Vascular endothelial growth factor: a neurovascular target in neurological diseases

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Abstract | Brain function critically relies on blood vessels to supply oxygen and nutrients, to establish a barrier for neurotoxic substances, and to clear waste products. The archetypal vascular endothelial growth factor, VEGF, arose in evolution as a signal affecting neural cells, but was later co-opted by blood vessels to regulate vascular function. Consequently, VEGF represents an attractive target to modulate brain function at the neurovascular interface. On the one hand, VEGF is neuroprotective, through direct effects on neural cells and their progenitors and indirect effects on brain perfusion. In accordance, preclinical studies show beneficial effects of VEGF administration in neurodegenerative diseases, peripheral neuropathies and epilepsy. On the other hand, pathologically elevated VEGF levels enhance vessel permeability and leakage, and disrupt blood–brain barrier integrity, as in demyelinating diseases, for which blockade of VEGF may be beneficial. Here, we summarize current knowledge on the role and therapeutic potential of VEGF in neurological diseases.

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The vascular endothelial growth factor (VEGF) family comprises several members, of which VEGF-A (also termed VEGF) has received the most attention[1]. Historically, VEGF was discovered in mammals as a growth factor for endothelial cells that is capable of stimulating the formation of blood vessels (angiogenesis). From an evolutionary perspective, however, this polypeptide originally emerged in the CNS of primitive organisms that lacked an established vasculature, suggesting a vessel-independent activity. Indeed, growing evidence indicates a diverse range of effects of VEGF and its family members on neural cells during development and in adulthood[2].

Here, we provide a brief overview of the functions of the VEGF family in the developing and adult nervous system. We then review current knowledge on the role of VEGF in neurological diseases, and summarize approaches that target VEGF in order to treat neurological disorders. The role of the VEGF family in CNS oncology is beyond the scope of this article, and we refer the reader to recent reviews on this topic[3–5].

The VEGF family

VEGF (also known as VEGF-A or vascular permeability factor, but henceforth referred to as VEGF in this Review) is the founding member of a family of growth factors. In mammals, the family comprises VEGF, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor (PlGF)[1] (FIG. 1). VEGF family members bind to cell surface receptor tyrosine kinases termed VEGFR-1, VEGFR-2 and VEGFR-3. Several members also bind to non-tyrosine kinase receptors of the neuropilin (NRP) family, NRP-1 and NRP-2 (also receptors for semaphorins), which function as co-receptors for the VEGFRs. VEGF binds to VEGFR-1, VEGFR-2, NRP-1 and NRP-2; VEGF-B binds to VEGFR-1 and NRP-1; PlGF binds to VEGFR-1, NRP-2 and NRP-1; and VEGF-C and VEGF-D interact with VEGFR-3, VEGFR-2, NRP-1 and NRP-2 (REF. 1) (FIG. 1).

VEGF is best known for its role in angiogenesis, stimulating endothelial cell proliferation and migration and increasing vascular permeability[2]. VEGF family members have been also implicated in lymphangiogenesis (formation of lymphatic vessels), monocyte recruitment, haematopoiesis, and proliferation or survival of non-vascular cell types expressing VEGFRs or NRPs, including neuronal cells[1,2,6] (FIG. 1). VEGF and VEGFR family members exert their effects via downstream signalling pathways, including the MEK–MAPK pathway (proliferation and migration), the PI3K–Akt pathway (survival), and the Src–eNOS pathway (permeability). Pathways involved in specific neurological diseases are indicated

Key points

- Vascular endothelial growth factor (VEGF) has been implicated in the aetiology and treatment of various neurological diseases
- VEGF exerts effects on multiple cell types in the nervous system, including endothelial cells, neurons, astrocytes, microglia, oligodendrocytes and Schwann cells
- VEGF protects neurons and fosters neurogenesis, and reduced VEGF levels contribute to neurodegenerative disorders
- VEGF can improve brain perfusion, partly by promoting angiogenesis, but pathological VEGF levels induce blood–brain barrier breakdown and vessel leakage
- Preclinical studies indicate that VEGF administration is beneficial in neurodegenerative diseases, peripheral neuropathies and epilepsy
- VEGF inhibition is approved as a treatment for neovascular ocular diseases, and might be beneficial in other neurological disorders involving BBB breakdown or excessive angiogenesis

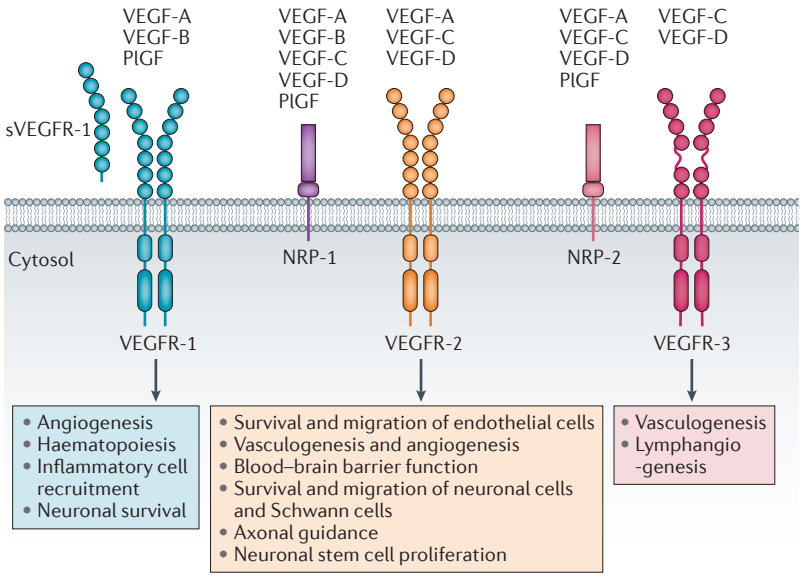


Figure 1 | **The VEGF family of growth factors.** The figure depicts the vascular endothelial growth factor (VEGF) isoforms VEGF (also known as VEGF-A), VEGF-B, VEGF-C, VEGF-D and placental growth factor (PlGF), and their binding to the receptor tyrosine kinases VEGFR-1, VEGFR-2 and VEGFR-3 and the co-receptors neuropilin-1 (NRP-1) and NRP-2. Major effects on receptor-expressing cell types in the vasculature and CNS are indicated. sVEGFR-1 can trap VEGF-A, VEGF-B and PlGF and reduce their biological actions. sVEGFR-1, soluble VEGFR-1.

in the respective sections below. For a general overview of downstream VEGF family pathway mediators, we refer the reader to recent reviews[1,7,8].

Besides the actions of VEGF and its family members on blood vessels, a growing body of literature describes direct effects of these molecules on neural cells during brain development and in normal brain function (see below). In addition, VEGF has numerous roles in the diseased nervous system, including multiple direct beneficial effects on various types of neural cells[2] (FIG. 2), as well as effects on the vasculature. The latter effects can be beneficial by promoting CNS perfusion[6] but, as a permeability factor[9], VEGF at high levels can compromise CNS homeostasis by inducing blood–brain barrier (BBB) dysfunction[2,10].

From an evolutionary perspective, VEGF homologues are present in cnidarians that lack a vascular system[11]. These organisms express VEGF during neural development, suggesting that VEGF originally arose as a neural factor and was later co-opted for vessel formation. Thus, VEGF is a prototypical neurovascular signal that regulates both vascular and neural systems.

VEGF in the developing nervous system

VEGF has a dual role in CNS development: it regulates the formation of blood vessels, and it also guides neuronal migration and axonal pathfinding[2,12,13]. VEGF is a master regulator of CNS blood vessel formation. Secreted by neural tube cells, VEGF first induces *de novo* formation of a perineural vascular plexus, and later in development it orchestrates the ingrowth of vessel sprouts into the neural tube to form the vasculature of the brain parenchyma[14,15]. Consistent with findings that VEGF is crucial for brain vessel formation and brain growth[16,17], inhibition of periventricular vessel growth via VEGF blockade during the third trimester of gestation was found to cause striatal periventricular apoptosis, depletion of cortical GABAergic interneurons, and ventricular enlargement[18]. These features are all hallmarks of periventricular leukomalacia, a form of white matter brain injury in preterm infants. During embryogenesis, VEGF also regulates the formation of brain collaterals, which determine the outcome of ischaemic brain insults (see below)[19,20].

VEGF signalling guides neuronal migration and axon pathfinding independently of its vascular effects. A gradient of extracellular matrix-bound VEGF controls the migration of cerebellar granule cells towards their final destination through VEGFR-2 signalling in granule cells[21]. In addition, migration of facio-branchial motoneurons in the hindbrain is regulated by VEGF via the co-receptor NRP-1. Notably, migration of these neurons is misguided in a mouse model of Charcot–Marie–Tooth disease type 2D (CMT2D), a peripheral neuropathy in which mutated glycyl-tRNA synthetase (GlyRS) aberrantly binds to NRP-1 and inhibits VEGF–NRP-1 signalling[22,23]. VEGF–NRP-1 can guide axons of retinal ganglion cells as they cross the midline at the optic chiasm[24], and VEGF–VEGFR-2 signalling in spinal commissural neurons regulates midline crossing of axons in the ventral spinal cord[25].

