Phase I Evaluation of a Fully Human Anti–α_v Integrin Monoclonal Antibody (CNTO 95) in Patients with Advanced Solid Tumors

Saifee A. Mullamitha,[1] Nhuan C. Ton,[1] Geoff J.M. Parker,[3] Alan Jackson,[3] Peter J. Julyan,[2] Caleb Roberts,[3] Gio A. Buonaccorsi,[3] Yvonne Watson,[3] Karen Davies,[3] Sue Cheung,[3] Lynn Hope,[1] Juan W. Valle,[1] John A. Radford,[1] Jeremy Lawrance,[1] Mark P. Saunders,[1] Mihaela C. Munteanu,[4] Marian T. Nakada,[5] Jeffrey A. Nemeth,[5] Hugh M. Davis,[6] Qun Jiao,[6] Uma Prabhakar,[6] Zhihui Lang,[7] Robert E. Corringham,[7] Robert A. Beckman,[7] and Gordon C. Jayson[1]
in association with the Biotherapy Development Association

Abstract

Purpose: A fully human monoclonal antibody to anti–α_v integrins (CNTO 95) has been shown to inhibit angiogenesis and tumor growth in preclinical studies. We assessed the safety and pharmacokinetics of CNTO 95 in patients with advanced refractory solid tumors.

Experimental Design: In this phase I trial, CNTO 95 (0.1, 0.3, 1.0, 3.0, and 10.0 mg/kg) was infused on days 0, 28, 35, and 42, and clinical assessments, dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), and [^{18}F]-2-fluorodeoxyglucose positron emission tomography (FDG-PET) were done. Patients achieving stable disease or better were eligible for extended dosing every 3 weeks for up to 12 months.

Results: Among the 24 enrolled patients, CNTO 95 was associated with one episode of grade III and four episodes of grade II infusion-related fever (all responded to acetaminophen). Of the six patients who received extended dosing, one patient (10.0 mg/kg), with cutaneous angiosarcoma, had a 9-month partial response. Pre- and post-treatment lesion biopsies confirmed tumor cell α_v integrin expression, as well as CNTO 95 penetration of the tumor and localization to tumor cells in association with reduced bcl-2 expression. A lesion in one patient (10.0 mg/kg) with stable ovarian carcinosarcoma was no longer detectable by FDG-PET by day 49. Exposure to CNTO 95 seemed to increase in a greater-than-dose-proportional manner; dose-dependent mean half-life ranged from 0.26 to 6.7 days.

Conclusions: CNTO 95 was generally well tolerated. Six patients received extended therapy, including one patient with a prolonged response. Biopsy data confirmed tumor localization and pharmacodynamic activity.

Authors' Affiliations: [1]Cancer Research UK and [2]Department of Medical Physics, Christie Hospital, Withington; [3]Imaging Science and Biomedical Engineering, University of Manchester, Manchester, United Kingdom; and [4]Department of Clinical Trial Management, [5]Oncology Discovery Research, [6]Clinical Pharmacology, and [7]Oncology Clinical Research and Development, Centocor Research and Development, Inc., Malvern, Pennsylvania

Received 11/27/06; revised 1/15/07; accepted 1/25/07.

Grant support: Centocor Research and Development, Inc., Malvern, PA. (The investigator analyzed and interpreted the data and approved the content of the manuscript before submission).

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.

Note: This work was previously presented at major American and European oncology conferences in 2004 and 2005.

Disclosures: Mihaela C. Munteanu, Marian T. Nakada, Jeffery A. Nemeth, Hugh M. Davis, Qun Jiao, Uma Prabhakar, Zhihui Lang, Robert E. Corringham, and Robert A. Beckman are or were Centocor employees at the time the study was conducted.

Requests for reprints: Gordon Jayson, Department of Medical Oncology, Christie Hospital, Wilmslow Road, Withington, Manchester M20 4BX, United Kingdom. Phone: 44-161-446-3606; Fax: 44-161-446-8565; E-mail: Gordon.Jayson@christie-tr.nwest.nhs.uk.

doi:10.1158/1078-0432.CCR-06-2779

Angiogenesis is crucial to tumor growth, as shown in recent randomized trials where vascular endothelial growth factor (VEGF) was inhibited (1–3). Angiogenesis is regulated by a range of pro- and antiangiogenic pathway targets, including the α_v integrins.

The integrins are a family of at least 24 $\alpha\beta$ heterodimeric glycoproteins, involved in cell-matrix binding and communication (4, 5). The α_v integrins are expressed by endothelial cells, particularly during angiogenesis (6) and are implicated in signal transduction by VEGF, fibroblast growth factor, and a variety of other cytokines (7). The α_v integrins are expressed on the surface of most epithelial tumors, and tumor progression and metastasis are often altered in relation to integrin expression (8). Signaling through integrin-associated networks affects cell proliferation and survival (9), and inhibition of integrins has been shown to have antitumor effects independent of antiangiogenesis (10, 11). The α_v integrins participate in metastasis, and their expression has prognostic significance in a variety of epithelial tumors (12, 13).

CNTO 95 is a fully human immunoglobulin $G_1\kappa$ monoclonal antibody that binds purified α_v integrins and α_v integrin–expressing cells with dissociation constants of 200 pmol/L and ≤24 nmol/L, respectively. CNTO 95 inhibited tumor growth directly in mouse models where there is no cross-reactivity with host vasculature and had a greater antitumor effect in rats where there is some limited cross-reactivity (11). CNTO 95 is an antibody, rather than a synthetic peptide (14), which binds multiple α_v integrins as opposed to $\alpha_v\beta_3$ alone (15). This first-in-human phase I study was conducted to determine the maximum tolerated dose (MTD; if applicable), dose-limiting toxicity (DLT; if present), pharmacokinetics, tumor response, and pharmacodynamic effects of CNTO 95.

Patients and Methods

The trial was a single-center, first-in-human, multiple-administration, open-label, dose-escalating study of CNTO 95 in cancer patients with solid tumors refractory to standard therapy or for whom no standard therapy was available. Hospital, local, and national ethics committees and the United Kingdom Administration of Radioactive Substances Advisory Committee (ARSAC) approved the protocol; all patients provided written informed consent. The study was conducted in accordance with Good Clinical Practice guidelines.

Patient eligibility criteria. Patients were enrolled into the trial between November 2002 and September 2004 at the Christie Cancer Center, Manchester, United Kingdom. Eligible patients were at least 18 years of age with histologically confirmed solid tumors that were refractory to standard therapy or for which no standard therapy was available. Eastern Cooperative Oncology Group performance status ≤2 was required. All patients had to have recovered from prior treatment and received no chemotherapy, hormonal therapy, or systemic steroids (except by inhalation) in the previous 4 weeks (2 weeks for irradiation, 6 weeks for mitomycin).

