

# A Phase II Clinical Trial of TRC105 (Anti-Endoglin Antibody) in Adults With Advanced/Metastatic Urothelial Carcinoma

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## Abstract

**TRC105 is a chimeric monoclonal antibody that targets CD105 (endoglin). Heavily pretreated patients with metastatic urothelial carcinoma received TRC105 at 15 mg/m<sup>2</sup> every 2 weeks on a 28-day cycle. Treatment was not associated with significant toxicities, but did not improve 6-month progression-free survival. Exploratory analyses suggest interplay between immunosuppressive subsets and TRC105, which warrants further study.**

**Background:** In this trial we assessed the efficacy and tolerability of TRC105, a chimeric monoclonal antibody that targets CD105 (endoglin) in patients with advanced, previously treated urothelial carcinoma (UC). **Patients and Methods:** Patients received TRC105 15 mg/kg every 2 weeks on days 1 and 15 of each 28-day cycle. The primary end point was progression-free survival (PFS) at 6 months. Secondary end points included safety, toxicity, and overall survival (OS). CD105 expression was evaluated using immunohistochemistry (IHC) in a separate cohort of 50 UC patients. Biomarker studies included immune subsets, circulating tumor cells (CTCs), circulating endothelial cells (CECs), circulating endothelial progenitor cells (CEPs), and osteopontin. **Results:** Of 13 patients enrolled, 12 were evaluable for OS and PFS. The 3-month PFS probability was 18.2% (median PFS, 1.9 months [95% confidence interval (CI), 1.8-2.1 months]). This met the criterion for ending accrual on the basis of the 2-stage design. Median OS was 8.3 months (95% CI, 3.3-17.0 months). IHC for CD105 scores was not associated with T stage ( $P = .26$ ) or presence of lymph nodes ( $P = .64$ ). Baseline levels of regulatory T and B cells, CEPs, and changes in CEC level after TRC105 exhibited trends toward an association with PFS or OS. CTCs pre- and post-TRC105 were detected in 4 of 4 patients. **Conclusion:** Although TRC105 was well tolerated, it did not improve 6-month PFS in heavily pretreated patients with advanced UC. CD105 staining was present in 50% of UC tumors at different intensities. Our observations on the pharmacodynamic significance of immune subsets, CECs, and CTCs warrant further study.

*Clinical Genitourinary Cancer*, Vol. ■, No. ■, ■-■ © 2016 Published by Elsevier Inc.

**Keywords:** Advanced urothelial cancer, Antiangiogenic therapy, CD105, Immune subsets, Metastatic urothelial cancer, Urothelial cancer

## Introduction

Urothelial carcinoma (UC), a common malignancy worldwide,<sup>1</sup> is sensitive to chemotherapy. In the first-line metastatic setting,

responses to standard combination cisplatin therapy are approximately 50%<sup>2</sup> and approximately 30% to 36% for noncisplatin combination therapy in cisplatin-unfit patients.<sup>3</sup> However, duration

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Submitted: Mar 8, 2016; Revised: May 6, 2016; Accepted: May 18, 2016

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of response is short, and median survival in patients with metastatic disease is approximately 14 months.<sup>2</sup> Currently there are no effective therapies for patients whose disease relapses after first-line combination chemotherapy. Therapies tested in this setting have shown discouraging response rates, with a median progression-free survival (PFS) of 2 to 3 months and a median overall survival (OS) of 6 to 9 months.<sup>4-6</sup> Clinical trials of immune checkpoint blockade with monoclonal antibodies to programmed death-1 (PD-1) and programmed death-ligand in patients with UC have yielded promising early results, but mature survival data from these trials are still pending.<sup>7-10</sup>

Endoglin/CD105 is overexpressed in vascular endothelial cells of soft tissues undergoing angiogenesis and in tumors.<sup>11,12</sup> Levels of CD105 correlate with endothelial cell proliferation, and CD105 is often used as a marker of tumor angiogenesis.<sup>12</sup> CD105 is therefore a potential therapeutic vascular target in oncology.

Urothelial carcinoma is a highly vascular malignancy that produces high levels of proangiogenic factors such as vascular endothelial growth factor, basic fibroblast growth factor, and interleukin-8.<sup>13</sup> Microvessel density, a histologic measure of angiogenesis, has been correlated with stage, recurrence, and survival in UC.<sup>14-21</sup> Clinical studies in UC with antiangiogenic agents have shown antitumor activity.<sup>22-26</sup> Although these studies validate the potential for an antiangiogenic approach to UC, they also indicate the need to investigate alternative ways to target the tumor vasculature. Directly targeting proliferating endothelial cells, a major component of tumor vasculature, by modifying CD105 signaling is a unique mechanism of targeting angiogenesis.

