Phase I Trial of a Monoclonal Antibody Specific for $\alpha_v\beta_3$ Integrin (MEDI-522) in Patients with Advanced Malignancies, Including an Assessment of Effect on Tumor Perfusion

Douglas G. McNeel,[1,2] Jens Eickhoff,[1,3] Fred T. Lee,[4] David M. King,[1,2] Dona Alberti,[1] James P. Thomas,[1,2] Andreas Friedl,[1,5] Jill Kolesar,[1,6] Rebecca Marnocha,[1,6] Jennifer Volkman,[1] Jianliang Zhang,[7] Luz Hammershaimb,[7] James A. Zwiebel,[8] and George Wilding[1,2]

Abstract At present, a variety of agents targeting tumor angiogenesis are under clinical investigation as new therapies for patients with cancer. Overexpression of the $\alpha_v\beta_3$ integrin on tumor vasculature has been associated with an aggressive phenotype of several solid tumor types. Murine models have shown that antibodies targeting the $\alpha_v\beta_3$ integrin can affect tumor vasculature and block tumor formation and metastasis. These findings suggest that antibodies directed at $\alpha_v\beta_3$ could be investigated in the treatment of human malignancies. The current phase I dose escalation study evaluated the safety of MEDI-522, a monoclonal antibody specific for the $\alpha_v\beta_3$ integrin, in patients with advanced malignancies. Twenty-five patients with a variety of metastatic solid tumors were treated with MEDI-522 on a weekly basis with doses ranging from 2 to 10 mg/kg/wk. Adverse events were assessed weekly; pharmacokinetic studies were done; and radiographic staging was done every 8 weeks. In addition, dynamic computed tomography imaging was done at baseline and at 8 weeks in patients with suitable target lesions amenable to analysis, to potentially identify the effect of MEDI-522 on tumor perfusion. Treatment was well tolerated, and a maximum tolerated dose was not identified by traditional dose-limiting toxicities. The major adverse events observed were grade 1 and 2 infusion-related reactions (fever, rigors, flushing, injection site reactions, and tachycardia), low-grade constitutional and gastrointestinal symptoms (fatigue, myalgias, and nausea), and asymptomatic hypophosphatemia. Dynamic computed tomography imaging suggested a possible effect on tumor perfusion with an increase in contrast mean transit time from baseline to the 8-week evaluation with increasing doses of MEDI-522. No complete or partial responses were observed. Three patients with metastatic renal cell cancer experienced prolonged stable disease (34 weeks, >1 and >2 years) on treatment. With this weekly schedule of administration, and in the doses studied, MEDI-522 seems to be without significant toxicity, may have effects on tumor perfusion, and may have clinical activity in renal cell cancer. These findings suggest the MEDI-522 could be further investigated as an antiangiogenic agent for the treatment of cancer.

The development of agents to target tumor vasculature rather than target tumor cells directly was first proposed as a possible means of treating cancers by Folkman in 1971 (1). Since that time, a number of agents have shown efficacy in preclinical models and have entered clinical trials, many with evidence of clinical benefit (2–7). The fruit of these labors has recently been realized by the Food and Drug Administration approval of bevacizumab, a monoclonal antibody (mAb) targeting the vascular endothelial growth factor, given its effect in combination with chemotherapy in prolonging survival in patients with metastatic colorectal cancer (8).

The $\alpha_v\beta_3$ integrin has been identified as a specific potential target of vasculature-directed antitumor therapies. Integrins are cell surface receptor molecules involved in extracellular matrix adhesion and cell-to-cell contact. Brooks et al. first showed that the $\alpha_v\beta_3$ integrin is overexpressed on new blood vessels, and that a mAb specific for $\alpha_v\beta_3$, LM609, could block angiogenesis in a murine model (9). They subsequently showed that treatment with this antibody could lead to regression of human tumors transplanted on a chick chorioallantoic membrane and induce apoptosis of the proliferating angiogenic vascular cells (10). Others showed that antibody blockade of $\alpha_v\beta_3$ blocked tumor formation and metastasis in a nude mouse model of

Authors' Affiliations: [1]University of Wisconsin Comprehensive Cancer Center; Departments of [2]Medicine, [3]Biostatistics, [4]Radiology, and [5]Pathology; [6]School of Pharmacy, University of Wisconsin, Madison, Wisconsin; [7]MedImmune Oncology, Inc., Gaithersburg, Maryland; and [8]Investigational Drug Branch, Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, Maryland

Received 2/3/05; revised 6/21/05; accepted 8/2/05.

Grant support: National Cancer Institute grants U01 CA62491 and N02-CO-124001 (22XSO82A) and National Center for Research Resources grant M01 RR03186.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Douglas G. McNeel, Department of Medicine/Medical Oncology, University of Wisconsin Comprehensive Cancer Center, K4/518 Clinical Science Center, 600 Highland Avenue, Madison, WI 53792. Phone: 608-265-8131; Fax: 608-265-8133; E-mail: dm3@medicine.wisc.edu.

doi: 10.1158/1078-0432.CCR-05-0262

human melanoma (11). Gasparini et al. have further shown that up-regulation of $\alpha_v\beta_3$ in tumor-associated vasculature of certain solid tumor tissues, notably breast cancer tissue (12), is associated with more aggressive disease.

The identification of $\alpha_v\beta_3$ as a marker of aggressive tumors, and the preclinical studies above, suggested that a mAb directed against $\alpha_v\beta_3$ could be investigated as an anticancer agent in human clinical trials. A humanized mAb, MEDI-523 (Vitaxin, Applied Molecular Evolution, San Diego, CA), derived from the LM609 murine mAb was the first such agent developed and evaluated in clinical trials. Gutheil et al. studied MEDI-523 in a phase I trial of patients with a variety of chemotherapy refractory tumors (13). The agent was given as a weekly i.v. injection with doses ranging from 0.1 to 4.0 mg/kg. The most common adverse events seen were infusion-related fever, chills, myalgias, nausea, and headaches. One patient with metastatic leiomyosarcoma showed a partial response with disease stabilization lasting 22 months. Two other phase I studies were also done at the University of Alabama and M.D. Anderson Cancer Center (14, 15). Although shown safe in these studies, no objective disease responses were seen.

