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A phase I study of TRC105 anti-endoglin (CD 105) antibody in metastatic castration-resistant prostate cancer

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Abstract

Objective—TRC105 is a chimeric immunoglobulin G1 monoclonal antibody that binds endoglin (CD105). This phase I open-label study evaluated the safety, pharmacokinetics and pharmacodynamics of TRC105 in patients with metastatic castration-resistant prostate cancer (mCRPC).

Patients and Methods—Patients with mCRPC received escalating doses of i.v. TRC105 until unacceptable toxicity or disease progression, up to a predetermined dose level, using a standard 3 + 3 phase I design.

Results—A total of 20 patients were treated. The top dose level studied, 20 mg/kg every 2 weeks, was the maximum tolerated dose. Common adverse effects included infusion-related reaction (90%), low grade headache (67%), anaemia (48%), epistaxis (43%) and fever (43%). Ten patients had stable disease on study and eight patients had declines in prostate specific antigen (PSA). Significant plasma CD105 reduction was observed at the higher dose levels. In an exploratory analysis, vascular endothelial growth factor (VEGF) was increased after treatment with TRC105 and VEGF levels were associated with CD105 reduction.

Conclusion—TRC105 was tolerated at 20 mg/kg every other week with a safety profile distinct from that of VEGF inhibitors. A significant induction of plasma VEGF was associated with CD105 reduction, suggesting anti-angiogenic activity of TRC105. An exploratory analysis showed a tentative correlation between the reduction of CD105 and a decrease in PSA velocity, suggestive of potential activity of TRC105 in the patients with mCRPC. The data from this exploratory analysis suggest that rising VEGF level is a possible compensatory mechanism for TRC105-induced anti-angiogenic activity.

Keywords

prostate cancer; metastatic; angiogenesis inhibitors; anti CD105; endoglin; TRC105

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Conflict of Interest
None declared.

Introduction

Prostate cancer is the most common malignancy in men in the USA, with an estimated 233 000 new cases diagnosed and 29 490 deaths in 2014 [1]. For patients with localized disease, the treatment intent is curative, but once the disease spreads beyond the prostate, treatment focuses on palliation. In 2004, two pivotal studies, TAX 327 [2] and SWOG 9916 [3], showed an overall survival benefit with docetaxel-based chemotherapy in metastatic castration-resistant prostate cancer (mCRPC). These trials established docetaxel and prednisone as first-line treatment for men with symptomatic mCRPC. Since that time, five additional agents have been approved for the treatment of mCRPC based on improved overall survival; however, responses are transient, indicating a need for additional treatment options. In castration-resistant prostate cancer (CRPC), anti-angiogenic approaches have shown potential for hindering tumour growth and metastasis [4].

Angiogenesis is a complex process involving multiple pathways that affect tumour growth, invasion and metastasis [5]. Angiogenic factors have been implicated in prostate cancer development and progression, and measurements of tumour angiogenesis have been shown to correlate with metastasis in invasive prostate carcinoma [6]. Angiogenesis inhibitors, either alone or in combination with chemotherapy, have been investigated as a therapeutic target in the treatment of prostate cancer [7]. Two phase III trials have explored the addition of bevacizumab or aflibercept to docetaxel and prednisone. In the CALGB 90401 study [8], 1050 patients with CRPC were randomized to treatment with docetaxel plus prednisone with or without bevacizumab, a humanized monoclonal antibody to vascular endothelial growth factor (VEGF)-A. The median overall survival did not improve statistically significantly in patients receiving bevacizumab compared with patients treated with placebo. Similarly, in the VENICE trial [9] the addition of aflibercept (a recombinant human fusion protein that binds A and B isoforms of VEGF and placental growth factor) to docetaxel and prednisone did not improve overall survival in men with mCRPC compared with placebo plus docetaxel and prednisone. Additionally, sunitinib plus prednisone showed no overall survival benefit when compared with placebo in men with mCRPC who have progressed on docetaxel [10].