Adequate bone marrow, renal, and coagulation function were required. Patients with reproductive potential were instructed to use contraception.

Major exclusion criteria included primary or secondary brain tumors, clinically detectable ascites, unstable systemic disease, history of thrombosis, recent surgery (within 4 weeks), a history of severe allergic reactions, and a history of anaphylaxis. Concurrent noninhaled steroids, aspirin therapy, or parenteral/p.o. anticoagulants (except to maintain preexisting indwelling lines) were prohibited during the trial. Any prior experimental therapy required a washout of at least 4 weeks or five half-lives before study entry. Patients previously treated with known modulators of $\alpha_v\beta_{3/5}$ were excluded.

Study design. Patients were sequentially allocated to cohorts of three patients per group receiving 0.1, 0.3, 1.0, 3.0, and 10.0 mg/kg of CNTO 95. No intrapatient dose escalation was permitted.

Pretreatment evaluations included a clinical assessment, complete blood count, biochemical and coagulation screen, hepatitis screen, dipstick urinalysis, 12-lead electrocardiogram (EKG), lung function tests, chest X-ray, and a restaging spiral computed tomography (CT) scan within 4 weeks of the first treatment. Patients also underwent (^{18}F)-2-fluorodeoxyglucose positron emission tomography (FDG-PET) and dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) within 7 days before the first infusion of the study agent (to rule out brain metastases) and on days 1, 7, and 49. From the third cohort onward, all patients had Doppler ultrasound of the lower extremities; upper extremities were included if there was an indwelling long line or suspicion of thrombosis.

Clinical and laboratory assessments were done immediately before each infusion and at weekly intervals throughout the study. A blinded, independent cardiologist evaluated EKGs, done immediately after the initiation and completion of each infusion, and at 6 to 8 and 24 h after study agent administration. Based on emerging nonhuman primate data associating CNTO 95 with transient asymptomatic uveitis, the last four patients received serial ophthalmic assessment, including slit lamp examinations.

Computed tomography was done within 2 weeks of study enrollment and repeated at day 49. Results were reported using the RECIST criteria (16). After achieving stable disease or better, a confirmatory CT scan was done at 1 month; extended dosing every 3 weeks (±7 days) at the same dose was permitted for up to 12 months.

Study agent administration. Study agent was supplied as a single-use, sterile, nonpyrogenic solution containing CNTO 95 at 20 mg/mL. Study agent was administered as an i.v. infusion over 2 h on days 0, 28, 35, and 42. The dosing schedule was designed to set a single-dose pharmacokinetic observation window for the initial period (up to 28 days), whereas subsequent weekly dosing is supported by Centocor internal safety data from cynomolgus monkeys.

Dose escalation and definitions of MTD and DLT. DLT was defined as any grade 3 or 4 toxicity considered to be possibly, probably, or definitely related to CNTO 95, according to the National Cancer Institute Common Toxicity Criteria. However, hypersensitivity reactions were defined as dose limiting when grade 2 or higher. Patients were considered to be evaluable for DLT if they had experienced a DLT or had received at least three administrations of CNTO 95 and undergone at least 7 days of follow-up after the third administration. This criteria prevented patients with minimal exposure to CNTO 95 (i.e., only one to two doses) and no DLT from being counted toward justifying dose escalation. All patients who received CNTO 95 were considered evaluable for safety. If a DLT was observed at any dose level, the cohort was expanded to six patients. If two or more patients within a cohort experienced a DLT, the previous lower dose was to be defined as the MTD. If no DLT was observed, the next higher dose cohort was opened after review of all clinical data by the Safety Data Monitoring Committee.

Pharmacokinetic evaluations. Serum samples were obtained before and at 2, 4, and 24 h after the start of study agent administration. Additional samples were obtained 7, 14, and 21 days after the first and fourth infusions. CNTO 95 concentrations were measured using a bead-based electrochemiluminescent immunoassay. Noncompartmental analysis was employed to calculate the pharmacokinetic parameters using WinNonlin (Version 4.0.1, Pharsight Corporation, Mountain View, CA) and standard methods.

Pharmacodynamic assays. Additional secondary objectives of the study were to assess tumor blood flow and vascular permeability with DCE-MRI and tumor metabolism using FDG-PET. DCE-MRI scanning was planned at days 0 (e.g., day−7 to pre-administration), immediately after administration (e.g., 24 h), and at days 7 and 49; whereas FDG-PET was to be done before study agent administration and at days 7 and 49.

DCE-MRI. Tumors were imaged by MRI on a Philips 1.5 T Intera system. The DCE-MRI protocol has been described previously (17). The location of the tumor to be studied using DCE-MRI was ascertained from pretreatment CT scans. The MRI protocol consisted of localizer acquisitions, followed by axial T_1-weighed fast-field echo (FFE; gradient echo) and T_2-weighed fast-spin echo volumetric acquisitions covering the tumor region. The DCE-MRI acquisition series was followed with a final axial postcontrast T_1-weighed FFE acquisition. The DCE-MRI protocol consisted of three-dimensional RF-spoiled FFE acquisitions with a temporal resolution of ~4 s. Omniscan (Amersham Health, Amersham, United Kingdom), 0.1 mmol/kg, was administered after the fifth dynamic time point as a bolus using a power injector (Spectris MR) at a rate of 3 mL/s.

During the first patient visit (predose), a screening examination was done to identify any possible metastatic deposits in the brain. This consisted of a postcontrast T_1-weighed FFE acquisition.

DCE-MRI data analysis. Tumor volumes were defined using two-dimensional regions of interest on each slice location containing the target tumor on the precontrast T_2-weighed images when images from

all visits were available. Each voxel within the tumor was identified as enhancing if the signal intensity rose above 3 SD of the data noise level during the dynamic series; otherwise, it was classified as nonenhancing, and no DCE-MRI parameters were extracted for that voxel. The proportion of each tumor that was classified as enhancing was recorded. Average DCE-MRI parameter values calculated within the enhancing volume included initial area under the tissue contrast agent concentration-time curve (IAUC) over 60 s from bolus arrival at the tumor, blood plasma volume (v_p), extracellular interstitial space volume (v_e), and volume transfer coefficient between these spaces (K^{trans}; refs. 18–21).

The change in tissue signal intensity due to contrast agent over time was converted into estimates of contrast agent concentration via estimation of T1 (22, 23). Reproducibility of the MRI and tumor volume parameters was estimated by measuring change between visit 1 (predose) and visit 3 (day 7) in dosing cohorts 1 to 4 (0.1-3.0 mg/kg; refs. 24, 25) when serum CNTO 95 levels were undetectable using either absolute or relative differences between visits depending on the parameter (26, 27). This method of estimating reproducibility may give an overestimate of the variance, in that delayed or residual effects of prior CNTO 95 administration may contribute to the variance.

