Targeted Antiangiogenic Therapy for Cancer Using Vitaxin: A Humanized Monoclonal Antibody to the Integrin $\alpha_v\beta_3$[1]

John C. Gutheil,[2] Thomas N. Campbell, Paul R. Pierce, Jeffry D. Watkins, William D. Huse, David J. Bodkin, and David A. Cheresh

Department of Clinical Oncology Research, Sidney Kimmel Cancer Center, San Diego, California 92121 [J. C. G., T. N. C., D. J. B.]; MedImmune, Inc., Gaithersburg, Maryland 20878 [P. R. P.]; Applied Molecular Evolution, Incorporated, San Diego, California 92121 [J. D. W., W. D. H.]; and Departments of Immunology and Vascular Biology, The Scripps Research Institute, La Jolla, California 92037 [D. A. C.]

ABSTRACT

Angiogenesis plays a central role in the growth and metastasis of cancers. Strategies aimed at interfering with tumor blood supply offer promise for new cancer therapies. Vitaxin (an anti-$\alpha_v\beta_3$ antibody) interferes with blood vessel formation by inducing apoptosis in newly generated endothelial cells. This Phase I study evaluates the safety and pharmacokinetics of Vitaxin in humans with cancer. Eligible patients demonstrated progressive tumors with stage IV disease and an Eastern Cooperative Oncology Group performance status ≤2. Treatment consisted of six weekly infusions of Vitaxin. Escalating doses from 0.1 and 4.0 mg/kg/week were evaluated based on the expectation that plasma levels would bracket the effective *in vitro* concentration. Escalation beyond 4 mg/kg/week was limited by drug availability. Adverse events were assessed weekly. Pharmacokinetics were performed weekly through week 9. Clinical response was assessed at week 9.

Of 17 patients treated, 14 were evaluable for response. Treatment was well tolerated with little or no toxicity. The most common side effect was infusion-related fever, which could be controlled with prophylactic antipyretics. Doses ≥1 mg/kg/week produced plasma concentrations sufficient to saturate the $\alpha_v\beta_3$ receptor *in vitro* (25 μg/ml). Vitaxin demonstrated a half-life in excess of 5 days at higher doses with no accumulation over 6 weeks of therapy. One patient demonstrated a partial response, and seven patients demonstrated stable disease. Three patients received Vitaxin beyond the first cycle of therapy. Each of these patients demonstrated disease stabilization that in one case lasted 22 months.

At the doses and schedule studied, Vitaxin appears safe and potentially active, suggesting that vascular integrin $\alpha_v\beta_3$ represents a clinically relevant antiangiogenic target for prolonged cancer therapy.

INTRODUCTION

Many disease states (cancer, psoriasis, rheumatoid arthritis, and diabetic retinopathy) are mediated, in part, by a pathological angiogenic response (1–4). In cancer, the progressive growth of solid tumors is strictly dependent on their ability to stimulate formation of new blood vessels to supply tumor cells with oxygen and essential nutrients. Under normal physiological conditions, the formation of new blood vessels is tightly regulated and most tumors persist in a relatively benign or dormant state (5). However, with neovascularization comes tumor growth and the ability to shed tumor cells into the circulation (2, 6). This can lead to metastases.

Inhibition of angiogenesis was first proposed as an anticancer strategy by Folkman (7) in 1971. Support for this comes from the observation that microvessel density can serve as a prognostic indicator in early-stage breast carcinoma (8). A similar correlation between microvessel density and prognosis has been made for patients with colon carcinoma, prostate carcinoma, and melanoma (8).

In view of this, antiangiogenic therapy appears an attractive and rational approach for the treatment of solid tumors. To date, a number angiogenic inhibitors have been identified (8), some showing promising antitumor effects (4, 9, 10). However, their molecular targets remain unclear (11), and many have been associated with undesirable side effects.

Another approach to antiangiogenic therapy is to inhibit the adhesive interactions required by angiogenesis vascular endothelial cells. The migration of endothelial cells is dependent on their adhesion to extra cellular matrix proteins, such as vitronectin, through a variety of cell adhesion receptors known as integrins. Recent evidence indicates that integrin $\alpha_v\beta_3$ plays a role in this process (12). Studies have shown the enhanced expression of $\alpha_v\beta_3$ on newly developing blood vessels in human wound tissue, tumors, diabetic retinopathy, macular degeneration, and rheumatoid arthritis. However, $\alpha_v\beta_3$ is not generally found on blood vessels in normal tissues. In fact, in various animal models, antagonists of $\alpha_v\beta_3$, such as the $\alpha_v\beta_3$ specific antibody, LM609, have been shown to decrease angiogenesis and induce tumor regression (3) or improve arthritic disease (13), and this was associated with the induction of apoptosis within the angiogenic blood vessels (4, 13).

