Amendment

Protocol Number: 11C0102-N **Reference Number:** 359988

Principal Investigator: Tim Greten NCI TGIB 301.451.4723 tim.greten@nih.gov

(NIH Employee Name, Institute/Branch, Telephone and e-mail)

Protocol Title: A Phase I/II Study of TRC105 in Combination with Sorafenib in Hepatocellular Carcinoma (HCC)

SIGNATURES

Principal Investigator (*):
Tim Greten - applied signature on 06/30/2016 1:10 PM EDT

Accountable Investigator:
PI is the Accountable Investigator

Branch Chief/CC Department Head (**):
Raffit Hassan - applied signature on 06/30/2016 2:46 PM EDT

Medical Advisory Investigator (if applicable):
N/A

Lead Associate Investigator signature:
N/A

Referral Contact signatures:
~~N/A~~ - Donna Mabry (see below)

Associate Investigators signatures:
Donna Mabry - applied signature on 07/01/2016 9:49 AM EDT

For Institute/Center Scientific Review Committee:
N/A

Other IC Clinical Director signatures:
N/A

APPROVALS

IRB Chair:
Michael Hamilton - applied signature on 07/07/2016 5:58 PM EDT

Clinical Director:
N/A

CONCURRENCE

OPS Protocol Specialist:

Royal Reed	AM N	07/12/16
Signature	Print Name	Date

* Signature signifies that investigators on this protocol have been informed that the collection and use of personally identifiable information at the NIH are maintained in a system of record governed under provisions of the Privacy Act of 1974. The information provided is mandatory for employees of the NIH to perform their assigned duties as related to the administration and reporting of intramural research protocols and used solely for those purposes. Questions may be addressed to the Protrak System Owner.

** I have reviewed this research project and considered the NIH Policy for Inclusion of Women and Minorities in Clinical Research. Taking into account the overall impact that the project could have on the research field involved, I feel the current plans adequately includes both sex/gender, minorities, children, and special populations, as appropriate. The current enrollment is in line with the planned enrollment report for inclusion of individuals on the basis of their sex/gender, race, and ethnicity and is appropriate and of scientific and technical merit.

IRB Meeting Date: Expedited

DEC Clearance Date: 06/22/2016

Protocol Version Date: 06/10/2016

Abbreviated Title: Sorafenib and TRC105 in HCC
NCI Protocol #: **11-C-0102**
Version Date: June 10, 2016
Amendment: N

Title: A Phase I/II study of TRC105 in combination with Sorafenib in Hepatocellular Carcinoma (HCC)

Principal Investigator: Tim Greten, MD
Thoracic and GI Oncology Branch
National Cancer Institute
Building 10, Rm 12N226
9000 Rockville Pike
Bethesda, MD 20892
301-451-4723
FAX: 301-480-8780
gretentf@mail.nih.gov

Lead Associate Investigator: Austin Duffy, MD, TGIB, CCR, NCI

Associate Investigators: William D. Figg, PharmD, GMB, CCR, NCI
Jane Trepel, DTB, CCR, NCI
Douglas K. Price, PhD, GMB, CCR, NCI
Peter Choyke, MD, CCR, MIP, NIH
Xin Wang, PhD, LHC, CCR, NCI
Brad Wood MD, OADRIS, DDR, CC, NIH
Liang Cao PhD, GB, CCR, NCI
Suzanne Fioravanti, RN, OCD, CCR, NCI
Melissa Walker, RN, OCD, CCR, NCI
Donna Mabry, NP, TGIB, CCR, NCI

Statistician: Seth Steinberg, PhD
Biostatistics and Data Management Section
National Cancer Institute
9609 Medical Center Drive 2W334
Rockville, MD 20892-9716
Phone: 240-276-5563
Fax: 240-276-7885
steinbes@mail.nih.gov

Referral Contact/ Donna Mabry, NP, TGIB, CCR, NCI

Study Coordinator: Suzanne Fioravanti, RN, OCD, CCR, NCI
Building 10, Room 12N226
9000 Rockville Pike
Bethesda, MD 20892-1906
Phone: 301-594-6544
Fax: 301-402-7901
Email: fioravas@mail.nih.gov

Investigational Agents:

Drug Name:	TRC105

IND Number:	110744
Sponsor:	Center for Cancer Research, NCI
Manufacturer	Tracon Pharmaceuticals, Inc.

Commercial Agent: Sorafenib

PRÉCIS

Background:

- Worldwide, hepatocellular carcinoma (HCC) is the fifth most common malignancy with a median survival of 6-9 months. The SHARP study established sorafenib as a standard consideration in this disease and set the bar for future studies of systemic therapy.
- TRC105 is a chimeric anti-angiogenic monoclonal antibody that binds CD105, a transmembrane receptor selectively expressed by proliferating endothelial cells. TRC105 binds to CD105-expressing endothelial cells and mediates growth inhibition, apoptosis and antibody-dependent cell-mediated cytotoxicity (ADCC).

Objectives:

Primary:

- Phase I: To establish the maximum tolerated dose (MTD) of TRC105 when given with standard-dose sorafenib for HCC.
- Phase II: To determine the estimate response rate according to RECIST criteria for the combination of TRC105 with sorafenib in HCC.

Eligibility:

- Histologically or cytologically confirmed diagnosis of HCC.
- Childs-Pugh A or B (7 points) cirrhosis is allowed.
- Patients must have disease that is not amenable to potentially curative resection, radiofrequency ablation, or liver transplantation.
- In phase I, prior systemic therapy is allowed.
- In phase II, prior systemic therapy for HCC (including sorafenib) is allowed.
- No history of bleeding varices in previous 1 year (unless subsequent liver transplant).
- No anti-coagulation (except low-dose aspirin).

***Design*:**

- TRC105 will be administered intravenously every two weeks, on days 1 and 15 of each 28 day cycle. Sorafenib will be self-administered twice daily by mouth.
- **Phase 1:** The first part of this study was a standard 3+3 dose escalation phase I study with the primary objective of establishing MTD for TRC105 when given in combination with standard-dose sorafenib. Sorafenib is taken orally at a dose of 400 mg twice daily. TRC105 is administered as an intravenous infusion every two weeks. Patients will be re-staged including imaging studies to assess for response and progression every 8 weeks. The TRC105 dose was escalated in cohorts of 3 to 6 patients up to a maximum of 15 mg/kg every two weeks (see table below). Intra-patient dose escalation was not allowed.
- **Phase II:** TRC105 will be administered as an intravenous infusion every two weeks at the recommended phase II dose, 15 mg/kg of TRC105 ever two weeks in combination with standard dose sorafenib, defined in phase I. The sample size and interim stopping rule will be determined using a Simon optimal two-stage design.

The first stage will initially enroll 6 evaluable patients, and if 0 of the 6 have a clinical response, then no further patients will be accrued. If 1 or more of the first 6 patients has a clinical response, then accrual would continue until a total of 23 patients have been enrolled. As it may take several weeks to determine if a patient has experienced a response, a temporary pause in the accrual may be necessary to ensure that enrollment to the second stage is warranted. If there are 1 to 2 clinical responses in 23 patients, this would be an uninterestingly low response rate. If there were 3 or more complete responses in 23 patients (13.0%), this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (5% response rate), the probability of early termination is 73.5%.

Cohort	**Sorafenib (mg PO twice daily)**	**TRC105 (mg/kg IV every two weeks)**
0	400 bid	1
1	400 bid	3
2	400 bid	6
3	400 bid	10
4	400 bid	15

TABLE OF CONTENTS

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective(s):

1.1.1.1 Phase I: To establish the MTD for TRC105 when given with sorafenib in HCC.

1.1.1.2 Phase II: To determine the estimateresponse rate according to RECIST criteria for the combination of TRC105 with sorafenib in HCC.

1.1.2 Secondary Objective(s):

1.1.2.1 To evaluate the safety of the combination of TRC105 and sorafenib in HCC.

1.1.2.2 To evaluate the immunogenicity of TRC105 as measured by human antichimeric antibody (HACA) and human antimouse antibody formation.

1.1.2.3 To evaluate overall response rate (ORR) as determined by both standard and EASL-modified RECIST criteria.

1.1.2.4 To determine progression-free survival (PFS) and overall survival (OS) for TRC105 and sorafenib in HCC.

1.1.2.5 To perform correlative studies assessing 1) potential biomarkers of response to angiogenic therapy, 2) changes in frequency and function of immune cells upon treatment and 3) molecular characterization of tumors.

