CLINICAL TRANSLATION OF ANGIOGENESIS INHIBITORS

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Angiogenesis inhibitors are a new class of drugs, for which the general rules involving conventional chemotherapy might not apply. The successful translation of angiogenesis inhibitors to clinical application depends partly on the transfer of expertise from scientists who are familiar with the biology of angiogenesis to clinicians. What are the most common questions that clinicians ask as they begin to test angiogenesis inhibitors in cancer clinical trials?

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Tumour growth depends on angiogenesis — the recruitment of new blood vessels[1,2]. Angiogenesis usually occurs during development, but, in the adult, it is involved in tissue regeneration and in chronic inflammatory conditions. Cancer cells begin to promote angiogenesis early in tumorigenesis. This 'angiogenic switch'[3] is characterized by oncogene-driven tumour expression of pro-angiogenic proteins[4], such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), interleukin-8 (IL-8), placenta-like growth factor (PLGF), transforming growth-factor-β (TGF-β), platelet-derived endothelial growth factor (PD-EGF), pleiotrophin and others[5–7]. Tumour-associated hypoxic conditions also activate hypoxia-inducible factor-1α (HIF-1α)[6], which promotes upregulation of several angiogeneic factors. Fibroblasts in or near the tumour bed begin to produce pro-angiogenic factors[7], and tumours also recruit progenitor endothelial cells from bone marrow[8]. The angiogenic switch also involves downregulation of angiogenesis suppressor proteins, such as thrombospondin[9].

Various angiogenesis inhibitors have been developed to target vascular endothelial cells and block tumour angiogenesis. Targeting cells that support tumour growth, rather than cancer cells themselves, is a relatively new approach to cancer therapy that is particularly promising because these cells are genetically stable, and therefore less likely to accumulate mutations that allow them to develop drug resistance in a rapid manner.

Direct and indirect angiogenesis inhibitors

There are two classes of angiogenesis inhibitors — 'direct' and 'indirect' (FIG. 1). Direct angiogenesis inhibitors, such as vitaxin, angiostatin and others, prevent vascular endothelial cells from proliferating, migrating or avoiding cell death in response to a spectrum of pro-angiogenic proteins, including VEGF, bFGF, IL-8, platelet-derived growth factor (PDGF) and PD-EGF (TABLE 1). Direct angiogenesis inhibitors are the least likely to induce acquired drug resistance, because they target genetically stable endothelial cells rather than unstable mutating tumour cells[10]. Tumours that are treated with direct-acting anti-angiogenic therapy did not develop drug resistance in mice[11].

Indirect angiogenesis inhibitors generally prevent the expression of or block the activity of a tumour protein that activates angiogenesis, or block the expression of its receptor on endothelial cells (TABLE 2). Many of these tumour-cell proteins are the products of oncogenes that drive the angiogenic switch[4,12,13] (TABLE 3). The activities of oncogene and tumour-suppressor gene products were initially studied in *in vitro* assays that monitored cancer-cell proliferation, apoptosis resistance, immortalization and anchorage independence[4,12]. Because the increased cancer-cell proliferation and decreased apoptosis that was associated with oncogene activation *in vitro* correlated so well with tumour growth *in vivo*, there was no reason to suspect that these new anticancer drugs (for example, signal-transduction inhibitors such as trastuzumab (Herceptin)) could also block the angiogenic output of a tumour[14]. But activating mutations in oncogenes, as well as in the anti-apoptotic factor BCL2 (REF. 15), have been shown to cause tumour cells to upregulate angiogenic proteins and to downregulate inhibitors of angiogenesis[4,15].

Summary

- Angiogenesis inhibitors are a relatively new class of cancer drugs. The biological and biochemical characteristics of angiogenesis inhibitors, however, differ from conventional cytotoxic chemotherapy.
- Basic research into the angiogenic process has revealed several ways by which the clinical efficacy of angiogenesis inhibitors can be improved. These include:
  - Differentiating between direct and indirect angiogenesis inhibitors.
  - Realizing that the microvascular endothelial cell is a genetically stable target of anti-angiogenic therapy.
  - Understanding that slowly growing tumours, which are more difficult to treat by chemotherapy, respond well to anti-angiogenic therapy.
  - An appreciation that rapidly growing tumours require higher doses of an angiogenesis inhibitor.
- Angiogenesis inhibitors are most effective when administered on a dose-schedule that maintains a constant concentration in the circulation instead of a schedule in which therapy is periodically discontinued. Chemotherapy seems to be angiogenesis dependent, in part, and a change in schedule to optimally target the endothelial cell instead of the tumour cell can overcome drug resistance in tumour-bearing mice.
- A current unsolved problem in anti-angiogenic therapy is the lack of surrogate markers for therapeutic efficacy. Whether quantification of circulating progenitor endothelial cells will become an indicator of efficacy remains to be shown.
- When various angiogenesis inhibitors become available for clinical use in cancer patients, these new therapeutic agents might be added to chemotherapy or to radiotherapy, or used in combination with immunotherapy or vaccine therapy.

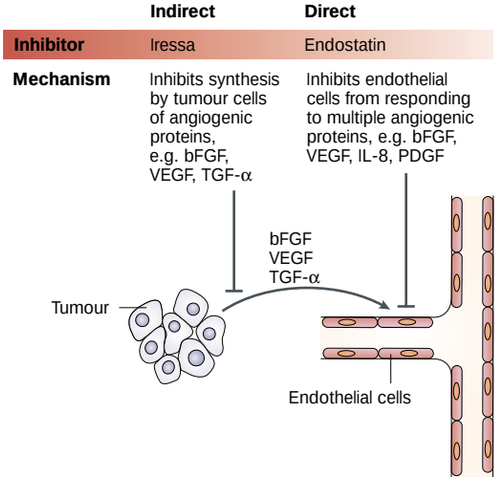


Figure 1 | **Direct and indirect angiogenesis inhbitors.** Direct angiogenesis inhibitors, such as endostatin, target the microvascular endothelial cells that are recruited to the tumour bed and prevent them from responding to various endothelial mitogens and motogens. Indirect angiogenesis inhibitors, such as ZD1839 (Iressa), target proteins — such as epidermal growth-factor tyrosine kinase and its products bFGF, VEGF and TGF-α, or their receptors, on endothelium — that are expressed by tumour cells. Among its many effects, tumour-cell signalling through this receptor induces the expression of vascular endothelial growth factor (VEGF), TGF-α and bFGF, which promotes angiogenesis. bFGF, basic fibroblast growth factor; IL-8, interleukin-8; PDGF, platelet-derived growth factor; TGF-α, transforming growth factor-α.

SIMIAN VIRUS 40 (SV40) TUMOUR ANTIGEN
A multifunctional phosphoprotein that is synthesized early in SV40 infection. It is required for virus DNA replication and for the regulation of viral gene expression in infected cells, as well as for the induction and maintenance of malignant transformation.