Vitaxin (Applied Molecular Evolution, San Diego, CA) is a humanized version of the LM609 monoclonal antibody that functionally blocks the $\alpha_v\beta_3$ integrin. This antibody has been shown to target angiogenic blood vessels (11) and cause sup-

Received 12/23/99; revised 5/2/00; accepted 5/2/00.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported by Applied Molecular Evolution, Inc. (San Diego, CA).
[2] To whom requests for reprints should be addressed, at Clinical Research and Development, Vical Inc., 9373 Towne Centre Drive, Suite 100, San Diego, CA 92121-3088; Phone: (858) 646-1178; E-mail: Jgutheil@vical.com.

Table 1 Patient demographics, treatment, and clinical outcome

Patient no.	Age	Sex	Diagnosis	PS	Vitaxin dose (mg/kg/wk)	Prior therapy	Response
1	44	M	Sarcoma	1	0.1	CHT[a]	PR
2	76	M	Colon	2	0.1	CHT	PD
3	52	M	NSCLC	1	0.1	CHT	PD
4	77	F	Ovarian	1	0.5	CHT	SD
5	50	M	Kidney	1	0.5	CHT, BIO	SD
6	58	F	Breast	1	0.5	CHT, RT	PD
7	59	F	Breast	2	1.0	CHT, RT	PD
8	66	M	Colon	0	1.0	CHT	SD
9	71	F	Colon	1	1.0	CHT	[b]
10	49	F	Cloacogenic	0	1.0	CHT, RT	PD
11	32	F	Cervix	1	2.0	CHT, RT	SD
12	64	F	Sarcoma	1	2.0	CHT, RT	SD
13	71	F	Colon	1	2.0	CHT	SD
14	82	M	Kidney	1	4.0	RT	PD
15	75	M	Kidney	2	4.0	CHT, RT, BIO	[b]
16	65	M	NSCLC	0	4.0	CHT, RT	[b]
17	42	M	Parotid	0	4.0	CHT, RT	SD

[a] CHT, chemotherapy; BIO, biologic therapy; RT, radiation therapy; PR, partial response; SD, stable disease; PD, progressive disease; NSCLC, non-small cell lung cancer.

pression of tumor growth in various animal models (9). Based on the selectivity of $\alpha_v\beta_3$ as a marker of angiogenic blood vessels and the effects of anti-$\alpha_v\beta_3$ in reversing disease in animal models, clinical trials were initiated to evaluate the safety and pharmacokinetics of Vitaxin in late stage cancer patients. Tumor response was assessed as a secondary end point.

Table 2 Vitaxin dose escalation schema

Dose (mg/kg/wk)	No. patients
0.1	3
0.5	3
1.0	4
2.0	3
4.0	4

PATIENTS AND METHODS

Eligibility. Adult patients (aged ≥18 years) with measurable late stage cancer were eligible for this study. Patients were required to have histologically proven, advanced (stage IV), incurable malignancies refractory to standard therapy. Pathology was determined by the treating oncologist and verified by central review. In an effort to evaluate Vitaxin therapy in a variety of different tumor types, study entry of patients with either breast, lung, or colon cancer was limited to three evaluable patients each. Inclusion criteria required patients to have a good PS[3] (Eastern Cooperative Oncology Group PS of ≤2) and adequate bone marrow, renal, cardiac, thyroid, and hepatic function. Additional entry criteria included a life expectancy of ≥3 months, a hemoglobin ≥8.0 mg/dl, a WBC count ≥2500/mm^3, an absolute granulocyte count ≥1000/mm^3, a platelet count ≥75,000/mm^3, a serum creatinine less than or equal to two times the upper limits of normal, a normal serum thyrotropin, an aspartate aminotransferase less than or equal to three times the upper limits of normal, a bilirubin less than or equal to two times the upper limits of normal, and a partial thromboplastin time within normal limits.

