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REVIEW

Clinical efficacy and safety of angiogenesis inhibitors: sex differences and current challenges

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Abstract

Vasoactive molecules, such as vascular endothelial growth factor (VEGF) and endothelins, share cytokine-like activities and regulate endothelial cell (EC) growth, migration, and inflammation. Some endothelial mediators and their receptors are targets for currently approved angiogenesis inhibitors, drugs that are either monoclonal antibodies raised towards VEGF, or inhibitors of vascular receptor protein kinases and signalling pathways. Pharmacological interference with the protective functions of ECs results in a similar spectrum of adverse effects. Clinically, the most common side effects of VEGF signalling pathway inhibition include an increase in arterial pressure, left ventricular dysfunction facilitating the development of heart failure, thromboembolic events including pulmonary embolism and stroke, and myocardial infarction. Sex steroids, such as androgens, progestins, and oestrogens and their receptors (ERα, ERβ, GPER; PR-A, PR-B; AR) have been identified as important modifiers of angiogenesis, and sex differences have been reported for anti-angiogenic drugs. This review article discusses the current challenges clinicians are facing with regard to angiogenesis inhibitor therapy, including the need to consider sex differences affecting clinical efficacy and safety. We also propose areas for future research taking into account the role of sex hormone receptors and sex chromosomes. Development of new sex-specific drugs with improved target- and cell-type selectivity likely will open the way to personalized medicine in men and women requiring anti-angiogenic therapy to reduce adverse effects and to improve therapeutic efficacy.

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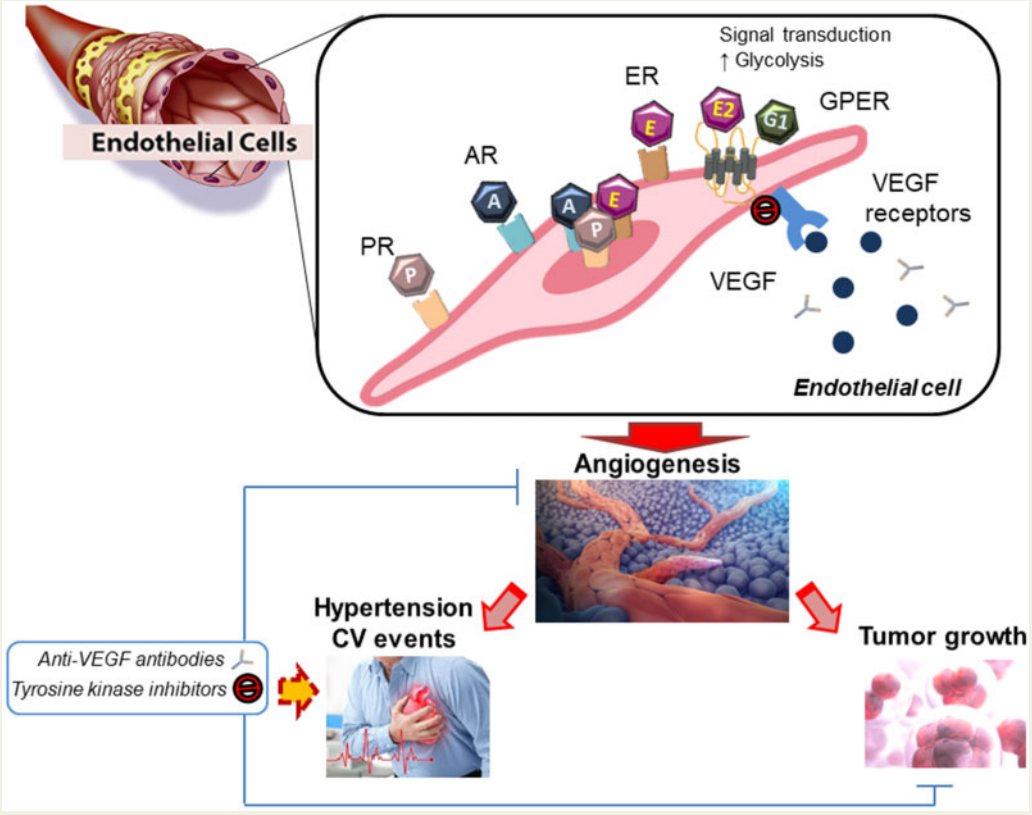
Graphical Abstract



Keywords Androgens • Cytokine • Cancer • Myocardial infarction • Progestins • Pulmonary embolism • Vascular endothelial growth factor A • Inflammation • Endothelial cells • Stroke • Heart failure • Angiogenesis inhibitor endothelins • Endothelium protein kinase • Drug safety • Sex chromosomes • Progestins • Steroid hormones • Steroids • Personalized medicine

1. Introduction

Vascular endothelial cells (ECs) form the thin layer of tissue covering the inner lining of blood vessels where they provide an anti-thrombogenic surface preventing inappropriate blood clotting.[1] ECs also maintain vascular tone and structure of the underlying vascular smooth muscle in the tunica media by releasing signalling molecules, such as nitric oxide (NO) and prostacyclin, as well as growth factors and pro-inflammatory molecules, such as angiotensin II (Ang II) and endothelin-1 (ET-1).[1] EC dysfunction resulting from vascular injury caused by chronic diseases, such as arterial hypertension and diabetes, and/or by physiological changes such as menopause and aging,[2,3] alters the protective functions of the endothelium.[4] These alterations promote inflammation, vasoconstriction, and cell proliferation, thus establishing a disease-prone prothrombotic environment.[1] EC injury initiates and propagates vascular smooth muscle cell proliferation, inflammation and formation of atherosclerotic plaques, which, through destabilization, are the main cause of acute coronary syndromes/myocardial infarction, ischaemic stroke, and acute aortic syndromes.[5,6] A specialized endothelium regulates the exchange of small molecules in and out of circulation in the blood-brain barrier and in renal glomeruli;[7] in the lymphatic system, ECs regulate interstitial fluid removal from tissues and transport of lymphocytes and antigen-presenting cells to lymph nodes.[8]

Angiogenesis is a physiological process that occurs during normal development, growth, and wound healing.[9,10] It involves sprouting of ECs into new capillaries from the pre-existing vessels formed in the earlier stage of vasculogenesis, and is essential for development of a normal embryo and for repair of damaged tissues.[11] In mammals and humans, vascular endothelial growth factor (VEGF) is a 45 kD peptide occurring in 4 main isoforms VEGF-A, VEGF-B, VEGF-C, and VEGF-D,[12] which bind to and activate VEGF receptors (VEGFRs), such as Flt1 (VEGFR-1),[13] Flk-1/KDR (VEGFR-2), and Flt-4 (VEGFR-3) respectively.[14] VEGFRs occur as membrane-bound or soluble forms, and have distinct binding profiles.[14] VEGFRs play key roles in EC function and angiogenesis, affecting EC proliferation, migration and survival, and contribute to tube formation and vascular permeability.[12,15] In contrast to other VEGFs, VEGF-B is predominantly expressed in the myocardium and has only weak angiogenic effects. Accordingly, cardio-selective VEGF-B gene therapy using an inducible *VEGF-B* transgene encapsidated into adeno-associated virus-based vectors improved cardiac function in a preclinical model of heart failure.[16,17]

Pathological angiogenesis is a feature of many diseases. In particular, it has been implicated in cancer growth owing to tumour's ability to release chemical signals that initiate mitogenic and antiapoptotic signalling pathways,[18] and to facilitate metastatic spread of cancer cells through haematogenic and lymphogenic diffusion. In addition, an interplay between factors causing abnormalities of angiogenesis in various tissues contributes to the development of chronic vascular complications in diabetes.[19] In diabetes mellitus, excessive neovascularization in the eye is a main cause of blindness.[20] Several vasoactive and growth-promoting factors, including VEGF, ET-1,[21] and various other tissue-specific factors, such as those found in endocrine glands,[22] are involved in both repair processes and pathogenesis (e.g. inflammation and tumour growth). Hence, vasoactive pro-angiogenic growth factors and their receptors represent current and potential future targets for drug development and new therapies.

