77. Pein F, Tournade M-F, Bergeron C, *et al.* Combination etoposide (VP16) and carboplatin (CBDCA) for resistant or relapsed Wilm's tumor (T). A phase II study conducted by the French Society of Paediatric Oncology (SFOP). *Proc Am Soc Clin Oncol* 1990, **9**, 293 (Abstract).
78. Martinez JA, Martin GM, Sanz GF, *et al.* A phase II clinical trial or carboplatin infusion of high-risk acute non-lymphoblastic leukemia. *J Clin Oncol* 1991, **9**, 39–43.
79. Vogler WR, Harrington DP, Winton EF, *et al.* Phase II clinical trial of carboplatin in relapsed and refractory leukemia. *Leukemia* 1992, **6**, 1072–1075.
80. Carli M, Flamant F, Treuner J, *et al.* High dose epirubicin (Epi) in combination with carboplatin (CBDCA) and vincristine (VCR) in stage IV malignant mesenchymal tumors (MMT). *Proc Am Soc Clin Oncol* 1992, **11**, 369 (abstract).
81. Piccart MJ, Nogaret JM, Marcelis L, *et al.* Cisplatin combined with carboplatin: a new way of intensification of platinum dose in the treatment of advanced ovarian cancer. *J Natl Cancer Inst* 1990, **82**, 703–707.
82. Doz F, Berens ME, Spencer DR, Dougherty DV, Rosenblum ML. Experimental basis for increasing the therapeutic index of carboplatin in brain tumor therapy by pretreatment with WR compounds. *Cancer Chemother Pharmacol* 1991, **28**, 308–310.
83. Doz F, Berens ME, Deschepper CF, Dougherty DV, Bigornia V, Rosenblum ML. Experimental basis for increasing the therapeutic index of cisdiamminodicarboxylatocyclobutaneplatinum (II) in brain tumor therapy by a high zinc diet. *Cancer Chemother Pharmacol* 1992, **29**, 219–226.
84. Glover D, Glick JH, Weiler C, Fox K, Guerry D. WR-2721 and high-dose cisplatin: an active combination in the treatment of metastatic melanoma. *J Clin Oncol* 1987, **5**, 574–578.
85. Schwachöffer JHM, Crooumans MA, Hoogenhout J, *et al.* Effectiveness in inhibition of recovery of cell survival by cisplatin and carboplatin: influence of treatment sequence. *Int J Radiat Oncol Biol Phys* 1991, **20**, 1235–1241.
86. Cohen JD, Robins HI, Javid MJ. Sensitization of C6 glioma to carboplatin cytotoxicity by hyperthermia and thymidine. *J Neuro-Oncol* 1990, **9**, 1–8.
87. Demchak PA, Mier JW, Robert NJ, O'Brien K, Gould JA, Atkins MB. Interleukin-2 and high-dose cisplatin in patients with metastatic melanoma: a pilot study. *J Clin Oncol* 1991, **9**, 1821–1830.

Pergamon

European Journal of Cancer Vol. 30A, No. 2, pp. 201–206, 1994

0959–8049/94 $6.00 + 0.00

0959-8049(93)E0040-W

Does Improved Control of Tumour Growth Require an Anti-cancer Therapy Targeting Both Neoplastic and Intratumoral Endothelial Cells?

Giampietro Gasparini and Adrian L. Harris

INTRODUCTION

MOST HUMAN metastatic solid tumours are not currently curable with any kind of systemic anticancer therapy (i.e. chemotherapy, hormone therapy and immune therapy), and the mortality caused by the "four major killer tumours" (lung, colorectal, breast and prostate cancers), has not significantly decreased in recent years [1]. This is due to the fact that some solid epithelial tumours are not sensitive to chemotherapy [2, 3], and that those which are initially sensitive may develop acquired resistance after exposure to drugs (see [3] and references therein). Thus, there is now a critical need for new therapeutic strategies in the hope of improving tumour growth control in the future. It seems appropriate to speculate that the search for the genetic and biochemical changes that lead to malignancy is an important strategy to identify the key stages of tumour transformation and the process of metastasis [4]. Identification of specific biological targets could also provide new opportunities for developing rationally designed biological therapies for cancer [4–6]. These new targets include oncogenes, growth factors and their receptors, signal transduction pathways, cell differentiation signals [6, 7], antisense inhibitors of gene expression [8], gene deletion [9] and tumour angiogenesis [10].

One of the most promising of these is the discovery of specific angiogenesis inhibitors. As stated by Marx [4], "researchers are sufficiently hopeful about the anti-angiogenesis approach to cancer therapy that they are beginning clinical trials".

THE BIOLOGICAL BACKGROUND

Before the 1960s, it was believed that the only relevant phenomenon related to tumour vascularisation was the dilation of blood vessels from normal tissues surrounding the tumour (hyperaemia) [11].