Other experimental studies support this concept. In mice, normal cells that have been immortalized with SIMIAN VIRUS 40 (SV40) TUMOUR ANTIGEN produced microscopic avascular tumours, which remained dormant[16]. However, when the immortalized cells were subsequently transfected with the *Ras* oncogene, tumours underwent neovascularization and grew rapidly. In a doxycycline-inducible *Hras* mouse neovascularized melanoma model, downregulation of the *Ras* oncogene led to endothelial apoptosis in the tumour bed, and this preceded tumour-cell apoptosis[17]. Activating mutations in *Kras* and *Hras* upregulate *Vegf* expression and downregulate expression of thrombospondin (an angiogenesis inhibitor)[13,18]. When the *Bcl2* oncogene was transfected into tumour cells, *Vegf* expression increased significantly[15].

In another study, human osteosarcoma cells that were implanted in mice formed only microscopic avascular, dormant tumours in which cancer-cell proliferation was balanced by apoptosis. When these cells were transfected with the *Ras* oncogene, *Vegf* expression doubled, expression of thrombospondin decreased significantly, and large neovascularized tumours grew within approximately 2 weeks[18]. So, targeting oncogene products not only affects cancer-cell proliferation and cell death, but also disrupts the production of angiogenic factors. Re-activation of tumour suppressors such as p53 can also inhibit angiogenesis by different mechanisms, which are discussed below[9,19–21]. These studies predict that certain anticancer drugs that were developed for their capacity to block an oncogene product (for example, inhibitors of the EGF receptor tyrosine kinase) would have indirect anti-angiogenic activity[4,14]. Indeed, RAS farnesyltransferase inhibitors block oncogene signalling pathways that upregulate tumour-cell production of VEGF and downregulate production of the angiogenesis inhibitor thrombospondin-1 (REF. 23).

It is important for clinical researchers to recognize that anticancer drugs that target an oncogene product can inhibit angiogenesis, as this can affect drug dose and schedule. A drug that inhibits angiogenesis indirectly might be discontinued prematurely because of 'resistance', which is determined by increased tumour angiogenesis. Instead, a second inhibitor could be added to the therapeutic regimen. For example, trastuzumab — an antibody that blocks ERBB2 (also known as HER2/neu) receptor tyrosine kinase signalling, suppresses cancer-cell production of angiogenic factors such as TGF-β, angiopoietin-1 and plasminogen-activator inhibitor-1 (PAI1)[22], and possibly also VEGF[14]. It also upregulates the expression of an angiogenesis inhibitor, thrombospondin. If the tumour, however, begins to express a different angiogenic protein[5,24], such as bFGF or IL-8 (REF. 25), the tumour under treatment might seem to have become 'resistant' to trastuzumab, and the therapy will be discontinued. But this practice might not be prudent for a drug with significant anti-angiogenic activity — it might be more effective to add a second anti-angiogenic drug to the regimen. A similar guideline could apply to EGF receptor inhibitors, such as ZD1839

Table 1 | Direct angiogenesis inhibitors

Drug	Endothelia-cell target	Clinical trials	References
Angiostatin	Binds to ATP synthase, angiomotin and annexin II on endothelial cells to inhibit endothelial-cell proliferation and migration	Phase I	69,156,157
Bevacizumab (Avastin)	Recombinant humanized monoclonal antibody against vascular endothelial growth factor (VEGF)	Phase II and III	158
Arresten	Believed to bind integrin-$\alpha_1\beta_1$ to inhibit endothelial-cell proliferation, migration, tube formation and neovascularization	No	159
Canstatin	Believed to bind integrin-$\alpha_v\beta_3$ to inhibit endothelial-cell proliferation, migration and tube formation	Should start this year	160
Combretastatin	Microtubules: induces reorganization of the actin cytoskeleton and early membrane blebbing in human endothelial cells	Completed Phase I	161
Endostatin	Believed to target integrin-$\alpha_v\beta_1$ to inhibit endothelial-cell proliferation and migration, and induce apoptosis of proliferating endothelial cells (R. Kalluri, personal communication); endostatin does not affect wound healing	Phase I and II	57,72
NM-3	An isocoumarin small-molecule inhibitor of VEGF. It was shown to selectively inhibit endothelial-cell proliferation, sprouting and tube formation *in vitro*	Phase I	162
Thrombospondin	Blocks endothelial-cell migration and neovascularization in the cornea, but might not be specific for endothelial cells	No	9
Tumstatin	Binds to integrin $\alpha_v\beta_3$ on endothelial cells; inhibits endothelial-cell proliferation and neovascularization	No	163,164
2-methoxyestradiol	Inhibits microtubule function in proliferating endothelial cells, resulting in endothelial-cell apoptosis	Phase I and II	97
Vitaxin	A humanized monoclonal antibody against integrin $\alpha_v\beta_3$	Phase I and II	165

(Iressa)[26], which prevent expression of VEGF, bFGF, TGF-α and IL-8 by tumour cells, as well as possibly other inhibitors of oncogene products (TABLE 3).

It is of interest that certain tumours, such as giant-cell tumours of the bone and angioblastomas, only or mainly produce a single angiogenic factor — bFGF. When patients with these tumours were treated with interferon (IFN)-α at low daily doses, drug resistance was not observed with therapy of 1–3.5 years duration[27,28]. At low doses, IFN-α inhibits tumour-cell production of bFGF production by tumour cells[29], as well as endothelial-cell motility, and therefore can be considered to have both direct and indirect anti-angiogenic activity[30].

Cells also produce their own endogenous inhibitors of angiogenesis, such as thrombospondins, which can be developed as therapeutics. Transgenic expression of matricellular glycoproteins thrombospondin-1 and -2 (TSP1 and TSP2) reduces the size and density of tumour vessels and reduces tumour growth in nude mice of human squamous-cell carcinoma[23]. Trastuzumab upregulates cancer-cell production of TSP1, so it blocks tumour-cell growth by a combination of anti-angiogenic mechanisms[22]. Angiogenesis inhibitors have therefore shown promise in animal studies, and clinical trials are underway. But what have we learned from animal studies about the best ways to use these drugs in the clinic?

Unique clinical aspects of angiogenesis inhibitors

The traditional methods of testing cytotoxic chemotherapeutic drugs in cancer patients who have failed conventional therapy do not always apply to the testing of angiogenesis inhibitors. Animal studies reveal that many angiogenesis inhibitors are most effective when administered by a dose and schedule that maintains a constant concentration of the inhibitor in the circulation, rather than a once-daily bolus therapy[31]. Cytotoxic drugs, by contrast, are usually administered at maximum tolerated doses followed by off-therapy intervals. In patients, some angiogenesis inhibitors (when used alone) can induce stable disease, whereas others, such as Iressa, IFN-α, endostatin and Takeda neoplastic product 470 (TNP-470), have been reported to cause tumour regression in some cases.

The anticancer drug TNP-470 is a synthetic analogue of fumagillin[32]. It selectively inhibits methionine aminopeptidase-2 (REFS 33,34), blocks the activity of the cyclin-dependent kinase CDK2, and inhibits phosphorylation and activation of the retinoblastoma (RB) protein. In the best results of early clinical trials with this drug, 18% of patients experienced a 50% regression of tumour volume. Furthermore, five patients experienced a "complete or dramatic and durable tumour regression, despite having failed all conventional therapy". These patients included those with carcinoma of the cervix that metastasized to lung[35], high-grade sarcoma of the kidney[36], renal-cell carcinoma[37], androgen-independent prostate cancer[38] and Kaposi's sarcoma[39]. Nevertheless, tumour regression by anti-angiogenic therapy is slow, and can take more than 1 year[27,28]. This is in contrast to the relatively rapid tumour regression that can be obtained by cytotoxic chemotherapy.