2. Endothelial pathophysiology: inflammation and beyond

Compelling evidence exists that oxidative stress, inflammation, hypoxia and angiogenesis are involved in a wide range of processes, from wound healing to cancer progression. Thus, peripheral immune tolerance and angiogenesis programmes are closely connected and cooperate to sustain tumour growth. The mechanisms underlying inflammation and repair show considerable overlap: both entail a coordinated series of events comprising cell growth and migration, angiogenesis, proliferation of connective tissue cells, synthesis of extracellular matrix, and finally remodelling—all coordinated in a tissue-specific fashion by cytokines, vasoactive and growth factors.[22,23] For instance, prostaglandin E_2 (PGE_2), which is now considered the main oncogenic prostanoid,[24] facilitates tumour initiation, progression and metastasis through cell proliferation and angiogenesis.[25] The latter involves epidermal growth factor receptor transactivation by PGE_2. In contrast, PGD_2, acting on the DP_1 receptor, may reduce angiogenesis and thereby tumour progression.[26] Interleukin 8, mainly produced by macrophages and ECs, is a major mediator of angiogenesis.[27] Toll-like receptor-2, a sensor of oxidation-associated molecular patterns, provides a key link connecting oxidative stress, inflammation, innate immunity, and angiogenesis.[28] Tumour hypoxia promotes recruitment of regulatory T cells by inducing expression of the chemokine CCL28, which in turn promotes immune tolerance and angiogenesis.[29]

In the last decade, intensive research focused on the role of innate and adaptive immunity in hypertension-mediated organ damage (HMOD), which has opened a new field of investigation.[30,31] There is now evidence to suggest that immune cells accumulate in blood vessels and communicate with ECs and the surrounding vessel wall through the production of cytokines, matrix metalloproteinases, and reactive oxygen species, the latter of which also reduce NO bioactivity.[31] Mediators released from multiple subsets of T cells, macrophages, and dendritic cells trigger inflammation in several organs, thereby promoting arterial hypertension and HMOD.[30–33] For instance, the angiogenic placental growth factor (PlGF), which activates VEGFR-1 (Flt-1),[13] is involved in T cell activation and infiltration in target organs including the kidneys and arterial wall, contributing to HMOD, renal failure, and atherosclerosis.[34–36] Thus, the innate and adaptive immune responses propagate the development of arterial hypertension and the resulting HMOD.

3. Current clinical applications of anti-angiogenic therapies

3.1 Age-dependent macular degeneration

Drugs interfering with angiogenesis have become a main pillar of the treatment of diabetic retinopathy and malignancies, and have been approved for clinical use (*Table 1*).[37] With regard to eye disease, aflibercept, a fusion protein combining the Fc portion of human IgG with the ligand binding domains of the VEGFR-1 and VEGFR-2 receptors, is licenced for intravitreal application to treat wet age-related macular degeneration (AMD), macular oedema, and choroidal neovascularization.[38] Aflibercept can also be administered intravenously in combination with other drugs in patients with metastatic colorectal cancer resistant to other drugs.[39] Ranibizumab is a VEGF-A-inhibiting biopharmaceutical that reduces angiogenesis, and hence is applied to treat excessive neovascularization in patients with wet AMD. Pegaptanib is a pegylated modified oligonucleotide that binds with high specificity and affinity to and inhibits the activity of $VEGF_{165}$, a soluble isoform that is mainly involved in pathological ocular neovascularization.[40] After being approved for the treatment of wet AMD, this drug has now been withdrawn in the European Union at the request of the marketing authorization holder because of a high rate of intraocular adverse events, in particular endophthalmitis.[41]

3.2 Cancer

Inhibition of angiogenesis represents a milestone among novel anti-cancer treatment strategies that have prolonged overall survival and progression-free survival.[42] This did not come unexpectedly as local blood supply resulting from tumour-driven angiogenesis is instrumental for both cancer cell proliferation and haematogenic and lymphogenic spread during metastasis.[43] Accordingly, anti-angiogenic drugs targeting angiogenic factors including VEGF-A, VEGF-B, fibroblast growth factor (FGF), transforming growth factor-β (TGF-β), and platelet-derived growth factor (PDGF) have become useful therapeutic weapons against certain cancers. Multiple preclinical and clinical studies have demonstrated that the judicious use of anti-angiogenic drugs can improve oxygenation and drug delivery in tumours, thus lowering their resistance to treatment (chemo-, radio- or immunotherapy).[44,45] Several new anti-angiogenic compounds and biopharmaceuticals are under active investigation for other forms of the neoplastic disease.[46]

VEGF is a key driver of EC proliferation and angiogenesis,[47] which explains why inhibitors of VEGF signalling comprise an important class of antitumor agents (*Table 1*).[48] Several orally active non-peptide small molecules that inhibit the protein tyrosine kinase function of VEGFRs (e.g. pazopanib, sorafenib, sunitinib, and axitinib), as well as monoclonal antibodies that target the VEGF receptor (e.g. ramucirumab), have been approved for clinical use.[49]

Sorafenib inhibits multiple receptor tyrosine kinases, including raf kinase, VEGFR-2, VEGFR-3, and PDGF receptor β (PDGFR β), and thereby interferes with angiogenesis, tumour invasion, and metastasis. Its current clinical applications include renal cell carcinoma and hepatocellular cancer.[50]

Sunitinib inhibits multiple receptor tyrosine kinases implicated in tumour growth, neoangiogenesis, and metastatic spread, including all VEGFRs.[51] Sunitinib is indicated for the treatment of unresectable and/or metastatic malignant gastrointestinal stromal tumours, advanced/metastatic renal cell carcinoma, as well as for pancreatic neuroendocrine tumours with disease progression in adult patients.[52] However, the

Table 1 Molecular targets, indications, and safety concerns of angiogenesis inhibitors approved for clinical use in the European Union and several non-EU European countries

Active substance	Target/mechanism	Current use/indications	Safety concerns and pitfalls
Aflibercept	Attaches to and inhibits VEGF and PlGF	AMD, impaired vision (intravitreal) Metastatic colorectal cancer (infusion)	Thrombocytopenia; arterial hypertension; venous thrombosis; pulmonary embolism; haemorrhage; GI perforation; fistula formation; proteinuria; neutropenia and neutropenic complications
Axitinib	Inhibitor of tyrosine kinases found in VEGF receptors	Advanced renal cell carcinoma	Arterial hypertension; bleeding; diarrhoea; fatigue; dysphonia; hypothyroidism; cough; constipation. A risk management plan including risk minimization measures for arterial and venous embolic and thrombotic events, haemorrhage and congestive heart failure/ cardiomyopathy has been included in the summary of product characteristics
Bevacizumab	Monoclonal antibody designed to recognize, attach to and inhibit VEGF	Metastatic colorectal cancer; metastatic breast cancer; non-small cell lung cancer; renal cell carcinoma; cancer of the ovary; cancer of the cervix	Arterial hypertension; bleeding; arterial thromboembolism; asthenia; diarrhoea; abdominal pain; GI perforation
Cabozantinib	Inhibitor of tyrosine kinases found in VEGF, MET (MET receptor tyrosine kinase (RTK) and its ligand hepatocyte growth factor or HGF, also known as scatter factor or SF), and glial cell-line derived neurotrophic factor receptor (RET)	Medullary thyroid cancer	Arterial hypertension, venous thrombosis, pulmonary embolism; diarrhoea; palmar-plantar erythrodysaesthesia syndrome; taste disturbances; increased liver enzymes; lymphopenia or neutropenia. A risk management plan including risk minimization measures for thromboembolic events and haemorrhages has been included in the summary of product characteristics
Lenvatinib	Inhibitor of tyrosine kinases found in VEGF, fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), stem cell factor receptor (KIT), and glial cell-line derived neurotrophic factor (RET) receptors	Advanced renal cell carcinoma; differentiated thyroid carcinoma	Arterial hypertension; peripheral oedema (swelling, especially of the ankles and feet); heart failure; diarrhoea; nausea; proteinuria; stomatitis; palmar-plantar erythrodysaesthesia syndrome; impairment of renal function / kidney failure; posterior reversible encephalopathy syndrome
Nintedanib	Inhibitor of tyrosine kinases found in VEGF, FGF, and PDGF receptors	Non-small cell lung adenocarcinoma	Diarrhoea; vomiting; increased liver enzymes; not to be used in patients who are hypersensitive to peanut or soya. A detailed risk management plan including risk minimization measures for venous thromboembolism, bleeding, and hypertension has been included in the summary of product characteristics.
Pazopanib	Inhibitor of protein kinases found in VEGF, PDGF, and KIT receptors	Advanced renal-cell carcinoma; certain forms of soft-tissue sarcoma	Arterial hypertension; dysgeusia; diarrhoea; nausea; skin hypopigmentation; exfoliative rash, headache, stomatitis; increased liver enzymes
Ponatinib	Inhibitor of the tyrosine kinase Bcr-Abl found on the surface of leukaemia cells	Chronic myeloid leukaemia and acute lymphoblastic leukaemia in patients who are Philadelphia-chromosome positive (Ph+).	Myocardial injury, myocardial infarction; atrial fibrillation; peripheral arterial occlusive disease; anaemia; angina pectoris; decreased platelet counts; arterial hypertension; coronary artery disease; heart failure; pneumonia; pancreatitis; fever; abdominal pain; acute kidney injury; urinary tract infection. Arterial occlusive adverse events reported in 25% of patients, with serious adverse events occurring in 20% of patients. Serious venous occlusive adverse events reported in

