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REVIEW

Angiogenesis inhibitors in cancer therapy: mechanistic perspective on classification and treatment rationales

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Angiogenesis, a process of new blood vessel formation, is a prerequisite for tumour growth to supply the proliferating tumour with oxygen and nutrients. The angiogenic process may contribute to tumour progression, invasion and metastasis, and is generally accepted as an indicator of tumour prognosis. Therefore, targeting tumour angiogenesis has become of high clinical relevance. The current review aimed to highlight mechanistic details of anti-angiogenic therapies and how they relate to classification and treatment rationales. Angiogenesis inhibitors are classified into either direct inhibitors that target endothelial cells in the growing vasculature or indirect inhibitors that prevent the expression or block the activity of angiogenesis inducers. The latter class extends to include targeted therapy against oncogenes, conventional chemotherapeutic agents and drugs targeting other cells of the tumour micro-environment. Angiogenesis inhibitors may be used as either monotherapy or in combination with other anticancer drugs. In this context, many preclinical and clinical studies revealed higher therapeutic effectiveness of the combined treatments compared with individual treatments. The proper understanding of synergistic treatment modalities of angiogenesis inhibitors as well as their wide range of cellular targets could provide effective tools for future therapies of many types of cancer.

Abbreviations

ANG2, angiopoietin-2; BV8, prokineticin-2; CAFs, carcinoma-associated fibroblasts; CAM, cell adhesion molecules; CSF-1, colony-stimulating factor-1; ECM, extracellular matrix; EGFR, EGF receptor; FGF, fibroblast growth factor; HIF-1α, hypoxia inducible factor-1α; HSPG, heparan sulfate proteoglycan; RTKs, receptor TKs; S1P, sphingosine-1-phosphate; TAMs, tumour-associated macrophages; TKIs, TK inhibitors

Introduction

Two major processes of blood vessel formation are implicated in the development of vascular system: vasculogenesis and angiogenesis. Vasculogenesis prevails in the embryo and refers to the formation of *de novo* blood vessels by *in situ* differentiation of the mesoderm-derived angioblasts and endothelial precursors. Angiogenesis is the formation of new capillaries from pre-existing vessels and circulating endothelial precursors (Polverini, 2002; Chung *et al.*, 2010; Ribatti and Djonov, 2012). Angiogenesis is a tightly controlled dynamic process that can occur physiologically in those tissues that undergo active remodelling in response to stress and hypoxia (Carmeliet, 2003; Folkman, 2007). However, it can be aberrantly activated during many pathological conditions such as cancer, diabetic retinopathy as well as numerous ischaemic, inflammatory, infectious and immune disorders (Carmeliet, 2003; Ali and El-Remessy, 2009; Willis *et al.*, 2011). Although the concept of proposing angiogenesis inhibitors as anticancer drugs received considerable skepticism when first presented by Dr. Folkman in the early 1970s (Folkman, 1971), active research in the field and subsequent clinical trials eventually resulted in US Food and Drug Administration (FDA) approval of bevacizumab for colorectal cancer in 2004 (Cohen *et al.*, 2007). Since then, several angiogenic inhibitors have been identified. This review will provide

an overview of the key mechanisms involved in tumour angiogenesis, classification of angiogenesis inhibitors as well as treatment rationales from the mechanistic point of view.

Sustained angiogenesis as a hallmark of cancer

Proliferating tumours tend to activate an angiogenic phenotype to fulfil their increased demand of oxygen and nutrients (Hanahan and Folkman, 1996; Carmeliet, 2005). Additionally, paracrine release of anti-apoptotic factors from activated endothelial cells in the newly formed vasculature supplies tumour cells with a survival privilege (Folkman, 2003). Consequently, in order to progress, tumours tend to activate an event called 'angiogenic switch' by shifting the balance of endogenous angiogenesis inducers and inhibitors towards a pro-angiogenic outcome. As a result, dormant lesion progresses into outgrowing vascularized tumour and eventually into a malignant phenotype (Hanahan and Folkman, 1996; Baeriswyl and Christofori, 2009). Hypoxia drives such imbalance through up-regulation of the transcription factor hypoxia inducible factor-1α (HIF-1α), which in turn increases the expression of many angiogenesis inducers as well as suppresses the expression of endogenous angiogenesis inhibitors (Pugh and Ratcliffe, 2003). In spite of that, accumulating evidence indicates that angiogenic cascade can be also driven by alternative HIF-1-independent pathways (Mizukami *et al.*, 2007; Arany *et al.*, 2008; Lee, 2013).

As summarized in Table 1, the angiogenesis inducers are a wide range of mediators that include many growth factors, a plethora of cytokines, bioactive lipids, matrix-degrading enzymes and a number of small molecules (Folkman, 1995; Folkman, 2003; Lopez-Lopez *et al.*, 2004; Bouis *et al.*, 2006; El-Remessy *et al.*, 2007; Bid *et al.*, 2011; MacLauchlan *et al.*, 2011; Murakami, 2011; Fagiani and Christofori, 2013; Qin *et al.*, 2013). Pro-angiogenic growth factors mostly activate a series of surface receptors in a series of paracrine and autocrine loops with the VEGF-A signalling representing the critical rate-limiting step, physiologically and pathologically. VEGF-A (traditionally known as VEGF) is the most potent VEGF isoform that acts mainly on VEGF receptor 2 (VEGFR2) to mediate vascular permeability, endothelial proliferation, migration and survival (Takahashi and Shibuya, 2005; Bouis *et al.*, 2006). In spite of the well-established master roles of VEGF signalling in literature, those processes are probably accomplished through a highly regulated interplay between VEGF and other pro-angiogenic factors. In this context, basic fibroblast growth factor (bFGF) activation of the endothelium is required for maintenance of VEGFR2 expression and the ability to respond to VEGF stimulation (Murakami *et al.*, 2011). Similarly, sphingosine-1-phosphate (S1P), a pleiotropic bioactive lipid that can directly contribute to tumour angiogenesis (reviewed in Sabbadini, 2011), is needed for VEGF-induced blood vessel formation, indicating the cooperation between S1P and VEGF in tumour angiogenesis (Visentin *et al.*, 2006). As a net result, the pro-angiogenic interplay of those ligands and others dominates over the activities of two dozen endogenous angiogenesis inhibitors that can be either matrix-derived inhibitors or non–matrix-derived inhibitors (Nyberg *et al.*, 2005).