Table 2 | Indirect angiogenesis inhibitors

Cancer-cell target	Pro-angiogenic proteins	Drug	Reference
EGF receptor tyrosine kinase	VEGF; bFGF; TGF-α	ZD1839 (Iressa); ZD6474; OSI774 (Tarceva); CI1033; PKI1666; IMC225 (Erbitux)	26
VEGF receptor	VEGF receptor on endothelium	PTK787; ZD6474; SU6668; SU11248	166,168
PDGF receptor	PDGF receptor	PTK787; SU11248	166
ERBB-2 (HER-2/neu receptor tyrosine kinase)	VEGF, angiopoietin-1, TGF-β, PAI1; upregulates thrombospondin-1	Herceptin	12,22
Interferon (IFN)-α receptor	Inhibits expression of bFGF by cancer cells	IFN-α	29

Production of pro-angiogenic proteins is blocked by the angiogenesis inhibitors. Interferon-α can be considered both a direct angiogenesis inhibitor, because it inhibits endothelial-cell migration[30], and an indirect angiogenesis inhibitor, because it inhibits synthesis of bFGF (mRNA and protein) by tumour cells[29]. bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; PAI1, plasminogen activator inhibitor 1; TGF-α/β, transforming growth factor-α/β; VEGF, vascular endothelial growth factor.

The clinical end points that are used to determine efficacy of cytotoxic agents, therefore, do not always apply to anti-angiogenic therapy. The term 'stable disease' illustrates this dilemma. If a cytotoxic drug brings about stabilization, but not tumour regression, the drug might be considered a failure by some oncologists because the tumour will eventually become resistant to the drug, and tumour growth will resume. But acquired resistance might not be as serious a threat with some angiogenesis inhibitors as it is with chemotherapeutics, so tumour growth can be arrested for long time periods. But how do anti-angiogenic drugs exert their anti-tumour effects, and what are the most reliable end points for determining therapeutic efficacy?

Table 3 | Pro-angiogenic oncogenes

Oncogene	Implicated pro-angiogenic activity	References
KRAS, HRAS	VEGF upregulation, TSP1 downregulation	150,169,170–172
SRC	VEGF upregulation, TSP1 downregulation	171,173–175
c-MYB	TSP2 downregulation	176
n-MYC	Angiogenic properties in neuroblastoma	177,178
c-MYC	Angiogenic properties in epidermis	179
ERBB2	VEGF upregulation	14
EGFR	VEGF, bFGF, IL-8 upregulation	14,180
PyMT	TSP1 downregulation	181
FOS	VEGF expression	182
trkB	VEGF downregulation	183
HPV-16	Secretion of VEGF and IFN-α	184,185
v-p3k	VEGF production and angiogenesis	186
ODC	Novel angiogenic factor	187
PTTG1	VEGF and bFGF upregulation	188
E2a-Pbx1	Induction of mouse angiogenin-3	189
BCL2	VEGF upregulation	15

bFGF, basic fibroblast growth factor; EGFR, epidermal growth factor receptor; IFN-α; interferon-α; IL-8, interleukin-8; TSP, thrombospondin; VEGF, vascular endothelial growth factor.

The biological effects of anti-angiogenic drugs

Tumour blood flow. Untreated tumours usually become hypoxic as their size increases. Radiological images or angiograms of large tumours (for example, renal-cell carcinoma) often reveal a dark ischaemic central area that is surrounded by a rim of vascularized tumour tissue. A clear line of demarcation exists between live tumour cells, which are within the oxygen diffusion limit of an open microvessel, and dead tumour cells that lie a few microns beyond this limit (FIG. 2). This is commonly interpreted to mean that the tumour has 'outgrown its blood supply'. It is more likely, however, that elevated tissue pressure that is secondary to vascular leakage has compressed the tumour vasculature[40]. These compressed areas become ischaemic, and foci of necrosis appear, surrounded by regions of hypoxia.

Tumours produce pro-angiogenic proteins to overcome hypoxia[41]. Hypoxic conditions allow activation of the transcription factor HIF-1 α, which induces expression of *VEGF* and other genes that are involved in angiogenesis induction[6,41]. HIF-1α activity is an unfavourable prognostic indicator in early-stage invasive cervical cancer[42] and in the response of oropharyngeal cancers to radiotherapy[43]. In normal tissues, VEGF is upregulated and its mRNA stabilized only under conditions of hypoxia[44]. In tumour cells, by contrast, *VEGF* is constitutively overexpressed, independently of the ambient oxygen tension, but can be further increased by hypoxia[41,42].

So, drugs that block signalling by VEGF and other angiogenic factors would seem to simply prevent tumours from inducing the new blood-vessel growth that is necessary to overcome hypoxia. But this is not as straightforward as it seems. Most anti-angiogenic drugs inhibit new microvessel growth in tumours, but they can also induce regression of recently developed microvessels — a phenomenon known as 'capillary drop-out'. Loss of vasculature causes tumour cells to undergo growth arrest or become apoptotic[45,46]. This tumour-cell death was initially thought to be caused by reduced delivery of oxygen to the tumour. In fact, because the sensitivity of hypoxic tumour cells to ionizing radiation is decreased, radiation oncologists initially expressed concern that angiogenesis inhibitors would reduce the efficacy of radiotherapy.

The effects of anti-angiogenics on other therapies. In 1995, however, Teicher *et al.* showed the opposite — that anti-angiogenic therapy actually increased tumour blood flow and oxygen delivery — at least during the first weeks of therapy[47]. This might explain why some tumours increase their size before decreasing it during anti-angiogenic therapy. Although these experiments were short term, the presumed mechanism was that anti-angiogenic agents decreased leakage of plasma proteins from tumour vessels, resulting in decreased intratumoral pressure[40]. Weischelbaum and colleagues subsequently reported that the efficacy of ionizing radiation was improved by co-administration of the angiogenesis inhibitor angiostatin, when administered at a dose that would be ineffective for angiostatin alone[48].

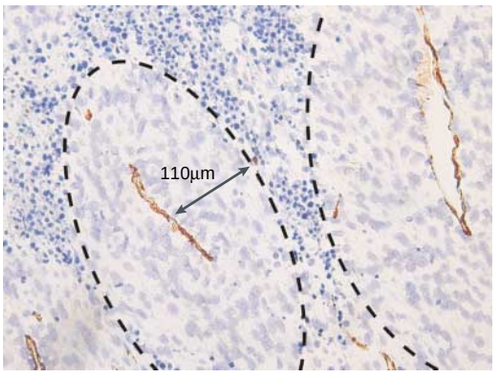


Figure 2 | **Tumour cells form cuffs around functional microvessels in a Dunning rat prostate carcinoma xenograft.** Tumour cells within 110 μm of a microvessel are viable (dashed line). Outside of this radius, tumour cells are dead (blue stain). Reproduced with permission from REF. 115 © (2002) Oxford University Press.