Research begun about 25 years ago by Folkman and associates, and successively carried out by several other groups, has revolutionised this concept. These studies show that angiogenesis, the fundamental process leading to the formation of new blood vessels by sprouting from pre-existing endothelium, is stimulated by tumour cells, and that it is of critical importance in the processes of tumour invasiveness, progression and metastasis [10].

It is now assumed that carcinogenesis and tumour progression

Correspondence to G. Gasparini at the Department of Radiotherapy and Oncology, St. Bortolo Medical Center, 36100 Vicenza, Italy.
A.L. Harris is at the Imperial Cancer Research Fund, Molecular Oncology Laboratory, Institute of Molecular Medicine, University of Oxford and John Radcliffe Hospital, Oxford, U.K.
Received 19 Oct. 1993; accepted 11 Nov. 1993.

are multi-step processes involving both the loss of normal control of cell proliferation (which may be the result of an activation of growth-promoting oncogenes or of a downregulation of tumour suppressor genes [12]) and the induction of tumour angiogenesis [13]. Tumour growth is a dramatic example of an angiogenesis-dependent disease [14]. Most solid tumours are first characterised by an avascular phase (such as early *in situ* carcinomas) during which tumour growth and invasiveness are low and confined to a few millimetres [15]. The switch from the prevascular to the vascular phase is followed by rapid tumour proliferation and progression, and contributes to the development of distant metastases [13, 14]. The induction of proliferation of the endothelial cells is probably due to an imbalance between positive and negative growth regulators [15]. The angiogenic phenotype may be achieved by tumour cells via several mechanisms, including paracrine production of angiogenic factors [such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF); but we now know several other angiogenic peptides]; by recruitment of host cells (macrophages) attracted by chemotactic stimuli and induced to release angiogenic molecules; or by mobilisation of specific peptides from the extracellular matrix [16, 17].

Alternatively, there are also both soluble molecules [such as activated transforming growth factor beta (TGF-β)] [18] and inhibitory genes [19] that act as negative regulators for endothelial cell growth.

Moreover, there is now increasing evidence that the determination of the angiogenic activity (quantified using immunocytochemical methods and counting microvessel density within the tumour) has clinical application in predicting development of metastasis and prognosis in several human solid tumours [20].

THE PHARMACOLOGICAL RATIONAL TO USING ANTI-ANGIOGENESIS DRUGS AS A NOVEL ANTI-CANCER STRATEGY

As early as 1971, Folkman wrote [21] that "the population of tumour cells and the population of capillary endothelial cells within a neoplasm may constitute a highly integrated ecosystem" and that "it seems appropriate to speculate that anti-angiogenesis may provide a form of cancer therapy worthy of serious exploration". However, only very recently has it become evident that intratumoral neovascularisation may be a new target for anti-cancer therapy. After the discovery, in 1975, of the first angiogenesis inhibitor in cartilage [22], Folkman and colleagues, and other groups, identified several classes of angiogenesis inhibitors, which showed antitumour efficacy both in *in vitro* and *in vivo* experimental models [10, 23].

As recently reviewed by Bicknell and Harris [24], there are several potential targets for intervention in tumour angiogenesis, including inhibition of production of angiogenic factors, neutralisation of angiogenic peptides by specific antibodies, inhibition of endothelial cell growth, migration or formation of differentiated tubes or initiation of blood flow. In Table 1, the known anti-angiogenic agents are classified by their mechanism of action. Interferon-α is the first of such drugs which showed activity in a clinical setting. In fact, it was used to successfully treat babies with life-threatening haemangiomas [25], a disease characterised by uncontrolled vessel proliferation. Moreover, at least three other more potent angiogenesis inhibitors (AGM 1470, recombinant platelet factor 4 and linomide) [26–32] have already been approved for clinical trials as novel forms of anti-cancer therapy.

Although endothelial cells may themselves be targets for chemotherapy if proliferating, they may also express drug resistance mechanisms such as glutathione transferases or multidrug resistance. Since endothelial cells represent the first protection barrier (to toxins and circulating foreign substances) of tissues and organs, it is quite likely that they may be relatively resistant to a range of conventional chemotherapeutic agents.

