Differentiation and Definition of Vascular-Targeted Therapies

Dietmar W. Siemann,[1] Michael C. Bibby,[2] Graham G. Dark,[3] Adam P. Dicker,[4] Ferry A.L.M. Eskens,[5] Michael R. Horsman,[6] Dieter Marmé,[7] and Patricia M. LoRusso[8]

[1]University of Florida, Gainesville, Florida; [2]University of Bradford, Bradford, West Yorkshire, United Kingdom; [3]University of Newcastle upon Tyne, United Kingdom; [4]Thomas Jefferson University, Philadelphia, Pennsylvania; [5]Erasmus Medical Centre, Rotterdam, the Netherlands; [6]Aarhus University Hospital, Aarhus, Denmark; [7]Tumor Biology Center, Freiburg, Germany; and [8]Karmanos Cancer Institute, Southfield, Michigan

ABSTRACT

The therapeutic potential of targeting the tumor vascular supply is now widely recognized. Intense research and development activity has resulted in a variety of investigational agents, a number of which are currently in clinical development. As these novel agents are quite distinct from the cytotoxic drugs conventionally used in the treatment of solid tumors, it will be particularly important to ensure early differentiation of these vascular-targeted therapies in order to encourage widespread understanding of their potential benefits and application in the clinic. Two distinct groups of vascular-targeted therapies have evolved: antiangiogenic agents and vascular-disrupting approaches. These differ in three key respects: their physiologic target, the type or extent of disease that is likely to be susceptible, and the treatment scheduling. Inhibitors of angiogenesis interfere with new vessel formation and therefore have a preventative action, require chronic administration, and are likely to be of particular benefit in early-stage or asymptomatic metastatic disease. Vascular-disrupting agents target the established tumor blood vessels, resulting in tumor ischemia and necrosis. These agents are therefore given acutely, show more immediate effects, and may have particular efficacy against advanced disease. It is essential that these agents can be readily distinguished from conventional therapies and that an understanding of key differences between the two types of vascular-targeted therapies is fostered. Here, a simple taxonomy and nomenclature is proposed in anticipation that the therapeutic potential of this novel class can be realized as these approaches advance in clinical settings and a new anticancer strategy becomes available in the clinic.

Received 8/13/04; revised 10/19/04; accepted 10/28/04.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Dietmar W. Siemann, Department of Radiation Oncology, University of Florida, P.O. Box 100385, Gainesville, FL 32610. Phone: 352-265-0287; Fax: 352-265-0759; E-mail: siemadw@ufl.edu.

INTRODUCTION

It is now well known that a functioning vascular supply is essential for solid tumor growth and metastasis, and that in the absence of angiogenic growth, tumors are unable to develop beyond a few millimeters and therefore remain dormant (1). Initiation of angiogenesis, however, allows rapid tumor growth, metastasis, and ongoing tumor progression. The therapeutic potential of targeting the tumor vascular supply is therefore apparent and rapid developments in this field have resulted in a large number of investigational drugs, many of which are now in advanced clinical development. If the anticipated clinical success is realized, clinicians may shortly be able to prescribe an entirely new class of anticancer drugs. These agents are quite distinct from radiation therapy and cytotoxic agents, therapies that along with surgery form the current cornerstone of cancer treatment. It is therefore essential that widespread understanding of this novel class of agents is established. To this end, an international group of scientists and clinicians with key expertise within the area of vascular-targeted therapies met with the objective of developing a clear and precise means by which to differentiate and describe the variety of agents designed to target the tumor vasculature.

VASCULAR-TARGETED THERAPIES

Current, cytotoxic, cancer therapies target the tumor cells directly. Whereas this approach is clearly successful, the limitations are evident and despite new agents and improved regimens, patient survival for most types of advanced cancer has not significantly improved for many years. Greater understanding of the mechanisms by which tumor cells grow and metastasize has led to the identification of a new range of more selective therapeutic targets. Therefore, hopes for therapeutic advancement are increasingly focused on the development of molecular-targeted agents (i.e., those agents directed against targets that are overexpressed or overactive in tumor cells). Rather than targeting the tumor cells per se, another strategy that has received a great deal of attention in recent years is to target the tumor's stroma, in particular the blood vessel support network (2–13).