Continued

Table 1 *Continued*

Active substance	Target/mechanism	Current use/indications	Safety concerns and pitfalls
			5% of patients. Venous thromboembolic reactions reported in 6% of patients.
Ramucirumab	Monoclonal antibody to VEGF receptor 2 (VEGFR2)	Gastric cancer; metastatic colorectal cancer; non-small cell lung cancer with mutated EGFR; hepatocellular carcinoma	Peripheral oedema; arterial hypertension; thrombocytopenia; arterial thromboembolic events; diarrhoea, abdominal pain, headache, proteinuria; GI perforation; severe gastrointestinal haemorrhage; not be used when lung cancer is close to a major blood vessel
Ranibizumab	Blocks VEGF-A	'Wet' form of age-related macular degeneration; macular oedema; proliferative diabetic retinopathy; sight problems associated with choroidal neovascularization; retinopathy of prematurity	Multiple ocular side effects; headache; arthralgia; nasopharyngitis
Sorafenib	Multikinase inhibitor of CRAF (RAF proto-oncogene serine/threonine-protein kinase, also known as proto-oncogene c-RAF), VEGFR-2, VEGFR-3, and PDGFR-β expressed in tumour vasculature	Hepatocellular carcinoma; advanced renal cell carcinoma; differentiated thyroid carcinoma	Myocardial infarction or ischaemia; bleeding; arterial hypertension or hypertensive crisis; diarrhoea; rash; infection; hand foot skin reaction; gastrointestinal perforation; drug-induced hepatitis
Sunitinib	Inhibitor of tyrosine kinases found in platelet-derived growth factor receptors (PDGFRα and PDGFRβ), VEGF receptors (VEGFR1, VEGFR2, and VEGFR3), stem cell factor receptor (KIT), Fms-like tyrosine kinase-3 (FLT3), colony stimulating factor receptor (CSF-1R), and the glial cell-line derived neurotrophic factor receptor (RET)	Gastrointestinal stromal tumour; metastatic renal cell carcinoma; pancreatic neuroendocrine tumours	Arterial hypertension; neutropenia; thrombocytopenia; anaemia; leucopoenia; heart and kidney failure; venous thrombosis; pulmonary embolism; internal haemorrhages; gastrointestinal disorders and perforation; shortness of breath and cough; skin discoloration, dryness of the skin and rash; dysgeusia; palmar-plantar erythrodysaesthesia syndrome; hypothyroidism; insomnia. A detailed risk management plan including risk minimization measures for torsade de points, left ventricular dysfunction/heart failure, pericardial events and cardiac ischaemic events has been included in the summary of product characteristics.
Tivozanib	Selective blocker of VEGF-ligand-induced phosphorylation of all VEGF receptors 1, 2, and 3	Advanced renal cell carcinoma	Arterial hypertension (which occurs in almost half of patients); voice changes, tiredness and diarrhoea; avoid St John's wort during treatment due to boosted tivozanib clearance and very serious risk of therapeutic failure
Vandetanib	Inhibitor of tyrosine kinases found in VEGF, EGF, and glial cell-line derived neurotrophic factor (RET) receptors	Medullary thyroid cancer	Arterial hypertension; pro-arrhythmic effects (QTc interval prolongation); diarrhoea, rash, nausea; headache

widespread use of such multitargeted agents has raised concerns about their cardiovascular safety,[53] which will be discussed below.

Ramucirumab is a fully human IgG1 mAb targeting specifically the extracellular domain of VEGFR-2 that has been approved for advanced gastric cancer or gastro-esophageal junction adenocarcinoma after prior fluoropyrimidine- or platinum-containing chemotherapy.[54] This drug is now also approved for the treatment of metastatic colorectal cancer, locally advanced or metastatic non-small cell lung cancer, and advanced or unresectable hepatocellular carcinoma.[55]

Bevacizumab, a monoclonal antibody that inhibits angiogenesis/neovascularization by neutralizing VEGF, is currently used as adjunct treatment for metastatic colorectal cancer, lung cancer and renal cell carcinoma, as well as for the treatment of AMD.[56] Bevacizumab is administered by intravenous infusion at a dose range of 5–15 mg/Kg every 2–3 weeks, depending on the therapeutic indication. Unfortunately, the clinical efficacy of bevacizumab is hampered by its nephrotoxic effects that can cause acute and chronic renal failure, overt nephrotic syndrome and also severe and/or drug-resistant hypertension, all of which are

reversible upon discontinuation of the drug. Indications, side effects, and toxicity of the above clinically approved angiogenesis inhibitors are summarized in *Table 1*.

4. Potential off-label use of licenced drugs with anti-angiogenic activity

Several drugs licenced for non-cancer indications display anti-angiogenic potential, which suggests the possibility for an off-label use. The oral hypoglycaemic agent metformin enhances bevacizumab activity in highly glycolytic tumours via inactivation of the liver kinase B1/AMP-activated protein kinase pathway.[57] Metformin also inhibits the mTORC1 pathway, which plays a pivotal role in metabolism, growth and proliferation of cancer cells.[58] Metformin also suppresses PCSK9 and the glucose sensor carbohydrate-responsive element-binding protein, which reduces intracellular glucose and glucose metabolite levels.[59] Notably, metformin down-regulates the expression of microRNAs such as miR-34a, modulates gene expression of *SIRT1* and its protein product, sirtuin1, and attenuates hyperglycemia-induced impaired angiogenesis and diabetes-associated EC dysfunction.[60]

Cardiac glycosides, such as ouabain, digitoxin and digoxin, inhibit Na^+/K^+-ATPase and are currently used to slow ventricular rate in rapid persistent atrial fibrillation.[61] These agents also attenuate angiogenesis *in vitro* and *in vivo*.[62–64] It has been previously reported that digitoxin, at concentrations in the therapeutic range, inhibits angiogenesis and focal adhesion kinase (FAK) activation induced by several pro-angiogenic stimuli.[62,63] Treatment with ouabain and digoxin inhibits expression of CD31 in tumour tissues more effectively than sorafenib.[65] While these findings suggest the possibility of repositioning of Na^+/K^+-ATPase inhibitors as broad-spectrum anti-angiogenic agents,[66] *ad hoc*-designed randomized clinical trials would be needed before these drugs can be considered further for cancer treatment.

