonstrated the safety and tolerability of a single intravitreal dose of ranibizumab[205], and a subsequent Phase I/II study in neovascular AMD showed that it has a good safety profile, offers improved visual acuity and decreases leakage from choroidal neovascularization[206]. Accordingly, in the Phase III MARINA trial of occult choroidal neovascularization (a type of neovascularization with angiographically indistinct margins), patients receiving monthly intravitreal injections of ranibizumab experienced significantly improved visual acuity compared to sham-injected patients, even following the first treatment[207] (TABLE 2). The incidence of serious adverse events was low, and quality of life was improved[207]. In a second Phase III trial, ANCHOR, ranibizumab was found to be superior to verteporfin in classic neovascular AMD (neovascularization with distinct angiographic margins), resulting in significantly greater improvements in visual acuity[208] and prevention of vision loss in 96.4% of patients. Monthly ranibizumab injections were well tolerated and the visual gains were maintained[209]. In addition, near vision, reading speed and overall quality of life were improved[210].

Clinical trials have also explored the efficacy of less frequent ranibizumab dosing. Results from the Phase IIIb PIER[211] and

Box 1 | **Impact of VEGFA inhibitors used in ophthalmology**

Age-related macular degeneration (AMD) and diabetic macular oedema (DME) are global health problems. AMD, a major cause of blindness worldwide, affects 10 to 13% of adults older than 65 in North America, Europe, Australia and Asia[247]. It is estimated that, globally, 196 million people will have some form of AMD in the year 2020 (REF. 248). Neovascular, or wet, AMD, accounts for only 10 to 20% of the cases of AMD but is responsible for much of the severe vision loss associated with the condition[249]. Wet AMD affected 1.75 million people in the United States in 2004 and is expected to reach 3 million by 2020 (REF. 250). Based on a pooled analysis of population studies around the world, diabetic retinopathy (DR) was estimated to have affected 21 million people globally in 2010 (REF. 251).

DME is increasing as the prevalence of diabetes is expected to rise by more than 50% from 2000 to 2030 (REF. 252). The increase in AMD and DME therefore has the potential to reduce the quality of life of an increasing number of individuals, with major social and economic implications. Retinal vein occlusion (branch and central) is estimated to affect more than 16 million people globally and is the second most common cause of vision loss due to retinopathies. The incidence increases with age, typically affecting people older than 50, and other risk factors include diabetes and hypertension[253].

With the discovery of the causal role of vascular endothelial growth factor A (VEGFA) in ocular neovascularization and vascular permeability in the 1990s, VEGFA inhibitors were developed for clinical use in ophthalmology. These have transformed the treatment of AMD, DME and other ischaemia-related retinopathies. Prior standard treatments for neovascular AMD relied on phototherapy with verteporfin (AMD) and thermal laser (AMD, DME and DR), which decreased the rate of vision loss, but had limited ability to restore vision. By contrast, the anti-VEGFA approach improved visual acuity in the average patient. Patients can also be treated on an as needed basis, reducing the number of clinical procedures and doctor's office visits.

Ranibizumab was approved for the treatment of AMD in 2006, following the success of the Phase III (ANCHOR)[208,209] and MARINA[207] trials, which showed that ranibizumab not only reduced vision loss but also improved visual acuity. By 2010, an estimated 450,000 AMD patients had been treated with ranibizumab[10], and its use has resulted in a 50% reduction in blindness due to AMD reported over 6 years, slowing down of vision loss and an improved quality of vision in patients (reviewed in REFS 10,197). Moreover, in southeast Scotland, the rate of blindness attributable to AMD was reduced from 9.1 to 4.8 cases per 100,000 in the period from 2006 to 2011 (REF. 254). Large numbers of people have also been treated with bevacizumab off-label. The results of the AURA Study, an international retrospective study in 6 European countries, Canada and Venezuela, indicate that visual acuity improvements are not maintained after 2 years in clinical practice settings. This may be due to insufficient treatment since mean change in VA at year 2 correlated with the number of injections administered over the 2-year treatment period[234]

The success of ranibizumab in a variety of pivotal clinical studies has led to its approval for the treatment of other conditions as well: DME, (Phase III RESTORE trial[255] and Phase III RISE and RIDE trials[218]); branch retinal vein occlusion (Phase III BRAVO trial[220]); central retinal vein occlusion (Phase III CRUISE trial[220]), choroidal neovascularisation (Phase III MARINA[207], ANCHOR[209] and HARBOR trials[213]); and DR with DME (Phase III RISE and RISE trials[218]).

