Therapeutic targeting of the angiopoietin–TIE pathway

Pipsa Saharinen[1], Lauri Eklund[2] and Kari Alitalo[1]

Abstract | The endothelial angiopoietin (ANG)–TIE growth factor receptor pathway regulates vascular permeability and pathological vascular remodelling during inflammation, tumour angiogenesis and metastasis. Drugs that target the ANG–TIE pathway are in clinical development for oncological and ophthalmological applications. The aim is to complement current vascular endothelial growth factor (VEGF)-based anti-angiogenic therapies in cancer, wet age-related macular degeneration and macular oedema. The unique function of the ANG–TIE pathway in vascular stabilization also renders this pathway an attractive target in sepsis, organ transplantation, atherosclerosis and vascular complications of diabetes. This Review covers key aspects of the function of the ANG–TIE pathway in vascular disease and describes the recent development of novel therapeutics that target this pathway.

Wet age-related macular degeneration
(wAMD). A form of ocular disease in which choroidal vessels grow through Bruch's membrane, which separates the retina from the choroid. This can cause fluid leakage and bleeding in the retina, and vision loss in the region of the macula.

[1]*Wihuri Research Institute and Translational Cancer Biology Program, Biomedicum Helsinki, University of Helsinki, Haartmaninkatu 8, P.O. Box 63, FI-00014 Helsinki, Finland.*
[2]*Oulu Center for Cell-Matrix Research, Faculty of Biochemistry and Molecular Medicine, Biocenter Oulu, Aapistie 5A, University of Oulu, 90220 Oulu, Finland.*

Correspondence to K.A.
kari.alitalo@helsinki.fi

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The blood and lymphatic vascular systems permeate most tissues of the body and are intimately involved in the pathophysiology of numerous diseases. The formation of new blood vessels (angiogenesis) is an essential step in the healing of wounds and in tissue regeneration. However, angiogenesis is also a key driver of pathogenesis in conditions such as cancer and neovascular eye diseases[1–3]. Increased vascular leakage from blood vessels occurs transiently during inflammation. Persistent vascular leakage in the eye can cause macular oedema, which is a common and difficult-to-treat vision-impairing complication in patients with diabetes. Moreover, vascular leakage can contribute to tissue hypoperfusion in severe infections and sepsis. During chronic inflammation, endothelial activation increases lipid and inflammatory cell accumulation in the arterial intima, causing atherosclerosis that can lead to insufficient blood flow and to ischaemic heart and peripheral arterial disease. In the lymphatic vasculature, insufficient function of lymphatic vessels leads to lymphoedema — a disfiguring swelling of the extremities — whereas the growth of new lymphatic vessels (lymphangiogenesis) facilitates tumour metastasis to distant organs.

Growth factor receptors expressed in endothelial cells of blood and lymphatic vessels control the development and functions of the cardiovascular and lymphatic systems. The identification of the vascular endothelial growth factor (VEGF) signalling pathway as the master regulator of developmental and pathological angiogenesis yielded the first clinical anti-angiogenic therapies for cancer and ocular neovascular diseases more than a decade ago[3]. However, therapy resistance complicates the use of inhibitors of the VEGF signalling pathway. For example, 20–30% of patients with metastatic renal cell carcinoma (mRCC) do not respond to sunitinib, a multi-kinase inhibitor (whose targets include all VEGF receptors (VEGFRs) and platelet-derived growth factor (PDGF) receptors) that is widely used as the first-line therapy in mRCC. Even patients who initially respond to sunitinib often show disease progression within a year[4]. VEGF inhibition has been more effective in controlling excess angiogenesis and preventing vision loss in neovascular eye diseases such as wet age-related macular degeneration (wAMD) and macular oedema, which are major causes of vision loss in adults[2]. Currently, wAMD is treated with ranibizumab (a VEGF-blocking monoclonal antibody antigen-binding fragment (Fab)) and aflibercept (a soluble recombinant VEGFR ectodomain that is also known as VEGF Trap-eye and ziv-aflibercept; see BOX 1 for details on VEGF-targeted therapeutics). However, VEGF inhibition results in suboptimal vision improvement in 40% of patients, and patients experience gradual vision loss over time[3].Therefore, there remains an unmet need for more effective anti-angiogenic therapies for both cancer and neovascular eye diseases.

Recently, the angiopoietin (ANG)–TIE signalling pathway has emerged as an attractive vascular drug target. The ANG–TIE pathway is required for lymphatic and blood vessel development during mid-gestation, and this pathway controls vascular permeability, inflammation and pathological angiogenic responses in adult tissues[5–7]. Investigational drugs that target the ANG–TIE pathway are expected to complement current anti-angiogenic strategies in the treatment of cancer and

Box 1 | **Therapeutics that target vascular endothelial growth factor**

Vascular endothelial growth factor (VEGF)-neutralizing biopharmaceuticals include the human monoclonal VEGF-targeting antibody bevacizumab, the VEGF-blocking monoclonal antibody antigen-binding fragment (Fab) ranibizumab, and aflibercept, a chimeric decoy receptor made of the VEGF receptor 1 (VEGFR1) and VEGFR2 ectodomains that is capable of neutralizing VEGF, placenta growth factor (PLGF) and VEGFB. Both bevacizumab and aflibercept are used in combination with chemotherapy for the treatment of various cancers, including colorectal cancer, and bevacizumab is also used to treat certain types of lung, kidney and brain cancer (glioblastoma)[3]. Ranibizumab, bevacizumab and aflibercept are currently used for the treatment of wet age-related macular degeneration and diabetic macular oedema[3]. Ramucirumab is a VEGFR2-blocking monoclonal antibody that has been approved for the treatment of metastatic renal cell carcinoma, non-small cell lung cancer and gastric cancer.

A great number of tyrosine kinase inhibitors (TKIs) that target VEGFRs and various combinations of other cellular kinases have been developed, the first ones being the multi-kinase inhibitors sunitinib and sorafenib. The clinical use of these inhibitors is associated with an anti-angiogenic effect and results in vessel regression in the tumours, but these inhibitors are likely to also target key tumour cell signalling pathways. Subsequently, more selective TKIs have been developed, including axitinib, pazopanib and cabozantinib (which inhibits VEGFR2 and MET (also known as hepatocyte growth factor receptor)). Notably, a new indication was recently discovered for axitinib, when it was found to inhibit a specific T315I mutant of the BCR–ABL1 fusion protein that is commonly found in imatinib-resistant chronic myeloid leukaemia and adult acute lymphoblastic leukaemia[194].

neovascular eye diseases in the future[8]. The ANG–TIE pathway has a unique role in the control of vascular stability. Manipulation of this axis may thus be beneficial in conditions in which excess vessel growth is not a problem but vascular stabilization is crucial, such as in diabetic vascular complications, sepsis, organ transplantation and atherosclerosis. In this Review, we summarize recent preclinical research into the functions of the ANG–TIE pathway in healthy and diseased vasculature, and describe the clinical development of drugs that target the ANG–TIE pathway in the context of cancer and vascular disease.

Signalling via the ANG–TIE pathway

The ANG receptors TIE1 and TIE2 (also known as TEK)[9,10], which were discovered 25 years ago, form a small subfamily of growth factor receptor tyrosine kinases (FIG. 1). TIE1 and TIE2 are almost exclusively expressed in the endothelium, but there is some expression also in haematopoietic cells[7,11,12]. Although this Review mainly focuses on the functions of the ANG–TIE pathway in the vasculature, TIE2 also marks a subset of M2 monocytes (TIE2-positive M2 monocytes (TEMs)), certain haematopoietic stem cell populations and muscle satellite cells[13–15]. The growth factors ANG1, ANG2 and ANG4 (the human orthologue of mouse ANG3) are ligands for TIE2, whereas TIE1 is an orphan receptor that can nevertheless be activated by ANG proteins via its interaction with TIE2 (REF. 16). ANG1 and ANG2 have been the focus of most studies so far, and less is known about ANG3 and ANG4 (BOX 2).

TIE1 and TIE2 show a unique mechanism of activation that depends on the cellular microenvironment. ANG proteins stimulate receptor translocation to cell–cell junctions in endothelial cell monolayers, whereas in motile, ANG-stimulated endothelial cells, TIE receptors translocate to the trailing cell–extracellular matrix (ECM) contacts[17,18]. The subcellular localization of the ANG receptors at endothelial cell–endothelial cell (EC–EC) and EC–ECM contacts determines, in part, the specificity of downstream signalling and the subsequent endothelial cell responses[17,18]. *In vitro* fluorescence lifetime measurement studies have indicated that ANG proteins stimulate TIE1 to directly interact with TIE2 at EC–EC junctions[19], and that TIE1 regulates TIE2 trafficking[19,20]. *In vivo* experiments have shown that TIE1 is required for full activation of TIE2 by ANG1 and ANG2 in the mouse vasculature[19,21].

ANG1 is a paracrine ligand expressed by mesenchymal cells and a strong TIE2 agonist that supports endothelial cell survival, vessel stability and endothelial barrier function[22]. TIE2 activation by ANG1 induces downstream signalling via the serine kinase AKT, which leads to inhibition of the transcription factor Forkhead box protein O1 (FOXO1) and repression of FOXO1 target genes, such as *Ang2* (REFS 23–26). On the one hand, transgenic expression of ANG1 in mice promotes non-leaky venous remodelling[27]. On the other hand, mutations in *TIE2* and *PIK3CA* (which encodes phosphoinositide 3-kinase catalytic subunit-α) that result in constitutive TIE2–AKT activation cause venous malformations in experimental mouse models and in patients[28–31]. Venous malformations are characterized by aberrant vascular remodeling, resulting in enlarged vein-like channels (BOX 3). By contrast, inactivating heterozygous *TIE2* mutations cause primary congenital glaucoma (PCG; FIG. 2). PCG is an important cause of childhood blindness worldwide. It results from abnormal development of Schlemm's canal, which normally maintains intraocular pressure via drainage of the aqueous humour in the eye[32–34].

The ANG1–TIE2 signalling axis promotes vascular stability via its effects on EC–EC junctions and on the actin cytoskeleton[35,36]. ANG1 stabilizes vascular endothelial cadherin (VE-cadherin; also known as cadherin 5 (CDH5)) in these junctions, thus inhibiting VE-cadherin loss in response to stimulation of endothelial cells by VEGF or inflammatory cytokines[35,37]. However, activation of TIE2 using pharmacological inhibition of vascular endothelial protein tyrosine phosphatase (VE-PTP; also known as PTPRβ), which dephosphorylates TIE2, has been shown to improve endothelial barrier function also in mice in which the *Cdh5* gene has been deleted[38]. In these studies, TIE2 activation, achieved via VE-PTP inhibition, stimulated the small GTPase RAP1 and decreased the phosphorylation of myosin light chain 2 (MLC2; also known as MYL2), which resulted in the stabilization of the cortical actin cytoskeleton[38].

ANG2 acts as a context-dependent weak TIE2 agonist or antagonist that can inhibit the ANG1–TIE2 signalling axis. ANG2 is expressed by endothelial cells and stored in their Weibel–Palade bodies[39]. ANG2 levels are greatly increased during vascular remodelling, which occurs, for example, during ovarian follicular development[40]. ANG2 expression increases in response to tumour necrosis factor (TNF) and VEGF stimulation of endothelial cells, and in response to hypoxia, which may partly explain its upregulation in the tumour

Schlemm's canal
A lymphatic-like vessel in the eye that drains aqueous humour from the anterior chamber into the blood circulation via aqueous veins.

vasculature[41–43]. Yes-associated protein 1 (YAP1), which is expressed in the growing vascular front of the developing postnatal retina, regulates ANG2 expression in a VE-cadherin-dependent and EC–EC contact-dependent manner, and promotes ANG2 expression in angiogenic endothelial cells[44]. During inflammation, ANG2 is secreted by endothelial cells in response to inflammatory cytokines. This attenuates ANG1–TIE2 signalling, which leads to increased FOXO1 activity and ANG2 expression[45]. ANG2 secretion by endothelial cells is required during acute inflammatory responses, which are impaired in ANG2-deficient mice[46]. In contrast to ANG1, ANG2 can destabilize endothelial monolayers by promoting the formation of actin stress fibres[47,48]. Although transgene-expressed *Ang2* or recombinant ANG2 proteins behave as context-dependent TIE2 antagonists in the inflamed vasculature, ANG2 acts as a weak agonist in the non-inflamed endothelium and in

