Published OnlineFirst January 11, 2011; DOI: 10.1158/1078-0432.CCR-10-1411

Clinical Cancer Research

Phase I and Pharmacokinetic Study of CT-322 (BMS-844203), a Targeted Adnectin Inhibitor of VEGFR-2 Based on a Domain of Human Fibronectin

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Abstract

Purpose: To determine the maximum tolerated dose (MTD), safety, pharmacokinetics, pharmacodynamics, immunogenicity, and preliminary antitumor activity of CT-322 (BMS-844203), a VEGFR-2 inhibitor and the first human fibronectin domain–based targeted biologic (Adnectin) to enter clinical studies.

Experimental Design: Patients with advanced solid malignancies were treated with escalating doses of CT-322 intravenously (i.v.) weekly (qw), or biweekly (q2w). Plasma samples were assayed for CT-322 concentrations, plasma VEGF-A concentrations, and antidrug antibodies.

Results: Thirty-nine patients completed 105 cycles of 0.1 to 3.0 mg/kg CT-322 i.v. either qw or q2w. The most common treatment-emergent grade 1/2 toxicities were fatigue, nausea, proteinuria, vomiting, anorexia, and hypertension. Grade 3/4 toxicities were rare. Reversible proteinuria, retinal artery, and vein thrombosis, left ventricular dysfunction, and reversible posterior leukoencephalopathy syndrome were dose limiting at 3.0 mg/kg. The MTD was 2 mg/kg qw or q2w. CT-322 plasma concentrations increased dose proportionally. Plasma VEGF-A levels increased with dose and plateaued at 2 mg/kg qw. Anti–CT-322 antibodies developed without effects on pharmacokinetics, VEGF-A levels, or safety. Minor decreases in tumor measurements occurred in 4 of 34 evaluable patients and 24 patients had stable disease.

Conclusions: CT-322 can be safely administered at 2 mg/kg i.v. qw or q2w and exhibits promising antitumor activity in patients with advanced solid tumors. The absence of severe toxicities at the MTD, demonstration of plasma drug concentrations active in preclinical models, and clinical pharmacodynamic evidence of VEGFR-2 inhibition warrant further development of CT-322 and suggest strong potential for Adnectin-based targeted biologics. *Cancer Res; 17(2); 363–71. ©2011 AACR.*

Introduction

CT-322 is the first in a novel class of targeted protein therapeutics (Adnectin) based on the 10th type III domain of human fibronectin (10Fn3) to enter clinical studies. Adnectins are genetically engineered variants of human fibronectin designed by redirecting the binding characteristics of fibronectin to specific disease targets, such as receptors, ligands, or proteins, while leaving the fibronectin backbone intact. CT-322 selectively binds and inhibits human, monkey, and rodent VEGFR-2 and has preclinical antitumor activity (1). The fibronectin-based protein moiety of CT-322 is linked to a 40-kDa branched polyethylene glycol (PEG) moiety to enhance drug exposure. Adnectins feature rapid speed of discovery with mRNA display, manufacturing ease via production in *Escherichia coli*, and the potential for highly targeted and multispecific (multidomain) molecules (2–4). On the basis of their inherent features, Adnectins may offer important advantages, including enhanced activity, more convenient dosing, improved tissue penetration, broad therapeutic application, and low immunogenicity, over other targeted therapies.

Tumor angiogenesis regulation by VEGFR-2 represents a clinically validated target for antiangiogenic therapies in multiple tumor types. Bevacizumab, a monoclonal antibody against VEGF-A, a ligand of VEGFR-2, is approved as a combination therapy for colorectal cancer (5–8), non–small cell lung carcinoma (NSCLC; refs. 9, 10), breast cancer

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

ClinicalTrials.gov Identifier: NCT00374179

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doi: 10.1158/1078-0432.CCR-10-1411

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Translational Relevance

We report the first investigation affirming the clinical activity of Adnectins, a novel platform of targeted proteins based on the 10th type III domain of human fibronectin. Adnectins are genetically engineered variants of fibronectin designed to bind specific targets while leaving the fibronectin backbone intact. The VEGF/VEGFR-2 pathway represents a clinically validated target for antiangiogenic therapies and is ideally suited to test the applicability of new therapeutic platforms such as the Adnectin platform. CT-322 (BMS-844203), the first Adnectin, was engineered to bind the extracellular domain of VEGFR-2, inhibiting VEGFR-2 signaling. In this phase I study, CT-322 exhibited class-specific adverse events, on target pharmacodynamic biomarker activity, and promising antitumor activity in patients with advanced solid malignancies. These findings suggest that Adnectins are an effective class of protein therapeutics to modulate extracellular targets that have acceptable tolerability, which support ongoing phase II trials with CT-322 and further development of Adnectins.