1.2 BACKGROUND AND RATIONALE:

1.2.1 Hepatocellular Carcinoma (HCC): Current standard of care.

Worldwide, hepatocellular carcinoma (HCC) is the fifth most common malignancy with a median survival of 6-9 months[1, 2]. There is a marked geographic heterogeneity with regard to the etiology of this disease and this complicates the design and interpretation of clinical trials. In addition, for the majority of patients with HCC, the presence of underlying cirrhosis is an additional non-cancer factor that is prognosis-determining and must be taken into account. The approach to treating HCC has traditionally comprised of locoregional strategies: surgery (partial resection or transplantation) or interventional radiologic procedures, such as chemoembolization or ablative techniques. These approaches still have primacy and the surgical and ablative techniques remain the only real possibility of cure. However, the publication of the landmark SHARP study established sorafenib as a standard consideration in this disease and set the bar for future studies of systemic therapy[3].

1.2.1.1 Cytotoxic chemotherapy.

A clearcut survival benefit for chemotherapy compared to best supportive care has never been satisfactorily established in HCC. Doxorubicin became the most widely studied chemotherapeutic agent based on older flawed studies that suggested good single-agent activity. A more reliable indicator of its efficacy was demonstrated in a phase III study comparing

doxorubicin to nolatrexed, a thymidylate synthetase inhibitor[4]. Median overall survival was better for the doxorubicin arm (32.3 v 22.3 weeks; P = .0068) with a response rate of 4% (v 1.4% in the nolatrexed which was considered inactive). Doxorubicin was then used to form the backbone of the so-called PIAF regimen, in combination with cisplatin, interferon and fluorouracil. This regimen appeared to be active based on a single-arm phase II study demonstrating a partial response rate of 26% and a median survival of 8.9 months[5]. What was particularly striking in that study was that of the 9 patients who were able to undergo resection after achieving a response, no viable tumor cells were found in 4 of the resected specimens. A phase III study was performed to compare the PIAF regimen with the de facto standard, single-agent doxorubicin[6]. Although there was a trend towards improved response rate (20.9% v 10.5%) and median survival (8.7 v 6.8 months) in the PIAF group, these were not statistically significant (P=.83). In addition the PIAF regimen had a significant increase in serious toxicity. One interpretation of the role of the PIAF regimen is for its very select use in patients of good performance status and hepatic function in whom cytoreduction is being attempted prior to potential resection[7]. There has been a paucity of phase III studies evaluating other chemotherapy combinations. Single-arm phase II studies have evaluated gemcitabine-based combinations, standard in pancreatico-biliary cancers, suggesting modest activity only. For example a small phase II study (N=34) of gemcitabine in combination with oxaliplatin revealed a response rate of 18% and a median survival of 11.5 months (95% CI: 8.5-14.3 months)[8]. There is a consensus in the field that chemotherapy combinations by themselves are unlikely to result in significant progress.

1.2.1.2 The SHARP and Asian-Pacific studies

The first systemic medical therapy to demonstrate a prolongation of survival in hepatocellular carcinoma was sorafenib - an oral multi-kinase inhibitor of vascular endothelial growth factor (VEGF) receptor, the platelet-derived growth factor (PDGF) receptor, and Raf[3]. A landmark phase III randomized, placebo-controlled study demonstrated – after closure of the study at the second pre-specified interim analysis – a median overall survival benefit in favor of the sorafenib arm (10.7 months v 7.9 months; HR 0.69; 95% CI, 0.55 to 0.87; P<0.001) and has resulted in the adoption of sorafenib monotherapy as the standard of care for patients with HCC who are not eligible for, or have had disease progression after, surgical or locoregional therapies. Of note, in this study was the inclusion of 'symptomatic progression' (measured by a questionnaire) as a co-primary endpoint. Also of note was the fact that 95-98% of patients had Child-Pugh class A cirrhosis. A second major study confirming the role of sorafenib – albeit with ostensibly less impressive results – was a study conducted in Asia in predominantly Hepatitis B patients[9]. In that study overall survival was 6.5 months versus 4.2 months in favor of the sorafenib arm (P=0.014).

1.2.1.3 Augmenting sorafenib by combining with chemotherapy.

Abou-alfa, et al. conducted a randomized placebo-controlled phase II study (N=96) evaluating sorafenib in combination with the prior (de facto) standard, doxorubicin[10]. Median OS was 13.7 months versus 6.5 months in favor of the sorafenib-containing arm, thus providing further evidence in favor of sorafenib, although unable to define what additional benefit, if any, was due to the contribution of doxorubicin. Shen and colleagues performed a phase II study combining metronomic UFT/tegafur chemotherapy with sorafenib in a predominantly BCLC stage C

(advanced stage) population demonstrating a 6% partial response rate, median progression-free survival of 3.7 months (95% C.I., 1.9- 5.5) and overall survival of 7.4 months (95% C.I., 3.4-11.4)[11]. Similarly Petrini and colleagues performed a study of continuous infusional 5-FU at 200 mg/m^2/day for 14 days of a 21 day cycle demonstrating a median TTP of 7.6 months (CI 95%=5.3–9.9) and median overall survival of 12.2 months (CI 95%=4.45–19.8)[12]. Other chemotherapy combinations of sorafenib currently being evaluated consist of capecitabine with oxaliplatin (XELOX) and gemcitabine with cisplatin (clinicaltrials.gov).

1.2.1.4 Augmenting the anti-angiogenic effects of sorafenib.

The difficulty in combining anti-angiogenic agents was demonstrated in a phase I study of sorafenib and bevacizumab in which overlapping toxicity resulted in an inability to dose-escalate, albeit with some evidence of clinical benefit[13]. As a result of this and a possible plateau effect with anti-VEGF therapy[14], interest has focused on overcoming the escape mechanisms employed by tumors rather than merely trying to saturate with anti-VEGF stategies. For example, fibroblast growth factor (FGF) levels have been shown to increase after anti-VEGF therapy and to modulate resistance to VEGF inhibition[15]. Brivanib is a dual inhibitor of VEGF and FGF receptors which is currently being compared to sorafenib in a phase III study[16]. An earlier phase II study demonstrated single-agent activity for brivanib in both sorafenib-naïve and pretreated patients with HCC[17]. Perhaps the most important escape mechanism employed by tumors in response to antiangiogenic therapy is hypoxia inducible factor (HIF)-1α, which plays a central role in tumor progression, acting as a master regulator of how cancer cells adapt to hypoxic conditions. HIF-1α therefore represents an attractive target in oncology, particularly in concert with anti-angiogenic strategies[18]. In preclinical models, anti-VEGF therapy with bevacizumab has been shown to cause an increase in intratumor-hypoxia – without the induction of apoptosis – and to result in a significant increase of HIF-1α-dependent gene expression[19]. Numerous strategies of targeting HIF-1α, including antisense oligonucleotides and use of topoisomerase-1 poisons (which appear to inhibit the translation of HIF-1α) are currently in the clinic[20].

1.2.2 CD105

CD105 (endoglin) is a 180 kDa homodimeric transmembrane receptor overexpressed by proliferating endothelial cells[21]. It appears that CD105 is specific to endothelial cells that are proliferating, as anti-CD105 monoclonal antibody reacts with activated endothelial cells in tumors but weakly or not at all in the endothelium of normal, stable vasculature[21]. The CD105 pathway is essential for angiogenesis during fetal development and cell-surface expression of this molecule is required for the formation of new blood vessels[22]. This is evidenced by CD105 null mice that die *in utero* as a result of impaired angiogenesis in the yolk sac[22].

CD105 acts as an accessory protein that interacts with the signaling receptor complex of the TGF- ß superfamily, thus modulating the effects of TGF- ß[23]. CD105 expression protects endothelial cells from the growth-inhibitory effects of TGF-β signaling[24]. When engaged by the TGF- ß/TGF-ßRII complex, CD105 preferentially activates Alk-1 which in turn phosphorylates Smad proteins 1, 5 and 8. This ultimately leads to stimulatory effects on endothelial cell proliferation, migration and transcription of pro-angiogenic genes. CD105 expression is induced by hypoxia through HIF-1α and protects hypoxic endothelial cells from apoptosis[25].

