A Phase I Study of Anti-Kinase Insert Domain-containing Receptor Antibody, IMC-1C11, in Patients with Liver Metastases from Colorectal Carcinoma

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ABSTRACT

Purpose: Angiogenesis plays an important role in colorectal cancer progression. Stimulation of vascular endothelial growth factor receptor (VEGFR), a transmembrane glycoprotein, results in endothelial mitogenesis. Within this family of receptors, VEGFR 2/kinase-insert-domain-containing receptor (KDR) appear to be principally upregulated during tumorigenesis. A chimeric anti-KDR antibody, IMC-1C11, blocks VEGFR-KDR interaction and inhibits VEGFR-induced endothelial cell proliferation. This trial seeks to assess the safety, tolerability and feasibility of targeting an important pathway in tumorigenesis.

Experimental Design: In a dose-escalation, single-agent study of IMC-1C11, we enrolled 14 patients with colorectal carcinoma and hepatic metastases. Safety-, pharmacokinetic-, immunogenicity-, and magnetic resonance imaging-assessed alteration of vascular effects of IMC-1C11 were evaluated in this trial. IMC-1C11 was infused weekly at 0.2 mg/kg (n = 3), 0.6 mg/kg (n = 4), 2.0 mg/kg (n = 3), and 4.0 mg/kg (n = 4) for 4 weeks, which constituted a cycle.

Results: No grade-3 or -4 IMC-1C11-related toxicities were observed. Minor grade-1 bleeding events were observed in four patients [0.2 mg/kg (n = 1) and 0.6 mg/kg (n = 3)]. Each resolved quickly and required no intervention. The starting dose of IMC-1C11 was selected to achieve a Cmax of ≈ 5 μg/ml. This concentration prevented KDR phosphorylation in vitro. Pharmacokinetic analysis demonstrated that the plasma t1/2 and Cmax were dose dependent with a plasma t1/2 of 67 ± 3 h at the 4-mg/kg dose level. Human antichimeric antibodies were detected in 7 of 14 patients. The antibodies to IMC-1C11 inhibited the circulation of the agent in two patients. One patient had prolonged stable disease for seven cycles (28 weeks). The mean changes in tumor-influx volume-transfer constant Kvr (min−1) and enhancement factor after 4 weeks of therapy were significantly decreased compared with pretreatment values in 11 patients.

Conclusion: IMC-1C11 was both safe and well tolerated. Drug levels of IMC-1C11 were reliably predicted. Further clinical investigation of anti-VEGFR/KDR agents is warranted.

INTRODUCTION

The inhibition of tumor-induced angiogenesis has become a major developmental strategy in cancer research (1, 2). Elucidation of the variety of molecular pathways involved in angiogenesis has provided targets for angiogenesis inhibition. Several potential angiogenic inhibitors against these therapeutic targets affect endothelial cell proliferation, migration, and survival (3–5). The inhibition of activated endothelial cells have been shown to abrogate tumor growth (6, 7).

VEGFR1 is thought to be the predominant angiogenic factor in malignant disease and one of the major survival factors for endothelial cells (8, 9). It is frequently detected in tumors and its expression has been correlated with poor prognosis in colon, breast, renal, and other primary tumors (10–13). VEGFR overexpression is frequently observed in colorectal carcinoma, in which it may play a role in the progression of metastatic disease (14). It can be synthesized by both normal and malignant cells and mediates angiogenic signals through interaction with one or more of its tyrosine kinase receptors (15). VEGFR2, also known as Flk-1/KDR, has been reported to be the most important receptor in VEGFR-stimulated tumor angiogenesis. KDR is found primarily on vascular endothelium (16) and several tumor cell types (17). Inhibition of VEGFR or KDR is reported to inhibit tumor growth (18).

