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Translational Relevance

Endoglin (CD105) is an endothelial cell membrane receptor that is highly expressed on hepatocellular carcinoma (HCC) vasculature and is upregulated by inhibitors of the VEGF pathway. This phase I study assessed the safety and efficacy of an endoglin antibody (TRC105) when combined with sorafenib in a sorafenib-naïve HCC patient population. Encouraging clinical activity was seen, suggesting a potential combinatorial approach to build on the initial promise of sorafenib in patients with HCC.

to mediating antibody-dependent cellular cytotoxicity (8, 9). We had previously assessed TRC105 in a phase II study in HCC, demonstrating lack of significant single-agent activity (10). However, based on a strong scientific rationale for combination with another antiangiogenic strategy and the finding of preclinical efficacy for endoglin antibody when combined with sorafenib in a murine syngeneic model of HCC, we conducted an open-label single-arm phase I study and expansion cohort to assess the safety and efficacy of TRC105 combined with sorafenib in a sorafenib-naïve HCC patient population.

Patients and Methods

Preclinical experiments

Female BALB/c mice, 6 to 8 weeks of age, were obtained from NCI–Frederick (Frederick, MD). BNL, a murine HCC cell line, was kindly provided from University of Navarra, Pamplona, Spain (11). Mycoplasma testing was performed by SAIC Frederick 2 months prior to experiments. Cells were routinely kept in culture for no more than 8 to 10 passages. Mice were injected subcutaneously with 1×10^6 BNL cells. One week after tumor inoculation when tumors were palpable, mice received a daily oral gavage of sorafenib (Bayer) at a dose of 10 mg/kg. Sorafenib stock solution (4×) was freshly prepared every 4 days using Cremophor EL/ethanol (50:50; Sigma). The final 1× dosing concentration was prepared by diluting with sterile water immediately prior to administration to mice. Control mice received vehicle. Endoglin antibody (clone MJ7/18), which binds murine endoglin, was purchased from the Developmental Studies Hybridoma Bank at the University of Iowa (Iowa City, Iowa) and purified at NCI (Rockville, MD). A total of 100 mg/mouse was given intraperitoneally every other day. Tumors were measured every other day using digital calipers. All mice were handled, fed, and housed in accordance with the U.S. Department of Health and Human Services institutional guidelines. Experimental protocol was approved by NCI Animal Care and Use Committee.

Clinical trial

Eligible patients were at least 18 years old with histopathologic confirmation of HCC by the Laboratory of Pathology of the NCI. Other eligibility criteria included: Eastern Cooperative Oncology Group performance status score 0–2; adequate bone marrow, liver, and renal function; disease not amenable to potentially curative liver transplantation, resection or ablative techniques, and progression following or not be amenable to transhepatic arterial chemoembolization (TACE). Prior progressive disease on sorafenib was excluded. If liver cirrhosis was present, patients must have had a Child–Pugh A or B (7 points) classification. In addition, patients with cirrhosis were required to have had esophagogastric endoscopy within 6 months prior to study entry for the assessment of varices. Concomitant treatment of underlying cancer was prohibited. All patients provided written informed consent. This study was approved by the NCI Institutional Review Board (ClinicalTrials.gov identifier: NCT01306058).

Study design

Patients who satisfied the eligibility criteria were enrolled in a $3 + 3$ dose escalation phase I study of TRC105 at dose cohorts of 3, 6, 10, and 15 mg/kg given every 2 weeks in combination with sorafenib 400 mg twice daily. A phase II cohort of the trial was opened afterwards at the MTD to establish the response rate to therapy. Prior to each TRC105 infusion, patients were premedicated with dexamethasone, acetaminophen, H2-blockade, and an anti-histamine prior to initial dosing, and dexamethasone was then discontinued in the absence of infusion reactions. Staging was performed by either contrast-enhanced CT or, in select cases, MRI scan every 8 weeks. Objective response and progression were evaluated in this study using the international criteria proposed by RECIST version 1.1. Dose-limiting toxicity (DLT) criteria included treatment-related grade 3 nonhematologic toxicities or grade 4 hematologic toxicities occurring within the first 28 days of treatment. First dose infusion reactions, a known toxicity of TRC105, were not considered DLT. Patients were considered evaluable for safety if they received any study treatment and were considered evaluable for efficacy if they received at least 1 week of treatment with sorafenib and TRC105.

Safety

All adverse events and serious adverse events occurring within 30 days of the last dose were reported according to the NCI Common Terminology Criteria for Adverse Events v4.0.