FDG-PET. Whole-body FDG-PET was done on fasted patients on a GE Advance (General Electric Medical Systems, Milwaukee, WI) PET scanner (28). Standardized uptake value and effective glycolytic volume (EGV, the product of average standardized uptake value and tumor volume) were determined on regions of interest as a measure of tumor cell viability (29). For repeat studies, normalized EGV values used the baseline region-of-interest threshold, and both the change in the sum of all lesions ($\Delta\Sigma EGV_{norm}$) and the mean of the changes across all lesions (mean ΔEGV_{norm}) were computed. Changes of $\pm 10\%$ were deemed significant.

Immunohistochemical analysis of angiosarcoma biopsy. Tumor biopsies were not required for study entry or during the study. However, on an ad hoc basis, one patient with cutaneous angiosarcoma underwent a biopsy following study agent administration that was compared with a previous diagnostic biopsy. The tissues were stained for CNTO 95, α_v integrin (CD51), β_3 integrin (CD61), β_1 integrin (CD29), and downstream signaling molecules, such as bcl-2, related to the α_v integrin complex.

Table 1. Baseline patient characteristics

	N = 24
Gender, *n* (%)	
Male	12 (50.0)
Female	12 (50.0)
Age (y)	
Median (minimum, maximum)	61.5 (37, 76)
Tumor types, *n* (%)	
Colorectal	9 (37.5)
Ovarian	6 (25.0)
Angiosarcoma	2 (8.3)
Ureteric	1 (4.2)
PNET	1 (4.2)
Pseudomyxoma peritonei	1 (4.2)
Endometrial	1 (4.2)
Melanoma	1 (4.2)
Renal	1 (4.2)
Carcinosarcoma	1 (4.2)
Prior chemotherapy, *n* (%)	
No therapy	3 (12.5)
1 line	4 (16.7)
2 lines	7 (29.2)
>2 lines	10 (41.7)
Prior radiotherapy, *n* (%)	8 (33.3)
Other prior treatments, *n* (%)	
Antiangiogenic (>1 y before)	1 (4.2)
Hormonal	4 (16.7)
Immunotherapy	1 (4.2)
Epidermal growth factor receptor inhibitors	1 (4.2)

Abbreviation: PNET, primitive neuroectodermal tumors.

Table 2. Number of patients with possibly or probably related adverse events by maximum toxicity grade

Event	Grade 1	Grade 2	Grade 3	Grade 4	Total
Fever*	8	4	1	0	13
Rigors*	10	1	0	0	11
Headache*	3	7	0	0	10
Somnolence	6	3	0	0	9
Vomiting	3	2	0	0	5
Nausea	3	1	0	0	4
Hematuria	3	0	0	0	3
Prothrombin decreased	3	0	0	0	3
Abdominal pain	1	1	0	0	2
Albuminuria	2	0	0	0	2
EKG abnormal	2	0	0	0	2
Hemorrhage nos	2	0	0	0	2
Anemia	0	1	0	0	1
Anorexia	1	0	0	0	1
Coagulation time Increased	1	0	0	0	1
Concentration impaired	1	0	0	0	1
Creatine phosphokinase Increased	1	0	0	0	1
Diarrhea	1	0	0	0	1
Fatigue	0	1	0	0	1
Myocardial ischemia	1	0	0	0	1
Thrombophlebitis deep	0	0	1	0	1
Tremor	1	0	0	0	1
Urticaria	1	0	0	0	1

Abbreviation: nos, not otherwise specified.
*Probably related.

Results

Patient characteristics. Twenty-four patients were enrolled into the study. Seven patients received 0.1 mg/kg; three patients each received 0.3 mg/kg, 1.0 mg/kg, or 3.0 mg/kg; and eight patients received 10.0 mg/kg. One subject in the 0.1 mg/kg cohort, one in the 1.0 mg/kg cohort, and two in the 10.0 mg/kg cohort were not evaluable for DLT because they received less than the required three infusions and did not experience DLTs. The 0.1 mg/kg cohort was expanded to six DLT-evaluable subjects as the first subject experienced a DLT. The 10.0 mg/kg cohort was also expanded to six DLT-evaluable subjects to further characterize the safety of the maximum dose used in the study, a potential phase 2 dose. The most common tumor types were colorectal and ovarian. The majority of patients had previously received at least two lines of chemotherapy (Table 1).

Toxicity. CNTO 95 was generally well tolerated. The number of patients with reasonably related adverse events (i.e., possibly, probably, or definitely related) are shown in Table 2. Except as discussed specifically below, these events were considered only possibly related. There was a dose-related increase in the

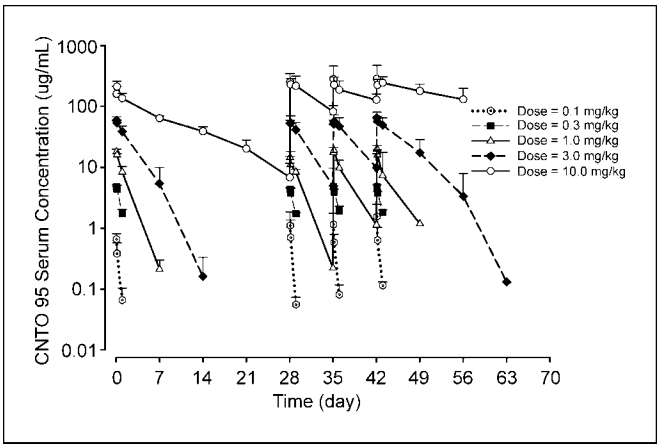

Fig. 1. Mean (SD) concentration-time profiles following CNTO 95 i.v. infusions on days 0, 28, 35, and 42.

occurrence of fever, chills, and headache within 8 h of study agent administration; grade 3 fever occurred in one patient in the 10.0 mg/kg cohort. However, these toxicities were easily treated with acetaminophen or ibuprofen. Due to the temporal association of these events with infusion and their increasing frequency and severity with increasing CNTO 95 dose, they were felt to be definitely related.

One patient developed a deep vein thrombosis of the leg 17 days after administration of the first dose of CNTO 95 (0.1 mg/kg) at a time when CNTO 95 serum concentration was below the limit of detection. This patient had an advanced ureteric carcinoma with pelvic disease that compressed the iliac vessels. This event was deemed possibly attributable to the study agent, despite the confounding iliac compression and timing after CNTO 95 was undetectable in serum. The cohort was subsequently expanded to six DLT-evaluable patients, with no additional episodes of related thrombosis. A second patient developed an axillary vein thrombosis within 4 days of the peripheral insertion of a central catheter and administration of 0.01 mg/kg (one tenth of the intended dose) of CNTO 95. This event was attributed to the catheter insertion.