Patients were excluded if they had known brain tumors, active opportunistic infection, serious nonmalignant disease, or were HIV-positive. Given that the effects of Vitaxin on embryogenesis are unknown, pregnant women were excluded. All patients of reproductive potential were required to practice birth control during the course of the study. Patients who underwent any major surgery, chemotherapy, or radiotherapy within 4 weeks of study entrance were also excluded. Concomitant treatment with other investigational drugs was not permitted.

This study was approved by the Institutional Review Board of the Sharp/Sidney Kimmel Cancer Center. All patients gave informed consent before participation (Table 1).

Study Design. The study was an open label, single center, dose escalating Phase I trial with sequential cohorts receiving increasing doses of Vitaxin (Table 2). Dose escalation followed standard Phase I criteria (<33% incidence of dose-limiting toxicity was required in prior cohorts before opening the next cohort for patient accrual).

Treatment. All patients received 6 weekly doses of Vitaxin at increasing doses (0.1–4 mg/kg). Given that dose limiting toxicity was not expected with Vitaxin, the doses chosen were anticipated to bracket the *in vitro* concentration of Vitaxin required to saturate the $\alpha_v\beta_3$ receptor and to block endothelial cell migration. No intrapatient dose escalation was allowed. Vitaxin was administered i.v. through a peripheral line as a 90-min infusion. Premedication was not given initially; however, with the appearance of fever in a subset of patients shortly after treatment, oral premedication with acetaminophen (650 mg) and diphenhydramine (50 mg) was given before each infusion. Tumor restaging was performed at week 9. Those

[3] The abbreviations used are: PS, performance status; VnR, Vitronectin receptor; AUC, area under the concentration curve.

patients demonstrating at least stable disease were considered for additional cycles of treatment (weekly for 6 weeks) repeated every 10 weeks. Tumor biopsies were performed before and after therapy to assess integrin $\alpha_v\beta_3$ expression and antibody binding.

Toxicity Assessments. Patients were evaluated weekly during treatment with a physical exam and blood work. Parameters followed throughout the study included serum chemistries, thyroid function, coagulation tests, and anti-Vitaxin antibodies. All toxicities were graded according to the National Cancer Institute Common Toxicity Grading System. Patients were assessed at week 12 for delayed toxicity.

Anti-Vitaxin Antibody Response. Samples were taken for determination of human antimouse antibody titers at baseline, and weeks 3, 6, and 9 after the start of treatment with Vitaxin. Patient samples were run in comparison to two murine antihuman antibodies. The first was a mouse antihuman κ. The second control was pooled sera from two mice inoculated with Vitaxin. Assays were run in plates coated with 200, 400, or 800 ng/ml of Vitaxin. Replicates were run in triplicate at 1:20, 1:40, 1:80, and 1:160 dilutions against each coating concentration.

Pharmacokinetics. Plasma Vitaxin levels were measured before and at 0.08, 0.5, 1, 4, 8, 24, 48, and 72 h after completion of each Vitaxin infusion. In addition, plasma Vitaxin levels were determined on day 1 of weeks 7, 8, and 9. Patient samples were prepared for each time point and frozen until the time of analysis.

Vitaxin levels were determined using an ELISA assay. This assay uses the VnR, specifically $\alpha_v\beta_3$, as the plate-bound ligand of Vitaxin. The VnR was purified from human placentas. Dynatech Immulon 2 U-bottomed microtiter plates were coated with 1.0 μg/ml VnR in Coating Buffer, sealed, and stored at 0°C-5°C from 1–21 days. After blocking and washing, the plates were incubated with freshly thawed standards and controls, plus appropriate dilutions of patient serum in blocking buffer with 5% human serum. After a 1-h incubation and washing, the plates were incubated for another hour with goat antihuman κ light chain conjugated with horseradish peroxidase. The washed plates were then loaded with ortho-phenylenediamine dihydrochloride substrate in Citrate buffer for 20 min and stopped with sulfuric acid. Absorbance was read at 490 nm and evaluated using SOFTmax PRO software. Standard curves were required to have a correlation coefficient >0.985, and the individual concentrations of standards were required to fall within 20% of their expected values. The absorbance values of the three highest concentrations of sample dilutions that fell within the range of the standards were selected in an Excel database, converted to concentrations, and averaged.