TRC105 is a human/murine chimeric anti-CD105 immunoglobulin (Ig)G1- $\kappa$  monoclonal antibody with an approximate molecular weight of 148 kDa.<sup>24</sup> It is composed of 2 light chains of 213 amino acids and 2 heavy chains of 448 amino acids. TRC105 binds with high avidity to human CD105, thus inhibiting angiogenesis and tumor growth.<sup>24,25</sup> TRC105 dosing has been established.<sup>26</sup>

We conducted a phase II trial of TRC105 to determine safety and toxicity and assess PFS in patients with advanced/metastatic UC. CD105 protein is expressed on the surface of CD4-positive (CD4<sup>+</sup>) T cells, activated monocytes, and macrophages.<sup>27,28</sup> Thus, TRC105, a monoclonal antibody targeting CD105, might modulate immune subsets, which might correlate with clinical outcome in patients with UC. In addition to assessing the clinical activity of TRC105, we also assessed its effect on immune subsets, mature circulating endothelial cells (CECs) and circulating endothelial progenitor cells (CEPs), circulating tumor cells (CTCs), and osteopontin.

## Patients and Methods

### Patient Selection

For this phase II study of TRC105 in advanced/metastatic UC that had progressed despite previous cytotoxic chemotherapy, eligible patients must have received  $\geq 1$  previous treatment with cisplatin, carboplatin, paclitaxel, docetaxel, or gemcitabine. The primary study objective was to determine the activity of TRC105 using Response Evaluation Criteria in Solid Tumors (RECIST). TRC105 was administered at a dose of 15 mg/kg every 2 weeks on days 1 and 15 of each 28-day cycle. Patients continued in the study as long as they tolerated therapy and showed no disease progression.

Patients were considered eligible if they were  $\geq 18$  years of age and had: (1) a diagnosis of UC of the bladder, urethra, ureter, or renal pelvis, with histologic confirmation by the National Cancer Institute (NCI) Laboratory of Pathology; and (2) progressive metastatic disease defined as new or progressive lesions on cross-sectional imaging. Patients had to have  $\geq 1$  measurable site of disease (according to RECIST) that had not been previously irradiated. If marker lesions had been previously irradiated, there had to be evidence of progression after irradiation or the appearance of 1 new bone lesion; (3) previous treatment with  $\geq 1$  previous cytotoxic agent (including cisplatin, carboplatin, paclitaxel, docetaxel, or gemcitabine), which might have been administered in the perioperative or metastatic setting, either sequentially (eg, first-line treatment followed by second-line treatment at time of disease progression) or as part of a single regimen; (4) Eastern Cooperative Oncology Group performance status of  $< 2$  or Karnofsky Performance Status of  $\geq 60\%$ ; (5) resolution of all acute toxic effects of previous chemotherapy, radiotherapy, or surgical procedures; and (5) adequate organ function. Patients were excluded if they had: (1) received an investigational agent within 4 weeks of the first dose of TRC105; (2) major surgery (including open biopsy) or systemic therapy within 4 weeks of the first dose of TRC105; (3) radiation therapy (except small field) within 3 weeks of the first dose of TRC105; (4) small field radiation therapy within 2 weeks of the first dose of TRC105; (5) minor surgical procedures within 2 weeks of the first dose of TRC105; (6) uncontrolled chronic hypertension (systolic  $> 140$  or diastolic  $> 90$  mm Hg despite optimal therapy); (7) brain metastasis or leptomeningeal disease; (8) unstable angina, myocardial infarction, symptomatic congestive heart failure, cerebrovascular accident, transient ischemic attack, arterial embolism, pulmonary embolism, deep vein thrombosis, percutaneous transluminal coronary angioplasty, coronary artery bypass grafting within the past 6 months; (9) a serious, nonhealing wound, ulcer, or bone fracture; (10) known active hepatitis; or (11) hemorrhage within 30 days of dosing or history of persistent gross hematuria. All patients gave written informed consent in accordance with federal, state, and institutional guidelines. The NCI's institutional review board approved the study.

### Study Design

This was a single-institution, single-arm, open-label, phase II clinical trial, using a 2-stage optimal design. On the basis of previous phase II trials with similar eligibility, the median PFS was ordinarily 2 to 3 months.<sup>22</sup> The objective of the present trial was to determine if TRC105 could produce outcomes consistent with 30% of patients being progression-free according to radiographic criteria at around 6 months ( $P_1 = .30$ ) while ruling out 10% of patients being progression-free at around 6 months ( $P_0 = .10$ ), using standard acceptable error probabilities:  $\alpha = 0.10$ ;  $\beta = 0.10$ . Initially, 13 patients were enrolled, and 12 were evaluable and followed for disease progression. According to the design, if  $\leq 1$  of the initial 12 patients were progression-free at 6 months, no more patients would be enrolled, and if  $\geq 2$  of the initial 12 patients were progression-free at 6 months, enrollment would increase to a total of 35 evaluable patients. If only 2 to 5 of those 35 patients were progression-free at 6 months, this would be deemed an inadequate treatment response, and  $\geq 6$  of the 35 patients progression-free at 6 months would indicate a PFS probability worthy of further investigation. Under the null hypothesis ( $\leq 10\%$  of patients were progression-free at 6 months), the

probability of early termination after the 6-month evaluation of the initial 12 evaluable patients was 66%.

The safety and toxicity of TRC105 in this patient population were also primary considerations of this study. All Grade 3/4 toxicities were reported according to the NCI Common Terminology Criteria for Adverse Events version 4.0.