As over 90% of all cancers present as solid tumors, reliant on a functioning vascular network to supply oxygen and nutrients, the broad-spectrum therapeutic potential of interfering with the vasculature holds great promise. Whereas conventional cancer treatments exert their antitumor effect by targeting the rapidly growing neoplastic cell population, vascular-targeted therapies elicit an indirect effect on the tumor cells. By targeting a component distinct from that targeted by cytotoxic agents, there is great potential for complementary activity. In addition, the cells of the tumor-associated vasculature are essentially normal and therefore have greater genetic stability than the neoplastic cells, possibly resulting in a lower risk of acquired drug resistance (4).

Historical evidence exists to implicate the vasculature as a possible "Achilles heel" of solid tumor growth. Indeed, many early cancer therapies such as the deliberate induction of

bacterial infections in cancer patients undoubtedly relied at least in part on action against the tumor's blood vessel network (14). Early attempts to inhibit the tumor blood supply have included vessel ligation and transcatheter arterial embolization (15). These invasive approaches are associated with a number of limitations, not least a nonselective effect that results in normal tissue damage. However, recent developments in this therapeutic area have now resulted in a wide variety of selective agents, which can be classified into two broad categories. One approach aims to inhibit key factors required for new vessel development, thereby inhibiting new vessel growth (antiangiogenic agents; refs. 2, 4, 6, 7, 11, 12). The second capitalizes on the inherent differences between the blood vessels of normal tissues and those of tumor tissues to target and destroy the existing tumor vasculature (vascular-disrupting agents; refs. 3, 5, 8, 9, 13, 16). With a number of novel agents now in advanced stages of clinical development, it is essential that the fundamental differences between the two approaches be clarified and widely understood. The way in which any successful drug candidates will be used, the appropriate stage of disease, and the expected outcome is likely to be quite distinct. The wealth of vascular-targeted therapies currently under development means that physicians will soon have such agents available in the clinic and a clear knowledge and understanding of the indirect nature of their effect and the key differences in the mechanism of action between the two classes of drug will be required to assure optimal usage.

AGENTS THAT AFFECT ANGIOGENESIS

The process of new vessel development is extremely complex, and is reliant on a delicate balance of biochemical signals and receptors in a variety of cell types working in concert to regulate the angiogenic "cascade" (2, 17, 18). A greater knowledge of this process has identified a number of points at which the cascade can be disturbed (Fig. 1). The objective of antiangiogenic therapies is therefore to interfere with new vessel formation, thereby preventing tumor growth and limiting metastatic potential (4, 6, 7, 11). The clinical outcomes are therefore likely to be quite distinct from those seen with conventional cytotoxic therapies, as inhibition of tumor progression, rather than destruction of existing disease, may be anticipated.

In tumors, vascular endothelial growth factor (VEGF) is considered to be the most potent and specific of the many angiogenic factors (19). It is not only crucial for endothelial cell proliferation and blood vessel formation, but also induces significant vascular permeability and plays a key role in endothelial cell survival signaling in newly formed vessels (19–22). VEGF is secreted by tumor cells and the expression can be increased by environmental triggers such as hypoxia, loss of tumor suppressor gene function and oncogene activation (23–25). Inhibition of VEGF functioning is therefore a key antiangiogenic approach. Targeting VEGF or its receptors with monoclonal antibodies (such as bevacizumab/Avastin) or small molecule inhibitors of VEGFR tyrosine kinase inhibitors (such as ZD6474 and PTK787) has confirmed the anticancer activity of these agents (26–28). Recent studies that combined Avastin with conventional

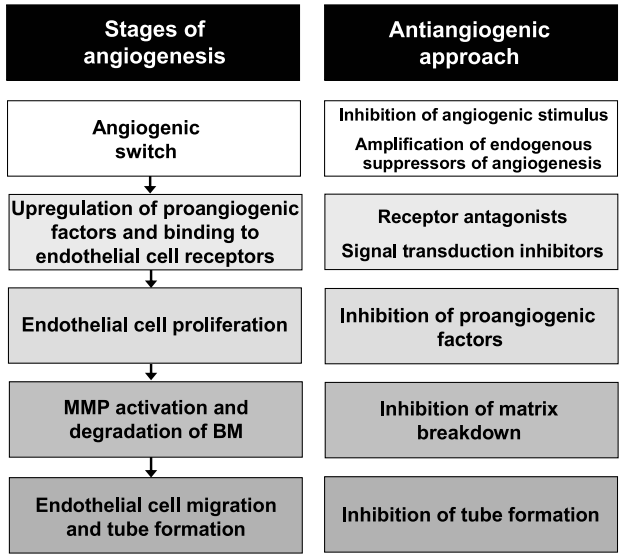

Fig. 1 Inhibition of the angiogenic process.

