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Angiogenesis and Cancer

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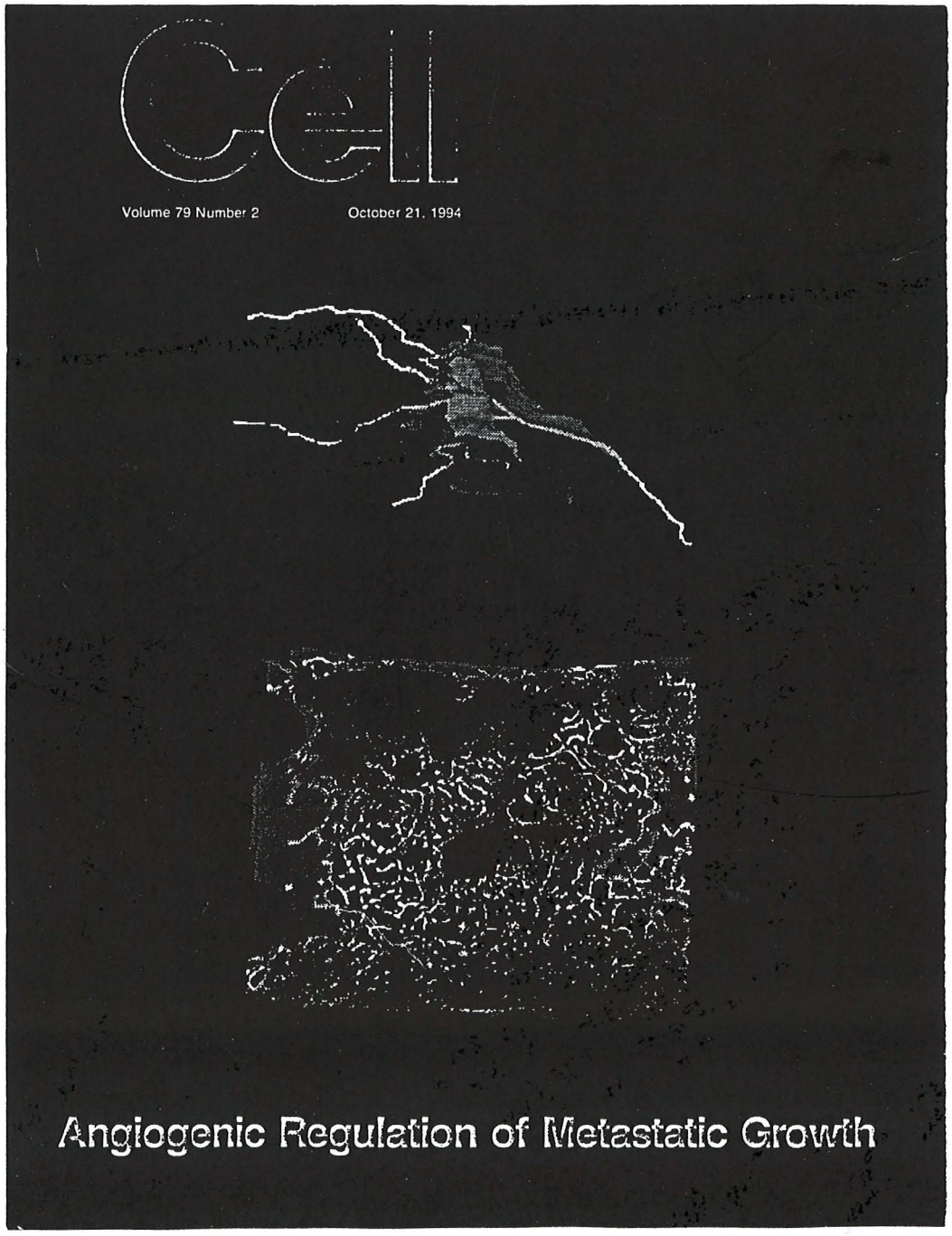
April 27, 1995



Angiogenesis (Neovascularization)

- The process leading to the formation of new blood vessels
- Plays a central role in embryonic development, reproduction, differentiation, ovulation and menstruation, repair of wound healing, peptic ulcers, and fractures

Angiogenesis

- Physiologic angiogenesis is highly regulated and activated for brief periods and then completely inhibited
- Under normal conditions, vascular endothelial cells are quiescent in adults
- Most dramatic angiogenesis-dependent disease is cancer
- Plays a role in pathophysiology of non-neoplastic diseases

Angiogenesis in the Pathophysiology of Non-neoplastic diseases

- Collateral blood vessels
- Ocular neovascularization
- Infantile hemangiomas
- Arthritis
- Psoriasis
- Duodenal ulcers
- Female reproduction
- Developmental angiogenesis

Collateral Blood Vessels (Ischaemic limbs, ischemic myocardium depend on hypoxic upregulation of VEGF production just as occurs in tumors.)

Ocular Neovascularization (VEGF increase in retina and vitreous, major mediator in diabetic ocular neovascularization so angiogenesis inhibitors developed for anticancer therapy may be used to treat ocular neovascularization)

Infantile Hemangiomas (VEGF and bFGF are overexpressed in infantile hemangiomas during proliferative phase and both decrease during involuting phase.)

Arthritis (Ingrowth of vascular pannus may be mediated by excessive production of angiogenic factors from infiltrating macrophages, immune cells or inflammatory cells; Angiogenesis inhibitor AGM-1470 is effective in preventing neovascularization of the joint in experimental immune arthritis in rats)

Psoriasis (Overexpression of angiogeneic polypeptide interleukin-8 and decreased expression of angiogenesis inhibitor thrombospondin. May result from "non-sprouting" angiogenesis - increased elongation and widening of dermal vessels.)

Duodenal Ulcers (Experimental duodenal ulcers in rats are deficient in microvessels, human gastric ulcers show a 23-fold decreased in bFGF content compared to normal mucosa; oral administration of bFGF to rats with duodenal ulcers induces angiogenesis in the ulcer bed and accelearates ulcer healing; Phase I clinical trials of patients with refractory duodenal ulcers treated with oral bFGF)

Female Reproduction (Physiologic angiogenesis in female reproductive tract mediated by angiogenic stimulators and inhibitors similar to mediators of tumor angiogenesis but under different regulation; growth of ovarian follicle and its corpus luteum may be governed by increased angiogenesis which occurs in the dominant follicle; dominant follicle, neovascularized during ovulation raises question if this inhibits the neovascularization of other follicles in a way analogous to a primary tumor inhibiting neovascularization of its metastases.)

Developmental Angiogenesis (e.g. neovascularization of the renal anlage is a paracrine process mediated in part by bFGF; VEGF also plays a role.)

Studies Demonstrating That Tumors are Angiogenesis Dependent for Growth, Progression, and Metastasis

Indirect Evidence

- Avascular tumors grow slowly and remain small, after vascularization grow rapidly and get big
- In transgenic mice induction of angiogenesis is an early important step in progression from normal to hyperplasia to neoplasia
- Angiogenesis is necessary but not sufficient for metastasis
- Degree of vascularization of the primary tumor predicts for metastasis

Direct Evidence

- Specific angiogenesis inhibitor, AGM-1470 inhibits tumor growth in vivo but not in vitro
- Monoclonal antibodies neutralizing bFGF or VEGF causes tumor remission in mice
- Monoclonal antibody neutralizing $\alpha v\beta 3$ integrin, an extracellular matrix protein necessary for vessel migration, blocks angiogenesis and tumor growth
- Antiangiogenesis therapy with interferon alfa-2a induces remission of life-threatening hemangiomas in infants, a disease characterized by uncontrolled endothelial-cell growth

Table 1. Studies Demonstrating That Tumors Are Angiogenesis-Dependent for Growth, Progression, and Metastasis

Reference	Brief Description of the Study
	Indirect evidence
Algire et al[34]	Avascular tumors implanted in subcutaneous transparent chambers in mice grow linearly and slowly. After vascularization the growth is rapid and nearly exponential.
Folkman et al[16]	Tumor growth in isolated perfused organs are limited to 1 to 2 mm^3 because blood vessels do not proliferate. After transplant into mice, tumors vascularize and expand rapidly.
Gimbrone et al[35]	Avascular tumors suspended in the acqueous fluid of the anterior chamber of the eye are limited in size (< 1 mm^3). When implanted in the iris they neovascularize and enhance their original volume by 16,000 times in a brief period.
Gimbrone et al[36]	In the rabbit cornea avascular tumors grow slowly and linearly, but growth develops exponentially after vascularization.
Knighton et al[37]	The diameter of tumors implanted on the chorioallantoic membrane of the chick embryo enhance more than 8 times after the switch from the avascular to the vascular phase.
Folkman et al[38]	In transgenic mice induction of angiogenesis is an important step for the progression from normal beta cells to hyperplasia to neoplasia. Tumor volume of insulinomas increased 100-fold after neovascularization.
Kandel et al[39]	The switch to the angiogenic phenotype during the multistep tumorigenesis of fibrosarcoma is associated with the export of an endothelial growth factor (basic fibroblast growth factor).
Lien & Ackerman[40]	Demonstrated that blood supply is essential for the growth of experimental liver metastasis in rabbits.
Starkey et al[41]	Decreased angiogenesis in mast-cell-deficient W/W^v mice is associated to a decreased rate of metastasis.
Liotta et al[42]	Angiogenesis is necessary but not sufficient "per se" for the development of metastasis.
Fidler and Ellis[43]	Reviewed that angiogenesis is necessary both at the beginning and at the end of the cascade of events that permit metastasis.
Srivastava et al[32]	The probability of developing metastasis in patients with intermediate-thickness skin melanoma is associated with the degree of vascularization at the base of the primary lesion.
Weidner et al[33]	The probability of axillary and distant metastasis in patients with early-stage breast carcinoma is related to the vascularization the primary tumor.
Gasparini et al[44]	The degree of vascularization of the primary predicts metastasis in patients with squamous-cell carcinoma of the head and nec
Weidner et al[45]	and prostate cancer.[45]
Gasparini et al[46]	In a series of node-negative breast cancer patients, vascularization was the only marker able to predict any site of distant metastasis.
	Direct evidence
Ingber et al[47]	A specific angiogenesis inhibitor, AGM-1470, inhibits tumor growth *in vivo* but not *in vitro*.
Hori et al[48]	A monoclonal antibody neutralizing bFGF causes a 70% remission of tumor size in mice.
Kim et al[49]	A monoclonal antibody neutralizing VEGF induces remission of three human tumor lines transplanted into nude mice.
Burrows et al[50]	A monoclonal antibody neutralizing endoglin, a cell-cycle-related protein expressed by proliferating endothelial cells, induces tumor remission.
Brooks et al[51]	A monoclonal antibody neutralizing the $\alpha_v\beta_3$ integrin, and extracellular matrix protein necessary for vessels migration, blocks angiogenesis and tumor growth.
Millauer et al[52]	Antiangiogenesis by a dominant-negative flk-1 mutant prevents growth of glioblastomas.
Ezekowitz et al[53]	First clinical evidence that antiangiogenesis therapy with interferon alfa-2a induces remission of life-threatening hemangioma in infants, a disease characterized by uncontrolled endothelial-cell growth.