Pharmacodynamic studies

Correlative biomarkers of TRC-105 effect were evaluated with radiologic techniques as well as assays performed on peripheral blood. All tests were performed at multiple time points including baseline and during the first and second 4-week cycles of treatment. Contrast-enhanced MRI was employed to detect effects on tumor vasculature. Imaging with MRI was performed at two time points (at baseline and during cycle 2 day 1 ± 2 days). Normalized signal intensities in unenhanced and enhanced MRIs were compared at each available time point with calculation of measured percentage of signal change to reflect tumor vascularity. MRI was performed on a 3T MR system (Philips Achieva) with a dedicated receive-only phased array coil.

Blood samples were collected in EDTA-containing Vacutainer at pretreatment (baseline), day 15 of the first cycle, day 1 of the second cycle, and following treatment discontinuation. After centrifugation, plasma samples were immediately frozen and stored at −80°C. Plasma biomarker tests were performed for VEGF and placental-derived growth factor (PlGF) using assay plates from Meso-Scale Discovery (MSD) according to the product manual. The concentrations of the cytokines were determined with recombinant standards and expressed as pg/mL.

ELISA (R&D Systems) was used to determine the specific concentrations of soluble CD105 in plasma samples. The addition of TRC105 *in vitro* inhibited the detection of soluble CD105,

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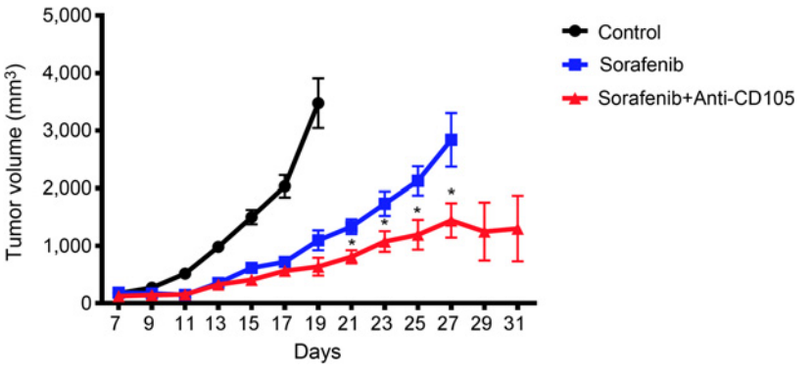

Figure 1.
Tumor volume over time following BNL tumor inoculation in BALB/c mice given daily oral gavage of sorafenib (10 mg/kg) with or without anti-CD105 antibody (100 mg/mouse injected intraperitoneally every other day) or no treatment. Tumor sizes are presented as mean ± SEM ($n = 14$ for control, $n = 10$ for sorafenib, $n = 10$ for sorafenib + anti-CD105).

and only plasma samples without detectable TRC105 serum concentrations were considered for analysis. ELISA was done per the manufacturer's instructions.

Pharmacokinetics

TRC105 serum concentrations were measured using a validated ELISA with a lower limit of quantification (LLOQ) of 200 ng/mL. Pharmacokinetic samples were drawn just prior to, and approximately 5 minutes following intravenous infusion of TRC105 on days 1, 15, 30, 45, and 60.

Statistical analysis

The primary objective of the phase I portion of the study was to determine the MTD for TRC105 when given with sorafenib in HCC. Once this was established, preliminary evidence of efficacy was assessed in an expansion phase II cohort to increase the experience with the combination and to determine whether it was associated with a response rate that was likely to exceed that of sorafenib alone. Results from the SHARP study suggested that an overall response rate for sorafenib alone in this patient population was 2% (1). The aim in the expansion phase II cohort was to rule out an unacceptably low partial response (PR) + complete response (CR) rate of 5% ($p0 = 0.05$) in favor of an improved PR + CR rate of 25% ($p1 = 0.25$). With $\alpha = 0.10$ (probability of accepting a poor treatment = 0.10) and $\beta = 0.20$ (probability of rejecting a good treatment = 0.20), the trial was designed to enroll 6 evaluable patients in the first stage and if at least one response was noted, to continue enrollment until a total of 23 patients were enrolled, in which case 3 or more responses would be considered adequate demonstration of efficacy. Other secondary objectives of this trial were to evaluate progression-free and overall survival by the Kaplan–Meier method as well as pharmacodynamic markers of drug effect. Paired data from angiogenic biomarkers obtained on study and following study treatment were compared with pretreatment results using a Wilcoxon signed rank test. All *P* values are two-tailed and presented without adjustment for multiple comparisons. Plasma biomarker analysis was performed with GraphPad Prism 7.0 as well as SAS Version 9.3.