VEGF in the adult nervous system

In the normal healthy nervous system, VEGF regulates microvascular density, controls vessel permeability, and maintains endothelial cell fenestration in the choroid plexus[10]. In addition, VEGF stimulates neural stem cell (NSC) proliferation and promotes neurogenesis (BOX 1). In neurological disease, VEGF affects various types of neural cells (FIG. 2): it safeguards stressed neurons via a neuroprotective survival effect, stimulates neurogenesis and neuronal differentiation (BOX 1), induces axon extension and branching, and promotes synaptic plasticity[2]. In addition, VEGF enhances migration of oligodendrocyte precursor cells, increases migration and proliferation of Schwann cells, stimulates

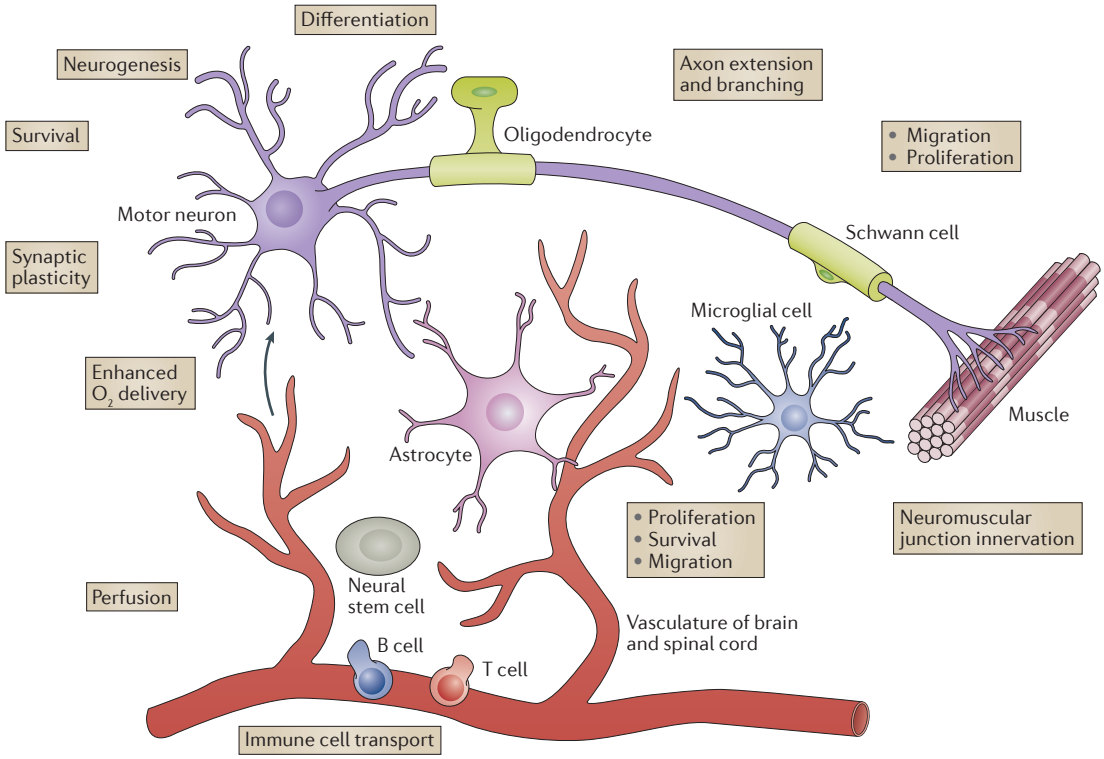


Figure 2 | **VEGF in the CNS.** Vascular endothelial growth factor (VEGF) has multiple roles in the CNS, both by affecting various neural cells directly, and by promoting vascular perfusion, transport of immune cells, and survival of cerebral blood vessel endothelial cells. VEGF also stimulates the production of neurogenic growth factors by endothelial cells. Permission obtained from Macmillan Publishers Ltd © Zacchigna, S. *et al. Nat. Rev. Neurosci.* **9**, 169–181 (2008).

expression of trophic factors by astrocytes, and triggers proliferation, survival and migration of astrocytes and microglia[2].

VEGF also has beneficial vascular effects, including improvement of perfusion via vasodilation or angiogenesis[6] (FIG. 2). Also, low maintenance levels of VEGF are necessary for endothelial cell survival and the integrity of the BBB, a vascular barrier that safeguards the brain against harmful blood-borne substances[6,26]. However, VEGF is a vascular permeability factor[9] and, at high levels, can compromise CNS homeostasis through disruption of the BBB[2,10]. BBB dysfunction can aggravate neurological diseases via several mechanisms, including leakage of neurotoxic proteins, which results in the production of reactive oxygen species and inflammation; accumulation of waste products due to defective disposal; deficient nutrient transport; and entry of inflammatory cells, leading to immune responses[27]. When its levels are highly elevated, VEGF can cause excessive formation of new leaky vessels, and bleeding[2]. VEGF also stimulates angiogenesis in conditions of inflammation or cancer in the brain (not discussed further here).

VEGF in neurodegenerative disease

Amyotrophic lateral sclerosis. Amyotrophic lateral sclerosis (ALS) is an adult-onset incurable disease characterized by progressive degeneration of motor neurons in the spinal cord, brainstem and motor cortex, leading to generalized paralysis, and death 3–5 years after diagnosis[28]. Around 10% of cases of ALS are caused by gene mutations, most commonly affecting the *C9orf72*, *SOD1*, *TARDBP* or *FUS* genes, although more than 40 ALS-associated genes have been identified.

The precise molecular mechanisms underlying ALS pathogenesis are elusive, but possible mechanisms include perturbations in protein stability and degradation, RNA biogenesis, cytoskeletal architecture and function, and mitochondrial function, along with excitotoxicity and insufficient neurotrophic signalling[28,29]. Emerging evidence indicates that motor neuron degeneration is caused not only by motor neuron-intrinsic defects, but also by alterations in surrounding cell types, including astrocytes, microglia, oligodendrocytes and endothelial cells[28,30,31]. VEGF was first implicated in ALS when mice with reduced VEGF levels ($Vegf^{\delta/\delta}$ mice) were found to develop adult-onset progressive motor neuron degeneration, reminiscent of ALS[32]. Lowering VEGF levels in $SOD1^{G93A}$ mice, a model of familial ALS, accelerates disease onset and shortens lifespan[33]. In humans, a single nucleotide polymorphism (−2578AA) in the *VEGF* gene, which is associated with low VEGF levels, increases ALS susceptibility[34].

The low VEGF levels in $Vegf^{\delta/\delta}$ mice could contribute to motor neuron degeneration via two possible mechanisms. First, reduced vascular perfusion of the CNS, possibly attributable to defective regulation of peripheral resistance arteries, might evoke chronic CNS ischaemia[32,35] (FIG. 3a). BBB breakdown due to endothelial cell

Box 1 | **VEGF in neuronal regeneration**

As neurogenesis in the adult brain contributes to the recovery of brain insults[173,174], efforts are underway to therapeutically increase this process. Vascular endothelial growth factor (VEGF) regulates adult neurogenesis through direct effects on neural stem cells (NSCs) and/or indirect effects on the vascular NSC niche[13]. Adult NSCs express the receptor VEGFR-2 (REFS 175–177), and administration of VEGF increases NSC proliferation *in vitro* and *in vivo*, as well as increasing the generation of newborn neurons[176–178]. In addition, VEGF expression in the hippocampal NSC niche increases after stroke or TBI[127,179], in association with increased NSC proliferation and neurogenesis. Knockdown of VEGF or blockade of VEGFR-2 in the niche attenuates the induction of adult NSC proliferation by TBI[127,180], suggesting that VEGF mediates this process, at least in part, and that therapeutic VEGF manipulation might further increase NSC proliferation and recovery. When VEGF is reversibly overexpressed in the hippocampal NSC niche, it increases angiogenesis, and blood vessels remain expanded after cessation of VEGF expression. Interestingly, NSC proliferation remains elevated for several weeks when VEGF expression ceases, suggesting that VEGF might control NSC proliferation via expansion of the vascular niche[181].

VEGF also regulates regrowth of damaged peripheral nerve axons to aid recovery of sensory and motor function after injury. On nerve damage, macrophages migrate into the axonal gap, which is devoid of axons, and secrete VEGF to induce blood vessel formation. Schwann cells then use these vessels as tracks to migrate into the axonal gap to facilitate subsequent axon regrowth[182]. Exogenous delivery of VEGF to injured nerves further enhances axon regeneration[183]. Thus, VEGF enhances neuronal regeneration after injury indirectly, via blood vessel-dependent mechanisms, and possibly also directly.

dysfunction, which could affect CNS homeostasis, has been detected in rodents and patients with ALS[31,36]. The idea that reduced VEGF levels contribute to this phenomenon is plausible, as maintenance levels of VEGF are required for endothelial cell function and survival, but it remains to be tested[6].