DC101, a rat antimouse monoclonal antibody that targets Flk-1 (the murine homologue of KDR), was shown to block VEGF-mediated endothelial cell signaling (19). Administration of DC101 produced dramatic antitumor effects in a variety of...
human tumor xenograft models (20), as well as in a hepatic metastasis colon cancer model (21). Because DC101 does not cross-react with human KDR, a chimeric monoclonal antibody, IMC-1C11, was developed for human trials (22). IMC-1C11 binds specifically to the endothelial cell surface extracellular domain of KDR, blocks VEGFR-KDR interaction and prevents VEGFR activation of the intracellular tyrosine kinase pathway (23).

This report describes the initial Phase I trial of IMC-1C11 that was carried out in patients with colorectal carcinoma metastatic to the liver.

PATIENTS AND METHODS

Eligible patients met the following criteria: (a) pathologically confirmed adenocarcinoma of the colon/rectum that is metastatic to the liver and is not curable with available treatment; (b) at least one liver metastasis had to be bidimensionally measurable; (c) age greater than 19 years; (d) a median survival of at least 12 weeks; (e) ECOG performance status of ≤1; and (f) adequate hematopoietic function (hemoglobin >9.0 g/dl; total leukocyte count >3,000/mm³; granulocytes >1,500/mm³; platelet count >100,000/mm³); hepatic function (alkaline phosphatase levels ≤4 times the upper limit of normal; bilirubin ≤1.5 upper limit of normal), and renal function (serum creatinine limit of normal). Patients may or may not have had extrahepatic sites of metastases. Major exclusion criteria included: (a) inability to assess vascular flow of hepatic metastatic lesion(s) by MRI; (b) therapy capable of inducing bleeding, such as warfarin, heparin or aspirin; (c) pregnancy or breast feeding; (d) major surgery, cytotoxic chemotherapy, or radiotherapy within the previous 4 weeks; (e) clinically significant cardiac disease; (f) inability to comply with or understand informed consent; and (g) brain metastases, uncontrolled seizure disorder, other active neurological disease, or serological evidence of chronic infection such as hepatitis or HIV. Signed informed consent was obtained from each patient before enrollment into the protocol. The protocol, informed consent, and informed consent procedures were reviewed and approved by the University of Alabama at Birmingham Institutional Review Board.

Treatment Plan. This study design was an open-label, single-arm Phase I trial designed to establish maximum tolerated dose and side effects of IMC-1C11 therapy. The study drug was infused in our outpatient General Clinical Research Center and patient were monitored with vital sign and symptom assessment for approximately 1 h post-infusion of IMC-1C11.

IMC-1C11 was given i.v. over ~1 h once a week for 4 weeks. This was considered a treatment cycle. Patients could continue treatment provided there was no evidence of disease progression or DLT. There were four cohorts of at least three evaluable patients per cohort. Patients were treated in cohorts of three to six patients at escalating doses of IMC-1C11 from 0.2 mg/kg to 4.0 mg/kg given weekly. Patients were enrolled at the next dose level 2 weeks after the last patient at the prior dose level completed a 4th week of IMC-1C11 in the absence of DLT. If one of the initial three patients in a cohort demonstrated a DLT, three more patients were entered into the cohort. The maximum tolerated dose was defined as one dose below the dose that induced a DLT in two or more patients in a cohort.

Toxicity was evaluated according to the expanded National Cancer Institute Common Toxicity Criteria Version 2. A DLT was defined as any grade-3 or -4 toxicity excluding allergic reactions, nausea, vomiting, or alopecia. Patients underwent evaluation of measurable disease by CT scan after completing four weekly treatments and subsequent scan were repeated after four weekly infusions. Measurable disease must have been bidimensionally ≥2 cm for lesions that are palpable, ≥1 cm and for soft tissue lesions followed by CT scan, and ≥2 cm for lytic bone lesions followed by CT or MRI. Patients without DLT who had either stable disease (defined as no change, or tumor response that has not increased by 25% or decreased by 50%) or responding disease could go on to receive additional therapy. Two exceptions to allow a second cycle of therapy were granted to patients 7 and 10, who each had a decrease in parameters of tumor perfusion at week 5, had a modest level of progression (26 and 35%, respectively), were asymptomatic, and had no FDA-approved treatment options. Patients underwent MRI for vascular flow/diffusion measurements ~1 week before the initial dose of IMC-1C11 and the week after the fourth dose. For patients who had stable or responding disease by CT, additional dynamic MRI for vascular flow/diffusion was performed 1 week after each four-dose treatment cycle.