These trials illustrate the need to investigate alternative methods of targeting tumour vasculature with either single agents or combination treatments. Agents that have unique mechanisms of action, have activity in tumours resistant to existing therapies, have better side-effect profiles, and could potentially be combined with anti-VEGF therapy are particularly promising candidates for further evaluation. One such alternative anti-angiogenic approach is to directly target proliferating endothelial cells by modifying endoglin (CD105) signalling in response to TGF- β [11] and bone morphogenetic protein-9 [12]. CD105 is involved in normal vascular development, is highly expressed on the surface of proliferating vascular endothelial cells [13–17], and is highly expressed on endothelial cells during tumour angiogenesis [18]. In hypoxic conditions, CD105 is upregulated through induction of hypoxia-inducible factor 1- α [14]. CD105 is also upregulated on tumour endothelial cells after inhibition of the VEGF pathway [14,19,20]. CD105, which is an accessory receptor for diverse TGF- β family cytokines, has been implicated in prostate cancer cell migration and invasion [21]. In prostate cancer, a high level of CD105 in tumour blood vessels is associated with decreased survival [18,22].

TRC105 is a human/murine chimeric anti-CD105 immunoglobulin G1- κ monoclonal antibody [23]. In preclinical models, SN6j, the murine parental monoclonal antibody of TRC105, inhibited tumour angiogenesis [14,24]. TRC105 binds to human CD105 and induces apoptosis of CD105⁺ endothelial cells and human umbilical vein endothelial cells via antibody-dependent cellular cytotoxicity [14,18]. In a phase I first-in-human study in patients with advanced cancer, treatment with TRC105 resulted in stable disease in 21 of 45 patients (47%) [14]. In the phase I trial reported in the present study, patients with mCRPC received i.v. infusions of TRC105 to define the maximum tolerated dose and to assess the safety, pharmacokinetics, pharmacodynamics and antitumour activity of TRC105. We also report on predictive markers, as well as the pharmacodynamic effects of TRC105 in regulatory T cells and CD8⁺ T cells.

Patients and Methods

Patient Selection

Patients were considered eligible if they had histopathological confirmation of mCRPC, defined as progressive disease despite surgical castration or ongoing androgen deprivation therapy and confirmed castrate levels of testosterone. Progressive disease was defined as two consecutively rising PSA values measured at a minimum of 1-week intervals, the appearance of ≥ 1 new lesions on bone scans, and/or progressive disease as measured by Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. There were no limitations on previous therapies, and previous chemotherapy was allowed. Patients were >18 years of age, had a life expectancy of >3 months, and an Eastern Cancer Oncology Group performance status of 0–2. All patients gave written informed consent in accordance with federal, state and institutional guidelines. The study was approved by the Institutional Review Board of the National Cancer Institute (NCI).

Study Design

This was a single-institution, single-arm, open-label clinical trial [25] to define the maximum tolerated dose of TRC105 given as an i.v. infusion in patients with mCRPC. The study included a phase I dose-escalation portion, which enrolled patients with progressive mCRPC. The study evaluated patients in six cohorts who received doses of TRC105 escalated according to a standard 3 + 3 design. Secondary endpoints of pharmacokinetic and pharmacodynamic parameters were also explored.

Treatment Plan and Toxicity Evaluation

Patients received i.v. infusions of TRC105 every 2 weeks on days 1 and 15 of each 28-day cycle (cohorts 1, 2, 3, 5 and 6) and every week on days 1, 8, 15 and 22 of each 28-day cycle (cohort 4) (Table 1). Doses were escalated per cohort; no inpatient dose escalation was allowed. Restaging bone scans and CT scans of chest, abdomen and pelvis were mandatory every 2 months for the first 4 months of the study (following cycles two and four), and then after every three cycles of treatment. Response and progression were evaluated according to RECIST 1.1 [26]. Radiographic progression was defined as (a) the first occurrence of two new lesions on bone scan or (b) progression of measurable disease by RECIST. Disease progression was determined by clinical and radiographic criteria without the use of PSA, in

accordance with recommendations from the Prostate Cancer Clinical Trials Working Group-2 [27]. Dose reductions and interruptions were allowed. On cycle 1 day 1 (C1D1) and cycle 1 day 15 (C1D15) patients were admitted for ~24 h to complete research studies, including pharmacokinetic measurements. Otherwise, treatment was administered on an outpatient basis. Premedications (acetaminophen, dexamethasone, H2 blocker, antihistamine) were given ~30 min to 2 h before the start of each dose of TRC105. Toxicities were graded using the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events. Before every other cycle of TRC105, patients were tested for proteinuria (urine protein-to-creatinine ratio).