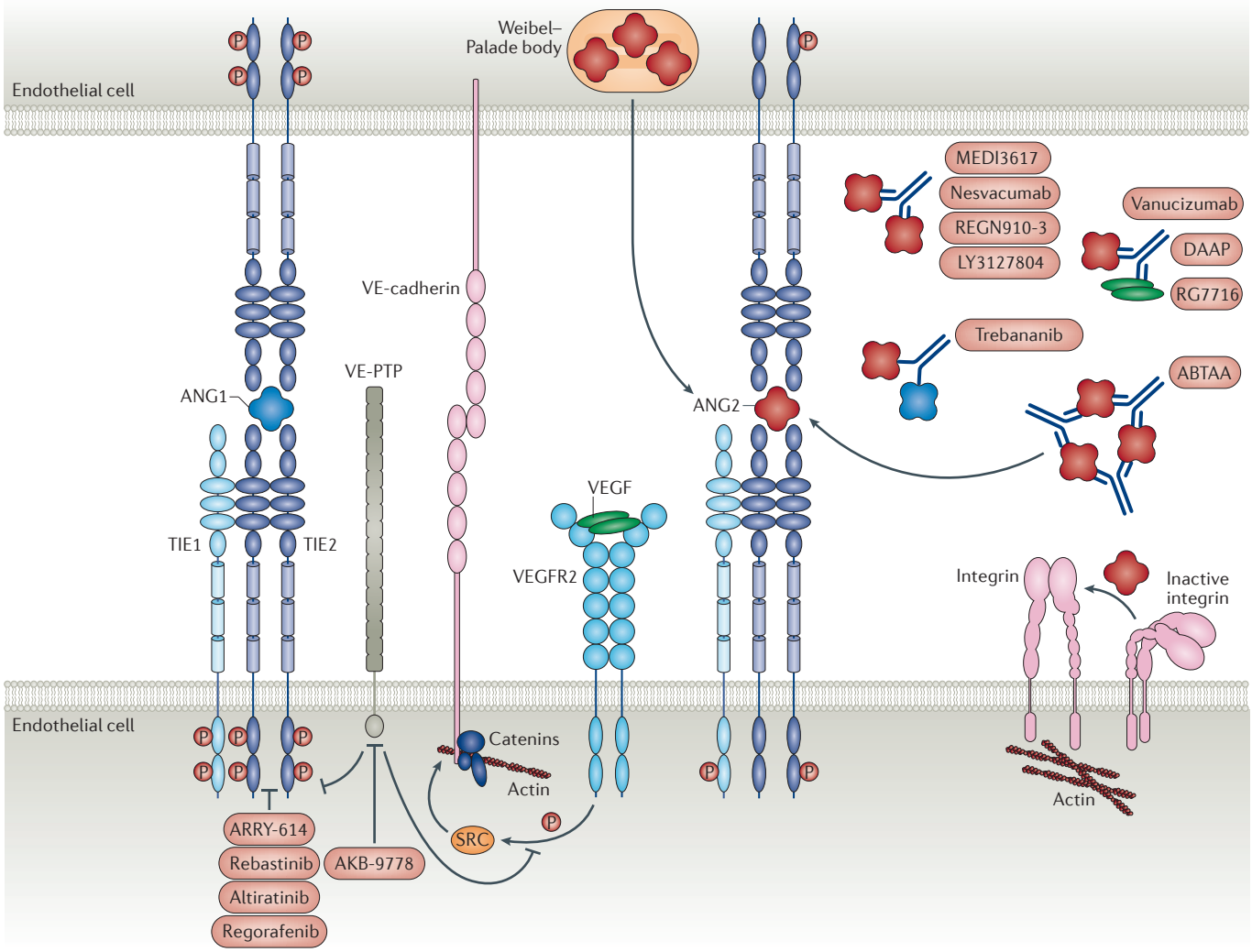

Figure 1 | **Signalling interactions and therapeutic targeting of the ANG–TIE pathway.** Angiopoietin 1 (ANG1) is a paracrine TIE2 agonist that induces the formation of TIE clusters at endothelial cell–endothelial cell (EC–EC) junctions and at endothelial cell–extracellular matrix (ECM) contacts (the latter not shown)[17,18]. TIE activation and the formation of the receptor complex containing TIE1 and TIE2 is dependent on α5β1 integrin, although the exact stoichiometry of the complex remains unknown[19,52,53]. Vascular endothelial protein tyrosine phosphatase (VE-PTP) dephosphorylates TIE2 and is found at EC–EC junctions, particularly after ANG1 stimulation[18,211]. Vascular endothelial cadherin (VE-cadherin) phosphorylation in response to vascular endothelial growth factor (VEGF) is dependent on the kinase SRC, resulting in VE-cadherin internalization[213]. VE-PTP also dephosphorylates VEGF receptor 2 (VEGFR2) and stabilizes the VE-cadherin–catenin complex that associates with the actin cytoskeleton at EC–EC junctions, thus promoting endothelial cell integrity[97,212,213]. Similarly, ANG2 induces TIE clustering at EC–EC junctions but activates TIE receptors only weakly[18]. ANG2 is released from endothelial cell Weibel–Palade bodies in response to various stimuli[39]. During inflammation, ANG2 function switches from agonist to antagonist; this is probably in response to TIE1 ectodomain shedding[19,49]. In endothelial cells with low TIE2 expression, such as in inflamed endothelium and endothelial tip cells in angiogenic vessels, ANG2 may also bind to and signal via integrin heterodimers[48,57]. The figure also shows inhibitors of the ANG2–TIE2 pathway that are currently in preclinical or clinical development. These drugs comprise ANG-targeted monoclonal antibodies (nesvacumab, MEDI3617, REGN910-3, LY3127804) and the peptibody trebananib, which interfere with ANG–TIE2 signalling, a chimeric decoy receptor (DAAP) and bispecific antibodies that additionally interfere with VEGF–VEGFR interactions (vanucizumab and RG7716). Included are small-molecule inhibitors of VE-PTP (AKB-9778); small-molecule inhibitors that have activity against TIE2 (ARRY-614, regorafenib, rebastinib and altiratinib); and ANG2-binding and TIE2-activating antibody (ABTAA). See TABLES 3,4 for further details.

Box 2 | **Angiopoietin 3 and angiopoietin 4**

After the cloning of angiopoietin 1 (ANG1) and ANG2, cDNAs encoding a third ANG were cloned from mice and humans[195–197]. Owing to the relatively high divergence between the mouse and human cDNAs, and species-specific effects that were initially observed in receptor activation experiments, the human orthologue of mouse ANG3 was named ANG4 (REF. 195). ANG3 and ANG4 are less well characterized, and their exact functions remain to be elucidated. However, similarly to ANG2, ANG3 expression is induced by hypoxia[198–200]. In the mouse corneal micropocket assay, treatment with recombinant ANG3 or ANG4 protein induced angiogenesis, and similarly to treatment with cartilage oligomeric matrix protein (COMP)–ANG1, expression of ANG3 and ANG4 via an adenoviral vector resulted in blood and lymphatic vascular remodelling in the trachea[73,201].

In the streptozotocin-induced mouse model of diabetes with erectile penile dysfunction, cavernous expression of ANG4 was found to be downregulated, and intracavernous injections of ANG4 protein improved erectile function by increasing cavernous endothelial cell content, activating the TIE2–AKT–endothelial nitric oxide synthase pathway, and decreasing reactive oxygen species production and apoptosis; these effects were similar to those observed after treatment with ANG1 (REFS 169,202).

certain vascular beds that have a low level of ANG1 signalling[19,49,50]. Gene deletion experiments have indicated that ANG2 is required as a TIE2 agonist for the development of the lymphatic vasculature[51]. Several antagonists that block the binding of ANG2 to TIE2 are in clinical development. These are discussed in more detail below.

Results from *in vitro* and *in vivo* models have shown that signalling via the ANG–TIE pathway is modulated by various endothelial integrins. Integrin α5β1 was found to enhance ANG1-induced phosphorylation of TIE1 and TIE2, their complex formation at the junctions of cultured endothelial cells, AKT phosphorylation and the nuclear exclusion of the transcription factor FOXO1 (REFS 19,52). The ectodomains of α5β1 integrin and TIE were shown to interact in a fibronectin-dependent manner in endothelial cells[52,53]. In addition, ANG proteins can directly interact with integrins in non-endothelial cells. ANG1 has been shown to interact with αvβ5 integrin in retinal astrocytes, and ANG2 interacts with α5β1 in some tumour cells and with α3β1 in vascular pericytes[54–56]. Low TIE2 levels seem to potentiate ANG2–integrin interactions in the endothelial tip cells of angiogenic sprouts[57], and to destabilize endothelial monolayers via ANG2 and α5β1 integrin[48]. Although multiple signalling interactions have been reported between the integrins and the ANG–TIE pathway, these interactions have not yet been targeted for drug development.

The ANG–TIE pathway in cancer

Tumour angiogenesis. Increased ANG2 expression has been associated with several types of human cancer, including melanoma, RCC, glioblastoma, and breast and colorectal cancer (CRC)[58–62]. Endothelial cells seem to be the primary source of ANG2 in human glioblastoma and RCC[62,63]. In a rat glioma model, ANG2 expression was induced in the endothelial cells of co-opted tumour blood vessels. In this model, ANG2 expression was associated with pericyte detachment and vascular regression, which led to the hypoxic upregulation of VEGF and ANG2, and the subsequent induction of angiogenesis at the tumour margin[64] (FIG. 3).

Several preclinical studies have demonstrated that monoclonal antibodies and peptide–Fc fusion proteins (peptibodies) that neutralize the ANG2–TIE2 interaction slow tumour growth and reduce tumour angiogenesis by decreasing the formation of new vessel sprouts and by inducing vessel regression[65–71]. Increased antitumour efficacy was observed in mouse tumour models when ANG2 blockade was combined with VEGF-blocking antibodies or aflibercept, or when a bispecific antibody that blocks both ANG2 and VEGF was used[50,67–69]. Blocking of ANG1 using peptibodies has been much less effective for the inhibition of angiogenesis and tumour growth in mouse xenograft models[70,71]. Thus, although ANG1 has angiogenic and lymphangiogenic activity during physiological vascular remodelling, as shown by impaired postnatal retinal vascularization in *Ang1*-deleted pups and by vessel enlargement after the administration of recombinant ANG1 in mice, ANG1 does not seem to promote vascular growth in tumours[54,72–75]. By contrast, increased ANG1 expression during anti-angiogenic tumour therapy seems to mediate the normalization of tumour blood vessels, which facilitates drug delivery[70,71,76].

Conversely, and in analogy to VEGF blockage, ANG2 blockade normalizes the abnormal phenotype of tumour blood vessels. Administration of ANG2-blocking peptibodies into tumour-bearing mice increased deposition of adhesion proteins such as VE-cadherin at EC–EC junctions and enhanced pericyte coating of the tumour blood vessels, which improved blood perfusion[70]. By contrast, increased ANG2 expression has been associated with tumour vessel destabilization and pericyte dropout. In orthotopic mammary carcinomas grown in mice that express a herpes virus thymidine kinase under control of the promoter of *Pdgfrb* (which encodes PDGF receptor-β), ganciclovir administration induced the depletion of PDGFRβ$^+$ pericytes from tumour blood vessels. This resulted in reduced tumour growth, but increased ANG2 expression, intratumoural hypoxia and lung metastasis[77]. The depletion of pericytes in combination with ANG2 blocking restored vascular stability, and decreased tumour growth and metastasis in this model[77]. In addition, the combination of ANG2-blocking antibodies with imatinib (which reduces pericyte coverage of the vessels) provided synergistic inhibition of tumour growth and decreased imatinib-induced intratumoural hypoxia, vascular leakage and lung metastasis.

Upregulation of ANG2 expression in the tumour vasculature and recruitment of TEMs to the tumour stroma have been suggested as potential mechanisms of resistance to inhibitors of the VEGF pathway. This was shown in the RIP1-Tag2 transgenic pancreatic neuroendocrine mouse tumour model, which represents a model of multistep tumour progression. Late-stage RIP1-Tag2 tumours developed resistance to VEGFR2-targeting antibodies, but the combination of these antibodies with ANG2-blocking antibodies overcame the resistance[78]. Although the combination treatment increased tumour hypoxia, this approach did not promote tumour invasion or metastasis[78]. However, dual VEGFR2–ANG2 blockade was less effective in the MMTV-PyMT mammary adenocarcinoma

Bispecific antibody
Engineered proteins that have the combined binding specificities of two antibodies, which allows the combinatorial targeting of two different molecules.

Imatinib
A tyrosine kinase inhibitor that inhibits the activity of ABL, KIT and platelet-derived growth factor receptor, and is used in the treatment of multiple cancers, most notably Philadelphia chromosome-positive chronic myeloid leukaemia and gastrointestinal stromal tumour.

RIP1-Tag2 transgenic pancreatic neuroendocrine mouse tumour model
A model in which mice express SV40 large T antigen in pancreatic islet β-cells under the insulin promoter. This drives the development of pancreatic tumours; hyperplastic or dysplastic islets develop into angiogenic, invasive carcinomas that can metastasize.

Box 3 | **Mutations that alter TIE2 signalling magnitude cause venous malformations and glaucoma**

Venous malformations are defects of vascular morphogenesis that result in the formation of distorted veins with irregular smooth muscle cell coverage and slow blood flow. More than 60% of venous malformations have *TIE2* mutations that result in ligand-independent tyrosine phosphorylation of TIE2, indicating a gain-of-function effect. The most common TIE2 mutation is the somatic amino acid substitution L914F[28]. Several other somatic and germline missense point mutations have been reported in the TIE2 kinase domains, kinase insert and carboxy-terminal tail (FIG. 2). In addition to single amino acid substitutions, lesions may also have *TIE2* double mutations that occur in *cis* (on the same allele) or have a premature stop codon near the C-terminal tail[28,203,204].

Recent genetic studies indicate that *TIE2* mutations cause a wide range of venous anomalies that differ in terms of heredity, number of venous malformation lesions and complications[205]. These venous anomalies include blue rubber bleb nevus syndrome, which is characterized by multiple cutaneous and gastrointestinal venous malformations. In contrast to unifocal venous malformations, which are most often caused by the somatic L914F mutation, multifocal forms of the disease are characterized by *TIE2* double mutations and increased endothelial cell dissemination capacity *in vitro*, which suggests that multiple isolated lesions carrying the same double mutation in an individual patient may have a common cellular origin[205].

Notably, *TIE2* mutation-negative venous malformations frequently have activating mutations in *PIK3CA*[29–31], including the 'hotspot' driver mutations (E542K, E545K and H1047R) that are also present in many types of cancer cells[206], in tissue overgrowth syndromes[207] and in more than 90% of lymphatic malformations that occur independently of *TIE2* mutations[208,209] (FIG. 2).

Inactivating heterozygous *TIE2* mutations are found in primary congenital glaucoma (PCG)[34] (FIG. 2). In PCG, decreased aqueous humour drainage from the anterior chamber of the eye via a dysfunctional Schlemm's canal increases intraocular pressure, which causes neurodegeneration and loss of vision. The *TIE2* mutations associated with PCG have a loss-of-function effect[34], in contrast to the gain-of-function mutations in venous malformations[210]. Collectively, the identification of different types of *TIE2* mutations in venous malformations and in PCG indicates that the extent of TIE2 activity is important for the development of normal vascular structures.

model, which showed resistance to VEGF-blocking agents in the absence of ANG2 overexpression[78]. Furthermore, in mouse models of glioblastoma, a combination of ANG1- and ANG2-blocking peptibody (AMG368) with aflibercept decreased vascular permeability and the presence of tumour-associated macrophages, and increased pericyte coverage, the number of intratumoural T lymphocytes and survival compared with anti-VEGF monotherapy[62]. In addition, the VEGF–ANG2 bispecific antibody as well as the combination of cediranib (which is a VEGFR-targeted tyrosine kinase inhibitor (TKI)) with MEDI3617 (which is an anti-ANG2-neutralizing antibody) altered tumour-associated macrophages, including the reprogramming of protumorigenic M2 macrophages towards the antitumorigenic M1 phenotype, and prolonged survival in mouse glioma models[79,80].

Interestingly, ANG2 has been reported to function as a TIE2 agonist in human tumour xenografts in immunocompromised mice in which it promoted endothelial cell survival via TIE2 activation and thereby limited the anti-angiogenic effects of VEGF inhibition[50]. Currently, the mechanisms that regulate the agonistic and antagonistic activity of ANG2 in tumours, and thus ANG2 effects on vessel stability, remain unknown, but recent results suggest that TIE1 ectodomain cleavage may have a role[19,49]. The various effects of ANG2 on vessel regression, angiogenesis and vascular integrity may, in part, depend on the degree of tumour vessel maturation and on the sites of ANG2 expression within the tumour vascular bed (FIG. 3). As ANG2 expression can induce resistance to inhibitors of the VEGF pathway, and combination of ANG2- and VEGF-targeted inhibitors has been better than monotherapy in mouse tumours, combined targeting of VEGF and ANG2 pathways is being tested in human cancer. The dual ANG2 and VEGFA inhibitor also elicits antitumour immunity that is enhanced by programmed cell death protein 1 (PD1) checkpoint blockade[306].

AKB-9778, a competitive small-molecule inhibitor of the catalytic activity of VE-PTP, was recently reported to promote TIE2 activation in cultured endothelial cells and in the mouse vasculature *in vivo*[81]. In mouse models of breast cancer, AKB-9778 normalized tumour blood vessels and increased perfusion, which resulted in an enhanced response to simultaneously administered cytotoxic drugs. In addition, AKB-9778 delayed tumour growth and metastatic progression by decreasing tumour cell extravasation[82]. However, somatic inactivating mutations of the gene encoding VE-PTP have been reported in about one-third of patients with angiosarcoma, which raises the possibility that VE-PTP inactivation could, under certain conditions, be involved in progression of vascular tumours[83,84].

TIE1 is also involved in tumour angiogenesis, and recent studies are beginning to elucidate the signalling mechanisms of this orphan receptor. TIE1 expression is increased in tumour blood vessels, and conditional *Tie1* deletion in the endothelium of adult mice reduced tumour angiogenesis and growth to a similar extent as was achieved with VEGF-targeting or VEGFR2-targeting antibodies[21]. Endothelial cell apoptosis was also increased in tumour isografts in *Tie1*-deleted mice[21]. Notably, treatment of *Tie1*-deficient mice with a soluble TIE2 ectodomain — which is capable of neutralizing both ANG1 and ANG2 — led to an additive effect on tumour growth inhibition, whereas VEGF inhibitors did not further decrease tumour growth in this model[21]. Similarly to treatment with ANG2-blocking antibodies,

MMTV-PyMT mammary adenocarcinoma model
A model in which the long terminal repeat of mouse mammary tumour virus (MMTV) drives the expression of mammary gland-specific polyoma virus middle T-antigen (PyMT), which leads to the rapid development of highly metastatic tumours.

