Analysis of Experimental Antiangiogenic Therapy

By Harold Brem and Judah Folkman
Boston, Massachusetts

• **Angiogenesis is a fundamental process by which new blood vessels are formed. Progressive tumor growth necessitates the continuous induction of new capillary blood vessels which converge upon the tumor. Suppression of tumor growth can be accomplished with the use of antiangiogenesis agents. AGM-1470 is a potent angiogenesis inhibitor in vitro and in vivo. In mouse studies, AGM-1470 has suppressed the growth and neovascularization induced by four murine tumors resulting in a 55% to 77% decrease in tumor growth. In these mice significant toxicity did not result from AGM-1470 therapy. AGM-1470 administered systemically to C57Bl/6 male mice for 20 to 28 days inhibited the growth of: (1) Lewis lung carcinoma resulting in a T/C (treatment/control = mean tumor volume of treated/mean tumor volume of control) of 0.38 ± 0.03 ($P < .001$); (2) colon adenocarcinoma 38 resulting in a T/C of 0.23 ± 0.02 ($P < .001$); and (3) fibrosarcoma 105 resulting in a T/C of 0.31 ± 0.05 ($P < .001$). To determine if antiangiogenic therapy was equally effective in mice of both sexes and in immunodeficient animals, we tested AGM-1470 in the treatment of fibrosarcoma 105 in both female mice and nude mice. For female mice T/C was 0.24 ± 0.06 ($P < .001$). For nude mice T/C was 0.27 ± 0.06 ($P < .001$). These results demonstrate that AGM-1470 suppresses the growth of a variety of different tumors. Furthermore, the antitumor effect of AGM-1470 therapy is independent of the immune system and sex.**

INDEX WORDS: Angiogenesis; antiangiogenic therapy; AGM-1470; fibrosarcoma; Lewis lung carcinoma; colon carcinoma.

ANGIOGENESIS IS a fundamental process by which new blood vessels are formed. It is a component of many physiological responses such as reproduction, development, and wound healing.[1,2] Each of these physiological functions encompasses numerous angiogenesis-dependent events. Many pathological processes are also angiogenesis dependent. For example, in children, retinopathy of prematurity often results in blindness[3] and some forms of hemangiomas are life-threatening.[4,5] However, the most lethal form of angiogenesis is induced by tumors. Considerable experimental and clinical evidence has been assembled over the past two decades to demonstrate that tumor growth and metastasis are both angiogenesis dependent.[6-13] Therefore, the potential use of an angiogenesis inhibitor to control these neoplasms is attractive.

Fumagillin is a secreted fungal product that was discovered in our laboratory and found to have potent antiangiogenic activity. However, fumagillin resulted in significant weight loss when administered to mice. Therefore, synthetic analogues were synthesized that were nontoxic. AGM-1470 is the most potent angiogenesis inhibitor of these analogues.[14] AGM-1470, in vitro, inhibits fibroblast growth factor induced stimulation of: (1) endothelial cell migration[13]; (2) endothelial cell proliferation[14]; and (3) capillary tube formation.[15] AGM-1470, in vivo suppresses angiogenesis when administered locally in the cornea[15] and on the chick chorioallantoic membrane.[14,15] Because tumor growth requires continuous angiogenic stimulation,[7] we tested AGM-1470 on a variety of subcutaneous tumors. Our goal was to elucidate further some of the biological characteristics of antiangiogenic therapy in general and AGM-1470 therapy in particular.

MATERIALS AND METHODS

Inhibitor

AGM-1470 was synthesized by Takeda Chemical Industries, Ltd and stored at −20°C. It was diluted in a 10% (w/v) solution of 100% ethanol and stored at 4°C. Immediately prior to treatment the stock solution of AGM-1470 was diluted in 0.9% sodium chloride solution (20 μL stock solution/1 mL of NaCl in a polypropylene tube). Treatment was 30 mg/kg by subcutaneous injection every other day at a site remote from the tumor. Control mice received the vehicle (2% ethanol solution in 0.9% saline) every other day.

Mice

All mice were housed one per cage and were fed ad lib with Purina chow and tap water. Male and female C57Bl/6 mice were obtained from Jackson laboratories (Bar Harbor, ME). Male nude mice (nu/nu) were purchased from the Massachusetts General Hospital (Boston, MA). The dorsum of these mice was shaved 1 day prior to tumor implantation. Treatment in all groups of 6- to 7-week-old mice began only after the tumors were visible and palpable (80 to 200 mm^3), eg, 4 to 7 days after implantation. On the 1st day of treatment the average tumor volume and weight in the treatment and control groups were the same.