Response Evaluation

Patients were allowed to stay on study until they showed radiographic disease progression or intercurrent illness requiring cessation of TRC105, or until patient withdrawal from the study.

Statistical Analysis

This study used a standard 3 + 3 design. The patient population evaluated for drug safety included all patients who received at least a portion of the TRC105 infusion. Descriptive statistics were used to summarize patient characteristics, treatment administration and safety. Pharmacokinetic data were analysed by a non-parametric unpaired two-tailed *t*-test (Mann–Whitney). Statistical analyses were performed and graphs created using Microsoft Excel v2010 and GRAPHPAD PRISM v6 (GraphPad Software, San Diego, CA, USA).

PHOENIX/WINNONLIN 6.3 software with non-compartmental analysis was used to calculate pharmacokinetic variables such as the maximum plasma concentration (C_{max}), area under the curve (AUC), clearance, half-life and volume of distribution at steady-state.

Pharmacokinetics

Patients received TRC105 as an i.v. infusion biweekly (cohorts 1, 2, 3, 5 and 6) or weekly (cohort 4). The first dose on C1D1 was a 4-h infusion. Pharmacokinetic measurements were assessed on serum collected on C1D1 before treatment, at the end of infusion, and 1, 2, 8, 24 and 48 h after the end of infusion. If there were no infusion-related adverse events (AEs), the next dose on C1D15 was infused over 2 h. Blood for pharmacokinetic measurement was collected on C1D15 at pretreatment, end of infusion, and 1, 2, 8, 24 and 48 h after the end of infusion. If no AEs were observed, all remaining doses were infused over 1 h. For doses given on cycle 2 day 1 (C2D1) and beyond, blood was collected just before infusion and again at end of infusion to obtain a trough and peak, respectively, during steady-state. TRC105 was measured using a TRC105-specific ELISA (WuXi AppTec, Philadelphia, PA, USA) with a validated lower limit of quantitation of 78 ng/mL.

Pharmacodynamics

Sample preparation—Blood was collected in an EDTA-containing vacutainer at pretreatment (baseline), and on C1D15 cycle 2 day 15 (C2D15). After centrifugation, samples were immediately frozen, and stored at -80°C .

Measurement of plasma biomarkers—The analysis was performed on VEGF, placental-derived growth factor, basic fibroblast growth factor and soluble VEGF receptor 1 using assay plates from Meso-Scale Discovery (MSD, Gaithersburg, MD, USA) according to the production manual. The concentrations of the cytokines were determined with recombinant standards and expressed as pg/mL values.

The CD105 immunoassay was developed and performed on the MSD assay platform. Briefly, 5 µL of the anti-CD105 capture antibody (R&D Systems, Minneapolis, MN, USA) at 36 µg/mL was coated in each well of an MSD 96 standard binding plate overnight at 4 °C. The plates were blocked with 3% BSA at 150 µL/well for 1 h. For detection, 25 µL /well of 10 × diluted plasma samples or serially diluted recombinant CD105 standards were added and incubated for 2 h, followed by 3 × washes with wash buffer (PBS + 0.05% Tween 20). Next, 25 µL /well of 800 ng/mL biotinylated anti-CD105 detection antibody (R&D Systems) was added and incubated at room temperature for 2 h. After the wash, 25 µL /well of 1 µg/mL SULFO-TAG streptavidin (1% BSA, 1 × PBS) was added and further incubated for 1 h. For detection, 150 µL/well of 1 × MSD Read Buffer was added and the assay plated analysed with an MSD Sector Imager.