Recent experiments provide additional evidence that angiogenesis inhibitors reduce plasma leakage from tumour vessels (Shay Soker, personal communication). When Evans blue dye was injected intravenously into mice, subcutaneous injection of VEGF or platelet-activating factor (PAF) caused a large blue stain to appear at the injection site (Miles test), which was prevented by prior treatment with endostatin or by TNP-470 — the same angiogenesis inhibitor used by Teicher *et al.*[49]. Angiogenesis inhibitors are also believed to enhance the effects of ionizing radiation by preventing repair of radiation damage to endothelial cells[50]. So, Teicher's original findings were correct — albeit counterintuitive.

A similar question is often raised about combinations of cytotoxic chemotherapy with anti-angiogenic therapy — wouldn't anti-angiogenic therapy prevent delivery of chemotherapy to tumour cells? Again, Teicher reported that angiogenesis inhibitors not only increased blood flow to the tumour, but also increased delivery of a cytotoxic agent, possibly due to decreased intratumoral pressure[47]. A study by Jain indicates that anti-angiogenic therapy might actually normalize the tumour vasculature[51].

Several clinical studies indicate that despite an increase in tumour blood flow during the early phase of anti-angiogenic therapy, chronic anti-angiogenic therapy causes total tumour blood flow to reach a steady state or to gradually decrease. When endostatin was continued for weeks or months in cancer patients, positron-emission tomography (PET) scans revealed a gradual, dose-dependent reduction in total tumour blood flow[52]. This could be caused by a drop-out of individual microvessels, followed by a loss of the surrounding tumour cells. Other studies of tumour blood flow during anti-angiogenic therapy have been reported by clinical investigators at the National Cancer Institute (NCI)[53–55]. In addition, there are several ongoing trials that are using non-invasive imaging techniques to assess changes in tumour blood flow following treatment with angiogenesis inhibitors. These include two clinical trials at the NCI that are evaluating the effects of anti-VEGF antibody — one in patients with renal cancer and the other in patients with breast cancer (S. Libutti, personal communication).

The optimal design of a clinical trial for an angiogenesis inhibitor would therefore be to provide for long-term therapy with assessment of tumour blood flow at consistent time intervals. If a patient's tumour progresses during therapy, a provision in the trial design to increase the dose of inhibitor might halt tumour progression or bring about the original stable state.

Apoptosis

When an angiogenesis inhibitor induces endothelial-cell apoptosis in a microvessel, tumour cells supported by that vessel (such as within the oxygen diffusion limit of up to approximately 150 μm) subsequently undergo apoptosis. In one experimental tumour system, endothelial apoptosis preceded tumour-cell apoptosis by 3–4 days[46] (FIG. 2). But do angiogenesis inhibitors induce tumour-cell apoptosis simply by cutting off the delivery of oxygen and nutrients, or by other mechanisms?

Several studies indicate that angiogenesis inhibitors can induce tumour-cell apoptosis by decreasing levels of endothelial-cell-derived paracrine factors that promote cell survival. At least 20 of these proteins have been reported to be produced by endothelial cells, PDGF, IL-6 and heparin-binding epithelial growth factor (HB-EGF), among others[56]. Production of paracrine factors is decreased, in part, because angiogenesis inhibitors can inhibit endothelial-cell proliferation[57]. It is unclear whether angiogenesis inhibitors also directly decrease endothelial-cell production of paracrine factors. Because anti-angiogenic therapy does not shut off DNA synthesis in tumour cells — at least at the beginning of therapy — these cells should remain susceptible to chemotherapy, unless they have acquired drug-resistance mechanisms. Although addition of an angiogenesis inhibitor to low-dose, metronomic chemotherapy had a synergistic effect on the inhibition of tumour growth in mice[46], it remains to be determined whether conventional chemotherapy (maximum tolerated dose) can be improved by combining it with an angiogenesis inhibitor.

Drug resistance

Hypoxia selects for tumour cells with diminished apoptotic potential[58]. This has been proposed as a mechanism by which tumour cells could become less responsive over time, or even resistant, to anti-angiogenic therapy[58,59]. Tumour cells also become resistant to chemotherapy-induced apoptosis[22,60,61]. For example, overexpression of the anti-apoptotic protein BCL2 allows prostate carcinoma to become refractory to cytotoxic chemotherapy[15]. Anti-angiogenic drugs, however, can also overcome this obstacle. Studies have reported that *in vivo* administration of the angiogenesis inhibitor TNP-470 overcomes the anti-apoptotic advantage that is conferred on the tumours by *BCL2* expression, and inhibits tumour growth.

How do anti-angiogenic drugs manage to kill apoptosis-resistant tumour cells? Even tumour cells that have adapted to survive under hypoxic conditions cannot withstand the ANOXIA that eventually accompanies microvessel dropout. There are several other therapeutic manoeuvres that might bypass tumour resistance to apoptosis. Inhibitors of HIF-1α suppress tumour growth and might also act synergistically with angiogenesis inhibitors[62]. In addition to HIF-1α, other transcription factors are involved in the hypoxia response, so targeting these factors might also promote the response to anti-angiogenic therapy.

The tumour suppressor p53. Mutations in *TP53* (the gene that encodes p53 in humans) also allow tumour cells to become resistant to apoptosis under conditions of hypoxia[61]. In mice, *Trp53* (the gene that encodes p53 in mice) mutations decrease the response of cancer cells to anti-angiogenic therapies[60]. Wild-type p53 normally suppresses tumour angiogenesis by upregulating TSP1 (REF. 9), inducing degradation of HIF-1α (REF. 19), suppressing transcription of VEGF[21] and downregulating bFGF-binding protein expression[21]. The increased neovascularization that occurs following the loss of p53 function, however, can be overcome by increasing the dose of anti-angiogenic therapy. For example, in mice bearing a mutant p53-associated human pancreatic tumour[62], there was a dose-dependent response to a single angiogenesis inhibitor, varying from 33% inhibition to 97% inhibition of tumour growth, leading to tumour regression[31].

It remains to be seen whether this result can be translated to patients with tumours that possess p53 mutations. In contrast to chemotherapy, which is administered at the maximum tolerated dose, anti-angiogenic therapy dosing might be optimized by titrating against the total angiogenic output of a tumour. This is analogous to the titration of insulin against blood sugar or coumadin against prothrombin levels. At present, however, there is no quantitative method for determining the total angiogenic output of a patient's tumour burden, so surrogate markers, such as circulating progenitor endothelial cells, are being studied[63].

