Tumor angiogenesis as a therapeutic target

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Angiogenesis – the formation of new blood vessels within a tumor (or many other tissue types) – has become a hotbed of pharmacological research as well as industrial drug discovery. This is the result of the efforts of a generation of scientists elucidating the complex (patho)physiological, biochemical and molecular events accompanying angiogenesis. It is estimated that >300 drug candidates are currently in various stages of testing, and it is, therefore, impossible to capture all of this in a brief review. Therefore, the emphasis here is on relatively advanced projects that are either in preclinical or clinical development, thus neglecting, to a large extent, the many exciting avenues being pursued in both academic and biotechnology laboratories. Although the potential of the approaches described cannot be overestimated, it is also important to note that there is still no drug on the market that achieves clinical benefit based on a selective modulation or inhibition of angiogenesis.

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▼ Tumor angiogenesis – the formation of new blood vessels within a tumor – is currently one of the most active, if not the most active, field in cancer medicine[1–6]. Its attractiveness stems from the fact that tumor angiogenesis is provoked by a tumor responding to hypoxia. Furthermore, in its downstream consequences it is mostly a host-derived process, thereby avoiding the formidable hurdles of other anticancer therapeutic modalities, such as the diversity of cancer types, heterogeneity within a given tumor, genetic or chromosomal instability and drug resistance. Tumor angiogenesis is, therefore, thought to be comparable for a large variety of solid cancers. If this is correct, in the majority of situations, elements such as the activated endothelial cell, remodelling of the basement membrane, and pericyte function can be approached in a systematic fashion, and therapeutic approaches can be designed that are based on a rapidly increasing body of evidence regarding the biology of tumor angiogenesis.

Therapeutic approaches

It is useful to divide therapeutic approaches based on tumor angiogenesis into two major classes[7]: (1) vasculostatic agents are agents that interfere with the process of forming new blood vessels, whereas (2) vasculotoxins comprise agents that use elements of newly formed blood vessels to target toxic principles, destroying these and thereby producing antitumor effects. Among the vasculostatic approaches, there are certain target families that are receiving a great deal of attention from many workers:

- ligand–receptor families that regulate neovascularization, primarily at the level of the endothelial cell, and which can be approached via various modalities, including small molecules (such as inhibiting kinase activity of receptor tyrosine kinases)[8];
- antibodies (blocking the receptors or neutralizing the ligands);
- soluble receptors (neutralizing the ligands);
- antisense molecules;
- gene therapy approaches proposing the use of antisense moieties; and
- dominant-negative mutants of receptors.

Many of these modalities are currently being tested in patients, and some are in advanced clinical trials. For example:

- the constantly growing metalloproteinase family that has a fundamental role in tissue and vessel remodelling accompanying angiogenesis. Also, this second target family has been the subject of intense activity by several academic and industrial competitors. This field has recently been dealt setbacks and several clinical projects have had to be terminated;
- stimulatory or inhibitory cytokines and proteinaceous endogenous inhibitors of angiogenesis, including interferons, angiostatin, endostatin and several others.

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Box 1. Complications in the angiogenic process

A complication in the angiogenic process is the fact that several other cytokines can also strongly influence angiogenesis[a]. For several years positive effects have been described for:

- Acidic and basic fibroblast growth factor (FGF-1, FGF-2) reacting with FGF-receptors (several isoforms)[b];
- Platelet-derived growth factor (PDGF) (AA, AB, BB), switching on PDGF receptors (α, β) (Ref. c);
- Thymidine phosphorylase (TP) (Ref. d), also known as PD-ECGF (platelet-derived endothelial-cell growth factor[e], homologous to TP;
- Hepatocyte growth factor (HGF) or Scatter factor[f,g];
- Interleukin-1 (IL-1) (Ref. h), IL-6 (Ref. i);
- IL-8 (representative of the angiogenic family of ELR$^+$CXC chemokines[j,k]);
- Angiogenin (ribonuclease A homologue)[l];
- Placenta growth factor (PIGF) (Ref. m);
- Insulin-like growth factor (IGF-I) (Refs n,o);
- Transforming growth factor (TGF)β family members[p];
- Tumor necrosis factor-α (at low doses[q,r]).

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Interferons have been clearly shown as active, albeit in narrow clinical indications, and other molecules have just entered, or are about to enter, the clinical arena;

- integrins, cadherins and other differentiation molecules at the endothelial surface that can be modulated in their activity by small molecules, peptidic structures and antibodies; and
- many other targets that are mostly defined by the anti-angiogenic activity exerted through a given molecule or drug candidate.

The formation of new blood vessels and their permeability is primarily regulated by (tumor-derived) vascular endothelial growth factor (VEGF), which acts via at least two different receptors (R; Ref. 9):

- VEGF-R1 (fms-like tyrosine kinase, Flt-1); and
- VEGF-R2 (kinase domain region, KDR/fetal liver kinase-1, Flk-1).

VEGF, and more specifically VEGF-A, exists in the human species in three isoforms (through alternative splicing), which are named according to the number of amino acid residues: $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$. These isoforms have distinct functional properties in terms of heparin binding and diffusibility. A related factor, placenta growth factor (PIGF), only binds VEGF-R1/Flt-1.

The membrane-bound VEGF receptors occur on the surface of activated endothelial cells and possess an intracellular tyrosine-kinase domain, which is necessary for intracellular signal transduction. It is thought that the VEGF dimer induces, upon binding, a dimerization of two receptor molecules, leading to autophosphorylation of the intracellular portion of the receptors and subsequent binding of SH2-containing proteins. Subsequent phosphorylation (activation) of phospholipase Cγ, phosphatidylinositol-3 kinase and Ras GTPase-activating protein (GAP) has been demonstrated[8].

There is an additional VEGF-R3 (Flt-4), which primarily regulates lymphangiogenesis and is activated by the newly discovered VEGF-C and D (which also both have weakly angiogenic activities)[9].

Recently, a second family of angiogenesis regulators – angiopoietins – have joined the VEGF family members. The angiopoietins bind to the Tie receptors (Tie-1, Tie-2; Ref. 10), which also transmit their signals via an intracellular tyrosine-kinase domain. There are two types of angiopoietins:

- Angiopoietin-1 (Ang-1; Ref. 11), which is required – in the presence of VEGF – for later stages of vascular remodeling, vessel maturation and stabilization. Ang-1 (and also the less-well understood Ang-4) have an agonist role at the level of the Tie-2 receptor;
- Angiopoietin-2 (Ang-2; Ref. 12), an antagonist for Ang-1 (at the Tie-2 receptor level), similar to its less-well characterized relative Ang-3.

Ligands for Tie-1 have not been described so far. The discovery of this second family of ligands and their receptors has led to a refined understanding of tumor angiogenesis. Tumors might not simply switch on the process of novel blood-vessel formation, but rather co-opt existing vessels and form a well-vascularized mass[13]. This initially co-opted vasculature then regresses, surprisingly, via vessel regression, leading to massive tumor apoptosis. At the periphery, however, the tumor can recover by initiating angiogenesis.

The process of angiogenesis, in adult neovascularization and in tumor angiogenesis, are currently understood as follows[14–16]: angiogenesis is primarily mediated by VEGF, driving endothelial cell proliferation, migration and tube formation. Subsequently, Ang-1, in physiological situations, leads to vessel maturation and stabilization. Such stabilized vessels can be destabilized, however, by Ang-2, and in the presence of VEGF a new round of angiogenesis can begin; in the absence of VEGF, vessel regression would ensue.