Additional VEGFA inhibitors have been developed and showed good results for a range of eye conditions. Pegaptanib, which specifically targets $VEGF_{165}$, was the first aptamer to be licensed for use in humans, specifically for use in neovascular AMD, in 2004[194]. Although results from the VISION trial demonstrated its efficacy[256], they were less impressive than those subsequently reported for ranibizumab and aflibercept in neovascular AMD. Aflibercept is a chimeric immunoglobulin G (IgG) Fc fusion protein, combining Ig-like domain 2 of VEGF receptor 1 (VEGFR1) and domain 3 of VEGFR2 (REF. 21). Aflibercept gained FDA approval for the treatment of neovascular AMD (Phase IIIVIEW 1 and VIEW 2 studies[231]); central retinal vein occlusion (Phase III COPERNICUS[257] and GALILEO trials[258]); DME (Phase III VIVID and VISTA trials[259]); and DR with DME (Phase III VIVID and VISTA trials)[259].

EXCITE[212] trials indicated that monthly injections of ranibizumab were more effective than quarterly injections (TABLE 2). However, the HARBOR study, which tested dosing on an as-needed basis, reported that 0.5 mg ranibizumab administered as needed, resulted in more clinically meaningful improvements in vision than when given monthly, requiring only 7.7 treatments over 12 months[213] (TABLE 2). These data led to the FDA approval of less-than-monthly dosing with ranibizumab. Higher monthly doses in HARBOR resulted in no additional visual acuity gains or adverse events[213]. More recently, results from a 'treat-and-extend' study, in which the treatment interval is gradually extended depending on the patient response, reported comparable results between monthly and progressively extended treatment intervals[214].

Bressler *et al.* recently modelled visual acuity outcomes in patients with neovascular AMD in the US population, based on data from the ranibizumab Phase III trials. Their analysis determined that ranibizumab has the potential to reduce the rate of legal blindness from neovascular AMD over 2 years by 72%[215]. Given the epidemiological importance of neovascular AMD in the United States and elsewhere, the potential impact of ranibizumab therapy on worldwide blindness is significant.

Subsequent large randomized clinical trials have demonstrated the efficacy of ranibizumab in several other vision-threatening diseases (BOX 1), resulting in the FDA approval for RVO in June 2010, for DME in August 2012 and for DR in patients with DME in February 2015. Ranibizumab also received EMA approval for the treatment of myopic choroidal neovascularization in July 2013. As in AMD, the average patient in the DME and RVO trials gained vision with monthly ranibizumab therapy, which were early and sustained over time[216–226]. In addition, data from the Diabetic Retinopathy Clinical Research Network (DRCR.net)[219,224–226] and SHORE[227] trials showed that as-needed dosing improved vision[226]. The DRCR trial also demonstrated that the need for therapy declined over time, with the average DME patient requiring 8–9 injections in the first year, 2–3 injections in the second year, 1–2 injections in the third year and 0–1 injections in years 4 and 5, with sustained gains in visual acuity after 24 and 36 months[224,225]. In the RISE and RIDE trials, approximately a quarter of DME patients were able to discontinue therapy after 3 years, suggesting that ranibizumab may be disease modifying[221] (TABLE 2).

Other VEGFA inhibitors in ophthalmology. Intravitreal bevacizumab has been used off-label in ophthalmology, initially because of the lack of availability of ranibizumab and later because of the relatively lower cost owing to the compounding of the anticancer agent. Although bevacizumab and ranibizumab showed comparable visual acuity benefits in the CATT[228] and IVAN[229] trials, bevacizumab was associated with increased systemic serious adverse events in CATT[228], possibly owing to the greater systemic exposure following intravitreal injection of this drug[230].

Aflibercept is also formulated for ocular use. It received FDA approval for the treatment of neovascular AMD in 2011, for DME in 2014, RVO in 2014 and DR with

Table 2 | **Selected Phase III clinical trial data for VEGFA-targeted therapies in ophthalmic diseases**