### **Treatment Plan and Evaluation of Toxicities**

TRC105 was administered on an outpatient basis at a dose of 15 mg/kg every 2 weeks on days 1 and 15 of each 28-day cycle. Thirty minutes to 2 hours before the start of each TRC105 infusion, patients were premedicated with 1 dose of acetaminophen 650 mg orally (p.o.), 1 dose of dexamethasone 20 mg intravenously (I.V.), 1 dose of famotidine (or similar H2 blocker) 20 mg I.V., and 1 dose of cetirizine (or similar oral or I.V. antihistamine) 10 mg I.V. or p.o. TRC105 was administered I.V. using an infusion pump. On cycle 1 day 1, TRC105 was infused over a period of 4 hours. If patients completed one 4-hour infusion without developing an infusion reaction, subsequent TRC105 infusions were reduced to 2 hours. If patients completed one 2-hour infusion without developing an infusion reaction, subsequent TRC105 infusions were reduced to a minimum of 1 hour. If this infusion rate proved to be safe, the dexamethasone dosage was gradually tapered, at the discretion of the investigator, with each subsequent infusion and eventually discontinued, if possible. If a patient experienced a Grade 2, 3, or 4 adverse reaction during infusion, the infusion was stopped and the patient was treated with an antipyretic, antihistamine, or other drug as indicated.

Treatment-limiting toxicities were defined as any Grade  $\geq 3$  hematologic or nonhematologic toxicity possibly, probably, or definitely related to TRC105. Any Grade 4 pulmonary embolus, including one without significant hypoxia and hemodynamic instability, was considered dose-limiting, and no more TRC105 was administered. If patients experienced a treatment-limiting toxicity at least possibly related to TRC105, the drug was withheld until the toxicity was resolved. For selected toxicities, TRC105 was reinitiated at a lower dose level (albeit not  $< 5$  mg/kg). Patients with a clinical Grade 4 nonhematologic toxicity were removed from the study.

### **Response Evaluation**

Restaging bone scans and computed tomography scans of the chest, abdomen, and pelvis were scheduled every 2 months for the first 4 months of the study (after cycles 2 and 4), then after every 3 cycles of treatment. Confirmatory scans were obtained 4 weeks after initial documentation of objective response, complete response (CR), or partial response (PR). Objective response and progression were evaluated using the new international criteria proposed by the RECIST version 1.1 Committee.<sup>29</sup>

### **Biomarker Evaluations**

Whole blood samples were collected in cell preparation tubes with sodium citrate (BD Vacutainer CPT Tubes; BD Biosciences, San Jose, CA) at baseline and after 6 treatments of TRC105 (C3D1). Peripheral blood mononuclear cells (PBMCs) were obtained using centrifugation and viably frozen until analysis. All analyses were performed using multiparametric flow cytometry (MACSQuant; Miltenyi Biotec, Bergisch Gladbach, Germany) and data were analyzed using FlowJo software version 10.0.7 (FlowJo,

LLC, Ashland, OR). PBMCs were analyzed for T regulatory (Treg) cells ( $CD4^+CD25^{hi}Foxp3^+$  T cells), T cells ( $CD3^+CD14^-CD19^-$ ), B cells ( $CD19^+CD3^-CD14^-$ ), natural killer cells ( $CD56^+CD3^-CD14^-CD19$ ), and monocytes ( $CD45^+CD14^+$ ). Only viable  $CD45^+$  cells were analyzed. All antibodies were purchased from BioLegend (San Diego, CA). CECs ( $CD146^+CD133^-CD31^+CD45^-$ ) and CEPs ( $CD146^-CD133^+CD31^+CD45^{dim/-}$ ) were analyzed as described previously.<sup>30,31</sup> CTCs were detected from 8 mL of peripheral blood drawn into BD Vacutainer CPT Tubes (BD Biosciences). Epithelial cell adhesion molecule (EpcAM)-positive CTCs were isolated using magnetic pre-enrichment and quantified using multiparameter flow cytometry, which is a novel method we recently developed and validated in cancer patients.<sup>31,32</sup> CTCs were identified as viable, nucleated, EpcAM<sup>+</sup> cells that did not express the common leukocyte antigen CD45, as described previously.<sup>31,32</sup> After enumeration of viable nucleated,  $CD45^-$ , EpcAM<sup>+</sup> cells, CTCs were further characterized for expression of Mucin 1, cell surface associated (MUC1), which plays a critical role in tumor growth and is being tested in clinical trials as a possible cancer vaccine target, and the stem cell marker CD133.

CD105 is part of the transforming growth factor beta (TGF- $\beta$ ) receptor complex, and osteopontin gene expression is upregulated by TGF- $\beta$ . Because circulating levels of osteopontin can be assessed using an enzyme-linked immunosorbent assay,<sup>33</sup> we used this platform to measure circulating osteopontin as a potential indicator of the effect of TRC105 on TGF- $\beta$  signaling. Plasma osteopontin was measured using an enzyme-linked immunosorbent assay (Human Osteopontin Assay Kit; IBL-America, Minneapolis, MN).