The balance of at least two biological systems (VEGF–VEGFR and Ang–Tie) regulate, in concert, the outcomes of vessel formation and vessel regression, and it is obvious that these complexities must be taken into account when designing and developing anti-angiogenic agents.

A third family comprising the eph receptors and their ligands, the ephrins, has joined the above receptor–ligand families. The various members of this new family appear to differentially regulate the interactions between arterial and venous endothelial cells. Because these receptors and ligands have not yet led to publicized drug discovery efforts, the reader is referred to these two reviews[17,18]. Such efforts could well be underway, however, benefiting from precise structural information on EphB2 receptors[19,20].

Box 2. Negative regulation of angiogenesis

Angiogenesis is negatively regulated by the following endogenous inhibitors[a]:

- Interferon-α, interferon-β;
- Platelet factor 4 and the interferon-γ inducible IP-10 gene (representatives of the angiostatic family of ELR⁻CXC chemokines[b,c]);
- IL-12 (Ref. d), upregulating interferon-γ and IP-10;
- Thrombospondin[e];
- Tissue inhibitors of metalloproteinases (TIMP 1, 2, 3, 4);
- Plasminogen activator inhibitors (PAI-1, -2);
- Angiostatin (internal fragment of plasminogen);
- Endostatin (C-terminal fragment of collagen XVIII);
- Canstatin (fragment of the α2 chain of collagen type IV);
- Antiangiogenic antithrombin (aaAT);
- Metallospondins (METH-1, METH-2) (Ref. f);
- Pigment epithelium-derived factor (PEDF) (Ref. g).

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In addition, and separately from the regulatory circuitry described in Boxes 1 and 2, the highly differentiated family of metalloproteinases (MMPs) and their natural inhibitors (tissue inhibitors of MMPs; TIMPs) have been shown to strongly influence angiogenesis[21,22]. This growing family of enzymes currently comprises 19 members, numbered MMP-1–MMP-19. Of particular interest are MMPs that digest non-fibrillar collagens, such as fibronectin, laminin (e.g. MMP-2 and -9), as well as four membrane-type MMPs (e.g. MT1-MMP) digesting progelatinase and activating MMP-2. Tissue remodeling is deemed to have a significant role in angiogenesis, invasiveness of tumors and their metastatic spread, and it was, therefore, logical to speculate that inhibitors of such enzymes might have beneficial effects.

Indeed, several broad-spectrum, small molecular-weight inhibitors of these enzymes have been demonstrated to

Table 1. Vascular endothelial growth factor tyrosine-kinase inhibitors

Compound	Mechanism of action	Stage of development	Company	Refs
SU5416	Inhibition of VEGF-R2 (IC_{50} = 200 nM)	Phase III (in Kaposi's sarcoma) and Phase I/II (metastatic colon cancer)	Sugen, Redwood City, CA, USA (subsidiary of Pharmacia-Upjohn, Bridgewater, NJ, USA)	–
PTK787/ ZK222584	VEGF-R2 inhibitor (IC_{50} = 97 nM) PDGF-R inhibitor (IC_{50} = 200 nM)	Phase I/II	Novartis Pharma AG, Basel, Switzerland	–
PD173074	Potent FGF-R1 antagonist; also has inhibitory activity against VEGF-R; inhibitor of bFGF and VEGF-induced angiogenesis	Preclinical development	Parke-Davis, Plymouth, MI, USA	–
SU6668	Potent PDGF-, VEGF- and FGF-receptor tyrosine-kinase inhibitor with anti-angiogenic activity; orally active	Phase I	Sugen, Redwood City, CA, USA (subsidiary of Pharmacia-Upjohn)	–
ZD6474	VEGF-receptor tyrosine-kinase inhibitor	Preclinical development	AstraZeneca, London, UK	Website[a] AstraZeneca, R&D presentation, 6 December, 1999
ZD4190	Substituted 4-anilinoquinazoline; KDR tyrosine-kinase inhibitor of the quinazoline type (IC_{50} ~ 50 nM); oral activity against Calu-6 lung carcinoma xenograft and other tumors at 100 mg kg^{-1}	Discontinued (website[a] AstraZeneca, R&D presentation, 6 December, 1999)	AstraZeneca, London, UK	104,105
CP564959, CP547632	Tyrosine-kinase inhibitors; anti-angiogenic agents	Preclinical development	Pfizer, Groton, CT, USA and OSI Pharmaceuticals, Uniondale, NY, USA	–
GW2286	VEGF-receptor tyrosine-kinase inhibitor	Preclinical	GlaxoSmithKline, Philadelphia, PA, USA and Research Triangle Park, NC, USA	106
Several compounds (see Fig.1)	VEGF-receptor tyrosine-kinase inhibitor	Preclinical	Merck Research Laboratories, West Point, PA, USA	107

Abbreviations: FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; KDR, kinase domain region
[a]http://www.astrazeneca.com

have dose-dependent anti-angiogenic effects. Whether the antitumor effects that have been noted are a result of, wholly or partly, these anti-angiogenic effects is far from clear. In experimental systems, an effect on invasion and metastasis has been demonstrated unequivocally. Tolerability of such broad-spectrum inhibitors in the clinics is mainly limited by musculoskeletal side effects, which are possibly because of the large spectrum of MMPs inhibited. The debate is far from closed on whether MMP-1, sheddase or any other MMPs are crucial targets, and more selective inhibitors are now emerging that allow various hypotheses to be put to the test (discussed later).

Finally, it has been recognized that the dynamics of angiogenesis and metastatic processes are also mediated, and to a large extent, by several additional surface receptors[23]. Many years ago it was recognized that integrins (most importantly $\alpha_v\beta_3$ and $\alpha_v\beta_5$) are important players in the angiogenic cascade[24,25]. An interesting bifurcation of the angiogenic cascade has been noted: basic fibroblast growth factor (bFGF) primarily induced $\alpha_v\beta_3$

Table 2. Metalloproteinase inhibitors[a]

Compound	Mechanism of action	Stage of development	Company	Refs
Clinical agents				
Marimastat (BB2516)	Broad spectrum metalloproteinase inhibitor	Phase III, most indications discontinued (March 2001)	British Biotech, Oxford, UK and Schering-Plough, Kenilworth, NJ, USA	–
AG3340 (Prinomastat)	Broad spectrum metalloproteinase inhibitor (less active against MMP-1) stage IV NSCL – discontinued (May 2001)	Phase III trials in advanced hormone refractory prostate cancer and F. Hoffmann-La Roche, Basel, Switzerland	Agouron, La Jolla, CA, USA (subsidiary of Warner-Lambert) and	–
Bay129566	More selective metalloproteinase inhibitor (no MMP-1 inhibitory activity)	Clinical development stopped	Bayer, West Haven, CT, USA	–
BMS275291 (D2163)	Metalloproteinase inhibitor without activity against sheddase	Phase I	Chiroscience R&D, Cambridge, UK and Bristol-Myers Squibb, New York, NY, USA	108
CGS27023A (MMI270B)	Broad spectrum metalloproteinase inhibitor	Phase I/II (on hold)	Novartis Pharma AG, Basel, Switzerland	–
Metastat (COL3, NSC683551)	Metalloproteinase inhibitor	Phase I (?)	CollaGenex Pharmaceuticals, Newtown, PA, USA	109,110
Selected examples of preclinical agents				
BPHA	Selective sulfonamide MMP inhibitor (potent activity against MMP-2 and -9, but not MMP-1, -3, -7)	Preclinical	Shionogi Pharmaceuticals, Osaka, Japan	111
BB3644	Broad spectrum metalloproteinase inhibitor plus sheddase inhibition	Preclinical	British Biotech, Oxford, UK	Oral presentation[b]
D1927	Follow up for BMS 275291	Preclinical	Chiroscience R&D, Cambridge, UK and Bristol-Myers Squibb, New York, NY, USA	
CH4815	Broad spectrum MMP inhibitor	Preclinical	Chiroscience R&D, Cambridge, UK	
OPB3206	Metalloproteinase inhibitor with activity against MMP-1, -2 and -9	Preclinical	Otsuka Pharmaceutical Co. Tokyo, Japan	112

