

Phase I Evaluation of CDP791, a PEGylated Di-Fab' Conjugate that Binds Vascular Endothelial Growth Factor Receptor 2

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Abstract Purpose: Specific blocking of vascular endothelial growth factor receptor 2 (VEGFR-2) is a novel therapeutic approach. Here, we report the first phase I clinical trial evaluation of CDP791, a PEGylated di-Fab' conjugate that binds VEGFR-2.

Experimental Design: Cohorts of patients received CDP791 at doses between 0.3 and 30 mg/kg every 3 weeks for the initial two doses.

Results: The compound was well tolerated with no dose-limiting toxicity. Dose-related hypertension was observed in patients receiving CDP791 10 mg/kg or more and several patients on the higher doses developed infusion-related cutaneous hemangiomas arising 28 to 106 days after the first drug administration and resolving 3 weeks after cessation. Biopsy and histologic evaluation showed that CDP791-bound VEGFR-2 is non-phosphorylated, suggesting that the drug is biologically active. Concentrations of CDP791 considered biologically relevant were sustained for 3 weeks when doses of 10 mg/kg or more were administered. Although no reductions in vascular permeability were recorded using dynamic contrast enhanced magnetic resonance imaging (DCE-MRI), there was a significant dose level-related reduction in tumor growth. While challenging the recent dogma that active VEGF inhibitors should modulate DCE-MRI measurements of vascular permeability, this highlights the potential of serial three-dimensional tumor measurements to detect tumor growth arrest. Twelve patients received drug for more than two treatments, although no partial or complete responses were seen.

Conclusion: The data show that CDP791 is biologically active and well tolerated, achieving appropriate plasma concentrations when administered at 10 mg/kg or more every 3 weeks.

Vascular endothelial growth factor (VEGF) has been validated as a target in oncology in randomized trials in colorectal cancer (1, 2), renal (3), breast cancer (4) and non-squamous, non-small cell lung cancer (5). Recent data suggest that anti-VEGF antibodies have an antivasular effect (6) that is associated with the inhibition of VEGF receptor 2 (VEGFR-2) phosphorylation (7). The drugs that have been developed as inhibitors of VEGF are largely not selective for VEGFR-1 or VEGFR-2, although most evidence suggests that the principal receptor that mediates angiogenesis is VEGFR-2. As VEGF inhibitors are associated with hypertension, proteinuria, thrombosis, and hemorrhage, it

is important to optimize the design of VEGF inhibitors to minimize these toxicities and to overcome any resistance mediated through the ability of VEGF-C and D to activate VEGFR-2 (8). One strategy to achieve this is to design drugs, such as CDP791, that inhibit VEGFR-2 specifically. In this trial, we administered CDP791 as monotherapy to patients with advanced cancer and evaluated the safety and tolerability, pharmacokinetics and dynamic contrast enhanced magnetic resonance imaging (DCE-MRI; ref. 9) effects of the drug.

CDP791 is a di-Fab' fragment polyethylene glycol (PEG) conjugate (40 kDa) that has a dissociation constant for VEGFR-2 of 0.049 nmol/L, inhibits VEGF-A and VEGF-C signaling *in vitro*, and has antiangiogenic activity *in vivo*. The rationale for designing CDP791 was that the most effective way to achieve blocking activity without any safety risk contributed by the Fc domain was to produce a di-Fab' molecule. However, anti-VEGFR-2 Fab' fragments alone would be cleared very quickly, and to improve the pharmacokinetics, a 40-kDa PEG group was attached, generating a molecule that has similar pharmacokinetics, *in vivo*, to those of an intact antibody. *In vitro* data suggested that an active concentration of CDP791 was 10 µg/mL. Following s.c. sponge insertion in dogs, the i.v. administration of 10 mg/kg CDP791 significantly inhibited both angiogenesis and fibrosis around the implant (10). VEGF regulates vascular permeability, and CDP791 significantly inhibited vascular leakage in dogs injected with intradermal

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VEGF (10). In cynomolgus monkeys, i.v. CDP791 dosed weekly at 400 mg/kg for up to 6 months was well tolerated (10). Human tissue binding studies showed that CDP791 bound to tissues known to express VEGFR-2 and *in vitro* studies showed inhibition of Ca^{2+} flux in response to VEGF stimulation (10). These data showed that CDP791 inhibits VEGFR-2 *in vitro* and angiogenesis *in vivo* and supported the following phase I clinical trial.