Anesthesia

Prior to shaving and at the time of tumor implantation the animals were anesthetized in a methoxyflurane chamber. Animals were killed by continuous inhalation of methoxyflurane.

From the Department of Surgery, Children's Hospital, and the Departments of Surgery and Anatomy and Cellular Biology, Harvard Medical School, Boston, MA.

Presented at the 23rd Annual Meeting of the American Pediatric Surgical Association, Colorado Springs, Colorado, May 13-16, 1992.

Supported in part by a grant to Harvard University from Takeda Chemical Industries, Ltd, and the Association for Academic Surgery Award (H.B.) and the Leon Hirsch Fellowship (H.B.).

Address reprint requests to Judah Folkman, MD, Children's Hospital, Hunnewell 103, 300 Longwood Ave, Boston, MA 02115.

0022-3468/93/2803-0028$03.00/0

Lewis Lung Carcinoma, Colon 38 Carcinoma, and Fibrosarcoma 105

Lewis lung carcinoma (LLC), dimethylhydrazine-induced murine colon adenocarcinoma 38 (MC-38),[16] and methylcholanthrene-induced murine fibrosarcoma 105 (MCA-105)[16,17] were grown individually as solid tumors to 1 cm^3. Each tumor was excised under sterile conditions in a laminar flow hood. A single cell suspension was obtained. Each subcutaneous injection was approximately 1 × 10^6 cells in a volume of 0.1 mL of normal saline.

Lewis Lung Carcinoma Cells-L1

This is a highly metastatic variant of LLC that was obtained as follows. LLC was grown in the dorsum of a mouse. One of the lung metastases was harvested and grown in culture for five passages. A suspension of these cells was then reimplanted subcutaneously, and found to have a significantly higher metastatic rate than its parent line. This line was termed LLC-lung-1 (LLC-L1). The tumor was passaged subcutaneously every 12 days.

Tumor Volume

Tumor dimensions were calculated every second or third day with calipers. Tumor volume was calculated by width^2 × length × 0.52. Tumor volume is also expressed as T/C (ie, mean tumor volume of treated/mean tumor volume of control). The T/C on the first day of treatment of all of the treated groups in all of the tumor models was 1.00 to 1.11.

Animal Weights

After treatment began the mice were weighed every 3 days.

Necropsy

Experiments were terminated between 20 and 27 days after treatment began. Autopsies were performed on all animals, at which time tumor weights were obtained. Animal weights at the time of necropsy were calculated by subtracting the tumor weight from the animal weight immediately prior to necropsy (Figs 1B and 2B).

Statistics

Statistical analysis was by Student's *t* test.

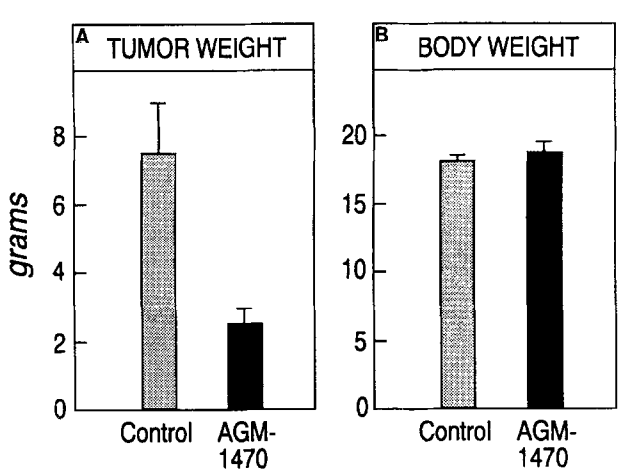

Fig 1. Treatment of LLC with AGM-1470 in male mice bearing LLC. (A) tumor weight; (B) animal weight.

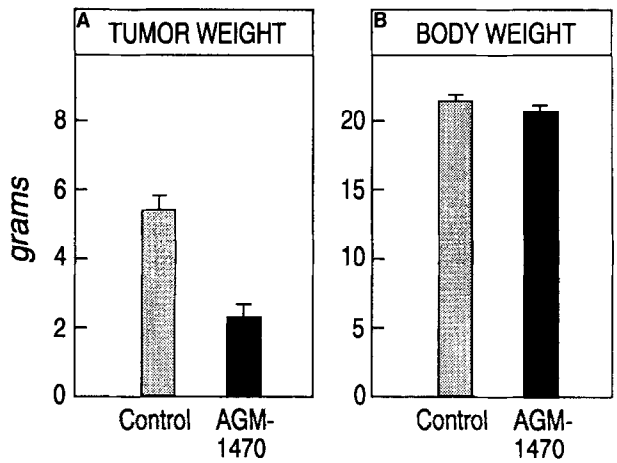

Fig 2. Treatment of MCA-105 with AGM-1470 in male mice. (A) tumor weight; (B) animal weight.