Abbreviations: MMP, matrix metalloproteinase; NSCL, non-small-cell lung.
[a]See Fig. 2 for structural formulae of some of these molecules.
[b]At *Therapeutic Control of Angiogenesis,* 17–18 January 2000, London, UK.

integrins on the surface of endothelial cells, whereas VEGF primarily induced $\alpha_v\beta_5$ (Ref. 26). These findings are at the roots of integrin-directed anti-angiogenic agents (discussed later). Cadherins, E-selectins and Ig superfamily adhesion molecules (in particular ICAM-1, -2 and -3, and VCAM-1) have been added in later years. These receptor families and their ligands offer many potential approaches, both in terms of vasculostatic and vasculotoxic approaches, as discussed in the remainder of this article. This whole area has been reviewed quite extensively[27].

Table 3. Other vasculostatic small molecular-weight compounds[a]

Compound	Rationale	Stage of development	Company	Refs
Doxorubicin, Paclitaxel	Anti-angiogenic activity exerted probably through antimitotic activity on endothelial cells	Marketed	Pharmacia-Upjohn, Peapack, NJ, USA and Bristol-Myers Squibb, New York, NY, USA	113
Non-steroidal anti-inflammatory agents	Indomethacin; NS398 (selective COX-2 inhibitor; PD98059 (selective inhibitor of the MAPK kinase MEK) are all potent inhibitors of tube formation *in vitro*, inhibitors of bFGF- and VEGF-induced ERK2 activity	Indomethacin commercially available; NS398 and PD98059: no development reported		114
Retinoids (Retinol, retinoic acid and derivatives)	Angiogenesis inhibitors; mediators of extracellular inactivation of MMP-2 by upregulation of TIMPs; modulators of CAM expression			115–119
EMD121974 (a_vb_3 antagonists)	Small molecular-weight antagonist of integrins of tumor endothelial cells suppressing angiogenesis	Phase II/III	Merck KgaA, Darmstadt, Germany; many other companies active [Merck, Hoechst Marion Roussel, G.D., Searle, Du Pont Pharmaceuticals, Wilmington, DE (SM256), SmithKline Beecham Pharmaceuticals.]	120–122
Zoledronate (Zometa®)	Bisphosphonate; bone resorption inhibitor with anti-angiogenic properties	Phase III	Novartis Pharma AG, Basel, Switzerland	123–125
IM862	Small peptide upregulating IL-12; inhibiting VEGF and bFGF production	Phase III (in Kaposi's sarcoma), Phase II in ovarian and metastatic melanoma	Cytran, Kirkland, WA, USA	126,127
Thalidomide	Inhibitor of bFGF- and VEGF-induced angiogenesis; inhibitor of TNFα	Phase II (in several cancer indications)	Celgene Corporation, Warren, NJ, USA	–
Linomide® (quinoline-3-carboxamide, Roquinimex)	Angiogenesis inhibitor with antimetastatic activity; inhibits endothelial cell migration and invasion	Phase II in several cancer indications; multiple sclerosis clinical trials suspended in 1997 because of myocardial infarctions	Pharmacia-Upjohn, Peapack, NJ, USA	128–131
CGP41251 (PKC412)	Benzoylated staurosporine derivative; protein kinase C inhibitor; inhibitor of angiogenesis *in vitro* and *in vivo*; orally active against a wide spectrum of murine tumors and human xenografts in nude mice	Phase IIa	Novartis Pharma AG, Basel, Switzerland	132,133
AGM1470 (TNP470)	*O*-(chloro-acetyl-carbamoyl)-fumagillol; anti-angiogenic agent with several proposed molecular targets	Phase I/II/III	TAP Pharmaceuticals (Takeda-Abbott), Deerfield, IL, USA	–
CAI (L 651,582; NSC609974)	Carboxyamido triazole; calcium influx inhibitor; inhibitor of the growth and invasion of head and neck squamous cell carcinoma	Phase II	National Cancer Institute, Bethesda, MD, USA/ Merck, Whitehouse Station, NJ, USA	134–136
Suramin (CI1003, Metaret)	Polysulfonated naphtyl urea compound; inhibits tumor angiogenesis and tumor growth through binding of several angiogenic proteins (bFGF, VEGF, HGF)	Phase II – preregistration (in prostate cancer)	Warner-Lambert, ACG, Osseo, MN, USA	137–142

Table 3. Other vasculostatic small molecular-weight compounds (continued)

Compound	Rationale	Stage of development	Company	Refs
Pentosan polysulfate (SP54®)	Anticoagulant; inhibition of angiogenesis through binding of growth factors (FGF)	Phase I/II	Lombardi Cancer Center, Georgetown University, Washington DC, USA	143–145
Squalamine	Angiostatic steroid; natural aminosterol purified from the dogfish shark	Phase I	Magainin Pharmaceutical, Plymouth Meeting, PA, USA	–
TAS102 (FTD plus TPI)	5-Trifluorothymidine (FTD) plus 5-chloro-6-(2-imino-pyrrolidin-1-yl) methyl-2,4 (1*H*,3*H*)-pyrimidinedione hydrochloride (TPI, an inhibitor of thymidine phosphorylase) in 2:1 ratio, as inhibitor of angiogenesis	Phase I	Taiho, Pharmaceutical, Saitama, Japan	146,147
NSC639366 (SPC100097, Adiaminoanthra-quinone) and analogs	Protein kinase C inhibitor; inhibits endothelial cell proliferation and migration and angiogenesis in the CAM assay	Preclinical research	Eli Lilly, Indianapolis, IN, USA	148,149
Thalidomide analogs	Derived from active metabolites of thalidomide (epoxides); Inhibitor of bFGF- and VEGF-induced angiogenesis	Preclinical development	EntreMed, Rockville, MD, USA	150 (Fig. 3)
2-Methoxy-estradiol (2-ME)	Inhibitor of bFGF- and VEGF-induced angiogenesis, microtubule inhibitor, inducer of endothelial cell apoptosis via activation of stress-activated protein kinase	Preclinical development	EntreMed, Rockville, MD, USA	151–153
ZD6126	Vascular targeting agent disrupting tubulin of growing endothelial cells	Preclinical development (Phase I?)	AstraZeneca, London, UK	Website[b] AstraZeneca, R&D presentation, 6 December, 1999
TAS202	Compounds based on the natural products magnosalin and magnoshinin; potent orally active angiogenesis inhibitors	Preclinical	Taiho, Pharmaceutical, Saitama, Japan	
NX1838	2′-Fluoropyrimidine RNA-based aptamer to VEGF165 with anti-angiogenic activity and antitumor activity in mice (same technology pursued also against PDGF and TGF-β)	Preclinical	NeXstar Pharmaceuticals, Boulder, CO, USA	154,155
PPARγ agonists	Natural ligand (15-deoxy-D12, 14-prostaglandin J2), BRL49653 and ciglitizone exert, among many other activities, powerful anti-angiogenic effects through inhibition of differentiation and proliferation of endothelial cells	Preclinical research	Genentech, South San Francisco, CA, USA	156
Tecogalan sodium (DS4152)	Sulfated polysaccharide-peptidoglycan complex inhibiting binding bFGF to its receptor	Discontinued	National Cancer Institute, Bethesda, MD, USA and Daiichi Pharmaceutical Corp., Fort Lee, NJ, USA	157,158

Abbreviations: CAM, chorioallantoic assay; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; VEGF, vascular endothelium growth factor.