Clinical Response Evaluation. Evaluation of tumor burden was performed by comprehensive scans (computed tomography, magnetic resonance imaging, X-rays) and physical examination at baseline (study entry) and 3 weeks after completion of therapy (week 9). Tumor responses were graded according to standard procedures based on at least two indicator lesions. A partial response was defined as less than a complete response but a $>50\%$ decrease in the sum of the products of the cross sectional tumor measurements of selected indicator lesions. Stable disease was defined as a $<50\%$ decrease and a $<25\%$ increase in the sum of the products of cross-sectional tumor measurements.

Statistical Considerations. A patient was considered evaluable for response after receiving six weekly doses of Vitaxin. Patients were considered evaluable for toxicity after having received one dose of Vitaxin.

RESULTS

Patient Characteristics. Seventeen adult patients (aged ≥18 years) were enrolled in this Phase I study (February 1997 to February 1998). The characteristics of these patients at study entry are presented in Table 1. The mean age was 61 years (range, 32–82) with eight females and nine males. Distribution of PS was PS 0–24% (4 of 17), PS 1–59% (10 of 17), and PS 2–18% (3 of 17). Pathological diagnoses included a range of tumor types, all with stage IV disease. All patients had received prior therapy for their cancer.

Vitaxin Toxicity. Overall, Vitaxin therapy was well tolerated. No significant toxicity was observed at any of the dose levels tested, and no patient was required to stop treatment or have therapy delayed because of an adverse event. Altogether, 88% (15 of 17) of patients experienced one or more adverse events during the course of the trial, the majority of which were grade 1 (68%) or 2 (32%). No toxicities greater than grade 2 were documented. The most frequent adverse events reported included commonly seen antibody infusion reactions: fever, chills, nausea, and flushing. Premedication was not given initially; however, several patients demonstrated a fever lasting up to 2 h after treatment. Oral premedication with acetaminophen and diphenhydramine before each Vitaxin infusion appeared to prevent fever in subsequent patients. Antibody infusion reactions decreased in incidence after the first infusion. No clinically significant cardiac, renal, hepatic, or hematological toxicity was observed.

Tumor Biopsy. Most patients underwent a tumor biopsy during week 9. No appreciable increase in bleeding or inhibition of wound healing was observed with these biopsies. One patient with a leiomyosarcoma (patient 1) underwent a scalp lesion biopsy during week 3 of treatment. This biopsy site did not achieve complete hemostasis until a week later. The same patient failed to demonstrate increased bleeding at the time of complete scalp lesion resection (week 6), or at the time of a tumor biopsy of a separate lesion (week 5).

Immunogenicity of Vitaxin. Vitaxin antibodies were not detectable in any patient at week 9. Thus, humanized Vitaxin does not appear to be immunogenic in any of the patients treated to date.

Pharmacokinetics. Vitaxin demonstrated a dose-dependent elimination half-life comparable with the elimination half-life of other humanized antibodies in man (Table 3; Ref. 14). The 14.6-h half-life at the lowest dose (0.1 mg/kg/week) was 4- to 9-fold lower than the 63–138-h half-life seen at higher doses (2–4 mg/kg/week). The volume of distribution for Vitaxin is consistent with distribution throughout the plasma volume (0.05–0.1 liters/kg). The AUC for Vitaxin increased with increasing doses in a manner that was not proportional to dose ranging from an AUC/dose of 412.3 μg·h/ml at 0.1 mg/kg/week to 2.1 mg·h/ml at 4 mg/kg/week.

Table 3 Vitaxin pharmacokinetics characteristics

Dose (mg/kg/wk)	C_{max} [a] (μg/ml)	*AUC168* (μg·h/ml)	V_z (liters/kg)	$T_{1/2}$ (h)	C_{max}/Dose (μg/ml)/(mg/kg)	*AUC168*/Dose (μg·h/ml)/(mg/kg)
0.1 (n = 3)						
Mean	2.0	41.2	0.052	14.63	19.6	412.3
SD	0.6	4.1	0.021	4.44	5.6	40.9
0.5 (n = 3)						
Mean	19.0	939.1	0.049	63.57	38.0	1878.3
SD	6.8	393.6	0.007	28.91	13.5	787.2
1.0 (n = 3)						
Mean	28.1	1400.9	0.042	86.89	281.4	1400.9
SD	6.1	579.2	0.039	8.74	6.1	579.2
2.0 (n = 3)						
Mean	46.9	3226.5	0.101	114.81	23.4	1613.2
SD	11.9	721.9	0.007	13.18	6.0	360.9
4.0 (n = 2)						
Mean	120.2	8209.0	0.097	138.45	30.0	2052.3
SD	31.9			1.43	8.0	

[a] C_{max}, maximum concentration detected; *AUC168*, area under the concentration curve from time = 0 to 168 h; V_z, volume of distribution, $T_{1/2}$, half life.