There are at least five lines of experimental evidence supporting the potential therapeutic usefulness of *specific* angiogenesis inhibitors for human anti-cancer therapy: (i) the switch from the prevascular to the vascular phase is a critical step for rapid growth of a primary solid tumour and for the development of metastasis [13]. In fact, both these phenomena are angiogenesis-dependent [14]; (ii) tumour angiogenesis may be induced by several different mechanisms, each one representing a potential target for the anti-angiogenic therapy (see Table 1); (iii) we are now probably only at the "dawn" of the discovery of the angiogenesis inhibitors, but several agents able to inhibit tumour angiogenesis *in vitro* and *in vivo* with specific and different mechanisms of action [10, 23] have recently been identified. Recently, research by Hori and colleagues [33] and Kim and colleagues [34] has documented that it is possible to inhibit vascular endothelial growth, and consequently to suppress tumour growth *in vivo* by using monoclonal antibodies able to neutralise specific endothelial growth factors (bFGF and VEGF, respectively). Such a strategy may be successful in those types of tumours secreting a single common angiogenic growth factor that may be targeted (for example, VEGF, which is secreted in most human breast and brain tumours) [24]; (iv) inhibitors of angiogenesis which are not effective against tumour cells *in vitro*, induce tumour regression *in vivo* [10, 23] and (v) the majority of angiogenesis inhibitors in animal models presents a good therapeutic index, having few and manageable systemic side-effects [23]. This is related to the fact that in adults endothelial cells represent a quiescent cell population, and that physiological angiogenesis occurs infrequently, under brief and highly regulated processes [35, 36]. Deneckamp and Hobson [35] suggested that the proliferating endothelial cells in tumours would be a target for therapy by direct killing of such cells (i.e. vascular targetting).

Since the most promising angiogenesis inhibitors are effective against specific angiogenic peptides (i.e. bFGF and VEGF) or proliferating endothelial cells, this constitutes the basis for their expected low systemic toxicity in adults. Table 2 shows the main differences between chemotherapy, presently the most widely used form of systemic anti-cancer therapy, and anti-angiogenic therapy, as extrapolated from experimental models.

THE HYPOTHESIS

The novel concept reported here is that most solid tumours are made up of two different target cell populations for therapy: (1) tumour proliferating cells to be killed by conventional therapies and (2) endothelial proliferating cells, the specific target for angiogenesis inhibitors. The main pharmacological differences between chemotherapy and anti-angiogenic therapy are summarised in Fig. 1, and they reveal an expected better therapeutic index for the angiogenesis inhibitors (systemic side-effects found in animal models are amenorrhoea, infertility and perhaps a delay in bleeding wound repair) and the lack of resistance to therapy by endothelial cells [23, 25]. Potential problems with maintenance therapy using anti-angiogenic agents may be wound healing, and the necessity for laparotomy or other surgical interventions. However, it may be that temporary cessation of the administration of these drugs would be accept-

Table 1. Anti-angiogenic drugs classified by their mechanism of action

General category*	General mechanisms	Drugs	
Anti-switch inhibitors	Block the production or export of angiogenic peptides (bFGF, VEGF)	No agent yet discovered	
Anti-in transit inhibitors	Inactivate angiogenic factors after their release from tumour cells, macrophage or extracellular matrix	(1) Antibodies against bFGF [33] (2) Antibodies against VEGF [34]	
Inhibitors of proliferating endothelial cells	Make endothelial cells unable to respond to angiogenic and growth stimuli	(a) Inhibitors of synthesis and turnover of collagen, and of vessel basement membrane: —Cartilage inhibitor [22] —Minocycline [47] —Medroxyprogesterone [48] —Sulphated carboxylmethyl chitin [49] —Angiostatic heparin steroids complexes [50, 51] —Beta cyclodextrin-tetra decasulphate + steroids (hydrocortison [50–52]) —Proline analogues [51] —Krestin† [53, 54] —Linomide† [26, 27] —Razoxane [55, 56] (b) Heparin-binding molecules: —Protamine [22, 57] —Thrombospondin [58, 28] —Recombinant platelet factor 4† [28–30]	(c) Direct inhibitors of endothelial cells proliferation: —Angiostatic antibodies [31, 32] (AGM-1470) —Linomide† [26, 27] —α-Interferon† [25, 59, 60] —D-Penicillamine [61] —Recombinant platelet factor 4† [28–30] —Tamoxifen [38] —Isoflavonoid genestein [62] (d) Molecular complexes inactivating bFGF: —Sulphonic derivatives of distamycin A [63, 64] —Suramin and analogues [65] (e) Cytokine activators —Krestin [53, 54, 66] —α-Interferon [25, 59, 60] —Tumour necrosis factor α [67]

*From [23] by Folkman and Ingber. †Drugs with multiple mechanisms of action.

able in such a situation. Regarding chemotherapy, it is well-known that systemic side-effects, mainly myelosuppression, are dose-limiting, and that primary or acquired chemoresistance is the major cause of treatment failure [2, 3].