MEDI-522 represents a second-generation humanized anti-$\alpha_v\beta_3$ mAb also derived from the LM609 murine antibody. The MEDI-522 antibody has been engineered with greater manufacturing stability and 7.2-fold greater affinity for the $\alpha_v\beta_3$ integrin than its predecessor MEDI-523 (16). It was hypothesized that the absence of significant clinical responses with MEDI-523 relative to preclinical studies might have been due to affinity and stability issues *in vivo* that could be overcome with the MEDI-522 antibody due to better tumor targeting and antibody retention. Preclinical studies have provided evidence that MEDI-522 may have significant activity *in vitro* and *in vivo* at clinically achievable concentrations. For example, the adhesion of M21 melanoma cells to fibrinogen following exposure to MEDI-522 was found to be inhibited in a dose-dependent fashion with maximal inhibition observed at a MEDI-522 concentration of ~50 ng/mL (16). The *in vitro* ability of MEDI-522 to inhibit endothelial cell migration was further assessed using an assay containing human umbilical endothelial cells exposed to human fibrinogen and the chemoattractant vitronectin. Maximal inhibition (80%) was observed at MEDI-522 concentrations of 1 to 10 μg/mL and did not increase further at concentrations as high as 100 μg/mL.[9] The *in vivo* antitumor activity of MEDI-522 was also investigated using the chorioallantoic membrane model described above. In this system, CS1 hamster melanoma tumor fragments were placed on the chorioallantoic membranes, following which various doses of MEDI-522 (5, 15, and 50 μg) were injected into the chorioallantoic membrane vessels. A dose-dependent reduction in tumor weight was noted with maximal inhibition at 50 μg/chorioallantoic membrane, equivalent to a MEDI-522 concentration of ~25 μg/mL.[9] Thus, preclinical data suggested that a continuous serum concentration at a minimum of 10 to 30 μg/mL of MEDI-522 was associated with *in vitro* and *in vivo* activity.

[9] Unpublished data.

MEDI-522 is currently being evaluated in clinical trials for cancer, such as metastatic melanoma and androgen-independent prostate cancer. The current study was conducted as an open-label phase I dose escalation trial to evaluate the safety and tolerability of MEDI-522 in patients with treatment-refractory solid tumors. Secondary end points were to determine pharmacokinetic variables for MEDI-522, determine if MEDI-522 has an antiangiogenic effect as measured by dynamic computed tomography imaging, and to define a recommended phase II dose based on either the maximum tolerated dose or optimal biological response dose.

Materials and Methods

Study agent and regulatory information. MEDI-522 was provided by the National Cancer Institute Cancer Therapy Evaluation Program through a Cooperative Research and Development Agreement with MedImmune Oncology, Inc. (Gaithersburg, MD). The study protocol was reviewed and approved by the Institutional Review Board of the University of Wisconsin, and all patients gave written informed consent for participation.

Patient population. Adult patients (ages >18 years) with incurable lymphomas or other solid tumors that were refractory to standard therapies were considered eligible for this study. Subjects with tumors amenable to multiple core biopsies were preferred; however, the donation of skin and/or tumor biopsies was elective and not a requirement for trial participation. Inclusion criteria required that patients have an Eastern Cooperative Oncology Group performance score of ≤2; a life expectancy of ≥12 weeks; and normal bone marrow, liver, and renal function as defined by a WBC of ≥3,000/μL, ANC of ≥1,500/μL, and platelet count of ≥100,000/μL; total bilirubin within normal institutional limits; aspartate aminotransferase and alanine aminotransferase ≤2.5 times the upper limit of normal institutional limits; and creatinine within normal institutional limits (or creatinine clearance of ≥60 mL/min). Patients were also required to have a thyroid-stimulating hormone level or thyroid hormone level (T_4) within normal institutional limits. Patients were excluded if they had been treated with chemotherapy or radiotherapy within 4 weeks of study entry, were being treated with any other investigational agents, or had a history of known brain metastases. Patients were also excluded if they had a history of allergic reactions to other mAb therapies or had other serious intercurrent illness, including HIV, which could limit study participation. Given the uncertain effects on embryogenesis, pregnant women were excluded, and patients of reproductive age were requested to practice birth control while on treatment. Finally, given the potential effects of MEDI-522 on neovasculature, patients with a history of any bleeding disorder or major surgical procedure within 4 weeks of treatment were excluded.

Study design. The study was an open-label, single-institution, phase I trial using a dose escalation schedule with sequential cohorts receiving increasing doses of MEDI-522 (Table 1). Given the previous phase I experience with MEDI-523 showing safety at high doses and to gather more biological response data, six subjects were accrued per dose level. Escalation to the next dose level was permitted if less than two dose-limiting toxicities were observed at the current dose level. A dose-limiting toxicities was defined as any adverse event grade ≥3 during the first month of treatment and given an attribution of at least possibly related to agent, or any adverse event resulting in delay of administration for >2 weeks. The maximum tolerated dose was defined as the dose level preceding a level at which more than one dose-limiting toxicity was observed.

Study procedures. Patients were treated weekly with MEDI-522 at doses ranging from 2 to 10 mg/kg administered i.v., as shown in Table 1. There were no intrapatient dose escalations. All patients received oral premedication with 50 mg diphenhydramine and 500 to

Table 1. Dose level assignment

Dose level	MEDI-522 (mg/kg)	No. patients
1	2	4
2	4	0
3	6	7
4	8	6
5	10	8

650 mg acetaminophen for 30 minutes before the first MEDI-522 administration. Patients receiving doses of ≥6 mg/kg of MEDI-522 received this premedication for the first two weekly doses. Weekly treatment was continued until one of the following occurred: (*a*) disease progression, (*b*) intercurrent illness preventing further administration, (*c*) unacceptable adverse events, (*d*) patient decision to withdraw from study, or (*e*) physician discretion. All patients completing at least 4 weeks of treatment (i.e., one cycle) were considered evaluable for response. Patients not completing 4 weeks of treatment (one cycle) were replaced. In patients who consented to undergo skin or tumor biopsies for additional drug-targeting correlative studies, random skin biopsies (or tumor biopsies) were obtained 1 to 2 weeks before the first treatment and 3 to 4 weeks after the first treatment.

Adverse event monitoring. All patients were evaluated weekly during treatment for symptomatic evidence of adverse events. Weekly blood tests included complete blood counts, serum electrolytes, calcium, phosphorus, creatinine, and liver function tests. In addition, the international normalized ratio and partial thromboplastin time were monitored weekly during the first cycle; urinalysis was done monthly; and thyroid function testing was done before study and at study conclusion. Patients were evaluated by a physician at least monthly. All toxicities were graded according to the National Cancer Institute Common Toxicity Criteria Grading System, version 2 and assigned an attribution of unrelated, unlikely, possible, probable, or definite in relation to treatment with MEDI-522.

Clinical response evaluation. Clinical response was not a primary end point of this study. However, all patients with measurable disease were assessed with computed tomography or magnetic resonance imaging scans obtained within 4 weeks before the first dose of MEDI-522 and at 8-week intervals after beginning treatment. Disease response and progression were determined using standard Response Evaluation Criteria in Solid Tumors (17).