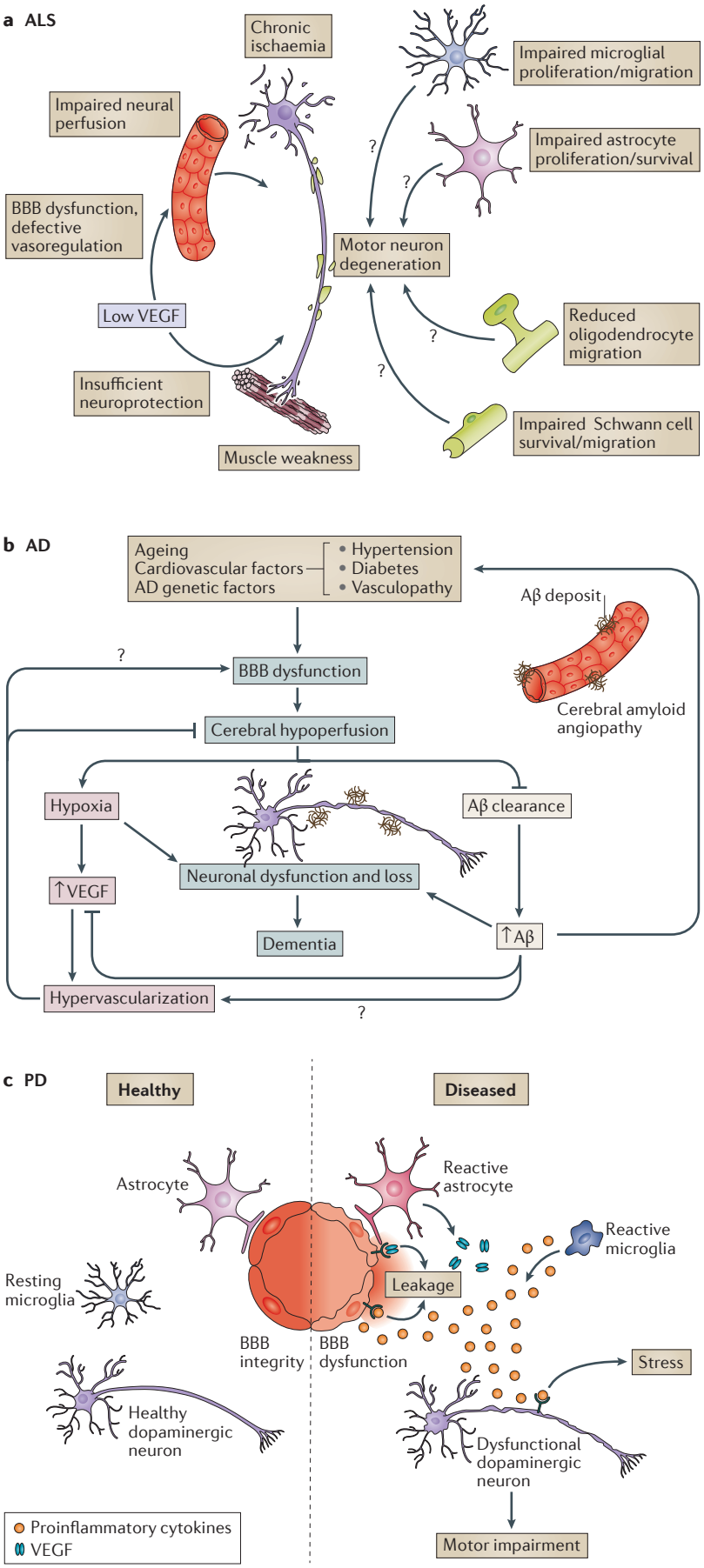
Second, VEGF exerts direct neurotrophic effects, so reduced levels of this protein deprive motor neurons of neuroprotection, thereby compromising survival (FIG. 3a). *In vitro,* VEGF stimulates motor neuron survival in both normal and stress conditions, including in response to *SOD1*^{G93A} expression[32,37–42]. These effects are mediated by VEGFR-2 activation, leading to PI3K–Akt signalling, which in turn inhibits p38 MAP kinase phosphorylation, thereby preventing Bcl-2 downregulation and inhibiting apoptosis[32,37,39,42]. In addition, VEGF induces expression of the AMPA receptor GluR2 subunit, thereby reducing motor neuron vulnerability to glutamate excitotoxicity[41]. VEGF, VEGFR-1 and VEGFR-2 are expressed by spinal motor neurons in mice and humans[32,42–44], and neuron-selective overexpression of VEGFR-2 delays disease onset and prolongs survival in *SOD1*^{G93A} mice, further illustrating the direct neurotrophic activity of VEGF[45]. VEGF also exerts pleiotrophic effects on astrocytes, microglia, oligodendrocytes and Schwann cells[30] (FIG. 3), all of which have been implicated in ALS pathogenesis. The relevance of VEGF's effects on these cell types in the context of ALS remains to be investigated.

A series of independent studies have highlighted the therapeutic potential of VEGF for ALS. Intramuscular administration of a VEGF-expressing, retrogradely transported lentiviral vector to *SOD1*^{G93A} mice, as well as intracerebroventricular (ICV) delivery of recombinant VEGF to *SOD1*^{G93A} rats, delayed disease onset, slowed disease progression, improved motor performance, mitigated axonal degeneration and motor neuron loss, and extended the lifespan[45,46]. Even when treatment was initiated at the onset of paralysis, disease progression was delayed and survival was prolonged. VEGF delivered via alternative routes, including neuronal overexpression, intrathecal transplantation of NSCs overexpressing VEGF, and adeno-associated virus (AAV)-mediated expression of VEGF in ventricular cells, was also found to be beneficial for *SOD1*^{G93A} rodents[47–49]. Interestingly, VEGF protein delivered via the ICV route is anterogradely transported along motor axons, a mechanism that may support preservation of neuromuscular junctions (NMJs) in *SOD1*^{G93A} rats[45]. Thus, VEGF may also act locally in the muscle to preserve NMJ integrity. Consistent with this idea, VEGF overexpression in muscle improves motor function and extends survival in *SOD1*^{G93A} rats[50,51].

Combinatorial VEGF treatment, in particular with neurotrophic factors that act on different signalling pathways, can result in synergistic therapeutic effects, as exemplified by combined intramuscular delivery of VEGF and glial cell line-derived neurotrophic factor (GDNF) to *SOD1*^{G93A} rats[49,51]. Although most preclinical studies on VEGF therapy for ALS have been performed in mutant *SOD1* rodent models, VEGF also reduces motor neuron death and prevents motor deficits in rat models of excitotoxic motor neuron death[40,52], suggesting that VEGF holds promise for the treatment of sporadic ALS. A phase I clinical trial to evaluate the safety of VEGF in patients with ALS has been conducted, but was terminated early owing to problems with the infusion delivery system (TABLE 1).

VEGF-B also exerts neurotrophic effects on motor neurons. *SOD1*^{G93A} mice lacking VEGF-B develop more-severe motor neuron degeneration, and interaction of VEGF-B with VEGFR-1 on motor neurons promotes neuronal survival[44]. Similar to VEGF, ICV infusion of VEGF-B in *SOD1*^{G93A} rats delays the onset of motor deficits, promotes motor neuron survival and increases lifespan[44]. The minimal angiogenic activity of VEGF-B may be beneficial in terms of avoiding undesired vascular effects.

Alzheimer disease. Alzheimer disease (AD) is a progressive disorder in which neurons of the cerebral cortex and hippocampus degenerate, resulting in loss of cognitive functions, memory, reasoning, movement coordination and pattern recognition. This common form of dementia in elderly individuals is characterized by extracellular deposition of amyloid-β (Aβ) in the brain parenchyma. However, Aβ deposits are also detected in arterial walls, a condition termed cerebral amyloid angiopathy (CAA) that is present in the majority (90%) of patients with AD[53–56]. Further aggravated by vascular dysfunction induced by ageing and cardiovascular risk factors (hypertension, diabetes and vasculopathy), CAA destroys microvascular structure and function, leading to loss of BBB integrity and cerebral hypoperfusion, along with an inflammatory response, all of which compromises neuronal viability. The resultant hypoperfusion not only causes hypoxia but also impairs Aβ clearance



(and, hence, further promotes Aβ accumulation), which may exacerbate neuronal dysfunction and loss[53–55,57–59] (FIG. 3b).

The effects of Aβ on VEGF-driven blood vessel function in AD remain unresolved, as both proangiogenic and antiangiogenic effects have been described[57,60–62]. On the one hand, Aβ inhibits VEGF binding to its receptor, and suppresses endothelial cell proliferation and survival responses to growth factors, including VEGF[61,63,64]. Also, in patients with AD, subnormal VEGF levels have been reported, which might aggravate vascular insufficiency[65–67]. On the other hand, VEGF, VEGFR-2 and NRP-1 are upregulated in the brains of mice and patients with AD[68], probably owing to the hypoxia that results from cerebral hypoperfusion[54,58]. VEGF might be upregulated to compensate for the hypoperfusion, explaining why patients and mice with AD show increased vascular density in regions around Aβ plaques[57,69]. These neovessels are leaky, however, which disturbs neural homeostasis and can cause neurotoxicity[62]. Whether these VEGF-driven vascular alterations are causally involved in the pathogenesis of AD requires further study.

Currently, therapy for AD is limited to symptomatic treatments[56,70,71]. To alleviate cerebral hypoperfusion in AD, proangiogenic strategies have been explored[57,62].