Slowly growing indolent tumours

Rapidly growing tumours are generally more sensitive to conventional cytotoxic chemotherapy than slowly growing, indolent tumours. In fact, the most slowly growing tumours (such as neurofibromas and indolent prostate carcinomas) are virtually unresponsive to chemotherapy. It has been assumed by some oncologists that slowly growing tumours would be as unresponsive to anti-angiogenic therapy as they are to chemotherapy[64]. However, when two types of human bladder cancers — a rapidly growing highly vascularized tumour and a slowly growing poorly vascularized tumour — were implanted into immuno-deficient mice, growth of both tumour types was inhibited by angiogenesis inhibitors. For a given dose of angiogenesis inhibitor, the slower the tumour was growing, the more effective the inhibitor[64]. Furthermore, in carcinogen-treated mice, breast cancers that arose spontaneously and grew very slowly were inhibited and/or regressed by treatment with mouse endostatin[65]. Finally, spontaneously arising carcinomas of the pancreatic β-cells in transgenic mice grew very slowly (compared with most transplantable tumours), but retained a high degree of sensitivity to angiogenesis inhibitors[66].

Poorly vascularized tumours

Another common misconception about anti-angiogenic therapy is that it is only effective in highly vascularized tumours. Some cancer patients who have failed conventional therapy lament that their physician told them they were not candidates for anti-angiogenic therapy because their tumour was not vascularized. A distinguished surgeon stated at an international meeting that "pancreatic cancer will not respond to anti-angiogenic therapy because it is a 'white' avascular tumour". On the basis of these assumptions, some angiogenesis inhibitor clinical trials require a pre-treatment biopsy for microvessel density to exclude patients whose "tumour microvessel density is too low". Another clinical trial for a new angiogenesis inhibitor is "restricted to renal-cell carcinoma and other highly vascularized tumours".

There are several flaws in these assumptions. First, virtually any tumour that is large enough to be visible or palpable has already undergone neovascularization to attain that size. Second, intensity of neovascularization cannot be determined by gross inspection of a tumour. A white neurofibrosarcoma of one or more cubic centimetres might have a microvessel density by microscopy that is similar to the microvessel density of a reddish hepatic carcinoma of similar size, except that in the whitish tumour the vessels are more compressed. Third, the lower the vascularity of a tumour, the more susceptible it seems to be to anti-angiogenic therapy[64]. Highly vascularized tumours might require higher doses of an angiogenesis inhibitor or combinations of angiogenesis inhibitors to achieve a tumour response.

Patients with multiple tumours

Occasionally, during anticancer therapy that employs surgery, radiotherapy or chemotherapy, a large tumour at one site regresses, while a tumour at a different site grows. This phenomenon, called 'mixed response' has not been adequately explained. In mice bearing two or more tumours, one tumour might suppress the growth of the other — a phenomenon known as 'concomitant resistance'[65,67,68]. Furthermore, removal of a tumour by surgery[69] or irradiation[70] often results in the vascularization and growth of dormant metastases. The phenomenon of 'concomitant resistance' can now be explained by the ability of one tumour to inhibit angiogenesis in the other[45,69]. Certain tumours produce enzymes that activate angiogenesis inhibitors such as angiostatin[69,71], endostatin[72–74] or anti-angiogenic anti-thrombin III[75,76], which in turn prevent the growth of remote tumours[72,73]. Administration of recombinant angiostatin to mice prevents growth of metastases that occurs after surgical removal of a primary tumour[69] or after reduction of the primary tumour by ionizing radiation[70].

ANOXIA
Complete lack of oxygen in tissues. This is different from hypoxia, which is defined as a low level of oxygen in tissues.

Another example of 'mixed response' was observed in a tumour-bearing animal model treated by TNP-470. TNP-470 has little or no effect on tumour cells *in vitro*, but has a wide spectrum of antitumour activity *in vivo*[77,78]. There are currently more than 60 reports from different laboratories on the ability of this drug to inhibit 33 different types of primary tumours and 23 different types of metastatic tumours in animal models. However, in a rat tumour model of Yoshida sarcoma, intravenous administration of TNP-470 suppressed the growth of primary tumours, but increased the growth of metastatic foci in the lymph nodes[79]. One explanation for this phenomenon is that when an angiogenesis inhibitor is not sufficiently potent to completely suppress metastases after it partially inhibits the primary tumour, the reduced production of endogenous inhibitor(s) from the primary tumour allows growth of the metastases[69].

Clinical effects of anti-angiogenic agents

Patients have been treated with direct angiogenesis inhibitors, such as IFN-α, for as long as 7 years[80], and with endostatin for more than 1 year[1]. These drugs were shown to have very low toxicity[28], and acquired drug resistance was rarely seen in animals that were treated for prolonged periods of time with angiostatin or endostatin. This does not mean that relapses, due to a loss of response to direct angiogenesis inhibitors, will never occur[11]. As recently described, there are epigenetic mechanisms of resistance that can compromise the efficacy of direct angiogenesis inhibitors over time, especially when these drugs are used as monotherapies[81]. Fortunately, there is a large and diverse number of angiogenic targets. Drug combination-therapy regimens in mice have resulted in prolonged antitumour responses, while avoiding or delaying resistance[46,82].

Here, we have selected a few trials, that have provided important information about the clinical applications of these drugs.

A prospective randomized trial of an antibody against VEGF, bevacizumab (Avastin), in 110 patients with metastatic renal-cell cancer — the first of its kind for an angiogenesis inhbitor — significantly prolonged the time to tumour progression with minimal toxicity, but tumour progression was rare[83]. Bevacizumab was also tested in a Phase III trial, in combination with capacitabine, as a third-line therapy for advanced relapsed metastatic breast cancer (see Genentech web site). The primary efficacy end point — an increase in the median time of progression-free survival — was not attained, nor was an increase in 12-month survival detected. The failure of this trial might have been due to the fact that only a small number of the patients' tumours actually expressed biologically active VEGF, highlighting the need to better characterize tumours before patients are included in trials of molecularly targeted therapeutics.

Chemotherapeutic agents have also been shown to have anti-angiogenic properties in animal models, in addition to their ability to induce direct cancer-cell death. Paclitaxel, which inhibits microtubule polymerization, inhibits vascular endothelial-cell proliferation, motility and invasiveness in a dose-dependent manner *in vitro* and tumour angiogenesis *in vivo*[84]. Secondary effects such as these could contribute to the antitumour efficacy of chemotherapy *in vivo* and might delay or prevent the acquisition of drug resistance by cancer cells.

Browder *et al.* proposed that the traditional dose-schedule regimen for chemotherapeutic agents cannot, however, provide the sustained blockade of angiogenesis that is achieved by angiogenesis inhibitors[46]. This could be because chemotherapy is usually administered at the maximum tolerated dose, followed by a treatment-free interval to allow recovery of bone-marrow and gastrointestinal-tract cells. During the treatment-free interval, microvascular endothelial cells in the tumour bed can resume their proliferation and support tumour regrowth[46]. Browder *et al.* experimented with changing scheduling and dose of a cytotoxic agent to augment its anti-endothelial activity — an approach called 'anti-angiogenic chemotherapy'[46].

Anti-angiogenic chemotherapy was first shown to be effective in tumour-bearing mice[46]. Administration of cyclophosphamide at more frequent intervals and at an overall lower dose with a brief treatment-free interval induced sustained apoptosis of endothelial cells in the vascular bed of the tumours, and more effectively controlled growth of drug-resistant tumours. This protocol also reduced side effects and avoided bone-marrow suppression. These results indicated that antitumour efficacy of cytotoxic drugs might be improved by changing the schedule and dose to provide optimum cytotoxic targeting of the microvascular endothelial cells in the tumour bed.