(11), and renal cell carcinoma (RCC; refs. 12, 13) and as monotherapy in glioblastoma multiforme (14, 15). Following bevacizumab, several small molecule VEGF receptor tyrosine kinase inhibitors (TKI) are in development and others have been approved for the treatment of RCC and hepatocellular carcinoma (16–18).

With VEGFR-2 therapies, irrespective of inhibition mechanism, class-related adverse events (AE) include hypertension, proteinuria, thrombosis, and hemorrhage (19–21). A pharmacodynamic characteristic of monoclonal antibodies is an increase in circulating plasma ligand that accompanies receptor inhibition (22). Thus, elevation of VEGF-A and class-related effects provide correlative biomarkers of clinical activity for protein-based drugs such as CT-322 that inhibit VEGF-A binding to VEGFR-2.

In vitro, CT-322 binds human VEGFR-2 with high-affinity (K_D = 11 nmol/L) without detectable binding to VEGFR-1 or -3 and inhibited proliferation of an engineered murine B-cell line induced by all known activators of VEGFR-2 (VEGF-A, -C, -D; ref. 1). CT-322 blocked migration, tube formation, and VEGF-induced phosphorylation of VEGFR-2 and downstream mitogen-activated protein kinase in human umbilical vascular endothelial cells. Finally, CT-322 inhibited VEGF-induced microvessel permeability *in vivo*, reduced blood vessel density and tumor growth of U87 tumor xenografts, and inhibited the growth of a broad spectrum of human tumor xenografts in mouse models.

Toxicologic studies were performed in rats and monkeys for up to 3 months. Principal target organ toxicity was in the kidney, with drug-induced nephropathy characterized by increased urea nitrogen, creatinine, and urinary protein levels. The no observable AE level for renal injury in 13-week repeat-dose toxicology was 5 and 10 mg/kg per week in rats and monkeys, respectively. VEGF inhibition effects were observed in rats as mean arterial blood pressure increases and in monkeys as dose-related epiphyseal growth plate thickening (23).

The basis for clinical development of CT-322 included both proof of concept and viability of the Adnectin class and assessment of CT-322 as a specific inhibitor of VEGFR-2. Specific VEGFR-2 blockade results in inhibition of all upregulated receptor activators, including VEGF-C and -D, and may have efficacy advantages over selective VEGF-A inhibition. The high specificity of CT-322 may provide a narrower toxicity spectrum and better patient tolerance than broad-spectrum small molecule VEGFR-2 TKIs with significant off-target effects. This phase I study of CT-322 in patients with advanced solid malignancies assessed safety, tolerability, and the maximum tolerated dose (MTD). Secondary objectives included pharmacokinetics, pharmacodynamics, determinations of biological activity, immunogenicity, and preliminary antitumor activity.

Materials and Methods

Patient selection

Eligibility requirements were pathologically confirmed solid malignancies refractory to standard therapy or for which no standard therapy existed, age 18 years or older, life expectancy of 12 weeks or greater, Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, previous chemotherapy completed $\geq$ 4 weeks prior (6 weeks for mitomycin C or nitrosourea), hemoglobin 9 g/dL or greater, absolute neutrophil count 1,500 mL or greater, platelet count 100,000/mL or greater, creatinine and bilirubin levels 1.5 $\times$ upper limit of normal (ULN) or less and calculated creatinine clearance greater than 60 mL/min, serum aspartate and alanine transaminase levels 2.5 $\times$ ULN ($\leq$ 5 $\times$ ULN if hepatic metastases present) or less, amylase and lipase levels less than 1.5 $\times$ ULN, intact coagulation, absence of pregnancy, and 1+ or less proteinuria by dipstick or less than 500 mg urinary protein per 24 hours. Patients were ineligible if they had brain metastases, squamous NSCLC with central chest tumor, major surgery within 28 days, known or active HIV or hepatitis B/C, prior anthracycline therapy or radiotherapy encompassing the heart if current left ventricular ejection fraction less than 50%, prior bone marrow transplant, or coexisting severe medical conditions. Prior anti-VEGF/anti-VEGFR therapy was allowed. All patients gave informed consent to participate in the study. This study was conducted in accordance with the Declaration of Helsinki and applicable guidelines on good clinical practice.

