Angiogenesis and current approaches to deal with its misregulation in related diseases

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Abstract: Angiogenesis, which is the formation of blood vessels from pre-existing vessels, normally supply nutrition and oxygen to cells and tissues. In medicine point of view, regulation of angiogenesis is disrupted in many diseases such as cancers, psoriasis, age related macular degeneration, diabetes, proliferative retinopathies and rheumatoid arthritis. How to suppress, control and regulate the angiogenesis have been very challenging tasks in order to provide better and more effective treatments for related patients. With this regard, anti-angiogenic therapy has been considered as a potential approach to do so. However, anti-angiogenic agents are not completely safe and present side effects. Therefore, many attentions have been paid to understand more about molecular and cellular mechanisms involved in angiogenesis in order to prevent many life-threating side effects of anti-angiogenic agents. It may lead to discovering more desirable drugs to tackle angiogenesis. This review aims to give an overview about what angiogenesis is as well as present the most important factors involved in angiogenesis. It also attempts to describe current approaches and challenges in controlling angiogenesis.
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Introduction

Angiogenesis is defined as forming new blood vessels from pre-existing vascular system (Logan-Collins, Lowy et al. 2008). Angiogenesis normally occurs in adult (e.g. wound healing) and during embryogenesis. However, angiogenesis is not always regulated properly, so that in many diseases such as cancers, psoriasis, age related macular degeneration, diabetes, proliferative retinopathies and rheumatoid arthritis misregulation of angiogenesis is seen (table 1) (Folkman 2007). Clinically speaking, improving and expanding knowledge regarding angiogenesis and in order to understand more about cellular and molecular mechanism which are involved in regulation and control the angiogenesis could lead scientists to provide more effective treatment for affected patients. This paper aims to address the most important factors involved in angiogenesis and present some current approaches impacting pathological angiogenesis in order to control the angiogenesis.

To date, many molecules have been recognized which are associated with the process of angiogenesis such as: vascular endothelial growth factors (VEGFs) and VEGF receptors (VEGFRs), immunoglobulin families (e.g. JAM-C) (Lamagna, Hodivala-Dilke et al. 2005), remodeling and guidance molecules including slit/robo, delta/notch, ephrin/eph (Huang, Xu et al. 2010), netrin/UNC-DCC (Freitas, Larrivee et al. 2008) and semaphorin/ plexin (Kim, Oh et al. 2011), extracellular matrix (ECM) proteins, such as collagens and fibronectin, adhesion molecules of the cadherin (VE and N-cadherin) (Gerhardt, Liebner et al. 1999) and integrin (e.g. αvβ3, α5β1) (Reardon, Neyns et al. 2011), homeobox gene products (e.g. HoxD3 and HoxB3) (Kodama, Sakai et al. 2009), transcription factors (e.g. HIF1a, NFjB) and inhibitors (e.g. Id1, 2), plasminogen activators/ inhibitors (uPA, PAI1) and 3 receptors(uPAR), matrix-degrading proteinases, in particular matrix metalloproteinases (e.g. MMP2, 9), MMP inhibitors (i.e. TIMPs) (Ruegg and Mutter 2007).

Angiogenesis has been considered as a multidisciplinary field of study for scientists. In other words, this subject encompasses various areas such as cell and molecular biology, physiology, experimental pathology, mouse genetics and drug development in order to understand the pathological and physiological aspect of angiogenesis (Ruegg and Mutter 2007). Imbalanced controlling angiogenesis can lead to aberrant blood vessel development (Gagne, Akalu et al. 2004). Even though angiogenesis therapy has been a target to treat cancer or many diseases related to angiogenesis process, in clinical trials the anticipated effects of anti- angiogenic therapy has been moderated (Wang, Cao et al. 2010).

1. Vascular endothelial growth factor (VEGF)

As mentioned earlier, there are various types of angiogenic factors which are correlated somehow in angiogenesis. However, vascular endothelial growth factor (VEGF) has been regarded as the most important factor in pathological and physiological status (Dong, Han et al. 2007). It has been stated that in approximately 30-60% of solid tumours VEGFs are expressed, while in other diseases (e.g. renal cell carcinoma) this number goes up to 100% (Longo and

Gasparini 2007). Detection of VEGFs routinely can be performed through various approaches such as ELISA and Immunohistochemistry (table 2).

The VEGF family is correlated with great number of diseases and consist of 6 members namely VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (PlGF). They are varied doe to either processing or alternative splicing. They bind to two types of receptors: VEGFRs and co-receptors (e.g. neuropilins) (Tugues, Koch et al. 2011). Among these six growth factors, VEGF-A is the most well characterized member so that due to alternate gene splicing, six isoforms of VEGF-A have been recognized (Sun and Schiller 2007) .VEGF promotes endothelial cell survival in newly formed vessels, stimulates endothelial cell proliferation (Wang, Li et al. 2008) and induces proteases which are engaged in the degradation of the extracellular matrix (ECM) required for endothelial cell migration (Sun and Schiller 2007). In addition, it functions as stimulator of vascular permeability (Zhang, Parangi et al. 2009).