Production of IMC-1C11. A single-chain antibody phage display library was constructed from spleen cells of mice immunized with a soluble form of KDR. After two rounds of biopanning, >90% of the clones recovered were specifically reactive to KDR (23). Subsequent selection identified two clones that blocked VEGFR binding to KDR. These two clones were expressed in Escherichia coli and purified as soluble scFv antibodies. One scFv, p1C11, was shown to inhibit VEGFR-induced KDR phosphorylation and VEGFR-stimulated DNA synthesis in human umbilical vein endothelial cells (23). This led to the development of IMC-1C11, a chimeric anti-KDR antibody (IgG1κ; Ref. 19).

IMC-1C11 was developed by immunization of BALB/c mice with KDR-alkaline phosphatase fusion protein. Purified mRNA from splenocytes of these mice was used to construct a scFv antibody phage display library. The library was used to select against immobilized KDR protein. A clone was selected, expanded in cell culture, and frozen in liquid nitrogen. Clone p1C11 scFv was selected for its high-affinity binding to KDR and for its ability to block KDR/VEGFR interaction and to inhibit VEGFR-induced biological activity on human endothelial cells. The gene segments encoding p1C11 scFv variable domains were amplified and subcloned into an expression vector that contained human IgG1κ constant domains for the expression of a mouse/human chimeric antibody, IMC-1C11. The IMC-1C11 expression vector was used to transfect cells of Chinese-hamster-ovarian origin and a stable expressing cell line was established. The cell line was expanded in cell culture and was frozen in liquid nitrogen.

Pharmacokinetics. Five-ml samples of blood were obtained from each patient during the first cycle and before each subsequent cycle. The serum from each sample was obtained preinfusion and at the end of infusion on day 1, and at 2, 6, 12, 24, 48, 96, and 168 h postinfusion. On days 8 and 15, blood samples were obtained preinfusion and 2 h postinfu-
sion. On day 22, samples were obtained preinfusion and at 2, 24, and 48 h postinfusion.

Briefly, 100 µl/well KDR-alkaline phosphatase [1 µg/ml in PBS (pH 7.2); ImClone Systems Incorporated, Somerville, NJ] was adsorbed to each well of a 96-well microtitr plate (Immulon 2, Dynatech) overnight at 2°C to 8°C. Plates were washed in washing buffer (PBS/0.05% Tween 20) and then were blocked for 2 h at room temperature with 10% HSBB [10% Horse Serum (Life Technologies, Inc.) in PBS/0.05% Tween 20]. The plates were washed with washing buffer, and a 100-µl volume of serum samples, standards, and internal controls were incubated in the wells for 2 h at room temperature. After washing with wash buffer, 100 µl of antihuman Fc-specific IgG1 horseradish peroxidase-conjugated antibody (Jackson ImmunoResearch) were added to each well. After a final washing, bound conjugate was then visualized by adding 100 µl of substrate tetramethylbenzidine (TMB) solution (Kirkgaard and Perry Laboratories). This gives a blue reaction product that turns yellow on the addition of the stopping solution (2 M H2SO4).

The plates were read on a Molecular Devices Thermomax 340PC ELISA reader using a wavelength of 450 nm. IMC-1C11 was quantitated by comparing with a standard curve prepared from reference standard IMC-1C11.