Materials and Methods

CDP791

CDP791 is an engineered di-Fab' fragment PEG conjugate, which binds to and blocks the activity of VEGFR-2. Each molecule of CDP791 has two constituents: a humanized antibody di-Fab' composed of two molecules of Fab' cross-linked covalently and site specifically at the hinge region using a maleimide cross-linker; and a 40-kDa PEG attached directly to the cross-linker. The addition of PEG is designed to increase the plasma half-life of the molecule. The molecular weight of CDP791 is ~140 kDa, with an affinity of 4.9×10^{11} mol/L for VEGFR-2, as measured by surface plasma resonance (Biacore).

Trial design

Aims of the study. The study examined safety, tolerability, and biological effects of CDP791 in patients with advanced, solid tumors for whom no standard treatments existed. The primary end points were safety and tolerability. Secondary end points were changes in tumor vascular parameters assessed using DCE-MRI, pharmacokinetics, and tumor response.

Patients. Patients with measurable solid tumors, more than 18 years of age, an Eastern Cooperative Oncology Group (ECOG) score between 0 and 2, and life expectancy of at least 3 months were eligible. They were required to have adequate liver (normal bilirubin and transaminases less than $2.5 \times$ the upper limit of normal), renal (creatinine less than $1.5 \times$ the upper limit of normal), and hematologic (hemoglobin >10 g/dL, white cell count $>3 \times 10^9$ /L, and platelet count $>100 \times 10^9$ /L) function, as well as normal coagulation (prothrombin time and activated partial thromboplastin time) and an electrocardiogram without significant abnormality.

Patients were excluded if they had an additional chronic disease affecting a major organ, infection requiring antibiotics, clinically significant ascites or pleural effusion, major surgery or treatment within the previous 4 weeks, previous history of reaction to biological agents or drugs containing PEG, a contraindication to MRI, history of infection with hepatitis B, C, HIV-1, or human T-cell lymphotropic virus-1, alcohol or drug addiction. Patients were not permitted to take drugs known to alter vascular flow, and they had to have recovered from the effects of previous treatments. Patients with known or suspected brain or central nervous system disease were excluded, as was anyone who had previously taken a VEGF inhibitor.

Study design. The study was an ascending dose cohort study. Patients were treated at the Christie Cancer Center (Manchester, United Kingdom). Sequential cohorts of patients received 0.3, 1.0, 3.0, 10, 20, and 30 mg/kg CDP791 dosed i.v. more than 60 min on days 0 and 21. A computed tomography scan was done during the 2 weeks before treatment and on day 35. Anatomic and DCE-MRI scans were done twice at baseline to establish reproducibility (11, 12) and on days 1, 7, and 20. Blood samples were taken to measure hematologic and biochemical toxicity at screening, before treatment, and on days 1, 7, 14, 21, 28, and 42. CDP791 concentration and anti-CDP791 antibodies were measured before treatment and 1, 2, 3, 4, 6, 8, and 24 h, 7, 14, and 21 days afterward. Adverse events were assessed before treatment at each study visit. Toxicity was assessed using the National Cancer Institute common toxicity criteria (NCI CTC; version 2.0). Response was assessed using RECIST. Any patient with stable disease or better at

day 35 was eligible to continue treatment for a further six 3-weekly cycles. During the continuation phase, standard laboratory safety and immunogenicity tests were measured in each cycle, and a sample for measurement of CDP791 concentration was taken before and at the end of each infusion of CDP791.