Results

Preclinical experiments

On the basis of the hypothesis that endoglin expression is upregulated by hypoxia and inhibitors of the VEGF pathway, such as sorafenib, and acts as a mechanism of resistance (6, 7), we explored the potentially complementary roles of combined VEGF pathway and endoglin inhibition in a preclinical experiment. Seven days after BNL tumor inoculation, BALB/c mice were given daily oral gavage of sorafenib (10 mg/kg). Endoglin antibody (100 mg/mouse) was injected intraperitoneally every other day. Tumor sizes are presented as mean ± SEM ($n = 14$ for control, $n = 10$ for sorafenib, $n = 10$ for sorafenib with endoglin). As shown in Fig. 1, the combination of sorafenib and endoglin antibody resulted in enhanced antitumor activity compared with sorafenib alone.

Patient characteristics

Twenty-six patients were enrolled in a clinical trial evaluating escalating doses of TRC105 in combination with sorafenib given at standard doses of 400 mg twice daily. One patient signed consent but developed rapid disease progression and did not receive any treatment. The baseline characteristics of the remaining evaluable ($N = 25$) patients are presented in Table 1. The

Table 1. Patient characteristics

Total	25
HCC/Fibrolamellar	24/1
Age	
Median (range)	60 (18–76)
Sex	
Male	19
Female	6
ECOG	
0	8
1	17
Liver cirrhosis	
Yes	15
No	10
Etiology of HCC	
HBV	3
HCV	15
Cryptogenic	6
Hemochromatosis	1
Baseline Child–Pugh score	
5	10
6	4
7	1
NA	10
Extrahepatic disease	
Yes	17
No	8
Prior therapies[a]	
No prior intervention	9
≥2 locoregional procedures	7
Previous TACE	8
Surgery/transplant	5/2
Ablation	2
Radioembolization	2

Abbreviations: ECOG, Eastern Cooperative Oncology Group; HBV, hepatitis B virus; HCV, hepatitis C virus.
[a]Of note, some patients received more than one prior therapeutic intervention.

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Table 2. Toxicity

	Treatment-emergent adverse events ($n = 25$)			
	Any grade	**Grade 3**	**Grade 4**	**Grade 5**
Headache	20 (80%)			
Epistaxis	19 (76%)			
Increased aspartate transaminase	18 (72%)	5 (20%)		
Rash, other	18 (72%)	3 (12%)		
Hypophosphatemia	18 (72%)	7 (28%)		
Hypoalbuminemia	17 (68%)	2 (8%)		
Anemia	16 (64%)	1 (4%)		
Fatigue	15 (60%)			
Increased alkaline phosphatase	15 (60%)	5 (20%)		
Diarrhea	15 (60%)	1 (4%)		
Increased blood bilirubin	14 (56%)	6 (24%)	1 (4%)	
Nausea	14 (56%)			
Increased alanine transaminase	13 (52%)			
Oral mucositis/pain	12 (48%)			
thrombocytopenia	10 (40%)	1 (4%)		
Amylase	10 (40%)	2 (8%)	1 (4%)	
Abdominal pain	9 (36%)			
Hand-foot skin reaction	8 (32%)	2 (8%)		
Infusion reaction	8 (32%)	1 (4%)		
Neutropenia	8 (32%)			
Weight loss	8 (32%)			
Hypertension	6 (24%)	1 (4%)		
Vomiting	6 (24%)			
Hypomagnesemia	5 (20%)			
Alopecia	5 (20%)			
Insomnia	4 (16%)			
Constipation	3 (12%)			
Intracranial hemorrhage		1 (4%)		
Myocardial ischemia				1 (4%)
Lipase			1 (4%)	
Hyperglycemia	1 (4%)	2 (8%)		
Hyperuricemia			2 (8%)	

majority were male (M:F 19:6) with a median age of 60 (range, 18–76). One patient had fibrolamellar variant HCC. Cirrhosis was present either by clinical or pathologic diagnosis in 15 patients with a median Child–Pugh score of 5. The most common etiology for HCC was viral hepatitis. Fifteen patients had hepatitis C. Three patients had hepatitis B, all of whom were on antiviral medication at the time of enrollment. All of the patients were Barcelona Clinic Liver Cancer stage C, for whom sorafenib was indicated. Seventeen of the patients had extrahepatic disease. Two patients had prior liver transplant and 4 had recurred following partial hepatectomy. Eight patients had prior locoregional therapies, which consisted of TACE, radioembolization, or radiofrequency ablation.