Immunogenicity. Human antibody response to IMC-1C11 was determined by a double-antigen radiometric assay procedure (24). In brief, 6.4 mm of polystyrene beads were coated with 2 µg/bead IMC-1C11 antibody in PBS by gentle agitation at 80 rpm overnight at room temperature. The beads were washed three times with phosphate buffer containing EDTA (PBE), blocked with PBE for 1 h at room temperature, and stored in PBE at 4°C. Patient sera or standards in PBE were added to a glass culture tube. After the addition of a single antibody-coated bead to the tubes, the tubes were gently agitated at 140 rpm for 1 h at room temperature. Beads were washed by adding and aspirating 4 ml of PBS. 125I-labeled IMC-1C11 antibody was added to the respective tubes and gently agitated at 140 rpm for 1 h at room temperature. Beads were washed again as above. The beads were transferred to clean tubes, then counted for 1 min to determine the 125I antibody-bound radiation. The assay result was calculated from the 125I antibody-bound radiation and known specific activity of 125I-antibody. Results were expressed as ng of IMC-1C11 antibody-bound/ml of patient serum.

Assessment of Vascular Perfusion. Dynamic contrast-enhanced perfusion MRI using Gd-DTPA (0.2 mmol/kg, supplied by Magnevist, Berlex Lab, Inc.) was performed on a GE Signa 1.5 Tesla MRI scanner (General Electric, Milwaukee, WI). A torso phased-array receiving coil was used. The vest-like coil was centered over the upper abdomen to maximize the signal:noise ratio in the liver. Two sequences were used to localize the lesion(s) for subsequent perfusion analysis: axial T1-weighted spoiled gradient echo, and axial T2-weighted single-shot fast spin echo. Each of these sequences acquired images of the entire liver in a single breath hold. The orientation of the plane of choice (axial, coronal, or sagittal) was dependent on the size and location of the lesion, to reduce the likelihood that respiration would significantly alter the lesion position during serial scanning from a selected 5-mm slice. We used multiphase fast spoil gradient echo (SPGR) pulse sequence with acquisition parameters: TR/TE = 8/4 ms, flip angle = 70°, FOV = 40 × 40 cm², slice thickness = 5.0 mm, image matrix 256 × 256, the delay between different phases was 50 ms. One hundred twenty images were acquired and each phase (image) required 1.1 s. The image data were transferred to a personal computer and were processed according to a two-compartment model4:

\[ k_{sp}^i = \frac{\text{slope}}{T_{10}^i A^i C_0^i} \]

where the slope is the initial rate of enhancement curve in the arterial phase (covers the first 20–35 images), A is the relativity and \( T_{10}^i \) = 1.433 s at 1.5 T and \( C_0^i \) = 8.77 D, D is the dose of Gd-DTPA, and the tumor influx volume transfer constant \( k_{in} \) is obtained. After the Gd-DTPA bolus injection, the nodules initial signal \( g \) is time point \( t_1 \) (set to 0 s) with signal height \( S(0) \) and reaches a maximum intensity at \( ~15–30 \) time points (an image was acquired for each time point) \( t \) with signal \( S(t) \). The maximum signal enhancement factor \( (EF) \) in arterial phase is defined as \( S(t) - S(0)/S(0) \) with \( t = 15 \) time points (at \( ~16.5 \) s). Signal beyond 15 time points likely has more portal vein blood flow, thus complicating the kinetic model analysis. MRI assessment was carried out before therapy and 1 week after the fourth infusion of each treatment cycle.

Statistical Analysis. Because of the noncomparative nature of this study, descriptive statistics were used to summarize toxicities and laboratory data. The frequency of adverse events was tabulated, as well as the proportion of patients exhibiting each adverse event. Pharmacokinetic analysis was conducted on 14 patients. Compartmental models were fit to each patient’s concentration profile using the nonlinear regression procedure in SAS (25). Pharmacokinetic parameters area under the curve (AUC), half-life (\( t_{1/2} \)), clearance (CL), and volume of steady state (V) were estimated using the fitted compartmental model. MRI assessments of vascular flow before and after treatment were examined, and the dose groups were compared descriptively. Comparisons were made before and after treatment overall, ignoring dose groups, using the paired \( t \) test and the signed-rank Wilcoxon test. For the dose group comparisons, the difference in vascular flow before and after therapy was computed for each patient, and the dose groups were compared using ANOVA and the nonparametric Kruskal-Wallis test. Spearman’s correlation coefficients were computed between vascular parameters \( (EF) \) and \( k_{in} \) and tumor growth using the CORR procedure in SAS.