Additionally, CD105 regulates components of the extracellular matrix to facilitate endothelial cell migration and promote formation of neo-vessels[26].

Anti-VEGF therapy up-regulates CD105 expression, indicating that a therapeutic strategy which targets CD105 may complement VEGF-inhibition[27].

Mutations in CD105 or its downstream signaling mediator leads to haplotype insufficiency causing a well-described syndrome known as hereditary hemorrhagic telangiectasia type 1 (HHT-1 or Osler-Weber-Rendu Syndrome). HHT-1 is a rare autosomal dominant genetic disorder characterized by vascular dysplasias, frequent episodes of epistaxis, mucocutaneous telangiectasias and arteriovenous malformations of the lung, brain, liver and gastrointestinal tract. The genotype is manifested *in utero*, but the phenotype does not become apparent for many years following birth. Affected patients most commonly present with epistaxis in the second decade of life. The phenotype of this disorder is limited to vascular effects, indicating the specific role of CD105 in the vasculature. Most patients affected with HHT-1 experience epistaxis by the age of 10 to 20 years, while telangiectasias of the skin and mucosa first appear by the age of 20 to 40 years. Visceral arteriovenous malformations are less common sequelae but may result in significant morbidity, including pulmonary shunting and intracranial hemorrhage.

1.2.2.1 CD105 in HCC

The specificity of CD105 expression in HCC tumor tissue was studied by Yang, et al. who analyzed CD105 expression levels in para-carcinomatous and tumor tissue in 113 HCC resected specimens. In addition, in 14 of these cases areas of distinct normal liver tissue were also resected. It was found that CD105 was expressed in 100% of the HCC levels but 0% of either the normal or para-carcinomatous tissue[21]. This was in contrast with the pan-endothelial anti-CD34 staining which - in addition to being present in 100% of tumor tissue – was also present in 94% and 87% of para-carcinomatous and normal liver tissue respectively.

Preclinical data in support of the concept:

CD105 and HCC: Studies performed in different laboratories using various antibodies to CD105 (endoglin) have revealed expression and upregulation in a wide range of tumor endothelia, but seldom found in the endothelia of normal tissues, suggesting that CD105 is highly related to tumor angiogenesis[28]. CD105 expression is an important prognostic factor in solid tumor patients including HCC[21]. In two mouse models of hepatoma - formed from Hepal-6 and H22 cell lines - tumor growth suppression, inhibition of angiogenesis, increased survival and tumor

apoptosis were observed on treatment with anti-CD105 antibody (Figure 1)[28].

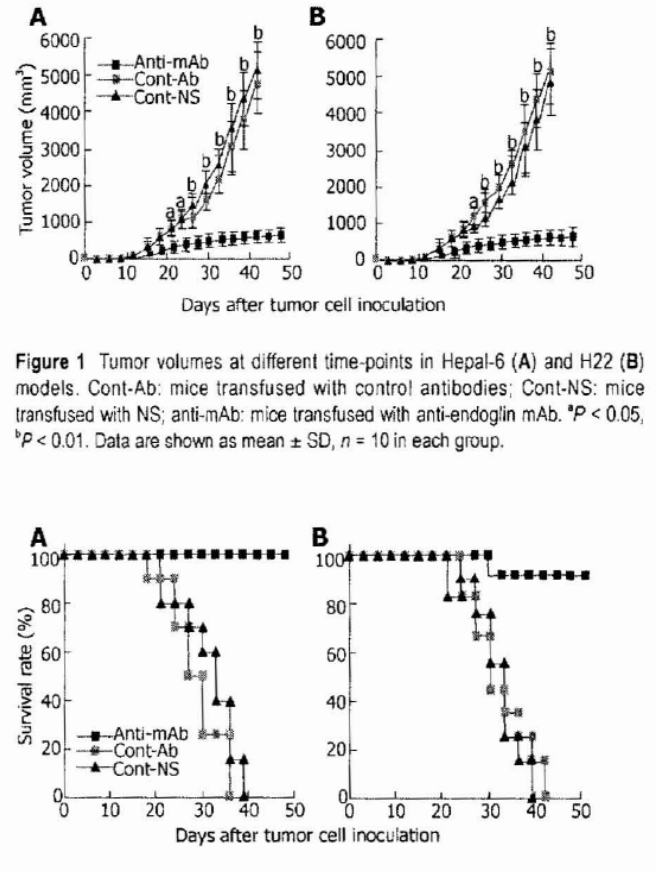


Figure 1 Tumor volumes at different time-points in Hepal-6 (**A**) and H22 (**B**) models. Cont-Ab: mice transfused with control antibodies; Cont-NS: mice transfused with NS; anti-mAb: mice transfused with anti-endoglin mAb. $^{a}P < 0.05$, $^{b}P < 0.01$. Data are shown as mean ± SD, $n = 10$ in each group.

Figure 1: Anti-murine CD105 antibody therapy delayed tumor growth (top panel) and extended mouse survival (bottom panel) in 2 xenograft models of HCC. In additional correlative studies, the therapy reduced tumor microvessel density and increased tumor apoptosis.

CD105 and VEGF: CD105 expression has been shown to be increased in certain tumors following inhibition of the VEGF pathway. CD105 expression increased more than two-fold in human pancreatic cancers grown in mice treated with an antibody that binds VEGF[27](Figure 2). Treatment of human bladder cancers grown in mice with an antibody that blocks activation of the VEGF receptor increased CD105 expression within the core tumor vasculature[29].

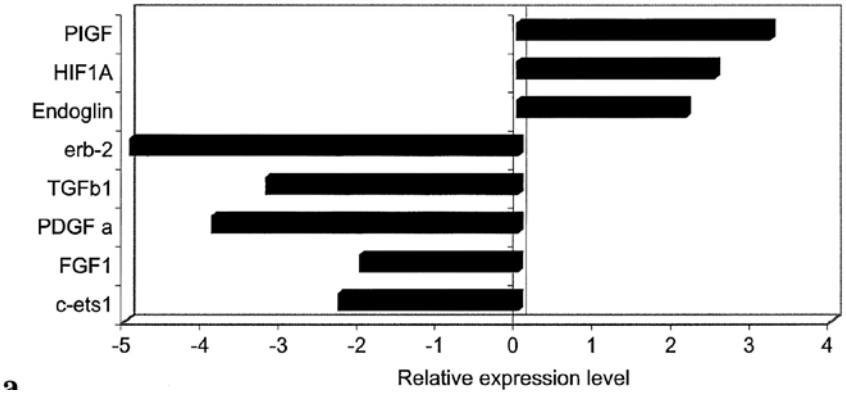


Figure 2: Effect of anti-VEGF antibody treatment on angiogenic gene expression: A microarray analysis of angiogenic genes. Genes altered more than 2-fold by anti-VEGF treatment are presented. Relative expression levels indicate positive or negative fold differences in anti-VEGF antibody-treated group compared with control antibody-treated group.

1.2.3 TRC105

All data cited in this section (unless otherwise noted) has been obtained from Tracon Pharmaceuticals, Inc and is found in the Tracon Pharma Investigator's Brochure, Project TRC105 Oncology, version 2.0: May 11, 2009. Edition 2.0.

TRC105 is a human/murine chimeric anti-CD105 IgG1 kappa monoclonal antibody consisting of human Cκ and Cγ1 constant regions with murine Vκ and V_H regions. TRC105 is composed of two light chains of 213 amino acids and two heavy chains of 448 amino acids and has an approximate molecular weight of 148 kDa. This monoclonal antibody binds with high avidity to human CD105 (endoglin), thus inhibiting angiogenesis and tumor growth.

1.2.3.1 Nonclinical Pharmacology, Efficacy and Toxicology

Pharmacology studies include *in vitro* and *in vivo* studies performed with the murine parent antibody (SN6j) and TRC105. These antibodies share identical variable regions. SN6j, the murine parent monoclonal antibody of TRC105, was generated by immunizing mice with an isolated CD105 preparation from human leukemia cells.

Pharmacology studies were conducted in murine and human models. Collectively, the studies demonstrate that TRC105 (or SN6j) binds with higher avidity to human than murine endothelial cells, and reacts more strongly with proliferating than quiescent endothelial cells. Studies also indicate that these anti-CD105 antibodies are able to mediate TGF-β dependent growth inhibition, induce apoptosis of human umbilical vein endothelial cells (HUVECs), mediate antibody dependent cell-mediated cytotoxicity (ADCC) of HUVECs, saturate CD105 binding at concentrations ≥ 250 ng/mL, and inhibit the growth of human tumor xenografts.