RESULTS

Colon Carcinoma

Tumor volume after 28 days of treatment (n = 24) was 8,544 ± 872 mm^3 in the vehicle-treated mice and 1,968 ± 193 mm^3 in the AGM-1470–treated mice (Figure 3A). T/C was 0.23 ± 0.02 ($P < .001$) (Fig 3B).

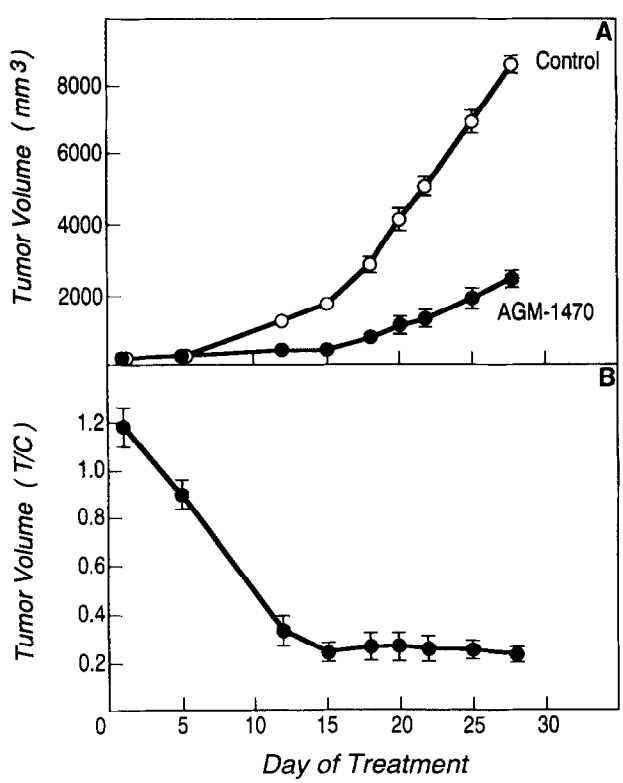

Fig 3. Inhibition of growth of MC-38 after administration of AGM-1470. (A) tumor volume; (B) T/C = mean tumor volume of treated/mean tumor volume of control for each day.

Lewis Lung Carcinoma

AGM-1470 inhibited the growth of LLC tumors in eight consecutive experiments (4 to 8 mice per group). The tumor volumes after 20 days of treatment were 6,717 ± 477 mm^3 in the control mice (n = 38) versus 2,533 ± 190 mm^3 in the AGM-1470–treated mice (n = 36). The T/C was 0.34 ± 0.06 ($P < .001$). The tumor weights after 20 days of treatment were 5.45 ± 0.40 g in the control mice versus 2.28 ± 0.40 g in the AGM-1470–treated mice ($P < .001$) (Fig 1A).

Fibrosarcoma 105

AGM-1470 inhibited MCA-105 growth in male mice (n = 43). The tumor weight after 27 days of treatment in the saline-treated mice (n = 7) was 7.52 ± 1.49 g compared with 2.51 ± 0.44 g in the AGM-1470–treated mice (n = 10) ($P < .003$) (Fig 2A). The tumor volumes were compiled from three separate experiments. The average tumor volume in the saline-treated mice was 4,995 ± 1,079 mm^3 versus 1,546 ± 267 mm^3 in the AGM-1470–treated mice ($P < .001$) (Fig 4A). The T/C was 0.31 ± 0.05 ($P < .001$) (Fig 4B).

Immune-Deficient Mice

AGM-1470 inhibited the growth of MCA-105 in nude mice. The average tumor volume in the control mice (n = 10) after 24 days of treatment was 11,587 ± 1,164 mm^3 versus 3,195 ± 579 mm^3 in the AGM-1470–treated mice (n = 10) ($P < .001$). The T/C was 0.28 ± 0.05 (Fig 4D).