Drug was supplied in 5-mL vials containing 50 mg of CT-322 in 10 mmol/L of sodium acetate, 100 mmol/L of sodium chloride, and 2% mannitol (w/v), pH 4.5, by Adnexus Each injection was prepared with 0.9% sodium chloride USP diluent.

CT-322 was administered intravenously (i.v.), weekly (qw), or biweekly (q2w) by 1-hour infusion without

Published OnlineFirst January 11, 2011; DOI: 10.1158/1078-0432.CCR-10-1411

premedication, with 1 cycle extending 4 weeks. The starting dose of 1.0 mg/kg was one tenth the no observable AE level in the primate toxicologic study and dose escalation did not exceed $^1/_2\log_{10}$. Once the qw MTD was determined, q2w administration was explored. To determine plasma VEGF-A biomarker dose response, 2 low-dose qw cohorts in which 3 and 4 patients received their first 2 doses of 0.1 or 0.3 mg/kg, respectively, were enrolled. These patients subsequently received 1 mg/kg beginning with their third qw dose.

Cohorts of 3 patients each were enrolled initially; however, if dose-limiting toxicity (DLT) occurred, the cohort was expanded to 6. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events, Version 3.0. DLT was defined as any grade 3 or higher hematologic toxicity and any grade 3/4 nonhematologic event except grade 3 hypertension responsive to antihypertensive therapy or grade 3 laboratory AEs that were asymptomatic and reversible to baseline or grade 1 within 7 days. Grade 3/4 proteinuria was considered a DLT, as was any treatment delay due to toxicity lasting 2 or more weeks. The MTD was the highest dose at which less than 33% of patients experienced treatment-related DLTs during cycle 1.

Pretreatment and follow-up studies

A complete history, physical examination, ECOG performance status, and laboratory studies including a complete blood count, urinalysis (including proteinuria), and standard blood chemistry analyses were performed qw (first 6 cycles). Vital signs were monitored before infusion, 15 and 30 minutes after beginning infusion, and 15, 30, and 60 minutes postinfusion. Creatine phosphokinase total, myocardial band, and cardiac troponin I or T analyses were performed q2w. HIV and hepatitis B/C serology (at entry), electrocardiogram (pre- and postinfusion on days 1 and 22 of cycle 1; preinfusion on day 1 of cycles 2, 4, and 6; and at termination), and left ventricular ejection fraction (baseline, day 1 of cycles 3 and 5, and at termination) by either multigated acquisition scan or echocardiogram were also completed. Relevant radiological imaging studies and tumor markers were performed before and during treatment, and radiological studies for disease status were repeated after every other cycle. Response was assessed by Response Evaluation Criteria In Solid Tumors (RECIST) 1.0 (24) or International Working Group Criteria for non-Hodgkin's lymphoma (25).

Plasma pharmacokinetic sampling and assay

On cycle 1 and 6, blood was collected prior to infusion; at midpoint; at the end of infusion; and at 10 and 30 minutes and 1, 2, 4, 6, 24, 48, 72, 96, 120, and 168 hours postinfusion on days 1 and 22 (qw) or 1 and 15 (q2w). Trough samples (immediately preinfusion) and peak samples (4 hours postinfusion) were taken on days 8 (qw) and 15 (qw and q2w) of cycle 1 and on day 1 of subsequent cycles. Separated plasma samples were stored at −70°C until assayed.

CT-322 plasma concentrations were determined using a validated ELISA sandwich method that captured CT-322 with a specific murine monoclonal antibody binding the drug receptor–binding region. CT-322, both active and intact, was detected with an anti-PEG antibody (see Supplementary Methods). The lower limit of quantification was 10.36 ng/mL.

Pharmacokinetic and pharmacodynamic analyses

Pharmacokinetic parameters of CT-322 were derived from plasma concentration versus actual time data via noncompartmental analysis using Kinetica software (Version 4.4.1 Thermo Electron Corporation). Dose proportionality was assessed using the power model described by Gough et al. (26). Accumulation, steady-state concentrations, and the relationship between dose and effects on circulating plasma VEGF-A concentrations were also explored.

Plasma evaluation of VEGF-A levels

Blood for plasma VEGF-A was collected preinfusion and 4 hours postinfusion for each infusion during cycle 1, at the first infusion of subsequent cycles, and at termination. VEGF-A plasma levels were determined with a quantitative sandwich electrochemiluminescence assay from Meso Scale Discovery, with a lower limit of quantification of 2.2 pg/mL.