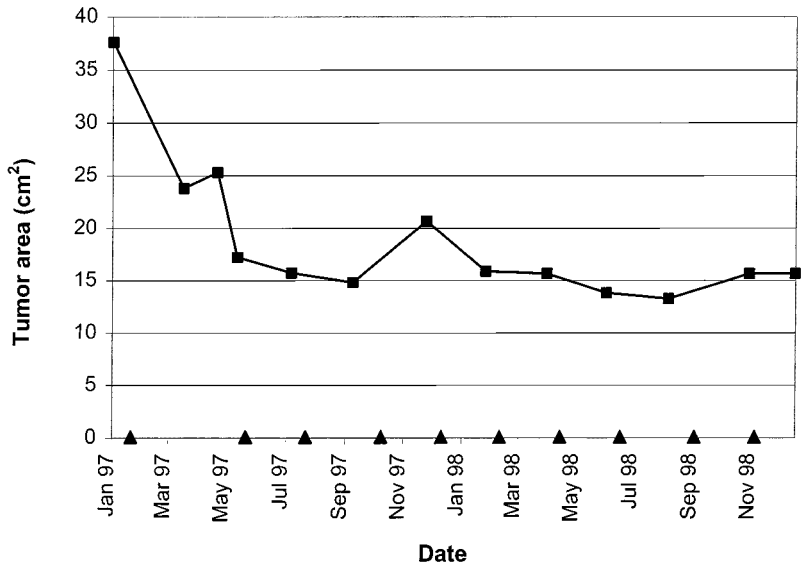

Fig. 1 Time course of indicator tumor measurements for patient 1 with a leiomyosarcoma. Sum of two measurable tumors. ▲, start date of each 6-week cycle of Vitaxin.

Clinical Outcome. After the first tumor evaluation at week 9, seven patients were shown to have at least stable disease in their indicator lesions. One patient (patient 1) with a widely disseminated leiomyosarcoma achieved a partial response (45% of baseline) based on assessment of his measurable disease (Fig. 1). This same patient had a tumor nodule removed surgically from his scalp during week 5, and had nonmeasurable metastatic disease to the liver that was judged to be stable.

Additional Cycles of Treatment. Three patients received treatment beyond the initial 6-week cycle of Vitaxin. Patient 1, with a leiomyosarcoma metastatic to the liver, initially demonstrated edema and reddening at the site of a cutaneous lesion on his scalp during week 3. This lesion (~1 × 1 cm in size) was removed and repaired with a small skin graft (~3 × 3 cm) from the patient's thigh. The patient's remaining measurable disease decreased in volume to 45% of baseline before his second cycle of treatment initiated on week 17. At week 29, a small cutaneous lesion appeared at the site of the prior scalp lesion. This lesion was limited to the scar surrounding the prior skin graft and was treated with local irradiation therapy resulting in complete resolution. Treatment with Vitaxin continued until week 93 based on the impression that Vitaxin was controlling a subset of the patient's metastatic disease. At week 55, his dose of Vitaxin was increased 3-fold (from 0.1 to 0.3 mg/kg/week). This increase in dose did not effect a further decrease in tumor size or a decrease in the growth rate of the patient's nonhepatic lesions. At week 93, his measurable lesions continued to remain stable, but progression was documented outside of the liver, and gastrointestinal bleeding felt secondary to progressive gastrointestinal disease persisted. Treatment with Vitaxin was discontinued at week 93.

Patient 6 with a diagnosis of metastatic breast cancer demonstrated stable disease with the first cycle of treatment and went on to receive three additional cycles of therapy. Although

the tumor growth rate during treatment with Vitaxin appeared to be less than that expected (as extrapolated form her prior tumor measurements), each tumor evaluation demonstrated slight tumor growth that eventually exceeded 25% of her baseline tumor measurements.

Patient 17, who was diagnosed with a parotid adenocarcinoma metastatic to the lung, initially demonstrated continued progression after his first cycle of treatment with Vitaxin. However, within 6 weeks after treatment, in the absence of further therapy, the patient's tumor demonstrated a modest decrease in volume. This suggested that Vitaxin was inducing a late response in the patient's tumor. The patient subsequently received a 24-week course of Vitaxin without evidence of tumor regression.