A recent experiment [37] demonstrates that the two targets (i.e. tumour cells and endothelial cells) may be succesfully attacked by the concurrent administration of chemotherapy and anti-angiogenic therapy. This supports the development of a new therapeutic strategy for the treatment of cancer. Teicher and colleagues [37] first found that an association of different angiogenesis inhibitors potentiates the efficacy of conventional chemotherapy in an animal model. In an elegant experiment they showed that the combination of minocycline + β-cyclodextrin-tetradecasulphate + tetrahydrocortisol (angiogenesis inhibitors) and cyclophosphamide induced a significantly higher growth delay of primary Lewis lung carcinoma in C57 BL mice, when compared both with angiogenesis inhibitors or with chemotherapy given alone. Toxic side-effects were not increased by the co-administration of the two modalities. Moreover, the combination significantly reduced volume and number of lung metastasis in tumour-bearing mice. These results clearly document that the concurrent administration of angiogenesis inhibitors can potentiate the efficacy of chemotherapy without an increase in toxicity. These results have been confirmed and duplicated using novel and more potent angiogenesis inhibitors, such as AGM-1470, co-administered with cyclophosphamide (Teicher, personal communication).

Gagliardi and Collins [38] documented that part of the action of the anti-oestrogens is due to a direct anti-angiogenic effect, which is dose-dependent. The angiostatic action of tamoxifen and related drugs does not seem to be altered by the presence of oestrogens [38]. Moreover, Noguchi and colleagues [39] found that tamoxifen may act also by decreasing transforming growth factor (TGF-α) production in oestrogen-positive and progesterone-positive breast cancers. Since TGF-α stimulates breast cancer growth through epidermal growth factor receptor [40] or by stimulating angiogenesis [41], its downregulation seems to be involved in the growth inhibitory effect of tamoxifen. Alternatively, tamoxifen may enhance TGFβ levels in oestrogen-negative breast cancers which is another way to inhibit growth and tumour angiogenesis [39]. These observations may, in part, explain why tamoxifen has been shown to be partially active in a percentage of patients with oestrogen-receptor negative breast carcinomas, as well as in other neoplasms which do not express large amounts of the receptor, such as metastatic malignant melanoma [42]. Indeed, Toi and colleagues [43] have docu-

Table 2. Comparison between chemotherapy versus anti-angiogenic therapy

Feature	Chemotherapy	Anti-angiogenic therapy
Mechanism of action	Various, but generally inhibit proliferation of tumour cells	Various, but generally inhibit endothelial cell growth or migration
Target-cell type	Proliferating tumour cells	Proliferating or migrating endothelial cells
Main effect	On both primary tumour and metastases	On metastases and primary
Specificity*	Low	High
Systemic toxicity	High and dose-limiting	Low
Therapeutic index	Low	High
Predictors for response	P-glycoprotein (?)	bFGF level in fluids; microvessel density (?)
Resistance	Yes	No
Duration of therapy†	Relatively brief	Long
Potentiation of the efficacy by combining agents of the same category‡	Yes, but with enhanced toxicity	Yes
Potentiation of the efficacy by combining the two different categories of drugs‡	Yes	Yes
Most appropriate therapeutic setting	Adjuvant	Adjuvant
Potential usefulness for chemoprevention§	No	Yes

*Chemotherapy also interferes with cell renewal of normally growing tissues whereas, in adults, endothelial cells are quiescent under physiological conditions [35]. †Speculated from data on α-interferon in life-threatening haemangiomas in infancy [25]. ‡Speculated from [37] and [43]. §By preventing the passage from the prevascular to the vascular and invasive phase (e.g. switch inhibition).

mented that in dimethylbenz[a]anthracene (DMBA)-induced rat mammary carcinomas, there is an additive inhibitory effect of AGM-1470 when given together with tamoxifen. This experiment suggests that the concurrent administration of two or more anti-angiogenesis drugs with different mechanisms of action or of an anti-angiogenic agent and hormone therapy may lead to potentiation of their antitumoral effect in hormone-sensitive neoplasias.

Possible hypotheses explaining the enhanced antitumour effect by combining chemotherapy (or hormone therapy) and anti-angiogenic therapy may be: (i) the simultaneous inhibition of growth of both proliferating tumour cells (by chemotherapy or hormone therapy) and of proliferating intratumoral endothelial cells (by angiogenesis inhibitors) may induce an inhibition of the production of cytokines (which stimulate endothelial cells) by the neoplastic popultion, and the arrest of the proliferation of their target cells (endothelium); (ii) if the courses of chemotherapy are intermittent, the tumour can grow back between courses. The continuous administration of anti-endothelial agents, by inhibiting revascularisation, may also inhibit tumour regrowth; (iii) reduced vascularisation may also lower interstitial pressure. There would be a balance between a low number of