TRC105 binds to purified human CD105 with high avidity by surface Plasmon resonance assay (5 - 35 pM). As expected, binding studies indicate that both SN6j and TRC105 bind with nearly identical avidity to human endothelial cells that express CD105 as determined by Scatchard analysis.

In Vitro Pharmacology of SN6j

Using Scatchard plot analyses performed by incubating fixed combinations of radiolabeled and native antibody with KM-3 cells, the avidity of SN6j was calculated by regression analysis to be 2.85×10^9 liter/mole (or 3.51×10^{-10} mole/liter).

TGF-β Dependent Growth Inhibition of HUVECs

SN6j was studied for its ability to inhibit the growth of HUVECs *in vitro* in the presence of TGF-β. HUVEC cells were cultured at 37 °C overnight prior to the addition of SN6j and TGF-β. Cells were then exposed to antibody or TGF-β for 72 hours, during which time the media containing antibody and/or growth factors was replaced every 24 hours. Cell growth was then quantified by assaying tritiated thymidine incorporation for 20 hours. The concentration of SN6j needed to inhibit HUVEC growth by 50% was 90 μg/mL in the absence of TGF-β. However, the

concentration of SN6j needed to inhibit HUVEC growth by 50% was much lower when cells were incubated with antibody in the presence of TGF-β. These data indicate that anti-endoglin antibody potently inhibits HUVEC growth in the presence of TGF-β. At physiologic concentrations of TGF-β (>100 pg/mL), the amount of SN6j needed to inhibit HUVEC cell growth was <10 μg/mL.

Induction of Apoptosis of Proliferating HUVECs

SN6j was studied for its ability to induce apoptosis of proliferating HUVECs *in vitro*. HUVEC cells were seeded and then incubated at 37 °C with SN6j (50 or 100 μg/mL) or 100 μg of MOPC, an IgG1-kappa isotype-matched control antibody. Following incubation, cells were washed and then lysed. The cytoplasmic and nuclear fractions were separated by centrifugation and nucleosomes contained in the cytoplasmic fraction were then detected via their histone components. Apoptosis was determined by assaying the fragmented nucleosome content of cytoplasmic fractions using a commercial ELISA (Roche Diagnostics). The degree of apoptosis induced by SN6j on proliferating HUVECs from multiple donors was 9.1% at 50 μg/mL and 25.6% at 100 μg/mL concentrations, relative to the value induced by camptothecin. Apoptosis of SN6j (100 μg/mL) treated HUVECs was 4.58 times as large as that of MOPC control IgG (100 μg/mL) treated HUVECs, while apoptosis of SN6j (50 μg/mL) treated HUVECs was 2.28 times as large as that of MOPC control IgG (100 μg/mL) treated HUVECs.

In a separate experiment using HUVECs from a single donor, the degree of apoptosis induced by SN6j was 31.5% at 50 μg/mL and 43.7% at 100 μg/mL concentrations, relative to the value induced by camptothecin. Apoptosis of SN6j (100 μg/mL) treated HUVECs was 2.53 times as large as that of MOPC control IgG (100 μg/mL) treated HUVECs, while apoptosis of SN6j (50 μg/mL) treated HUVECs was 2.10 times as large as that of MOPC control IgG (100 μg/mL) treated HUVECs. Overall, SN6j induced significant levels of apoptosis (although lower than those induced by camptothecin) on HUVECs at concentrations of ≥ 50 μg/mL.

In Vitro Pharmacology of TRC105

The constant regions of SN6j were humanized to yield TRC105 for full-scale development and evaluation in clinical studies. TRC105 (c-SN6j) is a chimeric IgG1 kappa antibody made by grafting the constant regions of human IgG1 heavy chain and kappa light chain onto the murine variable regions of monoclonal antibody SN6j that binds human CD105. Using Scatchard plot analyses performed by incubating fixed combinations of radiolabeled and native antibody with KM-3 cells, the avidity of TRC105 (c-SN6j) was calculated by regression analysis to be 2.98 x 10^{9} liter/mole (or 3.36 x 10^{-10} mole/liter).

TRC105 Avidity to Human CD105

A study of binding of TRC105 with human CD105 using a surface plasmon resonance assay indicated that avidity and affinity of TRC105 for human CD105 were approximately 5 x 10^{-12} and 35 x 10^{-12} mole/liter, respectively.

TRC105 Binding Potency to Human CD105

An ELISA assay was performed using plates coated with human CD105. TRC105 was found to half-saturate human CD105 at concentrations of approximately 34-50 ng/mL and to fully saturate human CD105 at concentrations of ≥ 250 ng/mL. TRC105 concentrations of ≥ 250 ng/mL are

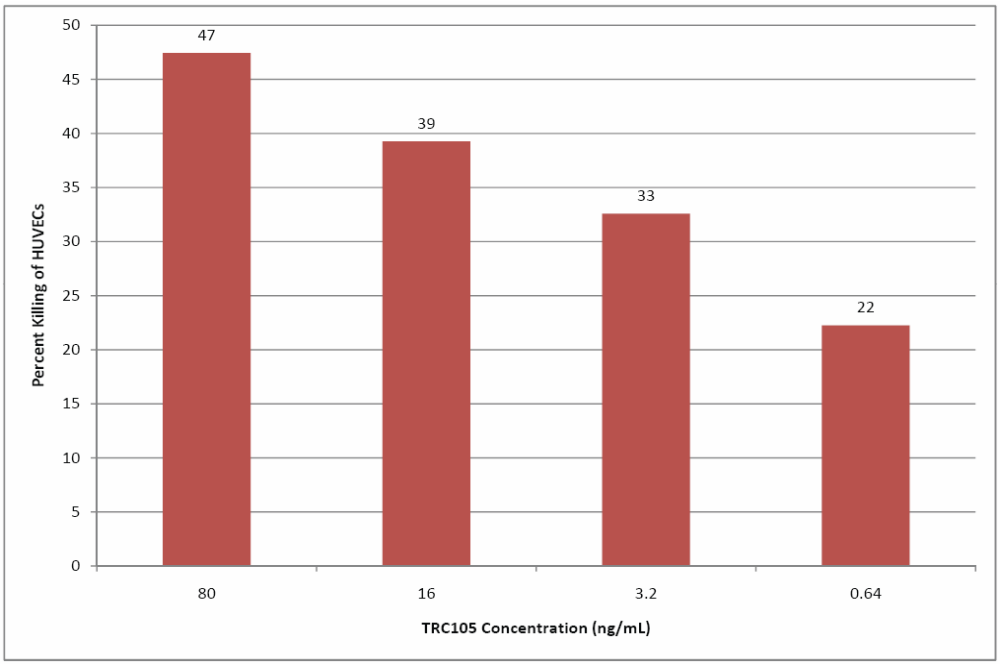
expected to saturate CD105 expressed on human endothelial cells and fully engage effector functions.

TRC105 Avidity to HUVECs In Vitro

A binding assay was performed to determine the avidity of TRC105 to cultured HUVECs. TRC105 was found to half saturate non-confluent (or proliferating) HUVECs at 2.3 ng/mL and to half saturate confluent (or non-proliferating) HUVECs at 22 ng/mL. Saturation was achieved at approximately 250 ng/mL in both cases.

TRC105 Induction of ADCC of Proliferating HUVECs In Vitro

TRC105 was investigated for its ability to mediate antibody-dependent cell-mediated cytotoxicity (ADCC) *in vitro*. In the presence of effector cells from three different donors, concentrations of ≥ 1 µg/mL of TRC105 lysed significant proportions of proliferating HUVECs, indicating that TRC105 exhibits ADCC activity *in vitro*. Lower concentrations of TRC105 were evaluated for ADCC using HUVEC cells and peripheral blood mononuclear cells that were activated with interleukin-2 (see graph below). TRC105 concentrations of < 1 ng/mL lysed significant proportions of proliferating HUVECs in the presence of interleukin-2 activated NK cells at an effector to target ratio of 10:1. TRC105 did not engage complement-mediated cytotoxicity (CDC) on proliferating HUVECs in vitro.