The multistep angiogenic process starts with vasodilation and increased permeability of existing vessels in response to tumour cell-secreted VEGF. This is accompanied by loosening of pericytes covering mediated by angiopoietin-2 (ANG2), a ligand of tyrosine kinase with immunoglobulin-like and EGF-

Table 1

Pro-angiogenic mediators implicated in tumour angiogenesis

Category	Examples	References
Growth factors	VEGFs	Bouis *et al.*, 2006
	FGFs	Bouis *et al.*, 2006
	TGFs	Bouis *et al.*, 2006
	PDGFs	Bouis *et al.*, 2006
	Insulin-like growth factors	Lopez-Lopez *et al.*, 2004; Bid *et al.*, 2011
	ANGs	Fagiani and Christofori, 2013
Cytokines	IL-8	Strieter *et al.*, 2004
	CSF-1	Lin *et al.*, 2006
Bioactive lipids	PGE2	Wang and Dubois, 2010
	S1P	Murakami, 2011
Matrix-degrading enzymes	MMPs	Bourboulia and Stetler-Stevenson, 2010
	Heparanases	Vlodavsky and Friedmann, 2001
Small mediators	NO	MacLauchlan *et al.*, 2011
	Peroxynitrite	El-Remessy *et al.*, 2007
	Serotonin	Qin *et al.*, 2013
	Histamine	Qin *et al.*, 2013

like domains 2 (TIE2) receptor (Bergers and Benjamin, 2003; Jain, 2003; Fagiani and Christofori, 2013). Meanwhile, many secreted matrix-degrading enzymes, such as MMPs and heparanases, function in concert to dissolve the basement membrane and to remodel the extracellular matrix (ECM) as well as to liberate more pro-angiogenic growth factors (bFGF and VEGF) from matrix heparan sulfate proteoglycans (HSPGs) respectively (Houck *et al.*, 1992; Whitelock *et al.*, 1996; Vlodavsky and Friedmann, 2001; Tang *et al.*, 2005; van Hinsbergh and Koolwijk, 2008). The overall chemotactic angiogenic stimuli guide endothelial cells to migrate, to align into tube-like structures and to eventually form new blood vessels. However, such blood vessels are characterized by being disorganized, chaotic, haemorrhagic and poorly functioning (Bergers and Benjamin, 2003).

The angiogenic phenotype in tumour micro-environment can further be sustained and extravagated by the recruitment of other types of stromal cells. Stromal cells such as fibroblasts, mesenchymal stem cells and various bone marrow-derived myeloid cells including macrophages, TIE2-expressing monocytes, neutrophils and mast cells contribute to tumour angiogenesis through their production of growth factors, cytokines and proteases (Murdoch *et al.*, 2008; Joyce and Pollard, 2009; Cirri and Chiarugi, 2011). For example, in response to cancer cell-derived TGF-β, PDGF or bFGF, fibroblasts are transformed to an activated phenotype with a higher proliferative activity and myofibroblastic characteristics (Kalluri and Zeisberg, 2006; Cirri and Chiarugi, 2011). Such carcinoma-associated fibroblasts (CAFs) were shown to promote angiogenesis and metastasis by secreting large amounts of MMP-2 and MMP-9 as well as by expressing many cytokines and chemokines that resulted in immune cell infiltration (Gerber *et al.*, 2009; Giannoni *et al.*, 2010). Furthermore, it has been shown that PDGF-C produced by CAFs is able to elicit VEGF production from tumour cells, thereby sustaining the angiogenic shift (Crawford *et al.*, 2009). Similarly, tumour-associated macrophages (TAMs), one of the bone marrow myeloid-derived cells, are induced to develop into polarized type II (alternatively activated or M2 macrophages), upon exposure to tumour hypoxia and tumour cell-derived cytokines (Leek *et al.*, 2002; Rogers and Holen, 2011). M2 macrophages tend to produce many pro-angiogenic growth factors, cytokines and matrix-degrading enzymes such as VEGF, PDGF, bFGF, TNF-α, COX-2, MMP-9, MMP-7 and MMP-12 (Lewis and Pollard, 2006).

From another perspective, angiogenesis may be dispensable for progression of some malignancies. For example, some tumours may co-opt pre-existent vessels as an alternative way to obtain blood supply. Vessel co-option was first described in the brain, one of the most densely vascularized organs, in which tumours may develop in earlier stages without the activation of angiogenic response (Holash *et al.*, 1999; Leenders *et al.*, 2002; Bergers and Benjamin, 2003; Hillen and Griffioen, 2007). In another example, hypovascularized tumours such as pancreatic ductal adenocarcinoma may involve certain adaptation to flourish in the absence of prominent angiogenesis (Bergers and Hanahan, 2008). Obviously, in both cases, tumours may be intrinsically indifferent to angiogenesis inhibitors. However, in most other cases, therapy directed towards the vasculature of solid tumours is being considered as an important direction in cancer treatment.

Classification of angiogenesis inhibitors

Growth of newly formed vessels in tumour micro-environment can be inhibited directly by targeting endothelial cells in the growing vasculature or indirectly by targeting either tumour cells or the other tumour-associated stromal cells. Therefore, angiogenesis inhibitors can be classified into direct and indirect inhibitors (Kerbel and Folkman, 2002; Folkman, 2007).

Direct endogenous inhibitors of angiogenesis

Direct endogenous inhibitors of angiogenesis, such as angiostatin, endostatin, arrestin, canstatin, tumstatin and others, are fragments released on proteolysis of distinct ECM molecules. Endogenous inhibitors prevent vascular endothelial cells from proliferating, migrating in response to a spectrum of angiogenesis inducers, including VEGF, bFGF, IL-8 and PDGF (Kerbel and Folkman, 2002; Abdollahi *et al.*, 2004; Mundel and Kalluri, 2007; Ribatti, 2009). This direct anti-angiogenic effect may be mediated by interference with endothelial integrins along with several intracellular signalling pathways (Mundel and Kalluri, 2007). For example, the ability of tumstatin-derived active peptide to inhibit angiogenesis and tumour growth is associated with the expression of the adhesion receptor, αvβ3 integrin, on tumour endothelial cells (Eikesdal *et al.*, 2008). Through binding αvβ3 integrin, full tumstatin was found to inhibit endothelial cell activation of focal adhesion kinase, PI3K, Akt, mammalian target of rapamycin (mTOR) and others (Maeshima *et al.*, 2002). Direct targeting of those signalling pathways by endogenous inhibitors was thought to be the least likely to induce acquired drug resistance because they target endothelial cells with assumed genetic stability rather than unstable mutating tumour cells (Kerbel and Folkman, 2002). However, endostatin has not yet led to any documented benefit to patients in randomized phase III trials, or even modest activity in phase II trials (Ellis and Hicklin, 2008).

Indirect inhibitors of angiogenesis

Indirect inhibitors of angiogenesis classically prevent the expression or block the activity of pro-angiogenic proteins (Folkman, 2007). For example, Iressa, an EGF receptor (EGFR) TK inhibitor (TKI), blocks tumour expression of many pro-angiogenic factors; bevacizumab, a monoclonal antibody, neutralizes VEGF after its secretion from tumour cells whereas sunitinib, a multiple receptor TKI, blocks the endothelial cell receptors (VEGFR1, VEGFR2 and VEGFR3), preventing their response to the secreted VEGF (Folkman, 2007; Roskoski, 2007). In addition, this class extends to include conventional chemotherapeutic agents, targeted therapy against oncogenes and drugs targeting other cells of the tumour micro-environment (Kerbel *et al.*, 2000; Ferrara and Kerbel, 2005).