Data and Statistical Analysis

Quantification was obtained with protein standards provided and software package on Sector Imager 2400. The data were subsequently entered into PRISM (GraphPad, San Diego, CA, USA) to generate median values with interquartile ranges. Comparisons between different time points were made using a paired t-test to obtain *P* values.

Results

Between April 2010 and August 2012, 21 patients with mCRPC were enrolled at the Clinical Centre of the NCI and treated with escalating doses of TRC105. Baseline characteristics are shown in Table 2. No patients were treatment-naïve. Fifteen patients had previously been treated with at least one chemotherapy regimen and three patients had been treated previously with the angiogenesis inhibitor bevacizumab. Three patients had also previously been treated with abiraterone.

All patients received a portion of the first dose of TRC105 and 20 of 21 completed the 28-day dose-limiting toxicity evaluation period. Patients received a median (range) of 4 (1–7) cycles of treatment. Of the 21 patients enrolled, 17 (81%) discontinued treatment because of disease progression, one because of intercurrent illness (development of a deep venous thrombosis requiring anti-coagulation), one because of an AE (vasovagal reaction) and two as a result of personal preference.

Safety

The maximum tolerated dose of TRC105 was the top dose level studied of 20 mg/kg every 2 weeks on a 28-day cycle. One patient in cohort 5 (15 mg/kg i.v. every 2 weeks) experienced a grade 4 vasovagal reaction, an AE that was the only dose-limiting toxicity to occur. In addition, there was one grade 3 fever and three instances of grade 3 hypotension, considered to be possibly related to TRC105. The most common AEs (Table 3) were infusion-related

reaction, defined as chills, flushing and/or hypotension experienced within the first 48 h after infusion (90%), grade 1 or 2 headache (67%) , anaemia (48%), epistaxis (43%), fever (43%), nausea (33%) , vomiting (24%), bone pain (19%) and oral haemorrhage (19%).

Response Analysis

Of the 21 patients enrolled, 20 were evaluable for response. Of these, 16 patients had measurable soft tissue disease; seven of these 16 had stable disease after two treatment cycles. Three patients had stable disease after four treatment cycles. Eight patients had reductions in PSA level, two of which were >50 %. Of four patients with bone-only disease, three had stable disease after cycle 2 and one elected to come off study.

Reductions in PSA level were observed across all dose levels without any clear dose response (Fig. 1). One patient in cohort 2 (3 mg/kg every 2 weeks) had a reduction in PSA of 6%. In cohort 3 (10 mg/kg every 2 weeks), two patients had PSA reductions of 51 and 17%, while in cohort 4 (10 mg/kg weekly), two patients had PSA reductions of 20 and 59%, respectively. In cohort 5 (15 mg/kg every 2 weeks), one patient had a PSA reduction of 41%, and in cohort 6, two patients had PSA reductions of 19 and 8% respectively.

Pharmacokinetics

Pharmacokinetic samples were collected from all 21 patients; however, only those on the 15 mg/kg and 20 mg/kg dose levels had their TRC105 serum levels quantitated by ELISA. Five patients who received 15 mg/kg and three patients who received 20 mg/kg of TRC105 had sufficient samples to be included in this analysis.

TRC105 follows an apparent biphasic distribution, which is consistent with published reports [14]. While there appeared to be no difference in the observed data between the two doses, there was a slight increase in the observable data from C1D15 compared with C1D1 (Fig. 2), which was to be expected as steady-state is approached. The accumulation factor calculated for each patient ranged from 1.001 to 1.15 , indicating that TRC105 did not accumulate significantly with subsequent doses.

A non-compartmental analysis was used to determine C_{max} , area under the plasma concentration vs time curve up to the last time point (AUC_{last}), half-life, clearance and volume of distribution at steady-state. Table 4 shows data (arithmetic mean $\pm$ SD) comparing dose levels (15 vs 20 mg/kg) and days (C1D1 vs C1D15). There were no statistically significant differences in either comparison for any variable calculated, except for clearance, which was lower in the 15 mg/kg dose compared with the 20 mg/kg dose.