Tumor Suppression of MCA-105 and LLC-L1 in Female Mice

To determine if the tumor inhibitory effect was sex dependent, male (n = 22) and female (n = 22) mice were implanted with MCA-105. The T/C after 26 days of treatment was 0.21 ± 0.03 ($P < .001$) in the male mice and 0.24 ± 0.06 ($P < .001$) in the female mice. The antitumor effect in males was nearly identical to its antitumor effect in females ($P > .05$) (Fig 4C).

Treatment for 23 days of LLC-L1 in female mice (n = 20) resulted in a tumor volume in the control mice of 4,200 ± 544 mm^3 versus 1,894 ± 124 mm^3 in the AGM-1470 mice (Fig 5A). The T/C was 0.45 ± 0.03 ($P < .001$) (Fig 5B).

Toxicity

Tumor-bearing mice that received AGM-1470 gained an average of 1 to 2 g throughout the course of the experiment, whereas untreated tumor-bearing mice gained an average of 5 g. This difference was accounted for in part by the difference in tumor weight. For example, in the treatment of mice with LLC, the average mouse weight after 20 days in control mice was 21.5 ± 0.4 g versus 20.7 ± 0.5 g in the AGM-1470–treated mice ($P < .001$) (Fig 1B). In

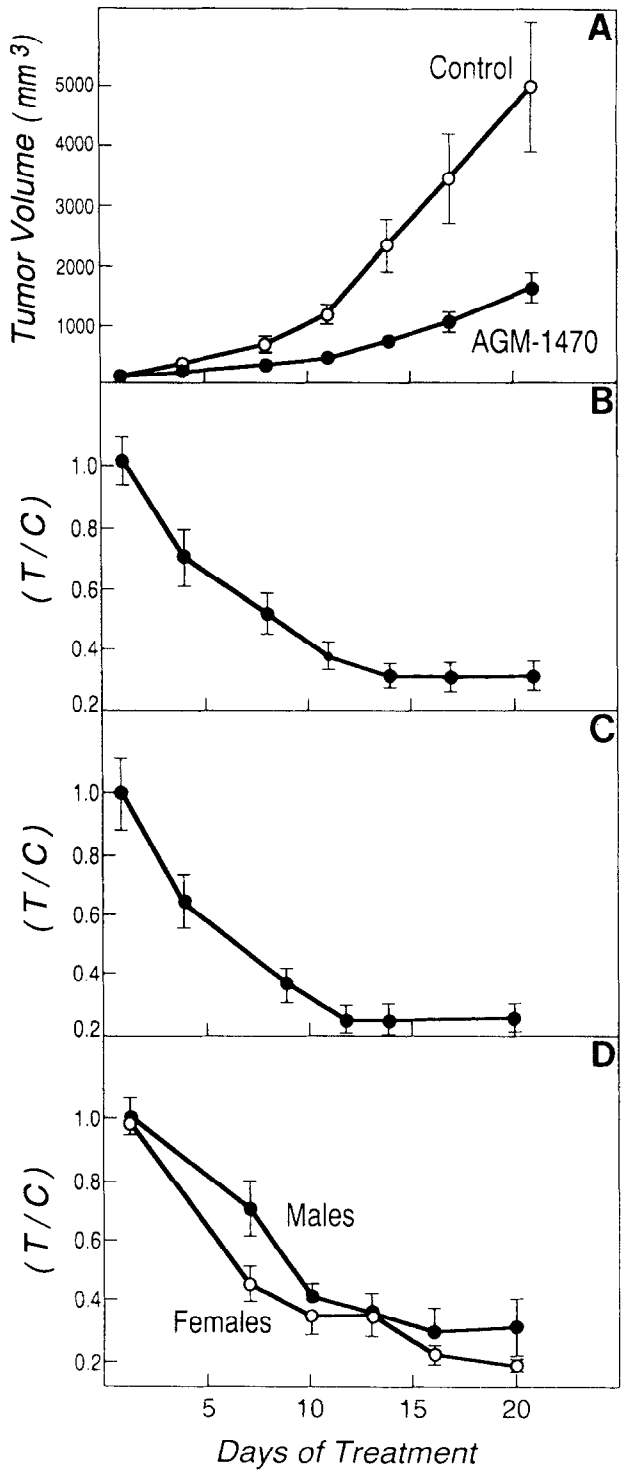

Fig 4. Inhibition of growth of MCA-105 after administration of AGM-1470. (A) tumor volume in male mice; (B) T/C in male mice; (C) T/C in male and female mice; (D) T/C in male nude mice.