Anti–CT-322 antibody assessment

Blood for determination of anti–CT-322 antibody levels was collected preinfusion on days 1, 15, and 22 of cycle 1; day 1 of subsequent cycles; and 30 days posttreatment. Anti–CT-322 antibodies were detected via ELISA (see Supplementary Methods). Patients with detectable CT-322 antibodies at termination were monitored for antibodies every 2 months as long as feasible.

Results

Patient characteristics and determination of MTD

Thirty-nine patients (Table 1) completed a total of 105 cycles of CT-322 (0.1–3.0 mg/kg i.v. qw or q2w). The number of patients treated, cycles administered at each dose, and the dose escalation scheme are listed in Table 2. The median number of cycles administered per patient was 2 (range = 1–23). Dose reduction occurred twice for AEs: grade 2 proteinuria in 1 patient (starting dose 3 mg/kg qw reduced to 1 mg/kg qw) and in a second patient after determination that 3 mg/kg exceeded the MTD, although no AE requiring reduction occurred in this patient.

Initially, 3 patients were enrolled at 1 mg/kg qw. One patient experienced grade 4 lipase elevation with grade 2 pancreatitis, and the cohort was expanded to 6 without further DLT. At the dose escalation of 3 mg/kg qw, 1 patient experienced grade 3 proteinuria and another grade 3 left ventricular systolic dysfunction (LVSD), indicating that the MTD had been exceeded. Therefore, 8 patients were enrolled at 2 mg/kg qw (2 patients discontinued because of

Published OnlineFirst January 11, 2011; DOI: 10.1158/1078-0432.CCR-10-1411

Table 1. Patient characteristics

Characteristic	No. of patients ($n = 39$ treated, 40 enrolled)
No. of cycles per patient, median (range)	2 (1–23)
Age, median (range), y	58 (31–85)
ECOG performance status	
0	9
1	28
2	2
Males/females	21/18
Previous therapy	
Chemotherapy	37
Radiation therapy	23
Prior antiangiogenic therapy	12
No. of prior chemotherapy regimens, median (range)	5 (1–20)
Tumor types	
Colorectal cancers	10
Prostate cancer	6
Neuroendocrine carcinoma	3
Renal cell carcinoma (collecting duct and clear cell)	2
Adenoid cystic carcinoma; anorectal squamous carcinoma; chondroblastic osteosarcoma; endometrial; gastric cancer-signet ring; Kaposi's sarcoma; laryngeal cancer; lung adenocarcinoma; non–Hodgkin's lymphoma; ovarian cancer, endometrial type; pancreatic cancer; paratesticular rhabdomyosarcoma; signet ring adenocarcinoma (unknown primary); thymus carcinoma; thyroid-squamous cell carcinoma; tonsil-squamous cell carcinoma; transitional cell carcinoma; uterine leiomyosarcoma	1 each

disease progression before the end of cycle 1). No DLT was observed at 2 mg/kg qw. To ascertain whether 3 mg/kg was tolerable with extended administration and to obtain pharmacodynamic biomarker information, a q2w schedule was initiated. One of 6 patients given 3 mg/kg q2w had a DLT of grade 3 proteinuria during the first 4-week cycle. Subsequently, 3 patients developed grade 3/4 AEs (2 with reversible posterior leukoencephalopathy syndrome and 1 with retinal artery and vein thrombosis) within cycles 2 or 3, prohibiting further exploration of this dose.

Table 2. Dose escalation scheme

Dose level, mg/kg	No. of patients			No. of completed cycles	Patients with DLT[a]	
	New	Modified to level[b]	Total		First cycle[c]	All cycles[d]
≤1.0 qw	13	2[c]	15	47	1/13	1/15
2.0 qw	8	1[c]	9	20	0/8	0/9
2.0 q2w	6	1	7	17	0/6	0/7
3.0 qw	6	0	6	10	2/6	2/6
3.0 q2w	6	0	6	11	1/6	4/6
Total	39			105		

[a]DLT or DLT equivalent (AE meeting DLT criteria but occurring past cycle 1).
[b]Patients whose doses were dose reduced for AEs.
[c]Patient 2003 started at 3.0 mg/kg per week for 3 cycles and was dose reduced to 2.0 mg/kg per week for 1 dose and then to 1.0 mg/kg per week thereafter due to AE.
[d]No. of patients with DLT/no. of patients treated at dose level.

