

Challenges of antiangiogenic cancer therapy: trials and errors, and renewed hope

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Abstract

Angiogenesis inhibition has been proposed as a general strategy to fight cancer. However, in spite of the promising preclinical results, a first generation of antiangiogenic compounds yielded poor results in clinical trials. Conceptual errors and mistakes in the design of trials and in the definition of clinical end-points could account for these negative results. In this context of discouraging results, a second generation of antiangiogenic therapies is showing positive results in phases II and III trials at the beginning of the twenty-first century. In fact, several combined treatments with conventional chemotherapy and antiangiogenic compounds have been recently approved. The discovery and pharmacological development of future generations of angiogenesis inhibitors will benefit from further advances in the understanding of the mechanisms involved in human angiogenesis. New styles of trials are necessary, to avoid missing potential therapeutic effects. Different clinical end-points, new surrogate biomarkers and methods of imaging will be helpful in this process. Real efficacy in clinical trials may come with the combined use of antiangiogenic agents with conventional chemotherapy or radiotherapy, and combinations of several antiangiogenic compounds with different mechanisms of action. Finally, the existing antiangiogenic strategies should include other approaches such as vascular targeting or angioprevention.

Keywords: angiogenesis • antiangiogenic therapy • bevacizumab

Introduction

Angiogenesis, the formation of new blood vessels by sprouting of pre-existing ones, is a main mechanism of vascularization during embryonic development, growth, formation of the corpus luteum and endometrium, regeneration and wound healing. However, deregulated, abnormal angiogenesis is involved in many pathological processes [1, 2]. The

complex sequence of events involved in angiogenesis is related to changes in endothelial cell biosignalling [3]. The relationships of angiogenesis with cancer have special relevance, since angiogenesis has been described as one of the hallmarks of cancer, playing an essential role in tumour growth, invasion, and metastasis [4]. Since tumour blood vessels

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show many differences from normal vessels and are not genetically unstable, they are potential targets for therapy of all types of cancer [1,5]. Due to the pivotal role played by endothelial cells in tumour angiogenesis, most previous efforts were devoted to the development of agents that could block their activation by an angiogenic signal (mainly VEGF), or to inhibit one or several specific functions of activated endothelial cells (proliferation, adhesion to extracellular matrix, proteolytic activities, migration, invasion or differentiation). The U.S. National Cancer Institute Database showed that in August 1999 a total of 20 angiogenesis inhibitors were being tested in clinical trials [6]. Remarkably, most of them were monotherapies with the antiangiogenic agent, and those compounds that had then reached the phase III, including several inhibitors of matrix metalloproteinases, were discontinued due to their lack of activity or the appearance of undesirable toxicities. In spite of the great number of angiogenesis inhibitors described so far (estimated to be >300 drug candidates in 2001), and the interesting results obtained in experimental models, even showing complete tumour regressions in pre-clinical studies, modest or even negative results emerged from the first generation of compounds entered in clinical trials [7]. Nevertheless, there is no reason for premature pessimism, as revealed by current ongoing trials and the clinical developmental status of anti-angiogenic drugs [8].

What can we learn from the previous failures?

A critical analysis of the disappointing results obtained in previous clinical trials points to different reasons for this failure. These include flaws in the methods used to select these inhibitors and in the design of the clinical trials to test their effects, as well as an oversimplified view of tumour vasculature pathophysiology.

Angiogenesis inhibitors are initially selected by means of *in vitro* assays that make use of endothelial cells from different sources. The results obtained in this primary screening can be dependent on the type of endothelial cell. Afterwards, the antiangiogenic activity of the selected compounds is usually tested with several *in vivo* assays (for a review, see

[9]). Although useful, these have limitations. Some of them do not take into account the tumour microenvironment (this is the case of vascularization assays in the chicken chorioallantoic membrane and in the mouse cornea). Other assays make use of rapidly growing tumours and/or animals that do not fit into the clinical reality. Most of the tumour systems used with laboratory animals show angiogenic responses much higher than those induced by human tumours [10].

Furthermore, the effectiveness of an angiogenesis inhibitor can be hampered by the intrinsic heterogeneity of human tumour angiogenesis. Animal and preliminary clinical trials have revealed that different tumours respond very differently to antiangiogenic therapy [11]. Thousands of patients have been submitted to clinical trials with antiangiogenic monotherapies. In fact, the responses have been extremely heterogeneous, most probably due to the randomized design of the trials. A previous selection of more homogeneous groups of patients would be highly desirable.