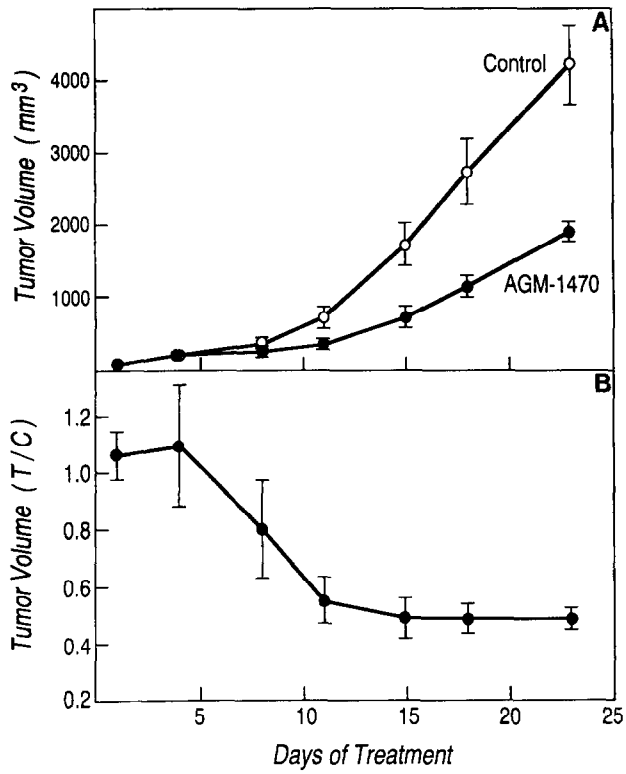

Fig 5. Inhibition of growth of LLC-L1 in female mice after administration of AGM-1470. (A) tumor volume; (B) T/C.

mice with MCA-105 the average mouse weight after 27 days of treatment in the control mice was 18.20 ± 0.48 g versus 18.72 ± 0.83 g in the AGM-1470 mice ($P < .001$) (Fig 2B).

Mice that received AGM-1470 were active throughout the course of therapy. There were no visible side effects (ie, hair loss, diarrhea, or seizure activity).

DISCUSSION

The inadvertent contamination of a culture plate of capillary endothelial cells by a fungus, *Aspergillus fumigatus* fresenius, led to the finding in our laboratory that fumagillin, the secreted fungal product that inhibited endothelial cell proliferation, was also an angiogenesis inhibitor.[14] AGM-1470 is a synthetic analogue of fumagillin selected for increased potency and decreased toxicity.

We show here that AGM-1470 has antitumor activity against two types of murine LLC, a colon carcinoma, and a fibrosarcoma. We further demonstrate that the antitumor activity of AGM-1470 operates independently of the sex of the host and is equally as effective in T-cell–deficient mice as it is in mice with an intact immune system. Prolonged therapy for 28 days is not associated with toxicity, as shown by continuous weight gain in treated animals.

These experiments do not prove that the antitumor effect of AGM-1470 is directly the result of its antiangiogenic activity. However, experimental data from our laboratory provide additional evidence that the antiangiogenic activity of AGM-1470 is responsible for its antitumor activity: (1) AGM-1470 is not cytostatic to Lewis lung cells in vitro as it is to endothelial cells (unpublished data); (2) the cytotoxic concentration of AGM-1470 for LLC cells in vitro is greater than 20,000 times the cytostatic concentration of this compound for endothelial cells in vitro[13]; thus, the tumor cells are virtually resistant to AGM-1470 before the animals are treated, yet potent tumor inhibition is obtained in vivo; (3) the concentration of AGM-1470 necessary to inhibit the chemotaxis of Lewis lung cells in vitro is approximately 300 times greater than the concentration of this compound necessary to inhibit endothelial cell chemotaxis in vitro[13]; (4) the intense, circumferential neovascularization of the cornea induced by an intracorneal implant of basic fibroblast growth factor is suppressed by systemic administration of AGM-1470 (subcutaneous injection)[18]; and (5) AGM-1470 has no effect against a leukemic tumor (P388) that grows as ascites in mice and is not dependent on angiogenesis.[14]

The significant antitumor effect of AGM-1470 in nude mice suggests that when this agent reaches clinical trial in cancer patients, and that if tried in patients with Kaposi's sarcoma, it should retain its efficacy even in the immunosuppressed patient.[19] AGM-1470 does not appear to require induction of the host immune system to manifest antitumor activity. This is in contrast to some other antitumor agents such as flavone acetic acid, which requires immune stimulation, ie, increased natural killer cell activity, to be effective.[20] Furthermore, as predicted, the antitumor activity of flavone acetic acid was less effective in nude mice.[21]

