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Anti-angiogenic agents: clinical trial design and therapies in development

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Abstract

Development of therapies aimed at inhibiting the growth of new blood vessels is among the most intensively studied approaches to the treatment of cancer. Deciphering the many biological processes involved in tumour angiogenesis has led to the development of new agents targeting either metalloproteases, angiogenic growth factors, endothelial cells or other components of the tumour neovasculature. More than 35 anti-angiogenic agents have already entered clinical trials in cancer patients and most of them are reviewed here. It has rapidly emerged from the preliminary results of these studies that the steps and endpoints classically adopted and used worldwide in developing new anticancer agents could be inappropriate to assess the efficacy of agents that do not target cancer cells directly. One of the major challenges for scientists and clinical researchers is to define new surrogate endpoints adapted to anti-angiogenic agents in the design of clinical trials. Once this has been achieved, the place of clinically active anti-angiogenic agents will need to be further refined in order to determine where they best fit in our current armamentarium, either as single agents or in combination with classical anticancer therapies. Finally, the use of these new agents may in the future encompass every aspect of cancer management, not only from palliative to curative treatment but also in the prevention of cancer.

Keywords: Angiogenesis; Neoplasms; Drug evaluation; Clinical trial

1. Introduction

Development of therapies aimed at inhibiting the growth of new blood vessels is among the most intensively studied approaches in the treatment of cancer. Such new anticancer agents, often referred to as anti-angiogenic agents, angiogenesis inhibitors or even angiosuppressors, are an example of what translational research and molecular medicine are capable of achieving. It has been 30 years since the first mention of tumour neovasculature as a potential therapeutic target [1]. Since that time our understanding of the intricate mechanisms leading to the formation of new blood vessels associated with tumour growth and the spread of metastases has greatly improved [2].

Angiogenesis — the growth of new blood vessels — takes place primarily during development of the embryo. It also occurs in adults, in a tightly controlled way, in physiological conditions such as ovulation and menstruation, hair growth and wound healing. This is achieved locally and only transiently through the action of finely balanced angiogenic inhibitors and stimulators [3]. Disregulated angiogenesis is seen in many pathological circumstances (e.g. diabetic retinopathy, cardiovascular diseases, rheumatoid arthritis, psoriasis, ... etc.) of which cancer has been the focus of interest in the recent years. The growth of tumours beyond a limited size of 0.2–2 mm (i.e. 10^5–10^6 cells) in experimental models depends on angiogenesis. In its absence tumours are unable to grow further although active cell proliferation, counter-balanced by apoptosis, is seen in those so-called dormant tumours [4,5]. The passage from the pre-angiogenic phenotype to the angiogenic phenotype, often referred to as the 'angiogenic switch', will allow the formation of a neovasculature that is indispensable for further tumour growth. The same processes will also favour metastatic dissemination [6].

Fig. 1 summarises some of the events leading to tumour growth and metastatic spread. Once a tumour is established, interactions between stromal cells, tumour

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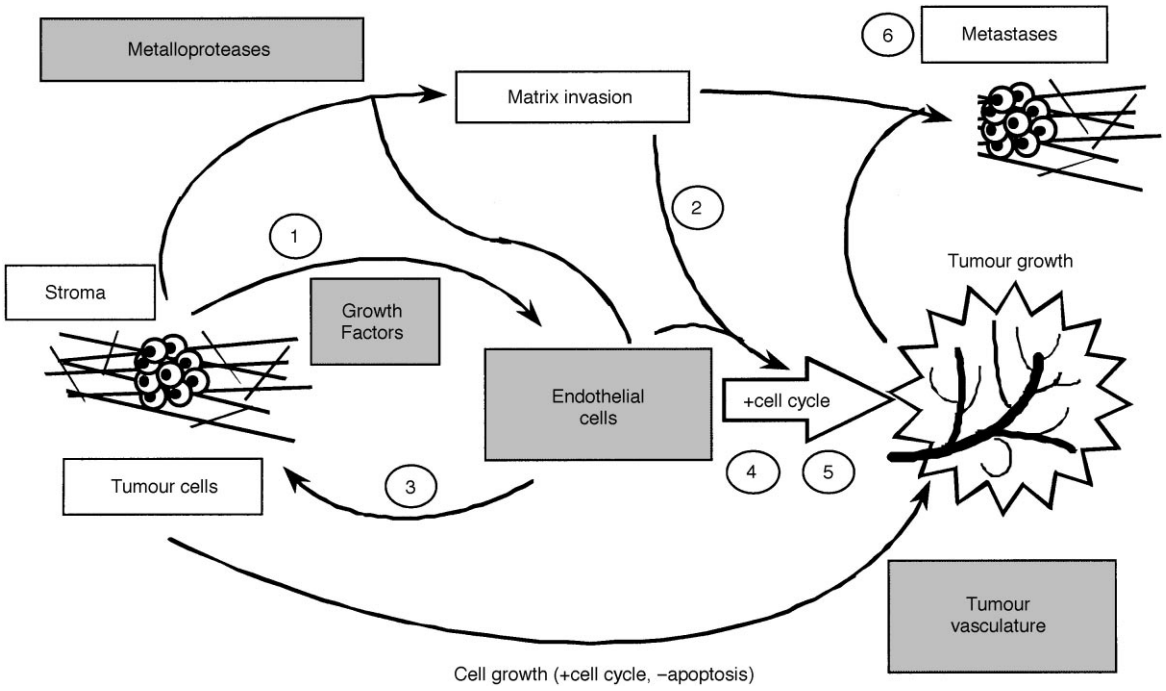