By Giampietro Gasparini and Adrian L. Harris

Journal of Clinical Oncology, Vol 13, No 3 (March), 1995: pp 765-782

Tumor Angiogenesis

- Solid tumors composed of tumor cells and stroma (supporting cells and connective tissue with vascularity for obtaining nutrients and oxygen)
- Onset of angiogenesis behaves as an event independent of other pathways of tumor progression
- Switch from avascular to vascular phase is accompanied by rapid primary tumor growth and progression
- Regulated by multiple biochemical and genetic mechanisms

- Paracrine system operates between tumor cells, stroma, and endothelial cells
- Neoplastic cells produce several angiogenic peptides
- Vascular endothelial cells secrete growth factors and cytokines that stimulate tumor cells to proliferate or atract and stimulate inflammatory cells
- Angiogenesis is necessary at the beginning (access of tumor cells to blood stream) and the end of the development of metastasis (vascularization of the metastatic deposit)

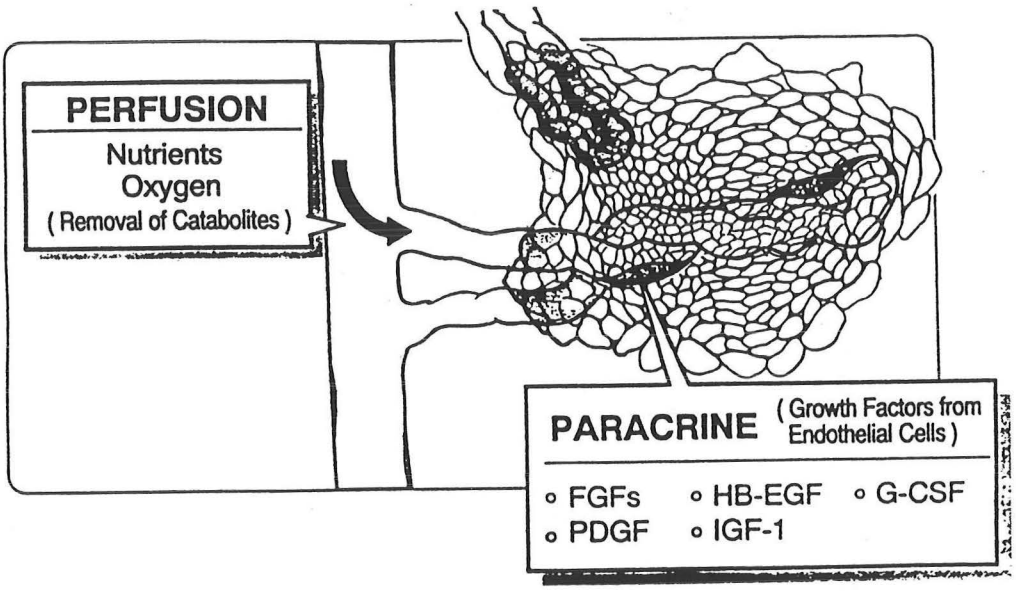


Figure 10–3. Tumor neovascularization. Diagram to illustrate the concept that the onset of tumor angiogenesis not only *perfuses* the tumor with nutrients as well as providing for removal of waste catabolites, but that capillary endothelial cells produce growth factors that can stimulate tumor cells in a *paracrine* fashion. Nicosia's studies[53] suggest that the subendothelial matrix also may stimulate adhesion and growth of tumor cells selectively.

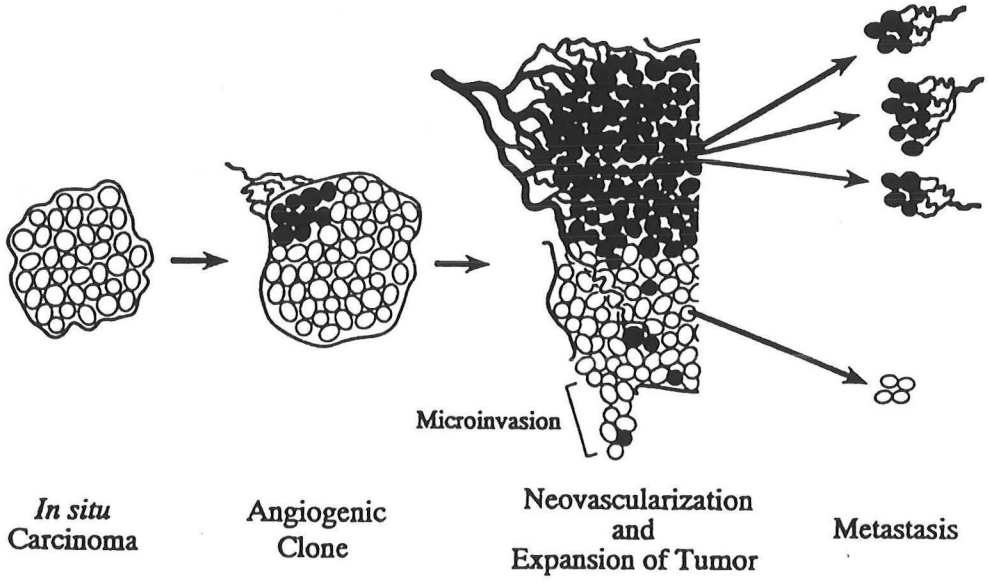


Figure 10–5. Model of the concept that subsets of tumor cells are angiogenic. (From Folkman J: Angiogenesis and breast cancer. J Clin Oncol 12:441, 1994, with permission.)

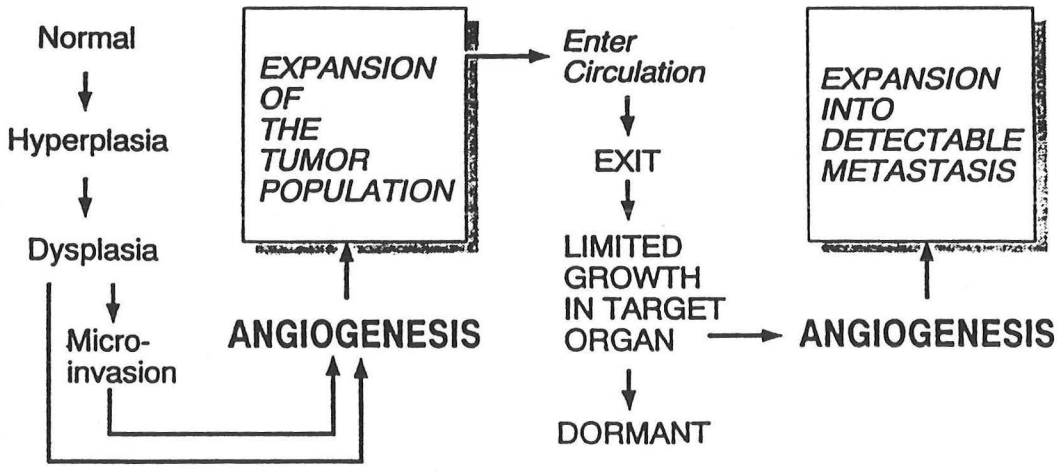


Figure 10–1. Model of role of angiogenesis in metastasis. At the beginning of the metastatic cascade, angiogenesis permits expansion of the primary tumor and provides increased vascular surface area for escape of tumor cells into the circulation. At the end of the metastatic cascade, angiogenesis permits expansion of the metastatic implant. (From Folkman J: Tumor angiogenesis. *In* Holland JF, Frei E, Bast RC, et al [eds]: Cancer Medicine, 3rd ed. Philadelphia, Lea & Febiger, 1993, pp 153–170, with permission.)

Correlation of Intratumoral Vascularization with Prognosis in Early-Stage (Stage I-II) Breast Carcinoma (Followup 2.5-9 years)

Number of studies	11
Number of patients	1,612
Number of node positive patients	510(32%)
Significant association with nodal metastasis	6/9 studies
Significant association with distant metastasis	6/9 studies
Significant adverse impact on relapse free survival	7/10 studies
Significant adverse impact on overall survival	5/7 studies
Independent factor in multivariate analysis	8/10 studies

Endothelial markers CD-31 (3 studies) fVIII-RA (7), both (1)

(Gasparini and Harris, J Clin Oncol 13:765-782, 1995.)

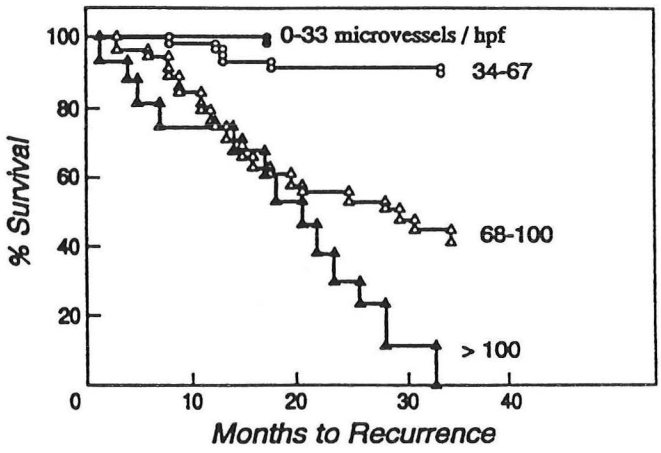


Figure 10–4. Correlation of microvessel count in breast cancer with recurrence-free survival for both node-negative and node-positive patients. hpf, high-power field. (From Weidner N, Folkman J, Pozza F, et al: Tumor angiogenesis: A new significant and independent prognostic indicator in early-stage breast carcinoma. J Natl Cancer Inst 84:1875, 1992, with permission.)