chemotherapeutic agent regimens in the treatment of patients with metastatic colorectal cancer provided the first unequivocal demonstration of the clinical value of inhibiting VEGF activity, resulting in improved survival (29). Confidence in the clinical potential of this approach is reflected by the wealth of antiangiogenic agents currently in development.

AGENTS THAT DAMAGE EXISTING BLOOD VESSELS

The goal of this therapeutic approach is to compromise the established tumor vasculature, thereby eliciting secondary tumor cell death due to ischemia. In contrast to the antiangiogenic treatment strategy, this approach has the potential to destroy existing tumor masses, as well as preventing progression. As such, these agents may therefore be particularly suitable for treating large tumors, which are typically resistant to conventional cytotoxic therapies (8, 30).

Historical evidence of the efficacy of this strategy can be found in the clinical application of colchicines, which in the 1940s was shown to have antitumor effects (31). Whereas this agent's side effects limited its utility, the concept of affecting solid tumors by destroying their vasculature has continued to hold significant appeal (5). Still, it was not until the recent development of a new generation of agents and approaches possessing the potential of selectively targeting existing tumor blood vessels that this strategy has significantly advanced. A range of strategies aimed at compromising the tumor vasculature is currently under investigation (3, 5, 8, 9, 13, 16). One approach encompasses the vascular targeted peptide therapies (3, 12). Such therapies are typically comprised of linked targeting and effector moieties that seek to induce thrombosis by targeting specific markers on tumor endothelium. Several approaches based on linking antibodies or peptides that recognize tumor-associated vasculature to effector molecules that can induce endothelial cell

damage have been tried. Antigenic determinants that are selectively and constitutively expressed on or around the tumor neovasculature including endoglin, VEGF receptors, αv integrins, the fibronectin EDB domain, and prostate-specific membrane antigen have been of particular interest in this strategy (3). Small molecule drugs that induce extensive necrosis in tumors as a result of vascular collapse, including flavanoids and tubulin depolymerizing/binding agents, represent another approach. 5,6-Dimethylxanthenone-4-acetic acid, the lead flavanoid under study, has a complex mechanism of action that is poorly understood. Its main action on vascular endothelial cells is thought to involve a cascade of direct and indirect events (which include release of vasoactive agents and cytokines) leading to induction of hemorrhagic necrosis (16). The principle mechanism of action of tubulin binding agents is believed to be the selective disruption of the cytoskeleton of proliferating endothelial cells that results in endothelial cell shape changes in tumor-associated endothelial cells. In turn, this results in tumor vessel occlusion and extensive necrosis (Fig. 2; refs. 9, 13, 32, 33).

Selective vascular destruction has now been shown in a large variety of experimental tumor models treated with therapeutic strategies aimed at the existing tumor blood vessel network. The resultant effect is extensive central tumor necrosis, leaving only a thin layer of viable cells at the tumor periphery, an observation common to all of these therapies and thought to arise as a result of diffusion of oxygen and nutrients to the peripheral tumor cells from the surrounding normal tissues (8).

CLARITY IN THE CLINIC

As vascular-targeted therapies are an entirely new clinical approach, it is perhaps not surprising that confusion exists between the various approaches taken to target tumor blood vessels in order to achieve a therapeutic benefit. With the advance of such novel agents into patients, the opportunity to clarify the key differences between agents that interfere with the angiogenic process and those that compromise established tumor blood vessels at an early stage should be taken in order to improve the general understanding of concepts associated with vascular targeting in cancer management. In order to simplify the dissemination of data and understanding of the potential benefits and application of these agents, a common terminology should be used in order to foster a common understanding. As all of the novel strategies discussed here focus on impeding the vascular supply of tumors, the term "vascular-targeted therapy" can be considered an appropriate "umbrella" term when describing this general therapeutic approach. It is also important that the vascular-targeted therapies are not referred to as cytotoxic therapy, a term that is inextricably linked with conventional anticancer agents and will only fuel confusion if used in this context.