Fig. 1. Schematic representation of the events leading to tumour angiogenesis and metastasis. Tumour cells release angiogenic factors directly (1) or indirectly (2) through extracellular matrix degradation. Endothelial cells may also feedback and stimulate tumour growth (3). Endothelial cells proliferate (4) and invade towards the tumour (5). These processes are repeated at sites of metastasis (6).

cells and endothelial cells will trigger degradation and invasion of the extracellular matrix. This is achieved through secretion and activation of various matrix metalloproteases [7], and will permit both intravasation/extravasation of tumour cells — metastases – and budding of new blood vessels. Activation and proliferation of endothelial cells are triggered by angiogenic stimulators, and more specifically growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) secreted by cancer cells. Therefore, events leading to angiogenesis and the formation of tumour vasculature will also favour the establishment of distant metastases, where the same events will take place to permit further tumour growth [6].

The potential targets for anti-angiogenic therapies stem from these biological processes and will therefore include: (1) metalloproteases; (2) angiogenic growth factors; (3) endothelial cells; and (4) tumour vasculature. Additionally, all agents known to target the cell cycle would, in theory, inhibit tumour growth by both cancer cell killing and indirect inhibition of angiogenic stimulation and inhibition of endothelial cell proliferation. These encompass classical anticancer agents, farnesyl transferase inhibitors, cyclin-dependent kinase inhibitors, ... etc. This has been experimentally verified for example for the taxanes, a recent new family of anticancer agents [8].

More than 35 anti-angiogenic agents are currently being investigated in clinical trials. These can be classified according to the target they inhibit in angiogenesis (Fig. 1). The mechanism of action of many investigational agents remains hypothetical or unknown. The design of clinical trials for anti-angiogenic agents and the therapies currently in clinical development will be discussed below.

2. Design of clinical trials for anti-angiogenic agents

As anti-angiogenic agents have moved from the laboratory to clinical trial, researchers have learned that the design of clinical trials may need to be specifically adapted to this new class of anticancer agents. The design used until recently has been the one developed to investigate the function of new cytotoxic drugs [9]. However, because the 'ideal' anti-angiogenic agent is expected to be devoid of any significant side-effects and to be given on a long-term basis to control neoangiogenesis and metastatic spread, without necessarily making the tumour shrink, classical clinical trials could prove to be inaccurate and miss some anti-angiogenic agents of interest [10].

The development of any new anti-angiogenic agent, once proved to be effective in preclinical models, should raise at least three questions.

2.1. What is the appropriate design for phase I? (Table 1)

The goals of phase I studies are to identify the toxicities, maximum tolerated doses (MTD) and pharma-

cokinetic properties of a novel compound. A dose below the MTD is then used for further developments in phase II studies for specific cancer types. If interesting objective tumour response rates (decrease superior to 50% for at least 4 weeks) are observed, then the new compound enters a phase III trial against the established standard treatment, if any.

Therefore, in phase I studies, the MTD for anti-angiogenic agents may be vastly in excess of the dose required to block blood vessel formation. For example, serum dose levels well above the IC_{50} inhibiting metalloproteases can be achieved without reaching the dose-limiting toxicity [11]. Therefore, the definition of an optimal biological dose (OBD) for which effective target inhibition is reached without noticeable toxicities could be a more suitable endpoint than the MTD. Similarly to the development of antimicrobial agents, simple laboratory anti-angiogenic assays could be designed to monitor the "serum anti-angiogenic property (SAP)" of patients. However, there are no such suitable assays currently available.

Because objective tumour responses are uncommonly seen with most of the anti-angiogenic agents in current development, surrogate endpoints need to be found and validated in assessing the potential efficacy for specific type of cancers in phase II studies. Using a pharmacokinetic endpoint based on animal data and inhibition of endothelial cells in culture is another possibility. In addition, in some cases there will be dose-limiting toxicities (DLTs), such as frozen shoulder with some matrix metalloproteinase inhibitors, which define a MTD. Finally, the toxicity profile of anti-angiogenic agents varies greatly and is certainly different from what oncologists are accustomed to with cytotoxic agents. Even if most side-effects for the drug in development are mild over a short period of time, these can prove significant and jeopardise the patient's quality of life with long-term use, making the grading of such toxicities sometimes difficult with the usual scales.