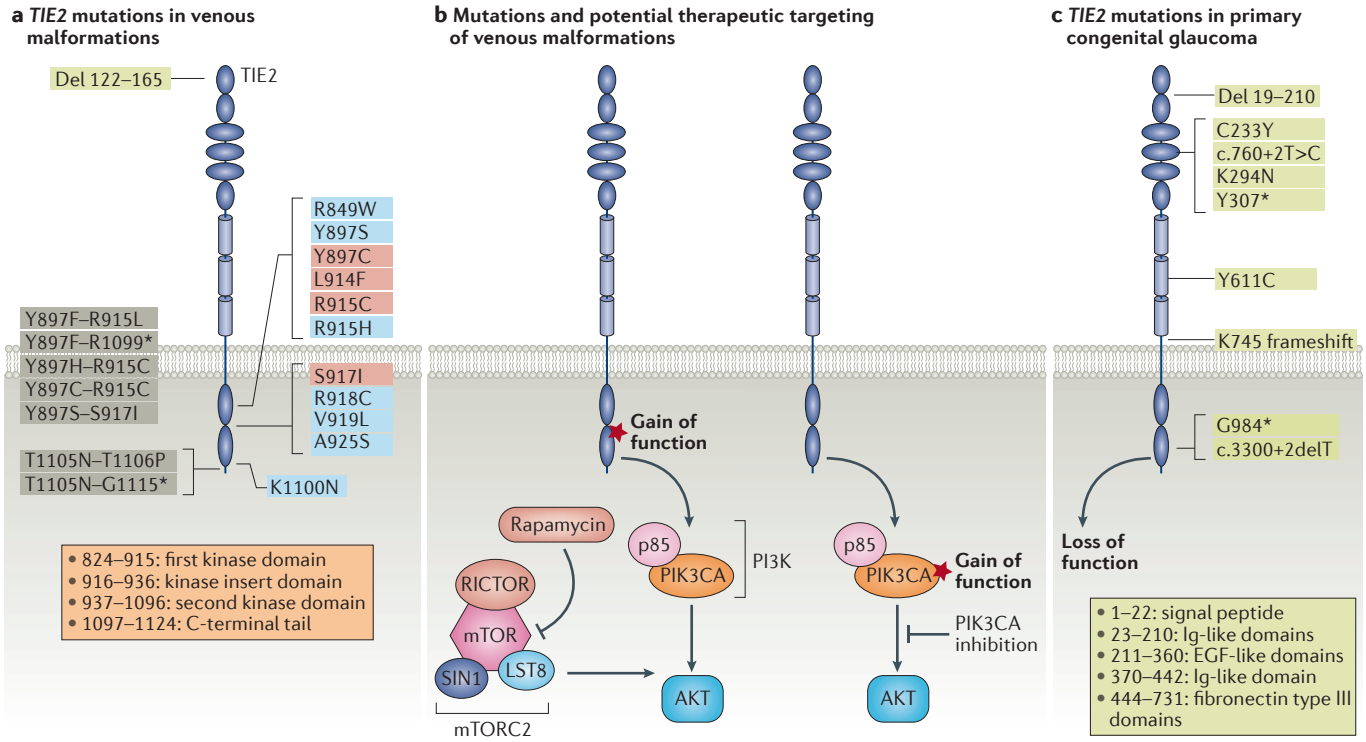

Figure 2 | ***TIE2* mutations in human disease. a** | *TIE2* mutations that cause human venous malformations are indicated in the schematic TIE2 domain structure. Highlighted in grey are double mutations that occur in the same allele (in *cis*), as observed in somatic venous malformation lesions. Highlighted in blue are mutations that have been found in the peripheral blood DNA of patients with hereditary venous malformations. R849W is a recurrent inherited substitution that may require a somatic second hit for the formation of venous malformation lesions[205] or loss of the wild-type allele due to an in-frame deletion of amino acid residues 122–165 of the extracellular immunoglubulin-like 2 (Ig2) domain, which results in intracellular retention of TIE2 (REF. 28). R849W has also been reported as a somatic mutation in one patient[204]. Highlighted in red are single mutations that have been found in somatic venous malformation lesions. L914F is a recurrent mutation. R918C has been reported as both a germline and somatic mutation, and Y897C has been reported as a germline mutation in one family[203,214]. Double mutations are enriched in patients who have multiple venous malformation lesions: T1105N–T1106P is recurrent in blue rubber bleb nevus syndrome and Y897C–R915C in sporadically occurring multifocal venous malformations[205]. The residues corresponding to amino acid substitutions and domain boundaries of human TIE2 are indicated. **b** | The majority of venous malformations are caused by somatic activating mutations (indicated by red stars) in either *TIE2* or *PIK3CA* (which encodes phosphoinositide 3-kinase (PI3K) catalytic subunit-α). Recurrent *TIE2* and *PIK3CA* mutations result in similar cellular and molecular dysfunctions[29], and *PIK3CA* mutations cause venous malformation lesions in mice and patients[29–31], which indicates that *TIE2* mutations and *PIK3CA* mutations participate in the same venous malformation-causative signalling pathway. Together, these mutations explain more than 80% of venous malformations. The first molecular treatment for venous malformations (rapamycin) decreases mutant TIE2-induced AKT phosphorylation — presumably via decreasing mechanistic target of rapamycin (mTOR) complex 2 (mTORC2)-mediated phosphorylation of AKT at S473 (REF. 215) — and inhibits the growth of venous malformation lesions caused by *TIE2* and *PIK3CA* mutations in mice[30,31,215]. In a prospective clinical pilot study, rapamycin improved the symptoms of six patients with severe venous malformations[215]. As PIK3CA functions downstream of TIE2, PIK3CA inhibitors could benefit most patients with venous malformations. A PIK3CA inhibitor (BYL719, which is currently in clinical trials in cancer) was more efficient than rapamycin in restoring molecular and cellular dysfunction caused by TIE2 and PIK3CA mutant proteins *in vitro*[29]. Thus, it could provide a possible therapy for venous malformations[29–31]. In addition to the TIE2–PI3K–mTOR pathway, mitogen-activated protein kinases (MAPKs) may also have a role in the pathogenesis of venous malformations[30,216]. **c** | Heterozygous loss-of-function mutations in *TIE2* cause human primary congenital glaucoma[34]. c.760 + 2T>C and c.3300 + 2delT refer to splice donor site mutations that result in a premature stop codon or exon skipping. EGF, epidermal growth factor; RICTOR, rapamycin-insensitive companion of mTOR; SIN1, SAPK-interacting protein 1. *indicates a premature stop codon.

Tie1 deletion induced at birth inhibited postnatal angiogenesis in the mouse retina, and this inhibition correlated with increased activation of the Notch pathway[21].

Metastasis. Tumour cells interact with endothelial cells during multiple steps of metastatic tumour progression. Metastasizing tumour cells can invade the abnormally leaky and unstable tumour vasculature, and subsequently arrest and extravasate through the endothelium at sites of distant metastasis[85].

The tumour microenvironment of metastasis (TMEM) consists of sites of the tumour vasculature where a sessile macrophage, endothelial cell and tumour cells make contact. TMEMs are found in both mouse mammary tumours and human breast cancer, and their presence predicts the risk of distant metastasis[86]. The TMEM contains pro-angiogenic TEMs that adhere to tumour vessels and induce a local VEGF-dependent increase in vascular permeability. This facilitates tumour cell intravasation and breast cancer metastasis[13,87,88] (FIG. 3).

The pre-metastatic niche is a predetermined site of distant organ metastases. ANG2 is upregulated in the lung endothelium during early metastatic growth[64]. In syngeneic B16 melanoma and Lewis lung carcinoma mouse tumour models, VEGF was shown to stimulate the activity of the transcription factor nuclear factor of activated T cells cytoplasmic 1 (NFATC1) in the pulmonary pre-metastatic niche, which resulted in the upregulation of ANG2 expression and increased metastatic lung colonization[89]. In immunodeficient mice, intravenously injected human tumour cells that invaded the lung decreased the integrity of pulmonary capillaries, whereas administration of ANG2-blocking antibodies improved the integrity of capillary endothelial junctions and decreased lung metastatic colonization[66]. In addition, the administration of ANG2-blocking antibodies decreased the formation of spontaneous lung metastasis in the MMTV-PyMT mammary adenocarcinoma model and of human tumour xenografts in mice. In the MMTV-PyMT model, ANG2-blocking antibodies impaired the association of TEMs with the tumour vasculature, TIE2 expression and the pro-angiogenic properties of TEMs[90]. ANG2 blockade also reduced tumour-associated lymphangiogenesis, thereby inhibiting lymph node metastasis of human lung carcinoma cells in immunocompromised mice[66]. The genetic deletion of *Ang2* in mice decreased pulmonary metastases of xenografted colon adenocarcinoma cells. By contrast, metastatic growth was increased in the liver of *Ang2*-deficient mice[91]. This increase in liver metastasis was associated with compensatory angiogenic mechanisms, including increased expression of granulocyte colony-stimulating factor (G-CSF) and CXC-chemokine ligand 1 (CXCL1), and infiltration of neutrophils and TEMs into the tumours. These results suggest that the function of ANG2 in metastasis is organ specific[91]. However, one should consider that the barrier properties of vascular beds in different organs vary from the fenestrated or discontinuous endothelium in organs such as the liver or in bone marrow sinusoids to the tight, contiguous endothelium of pulmonary capillaries and the highly impermeable blood–brain barrier[85].

The ANG–TIE pathway in vascular stabilization

Microvascular dysfunction and endothelial activation, which are associated with increased endothelial permeability and inflammation, present an important clinical problem in many pathological processes, including sepsis, vascular complications of diabetes — such as diabetic macular oedema (DMO) and impaired wound healing — ischaemia–reperfusion injury and atherosclerosis. The re-establishment of a structurally and functionally stable vasculature would be beneficial in these conditions.

Vascular permeability. ANG1 is a powerful vascular-stabilizing factor. Recombinant ANG1 protein or viral transgene delivery in mice and ANG1 stimulation of endothelial cells in culture inhibit vascular leakage induced by several inflammatory cytokines and growth factors, such as histamine, thrombin and VEGF[35,92,93]. By contrast, ANG2 increases vascular leakage in synergy with inflammatory cytokines, including histamine and VEGF[94], although a high dose of recombinant ANG2 was reported to reduce vessel permeability in mice[95]. Interestingly, *Tie2* silencing, but not *Ang1* deletion, increased vascular leakage in the lungs of adult mice, which indicates that TIE2 is essential for the maintenance of endothelial barrier function under basal conditions[38,96]. Furthermore, VE-PTP-blocking antibodies, the VE-PTP inhibitor AKB-9778 and deletion of the gene encoding VE-PTP (*Ptprb*), which results in TIE2 activation, inhibited lipopolysaccharide (LPS)-induced vascular leakage and leukocyte endothelial transmigration in mice[38]. Notably, upon *Tie2* silencing, VE-PTP inhibition promoted vascular leakage via destabilization of VE-cadherin[38], which suggests that it could activate the VEGFR2–VE-cadherin permeability pathway[97].

Inflammation and infection. In inflammatory conditions, the increased ANG2/ANG1 ratio attenuates the vascular-stabilizing ANG1–TIE2 axis[98]. Increased concentration of ANG2 in patient serum has been shown to correlate with a poor prognosis in sepsis, acute lung injury, acute respiratory distress syndrome and numerous other diseases[47,99,100] (TABLE 1). Inflammatory stimuli release ANG2 from Weibel–Palade bodies, which enables its binding to TIE2 (REF. 39). Decreased ANG1–TIE2–AKT signalling induces the translocation of FOXO1 into the nucleus, stimulating ANG2 expression[19,45,49]. ANG2 may act as an agonist to initially protect the endothelium via an autocrine mechanism[95,101]. However, during inflammation, ANG2 turns into a TIE2 antagonist in mice, and this switch between the agonistic and antagonistic activity seems to be regulated by TIE1 in addition to the inflammatory signals[19,49] (FIG. 4).

Under non-inflammatory conditions, transgenic ANG2 expression (controlled by the promoter of the gene encoding VE-cadherin or TIE1) stimulated TIE2 phosphorylation in mice[19,49]. Deletion of *Tie1* from the vascular endothelium of adult transgenic mice blocked the agonistic activity of ANG2. In addition, endothelial-specific *Tie1* deletion in mice decreased the agonistic activity of recombinant ANG1, which led to reduced ANG1-stimulated AKT activation, and increased FOXO1 nuclear translocation and the transcriptional activation of FOXO1 target genes[19,21]. Importantly, *Tie1* deletion blocked the capillary-to-venous remodelling that is induced by adenovirus-expressed ANG1 or ANG2 in non-inflammatory conditions[19].

The TIE2-agonistic activity of ANG2, produced either via a transgene or an adenoviral vector, was also lost in acute and chronic inflammation. ANG2 did not induce TIE2 phosphorylation during LPS-induced acute inflammation or during *Mycoplasma pulmonis*-induced chronic inflammation in mice[19,49]. The TIE1 ectodomain — which is responsible for interacting with TIE2 — was rapidly cleaved after LPS or TNF administration in mice[19,102] and more slowly in chronic *M. pulmonis* infection[49]. TIE1 cleavage may thus contribute to the loss of the agonistic activity of ANG2 during inflammation[19,49]. Overall, these results support a model in which TIE1 directly interacts with TIE2 to promote the agonistic functions of ANG proteins and vascular

Diabetic macular oedema (DMO). Oedema in the macula that causes vision impairment in patients with diabetes.