Conventional chemotherapeutic agents

Conventional chemotherapeutic agents have been shown to have anti-angiogenic properties in addition to the ability to induce direct cancer cell death. Such chemotherapeutic agents can affect the endothelial cell population in the

tumour bed during treatment cycles because they have significantly higher proliferation rates than resting endothelium outside a tumour, making them more susceptible to cytotoxic effect (Kerbel *et al.*, 2000; Folkman, 2003). However, the cyclic treatment rationale of cytotoxic drugs allows the potential damage to the tumour vasculature to be repaired during the long breaks. Thus, continuous low doses of chemotherapeutic agents were suggested as a way to reduce side effects and drug resistance (Drevs *et al.*, 2004). This modality is termed metronomic therapy, and clinically, it refers to the daily administration of 5–10% of the phase II-recommended dose of the chemotherapeutic agent (Penel *et al.*, 2012). The extended use of such low doses of cytotoxic agents elicits an anti-angiogenic activity through induction of endothelial cell apoptosis and decreasing the level of circulating endothelial precursors (Hamano *et al.*, 2004; Shahrzad *et al.*, 2008). In clinical investigations, metronomic dosing of cyclophosphamide and others showed promising efficacy in patients with advanced, multiple metastasized and/or multiple pretreated solid tumours (Lord *et al.*, 2007; Fontana *et al.*, 2010; Nelius *et al.*, 2011; Gebbia *et al.*, 2012; Briasoulis *et al.*, 2013; Navid *et al.*, 2013).

VEGF-targeted therapy

VEGF-targeted therapy includes neutralizing antibodies to VEGF (e.g. bevacizumab) or VEGFRs (e.g. ramucirumab), soluble VEGFR/VEGFR hybrids (e.g. VEGF-Trap) and TKIs with selectivity for VEGFRs (e.g. sunitinib and sorafenib; Baka *et al.*, 2006; Ellis and Hicklin, 2008; Hsu and Wakelee, 2009). Bevacizumab, a humanized monoclonal antibody against all isoforms of VEGF-A, has been approved for the treatment of colorectal, lung, glioblastoma and renal cell carcinoma (Hsu and Wakelee, 2009). Many other clinical trials with promising efficacy were also conducted in other cancers such as head and neck cancer, hepatocellular carcinoma, ovarian cancer, metastatic melanoma and gastric cancer (Argiris *et al.*, 2011; 2013; Burger *et al.*, 2011; Ohtsu *et al.*, 2011; Fang *et al.*, 2012; Minor, 2012; Schuster *et al.*, 2012; Van Cutsem *et al.*, 2012). However, for metastatic breast cancer, bevacizumab had been initially granted an accelerated FDA approval, which was later withdrawn due to lack of improvement evidence in disease-related symptoms or overall survival (Burstein, 2011; Montero *et al.*, 2012). Similarly, clinical trials showed that the addition of bevacizumab to the treatment regimens of advanced pancreatic cancer did not extend overall survival (Chiu and Yau, 2012). The neutralization of VEGF-A can also be achieved by soluble receptor construct (VEGF-Trap) that monomerically 'traps' the different isoforms of VEGF-A, in addition to VEGF-B and placental growth factor (Rudge *et al.*, 2007). VEGF-Trap showed clinical benefit in a phase III trial of oxaliplatin pretreated metastatic patients with colorectal cancer, and is currently being investigated in a prostate cancer phase III trial (Gaya and Tse, 2012). TKIs are small molecules with different chemical structures that have the ability to interact physically with the highly conserved kinase domain shared by different VEGFRs as well as PDGF receptors (PDGFRs), FGF receptors (FGFRs), EGFR, Raf kinases and c-Kit (a receptor of the pluripotent cell growth factor, stem cell factor). Such interaction directly inhibits tyrosine phosphorylation and the subsequent many downstream pro-angiogenic signalling networks (Baka *et al.*, 2006; Ivy *et al.*, 2009). Those multi-targeted TKIs demonstrated efficacy against various solid malignancies in different clinical trials, some of which have lead eventually to FDA approval of sunitinib and sorafenib. Sunitinib, known to inhibit several receptor TKs (RTKs) including VEGFR1–3, PDGFR-α, PDGFR-β, c-Kit, colony-stimulating factor-1 receptor (CSF-1R) and Flt-3, was approved for the treatment of renal cell carcinoma and gastrointestinal stromal cell tumours. Sorafenib that acts also by inhibiting VEGFR1–3 and PDGFR-β in addition to the serine–threonine kinases Raf-1, B-Raf, was approved for hepatocellular carcinoma in addition to renal cell carcinoma (Llovet *et al.*, 2008; Ivy *et al.*, 2009; Huang *et al.*, 2010).

FGF-targeted therapies

FGF-targeted therapies were recently reconsidered as promising anti-angiogenic and anti-tumour agents after a long period of little attention for drug development, partly due to redundancy (Bono *et al.*, 2013). The FGFR superfamily with its 18 ligands and four receptors has been involved in endothelial cell migration, proliferation and differentiation (Presta *et al.*, 2005). Therapeutic targeting of FGF/FGFR signalling was accomplished by either monoclonal antibodies that inhibit FGFs binding, small molecules that inhibit FGFR TK activity or allosteric modulators that bind the extracellular FGFR domain. Monoclonal antibodies against bFGF displayed potent anti-tumour and anti-angiogenic effects in different preclinical cancer models, which warrant further clinical evaluation (Zhao *et al.*, 2010; Wang *et al.*, 2012). Pan inhibitors of the FGFR TKs such as AZD4547 (blocks the activity of FGFR1–3) and ponatinib (blocks all the FGFR isoforms) elicited potent anti-tumour activities in preclinical investigations so they are currently being evaluated in clinical trials. Those inhibitors displayed the greatest potency in FGFR-driven cancer models, which may be attributed to the interference with the oncogenic functions of either amplified or constitutively active FGFR (Dutt *et al.*, 2011; Zhao *et al.*, 2011; Gavine *et al.*, 2012; Gozgit *et al.*, 2012). Accordingly, further studies are needed to evaluate the relative contribution of angiogenic versus oncogenic inhibitory mechanisms towards the overall anti-tumour activity. The allosteric antagonist of the FGFR, SSR128129E, showed a strong anti-angiogenic activity in addition to tumour growth and metastasis inhibitory effects in animal models of arthritis and cancer respectively. Because allosteric modulators leave a residual level of baseline signalling, they have the ability to fine-tune target biological responses. As a result, allosteric multi-FGFR inhibitors may have an improved benefit/risk ratio that is not attainable with the other TKIs (Bono *et al.*, 2013; Herbert *et al.*, 2013). However, preclinical findings suggest that long-term clinical outcomes may improve with blockade of additional pro-angiogenic RTKs that may also reduce the risk of drug resistance (Hilberg *et al.*, 2008). For example, dual inhibition of VEGFRs and FGFRs using brivanib produced enduring tumour stasis and angiogenic blockade following the failure of VEGF-targeted therapies (Allen *et al.*, 2011). Furthermore, triple inhibition of FGFRs, VEGFRs and PDGFR(s) using dovitinib (TKI258) or nintedanib (BIBF 1120) displayed broad-spectrum anti-tumour activities in several tumour xenograft models as well as promising data in clinical trials. Combined inhibition of FGFR/VEGFR/PDGFR targets not only tumour

cells, but also endothelial cells, pericytes and smooth muscle cells, resulting in an effective inhibition of tumour growth, angiogenesis and metastasis even in advanced tumour stages (Hilberg *et al.*, 2008; Ledermann *et al.*, 2011; Taeger *et al.*, 2011; Chen *et al.*, 2012; Angevin *et al.*, 2013).