Pharmacokinetics. Serum was collected for pharmacokinetic monitoring during the first and second cycles of treatment. Pharmacokinetic variables of MEDI-522 were estimated using noncompartmental methods. During cycle 1, samples were collected days 1 to 4 and day 6 of weeks 1 and 4 at the following time points: preinfusion; 5, 15, and 30 minutes after infusion; and 1, 2, 4, 24, 48, 72, 120, and 168 hours after infusion. Preinfusion samples were collected at weeks 2 and 3. During cycle 2, samples were collected preinfusion at weeks 5, 6, 7, and 8. Plasma MEDI-522 concentrations were determined by a vitronectin receptor binding ELISA at MedImmune Oncology, similar to the methodology as previously described (13). The limit of detection of this assay was 5 ng/mL. Similarly, detection of anti-MEDI-522 antibodies in patient sera was determined at these same time points by ELISA at MedImmune Oncology. The limit of detection for this assay was 6 ng/mL human anti-MEDI-522 antibodies.

Dynamic computed tomography imaging. Dynamic functional computed tomography was used as a method of assessing tumor blood flow in response to MEDI-522 treatment (18, 19). All patients with measurable disease underwent dynamic computed tomography imaging at baseline before treatment and after 8 weeks of treatment. All computed tomography examinations were done with a GE Lightspeed multirow helical scanner (GE Medical Systems, Waukesha, WI). Each examination was done in two parts. First, noncontrast images were obtained to localize the tumor of interest. Following this, a rapid bolus of iodinated contrast material (300 mg I/mL) was administered, with multiple scans acquired to assess tumor perfusion. Imaging software (CT Perfusion 2, GE Medical Systems) was used to generate a color-coded map of areas of differential perfusion, as well as a quantitative analysis of whole tumor blood flow in a single section, measured in mL/s/100 g (20). Other variables calculated in the software included blood volume, mean transit time, and permeability surface. For analysis, a single exemplary tumor site was assessed at the two time points and was chosen at the baseline evaluation based on the following: (*a*) large enough size to image based on anatomic landmarks, (*b*) within a region with little possible motion artifact, and (*c*) a solid tumor without a large cystic or necrotic component. To minimize repositioning and other potential technical errors, all scans were done by a single technologist with prescan knowledge of the area of interest. Specific regions of interest were defined by the software operator for the baseline evaluation that encompassed the entire tumor mass at four separate computed tomography sections. The perfusion variables (blood flow, blood volume, mean transit time, and permeability surface) collected at these sections were averaged and compared from baseline to the same regions of interest determined for the 8-week time point. A Pearson's correlation test was used to evaluate for changes in each variable with respect to treatment dose level. Of note, several patients (patients 1, 4, 5, 7, 8, 9, 10, 13, 19, 20, and 23) did not have tumors fulfilling the above criteria to warrant follow-up dynamic computed tomography assessment.

Statistical methods. Pharmacokinetic and dynamic computed tomography imaging variables are summarized by descriptive statistics. The area under the curve from time 0 to 168 hours ($AUC_{0\text{-}168}$) was calculated with the Lagrange method (21). The terminal half-life was determined by linear regression analysis of time versus the log-transformed concentration. The Jonckheere-Terpstra trend test was done to evaluate the association between increasing dose levels and each of the pharmacokinetic variables AUC and peak concentration. A Spearman's rank correlation analysis was also done to determine the relationship between the actual dose administered and the pharmacokinetic variable. Dose proportionality was examined by fitting the power model (22). Differences of pharmacokinetic variables between weeks 1 and 4 were evaluated using Wilcoxon signed rank tests. Accumulation was assessed by examining ratios of $AUC_{0\text{-}168h}$ of week 4 to $AUC_{0\text{-}168h}$ of week 1. Furthermore, a linear mixed-effects model with subject-specific random effects and trough concentrations as dependent variables was used to examine drug accumulation. Linear regression analysis was used to evaluate the relationship between dose and dynamic computed tomography imaging variables. The association between dose and duration of treatment was assessed by Cox regression analysis. All statistical analyses were done with SAS software, version 8.2 (SAS Institute, Inc., Cary, NC). All *P*s are two sided and were not adjusted for the number of variables evaluated. As such, they should only be interpreted as exploratory.

Results

Patient population. As shown in Table 2, 25 adult patients were enrolled in this study between November 2002 and May 2004 at the University of Wisconsin Comprehensive Cancer Center. The median age for all patients was 56 years (range, 32-74 years), and there were 15 treated males and 10 treated females. All patients had pathologic diagnoses of cancer as indicated, had received prior therapy, and had evidence of disease progression at the time of study entry.

Course of study. Patients were assigned to defined dose levels as shown in Table 1. Five dose levels were initially planned. Concurrent studies with this agent conducted at other sites, however, had shown safety at a 4 mg/kg dose. Consequently, after discussion with the National Cancer

Table 2. Patient demographics

	No. patients (%)
Total no. patients	25 (100)
Median age (y), (range)	56 (32-74)
Median duration of treatment (wks), (range)	8 (1-≥105)
Sex	
Male	15 (60)
Female	10 (40)
Diagnosis	
Breast	2 (8)
Colorectal	5 (20)
Melanoma	2 (8)
Non-small cell lung cancer	2 (8)
Ocular melanoma	1 (4)
Prostate	1 (4)
Renal	7 (28)
Sarcoma	3 (12)
Unknown primary	2 (8)

Institute Cancer Therapy Evaluation Program, a decision was made to not accrue the entire first dose level (2 mg/kg) and to skip the second dose level (4 mg/kg).

Adverse events. Table 3 shows all adverse events occurring in any course of treatment that were felt to be at least possibly attributable to MEDI-522. In this table, the highest grade of an event per individual patient is recorded. As can be seen, no significant toxicities were observed following treatment with MEDI-522. The major adverse events noted were low-grade constitutional symptoms (fatigue, anorexia, myalgias, and arthralgias), gastrointestinal symptoms (nausea, diarrhea, and emesis), and infusion reactions (rigors, chills, fever, flushing, tachycardia, and light-headedness). Of note, several patients experienced asymptomatic hypophosphatemia. One of these patients, patient 9, experienced grade 3 hypophosphatemia during the first cycle of therapy. Because a dose-limiting toxicity was defined as an event grade ≥3 occurring during the first cycle of treatment, the patient was removed from study. However, the first patient treated also experienced grade 3 hypophosphatemia during the first cycle of treatment. Given that this patient was otherwise asymptomatic, and the hypophosphatemia resolved spontaneously without treatment on repeat testing, and after discussion with Cancer Therapy Evaluation Program, it was elected to keep this patient on study. No further hypophosphatemia was observed in this patient until the 14th and 15th cycles when he again experienced asymptomatic hypophosphatemia resolving without specific treatment. No abnormalities were noted in the monthly urinalyses of this or any other patient. The only other dose-limiting toxicities observed was in a patient with extensive non–small cell lung cancer who experienced hypoxia during the first infusion of MEDI-522. Although it was felt that his underlying lung disease most likely contributed to this toxicity, a causal relation with MEDI-522 could not be excluded; therefore, the patient came off study. Only one grade 4 event was noted on study, a patient with prostate cancer who was hospitalized for disseminated intravascular coagulation. Although the progression of his underlying disease was felt to be the primary contributor to this event, a causal relation with MEDI-522 could not be excluded. No patients discontinued treatment due to intolerable adverse events.