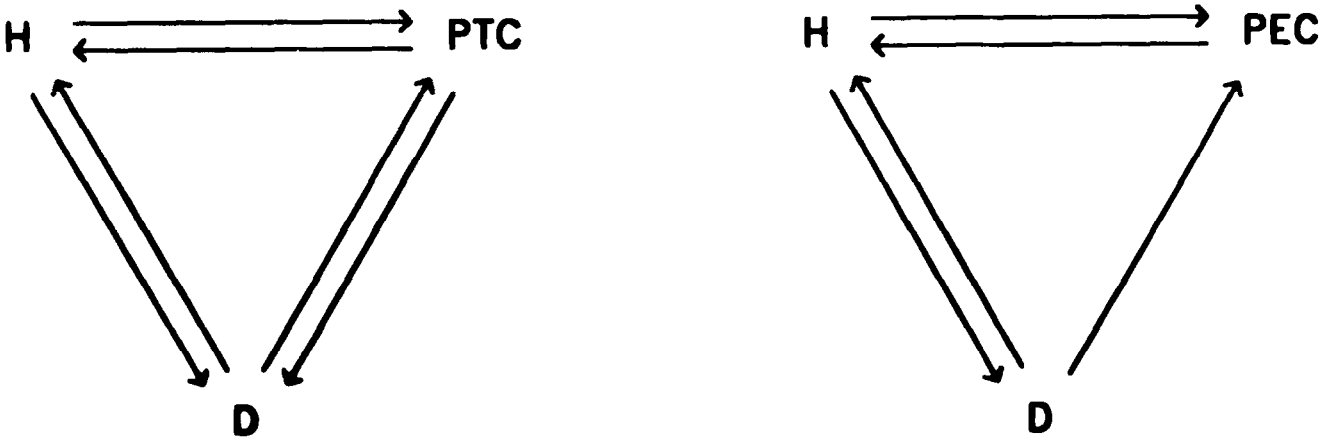

Fig. 1. Neoplastic and endothelial cells. Two different target cell populations in the tumour for the pharmacological effect. H, host; PTC, proliferating tumour cells; PEC, proliferating endothelial cells; D, drug. Pharmacological interactions *in vivo*: (a) D→H, therapeutic effect with high systemic toxic side-effects; H→D, possible drug metabolism, possible immune reactions, etc; D→PTC, antiproliferative effect on tumour cells (activity); PTC→D, chemoresistance; PTC→H, effects of disease on the host; H→PTC, natural defences of the host against the tumour, immune response. (b) D→H, therapeutic effect without relevant systemic toxicity; H→D, possible drug metabolism, possible immune reactions, etc; D→PEC, inhibition of intratumoral endothelial cell growth (activity); PEC→D, lack of resistance to therapy; PEC→H, effects of the proliferation of vessels on tumour growth and its effects on the host; H→PEC, possible natural controls on proliferating vessels, immune response.

blood vessels, which would lower drug delivery, and reduced pressure, which could result in a better delivery of chemotherapeutic or hormonal agents, depending on the relative importance of the two pathways; (iv) by increasing hypoxia, anti-endothelial agents could synergise with hypoxia-activated anticancer drugs [44]. This could greatly increase the differential toxicity by having two classes of agents which show marked differences between normal tissues and tumours; and finally, (v) duration of the efficacy of chemotherapy is generally brief in time due to the emergence of chemoresistance followed by tumour regrowth [3, 6], but this does not seem to occur for endothelial cells *versus* angiogenesis inhibitors [23, 25].

In conclusion, emphasis must be made on the importance of not using anti-angiogenic drugs alone, but by trying to kill cells in a synergistic approach, because either anti-angiogenic therapy and conventional anti-cancer therapy alone may have major disadvantages.

APPROACH AND PROSPECTIVES

Which are the most promising prospectives for the future therapeutic application of angiogenesis research? (i) From a hypothetical point of view, a drug able to prevent the switch from the prevascular to the vascular phase of tumour growth should be an effective *chemopreventive agent*, thus preventing tumour invasiveness and progression. In this setting Majewski and colleagues [45] have proposed that one of the possible mechanisms for the anti-neoplastic properties of retinoids and beta-carotene, which are now under clinical evaluation as chemopreventive agents, is the inhibition of tumour-induced angiogenesis, as found in a model of cutaneous angiogenesis in BALB/c mice. Testing a combination therapy of these compounds with a more specific angiogenesis-inhibitor agent [26–32] is warranted; (ii) a possible synergism between two or more angiogenesis inhibitors, with diverse mechanisms of action, may occur without enhancement of acute systemic toxicity or cumulative toxicity for these compounds [37, 43]. Thus, it seems appropriate to believe that such a combination may be given for long periods of time as a *maintenance therapy* in some solid tumours. This could be an advantage over chemotherapy which, in some instances, must be discontinued in responding patients due to the development of dose-dependent toxicities (i.e. cardiotoxicity for anthracyclines and nephrotoxicity for cisplatinum), (iii) because the main effect of some angiogenesis inhibitors (such as AGM 1470) is the antimetastatic action, one could expect that a combination of these compounds with chemotherapy or hormone therapy should be particularly effective in an *adjuvant setting*, for example, in breast and colon cancers with an elevated frequency of post-operative recurrence; and finally (iv) it will be of interest to verify in future studies if the immunocytochemical assessment of intratumoral vascularisation may be useful (predictive value) in selecting patients whose tumours have the highest microvessel counts for anti-angiogenic therapy [46].