Whereas the mechanisms underlying the two broad categories of vascular-targeted therapies are quite distinct, the nomenclature used to describe them has become confused. Review of the literature, congress reports and the lay and scientific press reveals a wide variety of descriptive terms and phrases used to discuss these novel agents. Agents that primarily act to inhibit tumor angiogenesis can simply and accurately be described as "antiangiogenic" drugs. The process of angiogenesis as a physiologic or pathologic process is widely understood and therefore inhibition of this process is a relatively straightforward concept. There is, however, less widespread understanding of the agents that target the established tumor vasculature, which we propose should be referred to as "vascular-disrupting agents," in order to communicate their key mechanism of action. These agents have often previously been referred to as "vascular-targeting agents" or "antivascular therapies." Whereas technically correct, it could be argued that antiangiogenic agents also target the tumor vasculature and have antivascular effects, hence the blanket term of "vascular-targeted therapies," suggested above.

Finally, it should be noted that conventional anticancer therapies such as certain chemotherapeutic agents and radiation can affect tumor vasculature. Indeed "antiangiogenic scheduling" of chemotherapy provides an intriguing application of cytotoxic agents to impair the tumor blood vessel network (34). However, these therapies differ from the vascular targeting therapies whose primary function targets the tumor endothelium

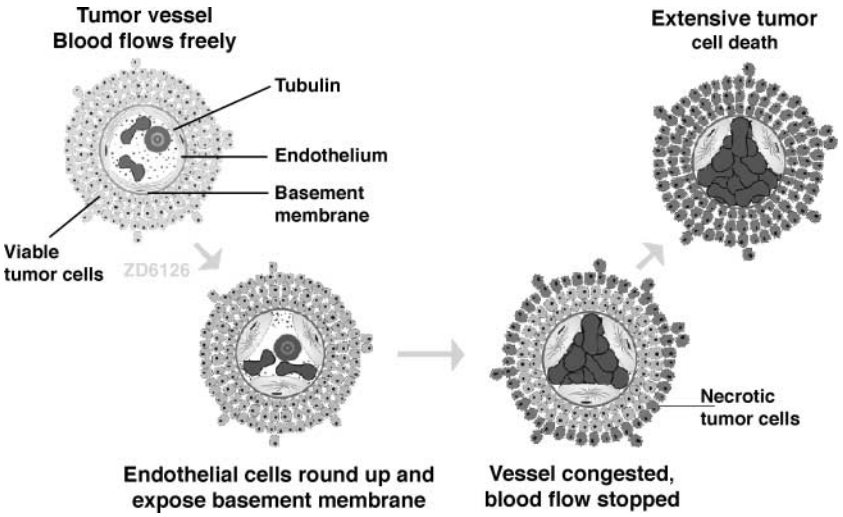

Fig. 2 Principal action of the microtubule destabilizing agent ZD6126.

with little direct effect on the neoplastic cell population. As such, they lie outside the central focus of the present discussion.

VASCULAR-TARGETED THERAPY: A TAXONOMIC CLASSIFICATION

In clinical practice, therapeutic decision-making will require initial differentiation between agents that inhibit the process of angiogenesis and agents that target established tumor blood vessels. Therefore, a precise, yet simple, means of describing vascular-targeted therapies is required. Such a classification, or taxonomy, should be capable of evolution and, above all, widely recognized and accepted. Therefore, a two-stage classification is proposed. Figure 3 shows how the antiangiogenic agents and vascular-disrupting agents are organized under the main class of vascular-targeted therapy. This simple diagram highlights the principal properties of antiangiogenic and vascular-disrupting agents as described above. Whereas agents that target the tumor vasculature may display both an antiangiogenic and a vascular-disrupting action, perhaps at different doses or schedules, it is acceptable to classify agents based on their *primary* action, because consideration of potential ''overlap'' in action would make even the broadest of differentiation difficult.