Preclinical investigations indicated transient asymptomatic anterior uveitis in nonhuman primates receiving i.v. or intravitreal administration of CNTO 95. However, slit lamp examination of four patients at the top dose level did not show ophthalmic toxicity. One patient reported transient blurring of vision at the start of treatment, but testing did not reveal any abnormalities; these symptoms did not recur with continuation of therapy for 10 months. This event was assessed as unrelated to the study drug.

Although there were some minor nonspecific EKG abnormalities (Table 2), none were deemed to be clinically significant changes by a blinded independent cardiologist. These events were judged as possibly related.

Pharmacokinetics. The mean serum concentration levels over time following all infusions are summarized in Fig. 1. The pharmacokinetic findings following the first infusion (Table 3) indicate that drug exposure increased in a greater-than-dose-proportional manner over the dose range 0.1 to 10.0 mg/kg. The initial rapid clearance from serum is probably due to tissue binding at low doses (≤3.0 mg/kg), whereas at the 10.0 mg/kg dose, the agent is cleared more slowly, indicating saturation of tissue binding. Following four infusions, apparent drug accumulation was observed only in the 3.0 and 10.0 mg/kg dose groups; however, steady state was not reached.

FDG-PET. Of the 19 patients that received all four doses of CNTO 95, 12 had FDG-PET scans done on days 7 and 49. The day 7 FDG-PET scan had 92% sensitivity for progressive disease detected by CT scanning at day 49. Positive and negative predictive values and specificity for disease progression could not be ascertained because nearly all patients progressed. Complete FDG-PET data were obtained in four patients in the 0.1 mg/kg cohort, and two, one, one, and four patients in the 0.3, 1.0, 3.0, and 10.0 mg/kg cohorts, respectively. In one patient with ovarian carcinosarcoma who had stable disease on CT scan (10.0 mg/kg), the lesion was no longer detectable on FDG-PET at day 49. One patient with a partial response in the 10.0 mg/kg cohort refused FDG-PET studies. No dose-dependent trends were observed.

DCE-MRI. Five patients contributed DCE-MRI data in the 0.1 mg/kg cohort, and two, three, three, and four patients in the 0.3, 1.0, 3.0 and the 10.0 mg/kg cohorts, respectively. Tumors assessed by DCE-MRI were classified as "MR stable" or "MR progressive," depending on whether the day 49 tumor volume by MR was within the 95% confidence interval for no change in tumor volume from baseline. Proportional enhancing tumor

Table 3. Mean (SD) pharmacokinetic parameters following the first CNTO 95 i.v. infusion

Dose	C_{max} (μg/mL)	AUC (μg·day/mL)	$t^{1/2}$ (day)	CL (mL/day/kg)	Vz (mL/kg)
0.1 mg/kg, *N*	6	4	4	4	4
Mean (SD)	0.65 (0.15)	0.32 (0.09)	0.26 (0.02)	326.19 (85.81)	122.67 (25.58)
0.3 mg/kg, *N*	3	3	3	3	3
Mean (SD)	4.76 (0.38)	4.66 (0.47)	0.62 (0.02)	64.90 (7.00)	58.57(7.75)
1 mg/kg, *N*	3	2	2	2	2
Mean (SD)	17.80 (1.72)	42.77 (2.23)	1.08 (0.08)	23.41 (1.22)	36.75 (0.94)
3 mg/kg, *N*	3	3	3	3	3
Mean (SD)	58.54 (8.67)	196.52 (62.11)	1.51 (0.23)	16.28 (4.91)	34.92 (9.89)
10 mg/kg, *N*	8	8	8	8	8
Mean (SD)	225.95 (21.91)	1,504.27 (171.09)	6.70 (2.42)	6.74 (0.83)	63.33 (17.65)

NOTE: C_{max}, maximum observed CNTO 95 concentration; AUC, area under the concentration-time curve for CNTO 95; $t^{1/2}$, half-life; CL, clearance; Vz, volume of distribution.

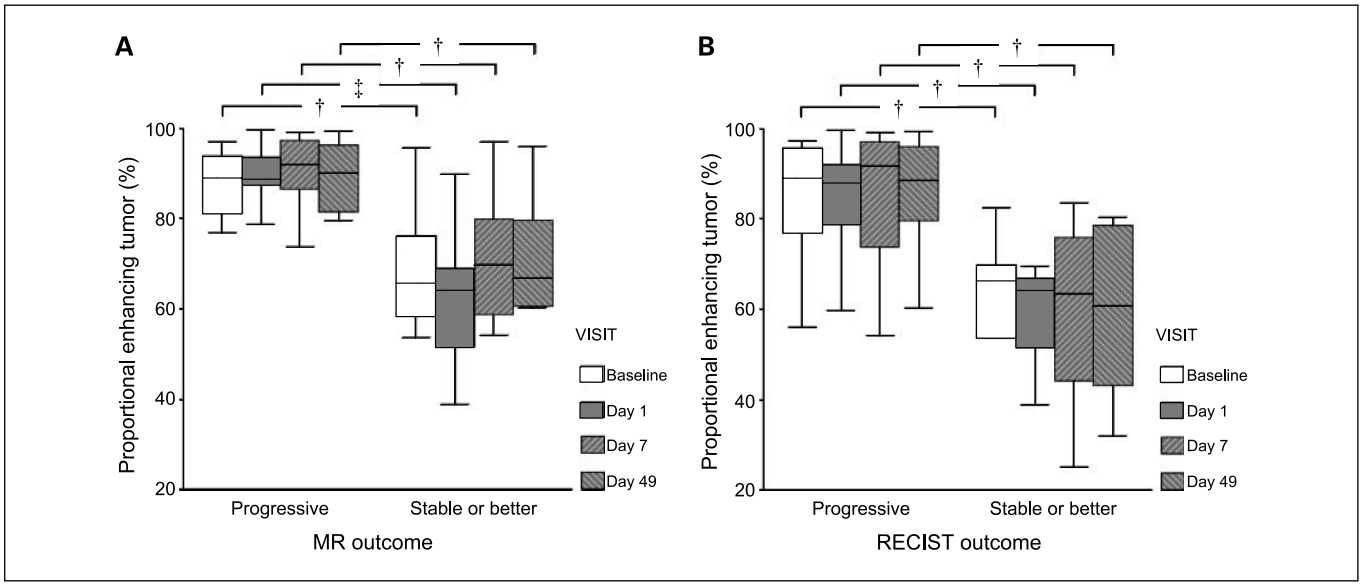

Fig. 2. DCE-MRI. Proportional enhancing tumor volume, as assessed using DCE-MRI, at each patient visit. Clear differences between patient groups demonstrating progressive and stable disease are shown. *A,* progression/stable disease, as assessed by MR at day 49. *B,* progression/stable disease, as assessed by CT, according to RECIST criteria. †, significant at $P < 0.05$; ‡, significant at $P < 0.01$ (Mann-Whitney two-tailed *U* test).

volume was significantly lower in the stable group than in the progressive group, whether classified using MR (Fig. 2A) or using RECIST criteria on CT (Fig. 2B). These findings support the potential prognostic value of this parameter. No significant differences or trends in outcome by dose were observed for any summary DCE-MRI parameter (K^{trans}, v_e, v_p, IAUC, or enhancing volume).