RESULTS

Patient Characteristics. The distribution of the fourteen patients among the four cohorts and their characteristics are listed in Table 1. There were six men and eight women, with an age range from 43 to 72 years. They all had excellent performance status, a median of two prior chemotherapy regimens, and five had liver as their only site of metastasis. Eight of the patients received one cycle of therapy (four infusions), whereas

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4 T. C. Ng et al., Arterial phase Gd-enhanced MRI perfusion in liver metastasis from colorectal cancer, manuscript in preparation.
five received two cycles (eight infusions), and one received seven cycles (twenty-eight infusions).

Toxicity. The antibody treatment did not produce acute infusion symptoms and produced no grade-3 or -4 toxicity (Table 2). All of the grade-1 or -2 symptoms were sporadic, lasting 1–3 days, were not repetitive with each infusion, and did not increase in frequency with increasing dose cohorts. The four bleeding episodes, possibly related to IMC-1C11, included a patient with 3 days of brief and scanty epistaxis, two patients with 1 day of blood streaking of sputum, one of whom also had 1 day of blood streaking in his stool; and one patient (patient 7) who had gross hematuria on four occasions over 8 weeks of therapy and who was documented to have tumor infiltration of her right kidney. Bleeding episodes were reported as history data and were not active at the time of visits. Platelet counts before and after episodes remained within the normal range, and coagulation profiles were not done because the patients were not actively bleeding. Overall, the antibody infusions were well tolerated, and in most patients, symptoms were related to the underlying disease process.

Antibody Response to IMC-1C11. Human antibody response to IMC-1C11 is presented in Table 3 over the initial 42 days of the trial. A total of 7 of 14 patients had evidence of detectable antibody on more than one occasion; this was observed in the two low-dose cohorts. In addition, one patient (patient 10) had a single borderline value of 34 ng/ml on day 22. The two highest antibody responses were seen on day 22 in patients 3 and 6 and are discussed below regarding IMC-1C11 plasma kinetics.

Pharmacokinetics. Table 4 summarizes the pharmacokinetic parameters of the initial infusion of IMC-1C11 in patients from the four-dose cohorts. The kinetics were assessed using both a one-compartment and a noncompartmental analysis with similar results. The one-compartment results are presented here. The two lower-dose cohorts were characterized by a short plasma t1/2, high plasma clearance rate (CL), and modest AUC. Multiple comparisons using ANOVA revealed significant differences between the dose cohorts (P = 0.01) for plasma t1/2. The dose cohorts of 2 mg/kg and 4 mg/kg had much longer plasma t1/2 (56 and 67 h) and lower plasma clearance rates (CL, 0.57 and 0.40 ml/h/kg), which were significantly different from the two low-dose cohorts. The two high-dose cohorts had much higher area under the curve (AUC) than the low-dose cohorts, reflecting both the larger dose administered and the longer
plasma half-life. Fig. 1 provides the plasma IMC-1C11 disappearance curves plotted as the percentage of maximum IMC-1C11 concentration, demonstrating the substantial differences in plasma kinetics in the low-dose cohorts. Fig. 2 presents the plasma disappearance curves in terms of IMC-1C11 mean plasma concentrations depicting the inability of the low-dose cohorts to maintain plasma levels of IMC-1C11 beyond 48-h postinfusion and the mean levels generated in the higher-dose cohorts. The ability of four weekly infusions to maintain continuously circulating IMC-1C11 levels was evaluated over the initial 4 weeks of treatment. Nadir blood levels before repeat infusion were either 0 or <1 μg/ml in the two lower-dose groups. In patients receiving 2 mg/kg IMC-1C11, the mean nadir level was 7.7 ± 6.7 (range, 1–19) μg/ml, whereas patients in the 4-mg cohort had values of 43.9 ± 23.9, with a range of 9–94 μg/ml. The fourth infusion plasma concentrations and kinetics of IMC-1C11 were compared with each patient’s initial infusion. In general, the fourth infusion had similar values as each patient’s initial infusion with two exceptions. Patient 3 had a fourth infusion C<sub>max</sub> that was 28% of the first infusion C<sub>max</sub>, and a 24-hour value that was below detection. This infusion took place on day 22 when he had an anti-IMC-1C11 level of 460 ng/ml.