Table 1
Clinical trial design

	Classical anticancer agents (cytotoxic)	Anti-angiogenic agents (non-cytotoxic)
Phase I	MTD DLT	OBD No toxicity
Phase II	Objective tumour response	? Surrogate end-point to be found and validated
Phase III	Survival TTP QOL	Survival TTP QOL

MTD, maximum tolerated dose; DLT, dose-limiting toxicity; OBD, optimal biological dose; TTP, time to progression; QOL, quality of life.

2.2. How to best assess the efficacy of the anti-angiogenic agent?

One of the challenges to clinical researchers in angiogenesis research is to identify in early phase human studies the most promising drugs from the many agents that have anti-angiogenic activity in preclinical studies. Assessment of tumour response by classical means (i.e. imaging, clinical examination) may be inappropriate but is still mandatory (Table 2). Initial interest has focused on the use of serum or plasma levels of growth factors important in angiogenesis to monitor treatment such as bFGF or VEGF [12,13]. However, several studies have failed to demonstrate a reliable parallel between tumour response, anti-angiogenic treatment and a decrease or increase in such markers [14]. A more direct approach that can be used in both phase I and II trials is non-invasive imaging to directly study tumour vasculature or blood flow. Positron emission tomography (PET) and magnetic resonance imaging (MRI) scanning can successfully be used to monitor tumour blood flow and leaky neo-vessels [15,16]. However, such facilities are sometimes not available easily. Doppler ultrasound can also be useful for certain type of tumours [17]. Measurement of contrast clearance by tumour with spiral computed tomography (CT) scan has been described recently and could provide an easier means of assessing tumour blood flow *in vivo*, given its wide availability [18]. More and more refined imaging techniques should be able to improve the early phase evaluation of new anti-angiogenic drugs.

From phase I to phase III: preclinical data show additive effects for chemotherapy with many anti-angiogenic agents [19]. Because most tumour types have an accepted standard first-line chemotherapy, randomised addition of the new agent may be a good design

Table 2
Assessing the efficacy of anti-angiogenic agents

Imaging of angiogenesis	MRI CT scan US doppler PET scan
Biology	Markers of angiogenesis (VEGF, bFGF, ... etc.) Tumour markers (e.g. CA125, CEA, PSA, ... etc.) Pharmacodynamics
Tumour response	Classical means (radiology, clinical examination, ... etc.)
Patient	QOL, benefit from therapy

MRI, magnetic resonance imaging; CT scan, computerised tomography scan; US, ultrasound; PET, positron emission tomography; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; QOL, quality of life; PSA, prostate specific antigen; CEA, carcinoembryonic antigen.

in phase II. The concern is that the dose of the anti-angiogenic agent may be inadequate, hence the importance of the choice of the dose based on several criteria. These would include response if it occurs, pharmacokinetics, MTD and pharmacodynamic endpoints [20]. Another approach is to move directly from phase I to phase III, assuming that angiogenesis is a common feature of every tumour and that the efficacy of an anti-angiogenic agent will therefore not depend on the cancer type. However, a randomised phase III study requires massive investment of resources and the recruitment of several hundred patients. Moreover, it is now clear that some tumours can gain access to a blood supply by other means (e.g. alveolar circulation in pulmonary primaries and secondaries [21], hollow channels substituting for blood vessels in melanoma [22]).

Finally, classical endpoints of phase III clinical trials, namely survival and quality of life (QOL) will need to ultimately remain the same [23].

2.3. What is the aim of the anti-angiogenic treatment?

One could certainly distinguish between three different clinical settings (Table 3).

2.3.1. Palliative

A palliative approach is the aim of treatment for any incurable advanced or metastatic cancer, providing the best QOL, usually linked to improved survival or time to tumour progression [23]. Most of the anti-angiogenic therapies are currently being developed in this setting. The potential to inhibit any further tumour growth, to avoid tumour spread and establishment of new distant metastases, or even to shrink the tumour, together with low side-effects and possible long-term use make anti-angiogenic agents very useful in this clinical situation. An important trial concept could be prevention of new secondaries, even if existing secondaries progress. In addition, a slower progression could be of value if the previous tumour growth rate was rapid. These endpoints are not accepted currently but could be of value to patients.

Table 3
The aims of anti-angiogenic therapy

Treatment	Setting	Aims
Palliative	Advanced or metastatic disease	Prolonged TTP Prolonged survival QOL
Curative	Adjuvant to surgery and/or radiotherapy	Cure
	Maintenance therapy after CR	Increased rate of DFS
Preventive	High risk population	Avoid growth of cancer

TTP, time to progression; QOL, quality of life; DFS, disease-free survival; CR, complete response.

2.3.2. Curative

A curative approach is certainly the ultimate goal of cancer treatment. Anti-angiogenic agents could prove useful in this setting by avoiding growth of established micrometastases, either in adjuvant treatment for early stage of cancer, or as maintenance therapy after completion of a complete response with chemotherapy/radiotherapy/surgery. Some anti-angiogenic agents are already being developed this way [24,25].