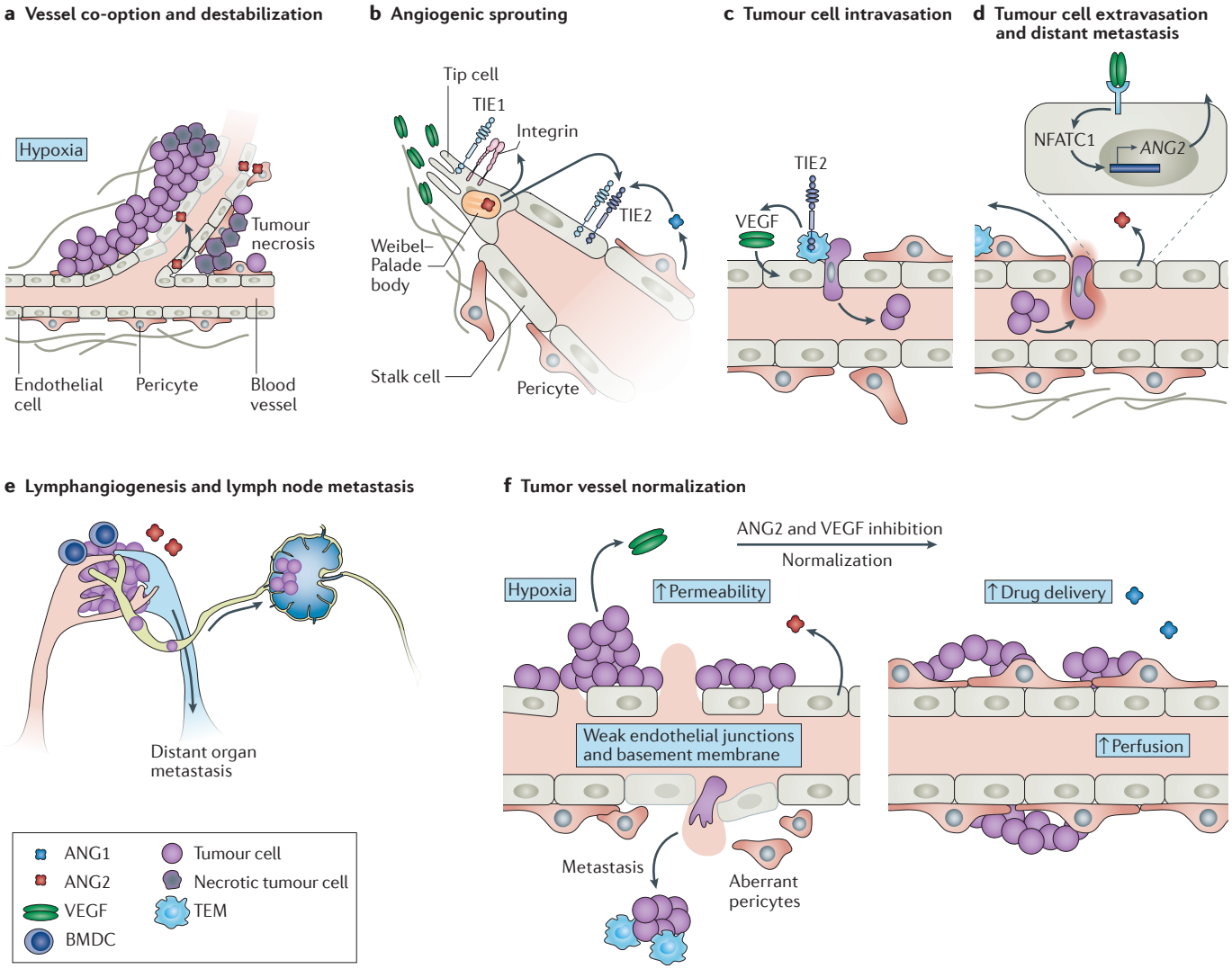

Figure 3 | **The ANG–TIE system in tumour angiogenesis and metastasis.** Angiopoietin 2 (ANG2) promotes several phases of tumour progression. **a** | ANG2 is upregulated in the vascular endothelium in a preclinical model of glioma and during vascular co-optive tumour growth in which a tumour grows along existing host blood vessels. In this model, ANG2 upregulation in endothelial cells is associated with the regression of the co-opted blood vessels. This regression results in augmented hypoxia, which increases the expression of vascular endothelial growth factor (VEGF) and ANG2, stimulating neoangiogenesis at the tumour margin (the so-called angiogenic switch)[64]. **b** | The upregulation of VEGF provides a strong signal for angiogenesis by regulating endothelial cell tip cell specification via tip cell-expressed DLL4 that stimulates Notch signalling in the stalk cells and by promoting endothelial cell migration[217]. Tip endothelial cells are characterized by high expression of ANG2, but low expression of TIE2, which may be downregulated via a TIE1-dependent mechanism in tip cells[20,218]. The high ANG2/TIE2 ratio may favour ANG2–integrin interactions in tip cells[57]. Stalk endothelial cells are likely to be exposed to paracrine ANG1, which stimulates phosphorylation of both TIE1 and TIE2 and promotes vessel stabilization. In tumour models, ANG2 blockade decreases vascular sprouting and induces vessel regression, acting in synergy with VEGF blockage[67,68,70]. Conditional deletion of *Tie1* in gene-targeted mice was associated with apoptosis of tumour endothelial cells and reduced tumour vascularization[21]. **c** | TIE2-expressing macrophages (TEMs), breast cancer cells and tumour endothelial cells make contact with each another at sites of the tumour microenvironment of metastasis. TEM-derived VEGF induces transient vessel destabilization, thereby promoting tumour cell intravasation in the primary tumour[87]. **d** | ANG2 promotes tumour metastasis to the lungs, and ANG2-blocking antibodies inhibit lung metastasis by improving the integrity of tumour vessels and by interfering with TEM function[66,90]. ANG2 is upregulated in the lung pre-metastatic niche in mouse tumour models via VEGF-mediated activation of the transcription factor nuclear factor of activated T cells cytoplasmic 1 (NFATC1), which stimulates *Ang2* transcription by directly binding to the *Ang2* promoter region in pulmonary endothelial cells[89]. **e** | ANG2-blocking antibodies inhibit tumour lymphangiogenesis and lymph node metastasis[66]. **f** | Tumour blood vessels are poorly organized and have functional and structural alterations, including leaky endothelial cell–endothelial cell (EC–EC) junctions, poor perfusion, an incomplete basement membrane and weak endothelial cell–pericyte interactions[219,220]. These alterations promote tumour progression, increase hypoxia and interstitial fluid pressure, and impair drug delivery to tumours. ANG2 blockade normalizes tumour vessels via increased deposition of cell junction adhesion proteins and pericytes on tumour vessels, increasing blood perfusion[70]. These effects were not observed in mice when ANG1-blocking agents were co-administered[70]. By contrast, TIE2 activation by treatment with the vascular endothelial protein tyrosine phosphatase (VE-PTP) inhibitor AKB-9778 normalized tumour blood vessels and increased perfusion in mouse models of breast cancer, thus delaying tumour progression by decreasing tumour cell extravasation[82]. Furthermore, the combined blocking of ANG2 and VEGF modulates the tumour microenvironment by promoting the macrophage M1 phenotype and anti-tumour immunity[62,79,80,306].

responses to these ligands under non-inflammatory conditions, whereas inflammation is characterized by TIE1 cleavage, low levels of TIE2 phosphorylation, and the loss of both the agonistic activity of ANG2 and vascular stability.

ANG2 blockade and stimulation of the ANG1–TIE2 signalling axis have both shown beneficial therapeutic effects in mouse models of acute inflammation, sepsis and acute lung injury. Treatment with ANG2-neutralizing antibodies or ANG2-targeting short interfering RNA, or reduction of the *Ang2* gene dosage in heterozygous mice, improved endothelial barrier function, attenuated endothelial inflammation and leukocyte infiltration into tissues, and increased survival in mouse models of sepsis and systemic inflammation[99,103–105]. In addition, treatment with recombinant ANG1 or the VE-PTP inhibitor AKB-9778 decreased vascular leakage in the caecal ligation and puncture model of sepsis and after LPS administration in mice, respectively[38,103]. More recently, a novel antibody that induces ANG2 clustering was shown to convert ANG2 into an agonistic ligand that activated TIE2 and improved vascular barrier function in mouse models of sepsis and endotoxaemia, which led to increased survival of the mice[106]. Interestingly, this new strategy of combining ANG2 blocking with TIE2 activation was more effective than neutralization of the ANG2–TIE2 interaction[106].

In addition to TIE1 ectodomain shedding, the mRNA and protein levels of ANG1, TIE1 and TIE2 are reduced in LPS-induced acute inflammation in mice[19,107]. Vessel dilation that occurs in inflammation in association with decreased laminar shear stress decreases TIE2 expression, but otherwise, the mechanisms that regulate these genes in inflammation remain to be investigated[108]. When TIE2 expression is reduced in pathological conditions, it is likely that more ANG2 binds to and signals via an alternative receptor. For example, in *Tie2*-silenced endothelial cells in culture, ANG2 was found to activate α5β1 integrin, and this resulted in the formation of actin stress fibres, changes in EC–ECM adhesion and destabilization of the endothelial monolayer[48].

Recently, *cis*-acting single-nucleotide polymorphisms in *TIE2* were observed to be associated with *TIE2* expression levels in human HapMap3 lymphoblastoid cell lines[109]. Notably, the haplotype with highest *TIE2* expression in patients was associated with a 28% reduction in the risk of acute respiratory distress syndrome, which suggests that genetic variation in the *TIE2* locus may contribute to the clinical outcomes of common infections[109]. Genetic variation resulting in reduced TIE expression in mice was also associated with susceptibility to the vascular complications of Ebola haemorrhagic fever[110]. Thus, low expression of the TIE receptors may predispose to vascular complications that are associated with infections and inflammation.

During chronic inflammation, the remodelling of capillaries to venules expands the vascular area, which facilitates plasma leakage and leukocyte emigration. Chronic infection of mouse airways by *M. pulmonis* increases ANG2 expression, decreases levels of phosphorylated TIE2 in the mucosal vessels and promotes venous remodelling[98,111]. ANG2-blocking antibodies decreased vessel remodelling, vascular leakage and leukocyte influx, and TNF-targeting antibodies further promoted these effects, suggesting that ANG2 inhibitors have potential for the treatment of sustained inflammation[98,111]. ANG2 may also be crucial in inflammatory lymphangiogenesis, which occurs in some corneal diseases and increases the risk of transplant rejection[112]. The administration of recombinant ANG1 stimulates vessel enlargement and capillary-to-venous remodelling in a TIE1-dependent manner; however, the ANG1-induced vessels have an extensive pericyte coating and enhanced blood flow, and are non-leaky[19,27,49,113,114]. Interestingly, although the injection of recombinant ANG2 into mice promotes similar changes in tissues, ANG2 switches from acting as an agonist to acting as an antagonist during the course of *M. pulmonis* infection and renders the vessels leaky, as described above[49].

In summary, increased FOXO1-driven *Ang2* expression, cleavage of TIE1, downregulation of TIE2 and ANG1 in inflammation-associated low-flow conditions, and a switch in ANG2 function from an agonist to an antagonist are likely to contribute to enhanced vessel permeability and vascular destabilization that occur during infection and inflammation (FIG. 4).

The role of the ANG–TIE pathway in organ transplantation and atherosclerosis. Ischemia-reperfusion injury that occurs during human organ transplantation may initiate a harmful inflammatory reaction, which leads to tissue injury, graft rejection and even death[115]. The concentration of circulating ANG2 was found to predict mortality in human kidney transplant recipients[116]. Furthermore, plasma ANG2 concentration was elevated during ischaemia–reperfusion injury in human and rat recipients of cardiac allografts but not in rat recipients of syngrafts[115]. A single intracoronary injection of ANG2-neutralizing antibodies was shown to prevent endothelial cell activation, leukocyte infiltration, transplantation-associated ischaemia–reperfusion injury and the development of chronic rejection[115]. This suggests that ANG2 neutralization could be used to suppress the inflammatory response and to prevent microvascular injury following organ transplantation.

Cardiac allograft vasculopathy (CAV) is a major late complication that causes transplantation failure. CAV lesions show similarities to atherosclerotic lesions in coronary arteries; however, CAV develops faster than does atherosclerosis, and it affects both arteries and veins. The development of intimal hyperplasia in transplanted hearts can cause narrowing of the vessel lumen and ischaemic heart disease. In rats, administration of ANG1 via an adenoviral vector reduced the incidence and extent of intimal lesions in the early phase of CAV[117,118]. The protective effect of ANG1 was associated with decreased circulating levels of ANG2, graft-infiltrating leukocytes, interstitial fibrosis and a reduced immune response[117].

Atherosclerotic lesion development involves lipid and inflammatory cell accumulation in the intima. This predominantly occurs at sites of turbulent blood

Table 1 | **Involvement of ANG–TIE pathway components in human disease and mortality**

Indication	Implications of the involvement of the ANG–TIE pathway	Refs
Retinopathies		
Proliferative diabetic retinopathy	Elevated ANG2 levels in the vitreous fluid correlate with increased ocular VEGF levels in active disease.	232
Diabetic retinopathy	An elevated ANG2/ANG1 ratio was found in the ocular fluids of patients with non-proliferative diabetic retinopathy and macular oedema who underwent vitrectomy. Elevated intravitreal ANG2 levels are associated with poor glycaemic control.	148
Retinopathy of prematurity	Vitreous levels of ANG1 and ANG2 are elevated in severe disease, and there is a negative correlation between ANG1 and ANG2 levels in moderately and mildly vascularized retinal lesions.	233
Rhegmatogenous retinal detachment	Elevated ANG2 levels are found in the vitreous.	234
Mortality in the general population and mortality due to complications		
Mortality in the general population	Elevated serum ANG2 levels are associated with an increased risk of all-cause and cardiovascular mortality.	235
Complications of haematopoietic stem cell transplantation	High plasma ANG2 levels after allogeneic haematopoietic stem cell transplantation are associated with an increased incidence of non-infectious transplant-related complications, endothelial cell damage and poor survival.	236
Complications in kidney transplant recipients	High serum ANG2 levels correlate with increased all-cause mortality.	116
Myocardial infarction complicated by cardiogenic shock	Elevated levels of ANG2 are associated with poor outcomes, reperfusion failure and complications, and predict mortality.	237
Major trauma	ANG2 is increased after trauma in patients with severe injury and systemic hypoperfusion. High plasma ANG2 levels are associated with early coagulation abnormalities, complement activation and a worse clinical outcome.	238
Children with severe bacterial infections	Low plasma ANG1 levels are associated with a poor prognosis.	239
Brain injuries		
Ischaemic stroke	Low plasma ANG1 levels are associated with poor outcomes.	240
Subarachnoid haemorrhage	ANG1 levels are reduced, particularly in patients with cerebral vasospasms and ischaemia.	241
Aneurysmal subarachnoid haemorrhage	High ANG1 levels predict good functional outcome after aneurysmal subarachnoid haemorrhage.	242
Arthritis		
Rheumatoid arthritis and associated cardiovascular disease	Serum ANG2 levels correlate with inflammation and disease severity in arthritis, and may be predictive of cardiovascular disease.	243,244
Fibrotic conditions		
Systemic sclerosis	Increased serum levels of TIE1 and ANG2. Serum levels of TIE2 are positively associated with oesophageal changes, and ANG1 levels are negatively correlated with the duration of Raynaud's phenomenon.	245
Liver cirrhosis and hepatocellular carcinoma	Elevated ANG2 levels are found in serum.	246
Liver cirrhosis	Increased serum ANG2 levels are found in the systemic and suprahepatic circulation.	247
Vascular calcification in chronic kidney disease	Plasma ANG2 levels correlate with the severity of arterial stiffness. ANG2 may promote inflammation and collagen expression.	130
Chronic hepatitis C	Serum ANG2 levels correlate with hepatic fibrosis.	248
Acute and chronic kidney disease		
Kidney injury as a complication of myocardial infarction	Elevated plasma ANG2 levels predict the development of acute kidney injury in patients with acute myocardial infarction.	249
Renal outcome, cardiovascular events and mortality in chronic kidney disease	Elevated plasma ANG2 levels predict adverse renal outcomes and are associated with an increased risk of major adverse cardiovascular events and all-cause mortality.	250

Table 1 (cont.) | **Involvement of ANG–TIE pathway components in human disease and mortality**