### Table 3 Human antichimeric antibody response to IMC-1C11<sup>a</sup>

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Dose (mg/kg)</th>
<th>Day 1 (ng/ml)</th>
<th>Day 22 (ng/ml)</th>
<th>Day 36 (ng/ml)</th>
<th>Day 42 (ng/ml)</th>
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<td>16</td>
<td>77</td>
<td>117</td>
<td>133</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>6</td>
<td>13</td>
<td>15</td>
<td></td>
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<tr>
<td>3</td>
<td>0.2</td>
<td>11</td>
<td>460</td>
<td>239</td>
<td>28</td>
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<tr>
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<td>8</td>
<td>71</td>
<td>52</td>
<td>46</td>
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<td>65</td>
<td>69</td>
<td>87</td>
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<tr>
<td>6</td>
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<td>21</td>
<td>86</td>
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<td>0.6</td>
<td>17</td>
<td>37</td>
<td>41</td>
<td>26</td>
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<tr>
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<td>8</td>
<td>7</td>
<td>51</td>
</tr>
<tr>
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<td>6</td>
<td>7</td>
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</table>

<sup>a</sup> Normal sera (n = 27) 10 ± 6 ng/ml (× ± 1 SD) and a positive value was >28 ng/ml (>3 SD).

### Table 4 IMC-1C11 pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Dose level (mg/kg)</th>
<th>Mean dose (mg)</th>
<th>AUC&lt;sup&gt;a&lt;/sup&gt; (μg/ml·h)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (μg/ml)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (hrs)</th>
<th>CL (ml/h/kg)</th>
<th>Volume (ml/kg)</th>
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<td>0.2</td>
<td>22</td>
<td>209 ± 102</td>
<td>10.6 ± 8.3</td>
<td>16 ± 7</td>
<td>1.13 ± 0.57</td>
<td>26.1 ± 14.1</td>
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<tr>
<td>0.6</td>
<td>48</td>
<td>394 ± 116</td>
<td>20.6 ± 8.4</td>
<td>13 ± 5</td>
<td>1.66 ± 0.64</td>
<td>32.2 ± 10.1</td>
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<tr>
<td>2.0</td>
<td>141</td>
<td>3,674 ± 1,031</td>
<td>45.2 ± 3.4</td>
<td>56 ± 12</td>
<td>0.57 ± 0.14</td>
<td>44.4 ± 3.3</td>
</tr>
<tr>
<td>4.0</td>
<td>306</td>
<td>10,434 ± 2,266</td>
<td>107.3 ± 27.2</td>
<td>67 ± 3</td>
<td>0.40 ± 0.9</td>
<td>39.1 ± 9.5</td>
</tr>
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</table>

<sup>a</sup> AUC, area under the curve; CL, clearance.

![Fig. 1](image1.png) Mean plasma IMC-1C11 levels expressed as percentage of C<sub>max</sub> achieved at the four treatment dose levels based on a one-compartment model.

![Fig. 2](image2.png) Mean plasma concentrations (μg/ml) of the initial day 1 infusion of IMC-1C11 for each dose-group based on a one-compartment model.
ng/ml. Patient 6 had an undetectable $C_{\text{max}}$ on his fourth infusion, when his anti-IMC-1C11 was 86 ng/ml.

**Alteration in Vascular Parameters.** Eleven of the 14 patients were evaluated for changes in vascular perfusion. Two parameters were measured before and after one cycle of treatment: the enhancement factor (EF) and tumor influx rate constant $k_{\text{in}}$ (min$^{-1}$), both of which are proportional to perfusion. Patients 1, 2, and 11 were not evaluated secondary to technical difficulty with the procedure or data acquisition. On the computer-generated image slice, a ROI was placed around index liver lesion(s). A detailed analysis of these studies will be published separately, but Table 5 provides a summary of the vascular parameters and tumor size change.