2.3.3. Preventive

Anti-angiogenic agents could also be used in a preventive setting, for example for populations at high risk of cancer. It has been shown recently that angiogenic competent tumour cells were able to stimulate and initiate angiogenesis after having reached only 20–50 tumour cells, long before reaching the supposedly critical size of 0.2–2 mm [26]. Moreover, the use of an angiogenesis inhibitor was effective in inhibiting tumour growth, even before the appearance of new blood vessels, highlighting the potential of such an approach [27].

In all three of the above situations, anti-angiogenic agents could be used in combination with other existing treatment approaches, such as radiotherapy, chemotherapy or hormonal therapy. Indeed, synergistic effects of combined modality treatment support this [19].

3. Anti-angiogenic therapies in development

3.1. Anti-angiogenic agents targeting matrix metalloproteases (Table 4)

Matrix metalloproteases (MMPs) are enzymes expressed by endothelial cells, tumour cells and stromal cells. There are now more than 20 enzymes that are classified as MMPs. MMPs will achieve the degradation of the extracellular matrix, permitting invasion and subsequent growth by blood vessels and cancer cells [7,28].

MMPs inhibitors (MMPIs) block angiogenesis, and therefore tumour growth, but also the ability of tumour cells to metastasise by invading blood vessels. MMPIs are in more advanced clinical development than most other anti-angiogenic agents.

3.1.1. Batimastat

Batimastat (BB94) is a low molecular weight hydroxamic acid derivative based on the structure of the natural MMP substrate collagen. It is a broad spectrum, competitive and reversible MMPI. Because of low solubility, restraining its administration to intraperitoneal or intrapleural routes, it has not been developed further than phase I–II trials [11].

3.1.2. Marimastat

Marimastat (BB2516) is the second compound from British Biotech to be tested in humans. It is the soluble equivalent of Batimastat, orally bioavailable. Phase I trials identified optimal biological doses and showed the main side-effects to be fatigue and cumulative reversible inflammatory polyarthritis. The results of the first ever completed randomised phase III study of a MMPI have been released by British Biotech in February 1999. This showed that Marimastat was at least as effective as gemcitabine for unresectable pancreatic cancer [29]. Two other phase III studies have since been reported, one showed a survival advantage over a placebo for inoperable gastric cancer treated by Marimastat [30]. A second compared the addition of Marimastat or a placebo to gemcitabine [31] and failed to show any benefit for the MMPI. Phase III randomised studies are ongoing in breast, colon, glioblastoma, ovarian and lung cancer [24].

3.1.3. Solimastat

Solimastat (BB3644) is again a broad spectrum inhibitor of MMPs. Like the earlier Marimastat, BB3644 is orally active. The distinguishing features that make it an attractive compound for further study are a reduced propensity relative to Marimastat to cause musculoskeletal side-effects, and also its potent inhibition of tumour necrosis factor-alpha (TNFα) release. BB3644 is in early development in phase I studies for solid refractory cancer.

3.1.4. AG3340

AG3340 is also a synthetic hydroxamic acid derivative, targeting more selectively inhibition of four different MMPs. Because it is not a broad spectrum MMP inhibitor, it is believed that its clinical toxicity will be more limited. Similarly, AG3340 causes time and dose-dependent inflammatory polyarthritis, involving shoulders, hips, hands and knees, that is reversible with discontinuation of the drug. AG3340 seems to have a synergistic effect in combination with several chemotherapeutic agents and has therefore been developed in this setting. It was safe and well tolerated in a phase I study with carboplatin and paclitaxel [32]. The drug is currently in two randomised phase III trials for non-small cell lung cancer (NSCLC) in combination with carboplatin/paclitaxel or cisplatin/gemcitabine versus a placebo. It is also being assessed for hormone refractory prostate cancer in combination with mitoxantrone/prednisolone.

3.1.5. BAY12-9566

BAY12-9566 is structurally distinct from other MMPIs. It is a butanoic acid analogue, oral selective inhibitor of MMP-2 and MMP-9. This may account for its different profile of toxicity, with no musculoskeletal side-effects. Phase I studies in patients with advanced solid tumours reported dose-related hepatic toxicity and thrombocytopenia [33–37]. Phase III randomised studies in small cell lung cancer (SCLC), NSCLC, pancreas and ovary cancer were ongoing with the drug being intended as a maintenance therapy for patients having achieved a tumour response. However, these studies were cancelled when preliminary analysis suggested that treatment with BAY12-9566 had a detrimental effect on survival for SCLC patients. This may reflect the narrow spectrum of drug activity and illustrate our lack of knowledge of the complex interactions of some pathways *in vivo*.