In patients with coronary artery and peripheral vascular disease, atherosclerotic plaque neovascularization contributes to inflammation and lesion instability, thus augmenting the risk of intraplaque haemorrhage, rupture and dissection, which are well-known determinants of acute coronary and cerebrovascular events.[67] Newly formed ECs following ischaemic events are structurally and functionally abnormal, in that they release more proliferative and vasoconstrictor factors and adhesion molecules, thus increasing cardiovascular risk.[68] Adventitial angiogenesis precedes intraplaque neovascularization and is the principal source of plaque microvessels.[69] Notably, treatment with the HMG-CoA reductase inhibitor rosuvastatin was reported to reduce adventitial and plaque neovascularization in patients with asymptomatic carotid atherosclerosis by about 30% after three months,[70] without changing LDL cholesterol and high-sensitivity C-reactive protein levels, plaque burden, or composition of the lipid-rich necrotic core, suggesting a possible anti-angiogenic action. Thus, statins that are being used for primary and secondary cardiovascular prevention in hypercholestolemic patients undergoing anticancer treatments[71,72] can counteract endothelial dysfunction induced by anti-VEGF drugs. Potential therapeutic effects of statins in cancer patients are also suggested by *in vitro* and *in vivo* studies demonstrating inhibition of colon or rectal cancer cell growth and proliferation.[73–75] According to a recent meta-analysis of 14 clinical trials involving 130 994 colon or rectal cancer patients, statin use before or after diagnosis was associated with a reduced all-cause and cancer-related mortality.[76] However, as meta-analyses have well-known limitations, properly designed randomized clinical studies are necessary to determine if statin treatment reduces angiogenesis and decreases mortality in cancer patients.

Finally, anti-angiogenic properties have been suggested for thalidomide, a sedative and teratogenic agent that was responsible of the so-called 'Contergan outbreak' in the late 1950s and early 1960s and later approved for the treatment of multiple myeloma owing to its anti-inflammatory and immunomodulatory effects.[77,78] In fact, inhibition of embryonic and foetal blood vessel formation is thought to be responsible for the major foetal abnormalities induced by thalidomide probably downstream celebron signalling, a protein that is part of an E3 ubiquitin ligase complex, which is involved in regulating protein degradation.[79]

Taken together, the above studies have revealed the complex effects of multiple drugs on angiogenesis. Whether such pharmacological effects have the potential to affect or improve clinical outcomes in cancer or eye diseases remains to be determined.

5. Toxicity issues of angiogenesis inhibitors

Currently approved angiogenesis inhibitors are intended to limit angiogenesis or prune abnormal vessels, thus reducing tumour growth and hypoxia; yet, one of their main limitations is that they also target healthy cells.[53] Intracellular signalling pathways of VEGF-A signalling via VEGFR-2 result in increased production of both PGI_2 and NO, which mediate enhanced vascular permeability, vasodilatation and improved EC survival.[53,80] Thus, disruption of physiological functions and endothelial homeostasis in the cardiovascular and renal systems, as well as interference with wound healing and tissue repair, are the main causes of side effects related to anti-angiogenic therapy.[81] Drug toxicity-associated side effects of VEGF inhibition by bevacizumab include not only arterial hypertension and nephrotoxicity,[80–83] but also thromboembolic events (transient ischaemic attack, stroke, venous thrombosis, pulmonary embolism, acute coronary syndromes, and myocardial infarction).[80] Although anti-VEGF therapies showed some promise in early trials and are now widely used in a variety of diseases, including cancer, the cardiovascular side effects associated with these treatments remain a concern.[84] The adverse effects of angiogenesis inhibitors may include off-target effects that are unrelated to angiogenesis pathways and may contribute to cardiovascular toxicity, including QT-prolongation and ventricular tachycardia or myocardial injury.[53,82–84] Interfering with the protective functions of ECs by different approaches targeting VEGF results in a similar spectrum of adverse effects (*Table 1*).[84] These untoward effects can become more relevant in the context of combination therapy using anti-angiogenic agents and immune checkpoint inhibitors approved for the treatment of some solid tumours.[85] Recent data for the latter agents indicate acceleration of atherosclerosis progression and coronary artery disease.[86,87]

Endothelin ET_A receptors have been implicated in angiogenesis in response to chronic ischaemia.[21,88] In the lung, activation of ET_A receptors by its predominant ligand ET-1 has been recently identified to contribute to the arterial hypertension associated with VEGF inhibition; this effect can be attributed to lack of the VEGF-dependent tonic inhibition of ET-1 release by NO, and to overproduction of ET-1 with ensuing activation of ET_A receptors.[89] Anti-angiogenesis induced kidney injury is also endothelin-dependent: treatment with selective ET_A and non-selective $ET_{A/B}$ receptor antagonists in rodents largely prevents sunitinib-induced arterial hypertension. However, sunitinib-induced albuminuria was prevented solely by the selective ET_A antagonist sitaxentan,[90] confirming

that angiogenesis inhibition-related renal injury is predominantly ET_A-receptor mediated.[21]

About 4% of patients on anti-angiogenic drugs, such as bevacizumab, may suffer toxicity-related side effects related to arterial thromboembolism.[83] Other toxicity-related side effects of bevacizumab may include congestive heart failure, wound healing complications, gastrointestinal perforations, and overt proteinuria due to its nephrotoxicity (*Table 1*).[91,92] Importantly, bevacizumab should be started only if wound healing is no longer an issue and therefore not before the patients have fully recovered from surgical procedures; [93] moreover, patients on bevacizumab should be closely monitored for potential haemorrhage, gastrointestinal perforations, renal function, and wound healing problems.[93,94] *Table 1* summarizes the clinically approved inhibitors of vascular signalling pathway and their main side effects as well as safety concerns.

6. Side effects of anti-angiogenic therapy in diabetes and eye diseases

Side effects of anti-angiogenic therapies often occur in the presence of diabetic retinopathy or diabetic macular oedema. Patients with diabetes experience an 'angiogenic paradox', i.e. defective formation of new blood vessels in the coronary and peripheral circulation, alongside an exaggerated angiogenesis in the retina.[19] This, albeit yet mechanistically unexplained, has clear therapeutic drawbacks as approaches aimed at stimulating collateral blood flow supply in patients with myocardial or peripheral ischaemia face the risk of inducing angiogenesis in the retina.[95] On the other hand, anti-angiogenic therapies targeting the proliferative stages of diabetic retinopathy carry the risk of aggravating myocardial or peripheral ischaemia owing to systemic spillover of locally administered anti-angiogenic drugs.[96] The same applies to anti-VEGF therapies used to reduce macular oedema in diabetic or aged patients,[97] which raises further safety concerns in such patients, as the presence of ocular complications is by itself a strong predictor of future adverse cardiovascular events such as myocardial infarction.[98,99] However, studies including meta-analyses on whether intravitreal administration of anti-VEGF agents increases the risk of adverse cardiovascular events, including atherothrombosis, venous thrombosis, and non-ocular haemorrhage,[100] were inconclusive.[101–104]

In addition, intravitreal anti-VEGF drugs, like bevacizumab, can reduce systemic levels of pigment epithelium-derived factor,[105] possibly contributing to the impaired regulation of vascular responses. The systemic consequences of such untoward effects of anti-VEGF drugs should be viewed with caution, especially in patients with diabetes, who present a complex derangement in VEGF signalling, including VEGF resistance, because of the hyperglycaemic chronic damage in multiple vascular beds, which implies a high prevalence and incidence of cardiovascular complications.[19]

7. Sex differences in the outcomes of anti-angiogenic cancer treatments

An emerging aspect with far-reaching implications is the sex difference of responses to anti-angiogenic drugs and related general and cardiovascular toxicities (*Table 1*). Of note, bevacizumab displayed longer overall survival in female than in male patients with non-small-cell-lung cancer and showed a sex- and dose-dependent progression-free survival.[106,107] The mechanism underlying such differences are currently unknown, but likely involve endogenous oestrogens and oestrogen receptors (ERs) and/or effects of androgens or its receptor.[106,107] It has been recently shown that in cancer tissue several genes involved in drug metabolism, including specific glutathione S-transferase and cytochrome P_{450} isoforms, are affected more strongly by oestrogen-responsive transcription factors in female than in male patients.[108] This important observation, however, would be consistent with sex-specific responses to small-molecule agent-based chemotherapy but not to biopharmaceuticals such as bevacizumab, which are cleared through different mechanisms.[109] Therefore, the mechanisms underlying sex-specific differences in bevacizumab efficacy and side effects remain to be determined. Along the same lines, a retrospective study revealed that with sunitinib treatment female renal cell carcinoma patients exhibit more toxicities in multiple organ systems compared to men.[110]