Clinical trial	Treatment	Visual acuity, loss of fewer than 15 letters (*p* value)*	Visual acuity, gain of 15 or more letters (*p* value)*	Visual acuity, mean changes in letters (*p* value)*	Refs
Occult choroidal neovascularization					
MARINA	0.3 mg ranibizumab	94.5% ($p<0.001$; 12 months)	24.8% ($p<0.001$; 12 months)	+6.5 ($p<0.001$; 12 months)	207
	0.5 mg ranibizumab	94.6% ($p<0.001$; 12 months)	33.8% ($p<0.001$; 12 months)	+7.2 ($p<0.001$; 12 months)	
	Sham injection	62.2%	5.0%	–10.4	
Neovascular age-related macular degeneration					
ANCHOR	0.3 mg ranibizumab	94.3% ($p<0.001$ versus verteporfin; 12 months)	35.7% ($p<0.001$; 12 months)	+ 8.5 ($p<0.001$; 12 months)	208
	0.5 mg ranibizumab	96.4% ($p<0.001$ versus verteporfin; 12 months)	40.3% ($p<0.001$; 12 months)	+11.3 ($p<0.001$; 12 months)	
	Verteporfin	64.3%	5.6%	–9.5	
PIER	0.3 mg ranibizumab	90.2% ($p<0.001$; 12 months)	13.1% (NS; 12 months)	– 0.2 ($p<0.0001$; 12 months)	211
	0.5 mg ranibizumab	83.3% ($p<0.001$; 12 months)	11.7% (NS; 12 months)	–1.6 ($p<0.0001$; 12 months)	
	Sham injection	49.2%	9.5%	–16.3	
EXCITE	0.3 mg ranibizumab quarterly	93.3% (NR; 12 months versus 0.3 mg monthly)	14.25 (NR; 12 months)	+3.3 ($p=0.0365$ versus 0.3 mg monthly)	212
	0.5 mg ranibizumab quarterly	91.5% (NR; 12 months versus 0.3 mg monthly)	17.8% (NR; 12 months)	–4.5 ($p=0.0867$ versus 0.3 mg monthly)	
	0.3 mg ranibizumab monthly	94.8%	28.7%	+8.3 (versus baseline)	
HARBOR	0.5 mg ranibizumab monthly	97.8% (all comparisons NS; 12 months)	34.5% (all comparisons NS; 12 months)	+10.1 (all comparisons NS; 12 months)	213
	2.0 mg ranibizumab monthly	93.4% (all comparisons NS; 12 months)	36.1% (all comparisons NS; 12 months)	+9.2 (all comparisons NS; 12 months)	
	0.5 mg ranibizumab PRN after 3 monthly loading doses	94.5% (all comparisons NS; 12 months)	30.2% (all comparisons NS; 12 months)	+8.2 (all comparisons NS; 12 months)	
	2.0 mg ranibizumab PRN after 3 monthly loading doses	94.9% (all comparisons NS; 12 months)	33.0% (all comparisons NS; 12 months)	+8.6 (all comparisons NS; 12 months)	
Diabetic macular oedema					
RIDE	0.3 mg ranibizumab monthly	98.4% ($p=0.0119$; 24 months)	33.6% ($p<0.0001$; 24 months)	+10.9 ($p<0.0001$; 24 months)	218, 221, 222
	0.5 mg ranibizumab monthly	96.1% (NS; 24 months)	45.7% ($p<0.0001$; 24 months)	+12.0 ($p<0.0001$; 24 months)	
	Sham injection	91.5%	12.3%	+2.3	
RISE	0.3 mg ranibizumab monthly	97.6% ($p=0.0086$; 24 months)	44.8% ($p<0.0001$; 24 months)	+12.5 ($p<0.0001$; 24 months)	218, 221, 222
	0.5 mg ranibizumab monthly	97.6% ($p=0.0126$; 24 months)	39.2% ($p<0.001$; 24 months)	+11.9 ($p<0.0001$; 24 months)	
	Sham injection	89.8%	18.1%	+2.6	
Branch retinal vein occlusion					
BRAVO	0.3 mg ranibizumab monthly	100% ($p<0.05$; 6 months)	55.2% ($p<0.0001$; 6 months)	+16.6 ($p<0.0001$; 6 months)	217
	0.5 mg ranibizumab monthly	98.5% (NS; 6 months)	61.1% ($p<0.0001$; 6 months)	+18.3 ($p<0.0001$; 6 months)	
	Sham injection	95.5%	28.8%	+7.3	

Table 2 (cont.) | **Selected Phase III clinical trial data for VEGFA-targeted therapies in ophthalmic diseases**