3.1.6. COL-3

In 1991, Golub and colleagues discovered that some tetracyclines could inhibit collagenases [38]. Manipulation of the tetracycline molecule resulted in the elimination of the antimicrobial properties without destroying the ability to inhibit MMPs. The resulting molecular compound, COL-3 (6-demethyl-6-deoxy-4 dedimethylaminotetracycline) is a high lipophilic, chemically modified tetracycline without antimicrobial properties. COL-3 directly inhibits several MMPs and is directly cytotoxic to a number of cell lines, possibly through the induction of apoptosis. Several phase I studies are being conducted with oral daily doses of up to 100 mg/m^2. A phase II trial is ongoing for brain tumours. The side-effects of photosensitivity and drug-induced lupus could limit its further development [39].

Table 4
Anti-angiogenic agents targeting matrix metalloproteinases

Drug	Sponsor	Stage of development
Batimastat (BB94)	British Biotech	Abandoned
Marimastat (BB2516)	British Biotech	Phase III in breast, gastric, colon, glioblastoma, ovarian, NSCLC, pancreas
Solimastat (BB3644)	British Biotech	Phase I
Bay12-9566	Bayer	Phase III lung, ovary, pancreas, "on hold"
AG3340	Agouron	Phase III lung, prostate
COL-3	Collagenex, NCI	Phase I, phase II brain
Neovastat (Æ-941)	Æterna	Phase III colon and NSCLC
BMS-275291	Bristol-Myers Squibb	Phase I/II
CGS27023A	Novartis	Phase I/II

NSCLC, non-small cell lung cancer.

Table 5
Anti-angiogenic agents inhibiting angiogenesis growth factors

Drug	Sponsor	Stage of development
Anti-VEGF antibody	Genentech	Phase II and III in breast, lung, prostate, renal and colorectal
SU5416	Sugen-NCI-NDDO Oncology	Phases I/II Kaposi's sarcoma, renal, melanoma, sarcoma, VHL Phases III NSCLC and colorectal
SU6668	Sugen-NCI-NDDO Oncology	Phase I
PTK787/ZK22584	Novartis	Phase I Phase I/II Kaposi's sarcoma, colorectal, VHL
Ribozyme (Angiozyme)	Ribozyme Pharmaceuticals Inc.	Phase I

NSCLC, non-small cell lung cancer; VHL, Von Hippel-Lindau syndrome; HCL, hairy cell leukaemia; CML, chronic myelogenous leukaemia; NCI, National Cancer Institute; NDDO, New Drug Development Office.

3.1.8. Neovastat

Neovastat (Æ-941) is an oral agent extracted from shark cartilage. It shows interesting inhibition of angiogenesis assays and MMP *in vitro* [40]. Phase III trials for colon and NSCLC are ongoing [41].

3.1.9. BMS-275291

BMS-275291 is a synthetic MMPI in phase I/II trial [42].

3.1.10. CGS27023A

CGS27023A (or MMI270B) is also a broad spectrum MMPI whose toxicities in a phase I trial were arthralgies, myalgies for 25% of patients and maculopapular rash in 20% for doses superior to 600 mg daily. DLT was not reached at doses up to 1800 mg daily. Plasma concentrations superior at 10 times the IC_{50} for the target MMPs were achieved at each dose level. No tumour response was seen in 36 patients [43,44]. Phase II trials are underway.

3.2. Anti-angiogenic agents inhibiting angiogenesis growth factors (Table 5)

3.2.1. Anti-vascular endothelial growth factor antibodies

VEGF is probably the most critical angiogenic growth factor [45,46]. The recombinant humanised monoclonal antivascular endothelial growth factor antibody (rhuMAb VEGF) developed by Genentech has shown no DLTs with weekly intravenous (i.v.) administration at doses up to 10 mg/kg [47]. Of the 25 patients who entered a phase I trial, one (4%) had a minor response and 13 (52%) others experienced disease stabilisation [48]. However, in a more recently completed phase II trial in hormone refractory prostate cancer, none of the 14 patients achieved a decrease in serum prostate specific antigen (PSA) superior to 50% at 70 days [49]. 3 patients (21%) had stable disease. Toxicity was mild with mostly asthenia in 40% of patients.

3.2.2. SU5416

SU5416 is a small peptide that acts as a potent tyrosine kinase inhibitor of VEGF receptor type 2 (Flk-1/KDR) that will block VEGF signalling. In a phase I trial involving 63 patients, DLTs were reached at a dose of 190 mg/m^2 given twice weekly over a 90-min infusion. These including projectile vomiting, headache and nausea, all reversible over 24–48 h. No tumour response was observed, but patients with various cancer types had stable disease for greater than 6 months (Kaposi's sarcoma, lung, colorectal, renal) [50,51]. Recommended doses for phase II trials are 145 mg/m^2 twice weekly, and are ongoing in renal cell cancer, soft tissue sarcoma and melanoma. Phase III randomised trials are ongoing in NSCLC in combination with gemcitabine/cisplatin and in colorectal cancer.

3.2.3. SU6668

SU6668 is a potent, broad spectrum tyrosine kinase inhibitor showing both anti-angiogenic and cytotoxic properties [52]. Furthermore, its bioavailability and pharmacokinetics support a once daily oral administration.