Pharmacokinetics. MEDI-522 serum concentrations were measured in all patients at weekly intervals before dosing and at multiple times during the first and fourth week of treatment, as described in Materials and Methods. The pharmacokinetic variables during weeks 1 and 4 are shown in Table 4. Mean serum concentrations of MEDI-522 per dose cohort are shown in Fig. 1. As shown, MEDI-522 serum concentrations declined in a biexponential manner with time. MEDI-522 exposures increased with escalating dose, with a nearly linear increase in C_{max} and AUC with respect to dose. The peak concentration, trough concentration, and AUC of MEDI-522 during weeks 1 and 4 showed a highly significant dose-dependent increase ($P < 0.001$). Peak concentration, trough concentration, and AUC were significantly higher during week 4 than during week 1 ($P < 0.001$). The observed half-life (59-106 hours) was similar to that seen with the first-generation MEDI-523 antibody (13). The half-life during week 1 was significantly shorter than the half-life during week 4 ($P < 0.001$). At doses of 6 mg/kg/wk, trough concentrations were consistently >25 μg/mL. The overall mean accumulation ratio was 1.96 (SD 0.39), which indicates drug accumulation of MEDI-522 from weeks 1 to 4. Drug accumulation was confirmed by the repeated measurement analysis of trough concentrations obtained before infusion on day 1 of weeks 1, 2, 3, 4, 5, 6, 7, and 8 ($P < 0.001$). In the ANOVA for accumulation from weeks 1 to 4, no statistical significant differences in accumulation among dose groups were found ($P = 0.161$). No human anti-MEDI-522 antibodies were detected at any time point (data not shown).

Nine patients consented to provide random skin biopsies before and after treatment for future studies to evaluate MEDI-522 targeting *in vivo*. Two of these patients also had skin metastases and consented to provide tumor biopsies at these same time points. Because two of these nine patients came off study before completing the first cycle, paired skin samples were ultimately obtained from seven patients, and paired tumor biopsy specimens were obtained from one patient. There were no complications, and results from studies obtained with these biopsy samples will be presented separately.

Biological response. Dynamic computed tomography imaging was done at baseline and after 8 weeks of treatment as a means of assessing tumor blood flow during treatment with MEDI-522, as described above. Color-coded maps were software generated from the acquisition data to show differential areas of blood flow, blood volume, mean transit time, and permeability surface, as illustrated in Fig. 2. Three regions of interest, corresponding to the entire tumor volume at three separate sections, were identified, and the corresponding regions of interest were identified on both the pretreatment and posttreatment scans. The mean blood flow, blood volume, mean transit time, and permeability surface were averaged across the three sections from the pretreatment sections and compared with the corresponding posttreatment average. The results from this analysis, analyzed with respect to patient dose level, are shown in Fig. 3. Although the number of patients in each group was small, a significant increase in mean transit time was observed with increasing dose of MEDI-522 ($P = 0.01$). No significant trend was observed with the other variables.

Table 3. Adverse events

Adverse event	Grade 1	Grade 2	Grade 3	Grade 4
Constitutional				
Fatigue	5	3	1	
Anorexia	3	1		
Pain	2	1		
Pruritus		1		
Arthralgias	2	1		
Myalgias	5	1		
Sweating	3			
Dry mouth	1			
Neurologic				
Headache	3			
Anxiety	1			
Muscle spasms	1			
Vivid dreams	1			
Dysesthesia	1			
Gastrointestinal				
Diarrhea	4			
Nausea	8			
Emesis	4			
Early satiety	1			
Altered taste	1			
Other gastrointestinal discomfort	2			
Pulmonary				
Cough	1			
Hemoptysis	1			
Hematologic				
Epistaxis	1			
Hemoglobin		2		
Lymphopenia			1	
Thrombocytopenia	1			
Bruising	1			
Hematuria			1	
DIC				1
Laboratory				
Hypocalcemia		1		
Hyponatremia	1			
Hypophosphatemia		3	2 (DLT)	
PTT	1			
Infusion reactions				
Rigors	5	3		
Fever	4	1		
Dyspnea		2		
Injection site reaction	2	1		
Light-headedness	2	1		
Hypoxia			1 (DLT)	
Hypotension	1			
Hypertension		1		
Rash	1			
Chills	1			
Facial flushing	3			
Sinus tachycardia	3			

NOTE: All adverse events by grade that were believed to be at least possibly related to treatment with MEDI-522 as of November 2004. The numbers represent the number of patients (of 25) experiencing a particular event at any point during multiple cycles of treatment, with the highest grade reported for any single individual.
Abbreviations: DIC, disseminated intravascular coagulopathy; DLT, dose-limiting toxicity; PTT, partial thromboplastin time.

Clinical response. Table 5 shows the duration of treatment and clinical response for all patients treated. No patients in the current study experienced a complete or partial response as defined by Response Evaluation Criteria in Solid Tumors (17). Three patients with metastatic renal cell cancer experienced either a minor response or prolonged stable disease (data not shown). At the time of this writing, two patients with metastatic renal cell cancer remain on treatment with stable disease for over 1 or 2 years, respectively. Eight of 25 patients total had stable disease noted at the 8-week radiographic staging. Figure 4 illustrates the duration of response with respect to either dose level (*A*) or disease type (*B*). As illustrated, four patients with metastatic renal cell cancer remained on study for multiple (16, 34, >50, >95) months with stable disease. There was no clear association between length of time on study with respect to dose level ($P = 0.2$, Cox regression analysis).

Discussion

This study reports the results of a phase I dose escalation, pharmacokinetic trial with a mAb, MEDI-522, targeting the $\alpha_v\beta_3$ integrin in patients with advanced-stage metastatic cancer. In addition, studies were done to assess the effects of treatment with MEDI-522 on tumor perfusion. Patients were treated at doses ranging from 2 to 10 mg/kg/wk. Our study shows that (*a*) treatment with MEDI-522 was without significant toxicity, and no maximum tolerated dose was identified by excessive or unacceptable dose-limiting toxicities; (*b*) treatment with MEDI-522 may have resulted in changes in tumor blood mean transit time as measured by dynamic computed tomography imaging; and (*c*) three patients with metastatic renal cell cancer had prolonged stable disease, suggesting that this agent could be further explored for the treatment of patients with metastatic renal cell cancer.