Clinical trial	Treatment	Visual acuity, loss of fewer than 15 letters (*p* value)*	Visual acuity, gain of 15 or more letters (*p* value)*	Visual acuity, mean changes in letters (*p* value)*	Refs
Central retinal vein occlusion					
Cruise	0.3 mg ranibizumab monthly	96.2% ($p<0.005$; 6 months)	46.2% ($p<0.0001$; 6 months)	+12.7 ($p<0.0001$; 6 months)	216
	0.5 mg ranibizumab monthly	98.5% ($p<0.005$; 6 months)	47.7% ($p<0.0001$; 6 months)	+14.9 ($p<0.0001$; 6 months)	
	Sham injection	84.6%	16.9%	+0.8	
COPERNICUS	2 mg aflibercept given as 6 monthly injections followed *pro re nata* weeks 24 to 52	5.3% (NR; 12 months)	55.3% ($p<0.001$; 12 months)	+16.2 ($p<0.001$; 12 months)	257
	Sham injection	15.1%	30.1%	+3.8	
GALILEO	2 mg aflibercept every 4 weeks for 24 weeks	7.8%‡ ($p=0.0033$; 24 weeks)	60.2% ($p<0.0001$; 24 weeks)	+18.0 ($p<0.0001$; 24 weeks)	258
	Sham injection	25.0%‡	22.1%	+3.3	

NR, not reported; NS, not significant; VEGFA, vascular endothelial growth factor A. **p* value versus baseline unless otherwise stated. ‡Indicates a loss of ≥10 letters.

DME in 2015. In the Phase III VIEW trials, aflibercept (2 mg) given every other month after three monthly loading doses achieved gains in visual acuity that were comparable to those following monthly 0.5 mg doses of ranibizumab[231].

A recent study by the DRCR.net, Protocol T, compared ranbizumab, bevacizumab and aflibercept in patients with DME involving the macular centre[232]. The results after 12 months indicated that all three treatments improved vision, although in eyes with poorer baseline vision (that is, 20/50 or worse) the mean differences favoured aflibercept. Additional data will be needed to validate the differences between these anti-VEGFA treatments[232].

Allergan has developed AGN 150998, an anti-VEGFA designed ankyrin-repeat protein that features antibody-like specificity and affinity for protein targets[233]. It is currently being tested in Phase II trials and may potentially offer dosing every three months. Other treatment strategies being developed in oncology have been examined for ophthalmic diseases, including various RTKIs, mTOR inhibitors and local radiation therapy[233].

Challenges, lessons learned and the future. Neovascular AMD, DME, DR and other ischaemia-associated retinal neovascularizations are global problems with major consequences. However, anti-VEGFA therapies have resulted in significant improvements in vision and quality of life.

The development of as-needed dosing regimens for ranibizumab and other VEGFA blockers has reduced the treatment burden for patients, potential treatment-related adverse events and healthcare costs. Nonetheless, some patients still require frequent injections to keep their disease under control. In these cases, longer-acting formulations or sustained-release technologies are needed. Although several technologies are in development, none has yet received approval.

Approximately 40% of patients with neovascular AMD show a suboptimal treatment response[207], defined as vision less than 20/40. Higher doses are not likely to be helpful, as data from Phase III studies indicate that the current approved doses are at or near the top of the dose response curves for AMD and DME[213]. Although 2 mg aflibercept recently demonstrated better outcomes than lower doses of bevacizumab or ranibizumab in patients with DME who have poor vision, those data await validation[232].

In addition, patients may not receive adequate treatment to experience maximal visual improvement. A recent multi-country, retrospective study of 2,227 patients with neovascular AMD indicated that, in actual clinical usage, patients receive fewer injections and have poorer outcomes than is observed in clinical studies[234]. The decline in visual acuity improvements over time suggests that some patients may have been under-treated. Long-acting delivery technologies, once available, may address the gap in visual outcomes observed in clinical trials and clinical practice.

Better outcomes may also require targeting multiple proteins or pathways. PDGFB inhibition, which may enhance the efficacy of anti-VEGFA by stripping pericytes from nascent vessels, making them more susceptible to vascular regression, is currently being investigated for the treatment of neovascular AMD. Preclinical studies have demonstrated improved regression of chroroidal neovascularization with an anti-PDGFB–anti-VEGFA drug combination[235]. A Phase II trial recently reported that the anti-PDGFB aptamer pegpleranib (Fovista; OphthoTech) combined with ranibizumab significantly improved visual acuity over ranibizumab alone[236,237], leading to the progression of this agent to Phase III trials. Regeneron is also clinically testing an anti-VEGFA–anti-PDGF-B combination.