No significant adverse events were noted in any of the three patients undergoing continued treatment with Vitaxin.

DISCUSSION

This study reports the use of targeted antiangiogenic therapy for cancer in patients with late stage cancer. At the doses used, Vitaxin can be safely administered over prolonged periods without toxicity. However, further dose escalation was limited by drug supply and as such, no statement can be made about the toxicity of Vitaxin at doses >4 mg/kg/week. Nonetheless, although this study did not determine the optimum dose or schedule for Vitaxin, the plasma concentration achieved at doses of ≥ 1 mg/kg/week was in excess of the concentration required for saturation of the $\alpha_\nu\beta_3$ receptors *in vitro* (25 μg/ml; Ref. 15).

Of 14 evaluable patients, 8 either demonstrated disease stabilization or a partial response. Furthermore, in one patient, treatment resulted in a partial tumor response that was maintained for 22 months as determined by measurements in the indicator lesions. In a second patient, slight tumor shrinkage was noted only after the first cycle of therapy was completed. These findings support the notion that targeting of the vascular $\alpha_\nu\beta_3$ integrin may provide clinical benefit to patients with various tumor types without causing significant side effects.

Because of the lack of toxicity, we were unable to determine a traditional maximally tolerated dose of Vitaxin when administered weekly for 6 weeks. Given that this study was not designed with adequate power to evaluate response as an end point, the single partial response seen at 0.1 mg/kg/week does not serve to define this as the optimal dose or schedule of Vitaxin. As with many biological therapies in clinical trials, subsequent studies with Vitaxin will require the establishment of a surrogate marker for clinically significant antiangiogenic activity or will need to evaluate other measures of patient benefit to define the optimum dose and schedule.

The pharmacokinetics of Vitaxin are similar to the pharmacokinetics reported for other humanized monoclonal antibodies (16). Vitaxin demonstrated a dose-dependent half-life that ranged from 14 h at the lowest dose evaluated to 138 h at the highest dose. Vitaxin appeared to demonstrate a trend toward nonlinearity, which has also been reported for other antibodies studied over a broad dose range. No accumulation of Vitaxin was seen over the 6 weeks of treatment. In addition, as with other humanized antibodies, Vitaxin failed to elicit a human-anti-human antibody response in patients.

Although significant promise exists for treatments that target tumor vasculature, issues remain that will require resolution before such therapy can be recommended for routine clinical use. One such issue is the effect of antiangiogenic therapy on wound healing and mucosal bleeding. During our study, one patient demonstrated bleeding after a tumor biopsy at week 3. This bleeding was judged to be consistent with biopsies of the scalp by the treating surgeon. Although this could be interpreted as representing an increased risk for bleeding with Vitaxin therapy, this same patient demonstrated no increased bleeding with a cutaneous tumor resection at week 6. Subsequent patients who underwent tumor biopsies both before and after treatment with Vitaxin demonstrated no increase in bleeding or an inhibition of wound repair. Although our preliminary experience suggests that treatment with Vitaxin does not increase the risk of bleeding, we cannot rule out an effect of Vitaxin on more significant wound healing, such as that associated with dental procedures and/or major trauma.

A second issue involves the ability of antiangiogenic therapies to effect a tumor response in all tumors present in a given patient. As was observed in patient 1, even at the time of discontinuation of therapy, several metastases remained stable, whereas other metastases progressed. Understanding the mechanism behind this observation may allow for the development of better therapies. One explanation of this observation is that differing sensitivity to antiangiogenic therapies exists between established as opposed to developing tumor vessels. An alternative explanation is that different metastases may elaborate different levels of angiogenic stimulation and therefore, may differ in their sensitivity to therapy (17).

Given these observations, antiangiogenic therapy may prove most useful in the treatment of patients with minimal residual disease or in patients with occult metastatic disease. Antiangiogenic therapy may also prove useful in combination with other classes of anticancer or antiangiogenic drugs that operate through distinct mechanisms. For these reasons, an ideal antiangiogenic therapy would possess minimal toxicity and the ability to be administered over a prolonged period of time. Vitaxin's lack of toxicity and ease of administration should allow for combination therapy with both chemotherapy and irradiation therapy. Development of Vitaxin appears justified with future trials evaluating the role of Vitaxin in longer treatment schedules and in combination with other anticancer agents.