At least three subjects were to be enrolled in the first cohort and at least five subjects in each subsequent cohort. Cohorts could be expanded or the study stopped if patients developed dose-limiting toxicities (DLT). DLT was defined as the development of grade 3/4 toxicity according to the NCI criteria in any of the safety tests, excluding plasma level of CDP791 or anti-CDP791, which was not available. Tolerability was assessed when at least three patients in the cohort had had a minimum of 24 h follow-up after the second dose of CDP791. A tolerated dose was defined as that in which zero of three or less than two of six patients developed a grade 3/4 toxicity during the above timeframe.

All patients gave written informed consent, and the study was approved by the South Manchester Local Research Ethics Committee and the Christie Hospital Research Committee.

Magnetic resonance imaging. All data were acquired on a 1.5-T Philips Intera system (Philips) following a previously described protocol (12). DCE-MRI parameters v_p (blood plasma volume), K^{trans} (trans-capillary transfer coefficient for contrast agent), and v_e (extravascular extracellular space volume) were derived using the Key model of tracer kinetics extended to account for blood volume (13–15). The initial area under the contrast concentration curve (IAUC) over the first 60 s post-contrast agent arrival was also calculated (16).

Tumors were outlined in three dimensions on the T_2 -weighted volume, with reference to the T_1 -weighted pre- and post-contrast volumes. DCE-MRI parameters were extracted from the enhancing tumor (17).

Pharmacokinetics. Concentrations of CDP791 were assessed using a sandwich ELISA consisting of a murine Fc-KDR fusion-coated plate and a goat anti-human κ -horseradish peroxidase conjugate as the detection layer. The limit of quantification for this assay (allowing for the minimum 1/100 dilution) was 0.53 $\mu\text{g/mL}$. Antibodies to CDP791 were assessed using a double antigen sandwich ELISA consisting of a CDP791-coated plate and a CDP791 biotin detection system. Samples were quantified against a rabbit anti-CDP791 high titer standard, and the limit of quantification was 0.45 units/mL (allowing for the minimum 1/10 sample dilution).

Statistical design. Five subjects per cohort were selected as an appropriate sample size for this study based on clinical rather than statistical considerations. These numbers were chosen to increase the power of the biomarkers studies. For the vascular parameters assessed by DCE-MRI, the percent change from baseline was summarized by dose level using descriptive statistics based on enhancing tumor volume average parameters. All DCE-MRI parameters were investigated in terms of the percentage change that may be ascribed to dose, effects of CDP791 on days 1, 7, and 20, and conversion to stable disease at the end of the trial. We also looked for evidence that any DCE-MRI parameter was related to the percentage change in tumor volume.

Immunohistochemistry. A formalin-fixed, paraffin-embedded (FFPE) hemangioma biopsy was sectioned and stained with H&E. Immunohistochemistry for VEGFR-2 was done using previously described antigen retrieval and staining procedures (7), with the minor modifications that the anti-human VEGFR-2 (New England Biolabs) and the phospho-KDR Y951 (Santa Cruz Biotechnologies) antibodies were used at 1:100 and 1:50 dilutions, respectively. FFPE normal human placenta was used as a positive control tissue for KDR and phospho-KDR immunohistochemistry, with rabbit immunoglobulin (Ig; DAKO) being used to control for nonspecific staining. PEGylated-CDP791 was detected using a mouse anti-PEG (AGP3) antibody (Academia Sinica) at 20 $\mu\text{g/mL}$ without the need for antigen retrieval. Liver hemangioma tissue (Cytomox) was used as a negative control tissue for anti-PEG staining, with a mouse IgM antibody (Immuno-Kontakt) as an isotype control. Dewaxed and hydrated tissue sections