### **Statistical Analysis**

Time to disease progression was defined as from the first date of study inclusion until the date of first observation of disease progression, death during the study, or removal from the study at the principal investigator's discretion. Other reasons for removal from the study, such as adverse events, patient decision, or concurrent illness, were used to censor time to disease progression. Patients remaining in the study or alive at time of analyses were censored at date of last follow-up. The probability of PFS or OS as a function of time was determined using the Kaplan–Meier method, with the statistical significance of the difference between Kaplan–Meier curves determined using an exact log rank test because of the small number of patients. The significance of the difference between 2 dichotomous parameters was determined using Fisher exact test, and the difference in continuous parameters between 2 time points was determined using a Wilcoxon signed rank test. Evaluations of laboratory and correlative parameters were performed as exploratory analyses and would require independent confirmation to be considered potentially definitive.

All *P* values are 2-tailed and reported without formal adjustment for multiple comparisons.

### **Immunohistochemistry**

Protein expression of CD105 (endoglin) was evaluated using immunohistochemistry with monoclonal mouse antihuman antibody, Clone SN6h (Dako, Dakocytomation; Carpinteria, CA) in a separate cohort of 50 UC patients with muscle-invasive disease.

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First, paraffin-embedded sections (5  $\mu$ m) were deparaffinized in xylene and rehydrated in graded alcohol. Tissue sections were microwaved in 10 mM sodium citrate pH 6.0 for 15 minutes, then allowed to cool. A high-sensitivity detection system (Catalyzed Signal Amplification, Dako) was used for antibody detection. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide in phosphate-buffered saline, followed by protein blocking. Sections were incubated for 1 hour at room temperature with the CD105 antibody (1:2000 dilution). Biotinylated link antibody was added, followed by streptavidin-biotin complex. Finally, the amplification reagent was visualized with streptavidin-peroxidase and 3,3'-diaminobenzidine as chromogen. Slides were counterstained with hematoxylin, dehydrated, and mounted. For negative controls, sections were incubated in parallel with their respective matched isotype nonimmune IgG. Vascular endothelial cells present in the tissue were positively stained as positive control. Level of CD105 expression was scored on the basis of intensity of staining in the cytoplasm of tumor cells. Intensity was recorded as 0 (no staining), 1 (weak staining), 2 (moderate staining), or 3 (strong staining), and the percentage of stained cytoplasmic/nuclear area was recorded.

### Results

#### Patient Characteristics

Thirteen patients were enrolled between April and October 2011 (see Table 1 for study demographic and baseline characteristics). One

**Table 1** Baseline Clinical Characteristics (n = 13)

Characteristic	Value
Median Age (Range)	67 (51-73)
<b>Sex</b>	
Male	8 (62)
Female	5 (38)
<b>Karnofsky Performance Status</b>	
60	1 (8)
70	0 (0)
80	2 (15)
90	10 (77)
<b>Primary Tumor Site</b>	
Bladder	11 (85)
Upper urinary tract	2 (15)
<b>Metastatic Sites of Disease</b>	
Lung	7 (54)
Liver	2 (15)
Bone	4 (31)
Any visceral metastases	10 (77)
Lymph node only	3 (23)
<b>Number of Previous Therapies for Metastatic Disease</b>	
1	3 (23)
2	4 (31)
3	3 (23)
4	2 (15)
5	1 (8)

Data are presented as n (%) except where otherwise stated.

patient enrolled in the study did not receive the study drug because of clinical disease progression and deterioration before the first dose. Patients received a median of 2 cycles (range, 1-4). Of 12 patients evaluable for disease, 11 (92%) discontinued therapy because of disease progression. One patient discontinued therapy because of deep venous thrombosis. No patients required dose reductions secondary to the management of TRC105-related toxicities.

#### Progression-Free and OS

Of 13 patients enrolled, 12 were evaluable for OS and PFS. The 3-month PFS probability was 18.2%, and all failures took place within 6 months (median PFS, 1.9 months; 95% confidence interval [CI], 1.8-2.1 months; Figure 1A). This met the criterion for ending accrual according to the 2-stage design, and the study was closed at that point. Median OS was 8.3 months (95% CI, 3.3-17.0 months; Figure 1B); 12-month survival probability was 33%. Patients were followed for up to 25 months. Nine patients subsequently received additional treatment after the study. There were no objective responses (PR or CR) according to RECIST version 1.1. Two patients had stable disease lasting 4 months.

#### Toxicities

All patients who received treatment were analyzed for toxicity. Interestingly, a common Grade 1 toxicity was asymptomatic telangiectasia. Table 2 shows the most common Grade 2 toxicities and all Grade 3 and 4 toxicities. Significant Grade 2 adverse events included anemia (42%). Grade 3 toxicities included anemia (8%) and skin infection (8%).