[a]See Fig. 3 for structural formulae of some of these molecules.

[b]www.astrazeneca.com

Table 4. Antibodies with vasculostatic properties

Antibody	Mechanism of action	Stage of development	Company	Refs
Monoclonal anti-VEGF antibodies (RhuMAb Anti-VEGF)	Neutralization of VEGF	Phase II/III (in NSCLC and colorectal in combination with standard chemo-therapy and in breast/ renal carcinoma as single agent)	Genentech, South San Francisco, CA, USA	159–162
Vitaxin™	Humanized antibody against $\alpha_v\beta_3$-integrin (murine parent antibody = LM609)	Phase II	The Scripps Research Institute, La Jolla, CA, USA; MedImmune, Gaithersburg, MD, USA and Ixsys, La Jolla, CA, USA	163,164
ABXIL8	Humanized anti-IL-8 antibody	Phase I	Abgenix, Fremont, CA, USA	–
IMC-1C11	IMC-1C11: chimerized anti-VEGF-R2 (KDR) monoclonal antibody based on single chain Fv antibody technology	Phase I	ImClone Systems, New York, NY, USA	165–168
DC101	DC101: anti-Flk-1 monoclonal antibody with potent anti-angiogenic activity against murine tumors and human xenografts in nude mice	Preclinical	ImClone Systems, New York, NY, USA	

Vasculostatic approaches based on small molecular-weight compounds

Vasculostatic approaches based on small molecular-weight compounds can be grouped conveniently into five classes:

- VEGF-R tyrosine kinase inhibitors (Table 1);
- metalloproteinases: small molecular-weight inhibitors (Table 2);
- other, small molecular-weight compound-based approaches (Table 3);
- antibodies with vasculostatic properties (Table 4);
- peptides and proteins with vasculostatic properties (Table 5);
- targeted approaches (vasculotoxins) (Table 6);
- gene therapy approaches (Table 7).

Several comprehensive and up-to-date reviews on anti-angiogenic therapy for cancer have recently been published[28–31].

VEGF-R tyrosine-kinase inhibitors

The important role of VEGF and its receptors in tumor-mediated angiogenesis has been validated by the early work of Kim and colleagues[32], working with anti-VEGF antibodies. It was, therefore, relatively obvious to search for inhibitors of the tyrosine kinase domain of VEGF-R1 and -2, building on the many years of experience acquired in the field of tyrosine kinase inhibitors in general[33–39]. This approach could also benefit from structural evidence because the group at Agouron had achieved the crystallization of human KDR (Ref. 37).

More recently, the group at Merck Research Laboratories showed the functional importance of valine in position 848 of KDR (rather than the previously reported glutamic acid residue)[40]. In addition, they demonstrated activation of KDR by autophosphorylation of two sites (tyrosine residues 1054 and 1059) in the activation loop of the catalytic site. The K_is of various inhibitors can vary dramatically, whether determined on the activated or unactivated form of the enzyme.

SU5416

The most advanced VEGF-R2 inhibitor is SU5416, which is in Phase III clinical trials. SU5416 is a potent ATP-mimetic VEGF-R2 inhibitor[41], both on the enzyme itself (IC_{50} =1.23 μM) and in cellular systems measuring receptor autophosphorylation (IC_{50} =1.04 μM). It also shows good selectivity with regards to EGF- and insulin-receptor tyrosine kinases and only weak activity on PDGF-receptor kinase (IC_{50} =20.26 μM). Surprisingly, VEGF-driven mitogenesis in human umbilical vein endothelial cells (HUVEC) is inhibited at much lower concentrations (IC_{50} =0.04 μM) than autophosphorylation of the receptor, which could be explained by inhibition of additional (PDGF-receptor, Flt-1 and/or unknown) targets. SU5416 inhibited dose-dependent A375 tumor growth almost completely at tolerated doses. A panel of tumor lines,

Table 5. Peptides and proteins with vasculostatic properties

Peptide/protein	Mechanism of action	Stage of development	Company	Refs
Interferon α, β	Angiogenesis inhibition clearly demonstrated in the clinic (hemangioendothelioma); inhibition of bFGF by downregulating its mRNA	Commercially available	IFN-α: Schering-Plough, Kenilworth, NJ, USA and F. Hoffmann-La Roche, Basel, Switzerland	169–171
Octreotide	Somatostatin analog acting via inhibition of IGF-I- and bFGF-stimulated growth of human endothelial cells; inhibition of corneal neovascularization	Commercially available	Novartis Pharma AG, Basel, Switzerland	172–175
Interleukin-12	Inhibition of angiogenesis through upregulation of IFN-γ and IP-10	Phase I	Genetics Institute, Cambridge, MA, USA	
Endostatin™	Not yet elucidated	Phase I	EntreMed, Rockville, MD, USA	–
Angiostatin™	Not yet elucidated; might involve binding of angiostatin to ATP synthase	Phase I	EntreMed, Rockville, MD, USA	–
Soluble, truncated Flt-1 (sFLT-1)	Neutralization of VEGF and inhibition of VEGF-mediated endothelial cell proliferation, inhibition of corneal neovascularization and antitumor efficacy in mice (R3230AC carcinoma)	Preclinical	Merck, Whitehouse Station, NJ, USA	176,177
TIMP	Endogenous inhibitor of MMPs	Preclinical	Amgen, Thousand Oaks, CA, USA	–
TIMP-2	MMP inhibitor	Preclinical	Aronex Pharmaceuticals, The Woodlands, TX, USA	–
Troponin-I	22 kDa angiogenesis inhibitor isolated from cartilage. Inhibitor of FGF- and VEGF-driven endothelial cell proliferation and angiogenesis in the CAM assay	Preclinical	Boston Life Sciences, Boston, MA, USA	178
AaATIII	Antiangiogenic antithrombin (cleaved serpin antithrombin); strongly growth inhibitory for bovine and human endothelial cells; efficacious against SK-NAS human neuroblastoma xenografts and murine Lewis lung carcinoma	Preclinical	Genzyme Molecular Oncology, Framingham, MA, USA	179
Canstatin	Human, 24 kDa endogenous matrix-derived inhibitor of angiogenesis, fragment of α-2 chain of type IV collagen; FLIP downregulation; antitumor effects (PC-3) comparable to Endostatin	Preclinical	ILEX Oncology, San Antonio, TX, USA	180
Inhibitors of Id1 and Id3	Two (intracellular) proteins involved in differentiation and cell cycle progression	Preclinical	Angiogenex, New York, NY, USA	181

Abbreviations: IFN, interferon; IGF, inhibitor growth factor; FGF, fibroblast growth factor; MMP, matrix metalloproteinase; TIMP, tissue inhibitor matrix metalloproteinase; VEGF, vascular endothelial growth factor.