The low toxicity of AGM-1470, despite prolonged therapy, is of interest because this finding is consistent with our previous results obtained from endothelial cells in vitro and from the chick embryo, which show that AGM-1470 inhibits proliferating and migrating endothelial cells but has little or no effect on nongrowing endothelium.[13,14] Thus, we did not see the side effects of diarrhea, anemia, weight loss, or hair loss expected from conventional cancer chemotherapeutic agents that are toxic to rapidly replicating normal cells. In fact, in preliminary unpublished data, we find little or no toxicity in mice treated (without tumors) for more than 150 days. The potential practical importance of this lack of toxicity is that the treatment of cancer patients by AGM-1470 (or by

other angiogenesis inhibitors) is likely to be long term (months to years). This idea is supported by our current experience with the successful treatment of life-threatening hemangiomas in babies by α-interferon.[22] The antiangiogenic activity of α-interferon,[23,24] although not sufficiently potent to abrogate tumor angiogenesis, is capable of causing regression of large hemangiomas. Therapy was usually started in newborns or in premature babies and continued for as long as 18 months without demonstrable toxicity.

While most conventional cancer chemotherapeutic agents are effective for one tumor type or for a restricted group of tumors, it could be argued that antiangiogenic therapy should be effective against a wide variety of tumors because this approach is targeted to the vascular endothelium.[12] Therefore, we can ask, is antiangiogenic therapy a form of generic antitumor therapy? The testing of this hypothesis, will require the demonstration of successful antitumor activity by an angiogenesis inhibitor against a wide variety of tumors. The efficacy of AGM-1470 against the four tumors reported here strengthens this hypothesis.

AGM-1470 is currently awaiting FDA approval for phase I clinical trial. It is too early to speculate on its potential role in anti-cancer therapy. Nevertheless, our experimental data leads us to think that such an angiogenesis inhibitor may begin to be used as: (1) an adjunct to conventional chemotherapy; (2) in combination with other angiogenesis inhibitors[25-36]; or (3) in the long intervals after initial chemotherapy, surgery, or radiotherapy.

ACKNOWLEDGMENT

The fibrosarcoma tumor line was prepared by the members of the Laboratory for Biological Cancer Therapy at the Brigham and Women's Hospital (courtesy of Drs J. Kusak, D. Schoof, J. Obando, and T. Eberlein). We greatly appreciate the technical expertise provided by Jeffrey Grosfeld, William Muirhead, Dipak Panigraphy, and Lawrence Sanders. We thank Wendy Foss for typing this manuscript.

REFERENCES

1. Folkman J, Brem H: Angiogenesis and inflammation, in Gallin JI, Goldstein IM, Snyderman R (eds): Inflammation: Basic Principles and Clinical Correlates (ed 2). New York, NY, Raven, 1992 (in press)

2. Brem H, Tsakayannis D, Folkman J: Time dependent suppression of wound healing with the angiogenesis inhibitor, AGM-1470. J Cell Biol 115:403a, 1991

3. Ben Sira I, Nissenkorn I, Kremer I: Retinopathy of prematurity. Surv Ophthalmol 33:1-16, 1988

4. Enjolras O, Riche MC, Merland JJ, et al: Management of alarming hemangiomas in infancy: A review of 25 cases. Pediatrics 85:491-498, 1990

5. Mulliken JB, Young AE: Vascular Birthmarks: Hemangiomas and Malformations. Philadelphia, PA, Saunders, 1988

6. Folkman J: Tumor angiogenesis: Therapeutic implications. N Engl J Med 285:1182-1186, 1971

7. Folkman J: What is the evidence that tumors are angiogenesis-dependent? J Natl Cancer Inst 82:4-6, 1990

8. Folkman J: Angiogenesis—Retrospect and outlook, in Steiner R, Weisz P, Langer R (eds): Angiogenesis Key Principles—Science-Technology-Medicine. Basel, Switzerland, Birkhauser Verlag, 1992, pp 4-13

9. Folkman J: Successful treatment of an angiogenic disease. N Engl J Med 320:1211-1212, 1989

10. Brem H, Tamargo RJ, Guerin C, et al: Brain tumor angiogenesis, in Kornblith PL, Walker MD (eds): Advances in Neuro-oncology. Mount Kisco, NY, Futura, 1988, pp 89-102