#### Biomarker Evaluations

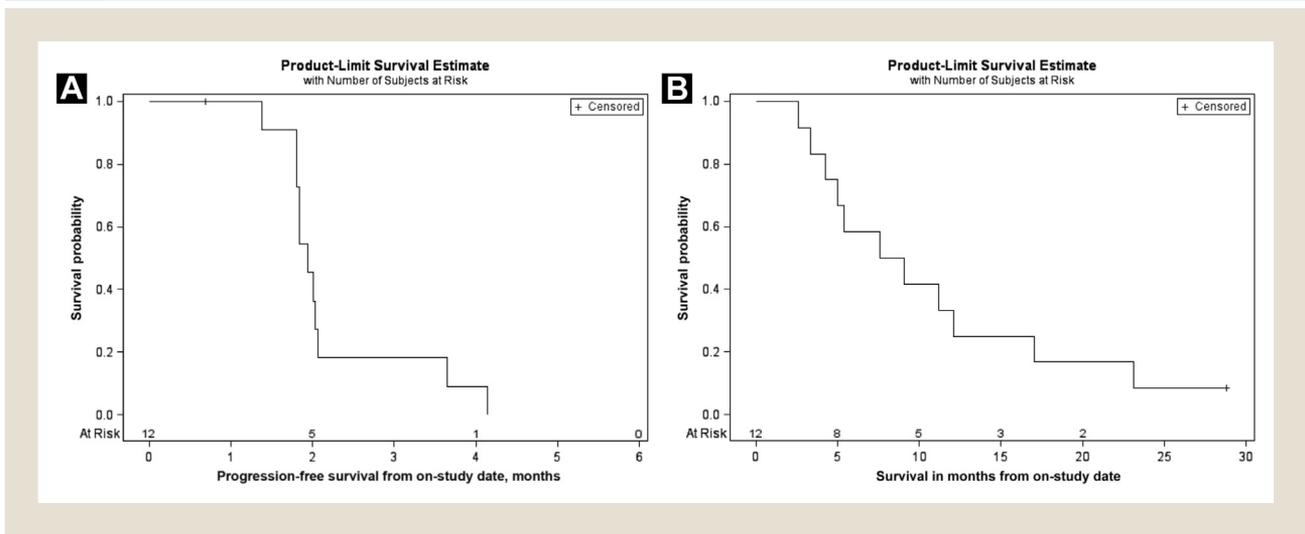
**Immunohistochemistry.** Immunohistochemistry for CD105 scores were 0 (50%), 1+ (10%), 2+ (36%), and 3+ (4%). There was no statistical association between CD105 staining (negative vs. positive) and T stage ( $P = .26$ ) or presence of lymph nodes ( $P = .64$ ).

**Immune Subsets.** In exploratory analyses, the level of Treg cells among CD4<sup>+</sup> T cells significantly decreased after treatment compared with baseline (n = 7,  $P = .016$ ; Figure 2A). Patients whose Treg cell level was lower than the median at baseline showed a trend toward improved OS compared with those whose Treg level was higher than the median at baseline (n = 7;  $P = .086$ ; Figure 2B). Patients whose B-cell level was lower than the median at baseline had somewhat improved OS compared with those whose B-cell level was higher than the median at baseline (n = 7;  $P = .057$ ; Figure 2C).

**Circulating Endothelial Cells and CEPs.** Patients in whom the percentage of apoptotic CECs among nucleated cells decreased after treatment showed a trend toward improved PFS compared with patients in whom the percentage of CECs increased after treatment (n = 7;  $P = .11$ ; Figure 3A). Patients whose CEP level was lower than the median at baseline had improved PFS compared with those whose CEP level was higher than the median at baseline (n = 7;  $P = .029$ ; Figure 3B).

**Circulating Tumor Cells.** We measured CTCs in 4 patients (3 with bladder cancer; 1 with ureter cancer) before and after treatment. We

**Figure 1** Progression-Free Survival and Overall Survival (n = 12). (A) Progression-Free Survival of Patients Treated With TRC105. (B) Overall Survival of Patients Treated With TRC105



detected CTCs in all 4 patients; however, the number of CTCs in 2 patients (1 with bladder cancer; 1 with ureter cancer) decreased after treatment with TRC105 (Figure 4A). Among the 4 patients tested, the 2 patients with bladder cancer who had < 5 CTCs after treatment with TRC105 showed longer OS (> 6 months) compared with those who had > 5 CTCs. One patient with bladder cancer who had < 5 CTCs before and after treatment had the longest OS (704 days; Figure 4A). CD133 and MUC1 expression levels showed dynamic

changes before and after treatment. CD133 expression was detected in all bladder cancer patients and the positive ratio increased after treatment, but CTCs in a patient with ureter cancer were negative for CD133 (Figure 4B). MUC1 expression was detected in all 4 patients (Figure 4C).

*Osteopontin.* Osteopontin increased significantly after treatment compared with baseline (n = 7;  $P = .016$ ; Figure 5).