◀ Figure 3 | **VEGF in neurodegenerative disease.** **a** | Amyotrophic lateral sclerosis (ALS). Low vascular endothelial growth factor (VEGF) levels are a risk factor for ALS in humans, and cause ALS-like disease in mice. Low VEGF levels impair spinal cord perfusion and cause chronic ischaemia of motor neurons, and also deprive these cells of vital VEGF-dependent survival and neuroprotective signals. Both mechanisms may contribute to the adult-onset progressive degeneration of motor neurons, with associated muscle weakness and paralysis and, ultimately, death. Other neural cell types, including microglia, astrocytes, oligodendrocytes and Schwann cells, are impaired in ALS, probably contributing to the motor neuron degeneration. Permission obtained from American Society for Clinical Investigation © Storkebaum, E. & Carmeliet, P. *J. Clin. Invest.* **113**, 14–18 (2004). **b** | Alzheimer disease (AD). Recent literature suggests multifactorial pathogenesis resulting from BBB dysfunction and chronic cerebral hypoperfusion, in combination with deleterious effects due to toxic amyloid-β (Aβ) accumulation in the brain parenchyma and blood vessel walls (cerebral amyloid angiopathy). Hypoxia enhances VEGF expression, possibly leading to a hypervascularization response to relieve hypoperfusion. Aβ is reported to inhibit VEGF receptor signalling, although some studies also indicate that Aβ promotes hypervascularization. We do not yet understand the relative importance of these opposing effects of Aβ, or whether hypervascularization co-contributes to disease progression. **c** | Parkinson disease (PD). The healthy brain (left) shows an intact BBB, functional dopaminergic neurons and normal astrocytes and resting microglia. Compromised blood–brain barrier (BBB) integrity contributes to the pathogenesis of PD by promoting reactive gliosis, resulting in release of VEGF and proinflammatory cytokines by activated astrocytes and microglia. These events further aggravate BBB disruption and promote dopaminergic neuron dysfunction and death.

Table 1 | **Trials of VEGF, or VEGF or PlGF blockade, in neurological diseases**

Disease	Intervention	Phase	Status	ClinicalTrials.gov identifier	Sponsor
Peripheral nerve injury	Neovasculgen ($VEGF_{165}$ plasmid)	I,II	Not yet recruiting	NCT02352649	Human Stem Cell Institute, Kazan, Russia
Amyotrophic lateral sclerosis	sNN0029 (containing $VEGF_{165}$)	I	Completed 2016*	NCT02269436	Newron Sweden AB, Stockholm, Sweden
Neuromyelitis optica	Bevacuzimab (anti-VEGF mAb)	Ib	Completed 2016	NCT01777412	Johns Hopkins University, Baltimore, Maryland, USA
Diabetic retinopathy, macular oedema	Ranibizumab (anti-VEGF mAb)	III	Recruiting	NCT02130024	Novartis Pharmaceuticals
Diabetic retinopathy	THR-317 (anti-PlGF antibody)	I	Announced for second half of 2016	Not applicable	Thrombogenics NV
	Ziv-aflibercept (VEGF-sequestering protein)	II	Recruiting	NCT02486484	University Hospital, Beirut, Lebanon
	Ranibizumab (anti-VEGF mAb)	II	Recruiting	NCT02328118	The First People's Hospital of Xuzhou, China
	Aflibercept (VEGF-sequestering protein)	II	Recruiting	NCT02320474	University Hospital, Poitiers, France
Diabetic macula oedema	Ranibizumab and bevacuzimab (anti-VEGF mAbs)	IV	Recruiting	NCT02462304	The University of Hong Kong, China
	Aflibercept (VEGF-sequestering protein)	IV	Recruiting	NCT07717142	University of Sydney, Australia
	Anti-VEGF mAb	IV	Recruiting	NCT02471651	California Retina Consultants
Macular degeneration	RTH258 (anti-VEGF mAb)	III	Recruiting	NCT02307682	Alcon Research
Rare VEGF-driven ocular diseases	Ranibizumab (anti-VEGF mAb)	II	Ongoing	NCT01908816	Novartis Pharmaceuticals
Non-neovascular AMD	Ranibizumab (anti-VEGF mAb)	I/II	Recruiting	NCT02140151	Southern California Desert Retina Consultants, MC
Neovascular AMD	OPT-302 (VEGF-C/D-sequestering molecule) and ranibizumab (anti-VEGF mAb)	I	Recruiting	NCT02543229	Opthea Pty Ltd
	X-82 (VEGFR/PDGFR inhibitor) and aflibercept (VEGF-sequestering protein)	II	Recruiting	NCT02348359	Tyrogenex
	Sirolimus (mTOR inhibitor) and anti-VEGF mAb	II	Recruiting	NCT02357342	Raj K. Maturi, Indianapolis, Indiana, USA
	Fovista (pegylated PDGF-BB aptamer) with aflibercept, ranibizumab or bevacizumab	II	Recruiting	NCT02387957	Ophthotech Corporation
	NT-503-3 (encapsulated cells expressing soluble VEGFR-1) or aflibercept	I/II	Recruiting	NCT02228304	Neurotech Pharmaceuticals
	LM324 (anti-VEGF mAb) or ranibizumab	I/II	Recruiting	NCT02398500	Alcon Research
Exudative AMD	Proton beam radiation and anti-VEGF antibody	I/II	Recruiting	NCT01213082	University of California, Davis, USA

*Trial terminated after completion of phase I owing to issues with the infusion delivery system. AMD, age-related macular degeneration; mAb, monoclonal antibody; PDGF, platelet-derived growth factor; PlGF, placental growth factor; VEGF, vascular endothelial growth factor.

Neuron-specific overexpression of VEGF in a mouse model of AD partially rescued cerebral vascular loss and restored memory behaviour[60]. Moreover, administration of VEGF-releasing nanospheres or VEGF-overexpressing mesenchymal stem cells to AD mice promoted neovascularization, reduced Aβ deposition and improved behavioural deficits[72,73]. Furthermore, in a prospective study that explored biomarkers for AD in relation to brain ageing in individuals with differing severities of cognitive decline, elevated VEGF levels in the cerebrospinal fluid were associated with improved brain ageing outcomes, suggesting that patients with early AD might benefit from VEGF treatment[74].

Huntington disease. Huntington disease (HD) is caused by mutations in the huntingtin gene, and is characterized by progressive dysfunction and neuronal death in corticostriatal circuits, resulting in motor impairment, cognitive decline, psychiatric disability and, ultimately, death[70,71]. Whether VEGF is functionally involved in HD remains unknown, but decreased VEGF levels are observed in peripheral mononuclear cells from individuals with this condition[75]. On the basis of its neurotrophic action, VEGF treatment has been explored in HD models. Injection of VEGF-releasing hydrogels into the striatum attenuated motor impairment and reduced striatal neuron loss in a rat model of HD[76]. Similarly, lentiviral

delivery of a low dose of VEGF reduced neuronal loss and mutant huntingtin aggregation in both *in vitro* and *in vivo* models of HD[77]. In the same study, however, a high dose of VEGF caused vascular leakage, astrogliosis and neuroinflammation, illustrating the importance of treatment dosage to elicit beneficial effects.

Parkinson disease. Parkinson disease (PD) is a degenerative disorder, primarily affecting movement, that results from degeneration and death of dopaminergic neurons[78]. Besides neuron-intrinsic causes, vascular perturbations might contribute to the pathogenesis of PD. VEGF expression by glial fibrillary acidic protein-positive astrocytes is increased in the substantia nigra and basal ganglia in PD[79–81]. Probably as a consequence, patients with PD exhibit increased microvascular density[80,81], but also have abnormally shaped blood vessels and a leaky BBB in the substantia nigra[82–86]. As a result of the BBB dysfunction, reactive astrocytes and microglia release proinflammatory, proapoptotic cytokines that compromise dopaminergic neuron survival and further aggravate BBB disruption in a positive feedback mechanism[80] (FIG. 3c).

In 6-hydroxydopamine (6-OHDA) neurotoxin-mediated preclinical models of PD, AAV-mediated VEGF overexpression or implantation of encapsulated VEGF-secreting cells in the striatum decreases amphetamine-induced rotational behaviour and preserves tyrosine hydroxylase-positive neurons in the substantia nigra, indicating a neuroprotective effect of VEGF[87–90]. This neuroprotection is attributable to a dual effect of VEGF on neural cells and blood vessels. VEGF promotes survival of dopaminergic neurons *in vitro*, and *in vivo* delivery of VEGF induces angiogenesis and glial proliferation, which may, respectively, improve perfusion and release of neurotrophic factors, in particular, GDNF[87–90]. However, the effects of VEGF are contextual and dose-dependent: the benefit is largest at low VEGF doses, and higher doses evoke excessive angiogenesis and brain oedema[89].

Combined delivery of VEGF and GDNF to the striatum of rats after induction of 6-OHDA lesions was found to be superior to monotherapy with either factor alone[91–93]. In addition, preclinical studies in animal models of PD have shown that transplantation of VEGF-expressing stem cells provides higher therapeutic benefits than transplantation of non-modified stem cells, by protecting damaged dopaminergic neurons and stimulating their regeneration[94,95]. In one such study, VEGF-expressing umbilical cord mesenchymal stem cells were infused into the striatum of rotenone-lesioned hemiparkinsonian rats[95]. This intervention ameliorated apomorphine-evoked rotations in the animals, and the transplanted cells showed evidence of differentiation into dopaminergic neuron-like cells in the substantia nigra.

A functional role for VEGF-B in PD has also been documented. VEGF-B has been shown to protect midbrain neurons from rotenone or 6-OHDA-induced neurotoxicity[96,97], but only when administered before lesion induction, suggesting a neuroprotective rather than a neurorestorative function[96,98].

In PD, blood vessels in the subthalamic nucleus (STN) degenerate, and STN neurons, which provide excitatory innervation to the substantia nigra dopaminergic neurons, become hyperactive[99,100]. Postmortem analysis of patients with PD who received STN deep brain stimulation showed enhanced levels of VEGF and increased density of microvessels with a tighter BBB in the STN[99]. High cervical spinal cord stimulation in PD rats induced VEGF expression in the striatum, concomitant with improved behavioural outcome and preservation of dopaminergic neurons that project to this region[101]. Although the precise molecular mechanisms remain unknown, VEGF-mediated angiogenic and/or neuroprotective activity might contribute to the beneficial effects of these interventions in PD.