Table 1. Baseline characteristics

Characteristic	Total subjects (N = 31)
Mean age (range)	62 (18-80)
Gender (males)	11
Race (Caucasians)	31
Mean body mass index (range)	28 (22-45)
Tumor type	
Ovarian	10
Colorectal	7
Renal	5
Cholangiocarcinoma	1
Unknown	2
Desmoplastic round cell	1
Endometrial	1
Gastric	1
Melanoma	1
Pancreatic	1
Small cell lung	1

were incubated with 3% (v/v) H₂O₂ to block endogenous peroxidases and then with the Vector labs endogenous biotin blocking kit before incubation with the primary antibodies. Following incubation with appropriate biotinylated secondary antibodies and ABC amplification (DAKO), antibody-antigen binding was visualized with 3',3'-diaminobenzidine (DAB; Vector Labs). Sections were counterstained with hematoxylin. Sections were imaged with a Leica DMRB microscope and Spot Insight color camera.

Results

Patient characteristics. A total of 31 patients with measurable, progressive disease and ECOG status 0 or 1 were recruited. There were four subjects in the 0.3-mg/kg cohort, six subjects in the 1-mg/kg cohort, five subjects each for

the 3-, 10-, and 20-mg/kg cohorts, and six subjects in the 30-mg/kg cohort. Table 1 summarizes the demographic data and tumor types of all patients. A total of 28 patients received at least two doses of CDP791, and five subjects received six or more infusions.

Safety. CDP791 was safe and well tolerated at all doses tested. The maximum tolerated dose of CDP791 is therefore ≥ 30 mg/kg. The majority of adverse events were NCI CTC grade 1 or 2 and judged mild or moderate (Table 2A). Nine subjects experienced 19 adverse events that were NCI grade 3 or 4 or severe. Table 2B summarizes the grades 3 and 4 adverse events. One event (subject 27, 30 mg/kg, had disease progression) was rated as severe but had no NCI grade reported.

Several adverse events stood out as not being typical of the population studied. These were hypertension, increase in activated partial thromboplastin time (APTT), hypocalcemia, and skin lesions. A total of 5 of 31 subjects experienced increased blood pressure. All but one were rated as being at least possibly related to CDP791. Four of the five had NCI CTC grade 1 (one at a dose level of 3 mg/kg, one at 10 mg/kg, and two at a dose level of 20 mg/kg), and one had grade 3 hypertension (at a dose level 20 mg/kg). The grade 3 event occurred in a patient with a past history of hypertension. The event was controlled with an increase in medications. The frequency and severity of increased blood pressure in this trial seem similar to that described with studies of other VEGF inhibitors (1).

Eight subjects developed grade 2 hypocalcemia, although only four were rated as being at least possibly related to CDP791. In all cases, the episodes were transient and asymptomatic. Six subjects developed grade 1 or 2 increased APTT, all at doses of CDP791 10 mg/kg or higher. Two of the six developed mild, self-limiting bleeding episodes, including one subconjunctival hemorrhage and one hemoptysis.

Table 2. Toxicities

(A) Adverse events related to study drug experienced by at least two subjects of all NCI CTC grades			
Event	Number of events (number of subjects)		
Skin lesions	12 (7)		
Headache	11 (9)		
Lethargy	9 (8)		
Nausea	8 (5)		
APTT prolonged	6 (6)		
Hypertension	5 (4)		
Pyrexia	6 (4)		
Myalgia	5 (4)		
Hypocalcemia	4 (4)		
Rigors	3 (2)		
Back pain	2 (2)		
(B) Subjects with grade 3 and 4 adverse events			
Subject	CDP791 dose (mg/kg)	Event	NCI CTC grade
3	0.3	Hypotension	3
4	0.3	Abdominal pain, hyponatremia, ascites, vomiting	3, 3, 3, 3
6	1	Rectal hemorrhage	3
9	1	Mandibular swelling (metastasis)	3
21	20	Hypertension	3
23	20	Abdominal pain, chest pain	3, 3
24	20	↑ γ GT, ↑ ALT	4, 3
27	30	↑ γ GT, ↑ Alk Phos/SGPT, vomiting, dehydration	3, 3, 3, 3
30	30	Enterococcal infection, tumor ulceration	3, 3