Only one other clinical pharmacokinetic study of TRC105 has been published [14] that can be used for comparison with the present first-in-human dose-escalation trial. The pharmacokinetic table from the present study (Table 4) shows a 1.4-fold greater C_{max} (456 mg/L) and a 2.8-fold greater AUC (33 240 h*mg/L, although the previous study used area under the concentration time curve with the last concentration extrapolated based on the elimination rate constant K_{el} from weekly dosing, not AUC_{last} from every 2-week dosing, as in the present study). Additionally, the drug used in the previous study had a 1.9-fold shorter half-life (42.8 h) than the drug used in the present study.

Pharmacodynamics Analysis

We selected free plasma CD105 as a pharmacodynamic biomarker for the evaluation of TRC105 activity. We reasoned that there could not be excessive free plasma CD105 protein, as it would bind to TRC105 and prevent it from reaching the targets on the endothelium surface. In the *in vitro* CD105 electrochemiluminescence assay that we developed, TRC105 competed for the binding to free CD105 and thus drastically inhibited the signal from CD105 (data not shown). The analysis of free plasma CD105 in the patients showed that there was an increase of plasma CD105 on C2D15 at three lower dose levels (Fig. 3). By contrast, plasma CD105 was reduced by ~95% in patients at dose levels 4–6, suggesting the depletion of free plasma CD105 by TRC105; therefore, there was sufficient TRC105 at dose levels 4–6 to deplete the circulating CD105 from plasma.

Examination of a panel of angiogenic molecules showed an induction of plasma VEGF at C1D15 and C2D15, an indicator of hypoxia and the anti-angiogenic activity of the agent (Table 5). Furthermore, it was interesting that VEGF induction was specifically associated with reduction of CD105 levels in plasma (Fig. 4), whereas the patients without reduced plasma CD105 levels exhibited no VEGF induction; thus, TRC105 was strongly associated with VEGF induction in the patients exhibiting plasma CD105 attenuation, providing evidence of anti-angiogenic activity of TRC105.

Further exploratory analysis in clinical endpoint association showed that the patients treated with TRC105 had a significant increase in PSA level at C2D15 ($P = 0.025$; Table 5). When the patients were further divided into subgroups based on the CD105 depletion by TRC105, the group without CD105 depletion were found to have a median increase in PSA level of 57%, which was significant ($P = 0.002$, Fig. 5). By comparison, the group with a significant reduction in plasma CD105 level had a median increase in PSA level of 16% that was nonsignificant ($P = 0.132$; Fig. 5). The exploratory analysis therefore suggests a potential association between the effects of TRC105 and decreased PSA velocity.

Immunological Markers

In an exploratory analysis, immune cell subsets were evaluated to examine the potential impact of TRC105 on these populations. Regulatory T cells decreased significantly after administration of TRC105 at C1D15 (Fig. 6A; $P = 0.032$) and showed a trend toward decrease at C2D15 ($P = 0.09$). The ratio of CD8⁺/CD4⁺T cells, however, did not change after treatment (data not shown). PD-1 on CD8⁺ T cells, which has been associated with T-cell exhaustion, immune escape and poor prognosis, had increased significantly at C2D15 (Fig. 6B; $P = 0.011$) [28–30]. Higher PD-1 expression levels on CD8⁺T cells, at baseline also correlated significantly with PSA increase (Fig. 6C; $P = 0.038$). It remains unclear if these immunological findings provide an explanation for the limited effectiveness of TRC105 which may have some, as yet undefined, immunological impact or if these findings were consistent with disease progression, as seen in many of these patients. In addition, osteopontin was evaluated in an attempt to evaluate the bone micro-environment which is critical in prostate cancer. The level of osteopontin in plasma increased significantly after treatment (Fig. 7, pre-cycle 1, day 1 vs C1D15, $P = 0.0056$; pre-cycle 1, day 1 vs C2D15, P

= 0.0001; C1D15 vs C2D15, $P = 0.012$), but changes in osteopontin after treatment were not associated with PSA response.