Safety

Overall treatment was well tolerated. One patient developed a severe infusion reaction on the first dose of TRC105 and continued on sorafenib alone. The most frequent toxicities were headache and also chronic, intermittent grade 1 oral cavity bleeding or epistaxis, a known toxicity of TRC105, reflecting mucocutaneous telangiectasia. One patient who remained on therapy for over 2 years at the higher dose level required multiple tooth extractions, possibly related to his chronic gingival bleeding. The headache tended to occur in the first few days following TRC105 infusion and was only moderately responsive to analgesics. Two patients required antimigraine medication. Three patients were treated at dose levels 1 (3 mg/kg TRC105) or 2 (6 mg/kg TRC105; see Supplementary Table S1 for dose level enrollment). One patient developed DLT (grade 3 AST elevation) at dose level 3 (10 mg/kg TRC105), and this dose level was expanded to 6 patients. One patient at dose level 2 developed a grade 3 cerebral hemorrhage attributed to a brain metastasis found on MRI scan, although a contributory effect of the investigational therapy could not be excluded. In addition, one patient at dose level 2 experienced fatal myocardial ischemia 6 weeks after starting on study, an event that was considered at least possibly attributable to therapy, although emergent angiography revealed extensive coronary artery disease. Dose escalation continued to dose level 4 (15 mg/kg TRC105) without further DLT, and this was determined to be the MTD. In total, 12 patients were treated at this dose level. Grade 3 or 4 treatment-related toxicities for all study participants are summarized in Table 2.

Efficacy

The overall response rate in 24 evaluable patients at all 4 dose levels was $5/24 = 21\%$ [95% confidence interval (CI), 7.1–42.2], and was $5/20 = 25\%$ (95% CI, 8.7–49.1) in patients with measureable disease. Four of the five responses occurred at the highest TRC105 dose level (dose level 4 at 15 mg/kg), and one response occurred at dose level 3 (Fig. 2A). To obtain a better estimate of efficacy once the MTD was established, we expanded dose level 4 so that a total of $N = 12$ patients were treated at this dose level, of whom 4 of 10 patients with measureable disease had response by RECIST (Fig. 2B). Duration of response ranged from 4.4 months to 27.6 months. Examples of clinical responses are shown in Fig. 3. One of the responses (Fig. 3E and

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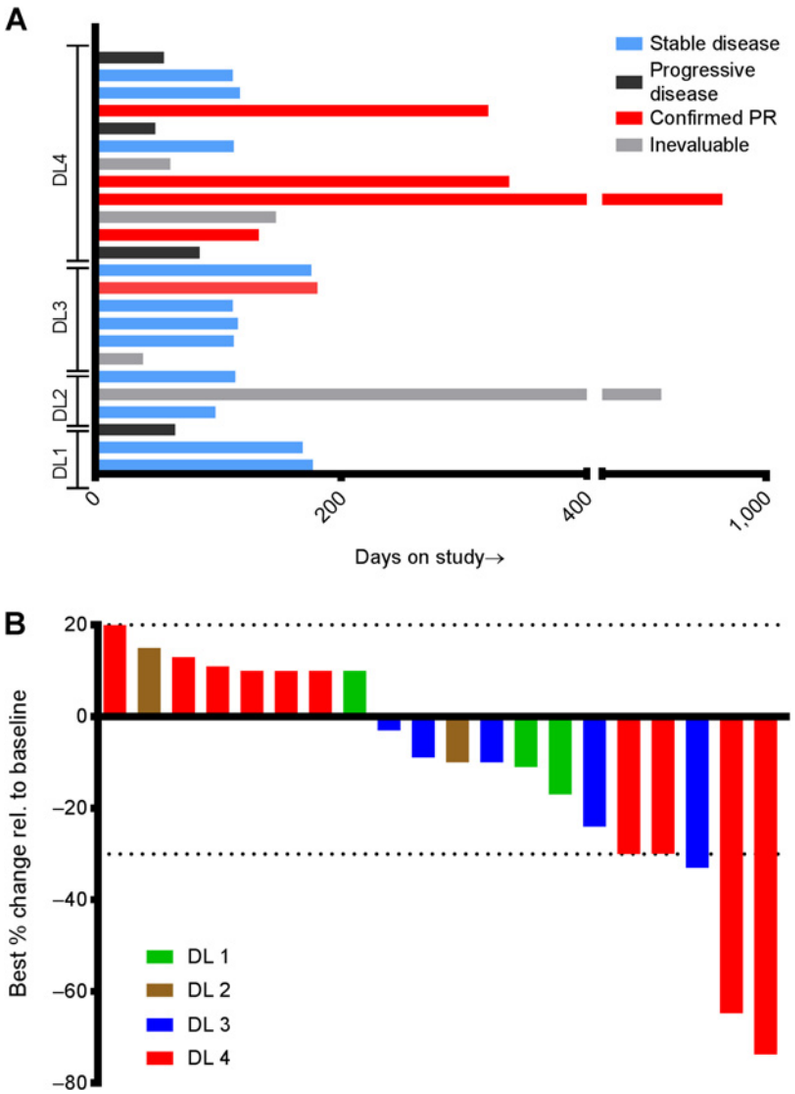