A clinical challenge in antiangiogenesis is the finding of biological markers that help to identify subsets of patients more likely to respond to a given antiangiogenic therapy, as well as to determine optimal dosing of therapy, to detect early clinical benefit or emerging resistances and to decide whether to change therapy in second-line treatments. [12, 13]. In this context, microvessel density has been proven to be a useful prognostic indicator but, at the same time, does not seem to be a good direct indicator of antiangiogenic treatment efficacy [14]. Surrogate biomarkers could include those related to the various steps of the angiogenic process, including variations in endothelial-cell survival, alterations in the endothelial-cell signaling, and variations in the number of circulating endothelial progenitor cells [3, 13, 15, 16]. Another possibility is the fractal analysis of the vascular network in tumour biopsies [17]. However, these approaches are far from an ideal biomarker for clinical practice. Although some authors consider taking biopsies repeatedly to be feasible [18], most physicians consider this to be very cumbersome for patients. Since tumour angiogenesis produces interstitial hypertension in tumours, the determination of interstitial pressure of tumours can be considered an alternative surrogate biomarker [19]. Easier to determine and less invasive approaches include the measurements of circulating levels of several angiogenic factors, but so far no growth factor has been validated for predicting

response to antiangiogenic therapy [12,13], as well as high resolution image analysis that requires expensive instrumentation that could not be available in all institutions [20].

On the other hand, the clinical end-points for dose-defining trials (phase I) and efficacy trials (phase II) should be reconsidered. The expected good tolerability and low toxicity of well selected antiangiogenic compounds give little relevance to the determination of maximum tolerated doses (MTD) and dose-limiting toxicity (DLT), which could be replaced by the determination of optimal biological dose (OBD) in phase I trials. More and better-designed pharmacokinetic studies are required not only to determine the OBD, but also to determine the optimal schedule of drug administration [21]. The case of suramin illustrates this issue. Some years ago, trials with this antiangiogenic compound were discontinued due to low responses and toxicity [22]. However, an improvement in the treatment regimen yielded enhanced response with decreased toxicity in phase I and II trials [23]. Probably, a readjustment of doses and/or schedule could contribute to diminish or even abolish some of the side effects produced by other previously tested antiangiogenic compounds.

In phase II trials, objective responses (*i.e.* the degree of tumour regression) might not be adequate end-points for angiogenesis modulators. Alternative parameters such as disease stabilization, progression-free survival and time to progression should be used. However, these parameters are more difficult to be evaluated properly and they require larger patient samples and more prolonged treatments. Validation and standardization of monitoring techniques for antiangiogenic therapy are urgently required.

The fact that tumour vasculature has been understood in an oversimplified fashion is another explanation for the poor results obtained in the first generation clinical trials. It is now known that different tumour types may acquire their blood supply by different mechanisms. Tumour vasculature is not necessarily derived from endothelial cell sprouting; instead cancer tissue can acquire its vasculature by a number of alternative mechanisms that could be the basis for developing effective clinical modalities using antivasular therapy of cancer [24]. The recruitment of circulating endothelial progenitor cells, mainly from bone marrow origin, can contribute to the tumoural neovascularization by vasculogenesis, a process that was initially thought to be limited to embryonic

development [25]. In fact, as recently reviewed, there is accumulating evidence that progenitor cells – as well as other stromal cells – are actively recruited into tumours and that this recruitment is essential for the proangiogenic environment of tumours [26]. Angiogenesis-independent tumour growth can occur along pre-existing blood vessels [27]. Another possibility is the 'co-option' of vessels during early growth of tumours in the absence of angiogenesis [11, 28]. On the other hand, vascular mimicry, the generation of microvascular channels by genetically deregulated and aggressive tumour cells, is an alternative way to provide blood supply to tumours and is independent of angiogenesis [29,30]. The potential of monocytes/macrophages to contribute to neovascularization has recently come into focus. Some experimental evidences indicate that infiltrating mononuclear cells may incorporate in the lumen microvessels and that peritoneal macrophages may form capillary-like lumens and branching patterns *in vitro* [31, 32]. Lymphangiogenesis has also been related to metastasis [33]. Pathological angiogenesis is characterized by structurally and functionally abnormal vessels and lymphatic vessels [1, 2]. These abnormalities result from an imbalance between levels of pro- and antiangiogenic molecules. As a result, the blood flow in tumour vessels is chaotic and the vessels are leaky [19]. This, in turn, compromises the delivery and effectiveness of conventional therapies, as well as molecular targeted therapies [34].