Peripheral neuropathies. Peripheral neuropathies are characterized by degeneration of peripheral motor, sensory and/or autonomic axons, leading to a plethora of symptoms, including muscle weakness and wasting, numbness, paraesthesia, pain and/or organ dysfunction, depending on the type of axon affected[102,103]. Peripheral axonopathy is a common complication in patients with diabetes mellitus and limb ischaemia, or in patients receiving chemotherapy, but it can also be genetic in nature.

In the case of diabetes, neuropathy is associated with basement membrane thickening and blood–nerve barrier breakdown[104]. *In vitro*, advanced glycation end products cause pericyte degeneration, and also induce transforming growth factor β release, which results in basement membrane thickening, thereby impeding oxygen diffusion to the surrounding pericytes and axons[105,106]. In diabetic neuropathy and models of chemotherapy-induced neuropathy, blood–nerve barrier disruption may be partially attributable to reduced levels of the tight junction protein claudin-5 (REFS 105,106) (FIG. 4). Moreover, the density of the vasa nervorum — the vessels that nourish axons in peripheral nerves — is reduced, thereby compromising nerve blood perfusion[102,107–109] (FIG. 4). Probably owing to the resultant hypoxia, VEGF expression is elevated in Schwann cells and neurons in a rat model of diabetic neuropathy[110,111]. High glucose concentrations increase production of VEGF but reduce production of soluble VEGFR-1 (a VEGF trap) by Schwann cells and dorsal root ganglion neurons *in vitro,* thereby increasing the levels of free VEGF[112].

In accordance with the idea of hypoperfusion of the peripheral nerves, several studies suggest that VEGF can exert a therapeutic effect through improvement of vascular function. Intramuscular VEGF-plasmid delivery was shown to alleviate peripheral neuropathy after hindlimb ischaemia by mitigating axonal degeneration and improving nerve recovery via enhanced vascularization and perfusion[113]. Similar beneficial effects were seen in rat models of diabetes or chemotherapy-induced neuropathy[102].

Besides its vascular effects, VEGF also exerts neuroprotective effects on peripheral nerve axons. *In vitro*, VEGF-activated signalling through VEGFR-2 was found to protect dorsal root ganglion sensory neurons from the

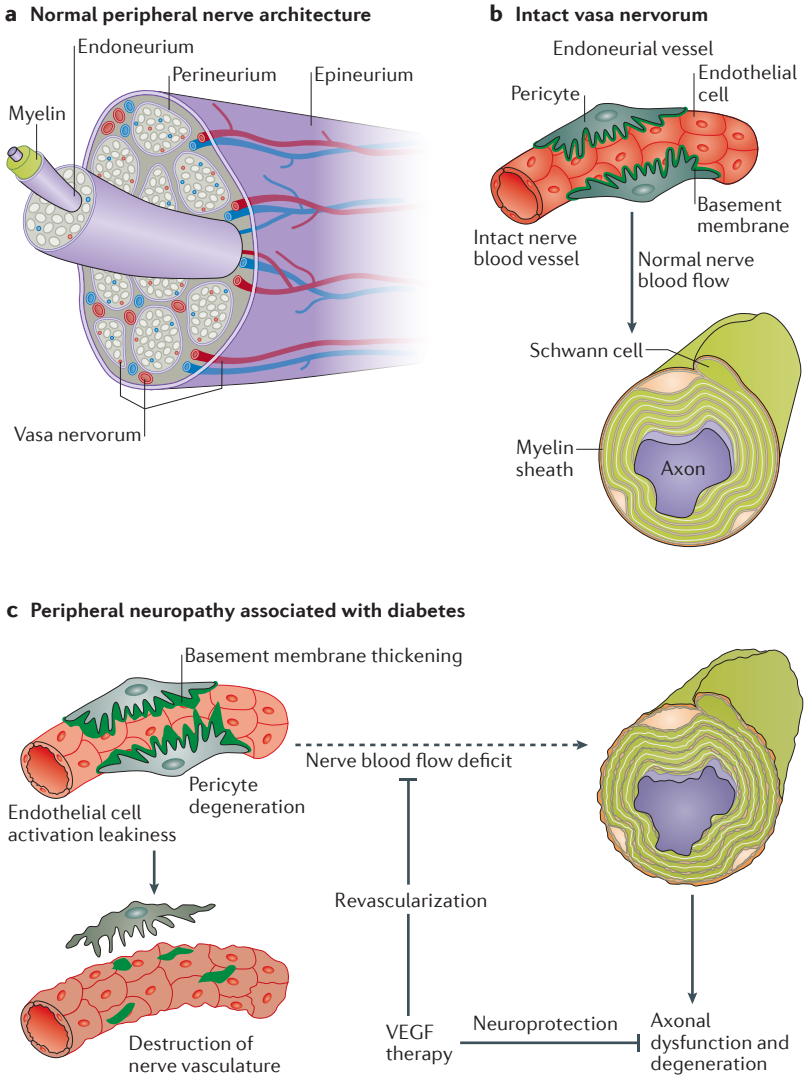


Figure 4 | **Vasa nervorum loss in diabetic peripheral neuropathy. a** | Normal peripheral nerve architecture with intact vasa nervorum. Permission obtained from Pocket Dentistry http://pocketdentistry.com/25-traumatic-injuries-of-the-trigeminal-nerve/. **b** | Intact vasa nervorum providing normal blood flow to a healthy nerve. **c** | Peripheral neuropathy associated with diabetes might result in part from destruction of the endoneurial vasa nervorum. Pericyte degeneration and basement membrane thickening impede oxygen transfer to perivascular axons, ultimately leading to destruction of the nerve vasculature. The resulting reduction in nerve perfusion leads to axonal dysfunction and degeneration. Vascular endothelial growth factor therapy alleviates diabetic peripheral neuropathy, possibly via a combination of direct neuroprotection and improvement of nerve perfusion by vasa nervorum revascularization.

effects of paclitaxel or hyperglycaemia, through induction of Hsp90 deacetylation and resultant elevation of anti-apoptotic Bcl-2 (REFS 114,115). Furthermore, neuronal overexpression of VEGFR-2 in mice reduced the sensitivity to paclitaxel-induced peripheral neuropathy, whereas a dominant-negative form of VEGFR-2 had the opposite effect, independently of vascular changes[114]. VEGF also stimulates the migration and survival of Schwann cells[113]. As already mentioned, defective VEGF signalling might also contribute to CMT2D, an inherited peripheral neuropathy caused by mutations in the gene encoding GlyRS. Through competitive binding of mutant GlyRS to NRP-1, VEGF–NRP-1 signalling in neurons is impaired, thereby promoting axonal degeneration[22]. Notably, the $VEGF_{165}$b isoform, which counteracts the vascular effects of $VEGF_{165}$a (REFS 116,117), protects neurons against chemotherapy-induced cytotoxicity via activation of VEGFR-2 and MEK1/2 and inhibition of caspase-3 (REF. 118). This isoform also alleviated pain in diabetic rats[119].

Similar protective effects are seen for VEGF-B: mice lacking VEGF-B or functional VEGFR-1 exhibit more-pronounced paclitaxel-induced neuropathy, and neuronal expression of VEGF-B or VEGFR-1, or treatment with recombinant VEGF-B, can protect against neuropathy[120]. A fraction of patients with cancer develop peripheral neuropathy as an adverse effect of chemotherapy or anti-VEGF therapy[114,121]. Administration of VEGF isoforms with neuroprotective but no vascular effects, such as VEGF-B or $VEGF_{165}$b, might be attractive for these patients, as these isoforms do not induce angiogenesis and, thus, are unlikely to promote cancer growth.

In the clinical setting, intramuscular injection of VEGF-encoding plasmid improved ischaemic neuropathy in patients with leg ischaemia[122], and alleviated the symptoms in patients with diabetic neuropathy[123]. Intramuscular delivery of VEGF-expressing plasmids is also being clinically evaluated for peripheral nerve injury (TABLE 1).

VEGF in other neurological diseases

Besides its neuroprotective effects in neurodegenerative disease, VEGF has roles in various other neurological diseases, including stroke, trauma, epilepsy and multiple sclerosis (MS). Pathologically elevated VEGF levels can contribute to disease pathology by inducing BBB breakdown and vascular leakage, thereby exposing the brain to harmful substances from the blood, increasing the influx of inflammatory cells and, potentially, exacerbating hypoxia, which can reinforce VEGF secretion in a feedback loop. In ocular neovascular diseases, increases in VEGF secretion in the retina cause vascular leakage and overgrowth as the primary pathological mechanism. The current state of VEGF targeting for the treatment of ocular neovascular diseases is described in BOX 2.