Figure 2.
Efficacy data for study population; **A,** Swimmer plot showing time on study and nature of response; red line, PR; Blue, stable disease; black = progressive disease; gray, nonevaluable. **B,** Waterfall plot showing magnitude of best response as reflected by change in target lesion sum over time as per RECIST.

F) manifested primarily as extensive necrosis. There were no objective responders to study treatment at the lower dose levels. With a median potential follow-up of 36.5 months, the median time to tumor progression in this study was 3.8 months (95% CI, 3.2–5.6 months) with a median overall survival of 15.5 months (95% CI, 8.5–26.3 months; Supplementary Fig. S1), including 1 patient who died at 33.5 months after initiating treatment.

Pharmacokinetics

Mean peak TRC105 serum concentrations were plotted over time by dose level to assess accumulation. Using data from all 24 evaluable patients, it appeared that there was a slight accumulation from day 1 (first dose) to day 15 (second dose), but none thereafter (Fig. 4A). Peak TRC105 serum concentrations were moderately well correlated (Spearman $r = 0.7$) with dose, increasing in an apparent linear (dose-proportional) manner (Fig. 4B). All TRC105 trough concentrations on dose level 1 (3 mg/kg) were below the LLOQ of 200 ng/mL. Only 1 of 3 patients at dose level 2 (6 mg/kg), 3 of 6 patients at dose level 3 (10 mg/kg), and 8 of 12 patients at dose level 4 (15 mg/kg) had measurable trough levels of TRC105.

Pharmacodynamics

Because TRC105 interfered with the R&D Systems ELISA, soluble endoglin was only assessed in patient samples without detectable TRC105 concentrations. Median soluble endoglin levels increased prior to dosing in cycle 2 compared with baseline (64.5 vs.versus 27.5 ng/mL; $P < 0.0001$; Fig. 5A). Median plasma levels of VEGF and PlGF increased after 4 weeks of therapy [243.4 vs. 202.5 ($P = 0.0025$) and 68.8 vs. 44.6 ($P = 0.0019$), respectively; Fig. 5B and C].

We evaluated the perfusion of the tumors with the analysis of normalized signal intensity in unenhanced and enhanced MRIs at each available time point with calculation of measured percentage of signal change to reflect tumor vascularity. Supplementary Figure S2 depicts a waterfall plot showing percentage of signal change in each evaluable patient compared with baseline. Although the majority of patients exhibited decrease in signal intensity following treatment, there was no correlation with dose

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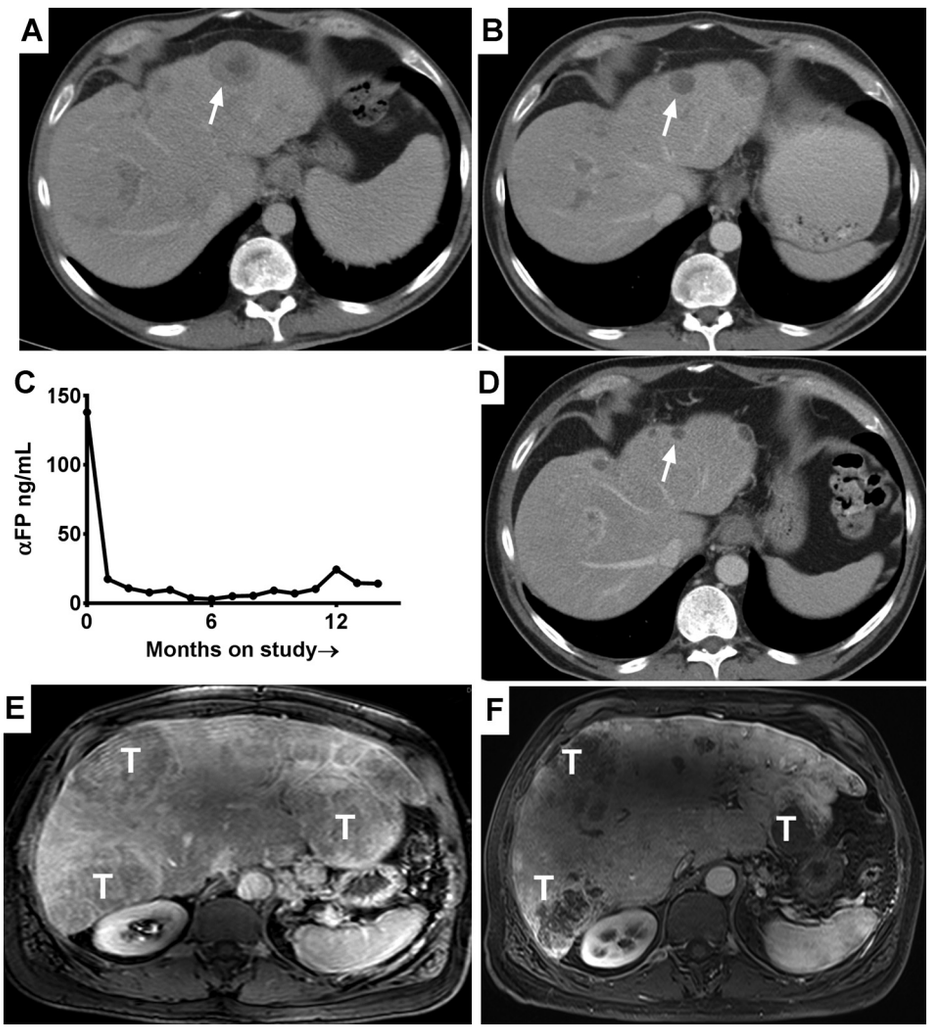