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Six patients were administered 2 mg/kg i.v. q2w with no DLTs. The MTD was therefore considered 2 mg/kg i.v. qw or q2w.

Safety and tolerability

Table 3 lists all treatment-emergent AEs (≥10% incidence). Grade 3/4 AEs were infrequent, occurring mostly at doses exceeding the MTD. As expected, the most common treatment-related AEs were proteinuria (15/39, 38.5%), hypertension (12/39, 30.8%), fatigue (11/39, 28.2%), and nausea (8/39, 20.5%). Proteinuria was not strictly dose related, although there was a trend for higher grades at higher doses, as grade 3 proteinuria was seen only at 3 mg/kg. Hypertension was observed in 1/13 (7.7%), 4/14 (28.6%), and 7/12 (58.3%) patients at 1, 2, and 3 mg/kg, respectively, and was dose related. Although only 12/39 patients (30.8%) were reported to have treatment-related hypertension, 24 (61.5%) had at least 1 systolic or diastolic blood pressure reading of 160 mm Hg or higher or 100 mm Hg or higher posttreatment, indicating that the frequency might have been underestimated. Infrequent class-related AEs included bleeding and thrombosis (epistaxis, hemoptysis, hemorrhage, deep vein thrombosis, central retinal artery, and vein occlusion), LVSD, reversible posterior leukoencephalopathy syndrome, and serum lipase elevation/pancreatitis. Two cases of LVSD were observed, both in elderly men (aged >78 years) with prior heart disease and in 1, prior mitoxantrone exposure. Two patients had serum lipase elevations. In 1 patient treated with 2 mg/kg q2w, elevation occurred without symptoms. In the other treated with 1 mg/kg qw, elevation was accompanied by abdominal pain and grade 2 pancreatitis, which resolved following treatment discontinuation. No systematic adverse effects were noted on serial electrocardiograms, serum creatine kinase, and/or troponin levels. No clinically significant myelosuppression was observed, although 1 patient had grade 3 neutropenia believed related to previous cytotoxic chemotherapy.

Table 3. All AEs (≥10% incidence)

Dose level, mg/kg	No. of patients	Fatigue			Nausea			Proteinuria			Vomiting			Anorexia		
		1/2	3	4	1/2	3	4	1/2	3	4	1/2	3	4	1/2	3	4
≤1.0 qw	15	4	0	0	7	0	0	6	0	0	5	0	0	3	0	0
2.0 qw	9	5	1	0	3	0	0	5	0	0	4	0	0	2	0	0
2.0 q2w	7	4	0	0	3	0	0	1	0	0	1	0	0	2	0	0
3.0 qw	6	5	0	0	4	0	0	2	1	0	3	0	0	4	0	0
3.0 q2w	6	0	0	0	1	0	0	1	1	0	0	0	0	1	0	0
		Hypertension			Dyspnea			Edema (peripheral)			Constipation			Pyrexia		
≤1.0 qw	15	1	0	0	1	0	1	2	0	0	5	0	0	4	0	0
2.0 qw	9	3	0	0	2	2	0	4	0	0	1	0	0	2	0	0
2.0 q2w	7	0	1	0	1	1	0	1	0	0	0	0	0	0	0	0
3.0 qw	6	1	1	0	2	0	0	1	1	0	4	0	0	2	0	0
3.0 q2w	6	5	0	0	1	0	0	2	0	0	0	0	0	0	1	0
		Anemia			Headache			Diarrhea			Arthralgia			Back pain		
≤1.0 qw	15	2	0	0	2	0	0	0	0	0	2	0	0	2	0	0
2.0 qw	9	1	1	0	0	0	0	2	0	0	1	0	0	2	0	0
2.0 q2w	7	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0
3.0 qw	6	1	0	0	2	0	0	3	0	0	1	0	0	0	0	0
3.0 q2w	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Epistaxis			Hypo-kalemia			Hypo-magnesemia			Hypo-tension			Urinary tract infection		
≤1.0 qw	15	1	0	0	0	1	0	1	0	0	1	0	0	1	0	0
2.0 qw	9	1	0	0	1	0	0	1	0	0	1	0	1	2	0	0
2.0 q2w	7	1	0	0	1	1	0	1	0	0	0	0	0	1	0	0
3.0 qw	6	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
3.0 q2w	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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Table 4. Summary statistics for CT-322 pharmacokinetic parameters on cycle 1 day 1