Finally, in this brief analysis of the rationale behind the failure of the first generation of antiangiogenic agents, concern should be given to the way in which scientists communicate their findings to society. Angiogenesis research is an especially competitive area in which the promising preclinical results have been very often prematurely amplified by mass-media releases. The high prevalence of cancer and the extremely high sensitivity of society towards this primary medical problem facilitate that great expectations could lead to deep disappointment.

Signs of hope

A second generation of antiangiogenic trials is beginning to show highly significant potential [8, 35]. The first study showing phase III data validating an antiangiogenesis strategy for treating human

cancers was obtained with bevacizumab, a humanized recombinant monoclonal antibody that neutralizes the biologically active forms of VEGF that interact with VEGF receptors 1 and 2. In a communication that received much attention in the 2003 ASCO Meeting [36], the authors reported that the bevacizumab/IFL (irinotecan/fluorouracil/leucovorin) combination led to significantly prolonged survival and had a better ability to shrink tumours than standard IFL alone, without statistically significant increases in adverse events in patients with metastatic colorectal cancer. The results were based on 412 patients in the IFL/placebo arm and 403 patients in the IFL/bevacizumab arm. The presence of bevacizumab in the treatment produced remarkable and statistically very significant increases in all the four determined survival and response parameters: median survival, progression-free survival, objective response, and duration of response. These impressive results led the FDA to approve the use of bevacizumab in patients with metastatic colorectal cancer and they have been finally published in the form of a research article in the *New England Journal of Medicine* [37]. However, as stated in the accompanying editorial, although it is tempting to attribute the effect of bevacizumab to a direct antiangiogenic mechanism, the validity of this assumption is presently uncertain [38]. Once more, mass-media releases have led to unrealistically high expectations. As commented in the aforementioned editorial, *'patients need to be informed that bevacizumab does not cure metastatic colorectal cancer and that there is no evidence as yet that the antibody has antitumour activity when administered as a single agent for this disease'* [38]. Although Hurwitz *et al.* did not measure surrogate markers of antiangiogenesis, they mention that bevacizumab may have altered tumour vasculature and decreased elevated interstitial pressures in tumours, thereby enhancing the intracellular delivery of chemotherapy agents [37]. Recently, combined therapies with bevacizumab have received two additional FDA approvals. In June 2006, bevacizumab plus FOLFOX4 (oxaliplatin/5-FU/leucovorin) treatment was approved for second-line metastatic colorectal cancer [39]. In October 2006, bevacizumab in combination with paclitaxel and carboplatin treatment was approved for the first-line treatment of patients with non-small cell lung cancer [40]. Currently, more than 100 clinical trials with bevacizumab are ongoing, including phase III trials in kidney, breast, prostate and ovarian cancer, among others.

Some promising results from clinical trials with other antiangiogenic compounds have already been published. In fact, successful clinical trials of multitargeted compounds have yielded two significant FDA approvals. In December 2005, sorafenib (BAT 43-9006), an inhibitor of the Faf/MEK/Erk and the VEGFR and PDGFR signaling pathways, received FDA approval for the treatment of renal cell carcinoma [41]. Sunitinib (SU11248), an oral inhibitor of VEGFR2, PDGFR, FLT-3 and c-KIT, received FDA approval in January 2006 for patients with gastrointestinal stromal tumours (GIST) and advanced kidney cancer, being the first time the agency had approved a new oncology product for two indications simultaneously [42, 43]. It seems that, after a period of flouting interest, antiangiogenic compounds have regained their place in the centre of anticancer treatment trials, as shown by the Eastern Cooperative Oncology Group Portfolio of clinical trials [44].