Seven subjects developed skin toxicity. The lesions seemed to be benign hemangiomas of ~5 to 10 mm diameter. They occurred at CDP791 doses of 10 mg/kg or higher and tended to appear after at least three cycles of therapy. Figure 1 shows a hemangioma on the thigh of a patient who had received 10 mg/kg CDP791. Histologic examination of a representative lesion showed that the cells stained with CD31 suggesting that endothelial cells were present (data not shown). The endothelial cells were arranged as disorganized vessels of varying diameter that bound proliferating cell nuclear antigen, suggesting that they were proliferating (data not shown). A muscular perivascular layer was not observed. The sections were stained for VEGFR-2, phospho-VEGFR-2, and PEG, demonstrating that VEGFR-2 was widely expressed. However, PEG was only present in parts of the sections that did not bind the anti-phospho-VEGFR-2 antibody, suggesting that the drug is biologically active, thereby inhibiting receptor activation and phosphorylation. Where phospho-VEGFR-2 was expressed, it seemed to be present internally, rather than at the cell surface. None of the patients discontinued CDP791 therapy as a result of skin toxicity. In general, lesions regressed after treatment was stopped.

CDP791 infusions were tolerated well. Of the adverse events reported within 2 h of the CDP791 infusions, or, if no time was recorded, on the dosing day, none was suggestive of an anaphylactoid reaction related to CDP791 infusion.

Pharmacokinetics. The pharmacokinetics were measured in all patients. At the lower doses, the clearance of CDP791 appeared as mono-exponential, but a rapid distribution phase, evident on individual profiles, appeared at the higher dose levels. Following the first administration of CDP791, dose proportionality was obtained for C_{max} but not for $AUC(0-t)$. C_{max} values increased from 5.53 $\mu\text{g/mL}$ in the 0.3-mg/kg dose group to 709.63 $\mu\text{g/mL}$ in the 30-mg/kg dose group. $AUC(0-t)$ values ranged from 81.50 $\mu\text{g h/mL}$ in the 0.3-mg/kg dose group to 102,158 $\mu\text{g h/mL}$ in the 30-mg/kg dose group. Moreover, the half-life increased from 17.4 to 203 h, with dose in the range of 0.3 to 30 mg/kg. At 30 mg/kg, the half-life was similar to that at 20 mg/kg. Increases were not dose proportional, which may have been due to expression of the antigen VEGFR-2 having a greater impact on CDP791 clearance in the lower dose groups (Table 3; Fig. 2).

After repeated dosing with CDP791, trough levels of CDP791 increased with successive doses in the 10-, 20-, and 30-mg/kg