In vitro analysis has shown that anti-CD105 antibodies are internalized following binding to CD105. SN6j and isotype-matched control IgG were individually labeled with FITC and then incubated with SVEC4-10 murine endothelial cells for four hours at room temperature. SN6j reacted with viable SVEC4-10 murine endothelial cells and was internalized into the cells as shown by strong FITC-SN6j staining within the intracellular portion of cells. In contrast, only weak background staining was seen with the cells treated with FITC-labeled isotype-matched

control IgG. Internalization of bound TRC105 is expected to limit the ability of the antibody to engage effector functions at low CD105 surface densities, such as those on quiescent endothelium.

1.2.3.2 *In Vivo* Nonclinical Toxicology Studies of TRC105

TRC105 Toxicology

TRC105 was administered by intravenous infusion over 30 minutes weekly for 5 doses to cynomolgus monkeys, at doses of 3 mg/kg, 10 mg/kg and 30 mg/kg. Three monkeys of each gender were treated at the two lower doses and six animals of each gender were treated at the high dose (30 mg/kg). Animals tolerated TRC105 at all doses tested. There was no clinical, clinical pathologic or histopathologic evidence of toxicity. The no observed event level (NOEL) was determined to be 30 mg/kg given by 30 minute intravenous infusion weekly for five doses.

A GLP repeat-dose toxicology study was undertaken in cynomolgus monkeys to determine the onset, reversibility, persistence, or delayed occurrence of toxic effects after five weekly doses of TRC105, followed by a four-week recovery period. Single-dose pharmacokinetic characterization of TRC105 was performed after Day 1 and multiple-dose pharmacokinetic characterization of TRC105 was performed after Day 29. In addition, the immunogenicity of TRC105 was determined. Doses were administered by a 30 minute infusion on days 1, 8, 15, 22 and 29 into 36 adult monkeys. There were three males and three females per dose at 3 and 10 mg/kg/dose and six males and six females at control (vehicle) and 30 mg/kg/dose. All animals were dosed on days 1, 8, 15, 22 and 29 via a 30 minute intravenous infusion. Three animals per sex per group in Groups 1 through 4 were euthanized on Day 30, and three animals per sex per group in Groups 1 and 4 were euthanized on Day 57. Toxicity was assessed by monitoring mortality, clinical condition, body weight, food consumption, ophthalmic examination results, electrocardiography (including heart rate), blood pressure, body temperature, respiratory rate, clinical pathology (hematology, coagulation, serum chemistry, urinalysis and fecal occult blood). All received a comprehensive necropsy; selected organs were weighed and tissues were evaluated microscopically. Survival, clinical condition, body weight, food consumption, ophthalmology examinations, physiologic parameters (electrocardiograms, blood pressure, body temperature, heart rate and respiratory rate), hematology and coagulation, clinical chemistry, urinalysis, fecal occult blood evaluations, organ weights, and macroscopic and microscopic findings were unaffected by TRC105 administration. Accordingly, the no observed effect level (NOEL) for intravenous TRC105 under the conditions of this study was 30 mg/kg. Tissue binding assays were conducted on selected tissues from the control and high-dose groups. Animals dosed with TRC105 demonstrated binding to endothelial cells consistent with internalization of bound antibody. While this staining pattern is consistent with CD105 distribution, internalized antibody may also reflect binding to Fc receptors known to be expressed by endothelial cells. Roswell Park Cancer Institute sponsored a non-GLP study in which cynomolgus monkeys were dosed twice weekly for three weeks with 1.0 mg/kg, 3.0 mg/kg or 10.0 mg/kg of TRC105 (c- SN6j). In summary, TRC105 was well-tolerated by nonhuman primates when dosed up to 30 mg/kg weekly for five doses as a 30 minute intravenous infusion in a GLP study or up to 10 mg/kg twice weekly for six doses as an intravenous bolus. The highest dose tested, 30 mg/kg weekly for five doses, produced no indications of a drug-related effect in a GLP study and is the NOEL.

1.2.3.3 TRC105 Nonclinical Pharmacokinetics and Immunogenicity

Toxicokinetics and immunogenicity were studied following the administration of TRC105 by intravenous infusion over 30 minutes weekly for five doses to cynomolgus monkeys, at doses of 3 mg/kg, 10 mg/kg and 30 mg/kg. The mean central distribution volume was 0.029, 0.040 and 0.038 L/kg in animals given 3 mg/kg, 10 mg/kg and 30 mg/kg of TRC105, respectively, by intravenous infusion over 30 minutes. The mean steady-state volume of distribution was 0.075, 0.087 and 0.076 L/kg in animals given 3 mg/kg, 10 mg/kg and 30 mg/kg of TRC105, respectively. As expected, the central volume of distribution for TRC105 was similar to the serum volume in monkeys. Both the central and steady-state distribution volumes were consistent with distribution volumes of other chimeric antibodies (e.g., rituximab and cetuximab). The mean terminal half-life of TRC105 was 133, 149 and 153 hours among the anti-TRC105 antibody negative animals in the 3, 10, and 30 mg/kg dose groups, respectively. Steady-state was attained by 5 weeks in each dose group. The mean serum TRC105 Cmax was 83, 135 and 104 µg/mL in the 3 mg/kg dose group on Study Days 1, 22 and 29, respectively. In the 10 mg/kg dose group the mean Cmax values were 212, 346 and 306 µg/mL, and were 834, 1113 and 957 µg/mL in the 30 mg/kg dose group on Study Days 1, 22, and 29, respectively. A single monkey dosed with 30 mg/kg of TRC105 tested positive for the formation of MACA (monkey anti-chimeric antibodies; i.e., antibodies reactive with the human portion of TRC105) on Study Days 36, 43, and 50, but not Study Day 57. All animals that tested negative for MAMA (monkey anti-murine antibodies; i.e., antibodies reactive with the murine portion of TRC105) before dose administration on Study Day 1 also tested negative when subsequent samples were obtained throughout the remainder of the study. Several other animals tested positive for MAMA prior to the first dose administration on Study Day 1 and also tested positive (at lower titers) at frequent times throughout the 57 day study period.

1.2.3.4 TRC105 Nonclinical Efficacy

TRC105 was studied *in vivo* for its ability to inhibit angiogenesis using a murine model of choroidal neovascularization (CNV). C57B/L6 mice were given an intravitreal injection of TRC105 in one eye and PBS in the contralateral eye. TRC105 demonstrated dose-dependent inhibition of CNV in C57B/L6 mice. The highest dose administered (5 µg in1 µL) inhibited CNV by over 50% versus control.