11. Klagsbrun M, D'Amore PA: Regulators of angiogenesis. Annu Rev Physiol 53:217-239, 1991

12. Folkman J: Oncology overview on antiangiogenesis, in Girardi AJ (ed): Oncology Overviews on Antiangiogenesis. Washington, DC, US Government Printing Office, 1991, pp vii-x

13. Brem H, Ingber D, Blood CH, et al: Suppression of tumor metastasis by angiogenesis inhibition. Surg Forum 42:439-441, 1991

14. Ingber D, Fujita T, Kishimoto S, et al: Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth. Nature 348:555-557, 1990

15. Kusaka M, Sudo K, Fujita T, et al: Potent anti-angiogenic action of AGM-1470: Comparison to the fumagillin parent. Biochem Biophys Res Commun 174:1070-1076, 1991

16. Massaro AF, Schoof DD, Rubinstein A, et al: Solid-phase anti-CD3 antibody activation of murine tumor-infiltrating lymphocytes. Cancer Res 50:2587-2592, 1990

17. Schoof DD, Massaro AF, Obando JA, et al: The biological effects of immunosuppression on cellular immunotherapy. Surg Oncol 1:27-35, 1992

18. Gonzalez EM, Adamis AP, Folkman J: Systemic administration of an angiogenesis inhibitor (AGM-1470), inhibits bFGF-induced corneal neovascularization. Invest Ophthamol Vis Sci 33:777, 1992

19. Levine AM: HIV-related malignancies. Proc Am Assoc Cancer Res 33:590-591, 1992

20. Ching L-M, Baguley BC: Induction of natural killer cell activity by the antitumor compound flavone acetic acid (NSC 347512). Eur J Cancer Clin Oncol 23:1047-1050, 1987

21. Bibby MC, Phillips RM, Double JA, et al: Anti-tumour activity of flavone acetic acid (NSC 347512) in mice—Influence of immune status. Br J Cancer 63:57-62, 1991

22. Ezekowitz RAB, Mulliken JB, Folkman J: Interferon alfa 2a therapy for life-threatening hemangiomas in infancy. N Engl J Med (in press)

23. Brouty-Boye D, Zetter BR: Inhibition of cell motility by interferon. Science 208:516-518, 1980

24. Sidky Y, Borden E: Inhibition of angiogenesis by interferons: Effects on tumor and lymphocyte-induced vascular responses. Cancer Res 47:5155-5161, 1987

25. Brem H, Folkman J: Inhibition of tumor angiogenesis mediated by cartilage. J Exp Med 141:427-439, 1975

26. Crum R, Szabo S, Folkman J: A new class of steroids inhibits angiogenesis in the presence of heparin or a heparin fragment. Science 230:1375-1378, 1985

27. Ingber D, Folkman J: Inhibition of angiogenesis through modulation of collagen metabolism. Lab Invest 59:44-51, 1988

28. Rastinejad F, Polverini PJ, Bouck NP: Regulation of the activity of a new inhibitor of angiogenesis by a cancer suppressor gene. Cell 56:345-355, 1989

29. Chen NT, Corey EJ, Folkman J: Potentiation of angiostatic steroids by a synthetic inhibitor of arylsulfatase. Lab Invest 59:453-459, 1988

30. Tamargo RJ, Leong KW, Brem H: Growth inhibition of the 9L glioma using polymers to release heparin and cortisone acetate. J Neurooncol 9:131-138, 1990

31. Moses MA, Sudhalter J, Langer R: Identification of an inhibitor of neovascularization from cartilage. Science 248:1408-1410, 1990

32. Brem SS, Zagzag D, Tsanaclis AMC, et al: Inhibition of angiogenesis and tumor growth in the brain. Suppression of endothelial cell turnover by penicillamine and the depletion of copper, an angiogenic cofactor. Am J Pathol 137:1121-1142, 1990

33. Oikawa T, Hirotani K, Ogasawara H, et al: Inhibition of angiogenesis by vitamin D_3 analogues. Eur J Pharmacol 178:247-250, 1990

34. Saiki I, Murata J, Nakajima M, et al: Inhibition by sulfated chitin derivatives of invasion through extracellular matrix and enzymatic degradation by metastatic melanoma cells. Cancer Res 50:3631-3637, 1990

35. Kerbel RS: Inhibition of tumor angiogenesis as a strategy to circumvent acquired resistance to anticancer therapeutic agents. Bio Essays 13:31-36, 1991