Table 6. Targeted approaches (vasculotoxins)

Compound	Mechanism of action	Stage of development	Company	Refs
5,6-Dimethyl-xanthenone-4-acetic acid (DMXAA, NSC640488)	Induction of hemorrhagic necrosis	Phase I	–	182,183
Combreta-statin A4 phosphate (CA4-P)	Plant extract (*Combretum caffrum*); selectivity for proliferating endothelial cells (prodrug?); disruption of actin/tubulin cytoskeleton leading to vessel occlusion; strong synergy with NO-synthase inhibitor; blood flow reduction demonstrated in a variety of solid tumors (proof of principles)	Phase I/II (intravenous, weekly infusions)	OXiGENE, Boston, MA, USA and Bristol-Myers Squibb, New York, NY, USA	184
CM101	Group B streptococcus polysaccharide exotoxin (GBS); binding to CM101 receptor HP59 (7tm receptor) on dedifferentiated endothelial cells; induction of apoptosis of tumor via complement-mediated local inflammation	Phase I	CarboMed, Brentwood, TN, USA	185
Exherin	Disruption of cadherins	Preclinical research	Adherex Technologies, Ottawa, Ontario, Canada and Fujisawa USA, Grand Island, NY, USA	186
Vasculature-targeted chemotherapy	Targeted RGD and other peptides linked to a cytotoxic agent (such as doxorubicin), binding to $\alpha_v\beta_3/\alpha_v\beta_5$ integrins of tumor endothelial cells	Preclinical development	Cancer Research Center, The Burnham Institute, La Jolla, CA, USA and Bristol-Myers Squibb, New York, NY, USA	187–189
TEC110 (coaguligand approach)	Tumor infarction by antibody-directed (truncated) tissue factor to tumor vasculature; vascular targeting towards VEGF/VEGF-R complex	Preclinical development	Techniclone International, Tustin, CA, USA	190
VEGF-mediated targeting of toxins	VEGF-mediated targeting of a diphtheria toxin conjugate to tumor endothelial cells	–	–	191

Abbreviations: RGD, asparagine-glycine-aspartate; VEGF, vascular endothelial growth factor.

either of human, rat or murine origin, was tested in efficacy models. With the exception of two out of ten tumors tested, all showed significant inhibition of tumor growth after treatment with SU5416. Importantly, the effects were not only seen when the tumor was injected subcutaneously, but C6 glioma cells, when injected into the colon, were also strongly inhibited. These findings were essentially confirmed in another system, the NF-1 neurogenic sarcoma xenografts[42]. On the basis of these findings, the compound was moved into development. The drug appears to be well tolerated in Phases I and II at the doses tested (4.4–15 mg m^{-2} day^{-1}), and, again based on apparently promising results, was recently promoted to Phase III clinical trials.

SU6668

SU6668 belongs to the same compound class as SU5416, and its structure has recently been disclosed (Fig. 1). The enzyme inhibitory profile of SU6668 appears to differ from SU5416 in that this compound is active against VEGF-, FGF- and PDGF-receptor tyrosine kinase. It displays comparable activity to SU5416 in a metastatic CT-26 colon cancer model[43].

PD173074

PD173074 is a potent inhibitor of both FGF- and VEGF-mediated signal transduction, shows good anti-angiogenic activity at low doses in the intact animal, and also a favorable pharmacokinetic (PK) profile without apparent toxicity.

Table 7. Gene therapy approaches

Compound	Mechanism of action	Stage of development	Company	Refs
Antisense compounds/ ribozymes	Antiangiogenic ribozymes targeting Flt-1 and KDR mRNA	Preclinical research	Ribozyme Pharmaceuticals, Boulder, CO, USA	192
	c-src antisense (downregulating VEGF expression	Preclinical research	–	193
	FGF-BP specific ribozome	Preclinical research	–	194
	Pleitrophin-specific ribozyme	Preclinical research	–	195
Vasculature-targeted gene therapy	(Re)targeted adenoviral vectors that are antiangiogenic	Preclinical research	GenVec, Rockville, MD, USA (TNFα gene therapy) and Selective Genetics, San Diego, CA, USA (FGF2-SAP, Pantarin®)	196–200
Adenoviral, retroviral and other transfection protocols	Retrovirus-mediated expression of a dominant negative mutant of VEGF-R2 suppressing tumor growth in mice	Preclinical research	–	201
	Transfection of RT-2 glioma cells with the platelet factor 4 (PF4) gene resulting in reduced tumor angiogenesis and increased survival of mice	Preclinical research	–	202
	Transfection of the Thrombospondin-1 (TSP-1) gene into breast carcinoma cells resulting in antitumor activity	Preclinical research	–	203
	TIMP-2 transfectants showing reduced decreased angiogenesis and metastasis	Preclinical research	–	204
	Angiostatin expression in a murine fibrosarcoma suppressing tumor growth	Preclinical research	–	205
	Suppression of angiogenesis and tumor growth by adenovirus-mediated gene delivery of a fragment of urokinase (ATF)	Preclinical research	–	206
	Suppression of tumor growth by adenovirus expressing a recombinant TIE-2 receptor	Preclinical research	–	207
	Systemic (i.v.) angiostatin gene therapy suppresses the growth of distant tumors	Preclinical research	–	208

It is an analog of its parent (PD166866), which is a selective FGF-receptor tyrosine-kinase inhibitor (discussed later). Interestingly, a marked additive effect on tumor growth (and to some extent survival) of mammary 16c tumor-bearing mice was seen when treatment with PD173074 was combined with photodynamic therapy[44].

PTK787/ZK222584

Formerly known as CGP79787, PTK787/ZK222584 is a potent inhibitor of VEGF receptor kinases at the submicromolar level[45–47]. It also inhibits other class III tyrosine kinases, such as PDGF-Rβ, c-Kit and c-Fms, but at higher concentrations. It is inactive against other kinases, including EGF-R, FGF-R1, c-Met and Tek, as well as intracellular kinases such as c-Src, c-Abl and protein kinase C-α (PKCα). The compound inhibits VEGF-induced autophosphorylation of KDR, endothelial cell proliferation, migration and survival in cellular assays. It also inhibits angiogenesis in *in vivo* models at doses of 50 mg kg^{-1} and is active against a range of murine tumors as well as human xenografts. Furthermore, it is active in an orthotopic metastasis model of renal cell carcinoma. It is currently in Phase I trials, but

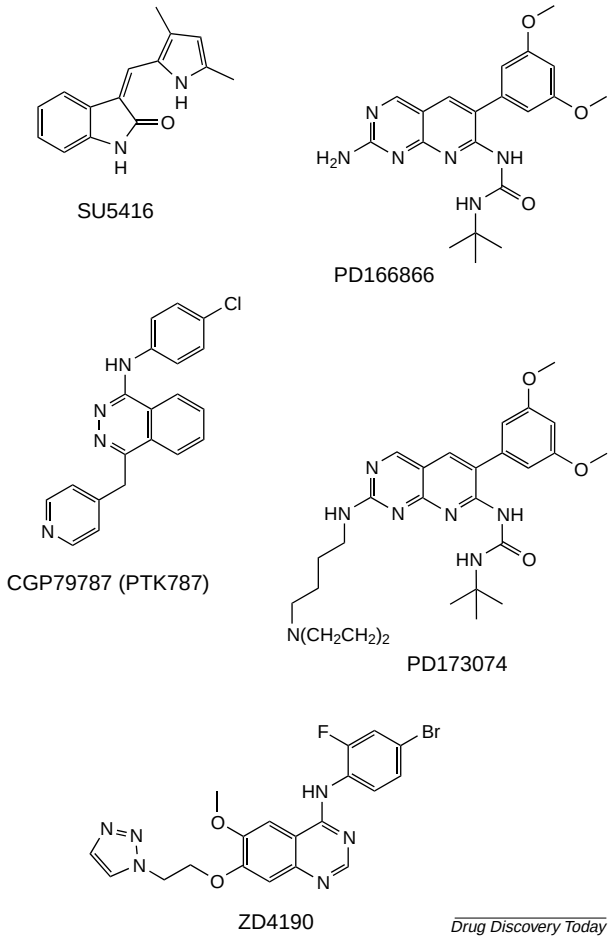

Figure 1. Vascular endothelial growth factor-receptor tyrosine-kinase inhibitors.

was on clinical hold because of rodent toxicity phenomena (hyperplastic changes leading to megaduodenum). This clinical hold was lifted in the fall of 2000 and clinical trials resumed because it could be shown that these changes were clearly of a benign nature, reversible and species-specific.