1.2.3.5 TRC105 Clinical Pharmacology, Toxicity and Efficacy

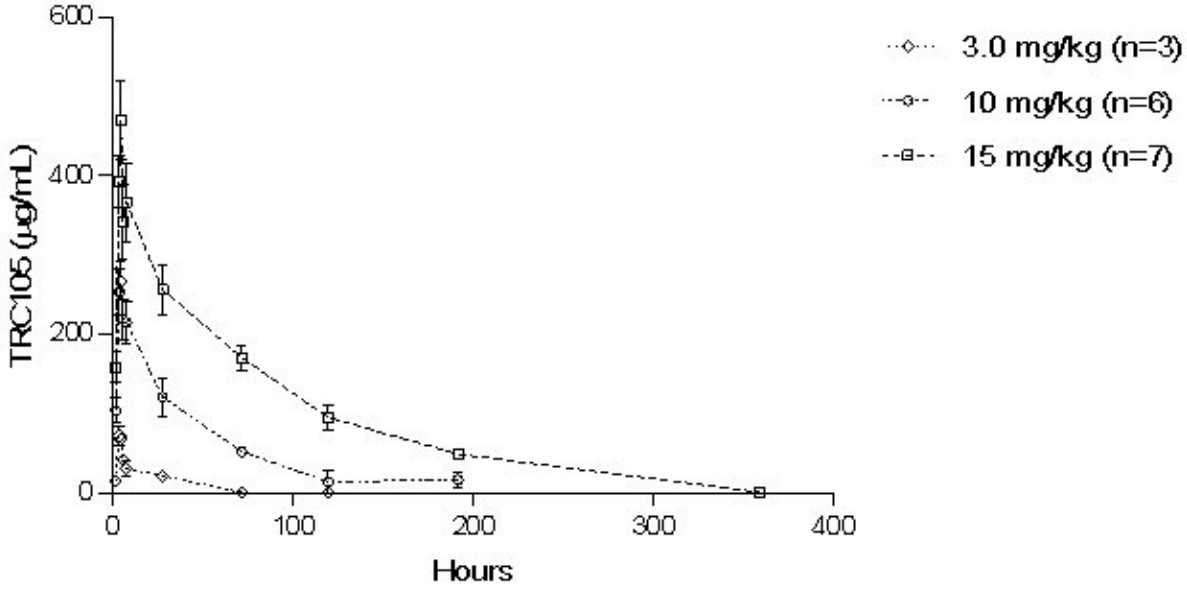
Clinical Studies of TRC105

Several studies with TRC105 are underway or have been completed. An open-label, phase 1, multicenter study of TRC105 (Study 105ST101) enrolled fifty patients, who were treated until disease progression with TRC105 at 0.01-15 mg/kg/q2wk or 10-15 mg/kg/wk. Studies of TRC105 in prostate, bladder, and ovarian cancer and a phase 1b study of TRC105 in combination with bevacizumab have also been completed. Ongoing studies besides this phase 1b study of TRC105 in combination with sorafenib in liver cancer include: a phase 1b/2 study of TRC105 in combination with axitinib in renal cell carcinoma, a phase 1b/2 study of TRC105 in combination with pazopanib, a phase 1b study of TRC105 in combination with bevacizumab in glioblastoma multiforme, and a single-patient study of TRC105 in combination with bevacizumab in choriocarcinoma.

TRC105 Clinical Pharmacokinetics

In Study 105ST101, TRC105 pharmacokinetics were assessed on patients enrolled at doses up to 15 mg/kg weekly. Circulating TRC105 was not measurable above the lower limit of quantitation of the assay (78 ng/mL) in patients receiving doses below 0.3 mg/kg. TRC105 was measurable above the target concentration based on preclinical data (200 ng/mL) for 4 hours at 0.3 mg/kg, 1 day at 1 mg/kg, 5 days at 3 mg/kg, 7 days at 10 mg/kg TRC105 dosed every two weeks. Serum concentrations expected to saturate CD105 binding sites (≥ 200 ng/mL) were achieved continuously at 15 mg/kg q2wk and 10 mg/kg weekly, and TRC105 accumulated at 15 mg/kg weekly (**Figure 1**).

Figure 1: Single-Dose and Multiple-Dose Pharmacokinetic Data from Study 105ST101



TRC105 Clinical Immunogenicity

In Study 105ST101, serum samples for evaluation of TRC105 immunogenicity, including HAMA and HACA, were collected pre-dose on day 1 of each 28 day cycle, at the end of study, and then at 4 and 12 weeks after the end of study visit.

HAMA and HACA data are available from the phase 1 monotherapy TRC105 trial. Neither HAMA nor HACA were detected in patients treated with CHO-produced TRC105, which will be used for all future clinical trials, including this study.*TRC105 Clinical Safety*

A total of 50 patients were treated on a phase 1 Study 105ST101 with escalating doses of TRC105 at 0.01, 0.03, 0.1, 0.3, 1, 3, 10 and 15 mg/kg every two weeks and then 10 and 15 mg/kg weekly. Dose escalation proceeded stepwise until the top dose was reached. The maximum tolerated dose was exceeded at 15 mg/kg weekly and the recommended phase 2 dose of TRC105 was therefore determined to be 10 mg/kg weekly. Three of 4 patients at 15 mg/kg weekly developed grade 3 hypoproliferative anemia (without leucopenia or thrombocytopenia) in

cycle 2, and one of the three progressed to grade 4 in cycle 3. Anemia was associated with accumulation of TRC105 and characterized by a low reticulocyte production index. Additional laboratory and clinical evaluations excluded common causes of anemia including blood loss, hemolysis, plasma volume expansion, inadequate erythropoietin, iron deficiency, and vitamin B-12 or folate deficiency. The anemia is believed to result from TRC105-mediated suppression of proerythroblasts, the only cells in the bone marrow known to express substantial levels of CD105. Anemia was reversible and manageable with dose reduction and standard supportive measures including erythropoietin and blood transfusion.

Infusion reactions, anemia, fatigue, epistaxis and headache were the most frequently observed adverse events considered related to TRC105. The majority of treatment-related adverse events were grade 1 or 2.

Infusion reactions, among the most common adverse events, were usually with the initial TRC105 dose and included one or more of the following signs or symptoms: rigors, bronchospasm, urticaria, hypertension, hypotension, tachycardia or bradycardia. Infusion reactions were initially reported at 1 mg/kg every 2 weeks for patients receiving TRC105 produced in NS0 cells without premedication. TRC105 produced in CHO cells was known to more potently engage ADCC *in vitro* than TRC105 produced in NS0 cells. Because of this, the initial dose level for patients receiving CHO-produced TRC105 was de-escalated to 0.3 mg/kg. Despite dose de-escalation, the first two patients at 0.3 mg/kg treated with CHO-produced TRC105 experienced grade 2 and grade 3 infusion reactions with the first dose in the absence of premedication. The protocol was therefore amended to require a glucocorticoids -based premedication regimen and extend the initial infusion duration from 1 to 4 hours.

The amendment mandating premedication and extended initial infusion duration successfully reduced the frequency and severity of infusion reactions and allowed dose escalation to continue. One additional patient who received CHO-produced TRC105 at 1 mg/kg developed a grade 3 infusion reaction with the third dose given over 2 hours. This patient had experienced a grade 2 infusion reaction when the dose was administered over 4 hours. In all three patients with grade 3 infusion reactions, TRC105 was not detectable in serum at the time of dosing, which allowed *de novo* binding of TRC105 to CD105 expressing endothelium within the vasculature. Grade 3 infusion reactions were not observed in patients dosed at 10 or 15 mg/kg who maintained TRC105 serum levels known to saturate CD105 binding sites for the full dosing interval. At dose levels where continuous TRC105 serum levels were achieved, glucocorticoids were safely discontinued and the infusion duration reduced to 1 hour.

Three patients developed grade 1 cutaneous telangiectasia on the trunk early in the course of therapy, all at dose levels of 10 or 15 mg/kg weekly that resulted in continuous serum levels of TRC105 known to saturate CD105 sites on human endothelium. Grade 1 or 2 hemorrhage was reported, including intermittent postcoital vaginal bleeding (that also occurred prior to TRC105 treatment), epistaxis, and superficial gingival bleeding.

Grade 1 or 2 headaches were observed, mainly in patients treated at doses of TRC105 above 3 mg/kg. Headaches began the day following infusion and were generally manageable with acetaminophen. However, grade 2 headache in one patient at 15 mg/kg weekly prompted discontinuation prior to completion of the dose-limiting toxicity evaluation period. Fatigue was

one of the more common adverse events attributable to TRC105 and was more prevalent at doses above 3 mg/kg.

One patient developed dose-limiting toxicity of grade 4 hemorrhage presenting as melena from a gastric ulcer within 5 days of the initial TRC105 infusion at 0.1 mg/kg. He discontinued TRC105 treatment, was transfused 2 units of packed red blood cells and the bleeding resolved with nonsurgical management by the time of upper endoscopy. Serious bleeding was not observed following protocol amendment to exclude patients with a history of peptic ulcer disease (unless healing was documented) and patients on ulcerogenic medications including non-steroidal anti-inflammatory drugs.

Classic toxicities associated with VEGF inhibition, including hypertension, proteinuria and thrombosis were not prominent. One patient with recurrent anal cancer treated at 0.1 mg/kg developed proteinuria considered possibly related to TRC105, but proteinuria was also noted prior to TRC105 dosing. Transient hypertension (156/112) without QT changes occurred in a single patient one day following infusion of 15 mg/kg, and was controlled by a single dose of oral antihypertensive medication. There were no arterial or venous thromboembolic events, nor gastrointestinal or other perforations in these patients.*TRC105 Clinical Efficacy*

In study 105ST101 stable disease ≥ 2 months was observed in 21 of 45 patients (47%) and stable disease ≥ 4 months in 6 of 44 patients (14%). Decreases in CEA, PSA, or CA-125 were noted in 7 of 21 patients (33%) and a global decrease in key angiogenic biomarkers was observed with treatment. One patient with castrate-refractory prostate cancer remains on TRC105 treatment after 6 years at a TRC105 dose of 0.01 mg/kg every 2 weeks. He has an ongoing complete PSA response, with resolution of bone pain and bone scan normalization. One patient with metastatic carcinosarcoma, manifested decreased tumor burden on computerized tomographic scanning and maintained stable disease for 20 months on therapy. The latter is especially notable when one considers that this patient had received three prior treatments -- carboplatin + paclitaxel for 4 months, anastrozole for 8 months, and ifosfamide for 2 months -- and had manifested tumor progression on each. In effect, TRC105 provided the most favorable clinical outcome and did so as a fourth-line therapy.