Dose level, mg/kg	C_{max}, geo. mean (CV), ng/mL	AUC_{0-t}, geo. mean (CV), μg h/mL	AUC_{∞}, geo. mean (CV), μg h/mL	$t_{1/2}$, mean (SD), h	Clearance, geo. mean (CV), L/h
0.1 qw[a] ($n = 3$)	2,452.67 (26)	69.68 (46)	78.27 (41)	21.71 (2.560)	0.0885 (33)
0.3 qw[a] ($n = 4$)	6,893.84 (18)	332.08 (23)	363.09 (22)	39.88 (5.350)	0.0703 (32)
1.0 qw[b] ($n = 6$)	2,9211.17 (18)	1,641.73 (20)	2,288.42[c] (19)	89.10 (31.102)	0.0381 (20)
2.0 qw[b] ($n = 5$)	5,2906.38 (16)	3,061.82[e] (31)	3,697.70[c] (6)	70.69 (12.686)	0.0379 (27)
2.0 qw[a] ($n = 3$)	5,3356.08 (19)	2,904.88 (8)	3,827.65[d] (.)	74.92 (10.020)	0.0430 (19)
2.0 q2w[a] ($n = 6$)	4,7767.50 (22)	3,407.48 (18)	3,742.00 (20)	98.45 (26.376)	0.0363 (33)
3.0 qw[b] ($n = 6$)	9,0202.65 (14)	5,926.05 (14)	6,835.03 (12)	53.55 (5.021)	0.0368 (22)
3.0 q2w[a] ($n = 6$)	8,5077.60 (18)	5,956.01 (15)	6,651.45 (16)	110.34 (18.788)	0.0392 (22)

[a]Analyzing laboratory was Adnexus.
[b]Analyzing laboratory was QPS.
[c]$n = 2$.
[d]$n = 1$.
[e]$n = 4$.

Pharmacokinetic results

A summary of the pharmacokinetic parameters for CT-322 after the first dose is presented in Table 4. CT-322 clearance was similar at all doses. The mean terminal half-life ($t_{1/2}$) for the 2.0 to 3.0 mg/kg q2w groups was 98.4 to 110.3 hours, whereas the mean $t_{1/2}$ for the 1.0 to 3.0 mg/kg qw groups was 53.6 to 89.1 hours. Linear regression of area under the plasma concentration–time curve from time zero to the last measurable concentration [AUC (0–t)] and maximum plasma concentration (C_{max}) versus dose indicated that exposure increased in approximate proportion to dose.

Supplementary Figure A1 shows CT-322 plasma concentration versus time curves post–cycle 1 dosing day 1. CT-322 did not accumulate substantially in plasma following repeated administration. The mean C_{max} of CT-322 increased by 45% and decreased by 13% in the 1.0 mg/kg qw and 3.0 mg/kg q2w treatment groups from cycle 1 day 1 to cycle 2 day 1, respectively. Changes observed in C_{max} were most likely due to intersubject variability. Similarly, the AUC (0–t) did not increase substantively over the first cycle (10% and 16% in 1.0 mg/kg qw and 3.0 mg/kg qw, respectively). Steady-state concentrations were generally achieved in the qw schedule by day 29 (cycle 2 day 1).

Pharmacodynamics

Administration of CT-322 resulted in increased plasma concentrations of VEGF-A in all dose groups. Figure 1A shows the fold increase in plasma VEGF-A from baseline to posttreatment troughs over time. A higher fold change in VEGF-A was observed qw versus q2w. VEGF-A increased with dose, with a maximum fold increase from baseline at the MTD.