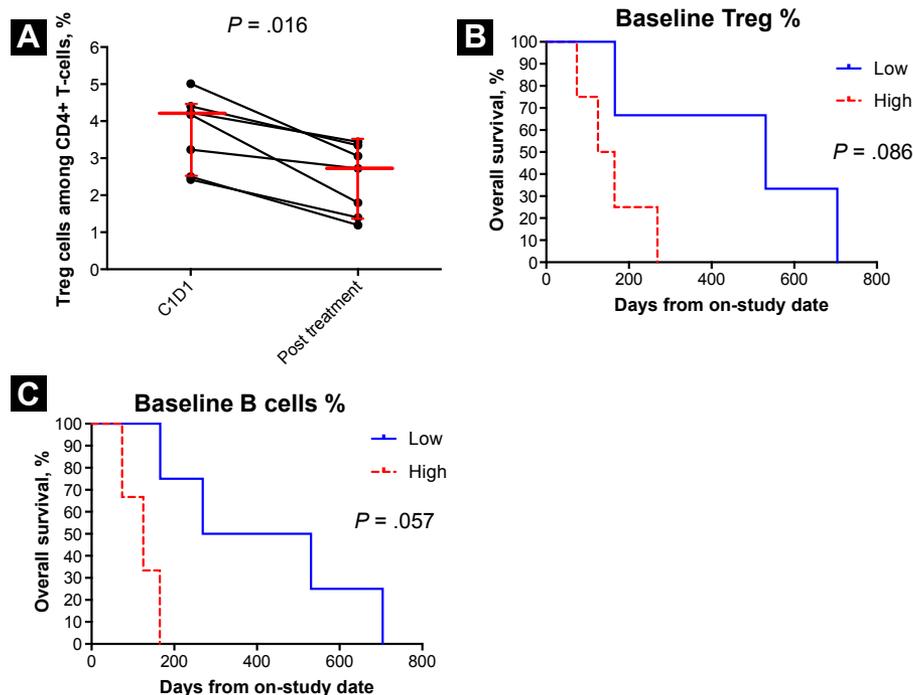
**Table 2** Incidence of TRC105-Related Adverse Events (n = 13)

Adverse Event	All Grades	Grade 1	Grade 2	Grade 3	Grade 4
Anemia	6 (46)	1 (8)	4 (31)	1 (8)	—
Anorexia	1 (8)	1 (8)	—	—	—
Cough	1 (8)	1 (8)	—	—	—
Edema (Limbs)	1 (8)	1 (8)	—	—	—
Epistaxis	6 (46)	6 (46)	—	—	—
Fatigue	1 (8)	1 (8)	—	—	—
Gum Bleeding	4 (31)	4 (31)	—	—	—
Headache	9 (69)	8 (62)	1 (8)	—	—
Hematuria	1 (8)	1 (8)	1 (8)	—	—
Hypoalbuminemia	1 (8)	1 (8)	—	—	—
Hypophosphatemia	1 (8)	—	1 (8)	—	—
Increased Alanine Aminotransferase	1 (8)	1 (8)	—	—	—
Infusion-Related Reaction	9 (69)	5 (38)	4 (31)	—	—
Maculopapular Rash	1 (8)	1 (8)	—	—	—
Nasal Congestion	3 (23)	3 (23)	—	—	—
Nausea	1 (8)	1 (8)	—	—	—
Proteinuria	1 (8)	1 (8)	—	—	—
Skin Infection	1 (8)	—	—	1 (8)	—
Telangiectasia	6 (46)	6 (46)	—	—	—
Vomiting	1 (8)	1 (8)	—	—	—
Xerostomia	1 (8)	1 (8)	—	—	—

Data are presented as n (%).

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**Figure 2** T Regulatory (Treg) and B-Cell Levels in Urothelial Carcinoma Patients Treated With TRC105 and Association With Clinical Outcome. (A) Change of Percentage of Treg Cells Among CD4<sup>+</sup> T Cells After TRC105 in Urothelial Carcinoma Patients. Percentage of Treg Cells Decreased After TRC105 (n = 7; Wilcoxon Signed Rank Test,  $P = .016$ ). Median and Quartiles Are Shown. (B) Kaplan–Meier Curves Showing Overall Survival of Patients With Baseline Percentage of Treg Levels Equal to or Above the Median (High) or Below the Median (Low) (n = 7; Exact Log Rank Test,  $P = .086$ ). (C) Kaplan–Meier Curves Showing Overall Survival of Patients With Baseline Percentage of B Cells Among CD45<sup>+</sup> Cells Above (High) or Equal to or Below the Median (Low) (n = 7; Exact Log Rank Test,  $P = .057$ )