36. Tamargo RJ, Bok RA, Brem H: Angiogenesis inhibition by minocycline. Cancer Res 51:672-675, 1991

37. Nakamura S, Sakurada S, Zaki S, et al: Inhibition of development of Kaposi's sarcoma-related lesions by a bacterial cell wall complex. Science 255:1437-1440, 1992

Discussion

D. Tapper (Seattle, WA): When Dr Folkman first proposed in 1971 a concept that angiogenesis or neovascularization was a critical element for the control point in tumor growth, it was immediately apparent that antiangiogenesis or the control of tumor growth by inhibiting neovascularization was his goal. Over the past 20 years, Dr Folkman and individuals in his laboratory have worked to provide early diagnosis for malignancy, identified the basic elements or factors associated with angiogenesis, and developed substances to treat malignancy which are antiangiogenic. I think you have heard a scholarly dissertation on the work by Dr Brem and his associates on these compounds. Fumagilin, which was brought up at last year's meeting and which Dr Brem mentioned today, appeared to be too toxic so with the work of pharmaceutical companies they developed these synthetic anologues which I believe Dr Brem has shown inhibit primary tumor growth, inhibit metastases, are not dependent on the immune system, and are not sex related. I would like to ask Dr Brem several questions. Do the tumors retain their tumorogenicity? That is, if you stop AGM-1470, will the tumors rapidly grow? Could you comment on the long-term use of this compound in animal tumor models? Have you enlisted patients for either a clinical toxicity study or a phase II therapeutic study? Do you know why the vascular endothelium does not become resistant to the drug? Finally, is there any chemical relationship between this angiogenic inhibitor and the others your group presented to this group in the past, specifically angiostatic steroids and cartilagen inhibitors?

M. Langham (Gainesville, FL): I also enjoyed the paper and would ask that the authors share their thoughts with us on the effects of the drug not necessarily in maintenance of weight but on growth in children, since presumably growth is going to be dependent on angiogenesis. Although there are no sex-related differences in the effectiveness of treating the tumor, there may be toxicity differences. Clearly one of the primary examples of angiogenesis is the menstrual cycle. Will the use of angioinhibitors in women affect fertility?

H. Brem (response): Thank you for the thoughtful questions. First, these tumors definitely retain their tumorogenicity. In experiments where we treat 10 consecutive passages of carcinoma, which amounted to 225 consecutive days of AGM-1470, then transplant the tumors and stop therapy, the tumors will grow. We think AGM-1470 is targeting the endothelium, so when the tumor cells are transplanted, they retain their ability to stimulate endothelial cell growth. When we are no longer inhibiting the endothelium, the tumors can then grow. We do think therapy will be long term and although we have not done formal studies at stopping and starting drugs, we do think that once the drug is stopped in the presence of tumor, the tumor will then grow. In answer to the question of why the vascular endothelium cells are nonresistant, one clue might come from work by Dr Gaudison's laboratory which has shown that endothelial cells, with the exception of endothelial cells in the testes, the brain, and portions of the skin do not make the multiple drug resistence gene. Clearly, there are other mechanisms to explain how a cell can become resistant. We have formal studies underway to determine if any resistance will develop. However, in preliminary studies in a year of treatment in some of these tumor models, no resistance has been seen.

Currently, the FDA is studying this compound and we are optimistic that in the near future clinical trials will begin at the National Cancer Institute. In response to the question of how AGM-1470 compares to other antiangiogenic agents discovered by Dr Folkman, it is structurely distinct and is neither a steroid nor a protein. It may be possible in the future to combine antiangiogenic agents for better efficiency. The question was asked about this drug's effect on menstruation. I would like to extend that question to how does it affect other physiological processes that are angiogenic dependent. The answer is it does affect certain physiological processes like wound healing. Interestingly, in studies that we have done, it only suppresses wound healing during a small window of time, for example when given in the first 4 days after the wound is made. If it is given for up to 100 days prior to wound formation or only 5 days after the wound is made, wound healing is not affected. It is possible that in menstruating women or pregnant women that these processes would be interferred with by this potent anti-angiogenesis inhibitor. In response to the question of "is growth affected," in preliminary studies of weaning mice (day 7 to 21) AGM-1470 did not significantly affect weight gain. Furthermore, it may have other applications in nonneoplastic, pathological angiogenic processes, for example, rheumatoid arthritis or diagetic retinopathy.