Discussion

The landscape in prostate cancer treatment has evolved over the past several years, resulting in new antiandrogen and immune-based therapies. These new therapeutic options have minimized the role of chemotherapy in the front-line setting in men with asymptomatic/minimally symptomatic CRPC. With greater emphasis on the role of the androgen receptor, the use of targeted antiandrogen molecules and agents that inhibit the conversion of testosterone to dihydrotestosterone has become more prevalent [31]. Resistance to antiandrogen therapy can develop as mutations occur in the androgen receptor ligand-binding domain [31], highlighting the need for optimized treatment regimens. As such, as the disease progresses, and resistance develops, optimized treatment regimens must be developed that can be used in combination with chemotherapy or with anti-angiogenesis agents. Interactions that are important in mediating resistance to VEGF inhibition are currently under investigation [32]. Development of resistance can occur in VEGF-dependent pro-angiogenic pathways [33]. As such, as the disease process progresses, combination therapies for CRPC using chemotherapy and agents targeting other pathways in tumour growth and metastases will need further evaluation. In particular, combinations may reduce tumours' resistance mechanisms to antiangiogenic treatments [34].

While results of trials with monotherapy antiangiogenic drugs such as bevacizumab and aflibercept in combination with chemotherapy have been disappointing, angiogenesis inhibitors may require dual-pathway blockade to optimize benefit [35]. Alternative combined anti-angiogenic strategies have shown promise in mCRPC. In a phase II trial, the addition of thalidomide and bevacizumab to docetaxel and prednisone resulted in higher response rates than using either agent alone with docetaxel [36]. The NCI is currently conducting a phase II study of the combination of docetaxel, bevacizumab, lenalidomide and prednisone in patients with chemotherapy-naïve mCRPC [37]. The activity of this regimen appears to be similar to the combination of thalidomide, docetaxel, bevacizumab and prednisone, with a superior toxicity profile.

Perhaps the most interesting correlative finding in the present study shows the potential anti-angiogenic activity of TRC105 in patients with prostate cancer. After treatment with TRC105, a significant increase in VEGF, a marker for hypoxia, was seen in patients with CD105 depletion. These data suggest that rising VEGF level is a possible compensatory mechanism for TRC105-induced anti-angiogenic activity and provides a rationale for TRC105 combinations with anti-VEGF therapies.

TGF- β is a purported target of TRC105 and important in regulatory T-cell function [38]. In the present study, TRC105 significantly decreased the percentage of regulatory T cells in CD4⁺ T cells, which correlated with reductions in PSA level; thus, TRC105 may affect regulatory T-cell production or survival, which may in turn affect PSA response. Additional findings suggest that PDL-1 expression on CD8⁺ T-cells either has a role as a marker of disease progression or may be associated with the efficacy of TRC105. Future randomized

trials that prospectively evaluate these questions are required. Given the emergence of immunotherapy in prostate cancer among other cancers, such findings could provide the rationale for immune-based combinations in future.

In the present study, TRC105 was a novel targeted therapy that was well tolerated. AEs were manageable and dissimilar to those commonly associated with VEGF inhibition. Toxicities such as hypertension, proteinuria and thrombi were infrequent, and there were no arterial or venous thromboembolic events, pulmonary emboli, serious bleeding episodes or perforations. Common AEs included headache, epistaxis and oral haemorrhage. Modest infusion reactions, often seen in treatments in which antibodies bind cellular targets, were also observed. Treatment with TRC105 resulted in stable disease and PSA declines in men with mCRPC. Activity has been seen with the combination of TRC105 and bevacizumab in solid tumours [39]. Further studies of TRC105 in combination with androgen receptor-targeted therapies, chemotherapy, VEGF inhibitors, or immune checkpoint inhibitors in patients with mCRPC could be valuable in defining the clinical potential of TRC105 in prostate cancer.