No significant toxicities were associated with treatment with MEDI-522 in this trial. The major adverse events noted during the study were mild constitutional and gastrointestinal symptoms, including fatigue, anorexia, myalgias, and nausea. The next most common events were infusion-related events, including rigors, chills, fever, flushing, tachycardia, and light-headedness. These events were more common at the 8 and 10 mg/kg doses (data not shown). Finally, grade 2 and 3 hypophosphatemia episodes were observed in several patients but seemed transient as it spontaneously resolved without treatment and was entirely asymptomatic. One patient with hypophosphatemia noted during the first cycle of MEDI-522 continued to be treated without recurrence of this hypophosphatemia until several months later. Urine electrolytes were not assessed; however, urinalysis done monthly in patients on study showed no evidence of proteinuria or other changes to

Table 4. Pharmacokinetic characteristics

Dose (mg/kg/wk)	No. patients	C_{max} (μg/mL)*†, mean (SD)	C_{min} (μg/mL)‡, mean (SD)	$AUC_{0\text{-}168h}$ (μg·h/mL)*§, mean (SD)	$T_{1/2}$ (h), mean (SD)
Wk 1					
2.0	4	41.6 (10.0)	8.4 (4.8)	2,337.6 (551.5)	59.09 (4.82)
6.0	7	130.2 (21.5)	34.3 (9.4)	7,750.6 (1817.3)	75.25 (24.93)
8.0	6	216.2 (62.9)	42.5 (21.1)	11,064.9 (2950.9)	61.23 (17.76)
10.0	7	387.3 (170.2)	54.5 (18.5)	15,371.1 (4849.8)	62.8 (20.87)
Wk 4					
2.0	3	53.5 (7.9)	17.4 (1.8)	3,597.0 (429.7)	88.83 (13.24)
6.0	6	205.7 (54.7)	68.5 (26.2)	14,374.4 (3726.4)	105.72 (29.32)
8.0	6	308.3 (73.4)	117.5 (62.1)	22,120.2 (7550.6)	97.11 (32.56)
10.0	5	470.6 (131.8)	140.4 (52.8)	28,223.8 (3883.3)	99.97 (40.75)

NOTE: Pharmacokinetic characteristics determined during weeks 1 and 4. C_{max}, peak concentration detected; C_{min}, trough concentration (on day 7 or 28 before next dose); $T_{1/2}$, half-life.

*For week 1, peak concentration and AUC were obtained at week 1 (0-168 h). Trough concentrations were measured immediately before week 2 administration. For week 4, peak concentration and AUC were obtained at week 4 (0-168 h). Trough concentrations were measured immediately before week 5 administration. Means and SD for all patients at each dose level.

†The association between peak concentration and dose was highly significant ($P < 0.001$).

‡The association between trough concentration and dose was highly significant ($P < 0.001$).

§The association between AUC and dose level was highly significant ($P < 0.001$).

suggest renal toxicity. In addition, no changes were noted in serum calcium or serum glucose levels. These findings, as well as the observation of the rapid resolution of the hypophosphatemia in the first patient with no evidence of recurrence until much later cycles of treatment, suggested that the hypophosphatemia observed was most likely secondary to intracellular phosphate migration. The $\alpha_v\beta_3$ integrin is known to be expressed in glomerular epithelial, endothelial, and mesangial cells of the kidney (23). In addition, there is extensive data in the literature to suggest that $\alpha_v\beta_3$ may participate in adhesion of renal-derived cell lines to extracellular matrix proteins (24). To our knowledge, however, there have been no reports of a role for the $\alpha_v\beta_3$ integrin in renal pathology, and no reports of integrin antagonists causing or exacerbating hypophosphatemia or other kidney diseases. Moreover, β_3 integrin–deficient mice show no signs of abnormal kidney function; hence again, we do not believe this was a sign of renal toxicity (25). Hypophosphatemia was not reported in previous phase I studies with the MEDI-523 antibody (13–15) and is not a generally recognized problem with other antibody-based therapies, suggesting that the hypophosphatemia observed was not due to effects of the IgG molecule itself. We have observed similar shifts in serum phosphate levels with an antibody-cytokine fusion protein therapy, EMD 273063, that were believed due to signaling effects mediated by the cytokine interleukin 2 (26). Integrin stimulation, including the $\alpha_v\beta_3$ integrin, is known to stimulate calcium mobilization and phosphatidylinositol 3-kinase–mediated signaling (27, 28). Hence, it is possible that inhibition of $\alpha_v\beta_3$ signaling by means of MEDI-522 blockade could result in transient intracellular phosphate shifts. There was no evidence of significant hypertension, headaches, or vascular events as have been observed with some other angiogenesis inhibitors in clinical development (3, 8, 29).

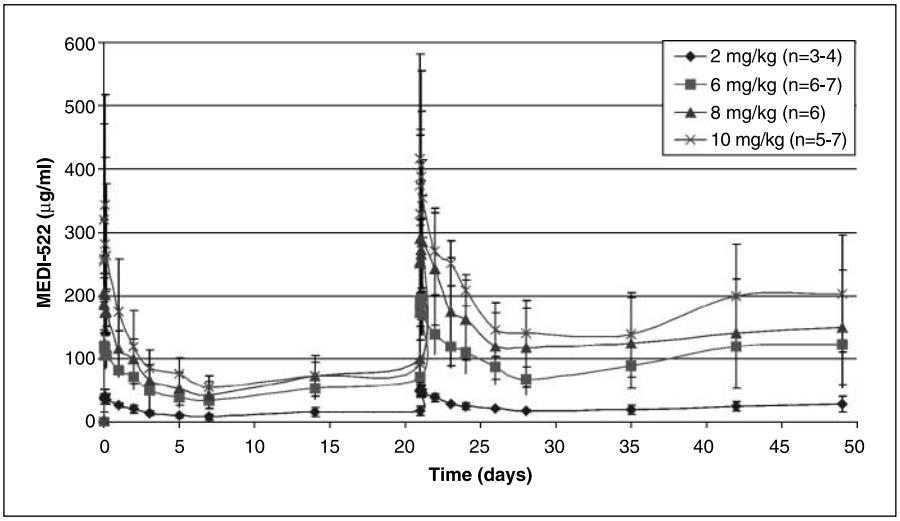

Fig. 1. Serum MEDI-522 concentrations. Serum concentrations of MEDI-522 were determined by ELISA at multiple time points for each patient over the first 8 weeks. Points, means of MEDI-522 serum concentrations per dose cohort at each time point collected; bars, SD.