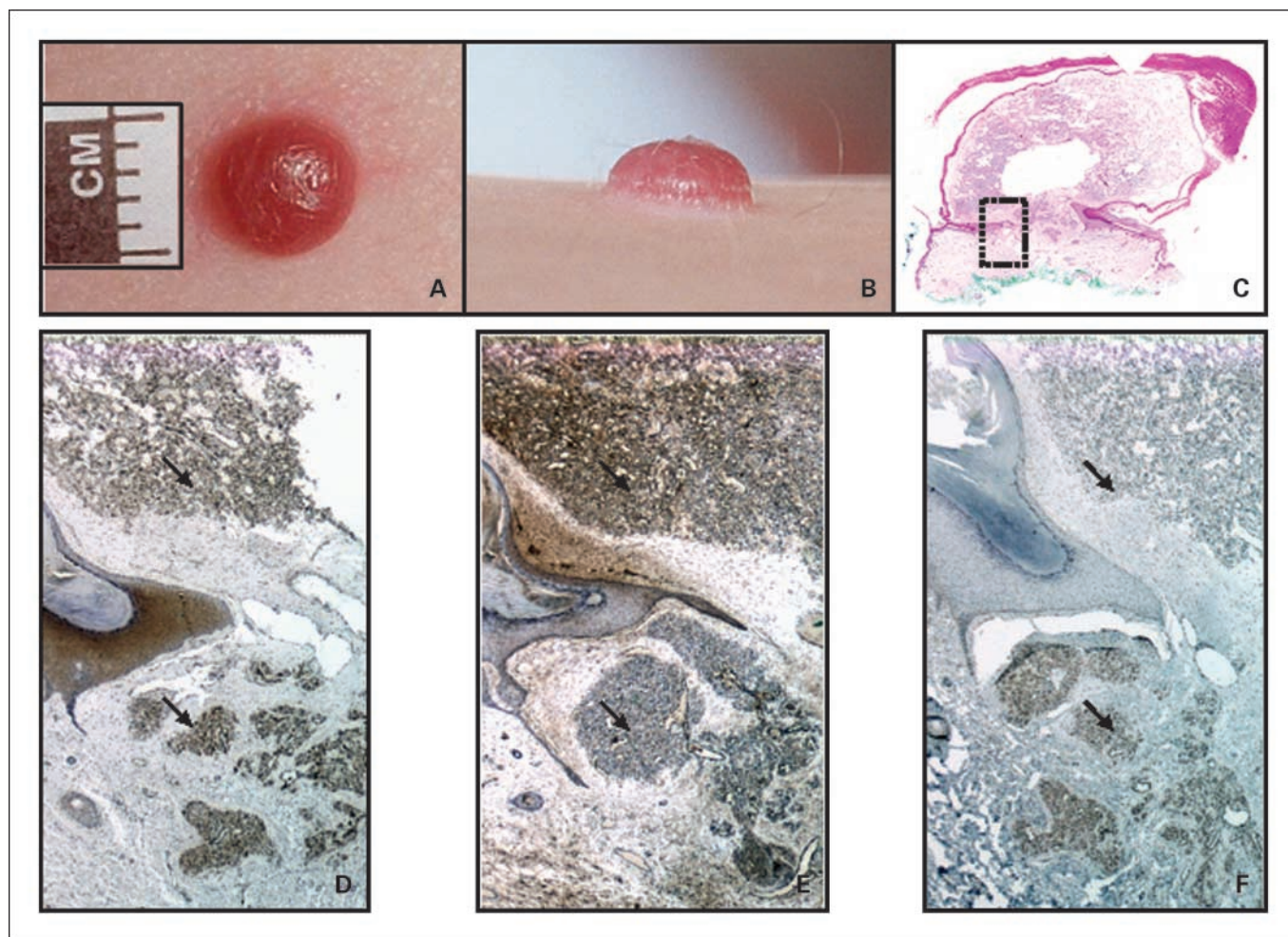


Fig. 1. Photograph and histology of hemangioma. *A* and *B*, macroscopic photographs of a typical hemangioma. *C*, low power H&E stain of the hemangioma. Dotted rectangle, the area studied in *D* to *F*. *D*, distribution of VEGFR-2. *E*, distribution of CDP791. *F*, distribution of phospho-VEGFR-2. Each immunohistochemical section shows two arrows corresponding to nests of endothelial cells. Top arrows, binding of CDP791 with absence of phosphorylated VEGFR-2. Bottom arrows, endothelial cells that did not bind the drug where intracellular phosphorylated receptor was present.