REFERENCES

1. Friedlander, M., Theesfeld, C. L., Sugita, M., Fruttiger, M., Thomas, M. A., Chang, S., and Cheresh, D. A. Involvement of integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ in ocular neovascular diseases. Proc. Natl. Acad. Sci. USA, *93:* 9764–9769, 1996.

2. Folkman, J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat. Med., *1:* 27–31, 1995.

3. Brooks, P. C., Clark, R. A., and Cheresh, D. A. Requirement of vascular integrin $\alpha_v\beta_3$ for angiogenesis. Science (Washington DC), *264:* 569–571, 1994.

4. Brooks, P. C., Montgomery, A. M., Rosenfeld, M., Reisfeld, R. A., Hu, T., Klier, G., and Cheresh, D. A. Integrin $\alpha_v\beta_3$ antagonists promote

tumor regression by inducing apoptosis of angiogenic blood vessels. Cell, *79:* 1157–1164, 1994.

5. Dameron, K. M., Volpert, O. V., Tainsky, M. A., and Bouck, N. Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. Science (Washington DC), *265:* 1582–1584, 1994.

6. Boehm, T., Folkman, J., Browder, T., and O'Reilly, M. S. Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance (see comments). Nature (Lond.), *390:* 404–407, 1997.

7. Folkman, J. Tumor angiogenesis: therapeutic implications. N. Engl. J. Med., *285:* 1182–1186, 1971.

8. Weidner, N., Semple, J. P., Welch, W. R., and Folkman, J. Tumor angiogenesis and metastasis—correlation in invasive breast carcinoma. N. Engl. J. Med., *324:* 1–8, 1991.

9. Brooks, P. C., Stromblad, S., Klemke, R., Visscher, D., Sarkar, F. H., and Cheresh, D. A. Anti-integrin $\alpha_v\beta_3$ blocks human breast cancer growth and angiogenesis in human skin (see comments). J. Clin. Invest., *96:* 1815–1822, 1995.

10. O'Reilly, M. S., Boehm, T., Shing, Y., Fukai, N., Vasios, G., Lane, W. S., Flynn, E., Birkhead, J. R., Olsen, B. R., and Folkman, J. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell, *88:* 277–285, 1997.

11. Sipkins, D. A., Cheresh, D. A., Kazemi, M. R., Nevin, L. M., Bednarski, M. D., and Li, K. C. Detection of tumor angiogenesis *in vivo* by $\alpha_v\beta_3$-targeted magnetic resonance imaging. Nat. Med., *4:* 623–626, 1998.

12. Auerbach, W., and Auerbach, R. Angiogenesis inhibition: a review. Pharmacol. Ther., *63:* 265–311, 1994.

13. Storgard, C. M., Stupack, D. G., Jonczyk, A., Goodman, S. L., Fox, R. I., and Cheresh, D. A. Decreased angiogenesis and arthritic disease in rabbits treated with an $\alpha_v\beta_3$ antagonist (see comments). J. Clin. Invest., *103:* 47–54, 1999.

14. Trang, J. M., LoBuglio, A. F., Wheeler, R. H., Harvey, E. B., Sun, L., Ghrayeb, J., and Khazaeli, M. B. Pharmacokinetics of a mouse/human chimeric monoclonal antibody (C-17–1A) in metastatic adenocarcinoma patients. Pharm. Res. (NY), *7:* 587–592, 1990.

15. Leavesley, D. I., Schwartz, M. A., Rosenfeld, M., and Cheresh, D. A. Integrin β_1- and β_3-mediated endothelial cell migration is triggered through distinct signaling mechanisms. J. Cell Biol., *121:* 163–170, 1993.

16. LoBuglio, A. F., Wheeler, R. H., Trang, J., Haynes, A., Rogers, K., Harvey, E. B., Sun, L., Ghrayeb, J., and Khazaeli, M. B. Mouse/human chimeric monoclonal antibody in man: kinetics and immune response. Proc. Natl. Acad. Sci. USA, *86:* 4220–4224, 1989.

17. Relf, M., LeJeune, S., Scott, P. A., Fox, S., Smith, K., Leek, R., Moghaddam, A., Whitehouse, R., Bicknell, R., and Harris, A. L. Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor β-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. Cancer Res., *57:* 963–969, 1997.