Clinical response. At day 49, stable disease was recorded in six patients (pt. 008, 009, 011, 014, 019, and 021). Four patients with ovarian cancer receiving CNTO 95 0.3 (008), 1.0 (011), 1.0 (014), and 10.0 (021) mg/kg had a stable disease for ~9, 4, 3, and 7 months, respectively, based on the CT findings. Two additional patients had stable disease, one with primary peritoneal adenocarinoma (009; 9 months at 0.3 mg/kg), and one with renal cell cancer (019; 5 months at 10.0 mg/kg). However, all but one patient (014 who showed disease progression) had stable disease or better for 1 to 7 months preceding study entry: 008, 011, 019, and 021 had stable disease for 1, 3, 2, and 5 months, respectively, and 009 had a partial response for 7 months.

One patient with previously untreated, progressive, cutaneous angiosarcoma responded to CNTO 95. The patient was a 61-year-old woman who had undergone a wide local excision in 1995 for a grade III infiltrating ductal carcinoma with no nodal involvement. She received adjuvant chemotherapy, radiotherapy, and tamoxifen for 5 years. In June 2003, the patient developed cutaneous bruising in the ipsilateral breast. Biopsies revealed Trojani grade III angiosarcoma and led to a mastectomy in December 2003. In July 2004, the disease recurred, and the patient was enrolled. A RECIST partial response was achieved, with a 66% reduction in the sum of the longest tumor dimensions. In February 2005, after 10 doses of CNTO 95, the main lesion was excised. The patient continued to receive CNTO 95 10.0 mg/kg every 2 weeks; the tumor progressed, relative to postexcision measurements, after 10.5 months of total treatment (9-month duration of response). Treatment with CNTO 95 was well tolerated by this patient.

Immunohistochemistry of angiosarcoma. A surgically removed post-treatment sample from the cutaneous angiosarcoma–responsive patient was compared by immunohistochemical analysis with the original diagnostic biopsy (Fig. 3) and with other control tumor samples for the expression of integrins, CNTO 95 penetration, and downstream integrin signaling markers.

Diffuse cytoplasmic tumor and vessel expression of the α_v integrins was observed in angiosarcoma tissue (Fig. 3C), in contrast to more variable α_v expression in a colon cancer sample (Fig. 3D). The expression of β_3 as shown in Fig. 3E indicates that tumor cells from the pretreatment angiosarcoma tissue were nonreactive, although polymorphonuclear cells were reactive in blood vessels within the tumor. Reactive polymorphonuclear cells were extremely abundant in angiosarcoma tissue treated with CNTO 95 (Fig. 3F) and were strongly positive for CD61 (glycoprotein IIIa [β_3]) staining. Diffuse staining surrounding these cells suggested that the target antigen might be secreted. No apparent differences in CD29 (β_1 integrin) were observed in untreated and CNTO 95–treated angiosarcoma tissue. Both samples showed strong membrane staining in a large percentage of tumor cells and in the stroma, including endothelial cells of the blood vessels.

CNTO 95 binding using a rabbit polyclonal anti-idiotype antibody in the angiosarcoma tissue showed no detectable staining in the pretreatment sample (Fig. 3G). However, in the post-treatment tumor sample (Fig. 3H), strong CNTO 95 cytoplasmic reactivity was detected in a subset of tumor cells, along with cells of possible macrophage or lymphoid origin. The cytoplasmic staining of CNTO 95 in post-treatment tumor samples is perhaps indicative of CNTO 95 internalization. Tumor cell staining was variable throughout the tumor and was generally present in focused areas positioned at the periphery of the tumor adjacent to large blood vessels often filled with polymorphonuclear cells.

Cell survival on extracellular matrices like fibronectin and vitronectin involves the expression of proteins such as bcl-2, a proto-oncogene that inhibits apoptosis. The integrin-mediated regulation of bcl-2 has been shown to be Shc adaptor protein and focal adhesion kinase dependent (30), and therefore, we examined the expression of bcl-2 in biopsies obtained before and post-CNTO 95 treatment to determine if the expression of bcl-2 would be altered. Bcl-2, a marker of integrin signaling, was distinctly stronger and present in a greater percentage of tumor cells in pretreatment samples (Fig. 3I) when compared with post-treatment samples (Fig. 3J). Lymphoid cells, in contrast, labeled strongly in both pre- and post-treatment samples.

Overall, these findings indicate that CNTO 95 is able to penetrate and bind to the α_v integrin target in the tumor, and its binding may be associated with a concomitant decrease in tumor cell–associated bcl-2 expression.

Discussion

This was the first phase I evaluation of CNTO 95, a fully human monoclonal antibody against α_v integrins. CNTO 95