These results might also help to explain why some patients who receive long-term maintenance or even palliative chemotherapy have stable disease beyond the time that the tumour would have been expected to develop drug resistance. Patients with slow-growing cancers who are on anti-angiogenic scheduling of chemotherapy involving continuous infusion of 5-fluorouracil[85–87], weekly paclitaxel[88,89] or daily oral etoposide[90–92] have shown an improved outcome — despite the fact that in some of these patients the tumours had already become resistant to conventional chemotherapy.

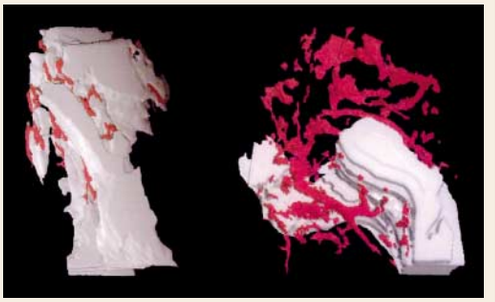
Anti-angiogenic chemotherapy has also been called 'metronomic' therapy[93], but the two terms do not have precisely the same meaning. Anti-angiogenic chemotherapy signifies that the target of the chemotherapy is the microvascular endothelium in the tumour bed. Metronomic therapy indicates that the schedule of administration is at very regular intervals.

Combining anti-angiogenics with chemotherapy. Some studies have indicated that direct angiogenesis inhibitors enhance the effects of anti-angiogenic chemotherapy. Xenografts of neuroblastoma cell lines were subjected to either continuous treatment with low doses of vinblastine, a monoclonal neutralizing antibody (DC101, which targets the FLK1/KDR receptor for Vegf) or both agents together to test whether the anti-vascular effects of the low-dose chemotherapy

Box 1 | Haematological malignancies

Although solid tumours are known to be angiogenesis dependent[1,114], it was assumed until 1993 (REF. 143) that leukaemias and other haematological malignancies did not induce angiogenesis to promote their own survival. In 1993, Brunner *et al.* observed that basic fibroblast growth factor (bFGF) was expressed by human bone-marrow and peripheral-blood cells[144], and the following year Nguyen *et al.* reported that bFGF was elevated in the urine of newly diagnosed leukaemic patients to higher levels than in most other malignancies[145]. Bone-marrow angiogenesis was subsequently found to correlate with multiple myeloma progression[146–148], and was also observed in the lymph nodes of patients with B-cell non-Hodgkin's lymphoma[149] and in bone-marrow biopsies from children with newly diagnosed untreated acute lymphoblastic leukaemia[114]. By 1999, cellular levels of another angiogenic protein — vascular endothelial growth factor — were reported to predict the outcome of patients with multiple myeloma[150]. The figure shows a comparison of normal versus leukaemic bone marrow, with blood vessels shown in red. These images are confocal microscopic sections of bone-marrow biopsies that have been stained with antibody to von Willebrand factor, which highlights blood vessels. In the left panel, normal bone marrow (from a child with a non-neoplastic disease) shows normal microvasculature of uniform-sized vessels. In the right panel, bone marrow from a child with newly diagnosed acute lymphoblastic leukaemia reveals intense neovascularization, with microvessels of variable diameters.

Angiogenesis inhibitors might therefore be useful in treating haematological malignancies. A recent study reported that the retroviral gene transfer of a vector encoding the direct angiogenesis inhibitors angiostatin and endostatin inhibits bone-marrow angiogenesis and tumour growth in a mouse model of leukaemia[137]. This therapy was shown to directly inhibit endothelial proliferation *in vitro*, but had no effect on leukaemia-cell proliferation. Mice that were inoculated with B-cell, T-cell or myelogenous leukaemias and treated with recombinant endostatin have also been observed to live significantly longer and experience fewer toxic side effects than with conventional chemotherapy (Timothy Browder *et al.*, unpublished observations).



could be enhanced when survival signals for endothelial cells, mediated by Vegf, were blocked[82]. Both DC101 and low-dose vinblastine treatment individually resulted in significant, but transient, xenograft regression, diminished tumour perfusion and direct inhibition of angiogenesis. The combination therapy, however, resulted in full and sustained regressions of large established tumours, without an ensuing increase in host toxicity or acquired drug resistance.

An anti-endoglin antibody was shown to act synergistically with cyclophosphamide in a skin tumour/severe combined immunodeficiency mouse model[94]. Endoglin (CD105) is a proliferation-associated cell-membrane protein that is expressed on the tumour-associated angiogenic vascular endothelium. It is required for angiogenesis and is a component of the TGF-β receptor complex. Furthermore, a combination of low-dose topotecan and anti-VEGF antibody therapy was shown to be more effective at suppressing angiogenesis, tumour growth and metastasis in an experimental Wilms' tumour model than either agent alone[95].

Thalidomide. In 1994, Robert D'Amato *et al.* reported that the anti-inflammatory drug thalidomide could inhibit angiogenesis induced by bFgf or Vegf in a rabbit cornea micropocket assay[96]. Thalidomide inhibited new blood-vessel formation in rabbits and mice independently of its ability to suppress infiltrating host inflammatory cells[97]. Thalidomide reduced the growth of carcinomas in rabbits[98] and Lewis lung carcinoma in mice. In 1999, thalidomide therapy was shown to be active, in humans, against advanced multiple myeloma[99] (BOX 1). Some 32% of patients who were treated with thalidomide had a positive response, as assessed by reduction of the serum levels of myeloma protein and urine levels of BENCE–JONES PROTEIN. These findings have been confirmed by other studies[100–105]. Thalidomide is now being tested in more than 160 clinical trials at more than 70 medical centres in the United States (Celgene Coporation, Warren, New Jersey, unpublished observations), and also in Europe for the treatment of various solid tumours. In fact, it has become one of the most effective drugs for treating patients with multiple myeloma — either as first-line therapy or for the treatment of patients who are resistant to conventional chemotherapy.

Thalidomide treatment suppresses the production of tumour necrosis factor (TNF)-α, which has been reported to be angiogenic[106–108]. However, other, more potent inhibitors of TNF-α, such as pentoxifylline and dexamethasone, have little or no activity in corneal angiogenesis assays[109]. Ibuprofen, which inhibits angiogenesis, actually increases serum levels of Tnf-α in mice[109]. Furthermore, TNF-α inhibitors are not effective in animal models of myeloma or in patients. TNF-α suppression therefore does not seem to be a main part of thalidomide's anti-angiogenic activity.

Thalidomide does have a direct anti-proliferative effect on multiple myeloma cells *in vitro*[108], although very high concentrations of thalidomide (up to 100 μM) are required. Nevertheless, this drug might be able to target both cancer cells and vascular endothelial cells.

BENCE–JONES PROTEIN
A monoclonal immunoglobulin that is produced by neoplastic plasma cells in patients with multiple myeloma. This protein can be detected in the urine and its concentration correlates directly with tumour volume.