### Table 5 MRI vascular parameters and tumor size change

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Patient no.</th>
<th>$K_{\text{in}}$ (min$^{-1}$)</th>
<th>EF</th>
<th>Tumor size change</th>
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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>$\Delta$</td>
<td>Pre</td>
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<td>14</td>
<td>0.58</td>
<td>0.36</td>
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$K_{\text{in}}$, influx rate constant; EF, enhancement factor; Pre, pretreatment; Post, posttreatment.

DISCUSSION

Tumor-induced angiogenesis seems to be critical to the establishment and growth of primary and metastatic cancer cells (27). The vascular response and support of tumors represents an interplay of proangiogenic and antiangiogenic factors produced by tumor cells and surrounding normal cells (28). VEGFR has been reported to be one of the most important of the proangiogenic factors involved in murine tumor models (21) and patients (29, 30). As a result, several VEGFR-targeted therapeutic strategies have been developed and there are ongoing clinical trials to evaluate clinical benefit. VEGFR angiogenic activity is mediated through receptor tyrosine kinases that are present principally on endothelial cells. Two of the three identified VEGFR receptor family members are expressed on endothelial cells (VEGFR1 and VEGFR2), and the VEGFR2 (KDR/flk-1) has been shown to play the central role in tumor angiogenesis (31, 32).

Four VEGFR-targeted treatment strategies have entered the clinic to date. These include a humanized monoclonal antibody directed to VEGFR (Bevacizumab), a chimeric monoclonal antibody directed to the VEGFR2 (IMC-1C11), several small molecule inhibitors of the VEGFR2 tyrosine kinase, and a ribozone (Angiozyme) specific for Flt-1/KDR mRNA. Although the two monoclonal antibody strategies would appear similar, i.e., inhibition of ligand or inhibition of ligand receptor,
they are likely to differ regarding alteration of different molecular pathways and possibly in vivo effects. It is clear that VEGFR can function as a ligand for a variety of receptors including three receptor/pathways in the VEGFR receptor family and at least one of two receptor/pathways in the neuropilin family (33). Thus, inhibition of VEGFR ligand has the potential to alter multiple signal transduction pathways in multiple cell types, whereas the targeting of the VEGFR2 (KDR) would be much more selective in that this receptor is found primarily on activated endothelial cells and some malignant cell types (34).

There are several parenteral and oral inhibitors of the VEGFR2 tyrosine kinase that are currently being examined in clinical trials, including SU5416, PTK 787, ZD6474, and CP547632 (35–38). These agents have been selected on the basis of relatively selective inhibition of the VEGFR2 ATP binding site. However, the large array of potential tyrosine kinase signaling elements in the human genome include many the functions of which are yet to be discovered. This may provide the potential for nonspecific (non-VEGFR2) effects that could account for side effects like refractory headaches, nausea, vomiting, and thrombotic events reported in early trials (39).

Thus, targeting the VEGFR2 (KDR) receptor represents a rather selective antiangiogenic strategy that has been demonstrated to have potent in vivo antitumor effects in murine models of multiple tumor types. This Phase I trial is the first clinical trial of a VEGFR2 (KDR)-specific monoclonal antibody, IMC-1C11. This reagent was very well tolerated over a dose range of 0.2–4.0 mg/kg weekly for a 4-week cycle, with total doses

**Fig. 3** Pre-therapy dynamic flow demonstrating two ROI in (A) with magnification of ROI (B and C). Post-therapy dynamic flow of the same ROI (D) with magnification views (E and F). Flow color bar, indicates intensity enhancement which is proportional to profusion.
administered at the highest-dose cohort of 920 to 1504 mg over 4 weeks. There were no DLTs, and most side effects reported were thought to be disease related. Several patients reported mild transient bleeding episodes (grade 1) with no particular pattern or dose effect. One patient had significant hematuria but had tumor invasion of the kidney as a contributing factor.