Table 3. Pharmacokinetics

Dose (mg/kg)	0.3	1	3	10	20	30
AUC ($\mu\text{g}\cdot\text{h/mL}$)	82	1,248	7,139	26,202	73,645	102,158
C _{max} ($\mu\text{g/mL}$)	5.5	22.4	69.8	206	463	710
Cl (l/h)	0.04	0.013	0.0079	0.0041	0.0034	0.0034
T _{1/2} (h)	17.4	53	88	169	203	203

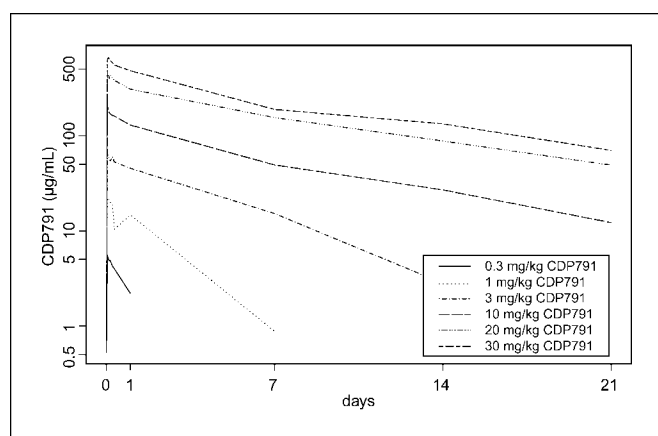
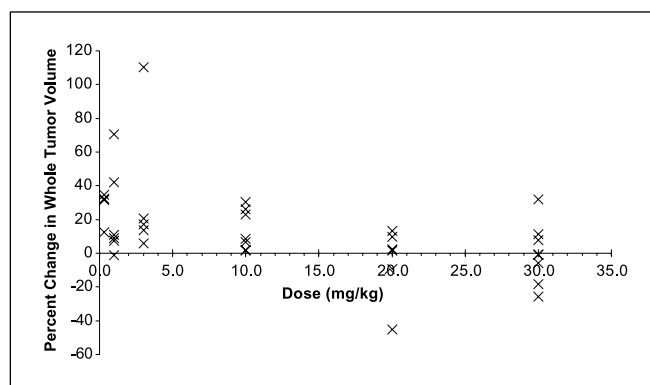
dose groups. In subjects who received several CDP791 cycles, trough and C_{max} levels appeared constant over time, indicating that steady-state conditions were achieved rapidly.

Immunogenicity. A low level (0.6 units/mL) of antibodies to CDP791 was observed in one sample (subject 32, day 63). In subsequent samples from this subject, antibodies to CDP791 were undetectable. Plasma concentrations of CDP791 were unaffected.

Anatomic and functional (DCE-MRI) imaging. An exploratory analysis of possible correlations between dose, progression status as determined by RECIST at day 35, and all DCE-MRI parameters revealed that dose of CDP791 correlated with the overall response [Spearman's ρ correlation coefficient (CC) = 0.459; $P = 0.005$ (two-tailed)]. The percentage change in whole tumor volume from pre-dosing, as measured on MRI at day 20, was also found to correlate with dose [CC = -0.553; $P < 0.001$ (two-tailed)], indicating a possible growth-arresting effect that could be ascribed to CDP791 (Fig. 3).

No evidence for a relationship between dose level and changes in any DCE-MRI parameter was observed. In particular, measurement of K^{trans} , which is related to endothelial permeability, did not show drug-related changes. Similarly, no changes were observed consistently across any of the dose groups on any of the posttreatment visits.

Disease control. No subjects achieved a complete or partial response, as defined by RECIST. A total of 12 of 29 evaluable subjects (two subjects had tumor assessments at screening only) had stable disease at day 35 and received additional cycles of CDP791. Five had stable disease after six cycles. These patients included two subjects with renal cancer and one subject each with colorectal cancer, endometrial cancer, and melanoma.

**Fig. 2.** Dose-level dependent pharmacokinetics of CDP791.**Fig. 3.** Dose-related percentage change in tumor volume. X-axis, dose level received by patients. Point, percent change from baseline to day 20 in the volume of a serially imaged tumor mass. A significant reduction in percentage tumor growth is observed as dose level increases.