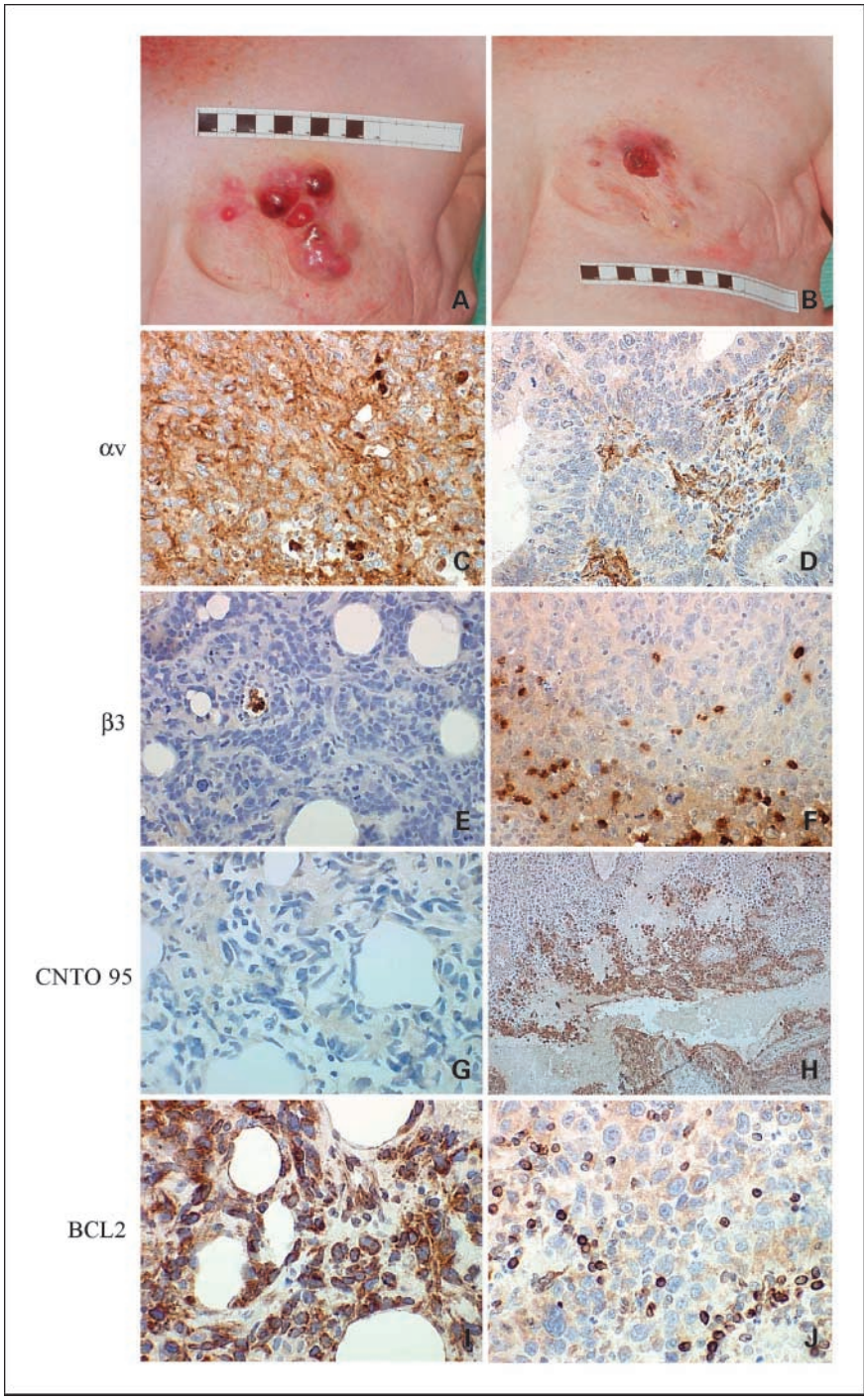

Fig. 3. Cutaneous response in angiosarcoma. Patient's disease before treatment (*A*) and after 4 months of 10 mg/kg of CNTO 95 (*B*). The visible lesion was then removed and examined via immunohistochemical analysis. The tumor expressed the α_v integrin uniformly (*C*) in contrast to a sample of colon cancer used as a control (*D*). Expression of β_3 integrin, a frequent heterodimeric partner of α_v integrin, was not seen in the pretreatment sample (*E*) and was only seen in infiltrating neutrophils after treatment (*F*). CNTO 95 was not found in the tumor tissue before treatment (*G*)*, but penetrated the tumor after treatment (*H*)*, and pretreatment levels of bcl-2 expression (*I*)*, were reduced with CNTO 95 treatment (*J*)*.
*, magnification 60×.

was generally safe and well tolerated. The most prominent toxicities were fever, chills, and headache, which were controlled with acetaminophen or ibuprofen. No other clearly related toxicities were observed. Fever has also been observed with a humanized monoclonal antibody directed against the $\alpha_v\beta_3$ integrins (31), but was not observed with the peptide α_v integrin antagonist cilengitide (15). In contrast to VEGF inhibitors, we did not observe any treatment-related hypertension, proteinuria, thrombosis, or hemorrhage. One episode of deep venous thrombosis, in the lowest dose cohort, was conservatively assessed as possibly related to study agent, despite CNTO 95 being no longer detectable in serum and tumor-related obstruction of the affected vessel. This cohort was subsequently expanded to include six DLT-evaluable patients, with no further DLTs observed during the study.

No MTD was determined in the study. This is a common occurrence with targeted agents, which are often less toxic than traditional agents. Drug exposure increased in a greater-than-dose-proportional manner over the range evaluated. At low dose levels (0.1-3 mg/kg), the initial rapid clearance from serum is probably due to saturable binding to a normal tissue sink, a phenomenon which has also been observed with the humanized monoclonal antibody directed against the $\alpha_v\beta_3$ integrins (15). Furthermore, the observed half-life (8-9 days) of CNTO 95 is consistent with other antibody therapeutics such as infliximab (32).

Although pharmacokinetic studies indicate saturation of the sink at the 10.0 mg/kg dose, pharmacodynamic studies did not provide a definitive answer as to whether this dose was optimal. In addition, the degree of tissue penetration into tumor masses cannot be inferred from serum pharmacokinetics. In a review of phase I studies of targeted agents, it was observed that pharmacokinetic data alone are rarely sufficient to determine an optimal biological dose (33). Additional clinical trials evaluating higher doses of CNTO 95 with systematic assessment of pharmacodynamic end points in tumor tissue are planned.

A prolonged partial response was observed in a patient with angiosarcoma, a tumor of malignant endothelial cells. As indicated by the immunohistochemical analysis data, this response may have been partially dependent on α_v integrins. The lack of β_3 integrin expression in these tumor cells suggests that another β integrin might interact with the α_v integrins in this tissue, but could also reflect expression of β_3 integrin below the level of detection. Additional evaluations are needed to determine whether CNTO 95, unique in that it binds to all α_v integrins, can be effective in α_v-expressing tumors irrespective of the type of β subunit expressed. Immunohistochemical analysis also showed that CNTO 95 penetrated into the tumor and decreased bcl-2 expression after treatment, which may impact caspase activation and apoptosis (34). In addition to evaluating the expression of $\alpha_v\beta_3$ and binding of CNTO 95, a number of markers of integrin signaling were evaluated from tumor biopsies using immunohistochemistry. The additional markers, associated with proliferation and apoptosis signaling, included bax, pMAPK, Ki-67, cleaved caspase-3, and intact caspase-9. Results of these analyses will be presented in a future publication.

The expression of these markers was also evaluated in various tumor types obtained from a tumor tissue bank. However, only one post–CNTO 95 treatment biopsy was available for evaluation. Tumor biopsies were not required during the study, and the immunohistochemical results were obtained from a single patient who provided the biopsy sample on an ad hoc basis. The pretreatment sample was obtained from a diagnostic biopsy that had been obtained at the time of the initial diagnosis of angiosarcoma; therefore, differences between the pre- and post-treatment biopsies may not solely reflect treatment effects, but could also reflect the evolution of the tumor between diagnosis and baseline just before treatment. Thus, the results from this biopsy are simply hypothesis generating and will be further confirmed in a planned study where paired tumor biopsies before and after treatment will be required. In addition, in ongoing phase II work, diagnostic biopsies are being collected and tested for target expression.