1. Boring CC, Squires TS, Tong T. Cancer statistics. *CA Cancer J Clin* 1992, **42**, 19–38.
2. Braverman AS. Chemotherapeutic failure: resistance or insensitivity? *Am Intern Med* 1993, **118**, 630–632.
3. Gasparini G, Bevilacqua P, Pozza F, Meli S, Weidner N. P-glycoprotein expression predicts response to chemotherapy in previously untreated stage III and IV breast cancer. *The Breast* 1993, **2**, 27–32.
4. Marx J. Cellular changes on the route of metastasis (commentary). *Science* 1993, **259**, 626–629.
5. Lippman ME. The development of biological therapies for breast cancer. *Science* 1993, **259**, 631–632.
6. Chabner BA. Biological basis for cancer treatment. *Ann Intern Med* 1993, **118**, 633–637.
7. Workman P, D'Incalci M, Berdel WE, *et al.* New approaches in cancer pharmacology: drug design and development (report of a European School of Oncology Task Force). *Eur J Cancer* 1992, **28A**, 1190–1200.
8. Wagner RW, Matteucci MD, Lewis JG, Gutierrez AJ, Moulds C, Frochler BC. Antisense gene inhibitor by oligonucleotides containing C-5 propyne pyrimidines. *Science* 1993, **260**, 1510–1513.
9. Frei E III. Gene deletion: a new target for cancer chemotherapy. *Lancet* 1993, **342**, 662–664.
10. Gasparini G, Weidner N. Tumour angiogenesis: from biology to the clinic. In Spandidos DA, ed. *Current Perspectives on Molecular and Cellular Oncology*, Vol.II. London, JAI Press Ltd, 1994, in press.
11. Coman DR, Sheldon WF. The significance of hyperemia around tumor implants. *Am J Pathol* 1946, **22**, 821–826.
12. Weinberg RA. Oncogenes, tumor suppressor genes, and cell transplantation: trying to put it all together. In Brugge J, Curran T, Harlow ED, McCormick F, eds. *Origins of Human Cancer: A Comprehensive Review*. New York, Cold Spring Harbor Laboratory Press, 1991, 1–16.
13. Folkman J, Watson K, Ingberg D, Hanahan D. Induction of angiogenesis during the transition from hyperplasia to neoplasia. *Nature* 1989, **339**, 58–61.
14. Folkman J. What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* 1990, **82**, 4–6.
15. Folkman J, Shing Y. Angiogenesis (mini review). *J Biol Chem* 1992, **267**, 10931–10934.
16. Iberg N, Rogelj S, Fanning P, Klagsbrun M. Purification of 18- and 22- KDa forms of basic fibroblast growth factor from rat cells transformed by the *ras* oncogene. *J Biol Chem* 1989, **264**, 19951–19955.
17. Folkman J, Klagsbrun M. Angiogenic factors. *Science* 1987, **235**, 442–447.
18. Antonelli-Orlidge A, Saunders KB, Smith SR, D'Amore PA. An activated form of transforming growth factor beta is produced by co-cultures of endothelial cells and pericytes. *Proc Natl Acad Sci USA* 1989, **86**, 4544–4548.
19. Rastinejad F, Polverini PJ, Bouck NP. Regulation of the activity of a new inhibitor of angiogenesis by a cancer suppressor gene. *Cell* 1989, **56**, 345–355.
20. Gasparini G. Quantification of intratumoral vascularization predicts metastasis in human invasive solid tumours (review). *Oncol Reports* 1994, **1**, 7–12.
21. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971, **285**, 1182–1186.
22. Brem H, Folkman J. Inhibition of tumor angiogenesis mediated by cartilage. *J Exp Med* 1975, **141**, 427–429.
23. Folkman J, Ingber D. Inhibition of angiogenesis. *Sem Cancer Biol* 1992, **3**, 89–96.
24. Bicknell R, Harris AL. Anticancer strategies involving the vasculature: vascular targeting and the inhibition of angiogenesis. *Sem Cancer Biol* 1992, **3**, 399–407.
25. Ezekowitz RAB, Mulliken J, Folkman J. Interferon alpha-2a therapy of life-threatening hemangiomas of infancy. *N Engl J Med* 1992, **326**, 1456–1463.
26. Ichikawa T, Lamb JC, Christensson PJ, Hartley-Asp B, Isaacs JT. The antitumor effects of the quinoline-3-carboxamide linomide on Dunning R-3327 rat prostatic cancers. *Cancer Res* 1992, **52**, 3022–3028.
27. Vukanovic J, Passaniti A, Hirata T, Traystman RJ, Hartley-Asp B, Isaacs JT. Anti-angiogenic effects of the quiniline-3-carboxamide, linomide. *Cancer Res* 1993, **53**, 1833–1837.
28. Folkman J, Taylor S, Spillberg C. The role of heparin in angiogenesis. In Nugent J, O'Connor M, eds. *Development of the Vascular System*. London, Ciba Foundation Symposium 100, Pitman 1983, 132–139.
29. Maione TE, Gray GS, Petro J, *et al.* Inhibition of angiogenesis by recombinant human platelet factor 4 and related peptides. *Science* 1990, **247**, 77–79.
30. Sharpe RJ, Scott CF, Donner AL. Recombinant human platelet factor 4 inhibits growth of B16 melanoma in a murine model. *Clin Res* 1989, **37**, 470a (abstract).
31. Ingber D, Frujita T, Kishimoto S, *et al.* Synthetic analogues of