3.2.4. PTK787/ZK22584

This is a relatively selective tyrosine kinase inhibitor, orally active, that blocks phosphorylation and therefore signal transduction by VEGF and platelet derived growth factor (PDGF) receptors. PTK787 is currently in phase I/II trials [53].

3.2.5. Anti-angiogenic Ribozyme

The anti-angiogenic Ribozyme — Angiozyme — inactivates mRNA for two VEGF receptors (Flt-1 and Flk-1). The disruption of the VEGF signalling pathway inhibits angiogenesis and tumour growth in animal models. Phase I trials are complete in humans [42].

3.3. Anti-angiogenic agents inhibiting activation of endothelial cells (Table 6)

3.3.1. TNP-470 (fumagillin analogue)

Multiple phase I and II studies of this agent have been completed and reported. Given as a 4 h weekly i.v. infusion, the DLT appears to be central neurological symptoms (encephalopathy and ataxia), reversible over 2 weeks after discontinuation of the drug [54].

Table 6
Anti-angiogenic agents inhibiting activation of endothelial cells

Drug	Sponsor	Stage of development
TNP-470 (fumagillin)	TAP Pharmaceuticals	Phase II lymphoma and advanced solid tumours
Squalamine	Magainin	Phase I/II NSCLC
Endostatin	Entremed	Phase I

NSCLC, non-small cell lung cancer.

Table 7
Anti-angiogenic agents targeting tumour vasculature

Drug	Sponsor	Stage of development
Vitaxin	Ixsys	Phase I/II
EMD121974	Merck	Phase I/II Kaposi's sarcoma, brain
CM101/ZDO101	Astra-Zeneca	Phase I
Combrestatin	Oxigen	Phase I

A recently completed phase II multicentre study in renal cell carcinoma reported one partial response (3%) of short duration and six stable disease (18%) for 6 or more months out of 33 treated patients. Long-term therapy with TNP-470 is achievable despite its toxicity [55]. Other responses have been reported in cancer of the cervix [56] and AIDS-related Kaposi's sarcoma [57].

3.3.2. Squalamine (MSI-1256F)

Originally derived from the liver of the dogfish shark, squalamine exhibits potent anti-angiogenic properties. This novel non-cytotoxic aminosterol prevents neovascularisation of tumours by inhibiting mitogen-induced proliferation and migration of endothelial cells. In a preliminary report of a phase I study [58], no grade 3–4 toxicities were observed at doses up to 357 mg/m^2/day of a 5 day continuous infusion every 3 weeks. In a second phase I study [59], no objective response was seen in 16 patients. A phase I–II study in NSCLC has started in combination with chemotherapy.

3.3.3. Endostatin

Endostatin, the C-terminal fragment of collagen XIII [60], specifically inhibits endothelial cell proliferation. Phase I trials have started at the end of 1999 in the USA [61] and preliminary results are eagerly awaited.

3.4. Anti-angiogenic agents targeting tumour vasculature (Table 7)

3.4.1. Vitaxin

Only low expression of the integrin $\alpha v\beta_3$ can be found in established blood vessels from the normal tissues while tumour neovasculature expresses high levels of this integrin. A humanised mouse monoclonal antibody directed to $\alpha v\beta_3$ has completed phase I trial at doses of 2 mg/kg/week for 6 weeks as a 90-min i.v. infusion. Of 12 evaluable patients for response, one partial response (8%) and 5 stable disease (42%) occurred [62]. Phase II studies are ongoing.

3.4.2. EMD121974

EMD121974 is a small molecule that will block integrins present on the endothelial cell surface. Phase I/II studies are ongoing in Kaposi's sarcoma, brain tumour and other solid tumours [63].

3.4.3. CM101/ZDO101

CM101 is a bacterial toxin derived from the *Streptococcus* group B that selectively targets proliferating blood vessels and activates complement. This results in severe vascularitis in new blood vessels, which is associated with tumour necrosis. In a phase I study, some tumour regressions were seen [64].

3.4.4. Combrestatins

Combrestatins are small natural molecules found in the bark of an African bush willow *Combrestum Caffrum*. The combrestatin A-4 prodrug is a derivative of combrestatin, selectively activated in proliferating endothelial cells overexpressing a phosphatase [65]. They induce vascular shutdown and can be classified as antivascular or vascular targeting agents. Promising clinical activity has already been reported, with a sustained complete response in a patient with an advanced anaplastic thyroid tumour [66].

3.5. Anti-angiogenic agents with multiple or unknown mechanisms of action (Table 8)

3.5.1. Thalidomide

Thalidomide, α-(N-phtalimido)glutarimide, was initially marketed in the late 1950s as a rapidly acting sedative and anti-emetic in Europe and Canada. It was withdrawn from the world market in 1961 because of severe teratogenic effects such as phocomelia and internal organ deformities associated with maternal use. However, there has been renewed interest in recent years in thalidomide and it is now under study as an angiogenesis inhibitor [67]. Its mechanisms of action are complex and poorly understood and include TNF-α inhibition and a variety of effects on the immune system and cell surface receptors.