Figure 3.
A, **B**, and **D,** CT scans in a 61-year-old gentleman with hepatitis C virus (HCV)-related HCC reduction in target lesion (white arrow) over time (**A**, baseline; **B**, 8 weeks; **D**, 12 months). **C,** Peripheral blood aFP measurement over time for the same gentleman. **E** and **F,** MRI scan at baseline and after 4 months of treatment for another responding patient with HCV-related HCC showing marked necrotic response in tumor (denoted T).

level and in only one case did a significant signal decrease correlate with objective response by conventional imaging.

Discussion

HCC has long been considered unique in terms of its reliance upon hepatic arterial blood supply and the presence of relative hypervascularity (12). Indeed, these very features are taken advantage of to aid both diagnosis and treatment. The sole proven drug treatments for advanced disease, sorafenib, and more recently regorafenib, have antiangiogenesis as their putative main mode of action (2). VEGF pathway blockade, either by specific inhibition with antibody or as a result of multikinase inhibition, causes intratumoral hypoxia, which in turn leads to upregulation of hypoxia-inducible factor-1a and the compensatory, even counteractive, transcription of many proangiogenic genes (13). There appears to be a close interplay between endoglin and VEGF levels (8). Endoglin expression is one of the responses to hypoxia induced by antiangiogenic or, more specifically, anti-VEGF pathway agents (8). It represents an attractive target in solid tumor oncology given this fact, and also because, by itself, endoglin is essential for endothelial cell proliferation and angiogenesis (7). Mice lacking endoglin die *in utero* from the absence of angiogenesis (5). Endoglin is densely expressed on the proliferating endothelial cells of many tumor types and has been correlated with a poor prognosis (7). In HCC, endoglin was found to be expressed in 100% of surgically resected specimens ($N = 113$) and highly specific for tumor areas in that neither the normal nor adjacent para-carcinomatous tissue stained positively for endoglin by IHC (4).

We found that endoglin-directed therapy, in combination with sorafenib, had enhanced antitumor efficacy in a preclinical mouse model. In a phase I clinical trial, we found that when the humanized antiendoglin mAb, TRC105, was similarly combined with sorafenib in patients with advanced HCC, there was evidence of efficacy, with objective, relatively durable, responses in a proportion of patients. The finding of objective responses was encouraging. In patients with advanced HCC, the objective response rate to sorafenib monotherapy, as reported by the SHARP study and the Asian-Pacific study, the two large phase III trials resulting in its early approval, was 2% and 3%, respectively (1, 14). In our study, 5 patients demonstrated confirmed PRs by standard RECIST criteria with an overall intention-to-treat objective response rate of 5/24 (21%) and a response rate of 5/20 =