Indication	Implications of the involvement of the ANG–TIE pathway	Refs
Cancer		
Multiple myeloma	ANG2 levels and ANG2/ANG1 ratio are increased in serum. These parameters correlate with the angiogenic process, and are prognostic for response to therapy and survival. Concomitant increases in the serum ANG2 and VEGF levels are predictive of a poor treatment response.	251, 252
Non-small cell lung cancer	Correlation between circulating *ANG2* mRNA levels and prognosis.	253
Breast cancer	Increased *ANG2* mRNA expression in biopsy samples correlates with lymph node invasion and short-term survival. Elevated serum ANG2 levels have prognostic value and potential for the early detection of breast cancer. Elevated serum TIE2 and bFGF levels are associated with a pathological complete response to bevacizumab and chemotherapy.	58, 254,255
Metastatic colorectal carcinoma	Serum ANG2 levels have prognostic value for overall survival in risk stratification.	256
Colorectal cancer	Low ANG2 expression is associated with a better response rate to anti-VEGF treatment.	60
Renal cell carcinoma	Plasma ANG2 levels are elevated in renal cell carcinoma when compared with various benign kidney diseases. Pre-operative plasma ANG2 levels correlate with increased tumour size and advanced grade.	257
Metastatic renal cell carcinoma	High ANG2 expression in the tumour vasculature is associated with clinical benefit in response to first-line sunitinib therapy.	63
Hepatocellular carcinoma	Elevated serum ANG2 levels correlate with shorter progression-free and overall survival in patients treated with sorafenib.	258
Melanoma	Increased serum ANG2 levels correlate with tumour progression and overall survival.	59
Chronic lymphocytic leukaemia	Elevated ANG2 plasma levels correlate with poor prognosis.	259
Acute myeloid leukaemia	Increased *ANG2* expression in bone marrow mononuclear cells indicates an unfavourable prognosis.	260
Glioblastoma	Low ANG2 levels predict increased overall survival.	62
Vascular leakage		
Wide range of leakage-associated infections	Decreased TIE2 expression worsens clinical outcomes.	109
Dengue virus infection	Elevated ANG2 and soluble VEGFR2 levels are associated with vascular leakage and may be surrogate markers for plasma leakage in patients with acute dengue virus infection.	261
Acute respiratory distress syndrome	Elevated plasma ANG2 levels are associated with mortality, particularly in paediatric patients who have undergone haematopoietic stem cell transplantation.	262
Sepsis	Low ANG1 levels, elevated ANG2 levels and higher ANG2/ANG1 ratio correlate with disease severity and clinical outcome.	47,99, 263–270
Acute lung injury	Higher baseline serum ANG2 levels are associated with increased mortality in non-infection-related disease, and a high plasma ANG2/ANG1 ratio predicts increased mortality.	271,272
Acute liver failure	Serum ANG2 levels correlate with surrogate markers of organ dysfunction and measures of disease severity.	273
Systemic capillary leak syndrome (also known as Clarkson disease)	Elevated ANG2 levels are detected in the sera of patients with episodic disease.	274
Disseminated intravascular coagulation	Patients have increased serum ANG2 levels and an elevated ANG2/ANG1 ratio.	275
Post-cardiac arrest syndrome	Increased serum ANG2 levels and an elevated ANG2/ANG1 ratio are found in non-survivors compared with survivors.	276
Acute kidney injury	Serum ANG2 levels predict mortality in patients with dialysis-dependent disease who were in intensive care.	277
Acute pancreatitis		
Acute pancreatitis	Elevated serum and plasma ANG2 levels predict severe acute pancreatitis associated with organ failure and also predict outcomes in severe acute pancreatitis with infectious complications better than do conventional markers used in the clinic.	278,279
Malaria		
Malaria	Plasma ANG2 levels and the ANG2/ANG1 ratio correlate with metabolic acidosis and clinical severity. Increased plasma ANG1 levels result in platelet activation that may dampen the effects of ANG2 on endothelial cells.	280,281
Cerebral malaria syndrome	Elevated levels of ANG2 and soluble TIE2 were found in children with cerebral malaria who did not survive. Plasma ANG1 levels are lower in survivors and non-survivors than in healthy controls at the time of hospital admission. Increased ANG2 levels correlate with malaria severity and predicted death.	282,283

Table 1 (cont.) | **Involvement of ANG–TIE pathway components in human disease and mortality**

Indication	Implications of the involvement of the ANG–TIE pathway	Refs
Cardiac conditions		
Congenital heart disease	ANG2 levels correlate with ventricular dysfunction, whereas levels of ANG1 and soluble TIE2 do not.	284
Acute decompensated heart failure	Elevated ANG2 levels are associated with peripheral oedema and predict poor outcome.	285
Obstructive lung disease		
Chronic obstructive pulmonary disease	Elevated serum ANG2 levels are present at the onset of disease exacerbations. High levels of ANG2 positively correlate with levels of serum C-reactive protein and are associated with unfavourable outcomes.	286
Paediatric asthma	Serum ANG1 levels and the ANG1/ANG2 ratio (but not ANG2 levels) are reduced in children with asthma. This may be related to inflammation and destabilization of the vasculature.	287
Severe asthma	Increased serum ANG2 levels correlate with respiratory function and asthma severity.	288
Diabetes		
Diabetes	Elevated plasma ANG2 and VEGF levels (but not ANG1 levels) are associated with metabolic indices and endothelial dysfunction. Reduced plasma VEGF and ANG2 levels may reflect an improved vascular status following treatment.	289
Angiopathy in type 2 diabetes mellitus	Elevated serum ANG2 levels (but not ANG1 levels) positively correlate with insulin resistance and levels of glycated haemoglobin, and are also associated with glucose metabolism disorders and vascular lesions.	290

ANG, angiopoietin; bFGF, basic fibroblast growth factor (also known as FGF2); VEGF, vascular endothelial growth factor; VEGFR2, VEGF receptor 2.

flow, which are commonly found at vessel bifurcations, whereas sites of high shear stress are protected[119]. TIE1 and ANG2 are expressed at sites of non-laminar flow in the mouse aorta[120,121]. In addition, atherogenic non-laminar flow has been shown to increase the expression of vascular cell adhesion molecule 1 (VCAM1), intercellular adhesion molecule 1 (ICAM1) and TIE1, whereas protective laminar flow suppresses *Tie1* promoter activity in endothelial cells[120,122,123]. Partial deletion of TIE1 in hypercholesterolaemic apolipoprotein E-deficient mice decreased the number of atherosclerotic lesions in the distal aorta, which suggests that TIE1 contributes to inflammation in the development of atherogenesis[123].

ANG2-blocking antibodies reduced plasma triglyceride levels and decreased the formation of intimal fatty streaks in another mouse model of hypercholesterolaemia-induced atherosclerosis, which suggests a beneficial effect of ANG2 depletion during the early phase of atherosclerosis[124]. However, ANG2 blockade did not affect pre-existing atherosclerotic plaques or plaque stability[124].

Cerebral cavernous malformations. Cerebral cavernous malformations (CCMs) are central nervous system-associated clusters of thin-walled, poorly pericyte-coated, dilated vessels that are vulnerable to leakage, which causes cerebral haemorrhages and neurological symptoms[125]. Recently, increased ANG2 secretion was linked to the development of CCM lesions in mice lacking *Pdcd10* (which encodes programmed cell death protein 10; also known as *Ccm3*) and in patients with CCM[125]. The PDCD10–UNC13B complex — which normally regulates the exocytosis of ANG2-containing secretory vesicles — was lacking in *Pdcd10*-deficient mice, and this led to increased secretion of ANG2, which has been associated with destabilization of EC–EC junctions and endothelial cell–pericyte interactions. The administration of an ANG2-blocking antibody reduced vascular destabilization and leakage in the mutant mice, which suggests that ANG2 inhibition may represent a novel approach for the treatment of CCM.

Diabetic vascular complications

Diabetes commonly results in vascular dysfunction that affects various parts of the body, including the heart, eyes, limbs, nerves, kidneys and wound healing. In the heart, diabetes and associated vascular dysfunction can impair cardiac function, induce tissue inflammation, cause interstitial and perivascular fibrosis, and promote capillary rarefaction. These changes contribute to atherosclerotic vascular disease, which is a primary reason for the cardiovascular dysfunction, in addition to an intrinsic heart muscle malfunction called diabetic cardiomyopathy. Retinopathy is another common complication of diabetes. It is characterized by pericyte dropout, the growth of abnormal blood vessels and vascular leakage, which result in DMO that may cause vision loss. Recent studies using genetically modified mouse models or injection of recombinant ANG proteins suggest that ANG1 protects against diabetic vascular dysfunction. Hyperglycaemia and dyslipidaemia suppress vascular protection by ANG1 and predispose to endothelial dysfunction and vascular disease[126]. At the cellular level, elevated glucose levels decreased ANG1-induced phosphorylation of TIE2 and AKT, but not phosphorylation of extracellular signal-regulated kinase 1 (ERK1; also known as MAPK3) or ERK2 (also known as MAPK1)[127].

Diabetic nephropathy is the most common cause of chronic kidney disease that leads to kidney failure. Vascular growth factors maintain the structure and integrity of the kidney glomerular filtration barrier. In healthy adult glomeruli, VEGF and ANG1 are constitutively expressed in glomerular podocytes, and ANG1

has been shown to have a protective role in preclinical models of diabetic nephropathy[126]. A widely used model of diabetes is induced in mice by injection of the pancreatic endocrine β-cell toxin streptozotocin (STZ), which results in hyperglycaemia. In the STZ-induced model system, glomerular ANG1 expression was decreased, whereas VEGF–VEGFR2 signalling was increased[128]. Inducible podocyte-specific expression of *Ang1* reduced albuminuria, decreased glomerular endothelial cell proliferation and VEGFR2 phosphorylation but increased phosphorylation of TIE2 and endothelial nitric oxide synthase[128]. By contrast, conditional *Ang1* deletion aggravated kidney pathology in STZ-induced diabetes, as shown by an increased urine albumin–creatinine ratio, glomerular leakage, increased mesangial ECM deposition and glomerulosclerosis, which are typically

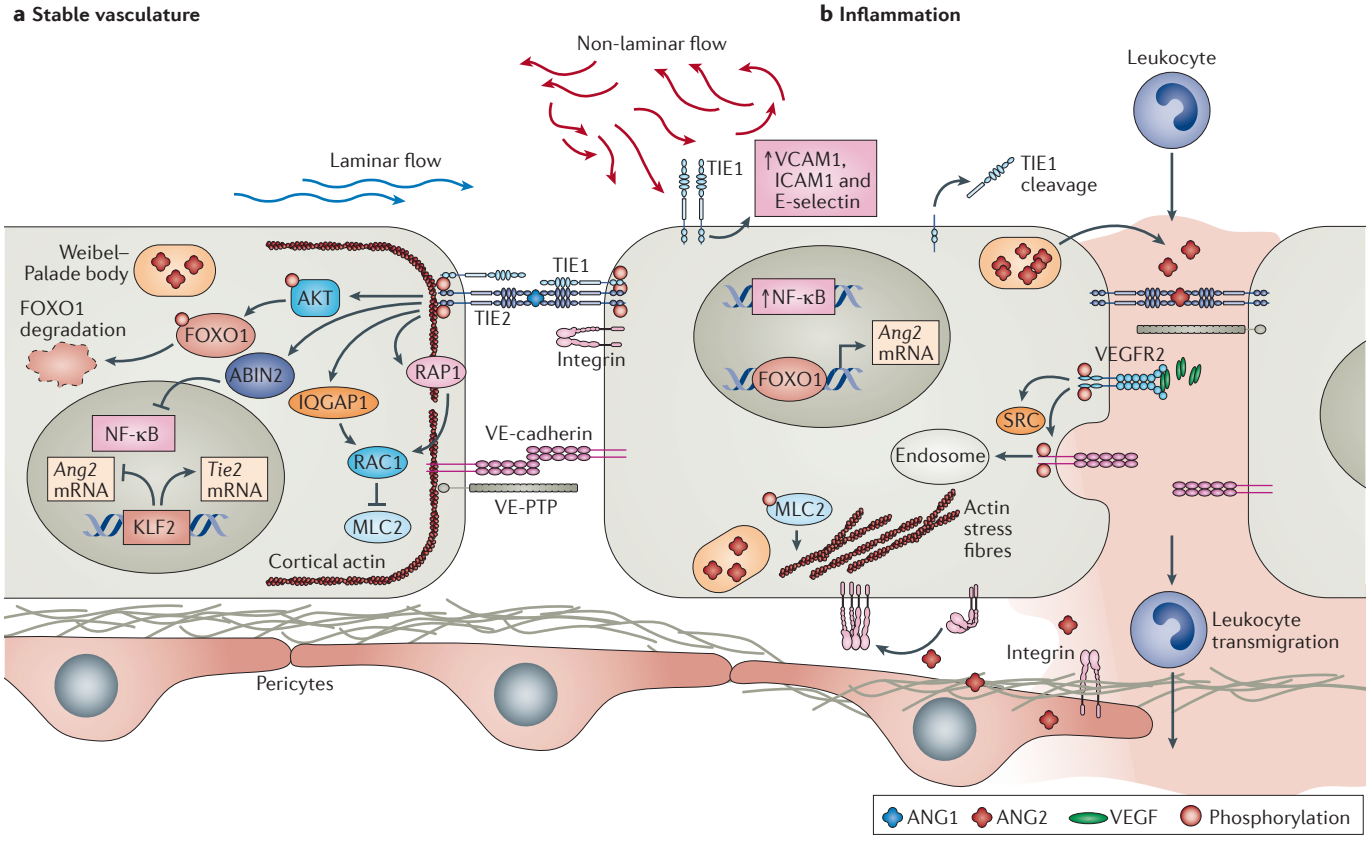

Figure 4 | **A model of ANG–TIE2 signalling in the regulation of vascular integrity in inflammation. a** | Angiopoietin 1 (ANG1) stabilizes newly formed blood vessels after pathological insults in mice[92,96]. ANG1 can stabilize the cortical actin cytoskeleton and promote cell junction integrity in cultured endothelial cells[35,36,38]. At endothelial cell–endothelial cell (EC–EC) junctions, ANG1 stimulates the formation of a TIE complex that contains both TIE1 and TIE2, and is dependent on α5β1 integrin[17–19,52,53]. ANG1 stimulates TIE phosphorylation (indicated by red circles), which leads to the activation of the GTPase RAS-related C3 botulinum toxin substrate 1 (RAC1) via the GTPase RAP1 or the GTPase-activating protein IQGAP1, and to stabilization of the cortical actin cytoskeleton in cultured endothelial cells[36,38]. In addition, ANG1 stimulates the activation of the serine kinase AKT in a TIE1-dependent and α5β1 integrin-dependent manner, which results in the phosphorylation of the transcription factor Forkhead box protein O1 (FOXO1), FOXO1 nuclear exclusion, and decreased expression of FOXO1 target genes in endothelial cells *in vitro* and *in vivo*[19–21,23–25]. TIE2-mediated activation of ABIN2 (also known as TNFAIP3-interacting protein 2) protects endothelial cells by inactivating the transcription factor nuclear factor-κB (NF-κB), which is a key transcriptional regulator of inflammatory responses in endothelial cells[221,222]. In addition, laminar flow and ANG1 stimulate the expression of the transcription factor Krueppel-like factor 2 (KLF2) and its target miR-30-5p, which results in decreased expression of *Ang2* and inflammatory genes[223,224]. **b** | During inflammation, ANG2 is secreted from Weibel–Palade bodies in response to inflammatory stimuli[39]. Although ANG2 can function as a weak TIE2 agonist in a TIE1-dependent manner during normal homeostatic conditions, ANG2 functions as a TIE2 antagonist during inflammation. This may involve the inflammation-induced cleavage of the TIE1 ectodomain[19,49]. ANG2 antagonism of TIE2 activates the FOXO1 pathway, which leads to increased expression of FOXO1 target genes, including *Ang2* (REF. 45). By contrast, inflammation-associated low flow decreases TIE2 levels[108]. Under these conditions, ANG2 may signal via endothelial integrins, which are upregulated, for example, during choroidal neovascularization in mice[225]. This signalling can lead to changes at endothelial cell–extracellular matrix contacts, stress fibre formation and vessel destabilization[48,226]. ANG2 can also induce pericyte apoptosis via p53 and α3β1 integrin, and retinal vessels show pericyte loss following intravitreal ANG2 injection[56,227]. The function of TIE1 seems to be more complex, as decreased laminar flow upregulates *Tie1* transcription in cultured endothelial cells. TIE1 can promote the expression of pro-inflammatory genes, including that encoding intercellular adhesion molecule 1 (ICAM1)[123,228,229], and deletion of *Tie1* protects apolipoprotein E-deficient mice from developing atherosclerosis[123]. During inflammation in mice, leukocyte transmigration induces the dissociation of vascular endothelial protein tyrosine phosphatase (VE-PTP) from vascular endothelial cadherin (VE-cadherin) and dephosphorylation of the constitutively phosphorylated Y731 of VE-cadherin[230], whereas vascular endothelial growth factor (VEGF)-induced permeability is mediated via Y685 phosphorylation and subsequent VE-cadherin internalization[213]. MLC2, myosin light chain 2; NF-κB, nuclear factor-κB; VCAM1, vascular cell adhesion molecule 1; VEGFR2, VEGF receptor 2.

observed in advanced-stage human diabetic nephropathy[96]. Upregulation of ANG2 and transforming growth factor-β (TGFβ) may promote the kidney pathology in these mice. Consistent with the renoprotective function of ANG1, adenoviral delivery of cartilage oligomeric matrix protein (COMP)–ANG1 (a soluble and stable form of ANG1) attenuated inflammation, fibrosis and proteinuria, and alleviated hyperglycaemia in a genetic model of diabetes, in the leptin receptor (*Lerp*)-mutant *db/db* mice[129].