Abbreviations:

mCRPC	metastatic castration-resistant prostate cancer
CD105	endoglin
VEGF	vascular endothelial growth factor
CRPC	castration-resistant prostate cancer
RECIST	Response Evaluation Criteria in Solid Tumors
NCI	National Cancer Institute
C1D1	cycle 1 day 1
C1D15	cycle 1 day 15
C_{max}	maximum plasma concentration
AUC	area under the curve
AUC_{last}	area under the plasma concentration vs time curve up to the last time point
AE	adverse event

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Fig. 1.
Percent change in PSA level from baseline.

Fig. 2.
Serum TRC105 concentrations vs time for all available data.

Fig. 3.
Endoglin (CD105) downregulation: associated with dose levels 4–6.

VEGF levels	Pretreatment				C1D15				C2D15			
Plasma VEGF (pg/mL)	Median	IQR	No. of Patients	P (paired)	Median	IQR	No. of Patients	P (paired)	Median	IQR	No. of Patients	P (paired)
Patients with CD105 reduction	195	122-276	9	NA	401	239-576	9	0.003**	551	151-680	9	0.008**
Patients without CD105 reduction	177	87-349	10	NA	242	89-392	10	0.852	181	110-432	10	0.204

Fig. 4.

Patients with endoglin (CD105) downregulation associated with induction of vascular endothelial growth factor (VEGF). C1D15, cycle 1 day 15 ; C2D15, cycle 2 day 15; IQR, interquartile range; VEGF, vascular endothelial growth factor.

	Pretreatment					C2D15			
Serum PSA (ug/mL)	Median	IQR	No. of Patients	<i>P</i>		Median	IQR	No. of Patients	<i>P</i>
Patients with CD105 reduction	1	NA	9	NA		1.16	0.81-1.65	9	0.132
Patients without CD105 reduction	1	NA	10	NA		1.57	1.36-1.91	10	0.002**

Fig. 5.
Patients with endoglin (CD 105) downregulation and changes in PSA level.

Fig. 6. (A) %Tregs among CD4+ T-cells. (B) PD-1 on CD8+ T cells. (C) PD-1 on CD8+ T cells at baseline. A Wilcoxon matched-pairs signed rank test was used in A and B. An exact Wilcoxon rank sum test was used in C. MFI, median fluorescence intensity; PD-1, programmed cell death protein 1; Tregs, regulatory T cells; C1D1, cycle 1 day 1; C1D15, cycle 1 day 15; C2D15, cycle 2 day 15.

Fig. 7.
Osteopontin levels in plasma. C1D1, cycle 1 day 1; C1D15, cycle 1 day 15; C2D15, cycle 2 day 15.

Table 1

Dose-escalation scheme and number of patients on each dose level.

Cohort	Phase I dose	<i>n</i>
1	1 mg/kg every 2 weeks	3
2	3 mg/kg every 2 weeks	3
3	10 mg/kg every 2 weeks	3
4	10 mg/kg weekly	2
5	15 mg/kg every 2 weeks	6
6	20 mg/kg every 2 weeks	4

Table 2Patient demographics and clinical characteristics (*N* = 21).

Characteristic	
Age, years	
Median	63.6
Range	47–87.5
Race, <i>n</i> (%)	
White	14 (67)
African-American	7 (33)
ECOG performance score, <i>n</i> (%)	
0	5 (24)
1	15 (71)
2	1 (5)
Gleason score at diagnosis	
Median	9
Range	6–10
Gleason score at diagnosis, <i>n</i> (%)	
8	18 (86)
7	3 (14)
On-study PSA, ng/mL	
Median	128
Range	0.14–2923
Metastases, <i>n</i> (%)	
Bone only	4 (19)
Soft tissue + bone	17 (81)
Liver	3 (14)
Lung and pleura	2 (10)
Previous chemotherapy regimens, <i>n</i> (%)	
1	3 (14)
2	7 (33)
3	5 (24)

ECOG, Eastern Cancer Oncology Group.