There are three receptors in the family, VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1) and VEGFR-3 (Flt-4). VEGF-A, VEGF-B and PIGF are able to bind VEGFR-1(Sun and Schiller 2007) . Inactivation of VEGFR-1 or VEGFR-2 is likely to be cause of embryonic lethality demonstrating the vital role of those receptors in the development of the vascular system. It is believed that VEGFR-2 is the main mediator of the permeability enhancing effects of VEGF, angiogenic and mitogenic (Ferrara, Hillan et al. 2004).

VEGFR-1 plays an important role in angiogenesis development (Ferrara 2001). It has been suggested that VEGFR-1 acts as a regulator in angiogenesis process. Nevertheless, the exact function of the receptor has not been completely defined. The larger part of angiogenic effects of VEGF-A including, migration and survival, endothelial cell proliferation, invasion and enhanced vascular permeability is mediated by VEGFR-2 (Sun and Schiller 2007). It is believed that VEGFR-2 is the main regulator of angiogenesis (Dvorak 2002). It has been proved that tumour cells, circulating endothelial cells (CECs), intratumoural endothelial cells and endothelial progenitor cell (CEPs) express VEGFR-1 and VEGFR-2 (Longo and Gasparini 2007).

In adults, expression of VEGFR-3 is restricted to the lymphatic endothelium, while during development it is expressed in all endothelial cells (Tammela, Zarkada et al. 2008). It is also indicated that the expression of such receptors may be restricted to some fenestrated vascular endothelium in adolescents (Mouawad, Spano et al. 2009). Up regulation of VEGFR-3 can be seen in wounds and tumours in angiogenic blood vessels which might contribute in solid tumour growth and tumour angiogenesis (Petrova, Bono et al. 2008). However, in terms of angiogenesis, Zhang and colleagues show that VEGFR-3 ligand-binding is not necessary for angiogenesis but is an essential element for lymphangiogenesis (Zhang, Zhou et al. 2010)

2. Neuropilins and heparan sulphate as two co-receptors

Neuropilins and heparan sulphate (HS) are known as two types of VEGF co-receptores (Sorensen, Emblem et al. 2012). Neuropilin 1 (NP1) acts as a receptor for the heparin-binding isoforms of VEGF so that VEGF-A165 is presented to VEGFR2 by NP1, resulting in VEGFR-2 signalling (Ferrara, Hillan et al. 2004). While, neuropilin 2 (NRP2), which is one homologous of two homologous of NRP, acts as a receptor for class3 semaphorins which are involved with development of nervous and vascular system (Sorensen, Emblem et al. 2012) . NPR 1 binds PIGF, VEGF-B, while NRP2 binds VEGF-C and VEGF-A 145 (Klagsbrun, Takashima et al. 2002). Due to anti-angiogenic activity of semaphorins, It has been shown that semaphorins (Class III) may be considered as a target for anti-angiogenc therapies (Neufeld, Sabag et al. 2012).

Heparan sulphate receptors are complex polysaccharides which are involved in variety of biological functions such asmetastasis, angiogenesis and tumourogeneis (Wegrowski and Maquart 2004). Due to binding of extracellular matrix proteins, growth factors (e.g. VEGFs) and enzymes to heparin sulphates and consequently triggering chemical response (Dredge, Hammond et al. 2010), heparan sulfate receptors are potentially considered as co-receptors for VEGFs in angiogenesis. In mice with expression of VEGF-A isoform, association of heparan sulphate in regulating retinal vascular development has been investigated (Tugues, Koch et al. 2011).

3. Integrins

Integrins are heterodimeric transmembrane receptors and consist of α andβ chains. Although various integrins such as α1β1, α2β1, α3β1, α5β1, αvβ3, αvβ5 are associated in angiogenesis, the role of αvβ5, as many studies have shown, is crucially important in new vessel formation (Gagne, Akalu et al. 2004). Integrins have been studied as factors which affect anticancer therapy. Integrins antagonists can be combined with cytotoxic anticancer therapy by which cancerous and endothelial cells can be destroyed (Huveneers, Truong et al. 2007). The role of integrin family in angiogenesis is critical. Integrin family regulates various important function related to solid tumours such as initiation, progression and metastasis (Desgrosellier and Cheresh 2010). They are considered as a crucial target in cancer therapy (Jin and Varner

2004). Various functions in tumor cell are regulated owing to integrin signaling. These functions include proliferation, migration, survival and invasion of tumour. Moreover, it has been determined that there is an association between expression of specific integrin and increase in disease development, resulting in reduction in patient survival (Desgrosellier and Cheresh 2010). Proliferation and survival of integrin are prevented by integrin antagonist activities through disruption of binding extracellular matrix (ECM) to their receptors. As a result, tumourgenesis can be suppressed by such integrin antagonists (Huveneers, Truong et al. 2007). Therefore, it may be worth to investigate how integrin antagonists affect tumour, as well as its microenvironment