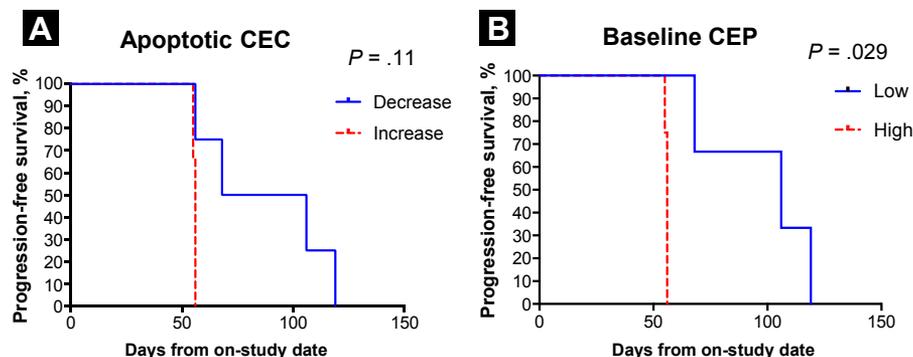


## Discussion

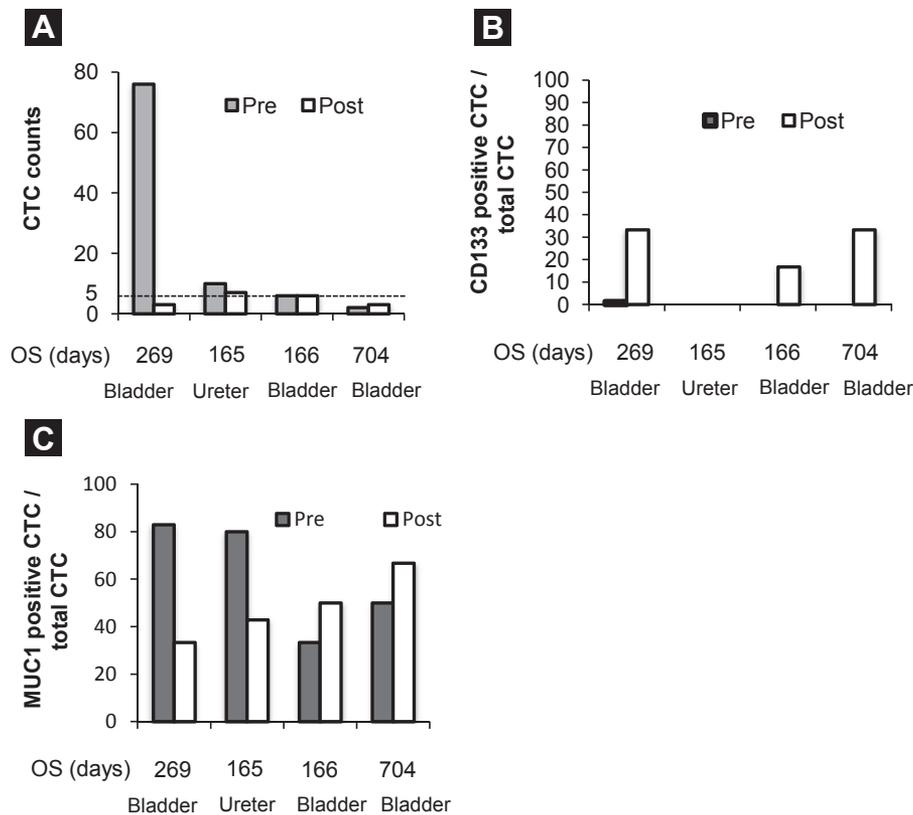
This study did not meet its primary end point of improving PFS at 6 months and closed early for futility. However, correlative

studies in this small group of patients suggest that TRC105 has immunomodulatory properties that might be exploited in future combination studies with immunotherapies.

**Figure 3** Circulating Endothelial Cell (CEC) and Circulating Endothelial Progenitor Cell (CEP) Levels in Urothelial Carcinoma Patients Treated With TRC105 and Association With Clinical Outcome. (A) Kaplan–Meier Curves Showing Progression-Free Survival of Patients With an Increase or Decrease of Apoptotic CECs From Baseline to Post-Treatment (n = 7; Exact Log-Rank Test,  $P = .11$ ). (B) Kaplan–Meier Curves Showing Progression-Free Survival of Patients With Baseline CEP Levels Equal to or Above the Median (High) or Below the Median (Low) (n = 7; Exact Log Rank Test,  $P = .029$ )



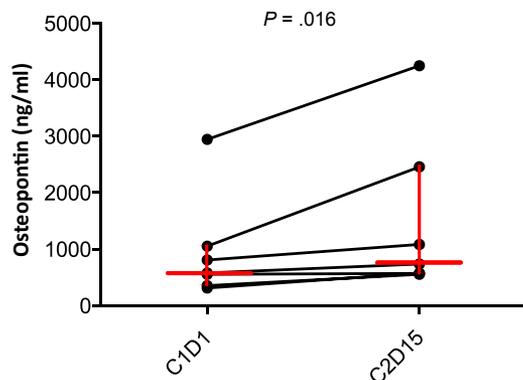
**Figure 4** Dynamic Change of Circulating Tumor Cell (CTC) Counts and CTC Phenotypes Pre- and Post-Treatment in Urothelial Carcinoma Patients and Association With Overall Survival (OS). (A) CTC Counts Pre- and Post-Treatment. (B) CD133<sup>+</sup> CTC/Total CTC Pre- and Post-Treatment. (C) Mucin 1, cell surface associated (MUC1)<sup>+</sup> CTC/Total CTC Pre- and Post-Treatment



Angiogenesis plays a complex role in tumor development. CD105 is essential in normal vascular development, and high tumor microvessel density assessed using CD105 staining has been

correlated with poor prognosis in solid tumors.<sup>34,35</sup> In vivo pre-clinical studies revealed that TRC105's parental monoclonal antibody inhibited tumor growth and angiogenesis.<sup>36,37</sup> This novel approach to antiangiogenesis identifies CD105 as a potential target in the treatment of solid tumors.