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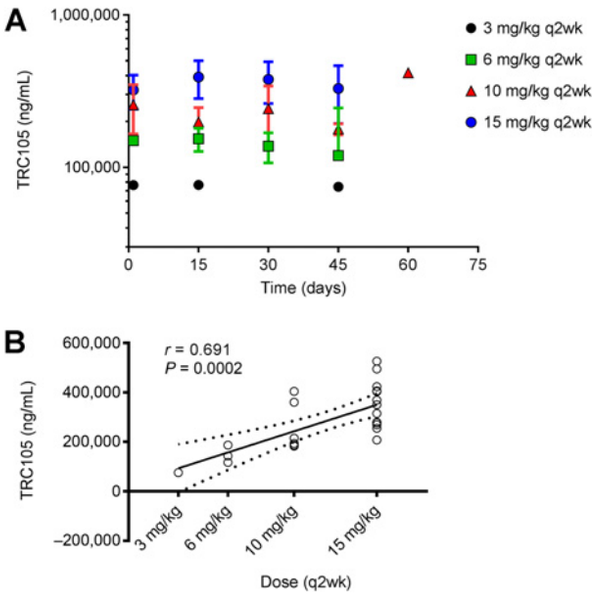

Figure 4.

Pharmacokinetics. **A,** Mean peak TRC105 serum concentrations. **B,** Dose proportional increases in mean per patient TRC105 peak serum concentrations. q2wk, every 2 weeks.

25% based on the 20 patients evaluable for response. The responses seemed to be dose dependent in that 4 of 10 evaluable patients (40%) treated at the highest dose level (TRC105 15 mg/kg) achieved PR. The other response occurred at dose level 3 (TRC105 10 mg/kg), with no responses observed at the lower dose levels. The combination regimen was relatively well tolerated. One concern with combining antiangiogenic agents is the bleeding risk, especially in a prone, generally thrombocytopenic HCC population who may have esophageal or gastric varices (15). In our study, we mandated an upper endoscopy to exclude those at risk, and perhaps as a result did not observe any high-grade bleeding events. One exception was a patient with a cerebral bleed in the setting of a brain metastasis, an event that may have been exacerbated by treatment. The main bleeding issue we experienced, as with sorafenib monotherapy, was chronic low grade, particularly gum bleeding, but also epistaxis, which did seem to impact on patients' quality of life, particularly at the higher dose levels. In the future development of this combination, any randomized design should include quality-of-life analysis to better assess this.

With regard to the correlative, pharmacodynamics studies, as expected, we did observe that median soluble endoglin levels increased compared with baseline. This was consistent with our prior study evaluating TRC105 monotherapy and was also reported by Liu and colleagues who evaluated different doses of TRC105 ranging from 0.3 to 15 mg/kg every 2 weeks as well as some patients receiving 10 and 15 mg/kg weekly (8, 10). The

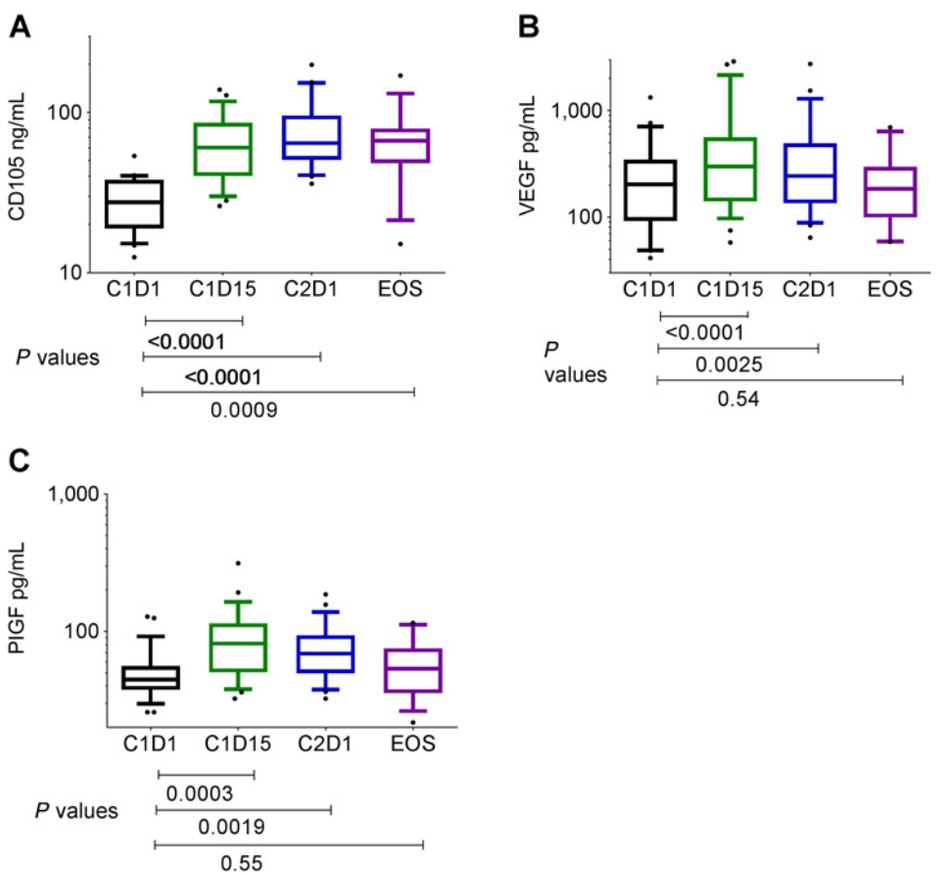

Figure 5.