A second level of differentiation allows description of the targets of each group of agents and the key mechanisms through which their action is elicited. Someone with a more limited knowledge of the new vascular-targeted therapies can clearly see from Fig. 4 that bevacizumab, for example, is an antiangiogenic agent that functions through inhibition of VEGF activity. Similarly, this flow diagram readily identifies combretastatin (CA4P) as a vascular-disrupting agent that functions by binding to tubulin. Whereas cancer is the focus of this taxonomy, it is clear that vascular-targeted therapies may have value in nononcologic settings such as ophthalmology and diabetes. The present, simple classification can readily be adapted to other such therapeutic applications.

CONCLUSIONS

It is proposed that the two simple schematics presented here can serve to differentiate and describe the current vascular-targeted therapies. Importantly in this fast-moving field, these figures can also be readily adapted to accommodate new developments. Appreciation of the key differences between the two broad groups of vascular-targeted therapies (antiangiogenic and vascular-disrupting) will be important in the clinical decision-making process, particularly as more agents become available. In addition to their use in combination with conventional treatment regimens, the potential therapeutic advantage of combining antiangiogenic and vascular-disrupting agents to target both aspects of the vessel network of growing neoplasia are apparent and no doubt will be elucidated in due course. Although a great deal of research is required to establish the clinical efficacy and ideal application of vascular-targeted strategies, this developing anticancer approach continues to generate great research interest and clinical optimism.

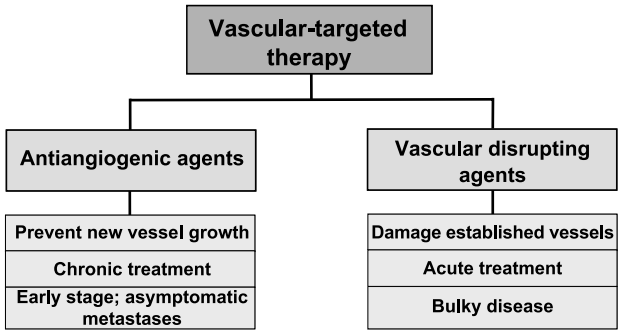

Fig. 3 Taxonomy of vascular-targeted therapies.

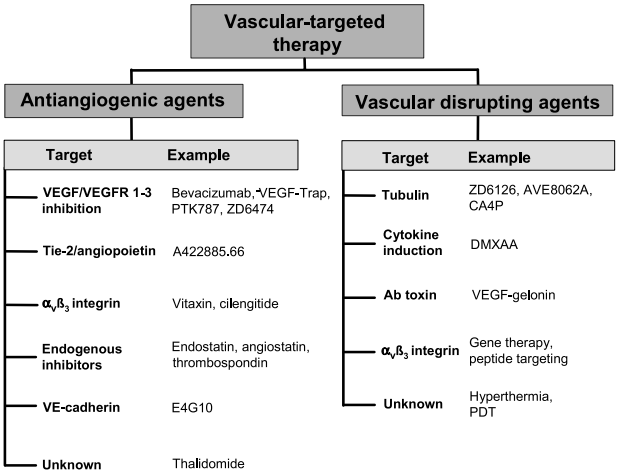

Fig. 4 Examples of targets of vascular-targeted therapies.

REFERENCES

1. Brem S, Brem H, Folkman J, Finkelstein D, Patz A. Prolonged tumor dormancy by prevention of neovascularization in the vitreous. Cancer Res 1976;36:2807–12.

2. Marme D. The impact of anti-angiogenic agents on cancer therapy. J Cancer Res Clin Oncol 2003;129:607–20.

3. Thorpe PE. Vascular targeting agents as cancer therapeutics. Clin Cancer Res 2004;10:415–27.

4. Kerbel R, Folkman J. Clinical translation of angiogenesis inhibitors. Nat Rev Cancer 2002;2:727–39.

5. Denekamp J. The tumour microcirculation as a target in cancer therapy: a clearer perspective. Eur J Clin Invest 1999;29:733–6.

6. Sridhar SS, Shepherd FA. Targeting angiogenesis: a review of angiogenesis inhibitors in the treatment of lung cancer. Lung Cancer 2003;42 Suppl 1:S81–91.