Oncogene-targeted therapy

Oncogenes, genes that cause the transformation of normal cells into cancerous cells, are thought to up-regulate many pro-angiogenic proteins. Therefore, anticancer drugs that were developed for their capacity to block an oncogene also have an indirect anti-angiogenic activity (Kerbel *et al.*, 2000; Bergers and Benjamin, 2003; Folkman, 2003). For example, dasatinib and other inhibitors of sarcoma (Src), an aberrantly activated non-RTK associated with many human malignancies, showed potent anti-angiogenic effects through the down-regulation of VEGF and IL-8 (Summy *et al.*, 2005; Han *et al.*, 2006; Haura *et al.*, 2010). Another example is to target the oncogenic Ras using farnesyl transferase (FT) inhibitors, which inhibit post-translational farnesylation of Ras that governs the latter's activity (Awada *et al.*, 2002). FT inhibitors were found to inhibit tumour VEGF expression and block FTase-dependent Ras activation, which is critically involved in VEGF-elicited angiogenic signal transduction and angiogenesis (Han *et al.*, 2005; Izbicka *et al.*, 2005; Kim *et al.*, 2010). In addition to classical oncogenes inhibition, interference with other tumour-deregulated signalling pathways would offer another approach in targeting angiogenesis. For example, inhibitors of heat shock protein 90 (HSP90), a chaperone molecule known to protect oncoproteins from misfolding and degradation in the protein-rich intracellular environment, were found to prevent VEGF production and to disrupt multiple pro-angiogenic signalling pathways in numerous cancer cells. They were also shown to inhibit tumour growth and vascularity of different human tumour xenografts (Sanderson *et al.*, 2006; Lang *et al.*, 2007; Eccles *et al.*, 2008; Trepel *et al.*, 2010; Moser *et al.*, 2012). Proteasome inhibitors, such as bortezomib (PS-341) or MG-132, were also shown to reduce tumour growth and vascularity of squamous cell carcinoma and pancreatic cancer xenograft probably through inhibition of NF–κB-dependent release of pro-angiogenic gene products, VEGF and IL-8 (Sunwoo *et al.*, 2001; Nawrocki *et al.*, 2002; Matsuo *et al.*, 2009). Similarly, inhibition of B-cell lymphoma 2 (Bcl-2), a prosurvival protein that regulates apoptosis by preventing the mitochondrial release of pro-apoptogenic factors, was shown to prevent NF-κB-mediated release of the pro-angiogenic factors IL-8 and CXC chemokine ligand 1 (CXCL1) as well as VEGF in tumour-associated endothelial cells and pancreatic cell lines respectively (Karl *et al.*, 2005; Wang *et al.*, 2008). Moreover, (–)-gossypol, a natural BH3 mimetic that inhibits BH3 domain of Bcl-2 as well as related prosurvival proteins (Bcl-xL and Mcl-1), was shown to remarkably decrease microvessel density in human prostate tumour PC-3 xenografts through decrease of VEGF and IL-8 release as well as blocking multiple steps in VEGF-activated biological events (Karaca *et al.*, 2008; Pang *et al.*, 2011).

Matrix degrading and remodelling-targeted therapy

Matrix degrading and remodelling are activated by tumours to modify local micro-environment, which in turn promote their angiogenic potential (Bergers *et al.*, 2000; Vlodavsky and Friedmann, 2001). Up-regulation of expression and activity of several endogenous MMPs including MMP-2, MMP-9 as well as MMP-3 and MMP-7 have been identified in invasive tumours (for a review, see Bourboulia and Stetler-Stevenson, 2010). Consequently, inhibitors of MMPs were extensively pursued as a therapeutic strategy for treating cancer. Unfortunately, MMPs intervention strategies had met with limited clinical success because of severe toxicities and associated metastasis-promoting effect (Coussens *et al.*, 2002; Devy *et al.*, 2009). Furthermore, the paradoxical roles of tissue inhibitors of metalloproteinases (TIMPs) may contribute to such failure depending on the net balance of TIMPs and MMPs in tumour stroma (Jiang *et al.*, 2002). As a result, efforts were directed at therapies exploiting endogenous MMP inhibitors, TIMPs or monoclonal antibodies against individual MMPs (Martens *et al.*, 2007; Jarvelainen *et al.*, 2009). For example, DX-2400, a highly selective fully human MMP-14 inhibitory antibody, was found to block pro-MMP-2 processing on tumour and endothelial cells, inhibited angiogenesis, and slowed tumour progression and formation of metastatic lesions (Devy *et al.*, 2009). Alternatively, in order to reduce toxicity and enhance drug delivery, polymeric nanoparticulate delivery systems could be used to target individual components of ECM. For example, targeted delivery of antisense inhibitors of laminin-8, a vascular basement membrane component, by conjugation to the natural drug carrier β-poly(L-malic acid) significantly reduced tumour microvessel density and increased animal survival in an experimental model of glioblastoma (Fujita *et al.*, 2006). Similarly, a nano delivery system that incorporate peptides against proteolytically processed type IV collagen significantly accumulated in tumours and blocked angiogenesis in experimental models (Mueller *et al.*, 2009). However, the highly sulfated oligosaccharides, Heparan (HS) mimetics highly sulfated oligosaccharides, were shown to have a heparanase-inhibiting effect sequestering, in turn, many heparan sulfate proteoglycan (HSPG)-binding factors (Johnstone *et al.*, 2010; Dredge *et al.*, 2011). In preclinical studies, HS mimetics have effectively targeted multiple HSPG-dependent functions and have resulted in decreased *in vivo* tumour growth, tumour invasion, tumour metastasis and angiogenesis (Johnstone *et al.*, 2010; Dredge *et al.*, 2011; Zhou *et al.*, 2011). Clinically, the heparanase inhibitor PI-88 showed preliminary efficacy as an adjunct therapy for postoperative hepatocellular carcinoma (Liu *et al.*, 2009).

Tumour-associated stromal cell-targeted therapy

Tumour-associated stromal cells crosstalk is a perquisite for the formation of a tumour vasculature, an essential step for tumour progression (Lorusso and Ruegg, 2008). Interference with those crosstalk circuits through intervention of cellular adhesion (highlighted in next paragraph) or tumour-induced recruitment of different stromal cells may be considered as an indirect way of anti-angiogenic therapy (Ferrara and Kerbel, 2005). The latter can be supported by studies in which inhibition of macrophage infiltration, for example, by either genetic ablation of the macrophage CSF-1 or liposomal clodronate-induced macrophage depletion, was shown to delay the angiogenic switch and malignant transition (Giraudo *et al.*, 2004; Lin *et al.*, 2006). Furthermore, CSF-1R

kinase inhibitors were found to reduce tumour-associated vascularity in two different tumour mouse models (Kubota *et al.*, 2009; Manthey *et al.*, 2009). In addition, clodronate and other related bisphosphonates, originally used to treat skeletal complications in patients with tumour-induced osteolysis, were shown to exert potent anti-tumour and anti-angiogenic effects in many other studies (Fournier *et al.*, 2002; Santini *et al.*, 2003; Stathopoulos *et al.*, 2008). Zoledronic acid, a third-generation bisphosphonate, was also found to reduce a number of tumour-associated macrophages and shift their phenotype from M2 to M1, resulting in a reduction in TAM-associated production of VEGF in murine models of spontaneous mammary carcinogenesis and mesothelioma (Coscia *et al.*, 2010; Veltman *et al.*, 2010). Clinically, repeated low-dose therapy with zoledronic acid, which maintains active drug plasma concentration, was able to induce an early remarkable and long-lasting decrease of VEGF levels in patients with cancer (Santini *et al.*, 2007). In another example, inhibition of mobilization of neutrophils, from bone marrow and their infiltration into tumour, using neutralizing anti–prokineticin-2, an antibody against a secreted protein known also as BV8, was shown to impair the initial angiogenic switch in a multistage pancreatic beta cell tumourigenesis model (Shojaei *et al.*, 2008). Furthermore, the neutralizing anti-BV8 was found to prevent myeloid cell-dependent tumour angiogenesis in several xenograft models (Shojaei *et al.*, 2007). Cancer-associated fibroblasts (CAF) can also be targeted with thapsigargin analogue coupled with peptides specific for fibroblast activation protein (FAP), a CAF membrane-bound protease whose catalytic site has access to the peritumoural fluid of the tumour micro-environment. This extracellular activation results in the death of CAFs as well as pericytes and endothelial cells within milieu of different human tumour xenografts (Brennen *et al.*, 2012).