Immunohistochemical Methods Used to Assess Angiogenic Activity

- Use markers fVIII-RA, CD-31, CD-34 to stain endothelial cells

- Monoclonal antibody CD-31 (anti-platelet/endothelial cell adhesion molecule or PECAM) is most sensitive and panspecific marker for endothelial cells. Stains one third more blood vessels, particularly smaller ones than fVIII-RA. However, not specific for intratumoral endothelial cells.

- Most clinical studies using CD-31 in primary breast cancer find a signficant correlation with prognosis and degree of staining

- Antibodies specific to endothelial-cell-proliferation antigens would improve specificity
  E-9
  TEC-11 (anti-endoglin)

- Simplicity, rapidity, low cost. But need reproducibility among pathologists (manual counts of microvessels in the areas of most vacularization of each tumor are made by two observers concurrently with a third observer consulted for discrepancies).

- Objective, multiparametric computerized image analysis under evaluation.

Tumor Microvessel Density, p53 Expression, Tumor Size, and Peritumoral Lymphatic Vessel Invasion Are Relevant Prognostic Markers in Node-Negative Breast Carcinoma

By Giampietro Gasparini, Noel Weidner, Pierantonio Bevilacqua, Sergio Maluta, Paolo Dalla Palma, Orazio Caffo, Mattia Barbareschi, Patrizia Boracchi, Ettore Marubini, and Franco Pozza

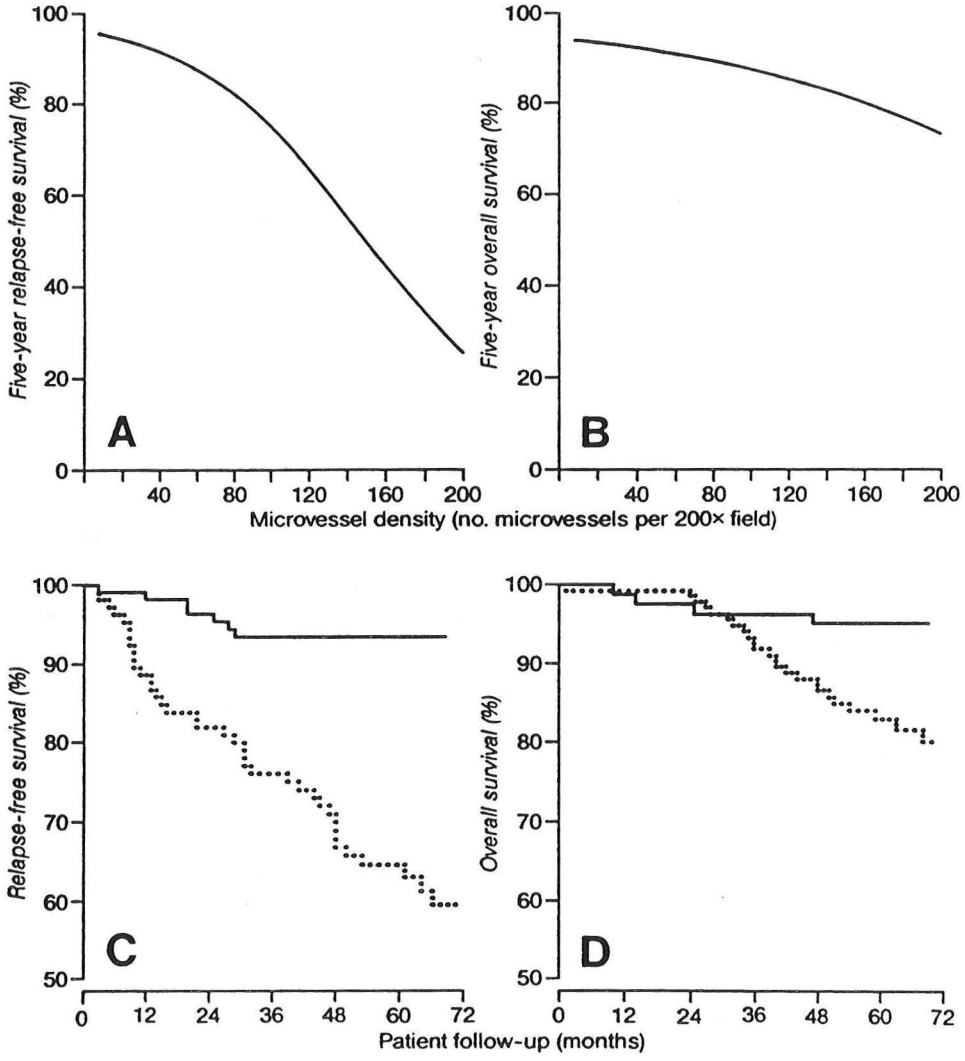


Fig 1. (A) Five-year RFS according to MVD as a continuous variable (number of microvessels per 0.74-mm² or 200× microscopic field) ($P = .0001$). (B) Five-year OS according to MVD as a continuous variable (number of microvessels per 0.74-mm² or 200× microscopic field) ($P = .0230$). (C) RFS for patients with MVD ≤ 80 (–) v those with MVD > 80 (····) ($P = .0001$). (D) OS for patients with MVD ≤ 70 (–) versus those with MVD > 70 (····) ($P = .012$).

Journal of Clinical Oncology, Vol 12, No 3 (March), 1994: pp 454-466

Tumor Microvessel Density, p53 Expression, Tumor Size, and Peritumoral Lymphatic Vessel Invasion Are Relevant Prognostic Markers in Node-Negative Breast Carcinoma

Journal of Clinical Oncology, Vol 12, No 3 (March), 1994: pp 454-466

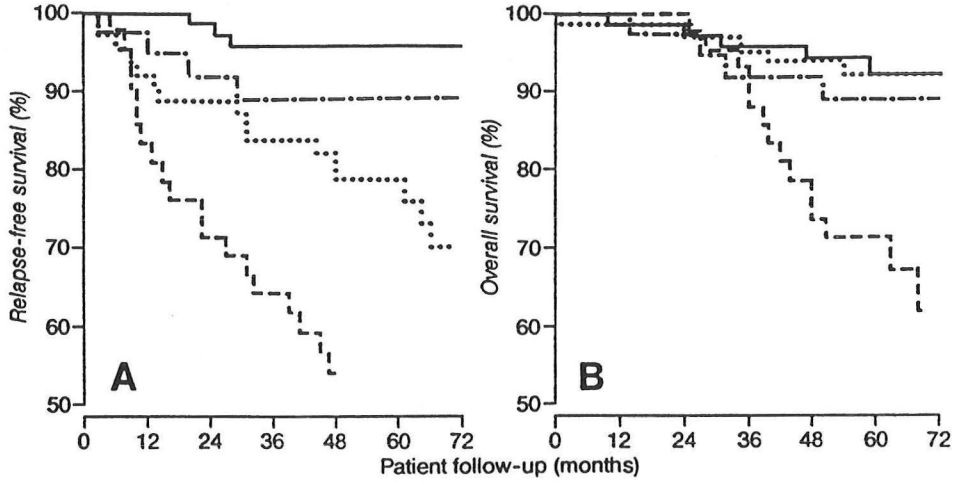
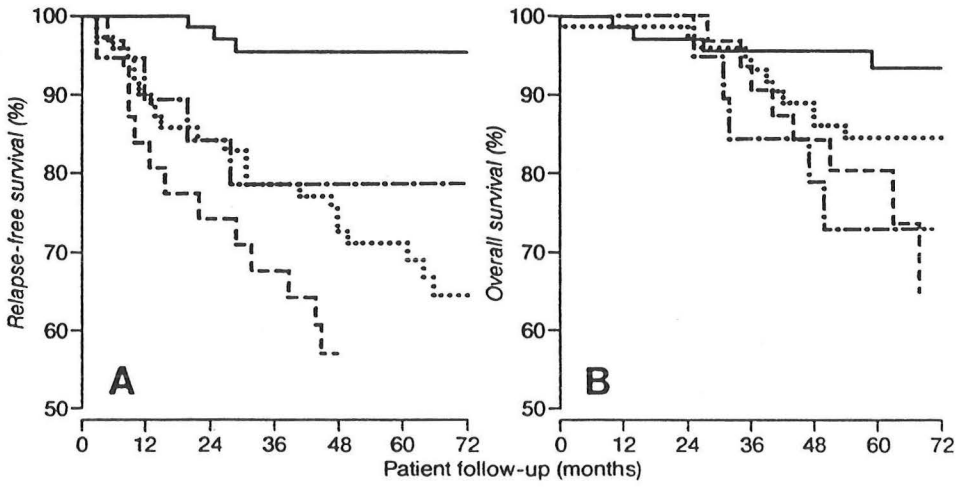


Fig 4. (A) RFS in patients stratified by MVD and tumor size (pT). (——) MVD ≤ 80 and pT1 (68 cases); (– · –) MVD ≤ 80 and pT2 (37 cases); (· · · ·) MVD > 80 and pT1 (64 cases); (– –) MVD > 80 and pT2 (42 cases). MVD ≤ 80 and pT1 versus MVD > 80 and pT2 tumors, *P* = .0001. (B) OS in patients stratified by MVD and tumor size (pT). (——) MVD ≤ 70 and pT1 (68 cases); (– · –) MVD ≤ 70 and pT2 (37 cases); (· · · ·) MVD > 70 and pT1 (64 cases); (– –) MVD > 70 and pT2 (42 cases). MVD ≤ 70 and pT1 v MVD > 70 and pT2 tumors, *P* = .01.