7. Eskens FA. Angiogenesis inhibitors in clinical development; where are we now and where are we going? Br J Cancer 2004;90:1–7.

8. Siemann DW. Vascular targeting agents. Horizons in Cancer Therapeutics 2002;3:4–14.

9. Chaplin DJ, Dougherty GJ. Tumour vasculature as a target for cancer therapy. Br J Cancer 1999;80 Suppl 1:57–64.

10. Arap W, Pasqualini R, Ruoslahti E. Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. Science 1998;279:377–80.

11. Ellis LM, Liu W, Ahmad SA, et al. Overview of angiogenesis: biologic implications for antiangiogenic therapy. Semin Oncol 2001;28:94–104.

12. Ruoslahti E. Specialization of tumour vasculature. Nat Rev Cancer 2002;2:83–90.

13. Siemann DW, Chaplin DJ, Horsman MR. Vascular-targeting therapies for treatment of malignant disease. Cancer 2004;100:2491–9.

14. Starnes CO. Coley's toxins in perspective. Nature 1992;357:11–2.

15. Kim DK, Kinne DW, Fortner JG. Occlusion of the hepatic artery in man. Surg Gynecol Obstet 1973;136:966–8.

16. Baguley BC. Antivascular therapy of cancer: DMXAA. Lancet Oncol 2003;4:141–8.

17. Carmeliet P. Angiogenesis in health and disease. Nat Med 2003;9:653–60.

18. Folkman J. The role of angiogenesis in tumor growth. Semin Cancer Biol 1992;3:65–71.

19. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003;9:669–76.

20. McMahon G. VEGF receptor signaling in tumor angiogenesis. Oncologist 2000;5 Suppl 1:3–10.

21. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 1995;146:1029–39.

22. Bates DO, Heald RI, Curry FE, Williams B. Vascular endothelial growth factor increases *Rana* vascular permeability and compliance by different signalling pathways. J Physiol 2001;533:263–72.

23. Zhang L, Yu D, Hu M, et al. Wild-type p53 suppresses angiogenesis in human leiomyosarcoma and synovial sarcoma by transcriptional suppression of vascular endothelial growth factor expression. Cancer Res 2000;60:3655–61.

24. Mukhopadhyay D, Knebelmann B, Cohen HT, Ananth S, Sukhatme VP. The von Hippel-Lindau tumor suppressor gene product interacts with Sp1 to repress vascular endothelial growth factor promoter activity. Mol Cell Biol 1997;17:5629–39.

25. Ferrara N. Role of vascular endothelial growth factor in physiologic and pathologic angiogenesis: therapeutic implications. Semin Oncol 2002;29:10–4.

26. Sorbera LA, Leeson PA, Bayés M. Bevacizumab Oncolytic. Drugs of the Future 2002;27:625–32.

27. Wedge SR, Ogilvie DJ, Dukes M, et al. ZD6474 inhibits vascular endothelial growth factor signaling, angiogenesis, and tumor growth following oral administration. Cancer Res 2002;62:4645–55.

28. Drevs J, Esser N, Konerding MA, et al. Effect of ZD6474, a VEGF receptor tyrosine kinase inhibitor, on primary tumor growth, metastasis, vessel density and microvascular architecture in murine renal cell carcinoma. Proc Am Assoc Cancer Res 2002;43:1082.

29. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004;350:2335–42.

30. Landuyt W, Ahmed B, Nuyts S, et al. *In vivo* antitumor effect of vascular targeting combined with either ionizing radiation or anti-angiogenesis treatment. Int J Radiat Oncol Biol Phys 2001;49: 443–50.

31. Seed L, Slaughter DP, Limarzi LR. Effect of colchicine on human carcinoma. Surgery 1940;7:696–709.

32. Blakey DC, Ashton SE, Westwood FR, Walker M, Ryan AJ. ZD6126: a novel small molecule vascular targeting agent. Int J Radiat Oncol Biol Phys 2002;54:1497–502.

33. Griggs J, Metcalfe JC, Hesketh R. Targeting tumour vasculature: the development of combretastatin A4. Lancet Oncol 2001;2:82–7.

34. Kerbel RS, Kamen BA. The anti-angiogenic basis of metronomic chemotherapy. Nat Rev Cancer 2004;4:423–36.