Cell adhesion molecules (CAMs)-targeted therapy

CAMs are cell surface proteins known to be involved in binding with other counter-receptors on adjacent cells or surrounding ECM macromolecules (Aplin *et al.*, 1998). Many CAMs, such as αv-integrins, E-selectin, N-cadherin and VE-cadherin, have been implicated in tumour angiogenesis (Bischoff, 1997; Tei *et al.*, 2002; Nakashima *et al.*, 2003; Weis and Cheresh, 2011). For example, αv-integrins are expressed on surface of endothelial cells and can determine whether cells can adhere to and survive in a particular micro-environment. A number of matrix-derived fragments have the ability to act as endogenous angiogenesis inhibitors through binding to integrins on endothelial cells, disrupting physical connections and suppressing signalling events associated with cell survival, migration and proliferation (Nyberg *et al.*, 2005). Consequently, integrins antagonism using peptidomimetics (e.g. cilengitide), monoclonal antibodies (e.g. volociximab) or oral small-molecule compounds have been investigated in a wide range of malignancies (Huveneers *et al.*, 2007). Cilengitide is a cyclized pentapeptide peptidomimetic designed to compete for the arginine-glycine-aspartic acid (RGD) peptide sequence, thereby blocking the ligation of the αvβ3 and αvβ5 integrins to matrix proteins (Hariharan *et al.*, 2007). Cilengitide is mainly under clinical development for glioblastoma; however, clinical trials of other malignancies such as head and neck cancer as well as lung cancer were also initiated (Reardon and Cheresh, 2011; Vermorken *et al.*, 2012; Manegold *et al.*, 2013). Alternatively, cyclic peptides containing RGD motif could guide nanoparticulate delivery system, which incorporates anti-angiogenic cytotoxic agents such as doxorubicin, paclitaxel or combretastatin A4, to accumulate specifically in tumour vasculature with no overt systemic toxicity (Murphy *et al.*, 2008; Ruoslahti *et al.*, 2010; Wang *et al.*, 2011). Volociximab, a chimeric humanized monoclonal antibody that selectively inhibits the αvβ1 integrin interaction with fibronectin, has been evaluated also in clinical trials for solid tumours such as renal cell carcinoma, recurrent ovarian cancer, advanced non–small-cell lung cancer and metastatic pancreatic cancer (Figlin *et al.*, 2006; Evans *et al.*, 2007; Jarvelainen *et al.*, 2009; Vergote *et al.*, 2009; Besse *et al.*, 2013). Cadherins constitute a superfamily of molecules that mediate calcium-dependent cell–cell adhesions. The intracellular domains of cadherins directly bind to β-catenin and link with cytoskeletal components, providing the molecular basis for stable cell–cell adhesion (Zhang *et al.*, 2010). Targeting cadherin signalling may also represent another way for tumour angiogenesis intervention. For example, ADH-1, a cyclic pentapeptide containing the cell adhesion recognition site (His-Ala-Val) required for N-cadherin adhesion, was shown to possess anti-angiogenic and anti-tumour activity (Blaschuk *et al.*, 2005; Blaschuk, 2012). Similarly, monoclonal antibody directed against specific region of VE-cadherin was able to inhibit tumour angiogenesis and growth with no side effects on normal vasculature (Corada *et al.*, 2002; May *et al.*, 2005).

Inflammatory angiogenesis-targeted therapy

Targeting inflammatory angiogenesis, responsible for a substantial part of tumour vascularization initiated by infiltrating leukocytes, may be considered as another indirect anti-angiogenic strategy (Albini *et al.*, 2005). Moreover, as mentioned before, tumour-infiltrating leukocytes contribute into malignant progression through production of many pro-inflammatory cytokines, chemokines and enzymes that can mostly induce angiogenic cascade (Balkwill *et al.*, 2005). Such vital roles have been supported by the early observation that nonsteroidal anti-inflammatory drugs can inhibit tumour angiogenesis and, in turn, tumour progression (Albini *et al.*, 2005). For example, ibuprofen was found to decrease tumour growth and metastatic potential in mice models through modulation of angiogenesis (Yao *et al.*, 2005). Moreover, selective inhibitors of COX-2, an inducible enzyme that catalyses the production of prostanoids from arachidonic acid, were also shown to inhibit angiogenesis (Tsujii *et al.*, 1998; Wei *et al.*, 2004). The anti-angiogenic effect of COX-2 inhibitors may be contributed, in part, by decreasing the COX-2 metabolic product PGE2, the predominant PG in solid tumours known to stimulate cancer cells to produce pro-angiogenic factors such as VEGF and bFGF as well as many other factors belonging to CXC chemokines family (Strieter *et al.*, 2004; Wang *et al.*, 2006; Wang and Dubois, 2010). Members of the CXC chemokine family are heparin-binding proteins that possess disparate regulative roles in angiogenesis. For example, the ELR+ CXC chemokines, characterized by highly conserved three amino acid motifs (Glu-Leu-Arg; 'ELR' motif), are potent promoters of angiogenesis, whereas

the IFN-inducible (ELR–) CXC chemokines are inhibitors of angiogenesis (Strieter *et al.*, 2004). The use of repertaxin, originally designed to target the ELR+ CXC chemokine receptors CXCR1 and CXCR2 on neutrophils to prevent their migration to sites of inflammation, was found to inhibit tumour angiogenesis, thereby suppressing tumour progression in a genetic model of pancreatic ductal adenocarcinoma (Ijichi *et al.*, 2011). It would be beneficial to explore other small-molecule CXCR2 antagonists that have already been developed for the treatment of inflammatory diseases in different preclinical models of cancer, especially inflammation-associated cancers (refer to Chapman *et al.*, 2009 for a list of newly developed CXCR2 antagonists used in the treatment of inflammatory diseases of the lung).

Treatment rationales of angiogenesis inhibitors

Angiogenesis inhibitors are used as either monotherapy or in combination with other anti-tumour drugs. Monotherapy using anti-angiogenic agents is mostly intended for prevention of cancer in susceptible individuals or for delaying disease progression in patients with cancer who have previously treated with first-line/second-line regimens. In combined therapy, anti-angiogenic drugs may be added to treatment regimens to increase their efficacy or to reduce developing drug resistance.