**Figure 5** Increase in Plasma Osteopontin Levels After TRC105 Treatment in Urothelial Carcinoma Patients (n = 7; Wilcoxon Signed Rank Test,  $P = .016$ ). Median and Quartiles Are Shown



Emerging evidence has revealed the interplay between the host immune system and many anticancer therapies that were not thought to have an immune target or to significantly affect immune cells.<sup>38-40</sup> However, how TRC105 might affect immune subsets in patients with UC remains to be shown. The CD105 protein is a TGF- $\beta$  coreceptor expressed on the surface of CD4<sup>+</sup> T cells,<sup>27,41</sup> activated monocytes, and macrophages in the tumor microenvironment.<sup>28</sup> It has been shown that TGF- $\beta$  is important in Treg cell induction.<sup>42,43</sup> In the current study, TRC105 significantly decreased the level of Treg cells in PBMCs in this small group of patients. Furthermore, patients with high baseline levels of Treg cells and B cells showed a trend toward poor OS. Interestingly, Treg decline in response to TRC105 was also observed in a phase I/II study of TRC105 in metastatic castration-resistant prostate cancer and correlated with prostate-specific antigen decline (NCT01090765).<sup>44</sup> These results suggest that TRC105 might regulate Treg cell levels directly or through CD105<sup>+</sup> monocytes or macrophages in cancer patients.<sup>45,46</sup>

## A Phase II Trial of TRC105 in Urothelial Carcinoma

Recent studies of immune checkpoint blockade have reported promising results in several cancer types, including UC.<sup>7-10</sup> Treg cells express PD-1 and cytotoxic T-lymphocyte-associated protein 4. However, anti-PD-1 and cytotoxic T-lymphocyte-associated protein 4 blockade increased Treg cell levels in patients with melanoma.<sup>47-49</sup> Combination therapy with immune checkpoint blockade and TRC105 might, therefore, have a synergistic effect and could potentially improve antitumor responses in UC patients.

CD19<sup>+</sup>CD24<sup>hi</sup>CD38<sup>hi</sup>CD1d<sup>hi</sup> and CD19<sup>+</sup>CD24<sup>hi</sup>CD27<sup>+</sup> regulatory B cells (Bregs) have been identified in humans and have been shown to play a critical role in immune tolerance.<sup>50-52</sup> In the current study, a lower baseline CD19<sup>+</sup> B-cell level was associated with a trend toward improved OS. Although we used only CD19 for B-cell phenotypic analysis, any CD19<sup>+</sup> B cells could differentiate into Bregs in response to environmental stimuli.<sup>52</sup> Thus, CD19<sup>+</sup> B cells detected in the current study might have an immunosuppressive function similar to Bregs. Although this hypothesis must be confirmed by further phenotypic and functional analyses, our results suggest that peripheral CD19<sup>+</sup> B cells might play an important role in disease progression in patients with UC treated with TRC105. Taken together, our data suggest an interplay between immunosuppressive subsets and TRC105.

It has been shown that CECs and CEPs are potential biomarkers of response to antiangiogenic therapies.<sup>53,54</sup> In this study, baseline CEP levels and changes in apoptotic CECs by TRC105 showed an association with PFS, although the number of patients analyzed was very small. CECs and CEPs might be predictive, pharmacodynamic markers in UC treated with TRC105.

Circulating tumor cell detection is a noninvasive diagnostic tool that can be used as a liquid biopsy for prognostic and predictive purposes, offering an opportunity for longitudinal, real-time tumor molecular characterization that can guide personalized cancer therapy.<sup>55,56</sup> It has been reported that CTCs have been detected in localized, high-risk nonmuscle-invasive, and metastatic bladder cancer, and that CTCs have potential prognostic value.<sup>56-59</sup> In the current study, we applied an integrated magnetic pre-enrichment and multiparameter flow cytometric analysis, a novel method that we recently developed.<sup>31,32</sup> Detection and characterization of CTCs before and after therapy showed dynamic changes in CTC number and expression of MUC1 and CD133 on CTCs, suggesting the presence of inpatient heterogeneity in UC. CTC analysis might be used to assess response to TRC105; however, larger patient samples will be needed to further elucidate the potential clinical relevance of CTC analysis.

### Conclusion

Treatment with TRC105 was not associated with significant toxicities. Grade 3 anemia was noted in 1 of 12 patients, but no dose reductions were required. Single-agent activity in this heavily pretreated population was not significant; however, combination studies are warranted. UC is a highly vascular, chemosensitive malignancy. Recently reported data suggesting UC's sensitivity to immune-targeted therapy provide a rationale for combining a novel antiangiogenic and immunomodulatory approach with chemotherapy or an immune-targeted therapy.

### Clinical Practice Points

- New, more effective therapies are needed in metastatic UC. Median OS in the second-line setting ranges only from 6 to 9 months.
- This trial assessed the efficacy and tolerability of single-agent TRC105, a chimeric monoclonal antibody that targets CD105 in patients with metastatic, heavily pretreated UC, a highly vascular malignancy.
- CD105 is a protein expressed in endothelial cells, CD4<sup>+</sup> T cells, activated monocytes, and macrophages.
- Treatment with TRC105 was not associated with significant toxicities.
- Although single-agent activity in this heavily pretreated population was not significant, exploratory analyses suggest that TRC105 might affect immune subsets, including Treg cells, in patients with UC.
- Recent data suggest that UC is sensitive to immune-targeted therapy, warranting future approaches that combine TRC105 with immunotherapeutic strategies.

### Acknowledgments

This work was supported by the Intramural Research Program of the Center for Cancer Research, NCI, National Institutes of Health, and TRACON Pharmaceuticals Inc, San Diego, California.

### Disclosure

The authors have stated that they have no conflicts of interest.

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