Debates about the role of integrins in angiogenesis are still controversial. Some believe that integrins activity can decrease angiogenesis if such receptors are blocked (Weis and Cheresh 2011), while others like Reynolds and colleagues support that integrins may play an opposite role in angiogenesis . They indicate that pathological angiogenesis is enhanced in mice lacking β3 and β5 integrins (Reynolds, Wyder et al. 2002). As many studies have proved, inhibition or blocking of integrins are affective strategies to cease the angiogenesis, Avaraamides and colleagues state that the ablation of αv integrins in mice has no effect in angiogenesis and present two possibilities accordingly. One is αv integrins functions as negative regulators of angiogenesis and second is angiogenesis occur in animal with lack of αv integrins as the result of compensatory changes in VEGF signaling (Avraamides, Garmy-Susini et al. 2008). Taken together, it seems that more investigations are required to understand the precise mechanism of such receptors.

4. Anti-angiogenic therapies and current challenges

There are three different ways that anti- angiogenic therapy may influence on tumour vessels: A) as the result of pruning of abnormal vessels, the structure of some remained vessels may be formed as normal vessel so that it leads to proper blood perfusion B) increasing necrosis, hypoxia and invasion due to excessive blood vessels abnormality and decrease in perfusion C) no effect. (Sorensen, Emblem et al. 2012). Additionally, three types of angiogenesis inhibitors mostly interact with growth factors (table 3) (Folkman 2007).Different anti-angiogenic agents are being used in clinical base. Table 4 and 5 show anti-angiogenic drugs in clinical development and clinical trials.

bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; HIF1α, hypoxia-inducible factor 1α; ID1, inhibitor of DNA binding 1, dominant negative helix–loop–helix protein; PDGF, platelet-derived growth factor; TIMP2, tissue inhibitor of metalloproteinase 2; TGFα, transforming growth factor-α; VEGF, vascular endothelial growth factor.

ALS, amyotrophic lateral sclerosis (or Lou Gehrig's disease); ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CLL, chroniclymphocytic leukaemia; CML, chronic myeloid leukaemia; CNS, central nervous system; GIST, gastrointestinal stromal tumour; MDS, myelodysplastic syndromes; NSCLC,non-small-cell lung cancer; NHL, non-Hodgkin's lymphoma; SCLC, small-cell lung cancer; VHL, von Hippel Lindau.

Gange and colleagues state that anti-angiogenic therapy provides many advantages such as elevated specificity for cancerous tissues. However, anti- angiogenic therapy is not "problem free", so that using such drugs can result to disruption of normal processes such as wound healing, pregnancy, the menstrual cycle, mucosal regeneration, bone remodelling may occur as consequences of anti-angiogenic therapy (Gagne, Akalu et al. 2004).

Treating patients with anti- angiogenic drugs may lead to some side effects such as hypertension, proteinuria, thrombosis,etc (Mitchell and Bryan 2010) (Table 6).

ECM, extracellular matrix; TF, tissue factor; TM, thrombomodulin; NO, nitric oxide; PGI2 , prostacyclin; VEGF, vascular endothelial growth factor.

Bergers and Hanahan propose that there are two modes of resistance to angiogenic therapy namely: pre-existing or intrinsic indifference and evasive resistance which is an adaption to circumvent the specific angiogenic blockade. Evasive (adaptive) resistance consists of accentuated invasiveness of tumour cells into local tissue to co-opt normal vasculature, revascularization consequent to upregulation of alternative pro-angiogenic signals, increased metastatic seeding and tumour cell growth in lymph nodes and distant organs, and protection of the tumour vasculature either by recruiting pro-angiogenic inflammatory cells or by increasing protective pericyte coverage (Bergers and Hanahan 2008). By contrast, in intrinsic resistance, even though molecular and cellular mechanism intrinsic resistance might be similar to previous resistance, it has been suggested that certain tumours seem to have a pre-existing tumour microenvironment which can transfer indifference due to treatment history, their stage of progression, host genotype or genomic constitution (Bergers and Hanahan 2008). A great number of patients (e.g., pancreatic cancer patients) are intrinsically resistant to anti- angiogenic therapy. A great deal of information from preclinical investigations has shown that the mechanism of resistance to anti- angiogenic drug in

evasive and intrinsic refractoriness is referred to the tumour cells themselves (Loges, Schmidt et al. 2010).