fumagallin that inhibit angiogenesis and suppress tumour growth. *Nature* 1990, **348**, 555–557.
32. Yanase T, Tamura M, Fujita K, Kodama S, Tanaka K. Inhibitory effect of angiogenesis inhibitor TNP-1470 on tumor growth and metastasis of human cell lines *in vitro* and *in vivo*. *Cancer Res* 1993, **53**, 2566–2570.
33. Hori A, Sasada R, Matsutani E, *et al.* Suppression of solid human basic fibroblast growth factor. *Cancer Res* 1991, **51**, 6180–6184.
34. Kim KJ, Li B, Winer J, *et al.* Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth *in vivo*. *Nature* 1993, **362**, 841–844.
35. Denekamp J, Hobson B. Endothelial cell proliferation in experimental tumours. *Br J Cancer* 1982, **46**, 711–720.
36. Hobson B and Denekamp J. Endothelial proliferation in tumours and normal tissues: continuous labelling studies. *Br J Cancer* 1984, **49**, 405–413.
37. Teicher BA, Sotomayor EA, Huang ZD. Antiangiogenic agents potentiate cytotoxic cancer therapies against primary and metastatic disease. *Cancer Res* 1992, **52**, 6702–6704.
38. Gagliardi A, Collins DC. Inhibition of angiogenesis by antiestrogens. *Cancer Res* 1993, **53**, 533–535.
39. Noguchi S, Motowura K, Inaji H, Imaoka S, Koyama H. Down-regulation of transforming growth factor-α by tamoxifen in human breast cancer. *Cancer* 1993, **72**, 131–136.
40. Dickson RB, Thompson SW, Lippman ME. Regulation of proliferation, invasion, and growth factor synthesis in breast cancer by steroids. *J Steroid Biochem Mol Biol* 1990, **37**, 305–317.
41. Schreiber AB, Winkler ME, Derynck R. Transforming growth factor alpha: a more potent angiogenic mediator than epidermal growth factor. *Science* 1986, **232**, 1250–1253.
42. Cocconi G, Calabresi F, Tonato M, *et al.* Treatment of metastatic malignant melanoma with dacarbazine plus tamoxifen. *N Engl J Med* 1992, **327**, 516–523.
43. Toi M, Yamamoto Y, Imazawa T, Takayamagi T, Akutsu K, Tominaga T. Antitumor effect of the angiogenesis inhibitor AGM-1470 and its combination effect with tamoxifen in DMBA induced mammary tumors in rats. *Int J Oncol* 1993, **3**, 525–528.
44. Hoffer FA, Taylor GA, Spevak M, Ingberg D, Fenton T. Metabolism of tumor regression from angiogenesis inhibition: ^{31}P Magnetic Resonance Spectroscopy. *Magnetic Resonance Med* 1989, **11**, 202–208.
45. Majewski S, Marczak M, Szmurlo A, *et al.* Inhibition of tumor-induced angiogenesis by systemic administration of retinoids and beta-carotene in Mice. *J Invest Dermatol* 1993, **100**: 602 (abstract 681).
46. Bicknell R, Harris AL. Novel growth regulatory factors and tumour angiogenesis. *Eur J Cancer* 1991, **27**, 781–785.
47. Tamargo RJ, Bok RA, Brem H. Angiogenesis inhibition by minocycline. *Cancer Res* 1991, **52**, 672–675.
48. Gross J, Azizkhan RG, Biswas R, Bruns R, Hseih D, Folkman J. Inhibition of tumor growth, vascularization and collagenolysis in the rabbit cornea by medroxyprogesterone. *Proc Natl Acad Sci USA* 1981, **78**, 1176–1180.
49. Saiki I, Murata J, Nakajima M, Tokura S, Azuma I. Inhibition by sulfated chitin derivatives of invasion through extracellular matrix and enzymatic degradation of metastatic melanoma cells. *Cancer Res* 1990, **50**, 3631–3637.
50. Folkman J, Ingber DE. Angiostatic steroids: method of discovery and mechanism of action. *Ann Surgery* 1987, **206**, 373–384.
51. Ingber D, Folkman J. Inhibition of angiogenesis through modulation of collagen metabolism. *Lab Invest* 1988, **59**, 44–51.