Discussion

We have shown that CDP791, a di-Fab'-PEG conjugate that binds and blocks VEGFR-2, is safe and well tolerated after repeat dosing in patients with advanced solid tumors. CDP791 was not immunogenic, and at doses of 10 mg/kg and above, concentrations likely to be biologically relevant were maintained throughout the 3-week dosing interval.

We (9) and others (18, 19) have shown in early clinical trials that VEGF inhibitors reduce the DCE-MRI parameter K^{trans} , which is related to vascular permeability. This is mechanistically plausible because VEGF is the principal mediator of vascular permeability in tumors. However, nearly all studies to date have investigated this parameter using drugs that inhibit both VEGFR-1 and VEGFR-2: anti-VEGF antibodies will inhibit the VEGF-mediated activation of both receptors, whereas receptor tyrosine kinase inhibitors, by virtue of their structure, have a range of inhibitory effects against VEGFR-1 and VEGFR-2. Thus, the data we present here are unique in that CDP791 is one of a very small class of pure VEGFR-2 antagonists. Because our studies have not shown a drug-related change in K^{trans} , the data therefore challenge the dogma that VEGF inhibitors should always modulate this parameter and have important implications for the interpretation of trial designs incorporating DCE-MRI.

One explanation might be that vascular permeability is regulated by both receptors, and this is why we have not seen an effect in the clinical trial. Although preclinical studies that investigated receptor-specific VEGF mutants implied that VEGFR-2 is the sole mediator of vascular permeability *in vitro* and *in vivo* (20), it is important to note that both the preclinical evaluation of the effects of CDP791 on vascular permeability and other studies (20, 21) were conducted in nonmalignant models. Thus, it is possible that in the malignant setting, the regulation of vascular permeability is dependent on perturbation of both receptors, accounting for the absence of change in K^{trans} in this study.

Several patients treated at the higher dose levels developed hemangiomas that resolved upon cessation of CDP791 administration. These have not been recorded before, and the mechanism through which they develop is unclear; however, biopsy of one showed that it contained endothelial cells, and that VEGFR-2 was expressed, but that CDP791 bound in areas

of nonphosphorylated VEGFR-2, implying that the drug achieved its pharmacologic function. Finally, there was a statistically significant association between dose level and tumor growth retardation and a significant negative correlation between tumor volume change and dosage. These data are compatible with CDP791 being pharmacologically and biologically active yet lacking an observable effect on tumor-related vascular permeability. These results contrast with the effect of the compound in the preclinical canine model of VEGF-induced vascular permeability. However, in that model, the vasculature is normal in contrast to the vessels in our patients' tumors. Finally, there has been one other clinical study of a specific VEGFR-2 inhibitor, the antibody IMC-1C11, which is a chimeric mouse anti-human-VEGFR-2 IgG₁ antibody (22). In that phase I trial, DCE-MRI studies were done, and relatively modest decreases in vascular parameters were observed after four, once weekly doses.

In summary, CDP791 was safe and well tolerated as monotherapy up to 30 mg/kg in patients with advanced solid

tumors. CDP791 is a potent inhibitor of VEGFR-2 signaling with exquisite specificity for that receptor and which appears to inhibit VEGFR-2 in patients. In this clinical trial, we have shown that it is well tolerated and pharmacologically and clinically active. Experimental studies showed that concentrations of 10 µg/mL or higher were sufficient for optimum biological activity *in vitro*. In this trial, doses of 10 mg/kg were sufficient to sustain this concentration for 3 weeks. Therefore, the recommended dose for phase II studies would be 10 mg/kg or higher. This is one of a very few pure VEGFR-2 antagonists, and our data challenge the dogma that all VEGF inhibitors will inhibit the vascular permeability-related constant, K^{trans} . On the other hand, our consecutive imaging studies have highlighted the possibility of serial three-dimensional growth measurements as a sensitive method to detect growth retardation for novel biological agents. CDP791 provides a novel form of attack on the VEGF pathway, and a phase 2 program is under way that will reveal how the differences observed in phase 1 translate into antitumor effects.

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