Rationale for monotherapy

Patients with cancer who have been treated with first-line or second-line regimens can proceed on a maintenance therapy using one anti-angiogenic drug to extend the progression-free survival (Giuliani *et al.*, 2010; Ledermann *et al.*, 2011; Fabi *et al.*, 2012). Furthermore, anti-angiogenic monotherapy may be beneficial in patients who are at high risk for developing cancer through interference with the angiogenic switch (O'Reilly *et al.*, 1997; Bergers and Benjamin, 2003). This kind of strategy is supported by the comparative safety of anti-angiogenic drugs allowing administration over extended periods (Ma and Waxman, 2008). Many natural and synthetic agents have been shown to significantly reduce the risk of cancer development. Aspirin, a non-selective inhibitor of COX-1 and -2, was associated with a reduced long-term incidence and mortality due to colorectal cancer (Rothwell *et al.*, 2010). The selective COX-2 inhibitor, celecoxib, was also shown to reduce the risk of developing colorectal adenomas in susceptible individuals (Bertagnolli *et al.*, 2006). However, many natural anti-angiogenic molecules present in numerous dietary sources can protect against cancer development and progression (Li *et al.*, 2012). For example, resveratrol, a natural phytoalexin and polyphenol, was found to prevent the onset of colon cancer in a mouse model of colitis-driven colon cancer (Cui *et al.*, 2010). Resveratrol also inhibited chemically induced mammary carcinogenesis in rats as well as tumour growth and metastasis in mice bearing Lewis lung carcinoma (Kimura and Okuda, 2001; Banerjee *et al.*, 2002).

Rationale for combinatory therapy

Crosstalk between angiogenic and oncogenic signalling pathways provides a strong rationale for combining angiogenesis inhibitors with chemotherapeutic and targeted anticancer agents. The consequent overall therapeutic response depends on appropriate designing of the combinatory treatment schedules (Ma and Waxman, 2008). Combination schedule of angiogenesis inhibitors can be neoadjuvant (before the chemotherapeutic drug), concurrent or adjuvant (after the chemotherapeutic drug) depending on the tumour type, anti-angiogenic drug and the chemotherapeutic agent itself (Li *et al.*, 2002; Ma and Waxman, 2009). Using such combinatory rationale has been proved to be more effective than individual treatments in many preclinical and clinical studies. For example, bortezomib, a proteasome inhibitor, was shown to enhance the growth inhibitory effects of IFN-α in the human bladder UM-UC-3 tumours, due to down-regulation of VEGF with ultimate decrease in microvessel densities (Papageorgiou *et al.*, 2006). In another study, combination treatment with vatalanib, a VEGF TKI, and dacinostat, a histone deacetylase inhibitor, was more effective than single agents in inhibiting *in vitro* and *in vivo* VEGF-induced angiogenesis, with a synergistic growth inhibitory effect on mouse models of subcutaneous prostate and orthotopic breast tumours (Qian *et al.*, 2004). A significant therapeutic improvement was also achieved when cyclophosphamide was included in the combination therapy with axitinib, another VEGF TKI, in prostate cancer PC-3 xenografts (Ma and Waxman, 2009). Clinically, the addition of bevacizumab to fluorouracil-based combination chemotherapy results in survival enhancement among patients with metastatic colorectal cancer (Hurwitz *et al.*, 2004; Giantonio *et al.*, 2007).

Mechanisms of enhanced therapeutic efficacy

Dual targeting of tumour vasculature

The activity of angiogenesis inhibitors on vascular cells could be potentiated when administered in combination with chemotherapeutic agents that themselves have vascular targeting properties (Naumova *et al.*, 2006). For example, the addition of paclitaxel to SU6668, a potent inhibitor of VEGFR2, FGFR1 and PDGF-β, was shown to inhibit ovarian carcinoma xenograft progression in the peritoneal cavities of nude mice (Garofalo *et al.*, 2003; Klenke *et al.*, 2007). This synergistic effect of paclitaxel may be attributed to its microtubule-binding properties that were known to correlate significantly with its anti-angiogenic and vascular-disrupting properties (Naumova *et al.*, 2006; Schwartz, 2009).

Targeting different cell types of tumour micro-environment

Enhanced therapeutic effect of anti-angiogenic and cytotoxic therapy combinations may be attributed to destruction of two separate compartments of tumours: cancer cells and endothelial cells (Teicher, 1996). The cytotoxic agents would destroy cancer cells directly, and the anti-angiogenic agents would kill cancer cells indirectly by depriving them of nutrients. Moreover, as mentioned before, chemotherapeutic agents may also have anti-angiogenic effects by targeting tumour endothelial cells and endothelial precursors, and thus enhancing the indirect killing of cancer cells (Hicklin and

Ellis, 2005; Jain, 2005). Similarly, dual pericytes and endothelial cell targeting was more effective when combinations of PDGFR(s) antagonists with a VEGFR2 inhibitor have been shown experimentally to greatly disturb pericyte–endothelial cell interactions with a resulted tumour regression (Bergers and Benjamin, 2003).

Normalization of tumour vasculature

During angiogenesis, VEGF induces microvascular permeability that increases deposition of fibrin and other plasma proteins in the tumour stroma leading to high interstitial fluid pressure within tumour micro-environment (Nagy *et al.*, 2006). The high interstitial fluid pressure limits chemotherapeutic drug delivery, a major limitation that was found to be ameliorated by co-treatment with angiogenic inhibitors through normalization of tumour vasculature and relieving local tumour oedema (Jain, 2001; Lammerts *et al.*, 2002; Tong *et al.*, 2004). For example, an anti-angiogenic antibody directed against VEGF was found to normalize tumour vasculature, creating an open therapeutic window during which the chemotherapeutic drug can be incorporated with a consequent maximum drug delivery (Tong *et al.*, 2004). To optimize the benefit of vascular normalization-enhanced tumour drug delivery, the duration of the open window during anti-angiogenesis treatment needs to be better defined by improving imaging techniques, which can measure the spatial and temporal changes in blood flow and other physiological parameters with higher resolution (Jain, 2005). An ongoing clinical trial is currently recruiting patients to test whether short course of low-dose sunitinib can normalize tumour vasculature and enhance tumour delivery of docetaxel, and whether that will improve treatment response and progression-free survival in several refractory solid tumours (ClinicalTrials.gov identifier: NCT01803503).