4.1. Anti-angiogenic drugs and chemotherapy

In order to enhance the effect of treatment in patients there is another strategy which is combination of anti VEGF drugs and chemotherapeutic agents. In this approach, anti-VEGF materials are responsible to normalize tumour vessels, resulting in improvement in blood flowing to tumours. Therefore, after normalisation of blood vessels, access of chemotherapeutic drugs to cancerous cell can be facilitated (De Bock, Cauwenberghs et al. 2011). It should also be noted that, however, anti- angiogenic drugs might close the blood barrier in tumour vessels, resulting in limitation in access of chemotherapeutic drugs to the tumours (van Kempen and Leenders 2006).In principal, the effects of chemotherapy should be decreased when an anti VEGF is administrated owing to reduction in drug supply by elimination of blood vessels (Sorensen, Emblem et al. 2012). For combination of anti- angiogenic drugs with chemotherapeutic drugs or radiation therapy, it is crucial to consider the mechanism of action of radiation or specific chemotherapeutic compounds (Gagne, Akalu et al. 2004). There is another point related to combination of anti-angiogenic therapy with chemotherapy which is about optimal scheduling such combination and its requirement to understand how long they remain in that state and the knowledge of the time window (the vessels are normalized) (van Kempen and Leenders 2006)

4.2. Molecular targets in angiogenesis

Tyrosine kinase inhibitors

Tyrosine kinases have been used as efficient therapeutic target to deal with angiogenesis process. Of many angiogenic receptors tyrosine kinase inhibitors (TKIs) can mention to Sarofenib and Sunitinib, which target VEGF path way and have been efficient to teat different types of cancers (Verheul and Pinedo 2007). One type of TKIs, for instance, is known as TKI-31. TKI-31 has been known as a tyrosine kinase inhibitor which inhibits c-Kit and c-Src on molecular base, platelet-derived growth factor receptor beta (PDGFRb), vascular endothelial growth factor receptor 2 (VEGFR2) (Zhong, Guo et al. 2006). A main problem correlated with progression of TKI is the "plethora of existing kinases". As a consequence, develop of drug for particular target is quite challenging which may lead to emerge of side effects (Ruegg and Mutter 2007). Another point related to TKIs which should be considered is about the relapse of disease in patients who undergo treatment with TKIs (Ellis, Hammers et al. 2009).

Peptide

High specificity and low toxicity of peptides have considered them as an crucial therapeutics which are being tested in diseases related to angiogenesis (Rosca, Koskimaki et al. 2011). Peptides also possess other advantages in this matter, including their solubility, lack of immune response in the host cell, stability and enhanced bio-avalibility. Of such peptides can mention to leucine rich repeat 5 of decorin, N terminal of parathyroid hormone, arginine rich N terminus of endostatin, pigment epithelium derived factor and peptides derived from type 1 repeat of thrombospondin, alpha 4 and beta 1 chains of laminin (Sulochana and Ge 2007). A research study, for instance, determines that P14 (residues 43-57 of PEDF molecule) and P18 (residues 39-57 of PEDF molecule) block endothelial cell chemotaxis. It also demonstrates that P 18 and P23 (residues 34-57 of PEDF molecule) stimulate apoptosis. This study proposes that P 18 can be regarded as novel agent for anti-angiogenic therapy for patients with renal and prostate cancer (Mirochnik, Aurora et al. 2009). Most of angiogenesis inhibitors are "endogenous short anti-angiogeneic peptides" such as tumstatin peptide, endostatin fragments and PF-4 peptide (Yi, Cho et al. 2009).

Monoclonal antibodies

One of the main advantages of monoclonal antibodies (mAbs) is their high specificity and binding their target with high affinity (Ruegg and Mutter 2007). Due to being produced of mAbs in mice, it is required that mAbs be humanised in order to decrease the risk of response from human immune system. In patients, antibodies are administrated every 2-3 weeks owing to their long half-lives (Ruegg and Mutter 2007). There are various research studies which have addressed the effect of monoclonal antibodies on angiogenesis. As an example, Nielsen and colleagues used a monoclonal antibody, Trastuzumab (Herceptin), which is used against extra cellular domain of the HER2 protein. This protein is involved in metastasis of breast cancer in women (Nielsen, Andersson et al. 2009). The exact mechanism of Trastuzumab as an anti-tumour drug has not been cleared yet. Nevertheless, in angiogenesis point of view, it has been stated that Trastuzumab reduce angiogenesis activity through activationg of angiogenic agents and reduction of VEGFs (Kaneda, Okamoto et al. 2012) . Monoclonal antibodies like other strategies have disadvantages. For example, a research study indicates that monoclonal antibody against PIGF has side effects in mouse due to minimal expression of PIGF of normal cells (unlike VEGF) (Kerbel 2008). Many of monoclonal antibodies have been approved by the FDA in order to be used in clinical trials (table 7) (Liu, Pop et al. 2008). Also, table (8) presents monoclonal

antibodies which are being tested in USA to be used for treatment of related diseases (Liu, Pop et al. 2008).