Fig 3. (A) RFS in patients stratified by MVD and p53 expression. (——) MVD ≤ 80 and p53-negative (66 cases); (– · –) MVD ≤ 80 and p53-positive (19 cases); (· · · ·) MVD > 80 and p53-negative (71 cases); (– – –) MVD > 80 and p53-positive (32 cases). MVD ≤ 80 and p53-negative v MVD > 80 and p53-positive tumors, *P* = .0002. (B) OS in patients stratified by MVD and p53 expression. (——) MVD ≤ 70 and p53-negative (66 cases); (– · –) MVD ≤ 70 and p53-positive (19 cases); (· · · ·) MVD > 70 and p53-negative (71 cases); (– –) MVD > 70 and p53-positive (32 cases). MVD ≤ 70 and p53-negative v MVD > 70 and p53-positive tumors, *P* = .05.



PURIFIED ANGIOGENIC FACTORS

GROWTH FACTOR	MOLECULAR WEIGHT	ENDOTHELIAL MITOGEN IN VITRO
Fibroblast growth factors		
Basic	18,000	+
Acidic	16,400	+
Angiogenin	14,100	o
Transforming growth factor α	5,500	+
Transforming growth factor β*	25,000	−
Tumor necrosis factor α	17,000	−
Vascular endothelial growth factor	45,000	+
Platelet-derived endothelial growth factor	45,000	DNA synthesis
Granulocyte colony-stimulating factor	17,000	+
Placental growth factor	25,000	+
Interleukin 8	40,000	+
Hepatocyte growth factor	92,000	+

*TGF-β inhibits endothelial proliferation in vitro, but a focal injection in vivo stimulates angiogenesis, possibly by recruiting active macrophages and possibly by mobilizing VPF/VEGF from extracellular matrix.

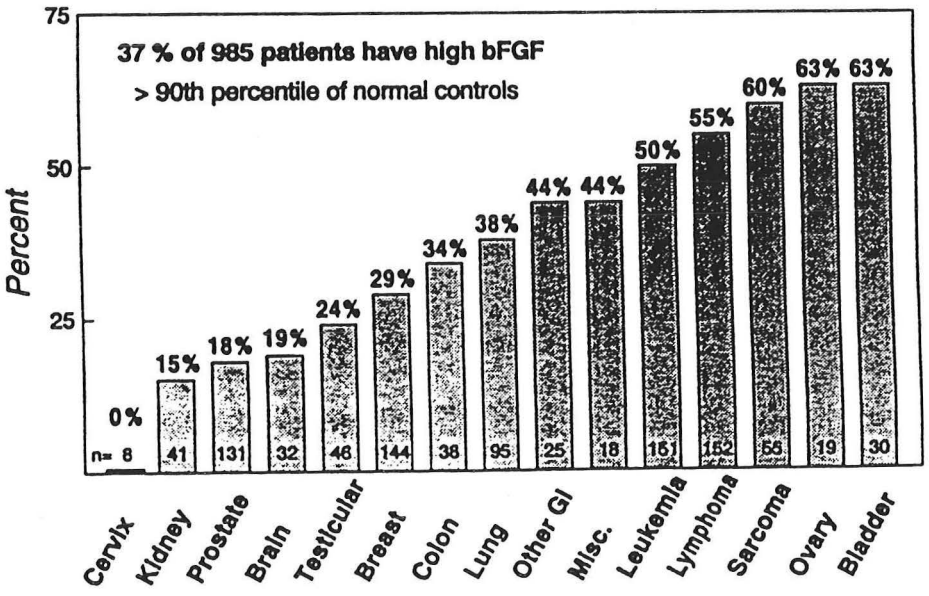


Figure 10–6. Distribution among patients with various types of cancer of abnormally elevated levels of the angiogenic peptide bFGF in urine. (From Nguyen M, Watanabe H, Budson AE, et al: Elevated levels of an angiogenic peptide, basic fibroblastic growth factor, in the urine of patients with a wide spectrum of cancers. J Natl Cancer Inst 86:356, 1994, with permission.)

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JUDAH FOLKMAN

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TUMOR ANGIOGENESIS

In: The Molecular Basis of Cancer, Mendelsohn J, Howley PM, Israel MA, Liotta LA, editors. W. B. Saunders, 206-232, 1995.

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Medical Sciences

Angiogenin antagonists prevent tumor growth *in vivo*

(angiogenesis/neoplasia/monoclonal antibody/athymic mice)

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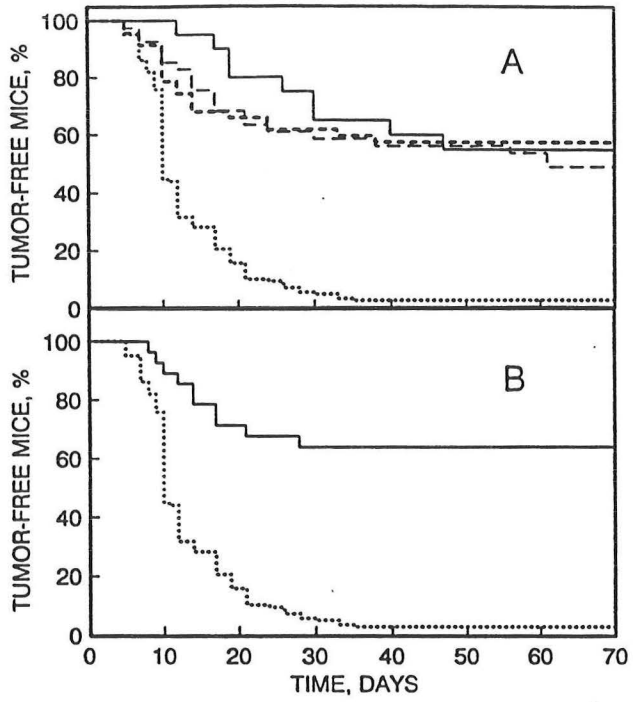
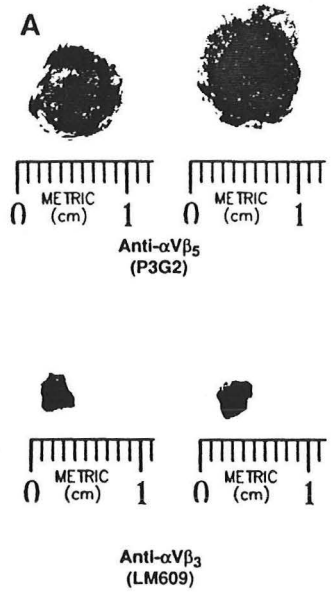


FIG. 1. Survivor functions showing prevention of HT-29 tumor growth in athymic mice by treatment with Ang antagonists. On day 0 HT-29 cells (1.25×10^5 per mouse) are mixed with PBS or the Ang antagonist and injected s.c. Daily injections of PBS or the Ang antagonist are then given for the next 35 days. (*A*) · · ·, PBS ($n = 145$); —, mAb 26-2F (60 μg; $n = 20$); – –, mAb 36u (60 μg; $n = 41$); - - -, mAb 26-2F + mAb 36u (30 μg of each; $n = 47$). (*B*) · · ·, PBS ($n = 145$); —, bovine actin (18 μg; $n = 28$).

Cell, Vol. 79, 1157–1164, December 30, 1994, Copyright © 1994 by Cell Press

Integrin $\alpha_v\beta_3$ Antagonists Promote Tumor Regression by Inducing Apoptosis of Angiogenic Blood Vessels

Peter C. Brooks,*† Anthony M. P. Montgomery,*
Mauricio Rosenfeld,*† Ralph A. Reisfeld,*
Tianhua Hu,‡ George Klier,‡ and David A. Cheresh*†



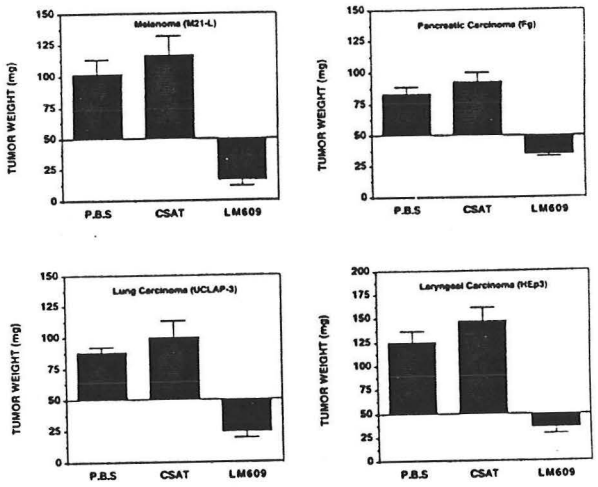


Figure 2. Inhibition of Tumor Growth by Antagonists of $\alpha_v\beta_3$ Integrin

Figure 1. IV Administration of $\alpha_v\beta_3$ Antagonists Inhibit Tumor-Induced Angiogenesis

Human M21-L melanoma tumor fragments (50 mg) were implanted on the CAMs of 10-day-old embryos. After 24 hr, embryos received a single IV injection of 300 μg/100 μl of either control peptide or cyclic RGD peptide. After a total of 72 hr, tumors were removed, analyzed,

(B) High magnification (4.33×) of peptide-treated tumors. The left panel represents normal vessels from control-treated tumors, and the right panel represents examples of disrupted blood vessels from LM609-treated tumors (arrows).