FGF-R tyrosine kinase inhibitors

The group at Sugen described a new group of tyrosine kinase inhibitors with high selectivity for FGF receptors, another important player in angiogenesis (see previously)[48]. Their best compounds (SU4984 and SU5402) inhibited the target enzyme with IC_{50} values of 10–20 μM. Inhibition of autophosphorylation in cellular systems was seen at a similar dose range. Furthermore, the structure of the catalytic domain, in particular the hydrophobic ATP-binding pocket of the FGF receptor, was also elucidated[49].

In addition, workers at Parke-Davis identified a highly selective FGF tyrosine-kinase inhibitor (PD166866), which belongs to the group of pyrido[2,3-*d*]pyrimidines[50,51] and shows anti-angiogenic activity[52], serving as a basis for the synthesis of the potent KDR–FGF-receptor inhibitor PD173074 (see previously).

MMP inhibitors

There are several recent reviews of MMP inhibitors in the literature[53–55]. Since the 1980s, potent compounds have been designed that are based on the substrate-cleavage site of the α-chain of type I collagen and a chelating moiety (such as hydroxamic acid, carboxylic acid or, in some cases, thiols) directed against the Zn^{2+} atom of the catalytic site. Once this was achieved, the remaining hurdle was oral bioavailability, which was generally high for inhibitors with peptidic design features. Systematic depeptidization led to compounds with good bioavailability, opening the way to effective clinical testing. Some of these compounds are discussed later. Further refinements that achieved various degrees of selectivity were secured by exploiting the variable depth of the S1' pocket in different MMPs. Exploiting this finding, it was possible to fashion compounds that were no longer active against fibroblast collagenase (MMP-1) and matrilysin (MMP-7). The group at British Biotech (Oxford, UK) recently published evidence that would favor the notion that neither MMP inhibitors that were selective to collagenase (MMP-1, -8, -13) nor gelatinase (MMP-2, -9) were causing tendinitis, but that both classes of compounds had lost their anticancer activity[55]. By contrast, a broad-spectrum MMP inhibitor that also inhibited sheddase (TNF-α convertase, TACE) was active in tumor models and did not cause tendinitis. This surprising result obviously requires independent confirmation.

Marimastat®

Marimastat is a broad-spectrum matrix MMP inhibitor with nanomolar activity against major enzymatic subtypes (MMP-1, MMP-2, MMP-3, MMP-7 and MMP-9; Ref. 56: Fig. 2). It works via competitive, reversible substrate inhibition and has improved pharmacokinetic properties when compared with its predecessor, Batimastat® (BB-94). Based on the fact that MMP inhibitors, as such, are not cytotoxic to tumor cells, the clinical trial design was primarily influenced by aiming at trough levels in patients achieving significant enzyme inhibition; 30–40 ng ml^{-1} will result in ~90% inhibition. Marimastat is in Phase III trials in patients with pancreatic, ovarian, colorectal and prostatic cancer. Surrogate endpoints such as a decrease in the rise of tumor markers were initially used to guide the design of the randomized Phase III studies. No clear-cut dose dependency could be detected, however. Recently, efficacy regarding one particular endpoint (progression free survival) has been acheived in gastric cancer. In pancreatic cancer,

the single agent, at the highest dose, showed a survival benefit that was comparable with the benefit achieved with gemcitabine alone; combination studies with gemcitabine, however, did not show additive effects in terms of patient survival rates and quality of life, although it should be remembered that in these combination studies Marimastat was used at a lower dose (10 mg). In March 2001, development of Marimastat in glioma, non-small-cell lung cancer, small-cell lung cancer and breast cancer was discontinued because of lack of efficacy.

The safety of the drug appears to be good; tolerability is limited by the (reversible) musculoskeletal side effects that are seen in ~30% of patients after several months of treatment and that require drug holidays to subside. These side effects are generally seen with all broad-spectrum MMP inhibitors.

CGS27023A (MMI270B)

CGS27023A (MMI270B), a hydroxamic acid derivative, is a potent, broad-spectrum inhibitor of MMP-1, -2, -3 and -9 (Ref. 57; Fig. 2). Similarly, as with other inhibitors of this type, it showed activity against human xenografts in the nude mouse and inhibited metastatic spread of orthotopically implanted murine tumors[58]. The compound was tested in a Phase I trial in patients with advanced solid tumors, and was found to induce disease stabilization in seven out of 36 patients. Musculoskeletal side effects were found to occur without clear dose dependency. Treatment was stopped because of a lack of clear-cut advantages with respect to other, more advanced MMP inhibitors.

AG3340 (Prinomastat)

This molecule is also an oral, non-peptide MMP inhibitor (Fig. 2) with selectivity (much less active against MMP-1/collagenase-1 and MMP-7/matrilysin)[59,60]. This inhibitor was shown to be active, when given orally at 50 mg kg^{-1}, against Lewis Lung tumors in mice, reducing growth of the primary tumor as well as metastatic spread. In early clinical trials (Phase I trials in lung, prostate, renal, colorectal, melanoma, sarcoma), disease stabilization was noted in more than a quarter of all patients. Phase II/III combination clinical trials in advanced hormone-refractory prostate and metastatic non-small-cell lung cancer have been discontinued as a result of lack of efficacy (May 2001).

Bay129566

Bay129566 is a second-generation MMP inhibitor (Fig. 2) with narrower selectivity (inactive against MMP-1). Nevertheless, this compound was active in preclinical studies, measuring the invasiveness of HT1080 tumor cells in a matrigel invasion assay[61], and also an orthotopic human colon xenograft (HCT116), where growth inhibition of the primary tumor was seen as well as inhibition of metastatic spread[62]. In Phase I trials the compound reached plasma levels at the 800 mg kg^{-1} dose bid (twice daily) that far exceeded the K_i values of MMP-2, -3 and -9 without causing musculoskeletal side effects[63]. Surprisingly, in Phase III trials the drug performed worse than placebo in small-cell lung cancer and was, therefore, stopped. The reasons for this unexpected outcome are yet to be elucidated. It could be speculated, however, that inhibition of MMP-2 might arrest production of angiostatin[64], thereby leading to enhanced tumor growth.