Thalidomide also inhibits angiogenesis induced by bFGF and VEGF. Its use at doses up to 800 mg daily in previously pretreated patients with refractory multiple myeloma gives an impressive 32% response rate with an overall survival of 58% at 1 year [68,69]. Thalidomide may also be of interest in the treatment of high-grade gliomas as a recently published phase II trial for previously irradiated recurrent patients showed a response in 12% of cases and additional stable disease in 33% [70], confirming the results of other phase I–II studies

Table 8
Anti-angiogenic agents with multiple or unknown mechanisms of action

Drug	Sponsor	Mechanism	Stage of development
Thalidomide	Celgene	– Inhibition of VEGF, bFGF, TNFα – Other?	Phase II glioblastoma, prostate, lung, breast, head and neck, Kaposi's sarcoma and others. Adjuvant metastatic colorectal
IM862	Cytran Inc.	– Activation IL-12 and NK – Other?	Phase I ovarian. Phase II melanoma, renal. Phase III Kaposi's sarcoma.
Suramin	NCI	– Blocks binding of growth factors to their receptors – Other?	Phase III prostate, phase II glioblastoma, myeloma, Castleman's disease.
PNU-145156E	Pharmacia & Upjohn	Suramin analogue	Phase I
Penicillamine	NCI	Copper chelation	Phase II glioblastoma
Tetrathiomolybdate	University of Michigan	Copper chelation	Phase I
Captopril	Not applicable	Copper chelation	Phase I/II
CAI	NCI	Inhibitor of calcium influx	Phase II ovarian, NSCLC, renal, prostate
ABT-627	Abbott	Endothelin receptor antagonist	Phase II prostate, glioblastoma
Interleukin-12	Hoffman Laroche	– Induction IFNγ -Other?	Phase II Kaposi's sarcoma, renal, ovarian
Interferon α	Not applicable	– Inhibition of VEGF and bFGF production – Other?	Approved for treatment of renal cell cancer, melanoma, multiple myeloma, lymphoma, HCL, CML. Multiple phase II and III trials
Taxanes (docetaxel, paclitaxel)	Aventis, Bristol-Myers Squibb	– Antimitotic – Other?	Approved for treatment of breast, ovary, lung. Multiple phase II and III trials
Razoxane	ICRF	– Topoisomerase II inhibitor – Other	Phase II renal

IFNγ, interferon gamma; NK, natural killer; ICRF, Imperial Cancer Research Fund; IL-12, interleukin-12; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; TNFα, tumour necrosis factor-α; NCI, National Cancer Institute; NSCLC, non-small cell lung cancer; HCL, hairy cell leukaemia; CML, chronic myelogenous leukaemia; CAI, carboxyamido-triazole.

[71]. Thalidomide can also be safely associated with carboplatin given at an AUC8, at doses up to 300 mg/m^2 in recurrent glioblastoma multiforme [72].

Thalidomide could also be useful in the treatment of cachexia associated with advanced cancer [73] and in the treatment of AIDS-related Kaposi's sarcoma [74]. Activity has also been reported in androgen-independent prostate cancer, where higher doses (1200 mg/day compared with 200 mg) of thalidomide seem to be most effective [75]. Heavily pretreated metastatic breast cancer patients do not seem to benefit from thalidomide in terms of response rate or disease stabilisation [76]. Interestingly, a substantial activity has recently been suggested in renal cell carcinoma with a 17% partial response rate and a further 17% of stable disease, confirmatory studies are under way [77].

3.5.2. IM862

IM862 is a dipeptide of L-glutamyl-L-tryptophan initially isolated from the thymus. IM862 inhibits angiogenesis in chorioallantoic membrane assays by inhibiting the production of VEGF. IM862 also has immunomodulatory properties and activates natural killer (NK) cell function. The intranasal administration route showed a bioavailability of 71% and this was chosen for human trials. A recent report showed activity in AIDS-related Kaposi's sarcoma with 36% of objective response and an additional 48% of stable disease. Response duration lasted a median of 33+ weeks (range 12+ to 95+) [78]. A very interesting feature is the apparent lack of severe toxicity. Further developments include a phase III randomised study in AIDS-related Kaposi's sarcoma, phase II trial in renal cell cancer and phase I trial in ovarian cancer.

3.5.3. Suramin

Suramin is a highly charged polysulphonated naphtylurea capable of binding a number of proteins including several growth factors such as bFGF. Numerous other mechanisms of action have also been advocated including its adrenolytic effects, inhibition of protein kinase C and topoisomerase II. It is in clinical trial in androgen-independent prostate cancer, brain tumours, multiple myeloma and Castleman's disease. A recently completed phase III trial in 460 hormone refractory prostate cancer patients demonstrates a benefit over a placebo in pain control and the proportion of patients achieving a 50% decline in PSA. Because of an allowed cross-over in the design of the study, benefit in overall survival could not be assessed [79]. Two phase II studies have reported its inefficacy in heavily pretreated metastatic breast cancer [80] and adult astrocytoma [81].