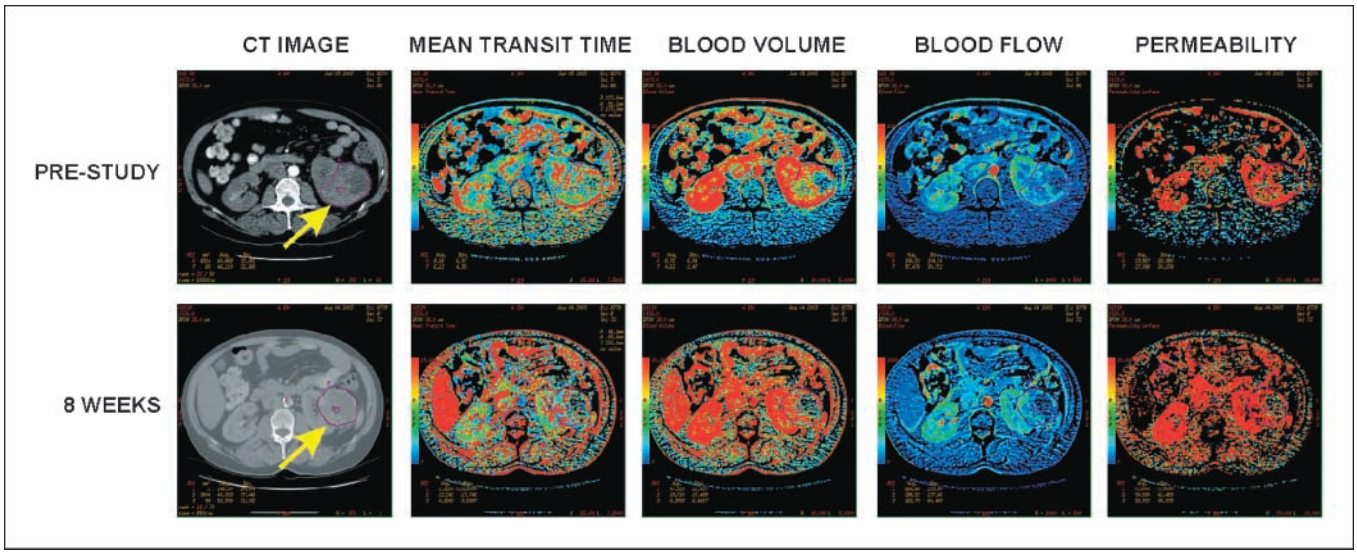

Fig. 2. Dynamic computed tomography (*CT*) analysis of tumor blood flow and perfusion. An example of a computed tomography image and perfusion data (blood flow, blood volume, mean transit time, and permeability surface) collected from a single patient (patient 15) before treatment with MEDI-522 (*top*) and at 8 weeks of treatment (*bottom*). This particular patient had metastatic renal cell cancer, was treated at dose level 4 (8 mg/kg), and serial perfusion imaging was done on the primary tumor. Arrows point to the circled region of interest used for comparison.

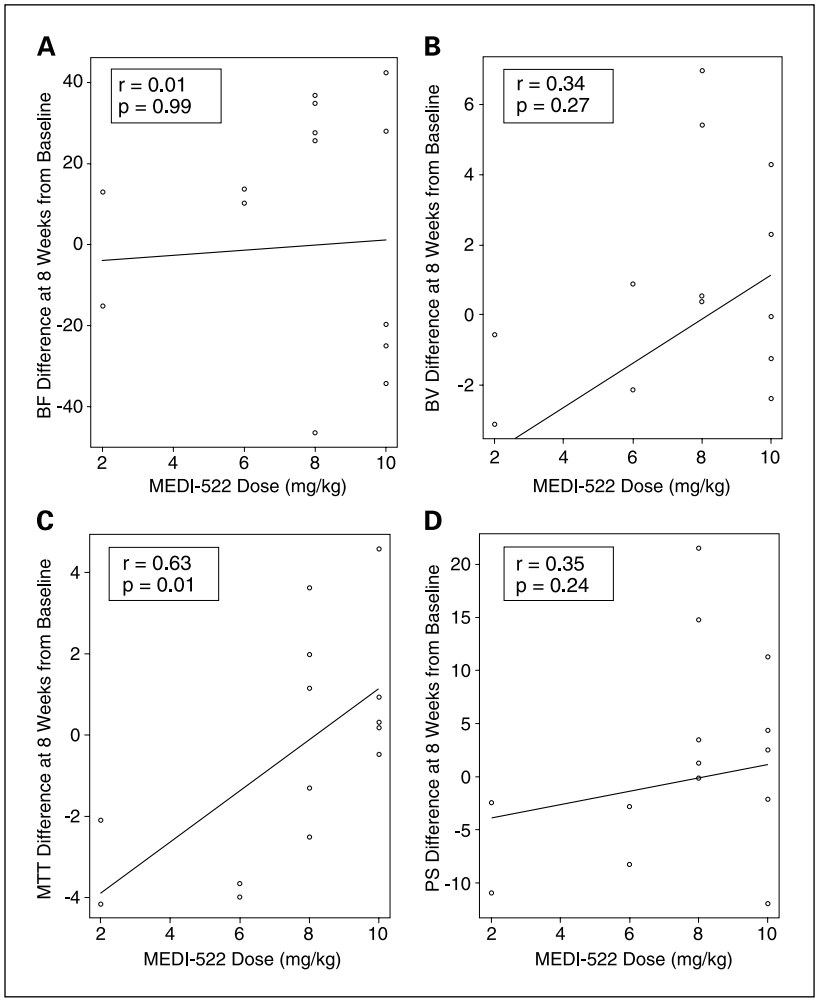

Fig. 3. Dynamic computed tomography tumor blood flow analysis with respect to treatment dose level. Points, mean blood flow (*BF, A*), blood volume (*BV, B*), mean transit time (*MTT, C*), and permeability surface (*PS, D*) from four sections of target tumor lesions. The difference in these variables at 8 weeks compared with baseline is plotted with respect to dose of MEDI-522. Correlation with respect to dose (*r*) and two-sided significance testing for each variable.