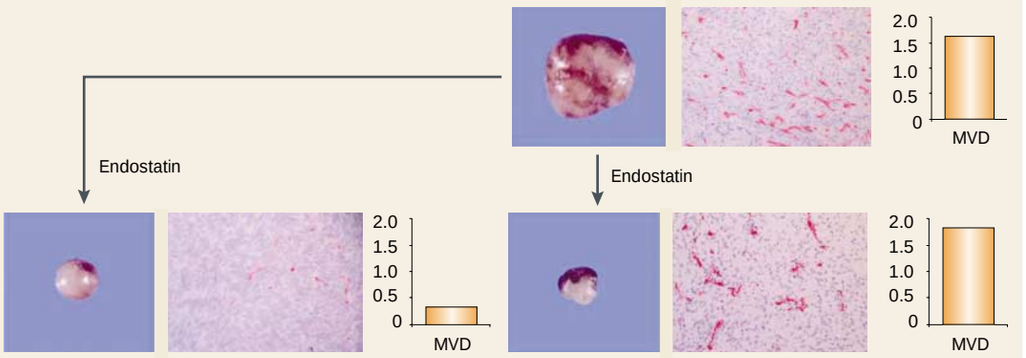
Box 2 | Predicting efficacy of anti-angiogenic therapy: is microvessel density helpful?

In 1972, a quantitative method for histological grading of tumour angiogenesis was developed that correlated the amount of neovascularization with tumour grade in human brain tumours[151]. Additional methods for quantifying the grade of tumour vascularization were developed[152], and followed by the first report of the use of tumour vascularity as a prognostic marker for cutaneous melanoma[153]. In 1991, Weidner *et al.* used anti-endothelial-cell antibodies to identify tumour vasculature, and showed that microvessel density (MVD) was a useful prognostic marker for human breast cancer[112] (image). Microvessel density measures the relative intensity of angiogenesis in angiogenic clones from one tumour to another. In the past 10 years, microvessel density quantification has become a reproducible prognostic factor for the risk of metastasis. The most plausible explanation for this correlation is that most human tumours contain angiogenic and non-angiogenic cells[154], both of which enter the circulation, but only the angiogenic cells can form metastases.

There are a few reports in which microvessel density did not correlate with risk of metastasis or mortality. This can be explained by other factors produced by the tumours. For example, if a primary tumour sample with high microvessel density also produced an angiogenesis inhibitor(s) that suppresses growth of distant metastases, vascularity would not be correlated with patient survival. For reviews of more than 50 publications of microvessel density and its prognostic value in more than 8000 patients, see REFS 114,115,155.

Although microvessel density is a useful marker of metastatic risk, it is not a good indicator of therapeutic efficacy, for several reasons. In normal tissues, degree of vascularization and oxygen/nutrient demand are tightly coupled. In tumours, however, degree of vascularization and tumour growth are loosely coupled, or even uncoupled. This might be because, in tumour cells, expression levels of angiogenic factors are no longer regulated by oxygen concentration. During tumour regression under anti-angiogenic therapy, microvessel density can decrease if capillary drop-out exceeds tumour-cell drop-out (autolysis), increase if tumour-cell drop-out exceeds capillary drop-out, or remain the same if disappearance of capillaries and tumour cells parallel each other.

The figure shows human osteosarcomas taken from mice that were treated with endostatin until there was more than a 50% inhibition of tumour growth[109]. Despite the fact that this drug inhibited growth of both tumours, the intensity of vascularization after treatment differed significantly between the tumours. MVD — expressed as percentage vascular area/tumour area — was quantified over the entire histological section rather than over vascular hot spots. The microvascular density dropped sharply in one treated tumour (left) but rose slightly in the second (right), even though both tumours were equivalently reduced in size by the treatment, relative to control. So, detection of a decrease in microvessel density during treatment with an angiogenesis inhibitor indicates that the agent is active. However, the absence of a drop in microvessel density does not indicate that the agent is ineffective[115]. Figure adapted from REF. 109 © (1991) Elsevier Science.



In patients with multiple myeloma or myelodysplastic syndromes, plasma levels of the pro-angiogenic proteins VEGF and bFGF were significantly decreased compared to pre-treatment levels. This decrease correlated with efficacy of thalidomide therapy[110].

In the original clinical reports of the antitumour effects of thalidomide against multiple myeloma[99], disease remission was not always accompanied by decreased microvessel density. This raised the question of whether the clinical efficacy of thalidomide was mediated entirely, or even partly, by its anti-angiogenic features[111]. However, although microvessel density, as measured by the method of Weidner *et al.*[112] (BOX 2), can determine cancer prognosis, it is not always useful for determining therapeutic efficacy. Although increased microvessel density in bone marrow is associated with relapse in multiple myeloma or with untreated multiple myeloma in virtually all reports so far, microvessel density has not been an effective measure of therapeutic response in many patients with multiple myeloma. For example, some studies reported that increased microvessel density persists in patients who have undergone a complete response to thalidomide[99] or to stem-cell transplantation[113]. Microvessel density also remains elevated in patients who have undergone remission after chemotherapy for acute lymphoblastic leukaemia[114]. Microvessel density might not therefore be a useful indicator of efficacy in solid tumours, although it has continued to be a valid prognostic indicator[114,115].

As a substitute for microvessel density, other surrogate markers of efficacy of anti-angiogenic therapy are being studied. Circulating bone-marrow-derived endothelial cells showed a tenfold reduction and returned to normal values after thalidomide treatment[110]. Decreased circulating levels of VEGF have been correlated with thalidomide's therapeutic efficacy in multiple myeloma and other haematological diseases[110]. However, serum levels of VEGF alone have not been a predictable marker of angiogenesis in other tumours. This is due, in part, to the high VEGF production levels by platelets[116] and because the levels of circulating angiogenesis inhibitors were not taken into account.

Interferon-α. IFN-α has been widely used not only as an antiviral agent to treat chronic hepatitis, but also as a cytotoxic agent to treat certain leukaemias and some bladder cancers[80]. The first evidence that IFN-α had anti-endothelial activity was reported in 1980 when it was found to inhibit the motility of vascular endothelial cells *in vitro* in a dose-dependent and reversible manner[30], and subsequently found to inhibit angiogenesis *in vivo*[117,118]. Experimental studies in mice showed that the anti-angiogenic efficacy of Ifn-α is optimal at low doses and declines at higher doses[119]. New blood-vessel growth in proliferating haemangiomas has been associated with increased expression of bFGF[27,28,120,121], and IFN-α has been shown to downregulate bFGF expression in human cancer cells[29].

The first use of anti-angiogenic therapy in a human being was in 1988, when PULMONARY HAEMANGIOMATOSIS in a 12-year-old boy was successfully treated with daily low-dose IFN-α therapy (3 million units/m^2)[122,123]. Subsequently, infants with life-threatening or sight-threatening haemangiomas were successfully treated with the same low doses of IFN-α for 1–2 years[124,125]. Durable complete regressions have been achieved in patients with solid tumours, such as angioblastomas and giant-cell tumours, who were treated with low-dose IFN-α[27,28,126]. Tumour angiogenesis was mediated mainly by bFGF in these tumours. IFN-α at low doses has also been used to successfully treat haemangio-endothelioma in patients with[127] or without[128] metastases.