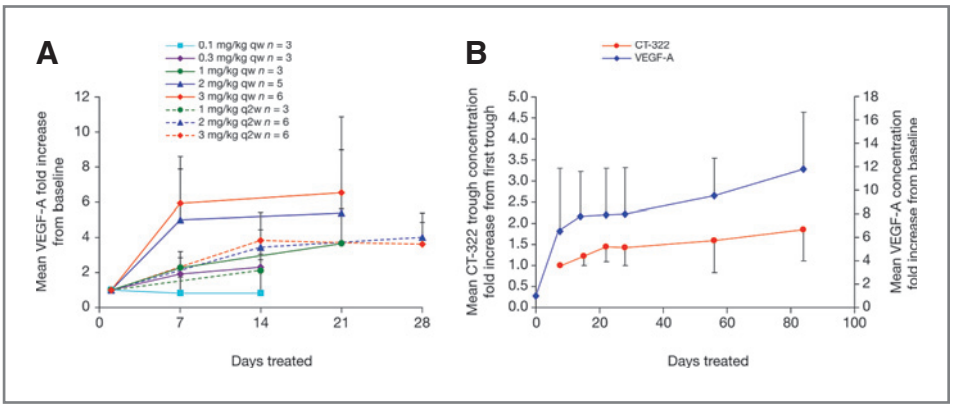

Figure 1. A, fold increase in plasma VEGF-A from baseline to posttreatment troughs over time. CT-322–induced increases in plasma VEGF-A concentrations plateau at 2 mg/kg qw. Patients treated with 0.1, 0.3, 1, 2, and 3 mg/kg qw and 1, 2, and 3 mg/kg q2w CT-322. Symbols and bars, mean ± SD. B, mean CT-322 trough levels and mean VEGF-A levels for 9 patients positive for anti–CT-322 antibodies over 12 weeks of treatment. Plasma VEGF-A and CT-322 trough levels in 9 patients treated for more than 12 weeks are not affected by antidrug antibodies.

Published OnlineFirst January 11, 2011; DOI: 10.1158/1078-0432.CCR-10-1411

Antibody response and immunogenicity

At baseline, no patients had detectable antibodies. Anti–CT-322 antibody formation was not dose or schedule dependent. Overall, 31 of 38 (82%) patients developed antidrug antibodies, with a median first positive at week 3. Among the antibody-positive patients, 17 (55%) had confirmed specific CT-322 antibodies. Competition binding with the human fibronectin-based wild-type Adnectin showed that the antidrug antibodies bind to the engineered binding loops of CT-322.

Of those with detectable anti-CT-322 antibodies, 19%, 39%, and 42% had maximum titers of 10^4, 10^3, and 10^2, respectively. CT-322 plasma concentrations and plasma VEGF-A biomarker responses were not substantially affected by the presence of anti–CT-322 antibodies and no AEs were associated with antibody response. In fact, patients remaining on therapy for 12 weeks or more developed antibodies with no substantive decrease in VEGF-A or trough CT-322 concentrations (Fig. 1B; see also Supplemental Methods)

Assessment of antitumor activity

Thirty-four patients had response-evaluable tumors. Among these, best response of stable disease was observed in 23 patients (67.6%) through 1 cycle, 11 (32.4%) through 2 cycles, and 5 (14.7%) through 4 cycles. Five patients remained stable through 6 cycles and entered a continuation study. Among these, 3 had prostate cancer, 1 non–HIV-related Kaposi's sarcoma, and 1 signet ring cell adenocarcinoma of unknown primary. Four evaluable patients experienced a decrease in RECIST sum of longest diameters; however, the decrease did not reach the 30% threshold required for partial response. Among these patients, 1 each had thymus carcinoma, RCC collecting duct variant, signet ring cell adenocarcinoma unknown primary (on study for 89 weeks despite prior progression on bevacizumab, irinotecan, and capecitabine and was 1 of the 5 patients stable beyond 6 cycles), and clear cell RCC (prior sorafenib). Patients achieving stable disease for more than 6 cycles and/or achieving tumor shrinkage less than RECIST 30% threshold were treated at all doses, suggesting that doses as low as 1.0 mg/kg qw may have activity.

Discussion

The VEGF/VEGFR signaling pathway is a clinically validated target of several tumor types, as multiple approved treatments that block VEGF-A (bevacizumab) or VEGFR-2 (sorafenib and sunitinib) are effective antitumor agents. In this study, CT-322, a novel engineered protein therapeutic based on 10Fn3 that specifically targets VEGFR-2 demonstrated class-specific AEs, pharmacodynamic biomarker (VEGF-A) on-target activity, and promising evidence of clinical activity consistent with specific inhibition of VEGFR-2.

At the highest dose of 3 mg/kg, AEs were clinically significant, requiring discontinuation. The appearance of reversible posterior leukoencephalopathy (RPLE) in this study may be a function of continued dose escalation to define a MTD of CT-322. This is in contrast to monoclonal antibodies directed to the ligand VEGF, for which a MTD was not defined. Therefore, in a phase I study with a small sample size, one cannot adequately conclude that the likelihood of this AE occurring is greater with this platform than antibody-based strategies administered at higher, and potentially equitoxic, doses. Moreover, CT-322 was administered safely, with mild to modest toxicities and promising antitumor activity at MTD of 2 mg/kg qw or q2w. On the basis of tolerable toxicities, increased plasma VEGF-A levels, and persistent stable disease in heavily pretreated and refractory patients, 1 mg/kg may also be an appropriate phase II dose. Future studies analyzing plasma biomarkers, clinical responses, and safety and tolerability will further define the optimal dose for specific tumor types.