Table 5. Duration of treatment and clinical response outcomes

Patient	Diagnosis	Age	Sex	Dose level	Duration of treatment (wk)	Response
1	Renal	60	M	1	>95	SD
2	Sarcoma	48	M	1	11	PD
3	Sarcoma	48	F	1	12	PD
4	Prostate	60	M	1	13	PD
5	Melanoma	47	M	3	6	PD
6	Renal	44	F	3	16	*
7	NSCLC	55	M	3	9	PD
8	Colorectal	61	F	3	4	†
9	Renal	32	M	3	5	‡
10	Colorectal	60	M	3	4	PD
11	Colorectal	60	F	3	7	PD
12	Sarcoma	47	M	4	16	§
13	Breast	57	F	4	9	PD
14	Renal	52	M	4	34	PD
15	Colorectal	59	F	4	8	PD
16	Unknown primary	71	F	4	5	PD
17	Colorectal	65	M	4	8	PD
18	Renal	53	M	5	>50	SD
19	NSCLC	37	M	5	1	‡
20	Renal	64	M	5	4	†
21	Unknown primary	54	F	5	8	PD
22	Ocular melanoma	56	M	5	8	PD
23	Breast	58	F	5	1	†
24	Melanoma	74	F	5	8	PD
25	Renal	53	M	5	8	*

Abbreviations: DLT, dose-limiting toxicity; PD, progressive disease; SD, stable disease; NSCLC, non – small cell lung cancer.
*Off study due to doctor discretion.
†Not evaluable, unable to complete course 1.
‡Off study due to adverse event (DLT).
§Off study due to clinical deterioration.

Dynamic computed tomography scans have been reported to provide quantitative information about arterial perfusion of tumors (20). In the current study, these scans were done as an exploratory study to determine if changes in blood flow could be detected in response to treatment. The number of patients enrolled with tumor volumes large enough to assess by means of dynamic imaging was small, and nearly half of the patients evaluated at baseline did not have tumors of sufficient size to evaluate by dynamic computed tomography imaging. Consequently, these studies were underpowered to detect small differences. However, with increasing doses of MEDI-522, a significant increase was observed in the mean transit time of blood through target tumor lesions after 8 weeks compared with baseline evaluations. This finding might suggest that a biological response was observed in impeding blood flow through small-caliber neovasculature following MEDI-522 treatment. No significant trends were observed with the other variables. At this point, it is unknown why one variable would show differences not reflected by the other variables but is likely due to small changes in each variable in a small sample population. Although it is possible that changes observed in only one variable are artifactual given the small sample size and

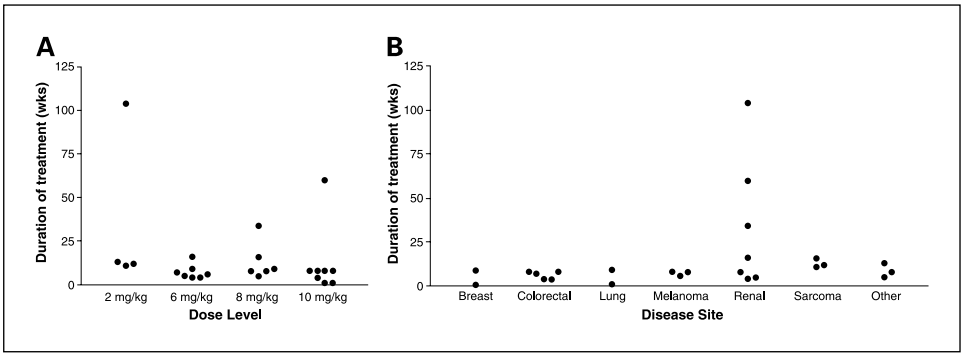

Fig. 4. Duration of treatment. Duration of treatment with MEDI-522 (weeks) by treatment dose level (*A*) or by the underlying malignancy of the treated patients (*B*).

possible variability in measurements, this seems less likely given the strong correlation with respect to dose. In addition, other studies exploring contrast enhanced magnetic resonance imaging as a tool for evaluating blood flow changes following vascular targeting agents have used dynamic imaging within days up to 8 weeks of beginning therapy (30, 31). We were interested to define possible changes in tumor perfusion after several weeks of treatment, at the time of routine tumor imaging. A possible disadvantage to our approach, however, was that changes in tumor size, larger or smaller, over an 8-week period might have affected interpretation of our results by introducing variability in the tumor size and computed tomography–defined regions of interest from one time point to the next. For future studies with MEDI-522, it might be preferable to consider dynamic imaging after a shorter interval of time to control for this possible variable. Not enough data were available from our current study to correlate the duration of treatment in those patients with prolonged stable disease with changes in computed tomography perfusion variables. In particular, the patient on study for the longest period of time had multiple lung metastases but none >2 cm, permitting computed tomography perfusion analysis. In addition, no correlation was observed between changes in computed tomography perfusion variables and pharmacokinetic measurements (data not shown).

Given the absence of significant adverse events and dose-limiting toxicities, a maximum tolerated dose was not truly defined for MEDI-522 in the traditional sense. Likewise, given the small number of patients and no evidence of a dose response in terms of number of patients with stable disease, no threshold dose was identified to recommend for phase II testing. The biological response data by dynamic computed tomography imaging suggest that at doses of $\geq$8 mg/kg/wk, there might have been effects on tumor perfusion, but these data are certainly limited by a small number of patients treated at lower doses. The pharmacokinetic data suggest that serum levels of $\geq$30 μg/mL were consistently achieved at a dose of 6 mg/kg/wk, and that at 8 weeks, trough levels of >100 μg/mL were achievable. Saturation was not observed, and the pharmacokinetic data suggested that drug accumulation, at least during the period of observation, occurred. Certainly, no significant toxicities were observed with peak plasma concentrations of >500 μg/mL, and the treatment of two subjects over 1 year without significant toxicity suggests that even if drug accumulation occurs beyond this time point, it might not pose significant risk at the doses studied. However, based on the preclinical studies described earlier in which continuous serum levels of 30 μg/mL were estimated to be a desired concentration, the further observation that one patient treated at 2 mg/kg/wk had prolonged stable disease over 2 years, and given that a dose of 6 mg/kg/wk in the current study consistently achieved a trough concentration of 30 μg/mL after several weeks, a dose of 6 mg/kg/wk will be the recommended dose for future phase II studies.

No objective responses were identified in the current study. Three patients with metastatic renal cell cancer, however, had prolonged stable disease for $\geq$34 weeks. Although the natural history of metastatic renal cell cancer is extremely variable, all three of these patients had evidence of disease progression before study entry. As of November 2004, two of these patients remain on study for over 1 and 2 years, respectively. Both of these patients had low-volume pulmonary and nodal metastases, biopsy proven to be metastatic renal cell cancer. In addition, both had evidence of disease progression in terms of increasing size of metastatic lymph nodes and/or pulmonary nodules by computed tomography scans obtained over a 3- to 6-month period before enrollment. The third patient with stable disease over 34 weeks of treatment had large-volume abdominal, nodal, and liver metastases. Two of these three patients, including the patient with bulky visceral disease, showed some decrease in size of the known metastases over the course of treatment, at least initially, but not meeting criteria for objective disease response by Response Evaluation Criteria in Solid Tumors (data not shown). Together, these findings suggest that MEDI-522 may have clinical activity in metastatic renal cell cancer. Given the success of other antiangiogenesis agents in clinical trials for metastatic renal cell cancer, including oral vascular endothelial growth factor tyrosine kinase inhibitors (2) and bevacizumab (4), this is not an unexpected finding. The safety and tolerability of MEDI-522 and its possible clinical efficacy in patients with metastatic renal cell cancer suggest that it could be further explored in patients with metastatic renal cell cancer, either alone or in combination with other agents.