From the results obtained so far in clinical trials, it can be concluded that the future clinical success of angiogenesis inhibitors could be related to their use in combination with chemotherapy or radiotherapy. Combined therapies can exert their effects on both tumour and endothelial cells simultaneously. Since abnormal angiogenic vessels compromise the delivery of drugs targeting tumour cells, the normalization of tumour vasculature with antiangiogenic therapy has emerged as a new paradigm for combination therapy [19, 45]. Synergic effects can be expected, since judiciously applied antiangiogenic therapy can increase the penetrability of chemotherapeutic agents, as well as the radiosensitivity of tumour cells. A detailed analysis of how antiangiogenic compounds reduce vessel density shows that these drugs reduce vascular permeability, destroy immature vessels and increase the recruitment of pericytes to stabilize other vessels. This transient stabilization has been termed the *normalization window*, defined as a period of time where tumour blood flow and oxygenation increases, thus providing an opportunity to better deliver chemotherapeutic drugs and radiation therapy [45].

As previously mentioned, the heterogeneity of blood vessel growth, the fact that angiogenesis differs among tumour types is a basis for the observed differences in response to antiangiogenic therapy in both animal and clinical trials [11]. Therefore, a multidrug approach might be more successful than monotherapy. The combined use of several antiangiogenic

Table 1 Antiangiogenic cancer therapy: past and future; clinical trial lessons

Past failures	Possible solutions
<ul style="list-style-type: none"> • Oversimplification of the pathobiology of tumour vasculature 	<ul style="list-style-type: none"> • Deeper knowledge of the molecular mechanisms of the angiogenesis pathways
<ul style="list-style-type: none"> • <i>In vitro</i> and <i>in vivo</i> models do not reflect the clinical situation 	<ul style="list-style-type: none"> • Development of more relevant <i>in vitro</i> and <i>in vivo</i> models
<ul style="list-style-type: none"> • Lack of selectivity in the design of clinical trials 	<ul style="list-style-type: none"> • Previous molecular characterization of the tumour to select the most convenient therapeutic strategy for each case
<ul style="list-style-type: none"> • Use of conventional clinical study design adopted for cytotoxic agents <ul style="list-style-type: none"> - Schedule of administration (MTD, DLT) - End-points reflecting tumour regression or cure 	<ul style="list-style-type: none"> • A new style of clinical trials <ul style="list-style-type: none"> - Long-term toxicity studies - Metronomic scheduling of therapy - Use of surrogate markers and imaging techniques to monitor the biological effect and efficacy of treatments - Selection of more homogeneous groups of patients
<ul style="list-style-type: none"> • Monotherapy with a single angiogenesis inhibitor 	<ul style="list-style-type: none"> • Combination therapies <ul style="list-style-type: none"> - Several antiangiogenic agents with different mechanisms of action - Antiangiogenesis and conventional chemo or radiotherapy
<ul style="list-style-type: none"> • Single purpose: inhibition of neovascularization as a therapy against developed tumours 	<ul style="list-style-type: none"> • Alternative approaches: <ul style="list-style-type: none"> - Vascular targeting against established tumoural vessels - Angioprevention

compounds targeting different steps of angiogenesis should be explored.

Another turn of the screw: a surrogate marker, at last

As stated before, reliable biomarkers are strongly needed to validate the efficacy of antiangiogenic therapy, to identify responsive patients and optimal doses, to predict efficacy of regimens that include anti-angiogenic agents, and to detect and prevent tumour escape. The lack of reliability of measurements of circulating levels of angiogenic factors has made the search for new biomarkers to shift away from measuring their levels to measuring their effects, such as the recruitment of endothelial progenitor cells from the bone marrow to the tumour where they contribute to neovascularization. Preclinical studies have shown that circulating endothelial cells, which are probably derived from blood vessel wall turnover, and circulating endothelial progenitors kinetics correlate well with several standard laboratory assays, that cannot be used in humans [25].

The initial suggestion that variation in the levels of circulating endothelial progenitor cells could be a use-

ful surrogate marker to monitor angiogenesis has been confirmed and extended in an outstanding report published in *Cancer Cell* [44]. This report provides evidence that the levels of circulating endothelial progenitor cells are genetically predetermined and regulated by regulators of angiogenesis, including VEGF, Tie-2 and thrombospondin-1. Moreover, antiangiogenic therapy can be optimized by monitoring the levels of both circulating endothelial cells and circulating endothelial progenitor cells [13, 25]. Therefore, the kinetics of these cells in peripheral blood is suggested to be useful surrogate markers of pathological angiogenesis with potential application for the monitoring of antiangiogenic therapy response.