Inhibition of Angiogenesis In Vivo by Interleukin 12

*Emile E. Voest, Bärbel M. Kenyon, Michael S. O'Reilly, Gary Truitt, Robert J. D'Amato, Judah Folkman**

Journal of the National Cancer Institute, Vol. 87, No. 8, April 19, 1995

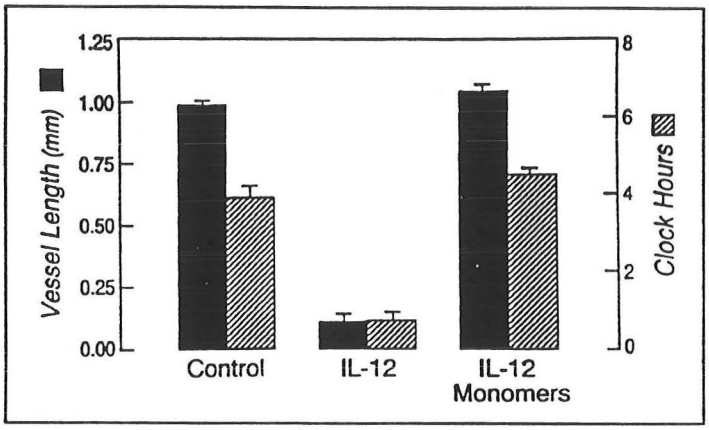


Fig. 2. Angiogenic response 5 days after implantation of the basic fibroblast growth factor pellets in C57BL/6 mice. Treatment consisted of either vehicle (control—21 corneas), IL 12 (30 corneas), or a monomeric mixture of IL 12 (10 corneas) as described in the "Materials and Methods" section. Vessel length and number of clock hours are presented as means ± SEM.

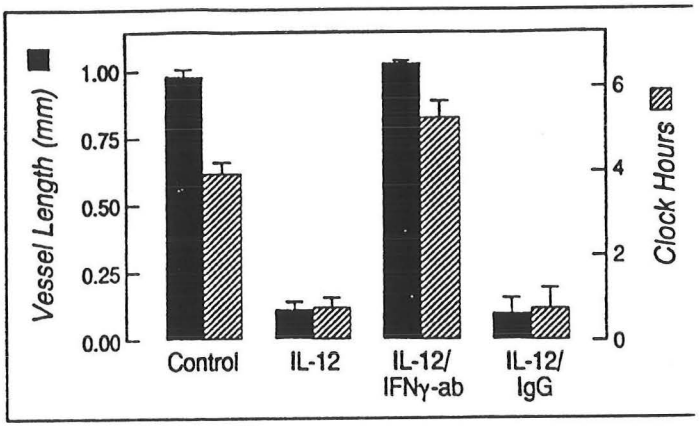


Fig. 3. Effects of IFN γ antibodies on IL 12-induced inhibition of mouse cornea neovascularization. C57BL/6 mice were treated with either a single in traperitoneal injection of rat IgG1 XMG1.2 IFN γ-blocking antibodies (IFNγ-ab or nonspecific rat IgG as described in the "Materials and Methods" section. Ves sel length and clock hours of neovascularization were measured on day 5. Thi experiment was repeated on two separate occasions, and similar results were ob tained. Data are presented as the means ± SEM of at least 13 corneas.

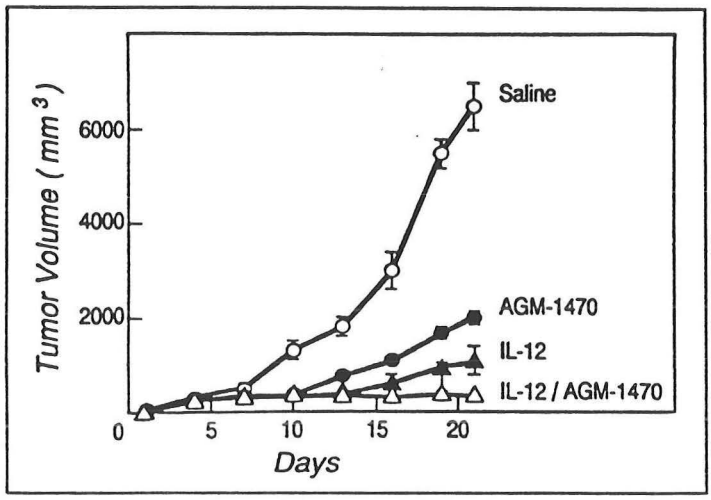


Fig. 5. Effect of IL 12 and AGM-1470 on growth of Lewis lung carcinoma. C57BL/6 mice were inoculated with Lewis lung carcinoma on day 0, and treatment with either saline, IL 12, AGM-1470, or simultaneously administered IL 12 plus AGM-1470 was started after the tumor became measurable. Treatment protocol and measurement procedures are described in the "Materials and Methods" section. Results are representative of a single experiment with four animals in each group.

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Medical Sciences

Thalidomide is an inhibitor of angiogenesis

(fibroblast growth factor/rabbit cornea)

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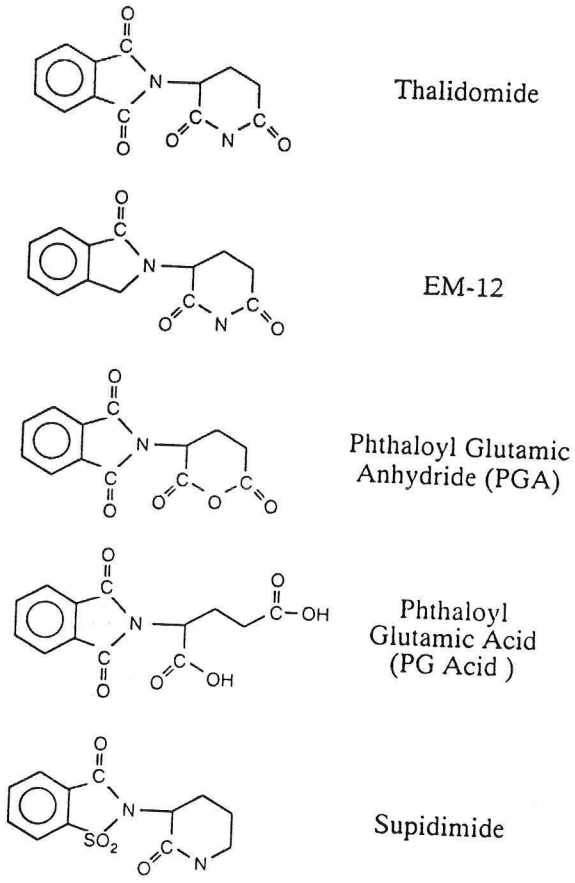

FIG. 1. Structure of thalidomide and related analogs.

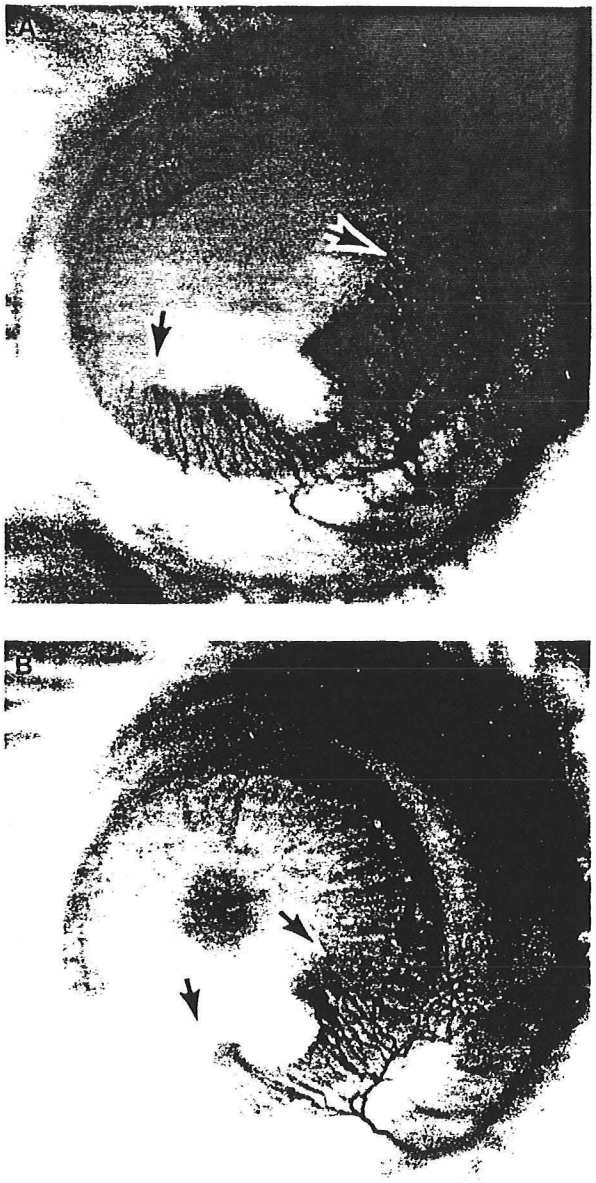

FIG. 3. Representative corneas at 8 days after implantation of bFGF pellets from control (*A*) and thalidomide-treated (*B*) rabbits. There is prominent corneal neovascularization (arrows) in the control with associated corneal clouding, which was demonstrated histologically to be stromal edema without inflammation. The thalidomide-treated animal has markedly less neovascularization with minimal corneal edema.

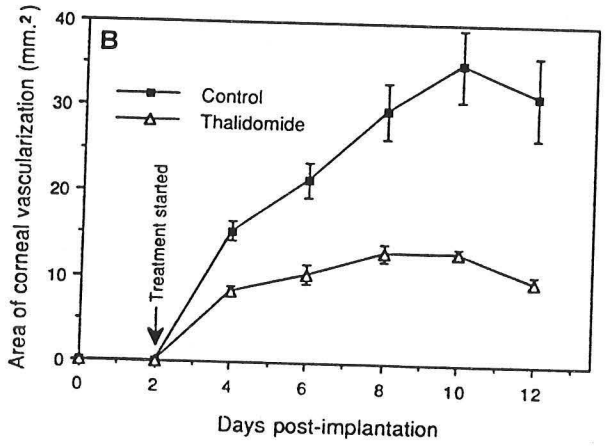

Table 4. Biologic and Pharmacologic Bases for Antiangiogenic Therapy of Tumors

The principal target is represented by proliferating endothelial cells. Since in normal tissues endothelial cells are quiescent, whereas in tumors they are activated/proliferating, this is the rationale for the specificity of the antiangiogenic therapy.
Endothelial cells are a target easily and directly accessible to specific inhibitors given by systemic administration. On the contrary, the delivery of conventional antitumoral therapies to tumor cells may be difficult and suboptimal under some circumstances (eg, necrosis, fibrosis, low blood flow, high interstitial fluid pressure).
Several angiogenic inhibitors have been discovered with different mechanisms of action, most of which are of low toxicity and present a favorable therapeutic index.
Antiangiogenic drugs act through four main mechanisms: (1) neutralization of endothelial growth factors (eg, suramin and monoclonal antibodies to bFGF and to VEGF); (2) inhibition of the proliferative endothelial cells (eg, AGM-1470; rhPF4; thalidomide); (3) block of the formation of new capillaries (eg, linomide) and, (4) inhibition of synthesis and turnover of vessel basement membrane (eg, angiostatic steroids).
All antiangiogenic agents act specifically on the vascular compartment. In fact they induce tumor remission in vivo, but have no direct cytotoxic effect on tumor cells in vitro.
Intratumoral endothelial cells are normal cells that are similar in the primary tumor and metastasis. They are unlikely to develop resistance to antiangiogenic drugs. Conversely, tumor parenchyma is made up by an heterogeneous cell population, which may create significative differences between the primary and metastasis. Tumor cell heterogeneity represents one of the most important causes of failure of conventional antitumor therapies.
Tumor cells and intratumoral neovessels may constitute two distinct targets for anticancer therapy. A therapeutic approach to both the targets may lead to a synergistic antitumor effect.
Several antiangiogenic agents are under early clinical evaluation, including AGM-1470, linomide, rhPF4, pentosan polysulphate, and suramin (Table 5).