Increased arterial stiffness associated with ageing, arteriosclerosis, endothelial dysfunction, inflammation and diabetes results in adverse changes in haemodynamics and a reduced ability to respond to blood pressure changes[130]. Increased arterial stiffness is also observed in the early stages of chronic kidney disease (CKD). In patients with CKD, plasma ANG2 levels correlate with the severity of arterial stiffness[131]. Nephrectomy increased ANG2 levels in mice, and ANG2-blocking antibodies decreased the expression of monocyte chemokines, profibrotic cytokines and collagen in the aortas of mice after partial nephrectomy, which suggests that ANG2 blockade can reduce the inflammation and collagen deposition that is associated with arterial stiffness[132].

Conditional *Ang1* deletion in adult mice limited pathological angiogenic responses such as excessive angiogenesis and fibrosis during wound healing[96]. By contrast, recombinant ANG1, delivered via an adenoviral vector, promoted the healing of cutaneous wounds in *db/db* mice[133,134]. ANG1 accelerated wound closure and epidermal and dermal regeneration, angiogenesis, lymphangiogenesis, and improved blood flow in the wound region[133,134].

Leptin-mutant *ob/ob* mice are obese and characterized by insulin resistance, hyperglycaemia, and alterations of peripheral nerve fibres and endoneural microvessels; these features are typical of the peripheral neuropathy observed in human type 2 diabetes. Intraperitoneal injections of COMP–ANG1 decreased the molecular biomarkers of neuropathy in *ob/ob* mice, promoted angiogenesis and suppressed inflammation in the sciatic nerves during a 3-week treatment period, which suggests that COMP–ANG1 provides neuroprotection in diabetic neuropathy[135].

The ANG–TIE pathway in ocular diseases

The ANG–TIE signalling pathway is essential for vascular development in the eye (TABLE 2), a process that involves regression of the embryonic hyaloid vessels in the vitreous and around the lens — which is a requisite for proper vision — and the simultaneous formation of the retinal vasculature. In mice, the superficial retinal vasculature develops during the first postnatal days. Subsequent regression of the hyaloid vessels and development of the deep and intermediate vascular plexuses continues for 3 weeks postnatally.

Pathological ocular neovascularization may affect the retinal, choroidal or corneal vasculature[2]. Retinal neovascularization, which may even cause retinal detachment, occurs in diabetic retinopathy, retinopathy of prematurity (ROP) and retinal vein occlusion. Choroidal neovascularization is observed in diseases that affect the outer retina and Bruch's membrane, such as wAMD. Hypoxia-inducible upregulation of VEGF, ANG2 as well as VE-PTP have been shown to govern the molecular pathogenesis of retinal and subretinal neovascularization in mouse models[2] (FIG. 5).

ANG2 is required for the postnatal development of the retinal vasculature[51,136]. ANG2 expression by endothelial cells is increased in the ischaemic retina and is necessary for ischaemia-induced retinal neovascularization, which is lacking in *Ang2*-deficient mice[136,137]. ANG2 is also required for VEGF-induced angiogenic sprouting in the retina. In adult mice, endothelial cells in the deep retinal capillaries continue to express ANG2, whereas ANG2 is not expressed in the superficial capillaries. Transgenic VEGF expression stimulated sprouting in the deep capillary plexus, whereas the superficial retinal capillary sprouts were stimulated only if *Ang2* transgene and *Vegf* via adenoviral vector were co-expressed in the retina, which indicates that ANG2 enhances retinal sensitivity to VEGF[138].

Transgenic mice with inducible ubiquitous or photoreceptor-restricted expression of ANG1 and ANG2 have revealed important functions of these growth factors in retinal and choroidal neovascularization. In a widely used model of oxygen-induced retinopathy (OIR), expression of *Ang2* at the onset of ischaemic retinopathy (upon return to normoxia, when VEGF levels are still high) was found to increase retinal neovascularization. By contrast, *Ang2* expression at a later time, when the ischaemia was reduced, resulted in the regression of the newly formed retinal vessels[139]. Inducible *Ang2* expression also caused the regression of choroidal neovascularization induced by laser-induced rupture of Bruch's membrane when VEGF levels were low[139]. By contrast, transgenic *Ang1* expression suppressed choroidal neovascularization without causing vascular regression and prevented retinal detachment in transgenic mice expressing VEGF in the photoreceptors[140]. Thus, ANG1 attenuates VEGF-induced neovascularization, whereas the sensitivity of the ocular vasculature to ANG2 depends on the VEGF/ANG2 ratio, such that vessel regression occurs when VEGF is low and stimulation of neovascularization occurs when VEGF levels are high.

The VEGF-blocking antibody bevacizumab, the Fab ranibizumab and the VEGF-trap aflibercept (BOX 1) have fundamentally altered the treatment of wAMD, and these therapeutics are also used to treat DMO[3]. VEGF-targeting drugs stabilize disease progression, prevent further loss of vision and improve mean baseline corrected visual acuity in patients with DMO. However, a considerable number of patients (nearly 50%) with neovascular eye diseases fail to respond to ranibizumab or bevacizumab[141–144]. It has been reported that VEGF inhibition is efficacious in retinal neovascularization until the endothelial cells become shielded by accessory cells and extensive ECM, which renders their survival less dependent on VEGF[2]. The choroidal neovessels then persist despite VEGF blockade, and they can grow and leak during treatment interruption. VEGFR2 expressed by retinal neurons functions mainly to titrate VEGF, thereby limiting neuronal vascularization[145].

Table 2 | **The ANG–TIE system in genetically engineered mouse models of ocular vascular disease**

Genetic model	Eye phenotype	Refs
Conditional *Ang1* deletion	• Decreased superficial and deep retinal vascular formation at postnatal day 5 (P5) and P17 • Increased vascular leakage and aggravated retinal neovascularization during OIR • Increased vascular leakage during laser-induced choroidal neovascularization	54,146
Ang2$^{-/-}$	• Persistent hyaloid vasculature at P10, and decreased postnatal retinal vascularization • No ischaemia-induced retinal neovascularization in the OIR model • Persistent proliferative retinopathy due to hypoxia-induced VEGF upregulation and characterized by endothelial proliferation, increased arteriolar and capillary diameters, and leakiness of the neovascularization front in the C57Bl/6 background	51,136, 291
Ang2$^{+/-}$	• Reduced pericyte loss and acellular capillaries in diabetic mice • Decelerated vasoregression in the retina with age • Reduced neovascularization and increased avascular zones in the OIR model	151, 292,293
Inducible transgenic retinal or ubiquitous ANG2 expression	• Impaired pericyte recruitment and abnormal retinal angiogenesis, mimicking diabetic retinopathy • Enhanced hyperglycaemia-induced vascular damage, vasoregression and pericyte destabilization • Induction of ANG2 expression during the ischaemic period of OIR increased retinal neovascularization, whereas ANG2 induction at a later, less ischaemic time point induced neovasculature regression • ANG2 stimulated regression of choroidal neovascularization induced by rupture of Bruch's membrane	139, 294,295
Inducible transgenic ubiquitous COMP–ANG1 or retinal ANG1 expression	• COMP–ANG1 expression suppressed retinal neovascularization in severe retinal ischaemia, which lead to improved vessel integrity and neuronal function during OIR, and decreased choroidal neovascularization and vascular leakage following rupture of Bruch's membrane • Transgenic ANG1 expression had no effect on established retinal or choroidal neovascularization • ANG1 suppressed ischemia-induced retinal and laser-induced choroidal neovascularization, and prevented retinal detachment in VEGF transgenic mice and vascular leakage induced by intravitreal VEGF injection	54,140, 146,296
Conditional *Ang1* and *Ang2* deletion, conditional *Tie2* deletion, *Tie2*$^{+/-}$	Impaired Schlemm's canal development, resulting in glaucoma	33,34
Conditional *Tie1* deletion	Impaired postnatal retinal vascular development in mice in which *Tie1* or both *Tie1* and *Tie2* were conditionally deleted	20,21

ANG, angiopoietin; OIR, oxygen-induced retinopathy; VEGF, vascular endothelial growth factor.

In wAMD, vascular leakage from nascent choroidal vessels is the key underlying cause of disease pathogenesis, and leads to vision loss and even blindness if left untreated. Intravitreal administration of COMP–ANG1 efficiently inhibited this condition, whereas inducible *Ang1* deletion aggravated laser-induced choroidal neovascularization in mice[146]. COMP–ANG1 suppressed vascular leakage by tightening endothelial cell junctions via increased expression of ZO1 and VE-cadherin, and by inhibiting the recruitment of angiogenic, VEGF-producing macrophages to the inflammatory lesions[146].

ROP occurs in premature babies who are nurtured in a hyperoxia chamber. Upon their return to normoxia, abnormal blood vessels grow in the retina, which can cause retinal detachment and lead to blindness. OIR in mice recapitulates the pathological features of human ROP. In the OIR model, *Ang1* expression or intraocular COMP–ANG1 administration normalized the vasculature, and prevented vascular leakage, abnormal angiogenesis and neuronal damage[54]. Interestingly, some of the vascular-protective effects of ANG1 seemed to be mediated via astrocytes, which are known to deposit fibronectin in the perivascular ECM, thereby guiding vessel growth. Blocking antibodies against astrocyte-expressed αvβ5 integrin decreased vessel sprouting and increased the retinal avascular area during OIR[54].

Diabetic retinopathy is the most frequent complication of diabetes. Intravitreal ranibizumab and aflibercept are currently used to treat of DMO[2]. For patients with proliferative diabetic retinopathy (PDR), laser photocoagulation remains the mainstay therapy, although this treatment is an inherently destructive procedure. Diabetic retinopathy is characterized by a loss of integrity of the retinal vasculature (including that of retinal pericytes), vascular leakage, ischaemia and neovascularization, which together cause vision loss[147]. High ANG2 levels have been detected in the vitreous of patients with diabetic retinopathy, and in rodent models of diabetes[148]. High glucose concentrations have been found to upregulate ANG2 expression in cultured retinal endothelial cells[149,150]. Importantly, the intravitreal administration of recombinant ANG2 increased retinal pericyte loss in non-diabetic rats, whereas diabetic mice with one *Ang2* allele missing showed decreased retinal pericyte dropout[151]. In addition, intravitreal injection of recombinant ANG2 protein in mice increased retinal vessel permeability by increasing the phosphorylation of VE-cadherin and its subsequent degradation[150]. These results suggest that ANG2, induced by hyperglycaemia, regulates integrity and pericyte coverage in retinal vessels in diabetic retinopathy. Interestingly, a recent report demonstrated that the depletion of retinal pericytes postnatally in mice using PDGFRβ-blocking antibodies induced signs of diabetic retinopathy, including hyperpermeability, hypoperfusion and neoangiogenesis that persisted in adult mice. In this model of diabetic retinopathy, pericyte loss was associated with endothelial inflammation, FOXO1 activation, ANG2 upregulation and cleavage of TIE1, whereas the

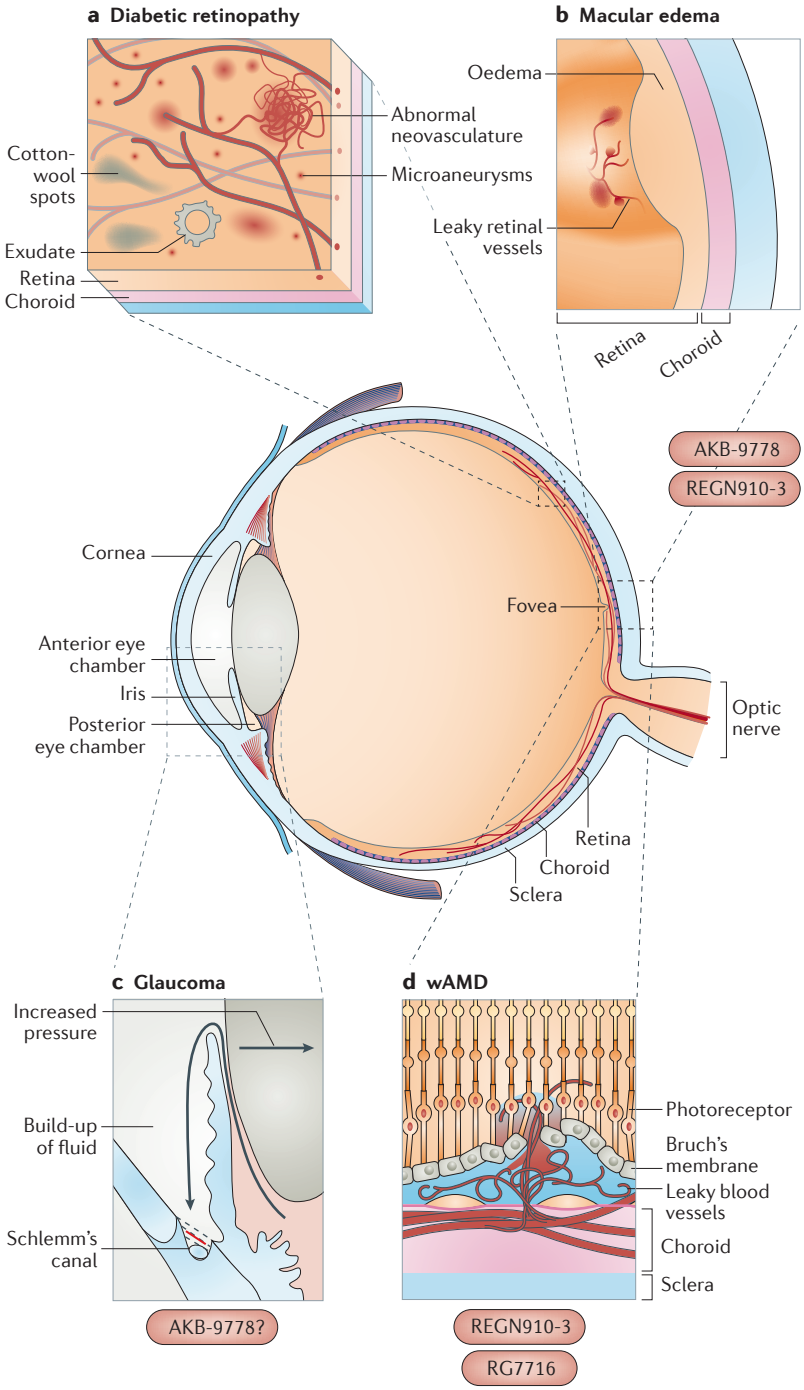

Figure 5 | **The ANG–TIE system in neovascular eye diseases. a** | Human diabetic retinopathy is characterized by microaneurysms, which are an early sign of the disease that result from the loss of capillary integrity and pericytes (not shown)[151]. Disease progression is accompanied by haemorrhages, retinal oedema (not shown), exudates of proteins and lipids that are leaked because of a breakdown of the blood–retina barrier, infarctions in the nerve fibre layer (cotton-wool spots) and venous nonperfusion (not shown). Increased retinal ischaemia and upregulation of vascular endothelial growth factor (VEGF) contribute to proliferative diabetic retinopathy that is associated with capillary remodelling. **b** | Diabetic macular oedema (DMO) is a leading cause of visual impairment in patients with diabetes and early diabetic retinopathy. DMO is characterized by vessel leakage, which leads to oedema and thickening of the central fovea, which are major factors that contribute to decreased vision. Currently, VEGF-targeted therapies, including ranibizumab and aflibercept, are used to treat DMO. A monoclonal antibody directed at angiopoietin 2 (ANG2; REGN910-3) and the small-molecule vascular endothelial protein tyrosine phosphatase (VE-PTP) inhibitor AKB-9778 are undergoing investigation for the treatment of human DMO[157,158]. **c** | Open-angle glaucoma is a common cause of increased intraocular pressure and subsequent damage to the optic nerve. Increased pressure is caused by impaired drainage of the aqueous humour via Schlemm's canal, which is a specialized vessel with lymphatic-like characteristics[32,231]. Mice with heterozygous deletion of *Tie2* or conditional deletions of both *Ang1* and *Ang2* have an impaired development of the Schlemm's canal and develop glaucoma[33,34]. Inactivating *TIE2* mutations cause human primary congenital glaucoma, which affects children under 3 years of age[34]. TIE2 activation (for example, via AKB9778-mediated inhibition of VE-PTP) may provide potential treatment for open-angle and congenital glaucoma. **d** | In wet age-related macular degeneration (wAMD), Bruch's membrane (which underlies the retina) thickens and breaks, which allows the growth of abnormal blood vessels in response to impaired oxygenation of the macula. Leakage and bleeding from the fragile vessels (exudate) cause scarring of the macula, which results in the rapid loss of central vision. The VEGF-targeting agents ranibizumab and aflibercept are effective in the early phase of wAMD progression. Other antibodies are in clinical development for wAMD, including the ANG2-targeted monoclonal antibody RGN910-3, and RG7716, which is a bispecific monoclonal antibody that targets both ANG2 and VEGF.

vasculature could be normalized by injection of a combination of antibodies against ANG2, VEGF and placenta growth factor (PLGF)[152].