mAb, monoclonal antibody; IgG, immunoglobulin G; RCC, renal cell cancer; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; CTLA-4, cytotoxic T lymphocyte antigen-4; CTCL, cutaneous T-cell lymphoma; CLL, chronic lymphocytic leukemia; NHL, non-Hodgkin's lymphoma; Ep-CAM, epithelial cell adhesion molecule; MUC1, mucin receptor; CEA, carcinoembryonic antigen; IL-6, interleukin-6; CNS, central nervous system; PSMA, prostate-specific membrane antigen; Her-1, epidermal growth factor receptor; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; DR5, death receptor; TRAIL-R1, tumor necrosis factor apoptosis-inducing ligand receptor 1; HLA DRB, human leukocyte antigen (Liu, Pop et al. 2008). Another example of using monoclonal antibody with significant effects is using those antibodies in patients suffering non-Hodgkin's lymphoma (NHL) (Fanale and Younes 2007). For this type of patients Rituximab (anti-CD20 antibody) has respond well. Whereas, rituximab has been used as front line treatment of funicular lymphoma, and in patients with intermediate and aggressive B-cell lymphomas, rituximab can be combined with chemotherapy (Fanale and Younes 2007).

Of main issues related to optimising the efficacy of monoclonal antibody therapy can mention to duration of use, continuing to refine its optimal timing with chemotherapy, whether it should be continued at disease progression and maintenance therapy (Fanale and Younes 2007).

Table 1 Angiogenesis-dependent diseases (Taken from Folkman 2007).

Disease	Symptoms
Diabetic retinopathy	Loss of vision
Rheumatoid arthritis	Pain and immobility from destroyed cartilage
Atherosclerotic plaques	Chest pain, dyspnoea
Endometriosis	Abdominal pain from intraperitoneal bleeding
Crohn's disease	Intestinal bleeding
Psoriasis	Persistent severe itching
Uterine fibroids	Vaginal bleeding, abdominal pain
Benign prostatic hypertrophy	Urinary retention
Cancer	Bleeding, thrombosis, anaemia, abdominal ascites, bone pain, seizures from cerebral oedema around a tumour and others
Benign prostatic hypertrophy	Urinary retention

Table 2 Common methods which are used to detect vascular endothelial growth factor (VEGF) detection methods (Adapted from Longo and Gasparini 2007

Method and description	Comments
Immunohistochemistry (IHC) detects VEGF protein expression in whole tissue sections (usually formalin-Wxed, paraYn-embedded tissue)	Possible to diVerentiate between tumor and non-tumor VEGF expression Simple to perform Most common detection method No standardized methodology or scoring procedure Results variable and subjective
Enzyme-linked immunosorbent assay (ELISA) and chemiluminescence immunosorbent assay (ICMA) detect VEGF protein expression in tissue homogenate (fresh-frozen tissue), serum, or plasma	Serum and plasma measurements convenient vs. tissue samples Can be automated for high throughput Cannot distinguish between tumor and non-tumor sources of VEGF Circulating VEGF may be bound to serum proteins and unavailable to ELISA antibodies Serum measurements may be confounded by release of VEGF from platelets
Western blotting detects VEGF protein expression in tissue homogenate (fresh-frozen tissue)	Cannot distinguish between tumor and non-tumor sources of VEGF Less simple to perform than IHC
In situ hybridization (ISH) detects VEGF mRNA in whole tissue sections (ideally, fresh-frozen tissue)	Can distinguish between tumor and non-tumor VEGF expression May not relate directly to VEGF protein expression Less simple to perform than IHC
Northern blotting detects VEGF mRNA from tissue homogenates (fresh-frozen tissue)	Cannot distinguish between tumor and non-tumor VEGF expression May not relate directly to VEGF protein expression Less simple to perform than IHC
Reverse-transcription polymerase chain reaction (RT-PCR) detects VEGF mRNA in tissue homogenates (usually fresh-frozen)	Quantitative method that can be automated for high-throughput Cannot distinguish between tumor and non-tumor sources of VEGF Sensitive to contamination May not relate directly to VEGF protein expression
RNase protection assay detects VEGF mRNA in cellular extracts (tissue or circulating)	Cannot distinguish between tumor and non-tumor VEGF expression May not relate directly to VEGF protein expression Relatively complex to perform

Table 3 | Three types of angiogenesis inhibitors (Taken from Folkman 2007).

Mechanism	Drug	Action
Type 1 Blocks one main angiogenic protein	Avastin (Avastin; Genentech) B	Blocks VEGF
	VEGF Trap (Regeneron Pharmaceuticals)	Blocks VEGF
Type II Blocks two or three main angiogenic proteins	Sutent (Sutent; Pfizer)	Downregulates VEGF receptor 2, PDGF receptor, cKIT receptor
	Tarceva (Tarceva; Genentech, OSI Pharmaceuticals, Roche)	Downregulates VEGF production, bFGF production, TGF by tumour cell
Type III Blocks a broad range of angiogenic regulators	Endostatin	Downregulates VEGF, bFGF, bFGF receptor, HIF1α, EGF receptor, ID1, neuropilin Upregulates thrombospondin 1, maspin, HIF1α, TIMP2
	Caplostatin	Broad anti-angiogenic and anticancer spectrum

Table 4 Current anti- angiogenic agents in clinical development (Taken from Sun and Schiller 2007).