7.1 Potential mechanisms underlying sex differences of anti-angiogenic therapy

A sexual dimorphism of sunitinib cardiotoxicity has also been demonstrated in isolated adult cardiomyocytes *in vitro*.[111] In the same study, sunitinib administration reduced left ventricular (LV) function and increased myocardial fibrosis and LV dilation in female mice only. Notably, sunitinib treatment increases *Mdr1* and *Cyp1A1* expression to a greater degree in males than in females.[111] As these genes are primarily involved in sunitinib disposal, these findings suggest that biological sex and sex hormones are major determinants of the pharmacokinetics and efficacy of tyrosine kinase inhibitors. Systems biology allows for the discovery of sex-specific molecular targets and biomarkers, and thereby may help to improve anti-angiogenic therapy.[112] Computational models suggest that systemic and tumour VEGF levels strongly depend on VEGFR expression, and tumours highly expressing plasma membrane VEGFR-1 could become resistant to anti-VEGF drugs, such as bevacizumab.[112] The tumour expression of VEGF-D shown on immunohistochemistry is a potential predictive biomarker of bevacizumab efficacy showing greater benefit in patients with low expression of VEGF-D.[113] Whether such biomarkers follow sex-specific patterns is another issue for future research, and possibly will allow to develop sex-specific personalized medicine.

8. Sex hormones and their receptors as novel determinants of angiogenesis

Arterial blood pressure values and arterial hypertension prevalence show distinct sex differences, being lower in women than in men before menopause, and exhibiting opposite trends when oestrogen production ceases.[114] Sex steroid hormones, such as oestrogens, progestins, and androgens, are critical regulators of vascular homeostasis, EC function, and cell growth (*Figure 1*). Men are at a higher risk to develop coronary artery disease and arterial hypertension than women.[115] In women, the risk for arterial hypertension, cardiovascular, cerebrovascular, and renal disease increases after natural or surgical menopause, which can be partly alleviated by hormone therapy.[114,115] In vascular disease processes that critically involve EC injury as the initiating step, such as atherosclerosis,[116,117] recent studies have highlighted the importance of X chromosome effects independent of ovarian sex hormones.[118–120] The role of sex chromosomes in physiological and pathological angiogenesis has

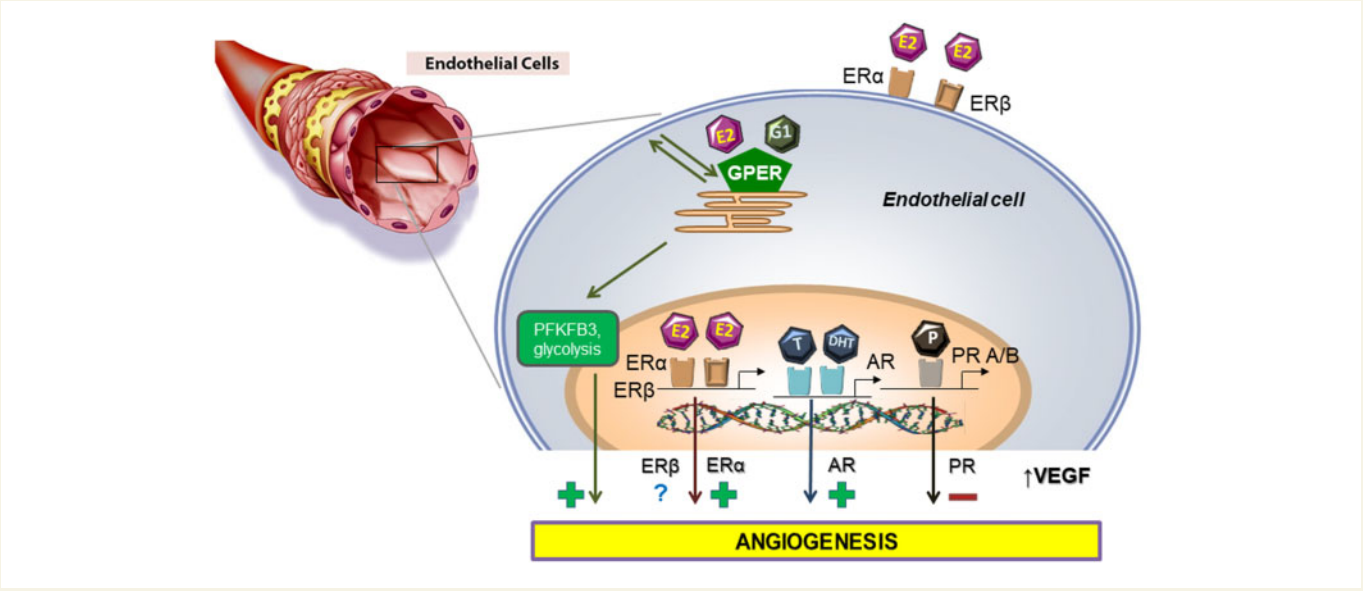


Figure 1 Sex steroids as modulators of angiogenesis. Sex steroids induce their biological effects through nuclear and membrane receptors. Membrane localizations of sex steroid receptors mediate rapid, non-genomic effects (which may also trigger sustained cellular responses), while nuclear receptors mediate sustained transcriptional (genomic) activities. Upon binding of their physiological ligands, i.e. testosterone (T), dihydrotestosterone (DHT), progesterone (P), and 17β-oestradiol (E_2), respectively, nuclear steroid receptors AR, PRs A and B, and ERs α and β translocate to the nucleus where they modulate transcription of target genes. The biological effects of E_2 are also mediated by membrane isoforms of ERα and ERβ as well as the G-protein-coupled oestrogen receptor GPER, an intracellular, yet membrane-bound transmembrane receptor located at the endoplasmic reticulum, which may shuttle to the cell membrane and back. Sex steroid hormones are critical regulators of vascular homeostasis, EC function and growth. AR activation transcriptionally regulates VEGF expression. Progesterone inhibits migration of vascular ECs *in vitro*, an effect that is mediated by PR signalling, but enhances the angiogenic potential of endothelial progenitor cells (not shown). Several mechanisms downstream ER activation mediate E_2-mediated angiogenesis. E_2 increases VEGF transcription largely via ERα. GPER activation by E_2 or the pharmacological agonist G-1 enhances angiogenesis via non-nuclear mechanisms involving up-regulation of the glycolytic activator PFKFB3.

hardly been studied. VEGF-D has been linked to the X chromosome,[121] while X chromosome-linked angiogenesis and VEGF expression[122,123] as well as inhibition of angiogenesis have been described.[124] Although the role of sex chromosomes defining health and disease in men and women is increasingly being recognized as recently discussed by the *ESC Working Group on Cellular Biology of the Heart*,[125] it is still unclear whether and how the X chromosome or the Y chromosome affect the outcome of anti-angiogenic treatments or how they contribute to adverse effects like hypertension. Similarly, sex-stratified gene regulatory networks are likely to play a role in the sex-specific therapeutic efficacy, safety profile and adverse effects of angiogenesis inhibitors.[126] *Figure 1* summarizes the main aspects of sex steroid hormone signalling and angiogenesis.