Chemosensitization of tumour cells

Co-administration of some other anti-angiogenic agents with conventional chemotherapy was found to increase tumour hypoxia and drug retention rather than vasculature normalization (Franco *et al.*, 2006; Ma and Waxman, 2009; Ma *et al.*, 2011). Although reduction in tumour drug uptake was detected in some studies, overall therapeutic response was found to be enhanced (Ma and Waxman, 2009; Bello *et al.*, 2013). The conflicting results regarding the aforementioned theories may highlight chemosensitization on the molecular level as a mechanism of enhanced therapeutic efficacy. In this context, anti-angiogenic drugs could augment the direct cytotoxic effect of chemotherapeutic drugs by interfering with survival pathways and/or enhancing drug sensitivity. Growing evidence supports chemosensitization as an important mechanism of enhanced therapeutic efficacy. Different types of tumour cells are capable of expressing the potent pro-angiogenic factor VEGF in addition to different types of VEGFRs on their surface, creating a survival autocrine loops that can be targeted directly by anti-angiogenic therapy (Hicklin and Ellis, 2005; Stacchiotti *et al.*, 2011). Moreover, cancer cells tend to overexpress some other RTKs, which can be targeted by dual anti-angiogenic drugs improving tumour response towards chemotherapy (Folkman, 2007). For example, triple-negative breast xenografts completely regressed when E-3810, inhibitor of VEGFR1–3 and FGFR-1 TKs, was combined with paclitaxel (Bello *et al.*, 2013). The addition of imatinib, inhibitor of BCR-ABL TK, c-Kit and PDGFRs, to 5-Fluoruracil or paclitaxel elicited an enhanced cytotoxic effects characterized by induction of apoptotic cell death and inhibition of tumour angiogenesis along with associated down-regulated tumour cell PDGF-BB and PDGFR-β (Kim *et al.*, 2005). Furthermore, dual inhibition of VEGFR and EGFR using vandetanib (ZD6474) resulted in significant tumour growth inhibition in a range of histologically diverse human xenograft models (Wedge *et al.*, 2002; Holden *et al.*, 2005; Yoshikawa *et al.*, 2009). When moved for clinical development, vandetanib demonstrated a high therapeutic efficacy in phases I–III trials against metastatic medullary thyroid cancer, which lead eventually to FDA approval (Leboulleux *et al.*, 2012; Wells *et al.*, 2012; Ton *et al.*, 2013). However, in many other clinical trials, vandetanib showed either acceptable clinical activities along with safety concerns or limited clinical activities that warrant pre-selection of patients (Blackhall *et al.*, 2010; Hsu *et al.*, 2012; Kreisl *et al.*, 2012; Lee *et al.*, 2012; Meyerhardt *et al.*, 2012).

Interference with the repair of cytotoxic drug-induced damage and resistance mechanisms

Anti-angiogenic therapy may interfere with cytotoxic drug/radiotherapy-induced damage repair, a protective mechanism that occur in response to chemotherapy or radiotherapy and considered as a main reason for disease recurrence (Ma and Waxman, 2008). For example, the addition of mTOR inhibitors to radiation therapy could prevent protection mechanisms against radiation-mediated cytotoxicity of tumour blood vessels (Gorski *et al.*, 1999; Shinohara *et al.*, 2005; Manegold *et al.*, 2008). Furthermore, it has been reported that radiotherapy promotes integrin-mediated survival signalling through PKB/Akt by up-regulating αvβ3 expression on endothelial cells. This escape mechanism can be circumvented by administering the αvβ3 integrin antagonist S247, which prevents radiation-induced PKB/Akt phosphorylation leading to enhanced anti-angiogenic and anti-tumour effects (Abdollahi *et al.*, 2005). Clinically, volociximab, an anti-αvβ1 integrin antibody, in combination with carboplatin and paclitaxel showed a preliminary evidence of efficacy in patients with advanced, untreated non–small-cell lung cancer (Besse *et al.*, 2013). Similarly, vascular-disrupting agents that interfere with tumour growth through occlusion of existing blood vessels, such as combretastin-A4 phosphate, can induce acute mobilization and homing of circulating endothelial precursors to tumour as a protective mechanism. Disruption of this circulating endothelial precursors spike by anti-angiogenic drugs or by genetic manipulation resulted in marked reductions in tumour rim size and blood flow as well as enhanced anti-tumour activity of the vascular-disrupting agents (Shaked *et al.*, 2006).

Consequences of anti-angiogenic therapy with other anticancer therapy

Contrary to initial expectations, treatment with angiogenesis inhibitors was associated with unexpected toxicities. The tox-

Table 2

Major categories of angiogenesis inhibitors and molecular targets

Category of angiogenesis inhibitors	Examples	Target/Mechanism	References
Chemotherapeutic agents	Cyclophosphamide	Induces EC apoptosis, decreases circulating EPC	Hamano *et al.*, 2004; Shahrzad *et al.*, 2008
	Paclitaxel	Microtubule	Naumova *et al.*, 2006; Schwartz, 2009
VEGF-targeted therapy	Bevacizumab	VEGF-A	Hsu and Wakelee, 2009
	VEGF-Trap	VEGF-A, VEGF-B and PlGF	Rudge *et al.*, 2007
	Sunitinib	VEGFR1–3, PDGFR-α, PDGFR-β, c-Kit, CSF-1R and Flt-3	Huang *et al.*, 2010
	Sorafenib	VEGFR1–3, PDGFR-β, Raf-1, B-Raf	Huang *et al.*, 2010
	Vatalanib	VEGFR1–3, PDGFR-β and c-Kit	Qian *et al.*, 2004; Jubb *et al.*, 2006
	Axitinib	VEGFRs, PDGFR-β and c-Kit	Jubb *et al.*, 2006; Ma and Waxman, 2009
	SU6668	VEGFR2, FGFR1 and PDGF-β	Klenke *et al.*, 2007
FGF-targeted therapy	AZD4547	FGFR1–3	Gavine *et al.*, 2012
	Ponatinib	FGFR1–4	Gozgit *et al.*, 2012
	SSR	FGFRs	Bono *et al.*, 2013
	Brivanib	VEGFRs and FGFRs	Allen *et al.*, 2011
	Dovitinib	FEGFRs, VEGFRs and PDGFR	Chen *et al.*, 2012
	Nintedanib	VEGFRs, FGFRs and PDGFR	Hilberg *et al.*, 2008
Oncogene-targeted therapy/signalling transduction-targeted therapy	Dasatinib	Src and indirectly VEGF, IL-8	Summy *et al.*, 2005; Han *et al.*, 2006; Haura *et al.*, 2010
	Tipifarnib	MMP-1	Izbicka *et al.*, 2005
	NVP-AUY922	Hsp90	Eccles *et al.*, 2008; Moser *et al.*, 2012
	Bortezomib	NF-κB-dependent release of VEGF and IL-8	Sunwoo *et al.*, 2001
	Gossypol	VEGF and IL-8 release	Pang *et al.*, 2011
	Dacinostat	Histone deacetylase	Qian *et al.*, 2004
Matrix degrading and remodelling-targeted therapy	DX-2400	MMP-14	Devy *et al.*, 2009
	PI-88	Heparanase	Liu *et al.*, 2009
Tumour-associated stromal cell-targeted therapy	JNJ-28312141	CSF-1R	Manthey *et al.*, 2009
	Zoledronic acid	TAM-associated production of VEGF	Coscia *et al.*, 2010; Veltman *et al.*, 2010
	Anti-BV8 antibody	Neutrophils recruitment	Shojaei *et al.*, 2008
CAMs-targeted therapy	Cilengitide	αvβ3 and αvβ5 integrins ligation to matrix proteins	Hariharan *et al.*, 2007
	Volociximab	αvβ1 integrin interaction with fibronectin	Evans *et al.*, 2007; Besse *et al.*, 2013
	ADH-1	N-cadherin	Blaschuk *et al.*, 2005; Blaschuk, 2012
Inflammatory angiogenesis-targeted therapy	Ibuprofen	COX1/2	Yao *et al.*, 2005
	Celecoxib	COX-2	Wei *et al.*, 2004
	Repertaxin	CXCR1 and CXCR2	Ijichi *et al.*, 2011

EC, endothelial cells; EPC, endothelial progenitor cells; Hsp90, heat shock protein 90; PlGF, placental growth factor; Src, sarcoma.