Brain injuries. Stroke and traumatic brain injuries (TBIs) are leading causes of death and long-term morbidity. Ischaemic stroke results from occlusion of a cerebral artery, causing infarction of the irrigated tissue. The necrotic core is surrounded by the penumbra, which is still viable but at risk of further decay without rapid reperfusion (FIG. 5a). Ischaemia also has a major role in haemorrhagic stroke and TBI. Pathophysiological responses to stroke and TBI include excitotoxicity, oxidative damage and inflammation, leading to neuron death. VEGF has contextual effects that can be both beneficial and deleterious in these conditions (FIG. 5). Beneficial effects include an increase in collateral vessel formation, vasodilation, angiogenesis, and neuroprotection[124,125] (FIG. 5b). By contrast, high VEGF levels, resulting from upregulation by severe ischaemia or from systemic VEGF administration, may aggravate tissue

Box 2 | **VEGF family members in ocular disease**

Vascular endothelial growth factor (VEGF) family members have prominent roles in neovascular ocular diseases. Diabetic retinopathy and the wet form of age-related macular degeneration (AMD) are characterized by vascular overgrowth and leakiness, and retinal oedema and inflammation. VEGF and placental growth factor (PlGF) levels correlate with ocular neovascularization in both conditions, and decrease on successful treatment[184,185]. Rodent and primate studies indicate that VEGF or PlGF delivery or overexpression in the eye induces choroidal neovascularization (CNV) and diabetic retinopathy-like vascular defects[186–188], whereas inhibition or loss of VEGF or PlGF reduces CNV and diabetic retinopathy in preclinical models[184,189,190].

Anti-VEGF therapies have emerged as treatments for AMD, diabetic macular oedema and proliferative diabetic retinopathy. Most clinical trials tested inhibitors of VEGF signalling, including pegaptanib, bevacizumab, ranibizumab and aflibercept (TABLE 1). However, nearly 50% of patients receiving intravitreal anti-VEGF exhibited residual ocular oedema, prompting trials of add-on therapies (TABLE 1). Several trials found beneficial effects of the broader-acting aflibercept in patients who became refractory to ranibizumab or bevacizumab[191]. Apart from a higher affinity for VEGF, the superior effect of aflibercept may relate to its inhibition of PlGF. In a mouse CNV model, genetic or pharmacological PlGF blockade inhibited CNV and enhanced the effects of VEGF-targeted inhibitors[192,193]. Unlike VEGF inhibitors, anti-PlGF antibodies can be safely administered systemically, and they inhibit ocular neovascularization without adverse effects[192].

In vitro experiments indicate a direct effect of VEGF on retinal neurons via VEGFR-2–PI3K–Akt signalling[194,195], and neuronal cell death has been observed after inhibition of VEGF signalling. In a streptozotocin-induced diabetic rat retina model, intravitreal injection of an anti-VEGF antibody led to increased numbers of apoptotic retinal ganglion cells, and of amacrine and bipolar cells[196]. Furthermore, chronic inhibition of VEGF function in adult mice resulted in significant loss of retinal ganglion cells[194]. Therefore, anti-VEGF therapies must be used with caution to treat ocular diseases.

damage through an increase in BBB leakage, leading to poststroke brain oedema and life-threatening intracranial hypertension[125,126].

In mice, VEGF is upregulated after injury, owing to tissue hypoxia, oxidative stress and inflammation[127–130]. Patients with stroke or TBI have elevated VEGF levels in serum and cerebrospinal fluid, respectively, and higher baseline VEGF levels are associated with increased risk of stroke[131–133]. Elevated VEGF levels contribute to early stroke pathology, including BBB breakdown, vascular leakage and oedema. Oedema increases intracranial pressure, which further increases ischaemia by obstructing blood vessels (FIG. 5c). Moreover, BBB disruption by VEGF increases extravasation of glutamate and albumin, which activates astrocytes and perturbs K^+ homeostasis in the brain parenchyma, leading to neuronal hyperactivity and stress[134] (FIG. 5c). Consistent with these observations, VEGF administration shortly after stroke increases vascular leakage and brain infarction, whereas VEGF blockade early after stroke reduces brain oedema and infarct size[125,126].

VEGF also has beneficial effects in stroke. By inducing vasodilation, VEGF improves perfusion and, hence, preservation of the penumbra[135,136]. At a later stage, VEGF is neuroprotective and ameliorates vascular dysfunction (FIG. 5b). Indeed, delayed VEGF treatment at 48 h after stroke stimulates angiogenesis without inducing vessel leakage, and improves recovery of neurological functions[125], probably by stimulating neuroprotection[137,138], reparative angiogenesis[125] (FIG. 5b) and neurogenesis (BOX 1). This finding could explain why VEGF is beneficial when administered after the acute phase[139], and administration of the anti-VEGF antibody bevacizumab exacerbates brain necrosis and neurological deficits[140]. Of note, VEGF levels during physiological growth influence stroke outcome in the adult, as elevated VEGF levels promote the formation of collaterals that bypass occluded vessels and reduce infarct areas[19,20] (FIG. 5b).

An outstanding question is how to improve the safety of VEGF therapy after stroke. Although similar beneficial effects of VEGF administration were confirmed in independent studies, adverse effects were also reported, particularly when VEGF was given systemically[124]. One attractive possibility is to use the VEGF homologue PlGF, which, on overexpression in the brain, improves angiogenesis without adverse effects[141]. Alternatively, moderate upregulation of endogenous VEGF expression by means of an engineered zinc finger transcription activator protein homing to the VEGF promoter (VEGF-ZFP-TF) can reduce neuronal apoptosis and improve recovery from neurological deficits after TBI[142]. Another possibility is the use of VEGF-derived molecules that separate vascular from neuronal effects. For instance, in rodent models of CNS ischaemia, QK, a VEGF-derived peptide that partly mimics the VEGF binding domain on VEGFR-2, and the VEGF splice isoform $VEGF_{165}b$, which is neuroprotective but lacks angiogenic activity, were both shown to be neuroprotective without causing oedema[118,143,144].

Neuroprotection in ischaemic brain diseases can be also achieved through chronic activation of hypoxia signalling, which results in increased expression of VEGF, among other protective factors. This is accomplished by reducing the activity of the cellular oxygen sensors of the HIF prolyl hydoxylase family (PHDs), by inducing mild hypoxia, or by pharmacological blockade or genetic deletion of PHDs. All three approaches confer neuroprotection after stroke, and several PHD inhibitors are in clinical development, but the mechanisms of neuroprotection remain unclear[145–150]. Genetic deletion or inhibition of PHD1 or PHD2 can protect neurons against ischaemic stroke, but via different mechanisms: HIF-independent metabolic reprogramming in the case of PHD1 inhibition, and HIF-dependent induction of hypoxia target genes in the case of PHD2 loss[146,150]. Inhibition of PHD2 also reduces neuronal activity and synaptic transmission, warranting caution for global PHD inhibition in the human brain[151].

Stimulation of NSC proliferation by VEGF contributes to its beneficial properties (BOX 1), mostly via a bystander effect, similar to that of bone marrow mononuclear cells (BM-MNCs) transplanted after stroke[152]. In this setting, VEGF increases the homing of BM-MNCs to the brains of rats with chronic hypoperfusion, and promotes functional recovery, despite vascular leakage[153]. Together, these data suggest a therapeutic window for VEGF-based therapies after brain injuries to safeguard neuronal survival and tissue regeneration, but care should be taken to avoid vascular leakage and inflammation, which exacerbate tissue damage. Dissociation of the neurogenic and neuroprotective properties of VEGF from its effects on vascular permeability might represent a therapeutic avenue for future investigation.