Suboptimal efficacy may also result from delayed diagnosis, after irreversible vision loss has set in, or from components of the disease that remain unaddressed by anti-VEGFA therapies. In addition to neovascularization and vascular leak, neovascular AMD and DME are also characterized by immune cell infiltrates and neural cell death[238], against which anti-VEGFA drugs are not effective. Moreover, in some animal models, VEGFA acts as a retinal neuroprotectant and its blockade under conditions of retinal stress accelerates retinal cell death[239].

Emerging clinical data suggest that anti-VEGFA drugs may be associated with retinal atrophy[240], although a causal relationship has not been established and the benefit–risk ratio for vision with anti-VEGFA therapy is still highly positive. The topic of neuroprotection remains controversial, as some preclinical models do not show retinal damage following VEGFA blockade[241].

Conclusions and perspectives

The identification of VEGFA as a major angiogenic mediator has revolutionized our understanding of the roles of angiogenesis in both normal physiological development and pathology. The achievements obtained during the past 10 years have not only supported VEGFA targeting in both ophthalmology and cancer but are opening up new opportunities for improved therapies in other diseases.

Despite the overall clinical success of anti-VEGFA agents, there remain several areas for further improvement. The impact of VEGFA inhibitors in cancer has not reached the dramatic efficacy anticipated in some early preclinical studies with other angiogenesis inhibitors[242]. Nevertheless, VEGFA inhibitors have shown benefits in patients with advanced and difficult to treat malignancies and are now a standard of care for the treatment of several metastatic cancers. However, there is heterogeneity in the clinical response. As already noted, much recent research has focused on the tumour microenvironment as a possible source of VEGFA-independent pathways mediating resistance to VEGFA inhibitors[175].

Anti-VEGFA therapy has been more transformative in ophthalmology. The visual gains seen early in therapy were maintained for at least 2–3 years in large randomized trials[218,221,222,243], possibly owing to the genetically stable nature of the retina, which resists the selective pressure to bypass VEGFA blockade. As mentioned above, modelling of visual acuity outcomes predicted a substantial reduction in legal blindness from neovascular AMD following anti-VEGF treatment[215]. Recent data, showing a marked reduction in the incidence rate of legal blindness due to AMD after the introduction of intravitreal VEGF inhibitors, are consistent with this prediction[244]. However, the cost and need for chronic therapy in some neovascular AMD and DME patients may require the development of long-acting delivery technologies, as noted above.

Thus, one major question is how to improve the efficacy of VEGFA targeting. The answer lies not only in the identification of predictive biomarkers, but also through better understanding of the mechanisms of action and resistance of currently used anti-VEGFA agents, as well as the elucidation of additional underlying disease mechanisms in cancer and ophthalmology.

As noted, combinations of anti-VEGFA agents with inhibitors of other pro-angiogenic pathways have not yet achieved much success. However, one approach that seems promising is combining anti-VEGFA strategies with inhibitors of unrelated pathways. For example, there is significant interest in combining anti-VEGFA treatments with immune checkpoint inhibitors such as those targeting cytotoxic T lymphocyte protein 4 (CTLA4) or programmed cell death ligand 1 (PDL1). This is because VEGFA inhibition was shown in preclinical studies to result in a significant increase in the number of tumour-infiltrating lymphocytes, which could be exploited in immunotherapeutic approaches[245]. Numerous clinical trials are currently testing this hypothesis and, although the data are immature, some promising hints of additive efficacy have been observed. However, the potential toxic effects of such combinations are unclear. Also, in a randomized Phase II study in women with recurrent platinum-sensitive ovarian cancer, the combination of the VEGFR RTKI cediranib with the PARP inhibitor olaparib (Lynparza; KuDOS, AstraZeneca) markedly increased PFS relative to olaparib alone[246]. These promising results, if validated in Phase III studies, may be paradigm-shifting.

The first decade of anti-VEGFA therapy has seen major advances in the treatment of certain cancers and intraocular neovascular disorders. Today's unanswered questions of resistance, refining molecular targeting, incorporating biomarkers and selecting appropriate combinations with other molecules, set the research agenda for how anti-VEGFA may be enhanced to improve patient outcomes in the next decade.

Napoleone Ferrara is at the University of California, San Diego, La Jolla, California, 92093, USA.

Anthony P. Adamis is at Genentech, Inc., South San Francisco, California 94080, USA.

Correspondence to N.F.
nferrara@ucsd.edu

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Competing interests statement
The authors declare competing interests: see Web version for details.