Table 5. Angiogenesis Inhibitors and Their Mechanisms of Action

Inhibition of endothelial-cell proliferation	AGM-1470† (angioinhibin or TNP-470)[47]
	quinoline-3-carboxamide† [129]
	Recombinant platelet factor 4† [130]
	Tamoxifen* [118]
	Pentosan polysulfate† [131]
	D-penicillamine[132]
	Genestein[133]
	Thalidomide[134]
	Bumetanide,* furosemide* [135]
	Interferons[56]
	Antibody TEC-11 to endoglin[50]
Blockage of endothelial-cell migration and formation of new capillaries	quinoline-3-carboxamide†
	Deoxymannojirimycin[136]
Neutralization of angiogenic peptides	Antibodies to bFGF[48]
	Antibodies to VEGF[49]
	Sulphonic derivatives of distamycin A[126]
	Suramin† [127] and analogs[128]
	Interferons[56]
Inhibition of the synthesis and turnover of vessel basement membrane	Minocycline* [137]
	Sulphated carboxylmethyl chitin[138]
	Angiostatic heparin-steroids complexes[139]
	Beta-cyclodextrin-tetra-decasulphate + steroids† [140]
	Proline analogs[141]
	Polysaccharide-K[142]
	Razoxane† [143]
	Protamine† [125,144]
Inhibition of extracellular matrix protein	Antibody anti $\alpha_v\beta_3$[51]
	Metallo-proteinase inhibitors (BB-94)†[145]
Stimulation of natural angiogenesis inhibitors	Retinoids[146,147]

*Drugs commercially available.
†Drugs under early clinical trials.

Table 6. Pharmacologic Characteristics of the Antiangiogenic Drugs Under Early Clinical Trials

			Experimental Studies		Clinical Studies				
Drug	Development	Mechanism(s) of Action	Antitumor Activity In Vivo	Toxicity	References	Maximum Dose Level Reached	Trial Status	Toxicity	Comments
Suramin	Polysulphonated nathyurea with antitryponosomal activity[157]	Blocks of several receptors to growth factors[158,159] Inhibition of the bFGF receptors, endothelial cell migration, and proliferation[158,159]	Yes, active also in vitro[160,164]	Severe systemic toxicity	Myers et al[162]	350 mg/m²/d continuous IV × 1 wk	Completed	See reference 164 for a review	Partial remissions observed in prostate cancer
					Stein et al[164]	1,400 mg/m²/wk IV	Completed		Remissions in cancer of the adrenal cortex, renal and T lymphoma observed
					La Rocca et al[166]	350 mg/m²/d continuous IV × 1 wk	Completed		Partial remissions in lymphomas observed
					Reed et al[167]	350 mg/m²/d continuous IV × 1 wk	Completed		Partial remissions in ovarian cancer Stabilization of tumor growth observed in patients with AIDS-related Kaposi's sarcoma
AGM-1470 (TNP-470)	Synthetic analog of fumagallin[47]	In vitro inhibition of endothelial cell proliferation[168] and migration[169] Inhibition of cyclin-dependent kinases and of DNA synthesis[169] Prevention of entry of endothelial cells onto the G_1 phase[170]	Yes, in particular antimetastatic effect[171,172]	Local ulceration and necrosis	Pluda & Yarchoan*	32.4 mg/m² 1-hr IV infusion every other day	Ongoing	Asymptomatic retinal hemorrhages	
r Platelet factor 4	Purified from *Escherichia coli*[173]	Dose-dependent inhibition of angiogenesis in the CAM-system[130] Inhibition of DNA synthesis in HUVE cells[174]	Yes[174]	Local pain	See Tai-Ping[175] for a review	2.5 mg/lesion daily or by continuous IV infusion	Ongoing	Local pain	Partial remissions observed in AIDS-related Kaposi's sarcoma
Pentosan polysulfate	Sulfated polysaccharide with anticoagulant activity[176]	Inhibition of proliferation and migration of HUVE cells stimulated by bFGF[177]	Yes[177]	Reversible anticoagulant activity	Pluda et al[178]	3 mg/kg body weight IV infusion or 270 mg/m² orally 3 times daily orally	Completed	Reversible anticoagulant effects	Stabilization of tumor growth observed in AIDS-related Kaposi's sarcoma
Linomide	Immunomodulator[179,180]	Induces a dose-dependent antiangiogenic effect[181]	Yes[129,181]	Low	Bengston et al[182]	0.3 mg/kg body weight orally	Completed	Fever	Study performed to test the immunologic effect of the drug in leukemic patients
Razoxane	Antimetastatic agent[183]	Antimetastatic effect[184]	Yes	Neutropenia	Hellman et al[183]	125 mg b.d. orally	Completed	Neutropenia	Phase III study demonstrating that adjuvant therapy with razoxane enhance relapse-free survival in Duke's group C colorectal cancers
BB-94	Metalloproteinase inhibitor[145]	Inhibition of the metalloproteinase enzyme	Decreases tumor volume and prolongs survival of mice bearing a human tumor[145]	Low	Harris (unpublished results)	300 mg/m² intraperitoneal	Ongoing	None	Tumor regression with reduction of the ascite

Abbreviations: HUVE, human umbelical vascular endothelium; CAM, chick embryo chorioallantoic membrane; IV, intravenously; b.d., twice per day.
*Personal communication, August 1994.

Inhibitors of Angiogenesis Produced by Human Cells

- Thrombospondin (produced by normal cells, down regulated by mutation in p53 gene)
- Glioblastoma angiogenesis inhibitory factor (turned on in glioblastoma cells by re-introducing a wild-type p53)
- Angiostatin (38 kd protein produced by some tumor cells; 98% homologous to internal fragment of plasminogen)

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Angiostatin: A Novel Angiogenesis Inhibitor That Mediates the Suppression of Metastases by a Lewis Lung Carcinoma

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Catherine Chen,* Rosalind A. Rosenthal,*
Marsha Moses,* William S. Lane,† Yihai Cao,*
E. Helene Sage,‡ and Judah Folkman*

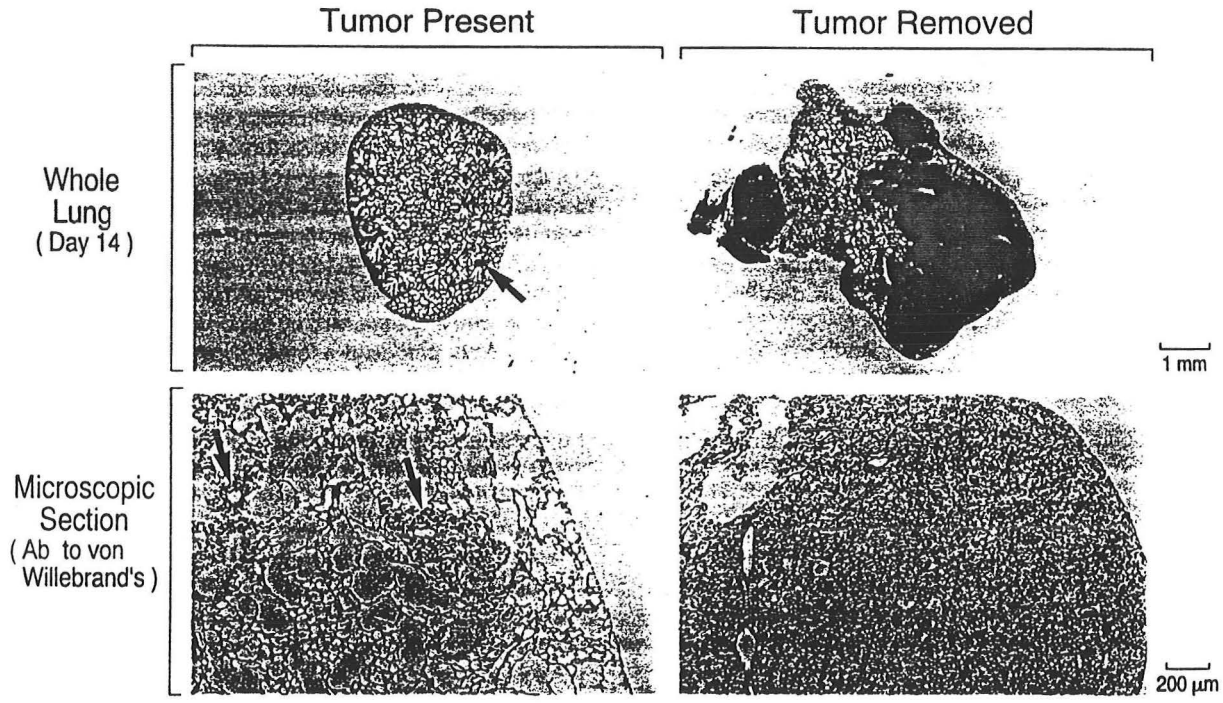

Figure 2. The Presence of a Primary Tumor Is Associated with an Inhibition of Neovascularization and Growth of Its Metastases

Mice were sacrificed 15 days after removal of primary tumors and their lungs compared with lungs of mice with an intact primary tumor. Hematoxylin and eosin staining of sections of lungs revealed the presence of metastases in both groups. Mice with a primary tumor present (left panels) had only small metastases (arrows) as compared with the growing and invasive metastases in the lungs of mice after primary tumor removal (right panels). Immunohistochemical staining with antibodies (Ab) against von Willebrand factor revealed neovascularization (brown stain) of the metastases after primary tumor removal. In contrast, when the tumor was left in place, there was only perivascular cuffing of metastases without neovascularization. Normal lung vessels were seen in both groups.