Table 3

Adverse events that were possibly treatment-related in > 1 patient or grade 3 or 4 toxicities ($N=21$).

Preferred CTCAE term	Grade 1	Grade 2	Grade 3	Grade 4
Infusion-related reaction	15	4	0	1
Headache	8	6	0	0
Fever	4	4	1	0
Nausea	3	4	0	0
Vomiting	4	1	0	0
Anaemia	0	6	4	0
Bone pain	0	4	0	0
Sinus tachycardia	2	0	0	0
Hypophosphataemia	0	1	0	0
Epistaxis	7	2	0	0
Fatigue	1	3	0	0
Dyspnoea	2	1	0	0
Cough	2	0	0	0
Oral haemorrhage	4	0	0	0
Back pain	2	1	0	0
Anorexia	1	1	0	0
Abdominal pain	2	0	0	0

CTCAE, Common Terminology Criteria for Adverse Events.

Table 4

Non-compartmental analysis of cycle 1 of TRC105.

	15 mg/kg (<i>n</i> = 5 [*])	Mean ± SD	20 mg/kg (<i>n</i> = 3 [*])	Mean ± SD	<i>P</i> [†]	CID1 (<i>n</i> = 8)	Mean ± SD	CID15 (<i>n</i> = 8)	Mean ± SD	<i>P</i> [†]
C_{max}¹, mg/L	322.85 ± 170.87		336.67 ± 47.32		0.3664	288.25 ± 104.99		367.81 ± 156.84		0.3231
AUC_{last}, mg[*]h/L	11 870 ± 7178		10 763 ± 1446		0.8649	9531 ± 4125		13 379 ± 6550		0.2317
T_{1/2}, h	80.99 ± 45.38		61.18 ± 29.02		0.5043	91.96 ± 46.37		56.54 ± 25.39		0.1203
Clearance, L/h	0.044 ± 0.020		0.077 ± 0.024		*0.0256	0.055 ± 0.026		0.060 ± 0.029		0.9203
VSS, L	4.83 ± 2.54		6.05 ± 1.36		0.0879	6.23 ± 2.65		4.52 ± 1.36		0.1883

CID1, cycle 1 day 1; CID15, cycle 1 day 15; C_{max}, maximum plasma concentration; AUC_{last}, area under the plasma concentration vs time curve up to the last time point; T_{1/2}, half-life; VSS, volume of distribution at steady-state.

* Includes data from both CID1 and CID15 for each patient on dose level.

[†] Non-parametric two-tailed unpaired t-test (Mann-Whitney) assuming equal distributions (homoscedasticity), significance at $\alpha = 0.05$.

Table 5

Biological effects: angiogenic factors in plasma.

Marker	Pretreatment, <i>n</i> = 19				C1D15, <i>n</i> = 19				C2D15, <i>n</i> = 19			
	Median	IQR	<i>P</i> (paired)		Median	IQR	<i>P</i> (paired)		Median	IQR	<i>P</i> (paired)	
Plasma CD105, ng/mL	15.5	{6.6–18.7	NA		12.7	0.8–36.4	0.109		1.6	0.4–42.6	0.106	
Plasma VEGF, pg/mL	195	93–286	NA		365	125–443	0.033*		365	131–586	0.005**	
Serum PSA, ng/mL	128	47–1050	NA		135	58–1417	0.158		149	65–1801	0.025*	
Plasma PlGF, pg/mL	29	20–35	NA		22	16–32	0.079		23	17–30	0.045*	
Plasma bFGF, pg/mL	71	40–133	NA		114	64–187	0.051		173	63–300	0.001**	
Plasma sVEGFR1 pg/mL	174	144–255	NA		223	199–262	0.651		239	176–293	0.684	

CD105, endoglin; C1D15, cycle 1 day 15; C2D15, cycle 2 day 15; IQR, interquartile range; VEGF, vascular endothelial growth factor, sVEGFR1, soluble VEGF receptor 1; PlGF, placental-derived growth factor; bFGF, basic fibroblast growth factor.