Levels of CD105 (**A**), VEGF (**B**), and PlGF (**C**) in plasma of study patients taken at cycle 1 days 1 and 15, cycle 2 day 1, and at the end of the study (EOS).

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increase in soluble endoglin levels following TRC105 treatment may be due to several factors, including prolonged stabilization of soluble endoglin due to TRC105 binding or increased shedding of soluble endoglin induced by TRC105 binding at the cell membrane. Hawinkels and colleagues have shown that endoglin shedding was mediated by matrix metalloproteinase (MMP)-14 and resulted *in vitro* in reduced spontaneous and VEGF-induced endothelial sprouting (16). Similarly, it would be expected that as a result of increased intratumoral hypoxia caused by antiangiogenic therapy that levels of angiogenic biomarkers would increase during therapy, and we did observe this for VEGF and PlGF. This finding has not been universal. For example, Liu and colleagues in a phase I trial of TRC105 noted a decrease in VEGF-A among other biomarkers at 4 weeks (8). Of note, in our study, there did not appear to be any difference in biomarker levels between responders and nonresponders, and it is therefore not clear whether these biomarkers will have predictive benefit. In our pharmacokinetic studies, we observed increases in peak TRC105 serum concentrations were moderately well correlated with dose, increasing in an apparent linear (dose-proportional) manner. There were no differences in TRC105 trough concentrations between doses however, suggesting lack of antibody accumulation.

Regarding the ongoing development of TRC105, this agent has been studied in combination with other VEGF pathway inhibitors in early-phase clinical trials. TRC105 combined with bevacizumab demonstrated activity in a bevacizumab refractory population in a phase I/II trial (17). The combination is being studied in ongoing phase II trials in patients with glioblastoma and choriocarcinoma. The combination of TRC105 and axitinib demonstrated preliminary evidence of activity, including a 29% PR rate per RECIST in antiangiogenic-refractory patients with renal cell carcinoma in a phase Ib study, and is being studied in the randomized phase II TRAXAR trial (NCT01806064). The finding of durable CRs in patients with angiosarcoma, when TRC105 was administered with pazopanib, has led to this combination being studied in the randomized global phase III TAPPAS trial (NCT02979899).

In summary, we found that the combination of TRC105, a human chimeric monoclonal endoglin antibody, and sorafenib was well tolerated and induced objective, durable, responses in a proportion of patients with HCC. This combination is currently under evaluation in a multicenter phase II study to confirm these encouraging early indications of efficacy.

Disclosure of Potential Conflicts of Interest

O.E. Rahma is a consultant/advisory board member for Bayer Pharmaceuticals Corp. No potential conflicts of interest were disclosed by the other authors.

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Authors' Contributions

Conception and design: A.G. Duffy, W.D. Figg, T.F. Greten

Development of methodology: S.V. Ulahannan, L. Cao, Y. Yu, J. Trepel, M.-J. Lee, W.D. Figg

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.G. Duffy, C. Ma, S.V. Ulahannan, O.E. Rahma, O. Makarova-Rusher, L. Cao, J. Trepel, M.-J. Lee, Y. Tomita, T. Heller, B. Turkbey, P.L. Choyke, B.J. Wood, T.F. Greten

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.G. Duffy, C. Ma, S.V. Ulahannan, L. Cao, Y. Yu, D.E. Kleiner, J. Trepel, M.-J. Lee, Y. Tomita, S.M. Steinberg, B. Turkbey, C.J. Peer, W.D. Figg, B.J. Wood, T.F. Greten

Writing, review, and/or revision of the manuscript: A.G. Duffy, S.V. Ulahannan, O.E. Rahma, O. Makarova-Rusher, L. Cao, D.E. Kleiner, J. Trepel, M.-J. Lee, Y. Tomita, S.M. Steinberg, T. Heller, B. Turkbey, P.L. Choyke, C.J. Peer, W.D. Figg, B.J. Wood, T.F. Greten

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.G. Duffy, S.V. Ulahannan

Study supervision: A.G. Duffy, T.F. Greten

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