Drug	Target	Phase of development	Toxicities
Monoclonal antibodies Bevacizumab (Avastin; Genentech)	VEGF-A	Approved for advanced NSCLC and metastatic CRC; phase II SCLC in development	Hypertension, proteinuria, arterial and venous thromboembolic events,and hemorrhage
Tyrosine kinase inhibitors • SU11248 (Sutent,sunitinib; Pfizer)	VEGFR-1–3, PDGFR, c-kit, FLT-3	Approved for mRCC and metastatic GIST; phase II NSCLC ongoing	Fatigue, asthenia, rash, yellow skin discoloration, de pigmentation, neutropenia, hypertension, stomatitis
• PTK787 (vatalanib; Novartis)	VEGFR-1–3, PDGFR-β, c-kit, cFms	Phase II NSCLC	Fatigue, nausea/vomiting, dizziness, hypertension, ataxia, dyspnea
• ZD6474 (Zactima; Astra-Zeneca)	VEGFR-2, EGFR	Phase II/III NSCLC	Diarrhea, rash, hypertension, proteinuria, QTc prolongation, transaminitis
• AZD2171 (Astra-Zeneca)	VEGF-1–3	Phase II/III NSCLC	Fatigue, nausea/vomiting, diarrhea, abdominal pain, hemorrhage, hypoglycemia, hypertension
• BAY 93-4006 (sorafenib; Bayer)	Raf-1, VEGFR-2, -3, PDGFR, c-kit	Approved for mRCC; phase II NSCLC ongoing	Fatigue, diarrhea, transaminitis, skin toxicity, hypertension
• GW-786034 (Glaxo-Smith-Kline)	VEGFR-1–3	Phase I	Fatigue, nausea/vomiting, anorexia, hypertension, hair depigmentation
• CP-547,632(Pfizer)	VEGFR-2	Phase I	Fatigue, nausea/vomiting, diarrhea, rash, dry mouth
• AG-013736 (Pfizer)	VEGFR-1–3, PDGFR, c-kit	Phase II NSCLC	Fatigue, nausea, hypertension, transaminitis, seizure, pancreatitis, stomatitis, hemoptysis

NSCLC: non-small cell lung cancer; CRC: colorectal cancer; mRCC: metastatic renal cell carcinoma; GIST: gastrointestinal stromal tumor.

Table 5 | Anti-angiogenic drugs which are approved for clinical use and phase of clinical trials for other indications (Adapted from Folkman 2007).

Drug (Trade name;company)	Approved	Phase III	Phase II	Phase I
Bortezomib (Velcade; Millennium Pharmaceuticals)	Multiple myeloma (2003)	NSCLC, multiple myeloma, NHL	Multiple myeloma, NHL, NSCLC, lymphoma, gliomas, melanoma, Waldenstrom's macroglobinaemia, prostate, head and neck, breast, liver, nasopharyngeal, gastric, pancreatic, colorectal, cervical/vaginal cancer, and others	Lymphoma, myelodysplasia, multiple myeloma, NHL, solid tumours, head and neck, cervical, colorectal, ovarian, prostate cancer, and others
Thalidomide (Thalomid; Celgene Corporation)	Multiple myeloma (2003)	Multiple myeloma, brain metastases, SCLC, NSCLC, prostate, kidney, ovarian, hepatocellular	Soft tissue sarcoma, multiple myeloma, ALS,melanoma, neuroendocrine tumours, leukaemia,glioma, glioblastomas, paediatric	Solid tumours, glioma