8.1 Receptors for androgens and progesterone

Androgen- and progesterone-mediated pathways contribute to angiogenesis, particularly in the context of disease, such as prostate and breast cancer, respectively.[127,128] Androgen receptor (AR) activation transcriptionally regulates VEGF expression.[129] Inhibiting VEGF-dependent angiogenesis by targeting androgen synthesis or AR signalling blocks two critical pathways in prostate cancer progression.[130] In addition, dual targeting of hypoxia-inducible factor 1α (HIF-1α) and AR pathways by HIF-1α inhibitors and enzalutamide, a second-generation AR antagonist, decreases VEGF-A levels and inhibits the growth of castration-resistant prostate cancer cell lines.[131] Progesterone and progesterone receptor (PR) are also involved in different aspects of angiogenesis in multiple tissues, including endometrial proliferation and tumour growth.[132] Progesterone at nanomolar concentrations inhibits migration of vascular ECs *in vitro*, an effect that is mediated by PR signalling.[133] However, progesterone increases VEGF secretion and the angiogenic potential of endothelial progenitor cells, resulting in neuroprotection following traumatic brain injury in rodents *in vivo*; these effects are blocked by ulipristal acetate treatment, suggesting a role for PR signalling in VEGF secretion and angiogenesis.[134]

8.2 The oestrogen receptors ERα and ERβ

Classically, oestrogen's signalling was held to occur through nuclear or membrane-bound ERs that are important for development, growth, and reproduction. ER signalling is also important in mammalian systems other than the reproductive organs.[135] ERs are involved in neurological development and metabolism, and contribute to protection against cardiovascular disease and osteoporosis.[135,136] The ER subtypes ERα and ERβ function as nuclear transcription factors interacting with a variety of co-regulators initiating target gene transcription, while cell membrane subpopulations of ERα and ERβ mediate non-genomic (rapid) signalling, or indirectly modulate genomic responses.[137] Sex-hormone receptors have both nuclear and cytoplasmic functions (*Figure 1*). In the case of oestrogen, ligand-bound receptors activate ERK, PI3K, and STAT signalling. These are the same pathways that are activated by growth factors, such as EGF and PDGF.[138,139] Hyperactivation of growth factor signalling can attenuate the responses to oestrogen signalling.[140] Oestrogen causes

several different cellular responses depending on the target cell.[135,136] While the presence or absence of ERs was held to account for this difference, the recent discovery of oestrogen-responsive microRNAs have been shown to shape and fine-tune oestrogen signalling. For instance, micro-RNA 375 regulates ERα by down-regulating RASD1, a known regulator of ERα expression in breast cancer cells.[141] ERα is an important therapeutic target in female reproductive cancers.[142] In breast cancer, oestrogens contribute to tumorigenesis by directly stimulating cancer cell growth and by promoting EC proliferation and thereby angiogenesis.[143,144] Oestrogen-dependent pathological capillary growth in breast cancer was found to correlate with nuclear ERα expression.[145,146] Accordingly, higher lymphangiogenesis and angiogenesis rates were observed in lung adenocarcinoma from young female than from male patients.[147] In preclinical models, the microenvironment in females sustains the lymphatic and vascular components of lung tumour development more efficiently than in males through ERα-dependent pathways; accordingly, treatment with an ERα antagonist or tamoxifen decreases lung tumour growth and lymph/angiogenesis in female but not male mice.[147]

8.3 G-protein-coupled oestrogen receptor

The G-protein-coupled oestrogen receptor (GPER) is a 7-transmembrane domain receptor that mediates both acute and chronic effects of oestrogens, and is activated by 17β-oestradiol (E_2), ER antagonists, phytoestrogens, and environmental pollutants.[148,149] In the adrenal cortex, GPER is involved in the regulation of aldosterone synthase and heterodimerizes with the angiotensin AT_1 receptor.[150] While tonically inhibiting aldosterone synthesis via ERβ, E_2 enhances aldosterone production in a GPER-dependent fashion in the human adrenal cortex.[151] This suggests a role for the latter receptor in raising arterial pressure.

GPER activation following treatment with E_2 can inhibit VEGF expression and angiogenesis in triple-negative breast cancer cells.[152] Conversely, activation of GPER has been shown to either stimulate[153] or inhibit[154] EC growth and angiogenesis *in vitro*. Also, available evidence points to oestrogens as key factors in promoting endothelial healing through endothelial progenitor cell mobilization, as well as angiogenesis.[155–157] The latter process also occurs physiologically in the female reproductive system.[158] In this regard, under hypoxia-reperfusion conditions resembling those seen in preeclampsia, treatment with E_2 prevents the failure of EC tube formation via GPER and downstream activation of eNOS and the Akt signalling pathway.[159] The soluble VEGFR-1 (sFlt-1) exerts anti-angiogenic effects by binding to and inhibiting the biological activity of VEGF and PlGF.[160] As a straightforward example of bench-to-bedside observations with regard to angiogenesis and cardiovascular complications, it was shown that in preeclampsia and pregnancy-associated hypertension excess placental secretion of sFlt-1 inhibits VEGF signalling in the vasculature, resulting in endothelial dysfunction.[160]

Several mechanisms downstream ER/GPER activation involved in E_2-mediated angiogenesis have been described.[161] A protective role of E_2 in ameliorating ischaemic damage has been demonstrated in myocardial ischaemia-reperfusion injury models[162,163] through mechanisms involving ERα and ERβ[164] as well as GPER.[165] EC- and vascular SMC-specific actions of E_2 and the anti-oestrogen tamoxifen have recently been shown to accelerate endothelial healing through an ERα-dependent mechanism in transgenic models. While E_2 action requires membrane-initiated ERα signalling, tamoxifen requires the presence of nuclear ERα in underlying SMC but not endothelial/haematopoietic ERα.[166] The mechanisms through which GPER contributes to angiogenesis remain to be further investigated and will also discussed below.

8.4 Drugs targeting oestrogen receptors

While E_2 acts as a non-selective ER/GPER agonist, drugs like selective oestrogen receptor modulators (SERMs) may evoke tissue-specific ER responses, inhibiting ERα and/or ERβ. However, SERMs as well as selective oestrogen receptor down-regulators (SERDs) inhibit ERα and ERβ, yet act as GPER agonists.[151,167,168] GPER binding and/or activation by diverse ER ligands including phytoestrogens (e.g. genistein), xenoestrogens (e.g. bisphenol A), and therapeutic anti-oestrogens (e.g. tamoxifen, fulvestrant, and raloxifene) has been documented.[148,149,169] Although SERMs and SERDs inhibit cell survival and proliferation through inhibition of ERα and ERβ, they simultaneously activate GPER. The stimulatory activity of tamoxifen and fulvestrant (ICI 182 780) on GPER signalling results in activation of downstream pathways including PI3K/Akt.[170] In our view, the observation that ER antagonists act as GPER agonists should require a reappraisal of the pharmacological selectivity of ER-targeting drugs and their positioning in the clinical treatment of different conditions. On the other hand, GPER may represent a new pharmacological target, and indeed a specific GPER agonist (G-1, Tespria™)[171] is currently tested in Phase 1 clinical trials for the treatment of some forms of cancer (E.R. Prossnitz, personal communication).

From what is herein summarized, the intricacies of steroid hormone biology and physiology with respect to the classical ERs and their role in a number of diseases are quite apparent. Since selective activation of GPER reduces arterial blood pressure and atherosclerosis in preclinical models,[172,173] modulating angiogenesis via this pathway using selective GPER agonists, such as Tespria™,[171] may help to reduce serious side effects, such as development of arterial hypertension in patients, possibly not only by increasing NO bioactivity but also by modulating GPER-mediated aldosterone production.[151]

8.5 Metabolic regulation in hypoxia-induced angiogenesis: a role for GPER

Abnormal metabolic regulation is a key factor contributing to arterial hypertension, particularly in the context of obesity, insulin resistance and diabetes.[174] Similarly, cancer cells can adjust their metabolism in response to anti-angiogenic treatment and may develop into a more aggressive and invasive phenotype,[175] or develop resistance to certain drugs as a result of anti-angiogenic agents-induced hypoxia.[176–178] During blockade of VEGF axis, hypoxia and infiltration of bone marrow-derived cells into tumour microenvironment are held to drive compensatory changes,[179] causing a shift to non-oxidative metabolism and accelerating the acquisition of an acid-resistant/glycolytic phenotype, which may account for the failure of anti-angiogenic therapies.[180,181] For instance, the anti-angiogenic effects of long-term sorafenib treatment involve a decrease in microvessel density augmenting tumour hypoxia, leading to HIF-mediated adaptive mechanisms to the hypoxic microenvironment that limit sorafenib efficacy.[182] Thus, targeting hypoxia-induced signalling may help overcome sorafenib resistance.[183] It should be noted, however, that not all cancer types are susceptible to anti-angiogenic treatment and that therapeutic resistance frequently occurs,[184] because tumour angiogenesis is a highly complex process involving an interplay of multiple, redundant signalling pathways. Furthermore, preclinical data