52. Folkman J, Weisz PB, Joullic MM, Li WW, Ewing WR. Control of angiogenesis with synthetic heparin substitutes. *Science* 1989, **243**, 1490–1493.
53. Fujii T, Sujita N, Kobayashi Y, *et al.* Treatment with Krestin combined with mitomycin C and effect on immune response. *Oncology* 1989, **46**, 49–53.
54. Kumar S, Saitoh K, Kumar P. Antiangiogenesis strategies in cancer therapy with special reference to Krestin. In Steiner R, Weisz PB, Langer R, eds. *Angiogenesis: Key Principles—Science, Technology, Medicine*. Basel, Birkhauser 1992, 463–470.
55. Karakiulakis G, Missirlis E, Maragoudakis ME. Mode of action of razoxane: inhibition of basement membrane collagen-degradation by a malignant tumor enzyme. *Methods Find Exp Clin Pharmacol* 1989, **11**, 255–261.
56. Hellman K. Angiogenesis. Time to review progress. In Steiner R, Weisz PB, Langer R, eds. *Angiogenesis: Key Principles—Science, Technology, Medicine*. Basel, Birkhauser, 1992, 26–28.
57. Potts M, Dopplet S, Taylor S. Protamine: a powerful *in vivo* inhibitor of bone resorption. *Calcif Tissue Int* 1984, **36**, 189–194.
58. Rastinejad F, Polverini PJ, Bouck NP. Regulation of the activity of a new inhibitor of angiogenesis by a cancer suppressor gene. *Cell* 1989, **56**, 345–355.
59. Brouty-Boye D, Zetter BR. Inhibition of cell motility by interferon. *Science* 1980, **208**, 516–518.
60. Sidky Y, Borden E. Inhibition of angiogenesis by interferons: effects on tumor and lymphocyte-induced vascular response. *Cancer Res* 1987, **47**, 5155–5161.
61. Matsubara T, Saura R, Hirohata K, Ziff M. Inhibition of human endothelial cell proliferation *in vitro* and neovascularization *in vivo* by D-penicillamine. *J Clin Invest* 1989, **83**, 158–167.
62. Fotsis T, Pepper M, Adlercreutz H, *et al.* Genestein, a dietary-derived inhibitor of *in vitro* angiogenesis. *Proc Natl Acad Sci USA* 1993, **90**, 2690–2694.
63. Tanaka NG, Sakamoto N, Inove K, *et al.* Antitumor effect of an antiangiogenesis polysaccharide from an arthrobacter species with or without a steriod. *Cancer Res* 1989, **49**, 6727–6730.
64. Sola F, Biasoli G, Pesenti E, *et al. In vivo* activity of novel sulphonic derivatives of distamycin A. In Steiner R, Weisz PB, Langer R, eds. *Angiogenesis: Key Principles—Science, Technology, Medicine*. Basel, Birkhauser, 1992 459–462.
65. Pesenti E, Sola F, Mongelli N, Grandi M, Spreafico F. Suramin prevents neovascularization and turnover growth through blocking of basic fibroblast growth factor activity. *Br J Cancer* 1992, **66**, 367–372.
66. Matsushima K, Hirose K, Oppenheim J. Induction of cytokine production by a plant-polysaccharide in human PBMC. *J Cancer Res Clin Oncol* 1990, **116**, 871–875.
67. Sato N, Goto T, Haranaka K, *et al.* Actions of tumor necrosis factor on cultured vascular endothelial cells: morphologic modulations, growth inhibition and cytotoxicity. *J Natl Cancer Inst* 1986, **76**, 113–116.

Acknowledgements—Dr Gasparini wishes to thank Prof. Judah Folkman, Professor of Pediatric Surgery, Harvard Medical School and Children's Hospital, Boston, Massachusetts, U.S.A. for having introduced him into the fascinating field of tumour angiogenesis and for his helpful and valuable comments. The authors wish to thank Miss Lucia Regolin and Mrs Daniela Mazzocco for the preparation of the manuscript. This research has been supported in part by a grant of the Associazione Italiana per la Ricerca sul Cancro (A.I.R.C.), Milan, Italy.