Future avenues for the vascular therapy of cancer

There are clear signs that during the last year antiangiogenesis research has entered a new age. Table 1 tries to summarize the trials and errors in past failures and possible solutions to them.

The development of new and better models for the *in vivo* assay of potential inhibitors of human angiogenesis should be considered a priority in this field of

research. There is increasing concern that by using approaches based on traditional end-points, potentially interesting angiogenic modulators might be rejected prematurely. Consequently, the extensive use of correlative studies in the early phases of drug development to establish surrogate biomarkers for use in efficacy trials is strongly recommended. Methods of imaging will be helpful to assess the efficacy of treatment [12, 13, 20]. A careful selection of the clinical setting for the investigation (for example, tumour type and stage of disease) and innovative statistical designs to optimize the selection of patients must be carried out before expensive, definitive phase III clinical trials. An example is the randomized discontinuation trial design (RDTD), aimed to select a subset of enrolled patients who are more homogeneous with respect to important prognostic factors than the group of patients that would otherwise be randomized in the trial.

Frequent administration of chemotherapy at low doses, ranging from one-tenth to one-third of the MTD, significantly increases the antiangiogenic effect. This 'metronomic scheduling' has shown impressive antitumour activity in animal models and is now being tested either alone or in combination with other antiangiogenic agents in clinical trials [35, 47, 48]. Furthermore, this metronomic approach is also used with radiation therapy, when administered at lower than normal doses, known as 'hyperfractionated radiation' [49].

The concept of vascular targeting is related to antiangiogenesis but involves a different approach. Juliana Denekamp outlined the concepts behind vascular targeting for cancer treatment in the early 1980s, showing that physical occlusion of the blood supply to tumours in rodents led to tumour regressions [50]. Vascular targeting agents exert their primary action on the pre-existing blood vessels of solid tumours. Vascular targeting therapies would share the advantages of antiangiogenic therapies and could offer some additional advantages. First, blood flow is a defined surrogate marker of biological activity that can be measured in the clinic. Second, temporary effects on vascular functioning may be sufficient. And third, unlike angiogenesis inhibitors, vascular targeting agents should require only intermittent administration to synergize with conventional treatments rather than chronic administration. Future research in this area should identify new, more specific tumour endothelial markers. The clinical studies

completed to date with vascular targeting agents are encouraging. Progression into combination studies has begun [51].

Recently, concerns have been raised on the possibility of resistance to antiangiogenic therapy [13, 21]. In fact, an effective antiangiogenic therapy could select for resistant and aggressive cancer cells during therapy-induced tumour regression [52]. Ideally, the most effective therapy would suppress all cancer cells, avoiding relapse. On the other hand, hypoxia is common in tumours, despite the increase in their vascularization, because of a poor perfusion caused by aberrant vessels [53]. Strategies that target hypoxic cells may therefore synergize with antiangiogenic treatments.

The contribution of inflammatory cells to tumour angiogenesis should also be kept in mind [54]. Cyclooxygenase-2 seems to play a key role. The inhibitors of this pathway exhibit high tolerability and they can be administered chronically. Their performance in clinical studies is currently being tested. However, the recent problems with cyclooxygenase inhibitors in cancer prevention treatment raise serious concerns for their use [55, 56].

Finally, the potentials of angioprevention should also be analyzed [57]. Inhibitors of angiogenesis could slow the progression of premalignant lesions and reduce the risk of developing invasive tumours. They have the potential to be used in primary, secondary or tertiary cancer prevention settings. In fact, monotherapies with antiangiogenic compounds could be useful as adjuvant treatments in situations of minimal residual disease following either cytoreductive surgery or cytotoxic treatment. However, in spite of the fact that past clinical studies have shown that many angiogenesis inhibitors can be given safely to patients, more long-term toxicity studies are needed.

How much antiangiogenic therapy will be incorporated in the future to the treatment of cancer patients depends on further advances in the understanding of the molecular mechanisms involved in tumour angiogenesis, the development of standardized methods to assess surrogate predictive markers of response, and the capability of performing a new generation of appropriately designed clinical studies. A convergence of the efforts carried out in basic, applied and clinical research would contribute to achieve these goals. This knowledge will be applied not only to cancer treatment but also to other diseases characterized by abnormal vasculature – such as hemangiomas, diabetic

retinopathy, macular degeneration and psoriasis, among others – for which antiangiogenic approaches have already shown benefits [1, 2].

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