icity profiles of those inhibitors reflect the systemic disturbance of growth factor signalling pathways that mediate their anti-angiogenic activity (Elice and Rodeghiero, 2010; 2012). In this context, disturbance of the tight endothelial cell-platelet interaction that maintains vascular integrity results in bleeding complications, gastrointestinal perforations, and disturbed wound and ulcer healing (Verheul and Pinedo, 2007). In general, the incidence of those adverse effects increases when anti-angiogenic agent is combined with chemotherapy. For example, bleeding complications have been observed in patients with colorectal cancer treated with chemotherapy in combination with bevacizumab

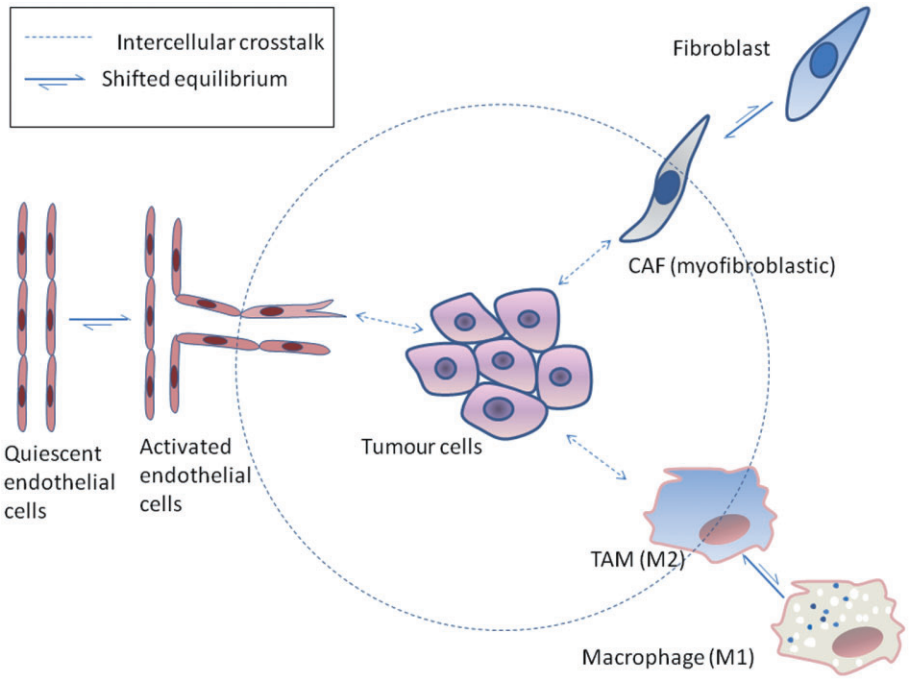

Figure 1

A representation of some cellular components of tumour micro-environment with shifted equilibria towards tumour-favouring phenotypes [CAFs with myofibroblastic characteristics; TAMs with M2 phenotype shift to M1 phenotype, a pro-inflammatory phenotype with a tumouricidal activity.

(Kabbinavar *et al.*, 2003; Giantonio *et al.*, 2006). In non–small-cell lung cancer, some patients treated with bevacizumab in combination with carboplatin and paclitaxel experienced severe or fatal pulmonary haemorrhage (Johnson *et al.*, 2004). Furthermore, a higher incidence of gastrointestinal perforation was observed in patients with colorectal cancer given bevacizumab in combination with chemotherapy compared with chemotherapy alone (Hurwitz *et al.*, 2004). Similarly, thrombotic events have been observed in patients treated with angiogenesis inhibitors, especially when these agents are given in combination with chemotherapy (Verheul and Pinedo, 2007). Treatment of patients with cancer with angiogenesis inhibitors is frequently associated with hypertension, which may require the addition of regular anti-hypertensive agent (Izzedine *et al.*, 2009). However, patients on anti-angiogenic therapy can develop resistance either via classical resistance mechanisms, such as increased drug metabolism or an increased number of drug efflux pumps, or via compensatory release of different angiogenic inducers (Kerbel and Folkman, 2002; Verheul and Pinedo, 2007). For example, VEGF-targeted therapies were shown to produce a period of stable disease followed by VEGF independent vascular regrowth, promoted cell invasiveness and metastasis (Ebos *et al.*, 2009; Loges *et al.*, 2010). Such resistance was thought to be mediated in part by pro-angiogenic ligands of the FGF family as well as BV8 secretion associated with enhanced recruitment of myeloid cells to hypoxic tumours (Casanovas *et al.*, 2005; Shojaei *et al.*, 2007; Loges *et al.*, 2010). Additionally, anti-VEGF refractoriness may be conferred to CAF-mediated release of PDGF-C that can direct the process of endothelial cell migration and angiogenesis, independent of VEGF as well as that can increase pericytes recruitment and vessels stabilizing minimizing their dependence on VEGF for survival (Crawford *et al.*, 2009; di Tomaso *et al.*, 2009). Consequently, targeting those angiogenesis signalling pathways may lead to enhanced response in anti-VEGF-resistant tumours (Hsu and Wakelee, 2009). Another possibility shown to circumvent this resistance scenario was to combine the VEGFR2 inhibitor cediranib or the monoclonal antibody bevacizumab with cilengitide, the integrin inhibitor (Reardon and Cheresh, 2011).

Summary and future directions

Angiogenesis is a critical process that occurs pathologically in many malignancies due to changing balance of endogenous angiogenesis inducers and inhibitors, leading to the activation of nearby endothelial cells to form new vasculature. Consequently, angiogenesis can be targeted to restrict initiation, growth and progression of most of angiogenesis-dependent malignancies. Numerous angiogenic inhibitors have been identified, some of which are currently being investigated in clinical trials and some others were even approved for cancer therapies. These angiogenesis inhibitors were classified based on their target into two main classes: direct and indirect inhibitors. Indirect angiogenesis inhibitors can be further subclassified based on their interference

mechanisms with the angiogenic cascade. A list of major categories and molecular targets for angiogenesis inhibitors is shown in Table 2.

Most angiogenesis inhibitors conferred clinical benefits mainly when combined with other chemotherapeutic/targeted therapies rather than being used as monotherapy. Unfortunately, many anti-angiogenic agents were shown to be associated with overt systemic toxicity as well as resistance emergence and disease recurrence. Drug resistance in anti-angiogenic therapy may result from a plethora of pro-angiogenic factors released by inappropriately functioning host cells in the tumour micro-environment as a compensatory mechanism. Therefore, the strategy of targeting endothelial cells alone may not be enough as explained in the previous texts, requiring the proposal of different rationales in which other cellular compartments of tumour micro-environment are targeted to attain proper anti-angiogenic and anti-tumour response. That highlights the importance of considering tumour micro-environment as a dynamic system, as depicted in Figure 1 in which interference with any of its components may be an approach to interfere with cancer hallmarks, including angiogenesis. Meanwhile, differentially expressed molecules on various types of tumour-associated stromal cells can be used as docking sites to concentrate drug conjugates and nanoparticles. That high local concentration of anti-angiogenic agents in tumour micro-environment may lead to increased sustained biological activities and/or reduced adverse effects. In conclusion, future anti-angiogenic treatment(s) should be designed as targeted delivery systems with the ability to accumulate selectively in tumours and/or an ability to shift micro-environmental equilibria towards tumour-unfavourable conditions. The consequent sustained disturbance of dynamic tumour micro-environment may lead eventually to tumour regression, hopefully without systemic toxicity and notable activation of resistance mechanisms.

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Conflict of interest

The authors declare that they have no conflict of interest.

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