1.2.3.6 TRC105 Physical, Chemical, and Pharmaceutical Properties and Formulations

Physical, Chemical and Pharmaceutical Properties

TRC105 is a chimeric anti-CD105 IgG1 antibody consisting of human Cκ and Cγ1 constant regions with murine Vκ and VH regions. TRC105 is composed of two light chains of 213 amino acids and two heavy chains of 448 amino acids and has an approximate molecular weight of 148 kDa.

Formulation

TRC105 is a sterile, clear colorless to slightly yellow opalescent solution for intravenous infusion. The solution may contain small amounts of visible particulates. TRC105 will be filtered through a 0.2 μm low protein binding filter at the clinical site prior to administration.

Each single-use vial contains a 7 mg/ml or 25 mg/ml solution may be provided in one or more of the following presentations:

(a) <u>Phosphate Buffered Saline Formulation (7 mg TRC105/mL)</u>

210 mg TRC105/30 mL single-use vial

or

(b) 20 mM L-Histidine/L-Histidine Monohydrochloride, 240 mM Trehalose, 0.01% Polysorbate 20 Formulation (25 mg TRC105/mL)

100 mg TRC105/4 mL single-use vial

400 mg TRC105/16 mL single-use vial

TRC105 is formulated as a preservative-free solution containing sodium chloride, monobasic sodium phosphate monohydrate, anhydrous dibasic sodium phosphate, and Sterile Water for Injection. Hydrochloric acid and/or sodium hydroxide may be added to adjust the pH. TRC105 is formulated to be isotonic with a pH of 6.2 – 8.2.

Storage Conditions

TRC105 should be stored refrigerated 2 °C to 8 °C (36 °F to 46 °F). Preparations of TRC105 in infusion containers are stable for up to 8 hours at room temperature.

Manufacturing Process

The TRC105 production cell line for initial phase I clinical testing was a NS0 cell line. The manufacturing process used bioreactors with media containing bovine sera from the US or New Zealand. A high producing CHO cell line was subsequently developed for phase I and later stage clinical development using media that is free of animal-derived components. The manufacturing process uses conventional purification and filtration steps and concludes with the formulation of TRC105 in phosphate buffered saline. The purity of TRC105 is greater than 95%. Only CHO derived material will be used in this study.

1.2.4 Sorafenib

Sorafenib (Nexavar®) is FDA-approved for the use of advanced HCC and renal carcinoma. A brief summary of the preclinical data including *in vitro* and *in vivo* activity, clinical pharmacology and toxicology is outlined below. A complete list of CAEPRs for sorafenib is also provided.

1.2.4.1 *In Vitro* Activity

The ability of sorafenib to inhibit a number of kinases was evaluated (Investigator's Brochure, 2003). The *in vitro* biochemical and cellular profile of sorafenib is summarized below:

Biochemical Assay	IC_{50} (µM)
c-raf[b]	0.002/0.006
b-raf wild-type	0.025

b-raf V599E mutant	0.038
VEGFR-2 (human)	0.090
VEGFR-2 (murine)	0.006
VEGFR-3 (murine)	0.010
PDGFR-β (murine)	0.028
Flt-3	0.058
c-KIT	0.068
FGFR-1	0.580
p38α	0.038
Cellular Mechanism[c]	**IC_{50} (μM)**
MDA-MB-231 MEK phosphorylation (Human Breast)	0.04
BxPC-3 MEK phosphorylation (Human Pancreatic)	1.00
LOX ERK phosphorylation (Human Melanoma)	0.80
b-raf ER MEK activation (Human Chimera, 3T3 cells)	2.30
VEGFR-2 phosphorylation (Human, 3T3 cells)	0.03
VEGFR-3 phosphorylation (Mouse, 293 cells)	0.10
PDGFR-β phosphorylation (Human, AoSMC)[d]	0.02
Cellular Proliferation	**IC_{50} (μM)**
MDA-MB-231 (10% FCS)[e]	2.60
MDA-MB-231 (0.1% FCS)	0.10
VEGF-HUVEC (2.0% FCS)[f]	3.00
PDGFR-β AoSMC[d] (0.1% BSA)[g]	0.23

a Recombinant enzyme assay
b Raf kinase activated with Lck (truncated/full length c-raf)
c Mechanistic cellular assays all performed in 0.1% BSA
d Human aortic smooth muscle cells
e Fetal calf serum
f Human umbilical vein endothelial cells
g Bovine serum albumin

In vitro kinase assays demonstrated that sorafenib is a potent inhibitor of wild type and mutant (V599E) B-Raf and c-Raf Kinase isoforms (Investigator's Brochure). In addition, sorafenib did not inhibit human EGFR or Her2 kinases at 10 μM. Nor were PKC-α, PKC-β, PKC-γ, and PKA (rat, rabbit and bovine sources) kinase activity inhibited *in vitro*. Sorafenib demonstrated an IC_{50} of 780 nM against p59 (bovine) Fyn kinase (Src family of protein tyrosine kinases). In non-kinase targets sorafenib had moderate potency against the adenosine A3, dopamine D1, and muscarinic M3 receptors with IC_{50} of 1.6 μM, 2.0 μM, and 3.1 μM, respectively. Sorafenib did not inhibit MEK-1, ERK-1, EGFR, HER2/neu, c-met, PKA, PKB, Cdk-1/cyclin B, pim-1, GSK

3-b, CK-2, PKC-α (r), PKC-β (r), PKC-γ at concentrations as high as 10 μM. In summary, sorafenib showed ≥100-fold more selectivity for raf kinase relative to other target proteins.

Sorafenib also inhibited *in vitro* several receptor tyrosine kinases (RTKs) that are involved in tumor progression, human VEGFR-2, murine VEGFR-2, murine VEGFR-3, murine PDGFR-β, Flt-3, c-KIT, and p38α (MAPK family). In cellular assays, sorafenib was found to be a potent inhibitor of human and murine VEGFR-2, murine VEGFR-3, and murine PDGFR-β receptor phosphorylation (Investigator's Brochure, 2003).

VEGF and PDGF receptors are involved in the mechanism of tumor angiogenesis[30, 31]. PDGF receptors may also play a role in patients with chronic myeloproliferative cancers[32]. Flt-3 is important in acute myelogenous leukemia [33] and c-Kit plays a critical role in gastrointestinal stromal tumors[34].

1.2.4.2 *In Vivo* Activity

Sorafenib has demonstrated *in vivo* anti-tumor efficacy as a single agent against a broad range of human tumor xenografts as summarized in the following table. The models evaluated include HCT-116 and DLD-1 colon tumor xenografts, MX-1 mammary tumor xenograft, NCI-H460 and A549 NSCLC xenografts, MiaPaCa-2 pancreatic tumor xenografts, and SK-OV-3 ovarian tumor xenografts. In this table, compound efficacy is expressed as percent tumor growth inhibition (TGI) and is calculated as ((1-(T/C)) *100, where T and C represent the mean tumor size in the Treated and Control groups respectively at the first measurement after the end of treatment. In the NCI-H460 (K-ras mutant) and A549 NSCLC tumor xenografts model, BAY 43-9600, at doses of 10mg/k, 30mg/k and 60mg/kg, induced a tumor growth inhibition of 27, 60 and 68%, respectively.