When administered early in disease pathogenesis, an adeno-associated viral vector encoding COMP–ANG1 normalized the retinal vascular changes in the diabetic Ins2^{Akita} mouse model. In this model, COMP–ANG1 reduced capillary vasoregression, vascular permeability and retinal hypoxia, which ameliorated neurovascular pathology, including the neural dysfunction associated with diabetic retinopathy, and improved vision[153]. VE-PTP antagonism using a VE-PTP-targeting antibody or the VE-PTP inhibitor AKB-9778 promoted vascular stability in several models of ocular neovascularization disease in mice. VE-PTP-targeting drugs suppressed retinal neovascularization in ischaemic retinopathy in the mouse OIR model, reduced subretinal neovascularization in rhodopsin-VEGF transgenic mice, decreased choroidal neovascularization after rupture of the Bruch's membrane and prevented retinal detachment in Tet-opsin-VEGF mice[81]. Furthermore, combined treatment with AKB-9778 and aflibercept provided even

better inhibition of neovascularization than did treatment with AKB-9778 alone[81]. In such contexts, the effects of long-term use of AKB-9778 on vascular parameters remain to be studied.

Collectively, these data suggest that agents that enforce the ANG1–TIE2 signalling axis may provide a potential therapeutic strategy for suppressing ocular neovascularization and vascular leakage, and imply that blocking ANG2 in addition to VEGF would be beneficial. Notably, *Ang1* and *Tie2* were the most strongly upregulated pro-angiogenic genes in late-stage corneal neovascular dystrophy in mice, which suggests that the ANG–TIE pathway has different functions in the various cellular environments of the eye[154].

Development of ANG–TIE-targeted drugs

ANG–TIE-targeted drugs. TABLE 3 and FIG. 1 summarize current ANG2- and TIE2-targeted biopharmaceuticals. ANG2-targeted investigational drugs include the peptide–Fc fusion protein trebananib (also known as AMG386), which blocks the binding of both ANG1 and ANG2 to TIE2, as well as MEDI3617, LY3127804 and nesvacumab (also known as REGN910), three human ANG2-targeting monoclonal antibodies that neutralize the interaction of ANG2 with TIE2. Although some of these agents were initially tested in combination with chemotherapy in clinical trials in patients with cancer, research has recently focused on their combination with VEGF-targeting anti-angiogenic drugs — including sunitinib, sorafenib, bevacizumab and aflibercept — in phase I and II clinical trials. In addition, some of the ANG2-targeted drugs have entered early clinical trials in combination with immune checkpoint inhibitors. Additional therapeutic agents in early clinical oncology trials include the bispecific ANG2–VEGF-targeted antibody vanucizumab (also known as RG7221; developed using CrossMab technology). RG7716, another bispecific antibody with dual specificity for ANG2 and VEGF, and REGN910-3, an ANG2-specific monoclonal antibody, are currently being tested in phase II clinical trials for wAMD and wAMD–DMO, respectively.

A few small-molecule TKIs, which inhibit TIE2 and some additional tyrosine kinases, are currently in phase I trials. Regorafenib, a multi-targeted TKI that inhibits TIE2 in addition to VEGFRs and some other kinases, is in clinical use for the treatment of gastrointestinal stromal tumours and previously treated metastatic CRC. However, as the TKIs that are in clinical development inhibit numerous other tyrosine kinases in addition to TIE2, the importance of TIE2 pathway inhibition for their therapeutic efficacy is unclear.

Trebananib was the first ANG-targeted drug to be tested in a phase III cancer trial. Although trebananib increased median progression-free survival (PFS) in combination with weekly paclitaxel in TRINOVA-1 (a randomized, double-blind phase III trial in patients with recurrent epithelial ovarian cancer), secondary end point analysis did not demonstrate a significant increase in overall survival[155]. However, subgroup analysis demonstrated that patients with baseline ascites formation achieved significantly prolonged overall survival compared with patients without ascites[156].

The VE-PTP inhibitor AKB-9778 was shown to be effective in a phase I trial in patients with DMO[157]. In several patients, AKB-9778 administration for 4 weeks reduced oedema and improved vision, demonstrating that increased TIE2 activity has therapeutic potential in diseases of the retinal vasculature[157]. In addition, positive results from a phase IIa study in DMO were recently reported[158]. The combination of AKB-9778 and ranibizumab reduced macular oedema to a significantly greater extent than ranibizumab monotherapy after 3 months of treatment. AKB-9778 is currently being tested for the treatment of DMO in phase IIb trials. Notably, in contrast to antibody-based and receptor ectodomain-based biopharmaceuticals, which require intravitreal injections, AKB-9778 is delivered via subcutaneous injections that result in systemic delivery of the drug[158].

Several ANG–TIE-targeted agents have been tested in preclinical studies. These include decoy receptors, TIE2 activity-modulating antibodies and recombinant ANG proteins (TABLE 4). A recent report described a novel mechanism of ANG2 targeting that combines ANG2 blockade with TIE2 activation[106]. ANG2-binding and TIE2-activating antibody (ABTAA) is an ANG2-targeting antibody that triggers ANG2 clustering, thereby resulting in TIE2 activation. In mouse models of sepsis and endotoxaemia, ABTAA was more potent than an ANG2-neutralizing antibody in protecting the vasculature[106]. ABTAA improved the endothelial glycocalyx in line with a previous report, which demonstrated that ANG1 strengthens the endothelial barrier function via increasing glycocalyx formation *in vivo*[106,159]. In addition, inflammatory cytokine storm, vascular leakage, vascular rarefaction and organ damage were alleviated, which resulted in improved survival of ABTAA-treated mice after inflammatory insult[106].

The TNF-targeting antibody adalimumab is used for the treatment of several inflammatory and autoimmune diseases, including rheumatoid arthritis, psoriasis and Crohn's disease. An inhibitor that combines ANG2-binding peptides with the adalimumab heavy chain neutralizing both ANG2 and TNF was more effective than adalimumab alone in an *in vivo* model of rheumatoid arthritis[160].

ANG1 as a therapeutic protein. Several studies have indicated that ANG1 can be used as a therapeutic protein. ANG proteins consist of a carboxy-terminal fibrinogen-like domain that is responsible for binding to TIE2, a central coiled-coil domain that mediates dimerization or trimerization of ANG monomers and an amino-terminal superclustering domain that promotes further multimerization of especially ANG1 into higher-order oligomers. The multimeric recombinant native ANG1 is poorly soluble, easily aggregated and heterogeneous. To overcome these problems while retaining the multimeric ANG1 structure that is necessary for efficient TIE2 activation[161,162], modified ANG1 proteins have been generated and tested in preclinical animal models; they have been administered by local injection, by implantation of ANG1-saturated resorbable sponges, or systemically via intravenous injection or via an adenoviral vector.

ANG1* is a chimeric protein in which the N-terminal region of ANG1 has been replaced by ANG2 sequences[163], and BowANG1 is a tetrameric ANG1 chimaera that contains four fibrinogen-like domains separated by two Fc domain dimers[161]. Both of these designed forms of ANG1 were shown to enhance vascular stability in a kidney tumour model[164], and they reduced blood–brain barrier leakage and ischaemic lesion volume after cerebral artery

Table 3 | **ANG–TIE-targeted therapies in clinical development (www.clinicaltrials.gov March 2017)**

Compound	Description	Phase	Indication	Co-treatment	Refs
ANG1-targeted and ANG2-targeted biologicals					
Trebananib (AMG386)	A peptide–Fc fusion protein (peptibody) that acts by binding both ANG1 and ANG2, thereby preventing their interaction with TIE2	III	Ovarian cancer	Paclitaxel	155,156, 297
		II	RCC	Sunitinib	188
		I	Solid tumours	Bevacizumab, motesanib	298
		Preclinical	Human xenografts	Bevacizumab	71
Nesvacumab (REGN910, SAR307746)	Fully human monoclonal antibody against ANG2 that blocks the binding of ANG2 to TIE2	I	Solid tumours	Aflibercept	299
		Preclinical	Human xenografts	Aflibercept	50
MEDI3617	Fully human monoclonal antibody against ANG2 that blocks the binding of ANG2 to TIE2	I	Solid tumours	Chemotherapy	–
		I	Melanoma	Tremelimumab	–
		Preclinical	Human xenograft and mouse glioma models	Cediranib	66,80, 300
		Preclinical	Cardiac transplantation	None	115
Vanucizumab (RG7221)	Bispecific monoclonal antibody. One arm binds ANG2 and the other binds VEGF (design based on bevacizumab and developed using CrossMab technology)	I	Solid tumours	Atezolizumab	–
		Preclinical	Orthotopic and syngeneic mouse tumours, human cell-line and patient-derived xenografts, transgenic mammary and pancreatic tumour models	Chemotherapy, PD1 antibody	68, 79,306
RG7716	Bispecific antibody targeting both ANG2 and VEGF (developed using CrossMab technology)	II	wAMD and DMO	None	–
		Preclinical	Spontaneous mouse CNV model, laser-induced CNV in non-human primates	None	301
REGN910-3	Fully human antibody against ANG2	I–II	wAMD and DMO	Aflibercept	–
AKB-9778	Competitive VE-PTP inhibitor	II	DMO	Ranibizumab	157,158
		Preclinical	Mouse models of choroidal neovascularization and ischaemic retinopathy	Aflibercept	81
LY3127804	Humanized monoclonal antibody against ANG2	I	Solid tumours	Ramucirumab	–
TIE2 TKIs					
Regorafenib	A small-molecule multi-kinase inhibitor of TIE2, VEGFRs, c-KIT, PDGFRβ, FGFR1, RET, RAF1, BRAF and p38 MAPKs	Approved	GIST and previously treated mCRC	None	302
Rebastinib	A TIE2 kinase inhibitor that also inhibits VEGFR1 and BCR–ABL	I	Breast cancer	Antitubulin therapy	–
Altiratinib (DCC-2701)	A small-molecule inhibitor of MET, TIE2, VEGFR2, TRKA, TRKB and TRKC	I	Advanced-stage solid tumours	None	–
		Preclinical	PyMT mammary tumour model and human xenografts	Bevacizumab	303
ARRY-614	Small-molecule inhibitor of p38 MAPKs and TIE2	I	MDS	None	304

ANG, angiopoietin; CNV, choroidal neovascularization; DMO, diabetic macular oedema; FGFR1, fibroblast growth factor receptor 1; GIST, gastrointestinal stromal tumour; MAPK, mitogen-activated protein kinase; mCRC, metastatic colorectal cancer; MDS, myelodysplastic syndrome; PD1, programmed cell death protein 1; PDGFRβ, platelet-derived growth factor receptor-β; PyMT, polyoma virus middle T antigen; RCC, renal cell carcinoma; TKI, tyrosine kinase inhibitor; TRKA, tyrosine kinase receptor A (also known as high-affinity nerve growth factor receptor); TRKB, tyrosine kinase receptor B (also known as BDNF/NT3 growth factors receptor); TRKC, tyrosine kinase receptor C (also known as NT3 growth factor receptor); VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; VE-PTP, vascular endothelial protein tyrosine phosphatase; wAMD, wet age-related macular degeneration.

occlusion in mice[165]. In cartilage matrix protein (CMP)–ANG1, the N-terminal domain of ANG1 was replaced with the coiled-coil domain of human CMP, and cysteine residues were mutated to generate a dimeric chimaera[166,167]. CMP–ANG1 activated TIE2 in an *N*-glycosylation-dependent manner to induce vascular enlargement in the mouse ear when intradermally injected as a recombinant protein, and it suppressed vascular leakage induced by topical LPS treatment[166]. Moreover, injection of matrilin 1 (MATN1; also known as cartilage matrix protein)–ANG1 reduced vascular leakage and inflammation in a mouse model of LPS-induced endotoxaemia[168].

The most widely tested, designed ANG1 variant is COMP–ANG1, which is generated by replacing the N-terminal domain of ANG1 with the pentameric coiled-coil domain of COMP. COMP–ANG1 is stable, more soluble than native ANG1 and a highly potent TIE2 agonist[167]. In various mouse models of diabetes, COMP–ANG1 has shown beneficial effects: it rescued penile cavernous blood vessels and erectile function[169,170], accelerated cutaneous wound healing[133], prevented neurovascular retinopathy[153] and glomerular pathology, increased blood flow in skeletal muscle, enhanced insulin sensitivity, improved metabolic status[129], increased insulin-stimulated glucose uptake and prevented high-fat-diet-induced insulin resistance[171,172]. Other mouse models have shown that COMP–ANG1 also protects against radiation-induced acute endothelial cell damage[173], endotoxaemia[174], kidney injury induced by reactive oxygen species[175], and ischaemic injuries in the muscle, heart and kidneys[114,176–179]. Furthermore, COMP–ANG1 was shown to prevent cardiac allograft dysfunction[118], improved the revascularization of skin grafts[180], and enhanced bone formation and angiogenesis alone and synergistically with bone morphogenetic protein 2 (BMP2) in unstressed mice[181], in a model of ischaemic necrosis of the femoral head[182], in distraction osteogenesis[183] and in cranial bone regeneration[184].

The chimeric molecule VA1 was generated to combine the strong angiogenic potency of VEGF and the vascular-stabilizing effect of ANG1 (REF. 185). VA1 was shown to activate VEGFR2 and TIE2, to promote angiogenesis in ischaemic skeletal muscle and to reduce the undesirable side effects of VEGF, including vascular leakage, inflammation and the formation of angioma-like structures[185].

Despite a wealth of preclinical data demonstrating vascular-protective effects of designed ANG1 ligands, it remains to be investigated whether and how ANG1 mimicry can be used in the clinic.