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Angiostatin: A Novel Angiogenesis Inhibitor That Mediates the Suppression of Metastases by a Lewis Lung Carcinoma

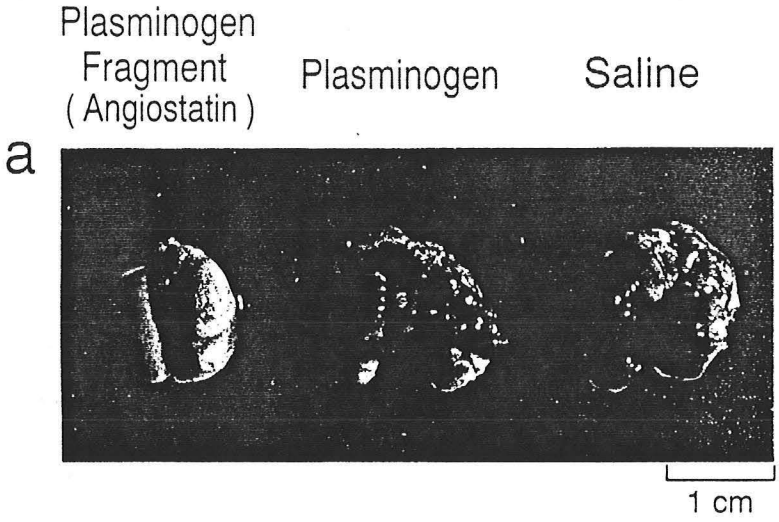


Figure 12. Systemic Treatment with Angiostatin Inhibits Neovascularization and Growth of Metastases after Primary Tumor Removal

(a) Lungs from mice 13 days after removal of a primary tumor. Treatment with angiostatin blocked the growth of metastases. Mice treated with plasminogen or saline had almost complete replacement of the normal lung tissue by growing invasive metastases.

(b and c) Hematoxylin and eosin staining and immunohistochemical staining with antibodies against von Willebrand factor of lungs of mice after removal of the primary tumor. In mice treated with intact plasminogen (shown in [c]), there was marked neovascularization (brown stain) and growth of the metastases. In contrast, mice treated with angiostatin (shown in [b]) had only perivascular cuffing of metastases without neovascularization. N, normal lung; T, tumor.

Dormancy of micrometastases: Balanced proliferation and apoptosis in the presence of angiogenesis suppression

LARS HOLMGREN, MICHAEL S. O'REILLY & JUDAH FOLKMAN

NATURE MEDICINE, VOLUME 1, NUMBER 2 FEBRUARY 1995

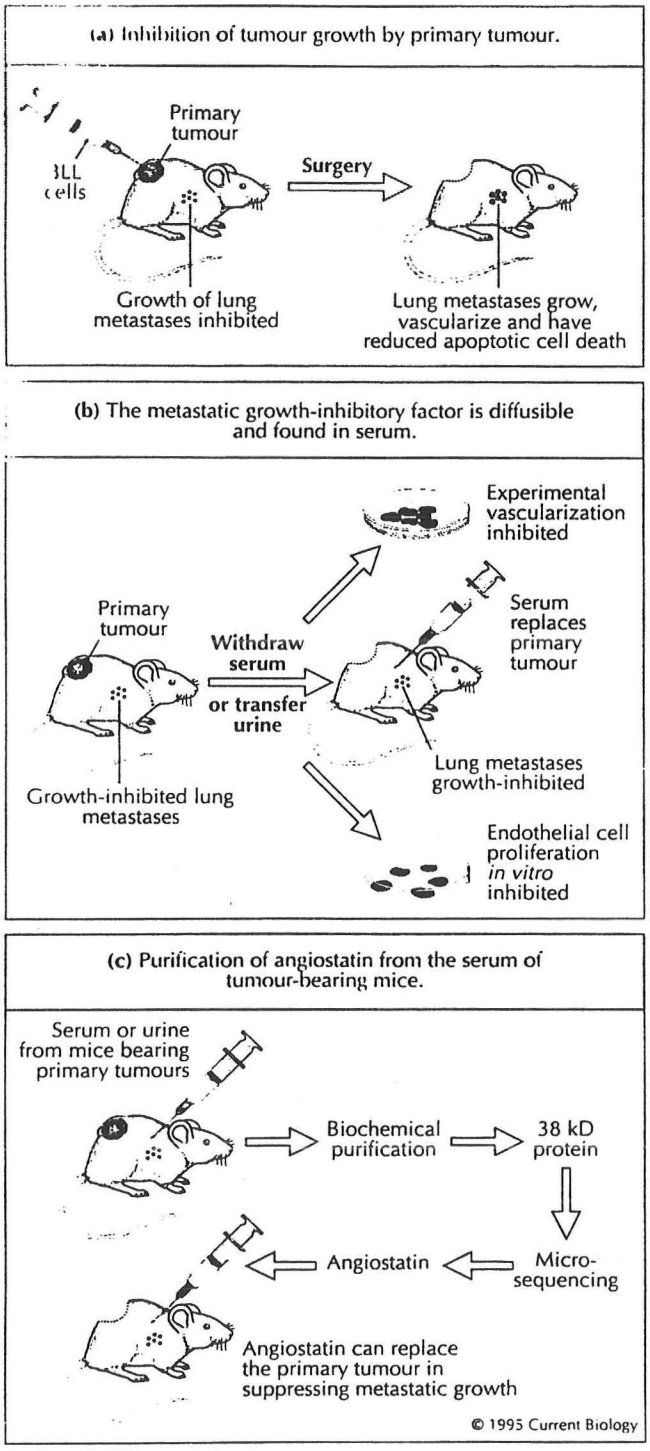
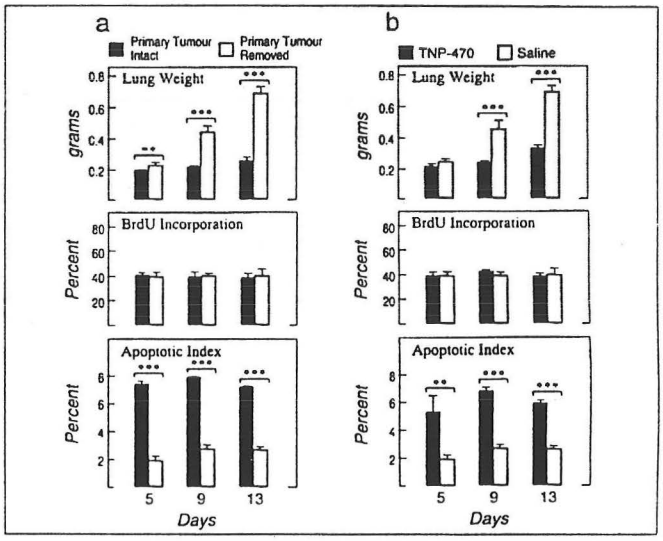


Fig. 2. Identification and purification of angiostatin.



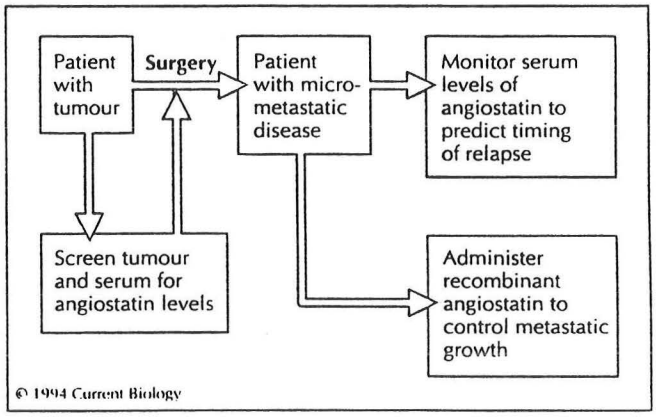


Fig. 3. Theoretical uses of angiostatin.

RICHARD VILE

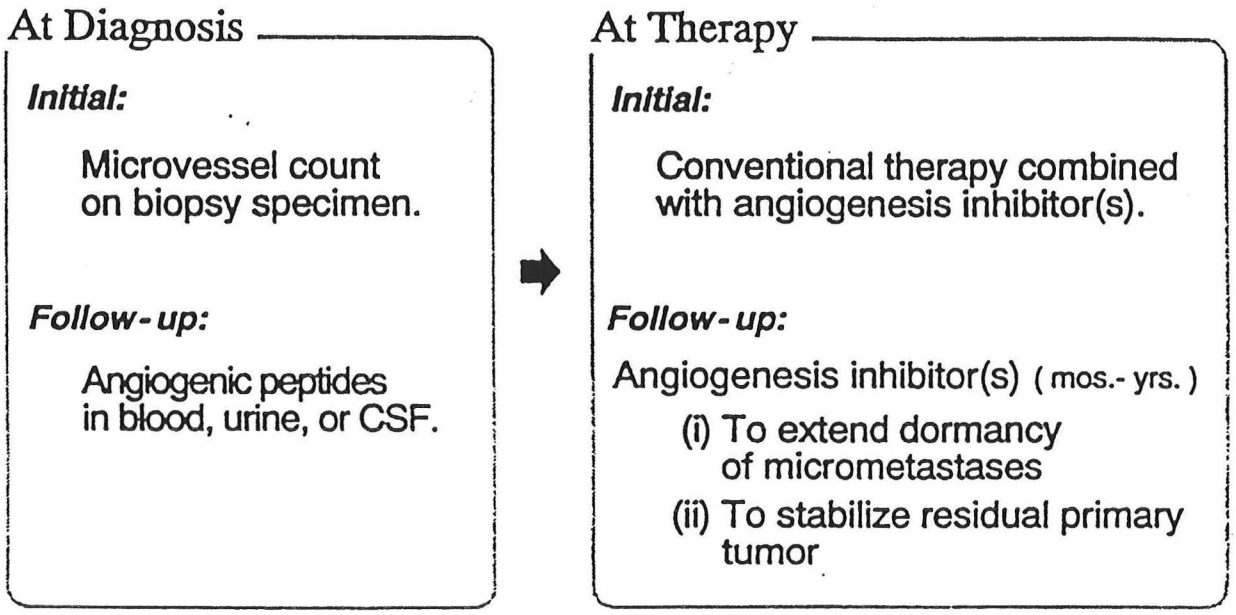


Figure 10–8. Proposed strategy for antiangiogenic therapy of neoplastic disease. CSF, cerebrospinal fluid.