3.5.4. PNU-145156E

PNU-145156E is a suramin analogue that blocks angiogenesis. Among 29 patients treated in a phase I study there was no tumour response and an unpredictable decrease in antithrombin III (ATIII) was associated with significant deep vein thrombosis and pulmonary embolism [82,83].

3.5.5. Copper antagonists or chelators

Some data suggest that copper is necessary for the proliferation of endothelial cells and that the activity of some angiogenic activators is copper dependent [42,84].

3.5.5.1. Penicillamine. In addition to copper chelation, penicillamine blocks endothelial cell migration and proliferation and is also an inhibitor of urokinase plasminogen activator. It is currently being used in a phase II trial for glioblastoma [42].

3.5.5.2. Tetrathiomolybdate. Tetrathiomolybdate was initially developed as an effective anticopper therapy for the treatment of Wilson's disease. In a recently completed phase I trial, stable disease was achieved in five of six metastatic cancer patients (83%) who were copper deficient for at least 90 days [85].

3.5.5.3. Captopril. Captopril is an oral inhibitor of angiotensin I converting enzyme (ACE) widely used in cardiology. Captopril could also function as a copper chelator or as a metalloprotease inhibitor. Some epidemiological data suggest that captopril may protect against cancer [86]. Captopril has been used in combination with other anti-angiogenic agents in a phase II study [87].

3.5.5.4. Carboxyamido-triazole (CAI). CAI is a low molecular weight synthetic compound that inhibits cellular calcium influx. It has shown anti-angiogenic and antimetastatic activity and can be given orally. In a phase I study of 21 patients with refractory solid tumours, DLTs were cerebellar ataxia and confusion. Minor response or stabilisation were observed for 47% of patients [88]. In a phase II study in androgen-independent prostate cancer, all patients progressed within two months and significant toxicities were observed [89].

3.5.6. ABT-627

ABT-627 is an endothelin receptor antagonist [90]. Preliminary reports suggest activity in hormone refractory prostate cancer with manageable toxicity [91]. Phase II studies are underway.

3.5.7. Interleukin-12 (IL-12)

Recombinant human IL-12 is a multifunctional cytokine with anti-angiogenic properties. IL-12 administered subcutaneously (s.c.) three times weekly has activity in renal cancer [92] and melanoma. IL-12 is also being investigated with low-dose intraperitoneal (i.p.) administration in patients with peritoneal carcinomatosis secondary to ovarian or gastrointestinal malignancies [93].

3.5.8. Interferon-α (IFN-α)

Many mechanisms of action account for the effectiveness of interferon-α in various clinical indications, including antiproliferative, immunomodulatory and anti-angiogenic effects [94]. Interferon-α has been the first anti-angiogenic agent to be proved of value in the treatment of life-threatening haemangiomas [95].

A recent report of the activity of IFN-α in advanced giant bone tumours emphasises the need for prolonged therapy with an impressive objective response rate of 50% and a median time to a maximal response of 3.1 years [96]. This observation further supports the theoretical lack of resistance to successful anti-angiogenic agents [97], and has also been reported in hairy cell leukaemia [98]. Interferon-α is already widely used in the treatment of cancer and continues to be tested in phase II and III trials.

3.5.9. Taxanes

The taxanes are cytotoxic agents stabilising microtubules, therefore inhibiting cell division and leading to cell death. More recently, anti-angiogenic properties have been supported by experimental data [8]. However, all antiproliferative agents could theoretically be anti-angiogenic by inhibiting endothelial cell proliferation and decreasing the tumour burden [99]. Indeed, it has recently been shown in an animal model that an alternative anti-angiogenic schedule for administration of cyclophosphamide could avoid the drug resistance associated with the administration of the same agent at conventional doses [100]. Similarly, Kerbel's team showed potent antivascular effects in mice with chronic vinblastine low-dose therapy [101].

3.5.10. Razoxane

Razoxane is an orally bioavailable topoisomerase II inhibitor with anti-angiogenic activity. In a phase II trial in renal cell carcinoma patients, 16% experienced stable disease for a minimum of 4 months [102].

4. Conclusions

Numerous anti-angiogenic agents are beginning to show antitumour effects in phase I/II trials with sometimes objective responses, although stable disease is more common, and preliminary results of some phase III trials are encouraging. The clinical data are beginning to accumulate that such approaches can produce an effective therapy but basic data such as response rate,

response duration, survival, long-term toxicity and synergy with radio- and chemotherapy is now the next major step of clinical research. This produces major challenges for trial design and also for adjuvant and preventive therapies. The medical and scientific community is aware that therapeutic approaches aimed at targeting tumour angiogenesis are only at their early stage of development. Further clinical investigation, together with new ways of assessing the efficacy of such agents, should provide valuable information and undoubtedly will give rise to a new powerful therapy to add to our anticancer armamentarium.

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