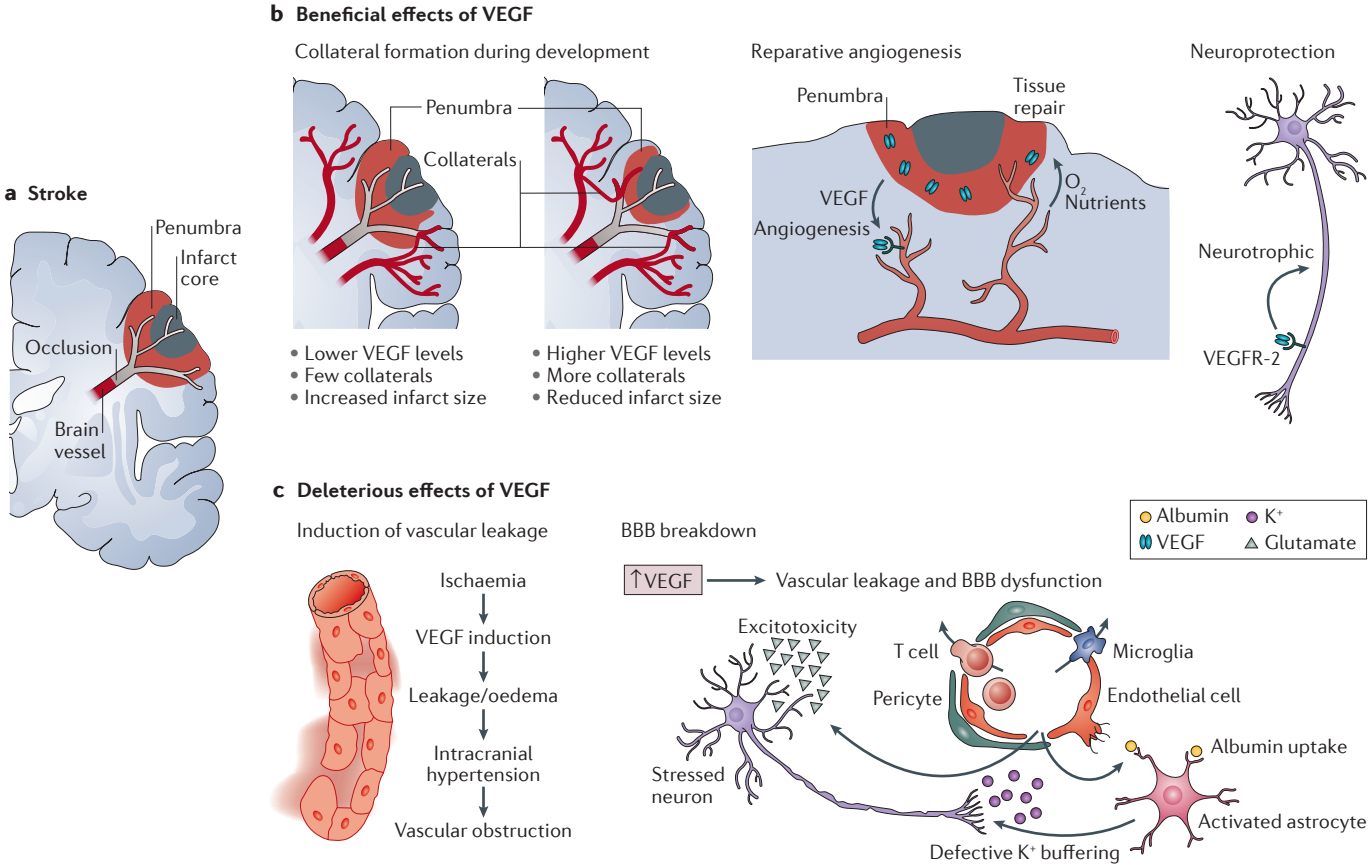


Figure 5 | **Role of VEGF in stroke. a** | Occlusion of brain vessels causes a brain infarct (black) that is surrounded by a still viable but at-risk penumbra (red). **b** | Beneficial effects of vascular endothelial growth factor (VEGF). VEGF levels during development determine the abundance of collaterals that can bypass occluded vessels and reduce infarct areas. Delayed treatment with VEGF can activate angiogenesis that supports tissue repair in the penumbra, or increase neuronal survival by directly activating VEGFR-2-mediated neuroprotection. **c** | Deleterious effects of VEGF. Excessive VEGF levels, particularly early after the infarct, might adversely affect stroke recovery via increased vascular leakage, oedema, obstruction of supply vessels through elevated interstitial pressure, and life-threatening intracranial hypertension. Increased leakage can transform the insult into a haemorrhagic infarct. Moreover, VEGF-induced blood–brain barrier (BBB) breakdown in the penumbra can damage neurons directly via disturbance of ion homeostasis and entry of inflammatory cells. EC, endothelial cell.

Epilepsy. Epilepsy is characterized by seizures due to synchronized neuronal activity after TBI, stroke, brain tumours or brain infection, or through genetic causes. Seizures induce VEGF expression and BBB breakdown in the rodent hippocampus and in patients with epilepsy[154,155]. The degree of BBB breakdown correlates with seizure burden in mice, and BBB disruption can provoke seizures in rodents[156,157]. BBB disruption allows diffusion of ions and neurotransmitters into the brain parenchyma, causing increased neuron excitability. In addition, leaked plasma albumin is taken up by astrocytes, triggering downregulation of K^+ channels, which impairs clearance of extracellular K^+. Elevated K^+ levels depolarize neurons, thereby increasing excitability. Furthermore, release of cytokines and VEGF by astrocytes and microglia, activated in response to BBB leakage, aggravates BBB disruption and sustains epileptogenic inflammation[158] (FIG. 6). Reduction of BBB breakdown through antivascular therapy might, therefore, represent a viable antiepileptogenic therapy. Indeed, analysis in hippocampal slices has shown that seizures stimulate VEGFR-2 signalling and angiogenesis while impairing BBB tightness — effects that are counteracted by anti-VEGF antibodies[159].

VEGF also seems to be neuroprotective in the epileptic brain, as VEGF administration reduces neuronal apoptosis after seizure induction[159–161]. Also, infusion of a low dose of VEGF that does not induce angiogenesis reduces neuronal loss after seizure induction, and improves learning[160,161]. Moreover, neuronal VEGFR-2 overexpression augments the discharge threshold and reduces seizure duration[162]. Conversely, neuronal loss is exacerbated when the VEGF-trapping VEGFR-1–Fc fusion protein is administered after seizure induction *in vivo*[161]. As in stroke, these data imply a dual effect of VEGF in epilepsy, on the one hand inducing BBB breakdown and vascular leakage, thereby contributing to seizures, but on the other hand offering neuroprotection.

Demyelinating diseases. Multiple sclerosis (MS) is an autoimmune disease of the CNS, characterized by an inflammatory attack by immune cells against

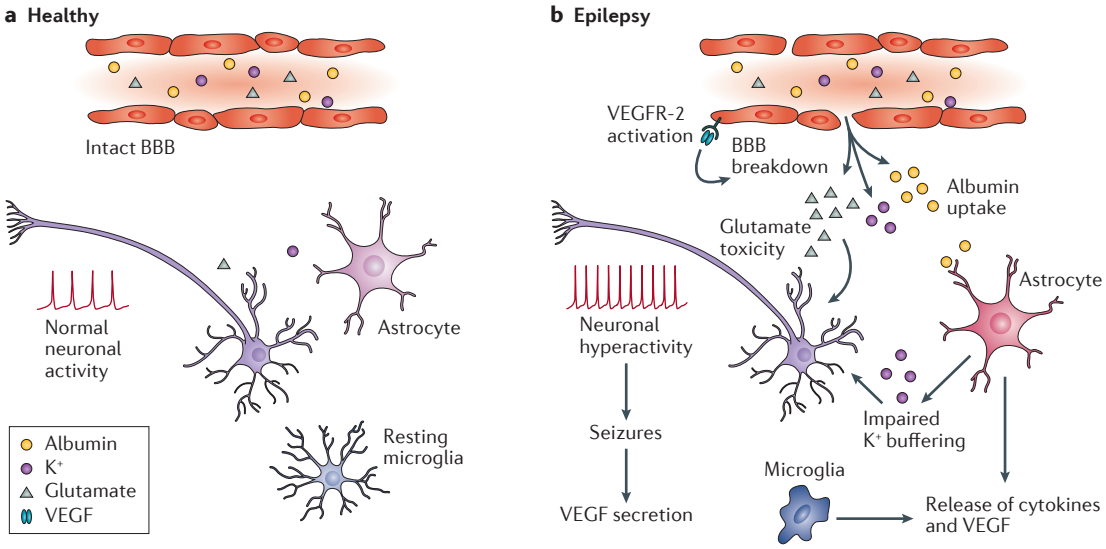


Figure 6 | **Vascular dysfunction in epilepsy. a** | An intact blood–brain barrier (BBB) prevents egress of blood-borne substances into the brain tissue, safeguarding normal neuronal activity and brain homeostasis in the healthy brain. **b** | BBB disruption in epileptic tissue allows K^+ ions and neurotransmitters such as glutamate to diffuse into the brain parenchyma, causing neuronal hyperactivity. Extravasated plasma albumin is taken up by astrocytes, leading to downregulation of K^+ channels, which results in impaired clearance of extracellular K^+. Release of cytokines and vascular endothelial growth factor (VEGF) by neurons, astrocytes and microglia further promotes BBB disruption.

oligodendrocytes, leading to demyelination, oligodendrocyte death and, ultimately, neuronal death in perivascular lesions (FIG. 7). Neuromyelitis optica (NMO) is another, more rare autoimmune disease, preferentially targeting aquaporin-4 (a molecule involved in water transport into the cell) in BBB astrocytes in the optic nerve and spinal cord (FIG. 7). Vascular leakage and BBB breakdown are hallmarks of MS and NMO[163,164], and they facilitate trafficking of immune cells and molecules into the brain parenchyma.

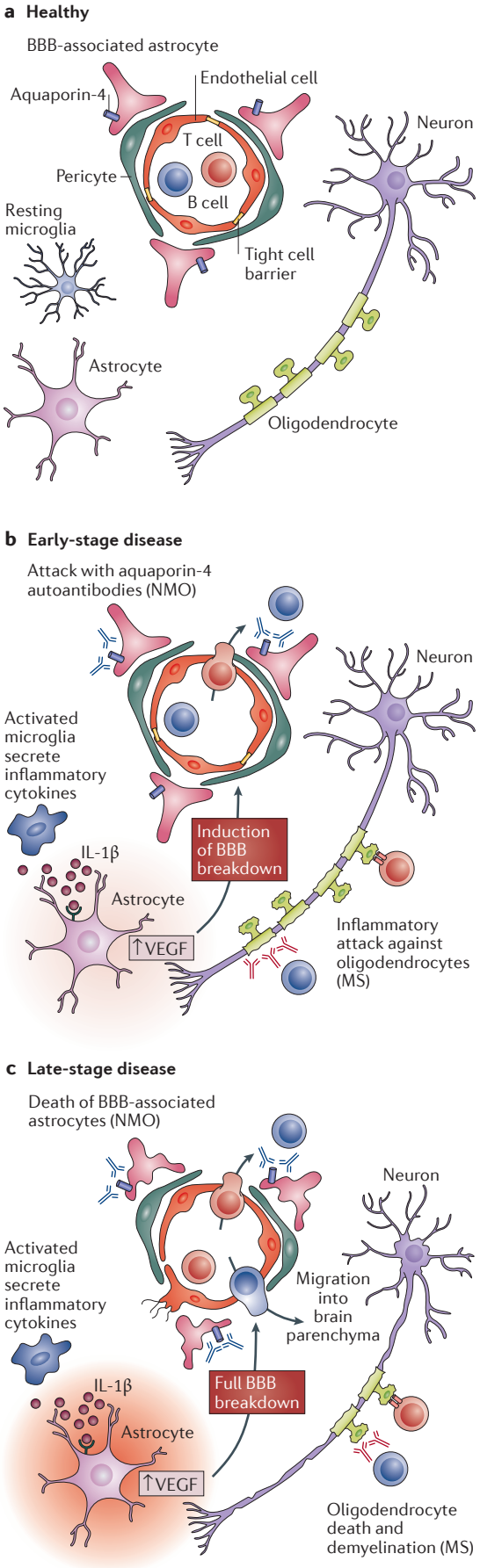
Patients with MS or NMO exhibit elevated serum VEGF levels[165–167]. As VEGF is a vessel permeability factor, increased levels of this protein render the BBB more leaky, thereby facilitating invasion of immune cells that target oligodendrocytes and BBB astrocytes in MS and NMO, respectively. Activated microglia secrete inflammatory cytokines, such as IL-1β, which augment VEGF release by astrocytes[168,169]. This process creates a vicious circle, whereby VEGF further increases vascular leakiness, thereby aggravating immune cell infiltration and inflammation, which, in turn, reinforce VEGF expression. Inflammation then kills oligodendrocytes or BBB astrocytes, leading to neurodegeneration owing to insufficient glial support (FIG. 7).