The pharmacokinetics of IMC-1C11 demonstrated a striking dose dependency with the two lower-dose cohorts (0.2 and 0.6 mg/kg) having a short plasma $t_{1/2}$, a high plasma clearance rate, and an inability to maintain circulating levels of antibody on a weekly infusion schedule. The two higher-dose cohorts were able to maintain circulating levels of IMC-1C11 on the weekly schedule, but the 4-mg/kg dose level was significantly better than the 2-mg/kg dose level. The plasma $t_{1/2}$ at 4 mg/kg was ~3 days, and, therefore, chronic schedules of administration could use weekly or biweekly schedules depending on the chosen target nadir levels. This pattern of dose dependence was also seen in our prior trial of anti-avβ3 monoclonal antibody (Vitaxin), which also targeted an endothelial cell-expressed antigen. It may well be that endothelial cell antigens that are up-regulated on tumor neoangiature have a baseline low expression on normal endothelial cells representing an intravascular antigen sink requiring higher dose administration to achieve adequate pharmacokinetics similar to that seen with anti-CD20 in lymphoma patients (“B-cell sink”) and anti-EGFR, (C225) with hepatic EGFR sink (40, 41).

Another characteristic of IMC-1C11 was its immunogenicity. Fifty percent of the patients developed a human antibody to chimera antibody (or HACA) response, primarily in the two low-dose cohorts. Two patients with the highest HACA response had an impairment of IMC-1C11 circulation at the time of their fourth infusion (day 22) when they had peak HACA levels. Some chimera mouse-human monoclonal reagents have had little or no immunogenicity in human trials, whereas others have induced significant HACA response. For example, C225 (anti-EGFR) has produced little or no HACA when assessed by the same HACA assay used in this trial (41).

Several of the antiangiogenesis trials have attempted to measure the vascular effects of antiangiogenesis strategies. This trial was our first experience with using dynamic contrast-enhanced perfusion MRI to assess the flow in hepatic metastases, and we were impressed with its potential. A detailed analysis of the data will be presented separately, but Table 5 provides a summary of the observations that demonstrated a significant decrease in two parameters ($k_{in}$ and $EF$) associated with the four doses of IMC-1C11. These are the same parameters that were reported to have decreased in a recent trial of a small-molecule inhibitor of VEGFR-mediated receptor kinase activation (42). However, the decreases in these parameters were transient, returned to baseline by week 8, and were associated with progressive disease in 8 of 11 patients. Three of four patients receiving a second cycle of therapy had vascular flow parameters that returned to pretherapy levels. It is unknown at this stage of antiangiogenesis clinical trials, what represents a clinically important change in MRI parameters of tumor-blood perfusion or of the duration of therapy and/or duration of flow inhibition required to prevent tumor growth. This trial would suggest that 4 weeks of therapy and/or 4 weeks of reduced tumor flow via MRI parameters are insufficient to prevent tumor growth, although it was interesting that a decline in flow parameters tended toward a decreased degree of tumor growth,
which might suggest that therapy trials of antiangiogenic agents be designed for longer treatment periods than 4 weeks as suggested by others (43, 44).

Finally, none of our patients had objective tumor regressions, although one patient had a long-term tumor stabilization despite progression documented prior to the institution of IMC-1C11 therapy. This experience is similar to that seen with other single-agent antiangiogenesis trials (45), in which tumor regressions are infrequent. This may reflect the need for long-term chronic treatment regimens to alter tumor growth rates or the need for combining antiangiogenesis strategies with other treatment modalities. The antimitmine VEGFR receptor reagent, DC101, has shown striking ability to enhance radiation (46) and chemotherapy (47) antitumor efficacy in animal models. Similar trials in human cancer are indicated.

This study provides evidence of the safety and low toxicity for an antibody blockade of VEGFR2, as well as insight into dose and schedule requirements. A fully human anti-VEGFR2 agent has been produced as a second-generation agent, which is anticipated to be nonimmunogenic for chronic administration as a single agent and in combination with chemotherapy or radiation.

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A Phase I Study of Anti-Kinase Insert Domain-containing Receptor Antibody, IMC-1C11, in Patients with Liver Metastases from Colorectal Carcinoma

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