Adverse effects of ANG–TIE pathway inhibition. Most of the published data about the potential adverse effects associated with ANG–TIE pathway modulation in patients with cancer originate from clinical studies of trebananib (an ANG1 and ANG2 inhibitor), which caused localized, low-grade oedema as the most frequent side effect[155,156,186,187]. In the TRINOVA-1 trial, serious adverse events were reported in 125 (28%) patients in the placebo group and in 159 (34%) patients in the trebananib-treated group. The oedema observed in the treatment group typically developed in the lower extremities during the first few months of trebananib administration; however, it was generally mild and, at least in some cases, reversible (within 4–12 months) after trebananib discontinuation[187]. It is not clear whether the oedema was linked to decreased oncotic pressure, lymphatic vessel damage, leaky blood vessels, impaired venous return or peripheral vasoconstriction. Thus, the risk factors that would predispose patients to trebananib-associated oedema remain unknown. Trebananib did not increase any of the known side effects of VEGF inhibitors, such as bleeding, thromboembolism, increased risk of hypertension or bowel perforation[187]. Furthermore, results from recently completed clinical trials indicate that the combination of trebananib and sunitinib for dual inhibition of ANG2 and VEGF, respectively, is tolerated in patients[188].

The administration of high-affinity VEGF-blocking agents to mice results in the pruning of quiescent vessels due to the inhibition of VEGF-mediated endothelial cell survival signals[189,190]. Blocking both ANG1 and ANG2 resulted in regression of healthy vessels in mouse trachea, whereas selective ANG2 inhibition did not affect healthy vessels[191]. ANG2 inhibition in combination with an antibody that specifically targets mouse VEGF did not aggravate the adverse effects of VEGF blockade on healthy vessels, although the combined use of treatments that inhibit ANG1, ANG2 and VEGF further reduced the number of capillary branch points in healthy tissues[68]. A dual ANG2–VEGF-blocking antibody generated using the CrossMab technology did not aggravate the vessel pruning induced by anti-VEGF monotherapy in mice[68], which suggests that ANG2 inhibitors and dual ANG1–ANG2 inhibitors may differentially affect the healthy vasculature when used in combination with anti-VEGF therapy. Such difference may be explained by the low expression of ANG2 in normal tissues under baseline conditions.

The ANG–TIE pathway as a biomarker in human disease. Increased levels of circulating ANG2 have been reported in multiple types of human cancer, including melanoma, RCC, breast cancer, CRC and glioblastoma[58–62]. Increased levels of ANG2 show some correlations with disease incidence, survival and response to cancer therapies (TABLE 1). However, baseline levels of circulating ANG2, ANG1 and TIE2 did not show a consistent predictive or prognostic association with PFS in a preliminary analysis of the phase III trial of trebananib in ovarian cancer[155]. It is currently not known if the level of circulating ANG2 is an appropriate surrogate biomarker for tumour-derived ANG2; thus, the potential of ANG–TIE biomarkers in cancer must be further investigated.

Relatively few studies have investigated ANG2 as a predictive biomarker of VEGF-based anti-angiogenic therapy. In patients with CRC and RCC who were treated with bevacizumab and sunitinib, respectively, circulating ANG2 levels correlated with poor outcomes[60,192]. In patients with mRCC who were treated with sunitinib as first-line therapy, strong ANG2 immunostaining in the tumour endothelium was associated with clinical benefit[63]. In glioblastoma, high expression of ANG2 in

Table 4 | **Preclinical development of angiopoietin–TIE-targeted therapies**

Preclinical compound	Description	Preclinical evidence	Preclinical indication	Refs
L1-7(N)	Peptide–Fc fusion protein that acts by binding to ANG2 and blocking its interaction with TIE2	Inhibits tumour growth (including the growth of established tumours), reduces the viable tumour fraction, inhibits tumour endothelial cell proliferation and has cooperative antitumor activity with inhibitors of the VEGF pathway	Human tumour xenografts	65, 70, 71
		Inhibits corneal and retinal angiogenesis	Rat corneal angiogenesis assay and OIR	65,71
mL4-3	Peptide–Fc fusion protein that binds ANG1, thus blocking its interaction with TIE2	Enhances tumour growth inhibition resulting from ANG2 blockage	Colo205 human colorectal tumour xenografts	70, 71
LC10 (ANG2-specific), LC06 (ANG2-specific) and LC08 (dual specificity for ANG1 and ANG2)	Fully human antibodies that block ANG binding to TIE2	Inhibit tumour growth, angiogenesis and lung metastasis, increase pericyte coverage and necrosis, and inhibit corneal angiogenesis	Human tumour xenografts	191
		Attenuate LPS-induced haemodynamic alterations and reduce mortality rate	LPS-induced systemic inflammation in mice	104
3.19.3	Fully human monoclonal antibody that blocks ANG2 binding to TIE2	Inhibits tumour growth, angiogenesis and metastasis, induces vascular regression; increased antitumour activity observed in combination with cytotoxic drugs or anti-VEGF agents	Human tumour xenografts, MMTV-PyMT tumour model	90, 310
COMP–ANG1	Pentameric ANG1 chimeric protein containing the coiled coil domain of COMP	Has greater TIE2-agonistic activity than native ANG1	Diabetic vascular complications, wound healing, ischaemic conditions, tissue regeneration and organ transplantation (see the main text for details)	167
CMP–ANG1	Dimeric ANG1 that contains a mutated coiled coil domain of CMP	Activates TIE2, inhibits LPS-induced vascular leakage and induces angiogenesis in mouse skin	Intradermal administration of CMP–ANG1 in mouse ear	166
DAAP	A chimeric decoy receptor that can simultaneously bind VEGF and ANG proteins, thus blocking their actions	Inhibits tumour angiogenesis and metastasis, and reduces ascites formation and vascular leakage in an ovarian carcinoma model	Mouse tumors, including the MMTV-PyMT mammary tumour	305
VA1	A chimeric molecule that consists of the receptor-binding parts of VEGF and ANG1, and activates both VEGFR2 and TIE2	VA1-induced angiogenesis was associated with less vessel leakiness, less tissue inflammation and better perfusion in ischaemic muscle than was VEGF-induced angiogenesis	Ischaemic vascular disease	185
ABTAA	ANG2-binding and TIE2-activating antibody that triggers clustering of ANG2, resulting in TIE2 activation	Protects the vasculature from septic damage by strengthening the endothelial glycocalyx and reducing cytokine storms, vascular leakage, rarefaction and organ damage. Increases survival. Normalizes tumour vasculature	Sepsis, MMTV-PyMT and orthotopic mouse tumour models	106, 309

ANG, angiopoietin; ABTAA, ANG2-binding and TIE2-activating antibody; CMP, cartilage matrix protein; COMP, cartilage oligomeric matrix protein; DAAP, double anti-angiogenic protein; LPS, lipopolysaccharide; MMTV, mouse mammary tumour virus; OIR, oxygen-induced retinopathy; PyMT, polyoma virus middle T antigen; VEGF, vascular endothelial growth factor.

the tumour was associated with poor overall survival of patients, and ANG2 expression increased during bevacizumab therapy[62]. The effect of ANG2 expression on the clinical response to anti-angiogenic therapy may depend on tumour type and the type of therapy. ANG2 blockade may promote a normalized vessel phenotype, which may be particularly important in cancers in which anti-angiogenic treatment is combined with chemotherapy.

The levels of circulating ANG2 and the ANG2/ANG1 ratio are increased in several other human diseases in which vascular function is compromised (TABLE 1). In sepsis, high plasma ANG2 concentrations in patients seeking medical care, or a continuous rise in ANG2 concentration, predict septic shock and death[47,99]. Thus, ANG2 may serve as a biomarker for diseases that are characterized by vascular leakage.

Recent work has also shown that cleavage of the extracellular domain of TIE1 is associated with both acute and chronic inflammation in mouse models[19,49]. Increased concentrations of the soluble TIE1 ectodomain were detected in patients infected with Puumala hantavirus, who often suffer from acute haemorrhagic fever with renal syndrome[19]. These new findings call for further studies of soluble TIE1 as a biomarker in human vascular diseases.

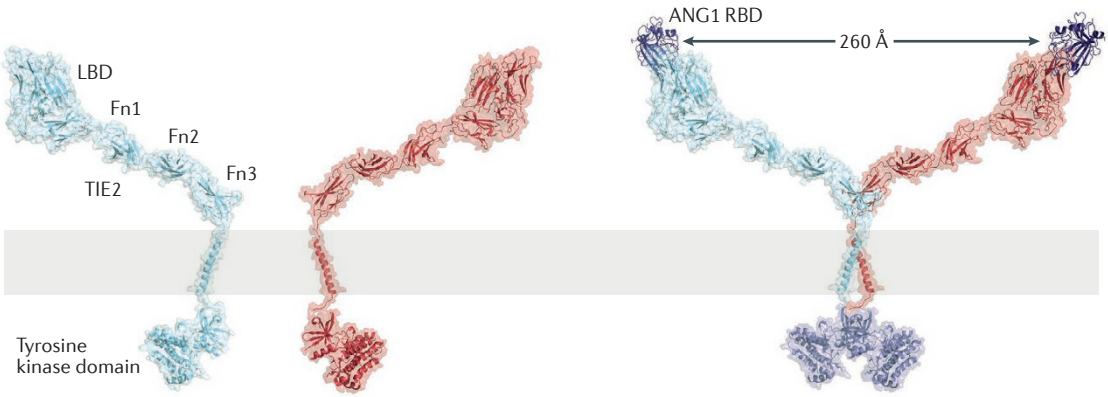

Figure 6 | **A model of TIE2 dimerization.** Unlike in other receptor tyrosine kinases, the ligand-binding domains (LBDs) of the TIE receptors are widely separated from each other in receptor dimers. The long 260 Å distance between the TIE2 LBDs suggests that oligomerization of angiopoietins regulates TIE2 dimerization in *cis*. The multimeric ANG1 agonist can extend to bind simultaneously to the two TIE2 receptors in the complex, whereas the antagonistic and agonistic ANG2 is a lower-order multimer and, at least ANG2 dimers, can bind to only one receptor monomer, which could explain the lower agonist activity of ANG2 compared with ANG1. Previous studies have indicated that ANG1 and ANG2 also mediate the association of TIE receptors in *trans* across endothelial cell–cell junctions[17,18]. The full-length TIE2 model is based on the crystal structures of TIE2 LBD (Protein Data Bank (PDB) identifier: 4K0V)[311], TIE2 fibronectin type III domains 1–3 (Fn1–3; PDB ID: 5MYA)[307] and the TIE2 tyrosine kinase domain (PDB ID: 1FVR)[312]. The transmembrane region was adopted from the NMR structure of the dimerization motif of epidermal growth factor receptor (PDB ID: 5LV6)[313]. Ligand-induced TIE2 dimerization in *cis* is mediated by homotypic interactions between the membrane-proximal Fn3 domains[307,308]. ANG1 receptor-binding domain (RBD) from the TIE2 LBD complex structure (PDB ID: 1K0V) is shown as a model in purple, and the two chains of TIE2 (blue and red) are shown as semitransparent surface models. Adapted with permission from Leppänen, V. M. *et al.* Structural basis of Tie2 activation and Tie2/Tie1 heterodimerization. *Proc. Natl Acad. Sci. USA* **114**, 4376–4381 (2017).

Clinical development of ANG–TIE pathway biopharmaceuticals. Results from preclinical studies provide strong evidence that targeting the ANG–TIE pathway has beneficial effects in several diseases that are associated with pathological vasculature. This concept is supported by results from early clinical trials. However, more evidence is needed to confirm the clinical potential of ANG–TIE-targeted therapies.

VEGF–VEGFR2 signalling stimulates ANG2 expression by endothelial cells[193]. Ongoing trials should answer the question of whether ANG2 blockade will improve VEGF-targeted anti-angiogenic therapy in cancer, as has been reported in preclinical models[67,68,78]. However, there is little information on which tumour types are the most responsive to ANG2-blocking therapy. *In situ* ANG2 protein expression has been measured in RCC and glioblastoma[62,63], but in many other tumour types, ANG2 concentrations have only been measured in serum. Although the circulating concentrations of ANG2 are increased in many human cancers, their systematic correlation with ANG2 within tumours has not been reported. Prospective studies are required to determine if circulating ANG2 can be used as a biomarker for ANG2-targeted therapies, and whether patient stratification for therapy based on ANG2 expression should be considered. In general, the effects of ANG2 blockade on tumour progression are likely to be affected by the levels of other growth factors in the tumour, such as VEGF, as well as by tumour dependence on angiogenesis or hypoxia pathway activation and tumour inflammatory cell status. In addition, further studies should clarify whether ANG2 provides a biomarker or target in therapeutic approaches that use immune checkpoint inhibitors for cancer treatment.

Recent work has provided further insight on the agonist-versus-antagonist function of ANG2 (REFS 307,308) (FIG. 6). Although ANG2-blocking antibodies normalize the tumor vasculature, a report on mice with human tumour xenografts suggests that ANG2 functions as a TIE2 agonist and thus provides endothelial cell survival signals that can compromise the efficacy of therapeutic VEGF pathway inhibition[50]. Accumulating evidence suggests that integrins and TIE1 modify the context-dependent functions of ANG proteins that affect vascular stability. TIE1 deletion inhibits tumour angiogenesis and growth in mice, but the therapeutic potential of ANG-integrin inhibition has not been explored[21].

The clinical development of drugs that target the ANG–TIE pathway has focused on ANG2-blocking therapies. ANG1 blockade on its own has limited efficacy in preclinical cancer models, but ANG1 neutralization inhibits the tumour vessel normalization that occurs during anti-angiogenic therapy, thereby decreasing drug delivery into tumours[70,76]. In addition, studies have demonstrated the therapeutic efficacy of AKB-9778 and ABTAA in preclinical tumour models in which treatment results in vascular stabilization via the activation of TIE2 (REFS 82,309). Similarly, AKB-9778 and ABTAA promoted vascular stability in patients with DMO and in murine models of sepsis, respectively[106,158]. For vascular-stabilizing therapies, potentiation of the ANG1–TIE2 axis seems to be a highly relevant strategy.

Conclusions

As discussed above, several investigational drugs — targeted at ANG2, both ANG2 and VEGF, both ANG1 and ANG2, TIE2 and VE-PTP — are in clinical development. Furthermore, various ANG1 ligands have been generated. ANG–TIE-targeted therapeutics differ in their mechanisms of action and primary clinical indications. VE-PTP inhibition, ANG2-targeting as well as dual ANG2–VEGF targeting have mainly been investigated in human neovascular eye diseases, whereas drugs that target ANG2, both ANG2 and VEGF, or both ANG1 and ANG2 were designed mainly for the treatment of cancer. TIE2-targeted investigational drugs are at an earlier stage of development; currently, TIE2 inhibition can only be achieved using multi-kinase TKIs. Although ANG1 variants that are under development as therapeutic proteins have promising vasculoprotective effects in preclinical models, these ligands have not yet been investigated in clinical trials.

ANG antagonists were originally developed as antineoplastic agents, yet their benefit for patients with cancer remains unclear. The current view is that ANG2 inhibitors require a combination with other targeted therapies. ANG2 inhibitors are currently being evaluated in clinical trials in combination with VEGF-based anti-angiogenic drugs or with immunotherapy. Further studies should reveal whether ANG2 can act as a biomarker or as a suitable anti-angiogenic target in combination with immune checkpoint therapy, such as PD1 inhibitors.

Outside of oncology, the clinical development of ANG–TIE-targeted therapeutics is most advanced for ocular vascular diseases. In DMO, VE-PTP inhibition has shown positive effects in combination with VEGF-targeted therapy. In addition, preclinical ANG–TIE targeting shows promise in numerous diseases involving compromised vascular function, including CCM, glaucoma, diabetic vascular complications, organ transplantation, and inflammatory and infectious diseases, for example, sepsis. It can also be envisioned that the identification and characterization of compounds to reduce uncontrolled TIE2–phosphoinositide 3-kinase (PI3K) pathway activation can be used for molecular therapies for venous malformations. Future work will establish the potential benefit of ANG–TIE targeting in numerous diseases in which endothelial cell activation, inflammation and failure of vascular barrier function have major roles.

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