		cancer	neuroblastoma, NSCLC, NHL, paediatric solid tumours, myelofibrosis, myelodysplastic syndrome, AML, CLL, SCLC, Hodgkin's disease, paediatric brain stem, liver, colorectal, kidney, neuroendocrine, endometrial,thyroid, uterine, ovarian cancer, and others	
Bevacizumab (Avastin; Genentech)	Colorectal cancer (2004), lung cancer (2006)	NSCLC, GIST, diabetic retinopathy, vascular occlusions, retinopathy of prematurity, colorectal, breast, ovarian, peritoneal, pancreatic, prostate, kidney cancer	Glioblastoma, glioma, mesothelioma, NSCLC, AML, CLL, CML, lymphoma, angiosarcoma, melanoma, billary tumours, SCLC, Kaposi's sarcoma, sarcomas, NHL, carcinoid, oesophagogastric, gastric, renal cell, head and neck, rectal, hepatocellular, bladder, pancreatic, gall bladder, breast, neuroendocrine, cervical, ovarian, endometrial cancer, and others	NSCLC, pancreatic, solid tumours, head and neck tumours, VHL, retinal tumours
Erlotinib (Tarceva; Genentech, OSI Pharmaceuticals, Roche)	Lung cancer (2004)	NSCLC, colorectal, pancreatic, ovarian, head and neck, oral cancer	NSCLC, mesothelioma, glioblastoma, glioma, gall bladder, GIST, biliary tumours, bladder cancer prevention, malignant peripheral nerve sheath tumours, endometrial, colorectal, pancreatic, breast, renal cell, prostate, ovarian, head and neck, gastric/oesophageal, liver cancer, and others	NSCLC, glioblastoma, solid tumours, colorectal, pancreatic, head and neck cancer
Pegaptanib (Macugen; OSI Pharmaceuticals)	Age-related macular degeneration (2004)			
Endostatin (Endostar)	Lung cancer (2005)			
Sorafenib (Nexavar;	Kidney cancer	Kidney, melanoma,	Melanoma, glioblastoma, GIST, SCLC, thyroid, neuroendocrine, mesothelioma, soft tissue	Solid tumours, melanoma, glioblastoma, NHL, glioma
Onyx Pharmaceuticals)	(2005)	hepatocellular cancer	sarcoma, NSCLC, CLL, multiple myeloma, cholangiocarcinoma, NHL, kidney, colorectal, prostate, ovarian, peritoneal, pancreatic, breast, gastric, head and neck, uterine, gall bladder, bladder cancer, and others	multiple myeloma, Kaposi's sarcoma, ALL, CML, MDS
Lenalidomide (Revlimid;Celgene Corporation)	Myelodysplastic syndrome (2005)	Multiple myeloma, myelodysplastic syndrome	NSCLC, NHL, multiple myeloma, CLL, myelofibrosis, myelodysplastic syndrome, glioblastoma, ocular melanoma, AML, mantle-cell lymphoma, Waldenstrom's macroglobinaemia, ovarian/ peritoneal, thyroid, prostate cancer	Multiple myeloma, prostate cancer, melanoma, myelodysplastic syndrome, solid tumours, paediatric CNS tumours
Sunitinib (Sutent; Pfizer)	GIST, kidney cancer (2006)	Renal cell cancer, GIST	Melanoma, VHL/solid tumour, NSCLC, GIST, hepatocellular, colorectal, prostate, breast, renal cell, gastric, neuroendocrine cancer, and others	Melanoma, solid tumours,colorectal, breast cancer
Ranibizumab (Lucentis; Genentech)	Age-related macular degeneration (2006)			

Table 6. Molecular mechanisms of toxicity of angiogenesis inhibition (Taken from Verheul and Pinedo 2007).

Toxicity	Possible underlying mechanism
Bleeding, disturbed wound healing	Platelet dysfunction; decreased expression of endothelial TF
Thrombotic events	Endothelial cell apoptosis; lack of endothelial cell renewal leading to exposure of the ECM to the circulating blood (results in platelet activation); increased TF expression; reduced TM and NO; direct platelet activation
Hypertension	Decreased NO and/or PGI2 production; inappropriate density of vessels (arterioles and capillaries); vascular stiffness; disturbed endothelin function
Hypothyroidism	Disturbed thyroid cell function; reduced vascularity of thyroid
Fatigue	Hypothyroidism
Proteinuria and oedema	Podocyte dysfunction owing to VEGF blockade; hypertension
Leukopenia, lymphopenia and immunomodulation	Inhibition of haematopoiesis and/or myelopoiesis; impaired dendritic cell function
Dizziness, nausea, vomiting and diarrhoea	Mucosa disturbance
Skin toxicity including rash and hand-foot syndrome	Epidermal cell apoptosis

Table 7. Monoclonal antibodies approved by the FDA for cancer therapy (Taken from Liu, Pop et al. 2008).

Generic (Trade mark)	Target antigen	Isotype	Species	Payload	Mechanism of action	Antitumor therapeutic activity	FDA year of approving
Rituximab (Rituxan™)	CD20	IgG1 k	Chimeric	–	Induction of apoptosis, ADCC, CDC,chemosensitization	Low grade B-cell NHL	1997
Trastuzumab (Herceptin™)	Her-2/neu	IgG1 k	Humanized	–	ADCC, chemosenzitation, CCA, inhibition of angiogenesis	Her-2 overexpressed metastatic breast cancer	1998
Alemtuzumab (Campath-1H™)	CD52	IgG1 k	Humanized	–	ADCC, CDC	B-cell CLL	2001
Cetuximab (Erbitux™)	EGFR (Her-1)	IgG1 k	Chimeric	–	Inhibition of angiogenesis, chemosensitization and radiosensitization, CCA, ADCC	Metastatic colorectal cancer, head and neck cancers	2004
Bevacizumab (Avastin™)	VEGF	IgG1 k	Humanized	–	Inhibition of angiogenesis	Colorectal cancer	2004
Panitumumab (Vectibix™)	EGFR	IgG2 k	Human	–	Inhibition of cell growth, induction of apoptosis, decreased proinflamatory cytokines and VEGF production	Metastatic colorectal cancer	2006
Gemtuzumab ozogamicin (Mylotarg™)	CD33	IgG4 k	Humanized	Calicheamicin	Double-stranded DNA breaks and cellular death induced by payload after intracellular hydrolysis	CD33+ relapsed AML	2000
Ibritumomab tiuxetan (Zevalin™)	CD20	IgG1 k	Murine	90-yttrium	Cellular death induced by β-emitter, induction of apoptosis, ADCC, CDC	Low grade or follicular, relapsed or refractory, CD20+ B-cell NHLs; Rituximab-refractory follicular NHL	2002
Tositumomab (Bexxar™)	CD20	IgG2a λ	Murine	131-iodine	Cellular death induced by γ-emitter, induction of apoptosis, ADCC, CDC	Relapsed CD20+ B-cell NHL; Rituximab-refractory NHL	2003