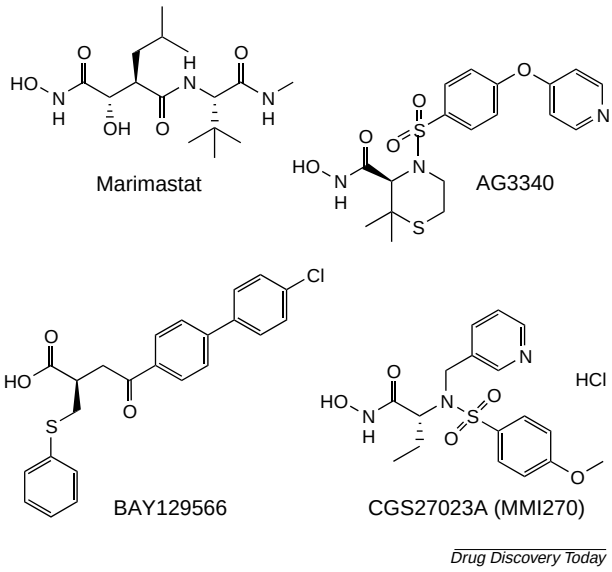

Figure 2. Metalloprotease inhibitors.

AGM1470 (TNP470)

AGM1470 (TNP470) is a semisynthetic fumagillin derivative (Fig. 3, Table 3) with anti-angiogenic properties[65], which has been found to strongly counteract the bFGF-induced proliferation of endothelial cells[66]. Recent literature has provided a comprehensive review of this area[29,67]. AGM1470 has been shown to be efficacious in several animal tumor models[68–70] and in a model of tumorigenesis in transgenic mice[71,72]. These findings correlate well with the reported decrease in tumor growth and increase in apoptosis in the 7,12-dimethylbenz(a)anthracine (DMBA)-induced rat mammary tumor model[73]. Potentiation of the efficacy of cytotoxic agents by AGM1470 has also been observed[74]. There is, however, a report that shows increased growth and incidence of lymph node metastasis as a consequence of AGM1470 treatment[75]. In terms of the

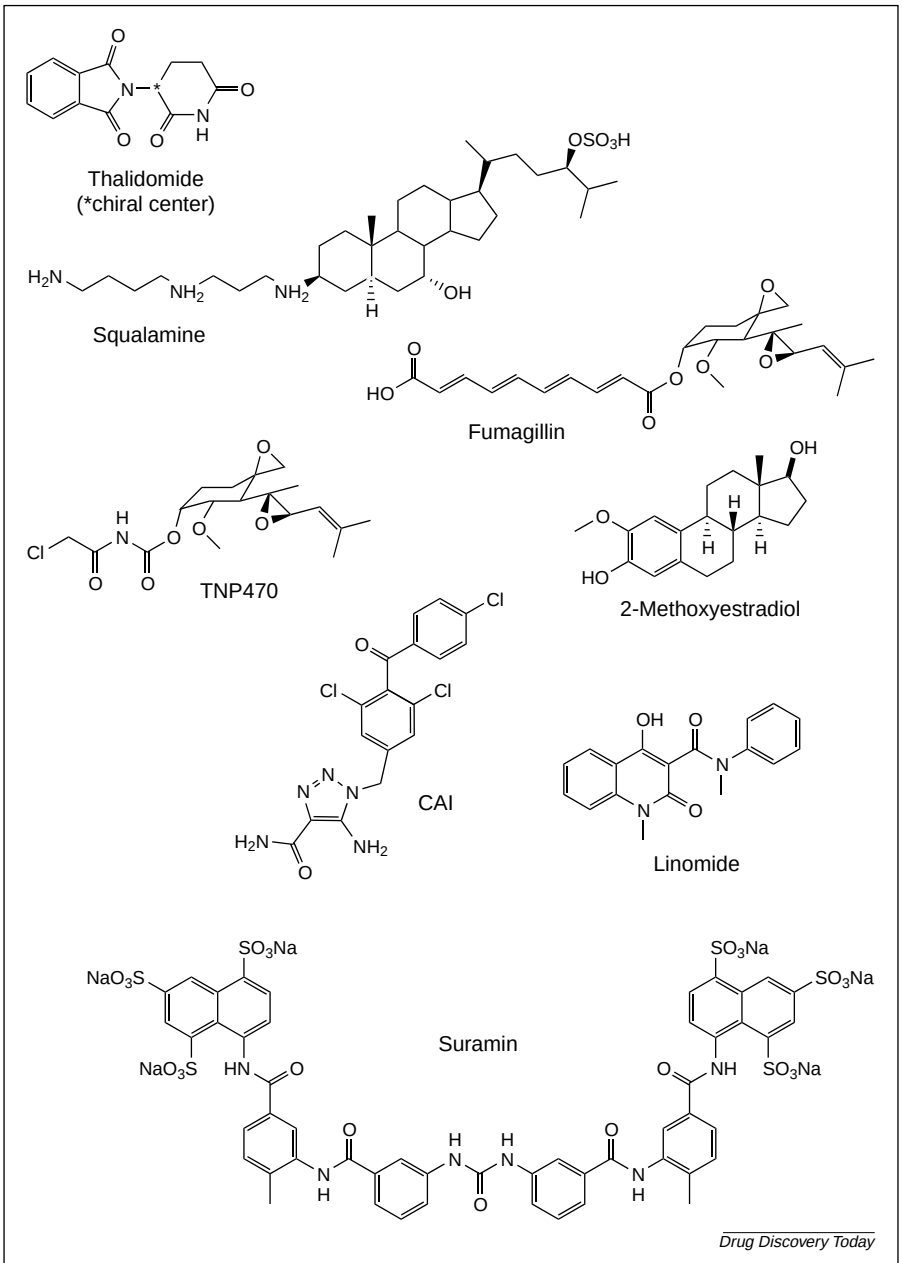

Figure 3. Other small molecular-weight agents.

mechanism of action, inhibition of methionine aminopeptidase (type 2) has been reported[76]; in HUVEC, AGM1470 inhibited the activation of cyclin-dependent kinases (cdks) and Rb phosphorylation[77].

Thalidomide

Thalidomide (Fig. 3, Table 3) has been shown to be an effective inhibitor of bFGF-induced angiogenesis[78,79]; it is a chiral compound and the S(–) enantiomer is strongly teratogenic, correlating with its anti-angiogenic effect. Complete cures in mice were achieved in combination with 5,6-dimethylxan-thenone-4-acetic acid (DMXAA, discussed later)[80]. Also, other combinations with other drugs, such as cytoxan and adriamycin[81] and sulindac[82], were effective. Thalidomide is being studied for several other indications, including AIDS because of its inhibitory effect on HIV replication[79]. Macrophages are likely targets of thalidomide because lipopolysaccharide-induced TNF-α production is inhibited[79]. Antiangiogenic effects and the suppression of TNF-α do not seem to be related. Thalidomide also inhibits expression of integrins, such as VCAM-1 (CD106) and E-selectin[83], and could also affect expression of $\alpha_v\beta_3$ and $\alpha_v\beta_5$. Effects on these integrins might not only be casually related to the anti-angiogenic, but also the teratogenic, effects of thalidomide.