In this study, a partial response was observed in one patient with angiosarcoma, suggesting that further investigation of CNTO 95 in this disease may be warranted. Several patients with ovarian cancer achieved a stable disease; however, because they entered the study with stable disease, this is not viewed as a compelling tumor-specific signal. Preclinical target expression data indicate potential utility for CNTO 95 in a broad range of epithelial tumors, and therefore, a broad phase II program is indicated to obtain preliminary efficacy data in multiple tumor types.

FDG-PET and DCE-MRI scans were done for the majority of patients. A complete FDG-PET response was observed in a patient with ovarian carcinosarcoma whose disease remained stable for 6 months while receiving CNTO 95. Results of FDG-PET scan indicated high sensitivity in early detection of disease progression in the current study. Several DCE-MRI parameters were measured and compared with single lesion MRI outcome and RECIST assessment of total disease.

No clear changes in DCE-MRI parameters were observed that could be related to CNTO 95 dose or therapy. Consistent with our findings, in a phase I study of an anti–$\alpha_v\beta_3$ antagonist employing dynamic CT, McNeel et al. (31) showed no changes in tumor vascular permeability. A greater proportional enhancing tumor volume on DCE-MRI imaging was noted in patients with progressive disease when compared with those with stable outcome. This was noted at all time points both before and following study agent administration (Fig. 2), indicating this was not a drug-related effect. We interpret the differences in proportional enhancing tumor volume between the groups as possibly reflecting differences in viable vascular supply to the tumors. This would imply that the progressive group had, on average, a greater proportion of well-perfused tumor tissue, which may relate to a greater growth potential.

Several other agents targeted to α_v integrins are in clinical development, including an antibody targeted to $\alpha_v\beta_3$ integrin (Abegrin, Medimmune, Inc.; refs. 15, 35–41) and Cilengitide (EMD 121974, Merck KGaA, Darmstadt, Germany), a cyclic peptide targeted to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins (14, 42–49). A phase II study of Abegrin, with or without dacarbazine chemotherapy, was recently completed in patients with melanoma. Median survival on the single-agent Abegrin arm was 12 months, compared with 8 to 9 months for historical controls. However, comparison to historical controls can be misleading due to potential effects of patient selection. Patients in the combination arm had survival comparable to historical controls and a response rate of 13%, similar to that achieved with dacarbazine alone. Abegrin is being studied in a variety of other solid tumors. Cilengitide has shown particular promise in malignant glioma where two complete responses, three partial responses, and four patients with stable disease for more than

6 months were observed in a phase I study of 51 adult patients with glioma. Cerebral perfusion data by MRI correlated with response status. In addition, a case report of a response in head and neck cancer has appeared (50).

Compared with these other agents, CNTO 95 is in an earlier phase of development and has a broader specificity for the α_v integrins. Given its broad specificity, it is encouraging that CNTO 95 is well tolerated. The partial response observed in this study occurred in a patient whose tumor did not express $\alpha_v\beta_3$ integrin, but did express $\alpha_v\beta_1$ integrin. A phase II program is required to determine if the broader binding specificity of CNTO 95 can translate into clinical activity.

In summary, CNTO 95, the first fully human monoclonal antibody with broad binding specificity for the α_v integrins, was well tolerated in this initial study and produced a 9-month partial response in an angiosarcoma patient. Immunohistochemistry findings indicated an association of CNTO 95 penetration in the tumor with a reduction in bcl-2 expression. Further studies of CNTO 95 are warranted in patients with malignancies involving α_v integrin expression.