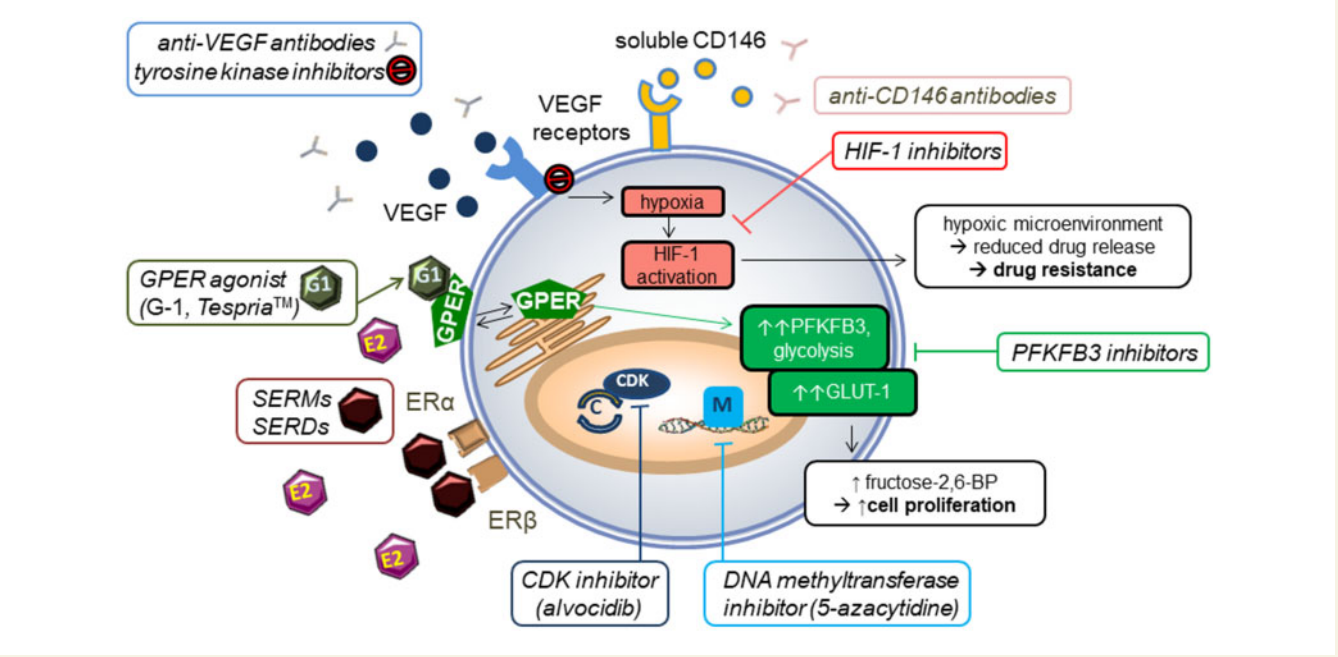


Figure 2 Current and emerging strategies of angiogenesis inhibition. Most angiogenesis inhibitors that are currently approved in cancer therapy target VEGF, its receptors or the activated signalling pathways. These medications include biopharmaceuticals—e.g. bevacizumab, aflibercept, and ramucirumab—and small-molecule tyrosine kinase inhibitors—e.g. sorafenib and sunitinib. However, the efficacy of these agents is heterogeneous across tumour types and some patients develop therapy resistance. These agents induce cardiovascular adverse events largely by blocking VEGF in the myocardium and the coronary and peripheral circulation. Sex steroid receptor pathways not only are involved in tumour growth and the development of anti-hormone resistance, but also appear to be emerging determinants of selective angiogenesis modulation, in part through metabolic regulation of glycolytic enzymes, such as PFKFB3 or glucose transporters, such as GLUT-1. SERMs may evoke tissue-specific ER responses, inhibiting ERα and/or ERβ. However, SERMs as well as SERDs, while inhibiting or degrading ERα and/or ERβ, act as agonists on GPER. Due to their roles in EC growth and function, a number of new targets have been considered to control pathological angiogenesis. These targets include CDKs, CD146, DNMT, and HIF-1α. Investigational agents targeting these pathways are at different stages of clinical drug development. Cell- and tissue-selective modulation of angiogenesis appears to be the way ahead to improve clinical outcome following treatment with angiogenesis inhibitors.

suggest that under certain conditions promoting rather than inhibiting vascular growth may eventually provide beneficial effects in terms of reduced tumour spread.[185,186] In this regard, novel agents targeting e.g. the p38 mitogen-activated protein kinase (p38 MAPK) signalling pathway may help enhance the tumour vasculature in a controlled manner and facilitate chemotherapeutic drug delivery.[187]

An emerging regulatory mechanism is suggested by the observation that angiogenic signalling pathways converge into metabolism.[188] A glycolytic cell phenotype is predominant in the tumour vasculature through up-regulation of the glycolytic enzyme activator 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3), which mediates the conversion of fructose-6-phosphate to fructose-2,6-bisphosphate, and the glucose transporter 1 (GLUT1).[189,190] Accordingly, pathological angiogenesis was found to be inhibited by blocking PFKFB3,[191] which could therefore represent a novel pharmacological target for anti-angiogenic drugs. Among other established E_2 effects on cell metabolism,[192] activation of nuclear ER signalling stimulates glucose uptake and glycolysis by inducing PFKFB3 expression and activity in ER-positive breast cancer cells, which is required for survival.[193] This might have implications for cancer treatment, as lowering glycolysis to the level detected in healthy endothelium could result in slowing down tumour EC proliferation.[194] In ECs, oestrogenic agents induce angiogenesis via GPER signalling by enhancing PFKFB3 stability.[153,195] This novel oestrogen-dependent mechanism couples to the endothelial glycolytic programme, which in turn may be operating in settings of vascular ischaemia where rapid metabolic and functional adaptation to environmental changes is required. Thus, PFKFB3 as a therapeutic target likely represents a double-edged sword, and emphasizes the need for developing pharmacological interventions that allow more specific and selective targeting in neoplastic diseases (*Figure 2*). As the side effects of current anti-angiogenic agents are substantial and these agents affect cell metabolism, it would seem, at this stage, that a better understanding of how to modulate favourably endothelial metabolism can help overcome at least in part the pitfalls of anti-angiogenic therapies.[196]

9. Angiogenesis inhibition: new approaches and targets

The pharmacological approaches to angiogenesis modulation under development along with current strategies as described in the above sections are summarized in *Figure 2*. Flavopiridol, also known as alvocidib, is an orally active small-molecule cell cycle inhibitor that targets the ATP-binding sites of cyclin-dependent protein kinases (CDKs) 2 and 4. This compound displays anti-angiogenic properties, and it is currently under

phase-2 clinical development for different forms of leukaemia and lympoma.[197,198]

The Notch signalling pathway contributes to multiple aspects of cancer biology including angiogenesis, given that the Notch target genes include VEGF.[199] Delta-like 4 (DLL4)-mediated Notch activation prevents excessive EC sprouting via down-regulation of VEGFR-2 and VEGFR-3 function.[200,201] The DLL-Notch signalling pathway has been implicated in anticancer drug resistance.[202,203] Furthermore, dysregulation of Notch signalling plays a role in vascular remodelling and mediates hypoxia-induced as well as neonatal pulmonary hypertension.[204–206]

The endothelial adhesion and signalling molecule CD146 occurs in two transmembrane isoforms and as a soluble protein.[207] Owing to its role in angiogenesis,[207] CD146 targeting may hold therapeutic potential for anti-angiogenic therapy in certain forms of cancer. The extracellular region of CD146 directly interacts with VEGFR-2, leading to activation of the p38/MAPK and FAK pathways.[208] Although antibodies targeting CD146 reduce angiogenesis in preclinical xenograft models, treatment may disrupt the physiological function of membrane CD146 and vascular integrity.[209] In contrast, antibodies targeting soluble CD146 may be beneficial in cancer and vascular disease.[210] Accordingly, the CD146-HIF-1α axis has been recently shown to play a crucial role in pulmonary vascular remodelling,[211] suggesting a further potential target for pulmonary hypertension treatment.