Evidence suggests that targeting of aberrant VEGF expression in brain autoimmune disease might augment immunosuppressive therapeutic strategies. In experimental autoimmune encephalomyelitis, an animal model of MS, VEGF blockade reduced brain inflammation and demyelination and suppressed angiogenesis, although effects on BBB permeability were not reported[170]. Though exciting, these findings require confirmation, as bevacizumab, an anti-human VEGF antibody that only minimally neutralizes mouse VEGF, was used to block VEGF in these preclinical experiments. Recently, bevacizumab has been evaluated in a clinical trial in patients with NMO (TABLE 1).

Therapeutic implications

Recent efforts to better understand the spectrum of effects of VEGF in the aforementioned neurological diseases have created novel therapeutic opportunities. However, any pro-VEGF or anti-VEGF therapeutic strategy should be carefully tailored to each neurological disorder, given that VEGF can either provide neuroprotection or promote BBB disruption, depending on the context. Nevertheless, as indicated in the sections above, administration or inhibition of VEGF and its family members has provided benefit in preclinical models of several neurological disorders.

Ocular diseases with neovascularization are the only neurovascular disorders for which VEGF inhibition has been clinically approved. Ongoing clinical trials are attempting to improve therapy based on VEGF inhibition, by testing novel VEGF blocking agents or combinations of VEGF inhibition with other treatments. By contrast, no established VEGF-based therapies exist for the treatment of other neurological diseases. In preclinical studies, VEGF or VEGF-B protein or gene therapy, alone or combined with neurotrophic factors, delayed disease onset or reduced neurological symptoms in rodent models of neurodegenerative diseases such as ALS[45–49,51], AD[72,73], HD[76,77] and PD[87–93,95,96,99], and also had beneficial effects in epilepsy[159–161] and stroke[125,135,136]. In addition, VEGF gene therapy improved peripheral neuropathy symptoms in patients with ischaemic or diabetic neuropathy[122,123]. The exact mechanisms underlying the beneficial effects in the aforementioned disorders



◀ Figure 7 | **VEGF as a mediator of neuroinflammatory disease. a** | Healthy brain vessels possess a functional blood–brain barrier (BBB) to support the cells of the surrounding brain parenchyma, while protecting it from immune cells and harmful substances in the blood. **b** | At early stages of neuroinflammatory disease, vessel barriers become weakened, allowing egress of immune cells that attack targets such as aquaporin-4-positive BBB-associated astrocytes in the case of neuromyelitis optica (NMO), or oligodendrocytes in multiple sclerosis (MS). Activated microglia secrete IL-1β, which induces vascular endothelial growth factor (VEGF) expression in astrocytes. **c** | Prolonged VEGF elevation increases angiogenic sprouting and causes breakdown of the BBB, a hallmark of advanced stages of neuroinflammatory disease. Inflammatory reactions against the respective target cells (BBB-associated astrocytes in NMO; oligodendrocytes in MS) cause death of the target cells and secondary neuronal apoptosis.

are not well defined, but improved vascular perfusion, neurotrophic effects and/or enhanced release of neurotrophic factors might be at play. In stroke, stimulation of neurogenesis could also contribute.

The efficacy and safety of pro-VEGF or anti-VEGF therapy will depend on clear definitions of the optimal dose, timing and delivery route for each indication. Dosing is critical, as a high VEGF dose poses risks of BBB disruption, brain oedema and neuroinflammation, as shown in models of HD and PD[77,89]. Similarly, timing of treatment initiation is relevant, as illustrated by the deleterious effects on BBB integrity of early but not delayed VEGF treatment in stroke[125,126,139]. The use of VEGF-B, which is less angiogenic than VEGF but still exerts neuroprotective effects, or of the VEGF splice isoform $VEGF_{165}b$, which has neuroprotective but no angiogenic activity, may partially circumvent these problems and allow more flexibility in dosing and timing of treatment initiation. Strategies that lead to upregulation of endogenous VEGF — for example, inhibition of prolyl hydroxylases[146,150], or engineered transcriptional activators that home to the VEGF promoter — might also be considered, as they yield normal relative expression of VEGF splice isoforms, and could avoid deleterious effects associated with overexpression of a single VEGF isoform.

Distinct administration routes may have to be considered for different neurological disorders, depending on the features of the particular disease, for example, ICV delivery for ALS and stroke[45], intramuscular delivery for ALS and peripheral neuropathy[46,50,107,122], and systemic administration for stroke[2]. Local and systemic delivery might yield different outcomes, as shown for VEGF treatment of stroke: ICV administration was beneficial, whereas systemic delivery had negative effects[2]. The delivery route must be tailored to the disease of interest, and will be co-determined by the ability of the drug to cross the BBB.

VEGF treatment for ALS in preclinical models illustrates the importance of identifying the optimal pharmacokinetics for VEGF treatment in neurological disease. Pharmacokinetic analysis and tracing experiments,

using ^{125}I-radiolabelled VEGF (^{125}I-VEGF), showed that VEGF failed to cross the BBB, and ICV administration resulted in diffusion: within 1 h, up to 70% of the injected VEGF had accumulated in the brain parenchyma, as confirmed by autoradiography[45]. ^{125}I-VEGF was also detected in the spinal cord, showing a rostrocaudal gradient that gradually diminished from the site of injection towards the lumbar spinal cord[45]. Clearance of VEGF from the cerebrospinal fluid occurred within 3 h, hence, continuous infusion of VEGF using osmotic mini-pumps was employed. Further experiments demonstrated that injected VEGF remained stable for several hours, and was anterogradely transported along motor axons, consistent with preservation of neuromuscular junctions in ALS rats[45]. Intramuscular injection of VEGF with demonstrable retrograde axonal transport produced beneficial effects in an ALS mouse model[47]. However, the pharmacological characteristics of pro-VEGF or anti-VEGF therapy are poorly defined for most other neurological disorders. More-detailed preclinical characterization of pharmacokinetics, distribution and stability will be instrumental for translation to human trials.

Patients with neurological disorders involving BBB breakdown or excessive angiogenesis, such as demyelinating diseases[170] or ocular disease (BOX 2), might benefit from anti-VEGF therapy. The VEGF-neutralizing antibody bevacizumab is being tested for safety in patients with NMO, and several advanced trials to optimize anti-VEGF approaches in retinal and other ocular diseases are in progress (TABLE 1). Administration of VEGF-neutralizing agents in ocular disease involves repeated intravitreal injection, but patient-tailored dosing regimens that attempt to maximize the risk:benefit ratio, thus alleviating therapy burden while retaining efficacy, are already being used with good clinical results[171,172]. Development of systemically deliverable agents, such as anti-PlGF, or orally available small molecules, such as the VEGFR and platelet-derived growth factor receptor inhibitor X-82, could further greatly reduce treatment discomfort for patients with ocular disease and other neurological conditions.

Overall, treatment of neurological diseases based on pro-VEGF or anti-VEGF strategies is relatively recent and still evolving. Critical issues regarding dose, timing, delivery route, frequency and treatment interval will need to be addressed to ensure optimal treatment efficacy and safety.

Conclusions

Research over the past 20 years has identified multiple roles for VEGF in brain function and pathology. In many cases, the role of VEGF not only depends on its canonical role as a regulator of angiogenesis and vascular permeability, but also suggests a direct role in neuronal protection. When considering the clinical use of VEGF as a neuroprotective agent, its ability to evoke BBB breakdown and vascular leakage should be taken into account. Conversely, when attempting to inhibit VEGF, attention should be paid to the possible deleterious effects of blocking its neuroprotective activity. In the future, it will be vital to unravel the downstream signalling mechanisms of VEGF that selectively regulate vessel permeability and neuroprotection, so as to therapeutically manipulate these processes separately. Investigation of VEGF homologues or isoforms with selective neuroprotective actions, such as VEGF-B and $VEGF_{165}b$, will also be important.

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All authors researched data for the article, made substantial contributions to discussions of the content, wrote the article, and reviewed and/or edited the manuscript before submission.

Competing interests statement
P.C. is named as inventor on patent applications WO 01/76620 and WO 2005/117946 and applicable resulting patents, which relate to results described in this article. The aforementioned patent application has been licensed, which may result in a royalty payment to P.C. The other authors declare no competing interests.