As anticipated for a PEGylated protein molecule, CT-322 is cleared slowly from the plasma, with $t_{1/2}$ of approximately 3 to 4 days, supporting qw dosing. However, because elevated VEGF-A and class-specific AEs were present with q2w dosing, this schedule might also be appropriate. Peak CT-322 concentrations may be responsible for intolerable toxicities because such toxicities were observed with both 3 mg/kg dose schedules.

The development of anti–CT-322 antibodies was not related to dose or schedule and did not seem to be associated with adverse safety outcomes or changes in pharmacokinetics or biological activity. "Furthermore, in preclinical studies antibody titers to CT-322 found in patients had no effect on the biologic function of CT-322" (Data on file, Adnexus).

Preclinical investigations documented that CT-322 is a potent and highly specific inhibitor of VEGFR-2. In this study, CT-322 was not associated with off-target toxicities such as skin rash observed with TKIs such as sorafenib. CT-322 may provide clinical advantages in tumor types such as colorectal cancer for which VEGF-C and/or -D signaling through VEGFR-2 is active and a narrower spectrum of toxicity is desired (27).

Targeting the VEGF/VEGFR-2 axis includes platforms that sequester the ligand VEGF such as antibody and receptor traps, inhibiting tyrosine kinase activity on the intracellular membrane domain of VEGFR-2, and targeting directly the receptor using antibodies (IMC18F/IMC1121B) or with CT-322. The 2 former platforms have met, in some circumstances, proof of concept and regulatory approval whereas the latter one, directly targeting the extra cellular domain of the receptor, remains investigational. To date, only preliminary data have been reported for strategies using antibodies to the receptor; therefore, comparisons of the receptor targeting platforms cannot be undertaken at this time. Furthermore, the use of receptor targeting strategies is not mutually exclusive of current treatments at either sequestering the ligand or targeting the receptor tyrosine kinase. Because receptor targeting increases the ligand due to direct competition at the receptor, a theoretical advantage for the combination of ligand sequestration with direct receptor targeting can be proposed and in preclinical models this indeed seems to be additive or synergistic (Data on File, Adnexus).

Published OnlineFirst January 11, 2011; DOI: 10.1158/1078-0432.CCR-10-1411

In conclusion, CT-322 can be safely administered at 2 mg/kg i.v. qw or q2w and exhibits promising antitumor activity in patients with a variety of advanced solid tumors. Furthermore, Adnectins are an effective class of protein therapeutics to modulate extracellular targets and exhibit a relatively safe and tolerable profile. These results support ongoing monotherapy and chemotherapy combination CT-322 phase II trials utilizing the qw schedule [Clinicaltrials.gov NCT00562419 (single agent in glioblastoma multiforme), NCT00850577 (with chemotherapy in NSCLC), NCT00851045 (with chemotherapy in colorectal carcinoma)] and further development of Adnectins, including the development of 2-domain Adnectins targeting dual receptors now under way.

Disclosure of Potential Conflicts of Interests

A. Tolcher, consultant/advisory board Bristol-Myers Squibb /Adnexus; A. Mita honoraria, Genentech; A. Patnaik, and K. Papadopoulos, commercial research support, START; B. Silver, employee of Adnexus, stock in Bristol-Myers Squibb, consultant to Adnexus; C. Eaton, J. Gokemeijer, and E. Furfine, employees of Adnexus, stock in Bristol-Myers Squibb; L. Iacono, employee of Bristol-Myers Squibb, stock in Bristol-Myers Squibb.

Acknowledgments

Data collection and processing were assisted by Gary Conner of Asclepius Research. Editorial support was provided by U.L. Prisco, PhD, and R. Rozich, PhD, of PAREXEL, and was funded by Bristol-Myers Squibb.

Grant Support

This work was supported by Adnexus, A Bristol-Myers Squibb R&D Company.

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Received May 25, 2010; revised August 9, 2010; accepted August 18, 2010; published OnlineFirst January 11, 2011.

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Published OnlineFirst January 11, 2011; DOI: 10.1158/1078-0432.CCR-10-1411

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Clin Cancer Res 2011;17:363-371. Published OnlineFirst January 11, 2011.