Squalamine

Squalamine (Fig. 3, Table 3) was isolated from the dogfish shark *Squalus acanthias* as an antimicrobial substance[84]. Squalamine is an angiostatic steroid and is purified as a natural aminosterol[85]. In contrast to other previously studied steroids, squalamine does not require heparin or suramin to exert its angiostatic activity. Squalamine can be shown to be active on newly formed, but not established, blood vessels in a three-day CAM (chick chorioallantoic membrane) assay over a dose range of 0.43–3.5 μg disk^{-1}. Similarly, statistically significant effects were obtained in a rabbit cornea model. In a collagen matrix assay employing HUVECs, VEGF and bFGF, squalamine could be shown to disrupt the endothelial connections, and induce the rounding up of many endothelial cells at lower concentrations than those required to inhibit endothelial cell growth and migration. Squalamine is active on several different types of endothelial cells and is inhibitory for angiogenesis induced by VEGF, bFGF, PDGF and hepatocyte growth factor (HGF). The mechanism of action is probably complex and unlikely to involve any known steroid receptors. Inhibitor effects on certain isotypes of Na^+/H^+ exchangers (NHE-3) has been described[86], but a direct link of these

effects with the angiostatic activity of squalamine has not been demonstrated. Squalamine, when delivered via intraperitoneal administration, is effective in inhibiting the establishment of H460 human tumor xenografts, but is ineffective as a single agent against existing tumors. Squalamine does, however, enhance the antitumor efficacy in combination with cisplatin or carboplatin, whereas other combination regimens were ineffective[87]. In any event, in those situations where squalamine was effective, a decrease in tumor vessel density was documented by immunocytochemistry.

Angiostatin™

The discovery of Angiostatin™ and several other anti-angiogenic proteins is based on the observation that some primary tumors, either in a preclinical or clinical setting, appear to inhibit growth of their metastases; removal of the primary tumor then leads to an increase in growth[88]. The hypothesis of this specific mechanism linked to angiogenesis in the primary tumor and to the production of long-lived, circulating negative-regulators of this process was vigorously pursued by the same group, and led to the identification of Angiostatin[89] and Endostatin™ (discussed later). According to Folkman's group, angiostatin is a potent endogenous inhibitor of endothelial cells, inhibiting growth of both primary and metastatic tumors. Angiostatin is an internal fragment of plasminogen, including the first three or four of the five kringle (K) domains. Its approximate mass in the mouse is 38 kDa, whereas in the human, active moieties of a mass of 40, 42.5 and 44 kDa have been described, including kringles K1–3 or K1–4. Differential glycosylation patterns contribute to the diversity of endogenous angiostatin proteins[90]. Endogenous angiostatin can be produced by various proteinases, such as serine elastase, macrophage-derived metalloelastase stromelysin-1 (MMP-3), human matrilysin (MMP-7), gelatinase A (MMP-2), gelatinase B (MMP-9) and metalloelastase (MMP-12).

Recombinant murine angiostatin has been produced successfully in the baculovirus system and has been shown to be active *in vitro* against bovine capillary endothelial cells as well as against Lewis lung carcinoma in mice at daily doses of 1 and 6 mg kg^{-1} (Ref. 91).

Recombinant human angiostatin K1–4 can be produced in the yeast strain *Pichia pastoris* as an active agent showing similar characteristics to an endogenous protein. It is active in the mouse at 1.5 mg kg^{-1} against metastases of Lewis lung carcinoma (LLC-LM) tumor, and suppressed the growth of primary tumors at 100 mg kg^{-1} (Ref. 92). Also, baculovirus-produced recombinant mouse angiostatin K1–4 was highly active in the mouse (somewhat more potent than human recombinant angiostatin, indicating a degree of species specificity). The specificity of angiostatins for endothelial cells appears attractive, and efficacy studies, using high doses of angiostatin at frequent intervals, have shown good tolerability. On these grounds, angiostatin has entered Phase I clinical trials (mid-2000). Many questions remain unanswered, including the precise cascade of events leading to production of angiostatin, as well as its receptor at the endothelial cell surface (annexin II) and downstream consequences of angiostatin binding. These points are clearly articulated in a recent review[90].

Endostatin™

Endostatin™ was first isolated from a murine hemangioendothelioma cell line, but can be successfully produced as recombinant protein in the baculovirus expression system; the *Escherichia coli*-derived material is more difficult to handle because of precipitation or refolding problems, but nevertheless clearly shows activity under certain conditions[93]. It is a 20 kDa fragment of the C-terminus of collagen XVIII (non-collagen domain, NC-1, that is non-triple-helical)[94]. Collagen XVIII is a type of collagen associated with many basement membranes. Cleavage of endostatin from collagen XVIII can be observed using cathepsin L. Endostatin is homologous to E-selectin and shows high-affinity binding to heparin; it can chelate a Zn^{2+} atom, which might or might not be important for its function. Endostatin blocks mitogen-activated protein kinase (MAPK) activation in endothelial cells and has activity in mouse tumor models at a dose range of 1.5–12.4 mg kg^{-1} day^{-1} (Ref. 95). This antitumor activity correlates with an increased apoptotic index and a decreased vessel density in endostatin-treated tumors, inducing, in the hands of O'Reilly and colleagues, a state of 'tumor dormancy'[93,96]. Long-term treatment does not appear to induce a state of resistance and tolerability of this agent does not seem to be a problem.

Clinical testing of anti-angiogenic agents

The clinical testing of anti-angiogenic agents (vasculostatins) is largely based on preclinical evidence demonstrating antitumor effects in murine tumor models (syngenic models, orthotopic models, human xenograft models, transgenic models)[97,98]. Essentially, these experiments usually show good tolerability of these agents in the mouse, and significantly decreased tumor growth. They generally do not lead to tumor regression or cure, unless used in combination with cytotoxic agents or other signal transduction inhibitors.

The relatively good tolerability in the mouse and in Phase I trials raises the problem of determining maximally tolerated doses (MTDs), which is probably not relevant as a

pharmacodynamic endpoint. In the absence of validated surrogate markers for efficacy, the clinician is then left with PK measurements that can be correlated with PK in the mouse, achieving efficacy. This situation is obviously not satisfactory and a broad range of experiments is underway to identify relevant serum markers (VEGFs, VEGF-Rs, bFGF-R, and so on), urinary markers (VEGFs, FGFs) or technologies based on non-invasive measurements of blood flow in dynamic magnetic resonance imaging, employing paramagnetic contrast agents such as gadolinium-diethylene-triaminepentaacetic acid (Gd-DTPA) that could have predictive quality. However, the true relevance of any biomarker will only be really clear once the efficacy of any antiangiogenic agent has been demonstrated.

Similar difficulties could arise in Phase II trials where regressions might be rare, but other endpoints such as disease stabilization, progression-free survival or time to progression might be more relevant. These parameters are obviously more challenging in terms of objective measurements and are likely to require larger patient populations. Also, treatment duration could be relatively prolonged before any measurable impact can be seen. The efficacy of interferon α in hemangioma patients is seen only after several months of treatment, and thus could be indicative of what is in store for the clinical cancer researcher[99,100,172]. The complexities of clinical testing of antiangiogenic agents is discussed in depth in the literature[67,101].

For vasculotoxins the situation is probably slightly less demanding because an effect is expected to be seen after a short treatment period (if preclinical experience can serve as a guide). Also, this type of modality might not require any combination partner.

Conclusion

All of the above-mentioned approaches rest on two assumptions: that angiogenesis is a basic and necessary feature of most, if not all types of solid cancer; and that murine tumor models are largely predictive for effects in man. However, both assumptions are subject to reservations. There are well-documented cases of non-angiogenic tumors in the lung (subset of 16% of non-small-cell lung carcinomas), which limits the generalization of the first assumption[102]. Also, the predictive value of murine models is limited by the fact that these tumors are growing much faster than average human cancers. This means that the percentage of actively dividing endothelial cells in human tumours is much lower than in a murine model[103], and that modality-directed active angiogenic processes will probably have an efficacy that is in line with these basic biological facts. These limitations do not in any way diminish the interest of anti-angiogenic therapy, but could be a useful reminder that clinical cancer therapy usually does not progress by quantum leaps but by incremental, yet immensely useful, steps.

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