Table 8. Monoclonal antibodies currently undergoing human clinical testing for cancer therapy in the USA (Adapted from Liu, Pop et al. 2008)

Name	Target antigen	Isotype	Specie	Payload	Antitumor therapeutic activity	Clinical trial phase
WX-G250 (Rencarex™)	Carbonic anhidrase IX	IgG1	Chimeric	–	RCC stages I–III	III
Ipilimumab (MDX-010)	CTLA-4	IgG1 k	Human	–	Malignant melanoma stages I–III Metastatic pancreatic cancer	III II
Zanolimumab (HuMax-CD4)	CD4	IgG1 k	Human	–	Malignant melanoma stages I–III, CTCL	III
Ofatunumab (HuMax-CD20)	CD20	IgG1	Human	–	Drug resistant B-cell CLL, low grade or follicular, relapsed or refractory, CD20+ B-cell NHLs; Rituximab-refractory follicular NHL	III
ch14.18	Ganglioside GD2	IgG	Chimeric	–	Neuroblastoma	III
Zalutumumab (HuMax-EGFr)	EGFR	IgG1 k	Human	–	Head and neck cancer (squamous cell type), NSCLC stage III	III
Oregovomab (B43.13, OvaRex™)	CA 125	IgG1	Murine	–	Advanced varian cancer (epithelial adenocarcinoma type)	III
Edrecolomab (IGN-101, Panorex™)	17-1A (Ep-CAM)	IgG2a	Murine	Colorectal cancer		III
131I-chTNT-1/B (Cotara™)	DNA/histone H1 complex	IgG	Human	131-iodine	Solid tumors	III
Pemtumomab (R-1549,Theragyn™	MUC1	IgG1	Murine	90-yttrium	Ovarian cancer	III
Lintuzumab (SGN-33)	CD33	IgG1	Humanized	–	AML	III
Labetuzumab (hMN14, CEAcide™)	CEA	IgG	Murine	–	Colorectal cancer	III
Catumaxomab (Removab™)	Ep-CAM and CD3	Trifunctional bispecifc	Rat × mouse quadroma	–	Gastric adenocarcinoma, advanced ovarian cancer, malignant ascites	II
CNTO 328 (cCLB8)	IL-6	IgG1 k	Chimeric	–	Hormone refractory prostate cancer, relapsed or refractory multiple myeloma, advanced RCC	II
3F8	Ganglioside GD2	IgG3	Murine	131-iodine	CNS or leptomeningeal neoplasms	II/III
177Lu -J591	PSMA	IgG	Murine	177-	Metastatic androgen-	II
Nimotuzumab	EGFR (Her-1)	IgG1 k	Humanized	–	Metastatic colorectal cancer, lung cancer, metastatic pancreas cancer	II
SGN-30	CD30	IgG1	Chimeric	–	Relapsed or refractory Hodgkin's lymphoma, anaplastic large cell lymphoma	II
Ticilimumab (CP-675206)	CTLA-4	IgG2	Human	–	Metastatic melanoma	II
Daclizumab (Zenapax™)	CD25	IgG1	Humanized	–	Adult T-cell leukemia/lymphoma	II
Epratuzumab (hLL2, LymphoCide™)	CD22	IgG1	Humanized	–	Relapsed CD22+ ALL, NHL	II
90Y-Epratuzumab	CD22	IgG1	Humanized	90-yttrium	NHL	II
Galiximab (IDEC-114)	CD80	IgG1 λ	Chimeric	–	Relapsed or refractory NHLs	II
MDX-060	CD30	IgG1 k	Human	–	Relapsed or refractory Hodgkin's lymphoma	II
CT-011	B7	IgG	Humanized	–	Diffuse large B-cell lymphoma	II
CS-1008	DR5	IgG	Humanized	–	Metastatic pancreatic cancer	II
SGN-40	CD40	IgG	Humanized	–	Diffuse large B-cell lymphoma	II
Mapatumumab (TRM-1)	TRAIL-R1	IgG1	Human	–	Relapsed or refractory multiple myeloma, NHL, colorectal cancer, NSCLC.	II
Apolizumab (Hu1D10, Remitogen™)	HLA DRB	IgG1	Humanized	–	B-cell NHL, refractory CLL	II
Volociximab (M200)	α5β1 integrin	IgG4	Chimeric	–	Advanced ovarian cancer	II

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