VASCULAR TARGETING AGENTS FOR THE TREATMENT OF SOLID TUMORS

VASCULAR TARGETING AGENTS

- Action
  - attack tumor vasculature, not tumor cells themselves
- Advantages
  - damage to a single blood vessel can result in the death of thousands of tumor cells
  - no need to kill all endothelial cells
  - avoid need for tumor penetration
  - endothelial cells are non-malignant, so mutants unlikely to emerge
  - applicable to many types of solid tumors

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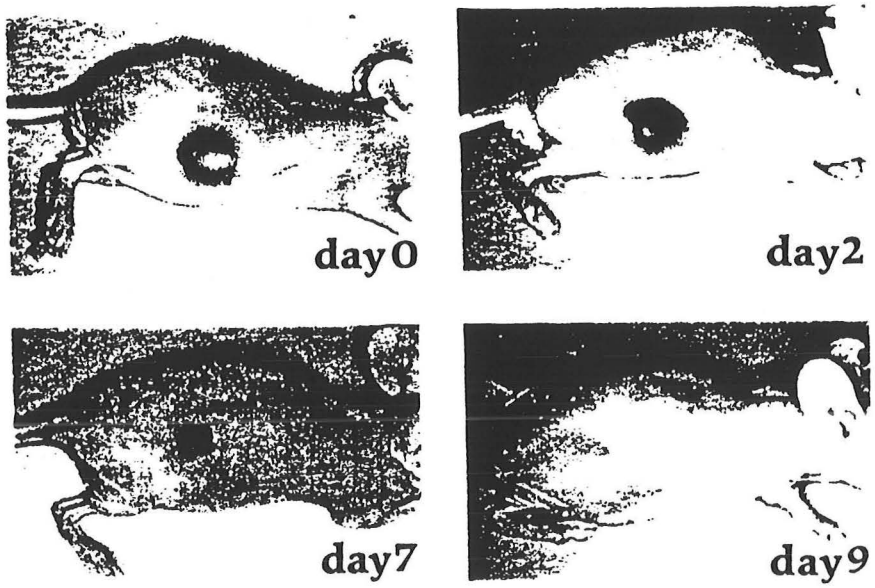
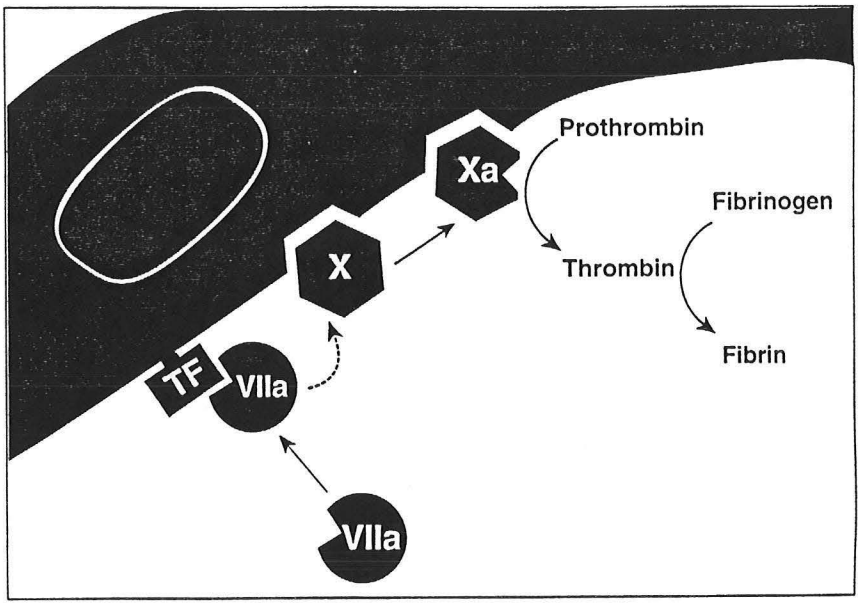


Mice bearing solid neuroblastoma tumors were given a single treatment with a vascular targeting that recognizes an experimentally-induced tumor endothelial cell antigen (MHC class II). Representative mice are shown various days after treatment.

TISSUE FACTOR-MEDIATED COAGULATION



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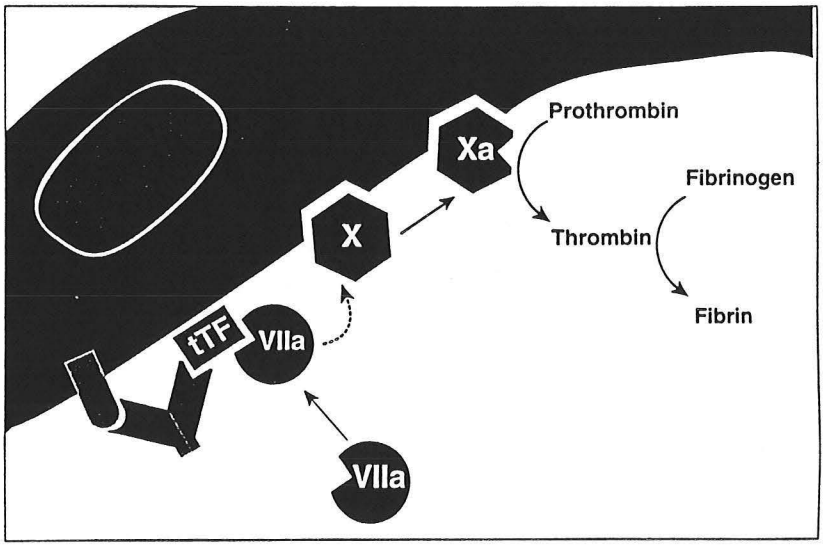
TARGETED TISSUE FACTOR-MEDIATED COAGULATION



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ANTIBODIES TO HUMAN TUMOR VASCULATURE

TARGETING TISSUE FACTOR TO TUMOR VASCULATURE

ADVANTAGES OF TARGETING TISSUE FACTOR

- Triggers the coagulation cascade ∴ have amplication, giving high potency
- Thrombosis of tumor vasculature should cause massive tumor cell killing
- Can use human tissue factor and human antibodies ∴ low immunogenicity

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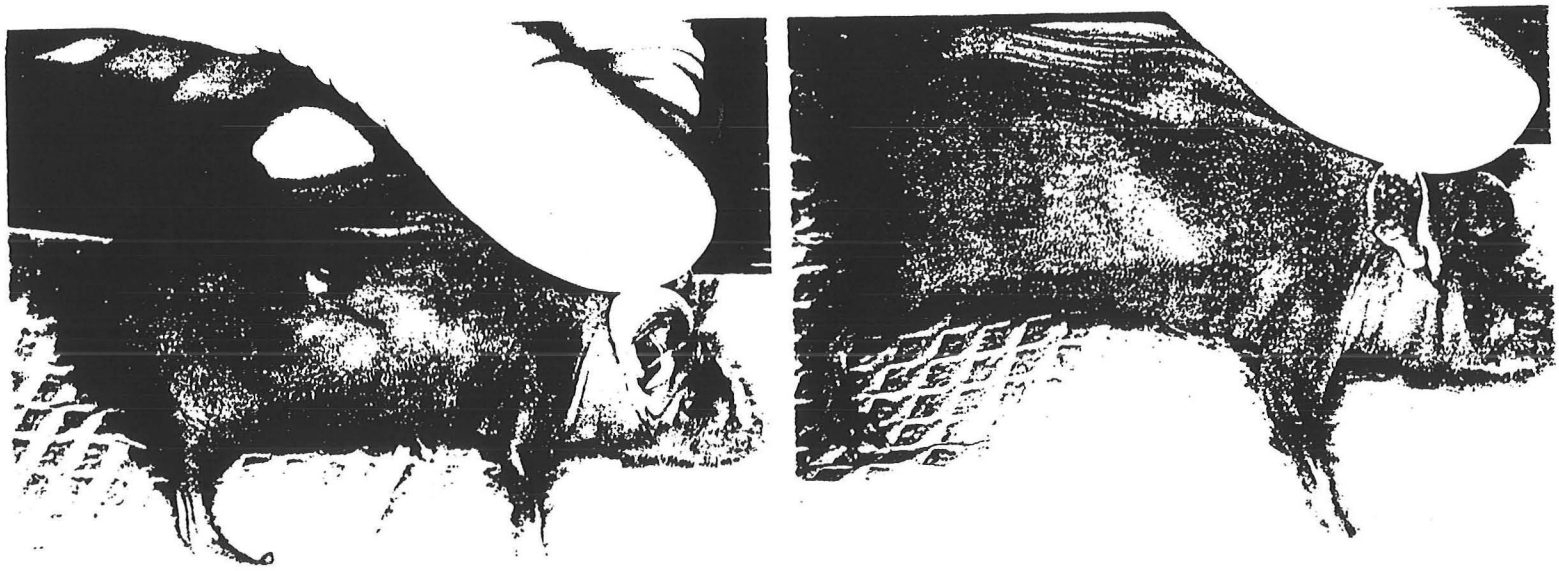

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