Future directions

There are several challenges that face the application of anti-angiogenic therapy to the clinic. These include the need for surrogate markers of efficacy and the requirement for long-term therapy. Using angiogenesis inhibitors in combination with other therapeutic approaches might increase the efficacy of both, but further research is required to uncover the mechanisms of action of different angiogenesis inhibitors, as well as of combinations of angiogenesis inhibitors with each other and with conventional anticancer therapies.

Surrogate markers of angiogenesis. Although bFGF and VEGF levels have been developed as useful surrogate markers for determining the response to thalidomide or IFN-α therapy, there is an urgent need for surrogate markers to determine efficacy of other types of anti-angiogenic therapies. For most tumours, it is unlikely that quantification of circulating factors will serve as useful surrogate markers. Tumours can generate various positive and negative regulators of angiogenesis. To determine whether a tumour is growing or regressing, it would be necessary to quantify the plasma or urine concentration of all of these mediators, which is not feasible at present. Quantification of microvessel density, although valuable as a predictor of future risk of metastasis or mortality, has not proven to be a useful indicator of efficacy of current anti-angiogenic therapy. Preliminary data indicates that quantification of circulating endothelial precursor cells might be used as a surrogate marker of tumour angiogenesis in animals and patients who are treated with endostatin (S. Soker and J. Heymach, unpublished observations) or with thalidomide[110]. The number of these cells has been correlated with the efficacy of endostatin therapy of experimental lymphoma. Other possible candidates include measurement of circulating proteins that are shed from endothelial cells in the tumour bed.

Anti-angiogenic therapy for suppression of tumour growth in patients is likely to require long-term (several years) administration of angiogenesis inhibitors, as was the experience in tumour-bearing animals. This is not a problem for inhibitors that can be administered orally. Most anti-angiogenic drugs, however, require intravenous administration. Recently, a sustained-release preparation of endostatin that can be administered subcutaneously at home — which would be more convenient for patients — has entered clinical trials[129].

Gene therapy. Anti-angiogenic gene therapy is also being tested in animal models[130]. Administration of a vector that constitutively expresses an anti-angiogenic protein allows for the persistence of the protein in the circulation — this has been shown to be more effective than the intermittent peaks of injected inhibitors in mice[31]. Several laboratories are studying anti-angiogenic gene therapy in tumour-bearing animals to determine feasibility for clinical use. There have been many successful experiments[129–140], as well as some negative results[135,141,142].

It is not yet clear why gene therapy of certain angiogenesis inhibitors is more effective than others in tumour-bearing mice. For example, systemic delivery of recombinant adenoviruses that express the ligand-binding ectodomains of the VEGF receptors FLK1 and FLT1 resulted in about 80% inhibition of tumour growth in animals bearing murine Lewis lung carcinoma, T241 fibrosarcoma or human BxPC3 pancreatic carcinoma[135]. By contrast, adenoviruses that are designed to express angiostatin, endostatin or neuropilin were significantly less effective[135]. But, when endostatin was transfected into tumour cells that were then implanted into mice, tumour growth was virtually completely inhibited[136].

The apparent difference in antitumour efficacy of endostatin when it is free in the circulation (low efficacy) versus when it is released locally in the tumour bed (high efficacy) is not clear. One possibility is that systemic gene therapy produces significantly higher plasma levels of endostatin than systemic protein therapy. If endostatin in

PULMONARY HAEMANGIOMATOSIS
Abnormal excessive growth of capillary blood vessels in the lungs, leading to haemorrhage and heart failure.

the circulation follows a U-shaped curve of efficacy, as does IFN-α[119], then very high concentrations of the protein in the circulation might be less anti-angiogenic than lower doses. We previously reported that endostatin — administered on a continuous intravenous schedule — induced 97% tumour regression of human BxPC3 pancreatic carcinoma when the dose reached 20 mg/kg/day (400 μg/20 g mouse) and the serum level reached a steady state at approximately 250 ng/ml (REF. 31). However, when a very high dose of endostatin was administered at 400 mg/kg/day (8000 μg/mouse), there was only a 49% inhibition of tumour growth (O. Kisker *et al.*, unpublished observations). Although these doses are far in excess of what a patient would receive, they remind us that at least for systemic endostatin therapy, serum levels might need to be carefully adjusted to generate blood levels in the range of 250–300 ng/ml. Recent successful studies of systematically administered endostatin gene therapy in mice support this speculation[138–140].

Preventive anti-angiogenic therapy. Because anti-angiogenic therapy is generally less toxic and less susceptible to induction of acquired drug resistance, we speculate that angiogenesis inhibitors could be used as prophylactic therapy in patients who have a high risk for cancer or for recurrence of cancer. An experimental study of spontaneous carcinogen-induced breast cancer in rats revealed that endostatin prevented the onset of breast cancer and also prolonged survival, compared with untreated controls[66]. At least one multicentre cancer-prevention clinical trial is underway (headed by the NCI), in which thalidomide is administered after complete surgical resection of metastatic disease in patients with rectal cancer.

Recurrent or metastatic medullary carcinoma of the thyroid gland might be one tumour type for which a 'preventive' anti-angiogenic strategy could be tested in a small clinical trial. After surgical removal of the primary tumour, secondary tumours sometimes appear in the patient's chest several years later — in teenagers or young adults. Recurrence might be preceded by a slow rise in circulating levels of calcitonin. Elevated calcitonin levels are specifically associated with medullary thyroid carcinoma and can be detected at 1 year or more before appearance of the tumour. After the cancer recurs, it is difficult to treat and is associated with a high mortality. In these patients, anti-angiogenic therapy could be administered at the onset of increased calcitonin levels, and decreased calcitonin levels could be used as an end point.

Because growth and regression of capillary blood vessels are controlled by several rate-limiting pathways, combinations of the approaches discussed in this article will probably be the most effective approach to controlling tumour angiogenesis. As different angiogenesis inhibitors are constantly developed and become more widely available, the number of these reagents that enter clinical trials will only increase. Improved understanding of the mechanisms of these drugs, which can only be gained by collaboration between basic and clinical researcher, should guide future clinical trials.

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Online links

DATABASES

The following terms in this article are linked online to:

Cancer.gov: http://www.cancer.gov/cancer_information/
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LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
angiopoietin-1 | anti-thrombin III | *Bcl2* | BCL2 | bFgf | bFGF | calcitonin | CDK2 | EGF | endoglin | endostatin | ERBB2 | FLK1 | FLT1 | HB-EGF | HIF-1α | *Hras* | Ifn-α | IFN-α | IL-6 | IL-8 | *Kras* | methionine aminopeptidase-2 | neuropilin | p53 | PAI1 | PD-EGF | pleiotrophin | PLGF | *Ras* | RB | TGF-β | Tnf-α | TNF-α | *Trp53* | TSP1 | TSP2 | *Vegf* | VEGF

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Angiogenesis inhibitors:
http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/A/Angiogenesis.html

Cancer clinical trials: http://clinicaltrials.gov/

Celgene site on thalidomide:
http://www.celgene.com/thalomid/index.htm

Access to this interactive links box is free online.