References

1. 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.
2. 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.
3. Giantonio BJ, Catalano PJ, Meropol NJ, et al. High-dose bevacizumab improves survival when combined with FOLFOX4 in previously treated advanced colorectal cancer: results from the Eastern Cooperative Oncology Group (ECOG) study E3200 [abstract]. J Clin Oncol 2005;23:2.
4. Jin H, Varner J. Integrins: roles in cancer development and as treatment targets. Br J Cancer 2004;90:561–5.
5. 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.
6. Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin $\alpha_v\beta_3$ for angiogenesis. Science 1994;264:569–71.
7. Friedlander M, Brooks PC, Shaffer RW, Kincaid CM, Varner JA, Cheresh DA. Definition of two angiogenic pathways by distinct α_v integrins. Science 1995;270:1500–2.
8. Hood JD, Cheresh DA. Role of integrins in cell invasion and migration. Nat Rev Cancer 2002;2:91–100.
9. Guo W, Giancotti FG. Integrin signaling during tumour progression. Nat Rev Mol Cell Biol 2004;5:816–26.
10. Mitjans F, Meyer T, Fittschen C, et al. *In vivo* therapy of malignant melanoma by means of antagonists of α_v integrins. Int J Cancer 2000;87:716–23.
11. Trikha M, Zhou Z, Nemeth JA, et al. CNTO 95, a fully human monoclonal antibody that inhibits α_v integrins, has antitumor and antiangiogenic activity *in vivo*. Int J Cancer 2004;110:326–35.
12. Rolli M, Fransvea E, Pilch J, Saven A, Felding-Habermann B. Activated integrin $\alpha_v\beta_3$ cooperates with metalloproteinase MMP-9 in regulating migration of metastatic breast cancer cells. Proc Natl Acad Sci U S A 2003;100:9482–7.
13. Natali PG, Hamby CV, Felding-Habermann B, et al. Clinical significance of $\alpha_v\beta_3$ integrin and intercellular adhesion molecule-1 expression in cutaneous malignant melanoma lesions. Cancer Res 1887;57:1554–60.
14. Eskens FA, Dumez H, Hoekstra R, et al. Phase I and pharmacokinetic study of continuous twice weekly intravenous administration of Cilengitide (EMD 121974), a novel inhibitor of the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ in patients with advanced solid tumours. Eur J Cancer 2003;39:917–26.
15. 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.
16. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000;92:205–16.
17. Parker GJM, Roberts C, Macdonald A, et al. An experimentally-derived functional form for a population-averaged high temporal resolution arterial input function for dynamic contrast-enhanced MRI. Magn Reson Med 2006;56:993–1000.
18. Evelhoch JL. Key factors in the acquisition of contrast kinetic data for oncology. J Magn Reson Imaging 1999;10:254–9.
19. Kety SS. The theory and applications of the exchange of inert gas at the lungs and tissues. Pharmacol Rev 1951;3:1–41.
20. Tofts PS, Brix G, Buckley DL, et al. Estimating kinetic parameters from dynamic contrast-enhanced T(1)-weighted MRI of a diffusable tracer: standardized quantities and symbols. J Magn Reson Imaging 1999;10:223–32.
21. Daldrup HE, Shames DM, Husseini W, Wendland MF, Okuhata Y, Brasch RC. Quantification of the extraction fraction for gadopentetate across breast cancer capillaries. Magn Reson Med 1998;40:537–43.
22. Haase A. Snapshot FLASH MRI. Applications to T1, T2, and chemical shift imaging. Magn Reson Med 1990;13:77–89.
23. Buckley DL, Parker GJM. Measuring contrast agent concentration in T1-weighted dynamic contrast-enhanced MRI. In: Jackson A, Buckley DL, Parker GJM, editors. Dynamic contrast enhancement techniques in oncology. Berlin: Springer-Verlag; 2004. p. 69–80.
24. Leach MO, Brindle KM, Evelhoch JL, et al. Assessment of antiangiogenic and antivascular therapeutics using MRI: recommendations for appropriate methodology for clinical trials. Br J Radiol 2003;76:S87–91.
25. Jayson G, Waterton JC. Applications of dynamic contrast-enhanced MRI in oncology drug development. In: Jackson A, Buckley D, Parker GJM, editors. Dynamic contrast-enhanced magnetic resonance imaging in oncology. Heidelberg: Springer; 2004.
26. Galbraith SM, Lodge MA, Taylor NJ, et al. Reproducibility of dynamic contrast-enhanced MRI in human muscle and tumours: comparison of quantitative and semi-quantitative analysis. NMR Biomed 2002;15:132–42.
27. Roberts C, Issa B, Stone A, Jackson A, Waterton JC, Parker GJM. Comparative study into the robustness of compartmental modeling and model-free analysis in DCE-MRI studies. J Magn Reson 2006;23:554–63.
28. DeGrado TR, Turkington TG, Williams JJ, Stearns CW, Hoffman JM, Coleman RE. Performance characteristics of a whole-body PET scanner. J Nucl Med 1994;35:1398–406.
29. Nakamoto Y, Zasadny KR, Minn H, Wahl RL. Reproducibility of common semi-quantitative parameters for evaluating lung cancer glucose metabolism with positron emission tomography using 2-deoxy-2-[^{18}F]fluoro-D-glucose. Mol Imaging Biol 2002;4:171–8.
30. Matter ML, Ruoslahti E. A signaling pathway from the $\alpha_5\beta_1$ and α_v β_3 integrins that elevates bcl-2 transcription. J Biol Chem 2001;276:27757–63.
31. McNeel DG, Eickhoff J, Lee FT, et al. 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. Clin Cancer Res 2005;11:7851–60.
32. Remicade [package insert]. Malvern, PA: Centocor, Inc; 2006.
33. Parulekar WR, Eisenhauer EA. Phase I trial design for solid tumor studies of targeted, non-cytotoxic agents: theory and practice. J Natl Cancer Inst 2004;96:990–7.
34. Kim R. Recent advances in understanding the cell death pathways activated by anticancer therapy. Cancer 2005;103:1551–60.
35. Mikecz K, Vitaxin applied molecular evolution. Curr Opin Investig Drugs 2000;1:199–203.
36. Faivre SJ, Chieze S, Marty M, et al. Safety profile and pharmacokinetic analysis of medi-522, a novel humanized monoclonal antibody that targets $\alpha_v\beta_3$ integrin receptor, in patients with refractory solid tumors [abstract 832]. Proc Am Soc Clin Oncol 2003;22.
37. Pizzolato JF, Sharma S, Maki R, Krug M, Hammershaimb L, Pluda J. Phase I study of medi-522, an $\alpha_v\beta_3$ integrin inhibitor, in patients (pts) with irinotecan-refractory colorectal cancer (CRC) [abstract 983]. Proc Am Soc Clin Oncol 2003;22.
38. Wilder RL. Integrin $\alpha_v\beta_3$ as a target for treatment of rheumatoid arthritis and related rheumatic diseases. Ann Rheum Dis 2002;61:ii96–9.
39. Hersey P, Sosman J, O'Day S, et al. A phase II, randomized, open-label study evaluating the antitumor activity of medi-522, a humanized monoclonal antibody directed against the human $\alpha_v\beta_3$ (avb3) integrin, ± dacarbazine (DTIC) in patients with metastatic melanoma (MM). J Clin Oncol, 2005. American Society of Clinical Oncology Annual Meeting Proceedings 2005;23:16S part I of II: 7507.
40. 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.
41. 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.
42. Nabors LB, Rosenfeld SS, Mikkelson T. A phase I trial of EMD 121974 for treatment of patients with recurrent malignant gliomas [abstract 236]. J Neuro Oncol 2002;4.
43. Tucker GC. α_v integrin inhibitors and cancer therapy. Curr Opin Investig Drugs 2003;4:722–31.
44. Lode HN, Moehler T, Xiang R, et al. Synergy between an antiangiogenic integrin α(v) antagonist and an antibody-cytokine fusion protein eradicates spontaneous tumor metastases. Proc Natl Acad Sci U S A 1999;96:1591–6.
45. MacDonald TJ, Taga T, Shimada H, et al. Preferential susceptibility of brain tumors to the antiangiogenic effects of an α(v) integrin antagonist. Neurosurgery 2001;48:151–7.
46. Raguse JD, Gath HJ, Bier J, Riess H, Oettle H. Cilengitide (EMD 121974) arrests the growth of a heavily pretreated highly vascularised head and neck tumour. Oral Oncol 2004;40:228–30.
47. Smith JW. Cilengitide Merck. Curr Opin Investig Drugs 2003;4:741–5.
48. Nabors LB, Fiveash J. Treatment of adults with recurrent malignant glioma. Expert Rev Neurother 2005;5:509–14.
49. Nabors LB, Rosenfeld SS, Mikkelsen T, et al. TA-39. NABTT 9911: A phase 1 trial of EMD 121974 for treatment of patients with recurrent malignant gliomas. Neuro-oncol 2004;6:307.
50. Raguse JD, Gath HJ, Bier J, Riess H, Oettle H. Cilengitide (EMD 121974) arrests the growth of a heavily pretreated highly vascularised head and neck tumour. Oral Oncol 2004;40:228–30.

Clinical Cancer Research

Phase I Evaluation of a Fully Human Anti–α_v Integrin Monoclonal Antibody (CNTO 95) in Patients with Advanced Solid Tumors

Saifee A. Mullamitha, Nhuan C. Ton, Geoff J.M. Parker, et al.

Clin Cancer Res 2007;13:2128-2135.