Dunn *et al.*[212] reported that mechanical changes disturbing normal blood flow also affect genome-wide DNA methylation patterns in arterial ECs. These investigators found that DNA methylation induced by changes in blood flow requires DNA methyltransferase (DNMT) activity, and that treatment with the DNMT inhibitor 5-azacytidine largely restores DNA methylation to normal. This suggests a mechanism linking DNA methylation changes to differential gene expression characteristic of EC dysfunction and atherosclerosis.[213] Bromodomain proteins are epigenetic readers, which bind acetylated histone residues and form complexes with transcription factors to regulate gene expression.[214] The bromodomain inhibitor JQ1 in combination with romidepsin, which is used for cutaneous and other peripheral T cell lymphomas, holds potential to reduce tumour size, cell proliferation rate, and angiogenesis.[215]

10. Angiogenesis inhibitors in clinical medicine: has efficacy met safety yet?

Biologics such as antibodies as well as protein kinase inhibitors prevent biological actions of growth factors, such as VEGF, or inhibit the downstream signalling of receptor tyrosine kinases. While these treatments are effective in different types of cancer,[216,217] overall outcomes have been rather heterogeneous. In fact, some tumours are more sensitive than others and some patients develop resistance to these therapies, suggesting that individualized treatment regimens would be required.[218] Solid tumours may use multiple vascularization mechanisms alternative to angiogenesis, such as vessel co-option, intussusception or vascular mimicry, for adequate blood supply, which has been demonstrated in many tumour types.[219] In addition, anti-VEGF agents cause cardiovascular side effects largely by blocking VEGF in the myocardium and peripheral circulation (*Figure 3*). The mechanistic and clinical aspects of hypertension and vascular disease following pharmacological VEGF inhibition have been reviewed elsewhere,[82–84,220–222] yet remain partially

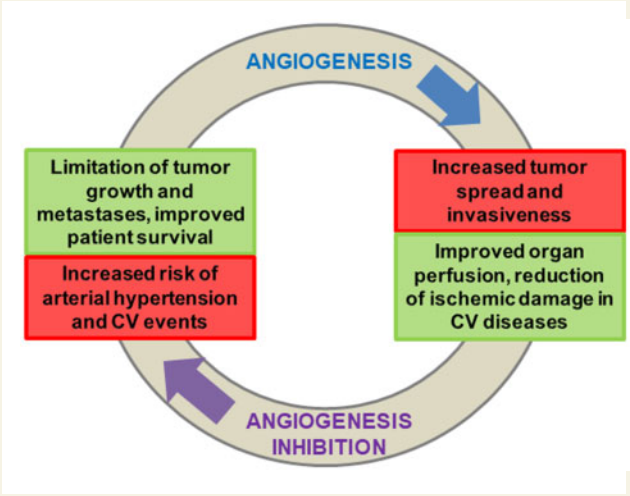


Figure 3 Efficacy and safety of angiogenesis inhibitors: a delicate balance. Angiogenesis is characterized by sprouting of ECs into new capillaries from the pre-existing vasculature, and is essential for repair of ischaemic tissues. Pathological capillary growth is associated with conditions such as cancer, diabetes, eye diseases and inflammatory diseases. Thus, while activation of angiogenesis in cancer tissue fosters tumour progression, it may contribute to ameliorating ischaemic damage in target organs, such as the heart, the kidney and the brain. Angiogenesis inhibitors are currently approved for treatment of certain forms of cancer and eye disease. However, such medications are not always specific enough to eliminate tumour vascularization, and may also damage healthy ECs in non-cancerous tissues. As a consequence, angiogenesis inhibition causes multiple cardiovascular side effects, including hypertension, thromboembolic events including pulmonary embolism, myocardial ischaemia and infarction, among others.

elusive. In clinical practice, the balance between efficacy and safety can be difficult to achieve. In fact, the therapeutic benefit on survival is outweighed by adverse effects including arterial hypertension (which is often resistant to antihypertensive treatment),[223] nephrotoxicity, myocardial dysfunction, thrombosis, pulmonary hypertension, and an increased risk of coronary and cerebrovascular events (*Figure 3* and *Table 1*). Further investigation into the diverse effects of angiogenesis inhibitors on vascular biology may help to improve the clinical application of these agents as cancer therapeutics.

For the clinical management of patients on anti-angiogenic drugs and to reduce the side effects related to their cardiovascular toxicity, combination therapies including lower doses of several VEGF inhibitors may limit compensatory up-regulation of other angiogenic pathways and may be beneficial, at least in some patients.[224] More targeted interventions on endothelial function are being investigated.[18,186,196] A recent single-cell RNA-seq analysis has highlighted the tissue specialization of ECs and their remarkable heterogeneity, including sex-specificity of ECs within organs, such as brain, heart, and lung. This specificity is preserved during development and conserved across species.[225] Such a heterogeneous and sex-dependent cellularity strongly emphasizes the need for future sex-specific precision/personalized medicine strategies targeting the vascular endothelium. In view of recent advances in understanding the complexity of angiogenesis, sex steroid receptor - which are also involved in the development of anti-hormone resistance[142,167]- and sex chromosomes appear to be emerging determinants of selective

angiogenesis modulation, possibly through metabolic regulation of glycolytic enzymes.[153,195] Since activation of GPER acutely[172] and chronically[226] reduces blood pressure, GPER ligands under clinical development might also help to reduce 'onco-hypertension',[227] the main side effect of anti-angiogenic drugs (*Table 1*).

11. Conclusions

Anti-angiogenic therapy has become a cornerstone in oncology and ophthalmology. Despite therapeutic efficacy, the side effects of these drugs can be considerable (*Table 1*). Therefore, anti-angiogenic therapy requires careful clinical monitoring and, if necessary, early additional intervention to reduce the risk of organ injury and failure.

The involvement of sex hormones and their receptors has just started to be unravelled in the progression of non-reproductive cancers, particularly by modulating angiogenesis facilitating haematogenic and lymphogenic metastasis.[228] Sex differences in the efficacy and safety of anti-angiogenic medications have become recognized only recently,[106,107] and the underlying mechanisms are still poorly understood. In addition to a potential role of sex chromosomes,[125] sex-stratified gene regulatory networks[126] and steroid hormone receptors may therefore represent novel targets for selective pharmacological modulation and personalized medicine in patients undergoing anti-angiogenic therapy. Systems biology represents a promising new way to identify sex- and sex chromosome specific and sex-hormone-dependent targets and mediators. Future research efforts should be directed towards the development of selective sex- and cell-type-specific inhibitors of angiogenesis.

Authors' contributions

Conception and design of the work: A.C. and M.B.

Drafting the work: A.C. and M.B.

Acquisition, analysis, or interpretation of data for the work: all Authors.

Revising the work critically for important intellectual content: all Authors

Agreement to be accountable for all aspects of the work: A.C. and M.B.

Final approval of the version to be published: all Authors.

Disclosures: M.B. has served on the Diabetic CKD Advisory Board and the Steering Committee of the SONAR (Study of Diabetic Nephropathy with Atrasentan) randomized clinical trial conducted by AbbVie, Inc., and has been a consultant to AbbVie, Inc. and Pharmazz, Inc. M.B. is an inventor on U.S. patents No. 10,251, 870 B2 and 10, 682, 341 B2 owned by the University of New Mexico, for the therapeutic use of GPER-selective ligands. The other authors have no disclosures to report.

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