References

1. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med 1971;285:1182–6.
2. Motzer RJ, Rini BI, Michaelson MD, et al. SU011248, a novel tyrosine kinase inhibitor, shows antitumor activity in second-line therapy for patients with metastatic renal cell carcinoma: results of a phase 2 trial. Proc Am Soc Clin Oncol 2004;22:382s.
3. Ahmed SI, Thomas AL, Steward WP. Vascular endothelial growth factor (VEGF) inhibition by small molecules. J Chemother 2004;16 Suppl 4:59–63.
4. Yang JC, Haworth L, Sherry RM, et al. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N Engl J Med 2003;349:427–34.
5. Johnson DH, Fehrenbacher L, Novotny WF, et al. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. J Clin Oncol 2004;22:2184–91.
6. Miller KD, Chap LI, Holmes FA, et al. Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. J Clin Oncol 2005;23:792–9.
7. Herbst RS, Johnson DH, Mininberg E, et al. Phase I/II trial evaluating the anti-vascular endothelial growth factor monoclonal antibody bevacizumab in combination with the HER-1/epidermal growth factor receptor tyrosine kinase inhibitor erlotinib for patients with recurrent non-small-cell lung cancer. J Clin Oncol 2005;23:2544–55.
8. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004;350:2335–42.
9. Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin $\alpha_v\beta_3$ for angiogenesis. Science 1994;264:569–71.
10. Brooks PC, Montgomery AM, Rosenfeld M, et al. Integrin $\alpha_v\beta_3$ antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. Cell 1994;79:1157–64.
11. Mitjans F, Sander D, Adan J, et al. An anti-α_v-integrin antibody that blocks integrin function inhibits the development of a human melanoma in nude mice. J Cell Sci 1995;108:2825–38.
12. Gasparini G, Brooks PC, Biganzoli E, et al. Vascular integrin $\alpha(v)\beta_3$: a new prognostic indicator in breast cancer. Clin Cancer Res 1998;4:2625–34.
13. Gutheil JC, Campbell TN, Pierce PR, et al. Targeted antiangiogenic therapy for cancer using Vitaxin: a humanized monoclonal antibody to the integrin $\alpha_v\beta_3$. Clin Cancer Res 2000;6:3056–61.
14. Posey JA, Khazaeli MB, DelGrosso A, et al. A pilot trial of Vitaxin, a humanized anti-vitronectin receptor (anti $\alpha_v\beta_3$) antibody in patients with metastatic cancer. Cancer Biother Radiopharm 2001;16:125–32.
15. Patel SR, Jenkins J, Papadopolous N, et al. Pilot study of vitaxin: an angiogenesis inhibitor-in patients

with advanced leiomyosarcomas. Cancer 2001;92:1347–8.

16. Wu H, Beuerlein G, Nie Y, et al. Stepwise *in vitro* affinity maturation of Vitaxin, an $\alpha_v\beta_3$-specific humanized mAb. Proc Natl Acad Sci U S A 1998;95:6037–42.

17. Tsuchida Y, Therasse P. Response evaluation criteria in solid tumors (RECIST): new guidelines. Med Pediatr Oncol 2001;37:1–3.

18. Miles KA, Charnsangavej C, Lee FT, Fishman EK, Horton K, Lee TY. Application of CT in the investigation of angiogenesis in oncology. Acad Radiol 2000;7:840–50.

19. Kanematsu M, Osada S, Amaoka N, et al. Expression of vascular endothelial growth factor in hepatocellular carcinoma and the surrounding liver: correlation with angiographically assisted CT. AJR Am J Roentgenol 2004;183:1585–93.

20. Tsushima Y, Funabasama S, Aoki J, Sanada S, Endo K. Quantitative perfusion map of malignant liver tumors, created from dynamic computed tomography data. Acad Radiol 2004;11:215–23.

21. Yeh KC, Kwan KC. A comparison of numerical integrating algorithms by trapezoidal, Lagrange, and spline approximation. J Pharmacokinet Biopharm 1978;6:79–98.

22. Gough M, Hutchison M, Keene O, et al. Assessment of dose proportionality. Drug Information Journal 1995;29:1039–48.

23. Patey N, Halbwachs-Mecarelli L, Droz D, Lesavre P, Noel LH. Distribution of integrin subunits in normal human kidney. Cell Commun Adhes 1994;2:159–67.

24. Yi XY, Wayner EA, Kim Y, Fish AJ. Adhesion of cultured human kidney mesangial cells to native entactin: role of integrin receptors. Cell Commun Adhes 1998;5:237–48.

25. Hodivala-Dilke KM, McHugh KP, Tsakiris DA, et al. β_3-Integrin-deficient mice are a model for Glanzmann thrombasthenia showing placental defects and reduced survival. J Clin Invest 1999;103:229–38.

26. King DM, Albertini MR, Schalch H, et al. Phase I clinical trial of the immunocytokine EMD 273063 in melanoma patients. J Clin Oncol 2004;22:4463–73.

27. Cooke ME, Sakai T, Mosher DF. Contraction of collagen matrices mediated by $\alpha_2\beta_1$A and $\alpha(v)\beta_3$ integrins. J Cell Sci 2000;113:2375–83.

28. Paulhe F, Perret B, Chap H, Iberg N, Morand O, Racaud-Sultan C. Phosphoinositide 3-kinase C2α is activated upon smooth muscle cell migration and regulated by $\alpha(v)\beta(3)$ integrin engagement. Biochem Biophys Res Commun 2002;297:261–6.

29. Thomas AL, Morgan B, Drevs J, et al. Vascular endothelial growth factor receptor tyrosine kinase inhibitors: PTK787/ZK 222584. Semin Oncol 2003;30:32–8.

30. Wilmes LJ, Hylton N, Wang DH, et al. AG-013736, a novel VEGFR TK inhibitor, suppresses tumor growth and vascular permeability in human BT474 breast cancer xenografts in nude mice. Proc Am Assoc Cancer Res 2003;44:3772.

31. Morgan B, Thomas AL, Drevs J, et al. Dynamic contrast-enhanced magnetic resonance imaging as a biomarker for the pharmacological response of PTK787/ZK 222584, an inhibitor of the vascular endothelial growth factor receptor tyrosine kinases, in patients with advanced colorectal cancer and liver metastases: results from two phase I studies. J Clin Oncol 2003;21:3955–64.