Sorafenib Demonstrates Broad Spectrum Anti-Tumor Efficacy in Preclinical Xenograft Models

Tumor Type	Model	Dose (mg/kg/dose free base equiv.)[1]	Percent TGI ((1-(T/C))100)
Colon	HCT-116	10 30 100	45 64 68
Colon	DLD-1	15 30 60	31 66 75
NSCLC	NCI-H460	10 30	27 56
NSCLC	A549	30 60	60 68
Mammary	MX-1	30 60	51 67
Pancreatic	Mia-PaCa-2	10 30	45 66

		100	73
Ovarian	SK-OV-3	10 30 100	58 64 81

The majority of the initial anti-tumor efficacy evaluations *in vivo* were conducted in the HCT116 colon tumor model since the tumorigenicity of this cell line was previously shown to be dependent on K-ras activation. Additional studies indicated that prolonged anti-tumor efficacy could be attained by extending the duration of treatment and that, in this tumor model, sorafenib was able to arrest tumor growth even if therapy was initiated against a substantially greater tumor burden.

Sorafenib also showed significant oral activity against two additional human tumor xenograft models that contain K-ras mutations: MiaPaCa-2 pancreatic carcinoma and H460 non-small cell lung carcinoma. The anti-tumor efficacy of sorafenib was also evaluated against the human SKOV-3 ovarian tumor cell line that contains a wild-type Ras but over-expresses both the EGF and Her2 growth factor receptors. These receptors also signal through the Ras/Raf/Mek pathway.

In human tumor xenografts, MDA-MB-231 (breast) and Colo-205 (colon), there was a dramatic reduction of tumor neo-vascularization (Investigator's Brochure). Recent data also indicated that inhibition of c-raf may promote cell death in endothelial cells as a downstream event of VEGFR-2 stimulation[35].

Taken together, data suggests that sorafenib may be of therapeutic value not only in human tumors containing ras gene mutations, but also in tumors over-expressing growth factor receptors in the Ras/Raf/Mek pathway, and by inhibiting tumor angiogenesis or neo-vascularization through inhibition of VEGFR-2, VEGFR-3, and/or PDGFR-β.

The ability of sorafenib (or its tosylate salt, BAY 54-9085) to be combined with paclitaxel, irinotecan, gemcitabine, or cisplatin was evaluated in preclinical *in vivo* models. In these studies, the focus was to evaluate if the co-administration of sorafenib would adversely affect the tolerance or anti-tumor efficacy of the 'standard of care' agent. The general health of mice was monitored and mortality was recorded daily. Tumor dimensions and body weights were recorded twice a week starting with the first day of treatment. Treatments producing greater than 20% lethality and/or 20% net body weight loss were considered 'toxic'. The results of these combinability analyses are summarized below:

Combinability of Concurrent Treatment with Sorafenib and Clinically Established Agents

Combination Agent	Tumor Model	**Combinability Y/N**
Paclitaxel	NCI-H460 NSCLC MX-1 Mammary	Yes Yes
Irinotecan	DLD-1 Colon	Yes

Gemcitabine	MiaPaCa-2 Pancreatic	Yes
Cisplatin	NCI-H23 NSCLC	Yes

Sorafenib can be safely combined with a variety of standard cytotoxic cancer chemotherapy agents, including paclitaxel, irinotecan, gemcitabine and cisplatin with no significant increase in the toxicity associated with those agents and without diminishing their anti-tumor efficacy in preclinical models.

1.2.4.3 Preclinical Pharmacology

Sorafenib's absorption in mice and dogs is 92% and 69%, respectively. The bioavailability in mice and rats was 79% and in dogs was 61%. Plasma clearance in mice, rats and dogs was low (CL<0.21 l/h/kg) and the Vss was also low (~0.7 – 0.9 l/kg). Excretion of the compound is primarily via the biliary/fecal route in rats and dogs. Protein binding was high; the mean free fraction was 2.5% in mice, 1.6% in dogs, 1.4% in rats, and 1.2% in humans. Sorafenib showed a market potency to inhibit several CYP isoforms involved in drug metabolism and clinically relevant drug-drug interactions with substrates of different isoforms appear to be possible. *In vitro* model of enzyme induction using human hepatocyte showed no significant induction of CYP 1A, 2C9, 2C19 and 3A enzymes, but CYP 3A4 is the enzyme responsible for the metabolism of sorafenib *in vitro* in man.

1.2.4.4 Clinical Pharmacology

Evaluation of data across four different studies has indicated that in consistence with its half-life, plasma sorafenib accumulates upon multiple dosing. There is no further increase in sorafenib Cmax and/or AUC values beyond 7 days of multiple dosing. There is a less than proportional increase in sorafenib Cmax and AUC values with increasing doses from 100 mg bid to 800 mg bid. Data from four ascending dose studies show that sorafenib exhibits high inter-patient pharmacokinetic variability that is not explained by age, race, gender, or body weight. Sorafenib generally accounts for approximately 64-86% of the circulating analytes after at least 7 days of multiple dosing. Approximately 19% of the dose is excreted in urine and 76% in feces. BAY 68-3472 is the major metabolite in plasma. The relationship between dose and anti-tumor activity cannot be accurately estimated based on the available data. The dose of 400 mg bid exhibits a similar safety profile in hepatoma patients with either Child's Pugh A or Child's Pugh B hepatic function status even though preliminary data shows numerical differences in PK.

1.2.4.5 Preclinical Toxicology

Single-dose oral toxicity/tolerance studies were conducted in the rat, mouse and dog. Repeated-dose toxicity was covered by studies with daily oral treatment of up to 6 months in rats, 3 months in mice, and up to 12 months in dogs. Subacute studies in rats and dogs included a 4-week reversibility segment for the high dose level. The preclinical toxicity profile of sorafenib short-term high-dose treatment was well-tolerated clinically by rats, mice and dogs. Exposure-dependent mortality (mean time to death ≥ 3 weeks) occurred without preceding specific signs of morbidity in rats. Dose-limiting toxicity in dogs was gastrointestinal (emesis, bloody diarrhea) at 30 mg/kg bid or 60 mg/kg bid. Aside from effects on the GI tract (bloody diarrhea) and marked effects on the skin (hair loss, inflammation) once daily dosing of up to 60 mg/kg for 3 months was clinically well tolerated by dogs. In rats, repeated-dose toxicity studies were all

conducted with once daily oral administration. Mortality was induced at a dose of 5 mg/kg/d sorafenib in a 3-month study. In a chronic 6-month study, no mortality was induced at the highest dose tested of 2.5 mg/kg sorafenib; this dose is considered the MTD. Histopathology revealed degeneration/regeneration processes in multiple organ systems including liver, kidneys, lymphoreticular/hematopoietic system, GI tract, pancreas, adrenals, reproductive organs, skin, teeth, and bone. No main target organ of toxicity could be identified. Some morphological lesions were not reversible within 4 weeks (e.g., bile duct proliferation, liver fibrosis, adrenal necrosis, effects on lymphoreticular system) following a 4-week course of treatment. The morphological no-effect-level after 3 months of treatment was below 10mg/kg/d and 1 mg/kg/d for dogs and rats, respectively. Some changes, notably in the liver (bile duct proliferation) and skin (degeneration of hair follicles), were observed at this dose level. Changes in hematology and clinical chemistry were poor predictive indicators for specific organ lesions. Serum levels of hepatic transaminases were markedly, but not consistently, elevated. At the repeated MTD in rats (2.5 mg/kg/d) and dogs (30 mg/kg/d), AUC 0-24 was about 35 mg/h/l at steady stat, indicating no substantial difference in toxicity in the two species when compared at systemic exposure. Based on genotoxicity assays, SORAFENIB does not demonstrate a significant risk of genotoxicity to patients. Results from the general (repeated dose) toxicity studies indicate that BAY43-9006 has the potential to adversely impair reproduction performance, as well as embryonic and fetal development. Various effects, including retardation, were observed in male and female reproductive organs of rats.

1.2.4.6 Clinical Toxicology

Preliminary anti-tumor activity has been reported in a variety of tumor types with tumor shrinkage seen in colorectal carcinoma, melanoma, thyroid, sarcoma, pancreatic cancer, and renal cell cancer. Sorafenib is well tolerated at 400 mg bid. Dose limiting toxicities occurring more frequently at higher doses include hand-foot syndrome, diarrhea, fatigue, hypertension, pain, and rash. ≥Grade 3 and 4 drug-related adverse events were uncommon. Significant events reported included grade 3 or 4 amylase and lipase elevation (not associated with symptoms of pancreatitis), and hand foot syndrome. In addition, mild to moderate drug-related adverse events reported included flu-like symptoms, fever arthralgia, hypertension, weight loss, anorexia, abdominal pain, vomiting, elevated liver function tests, pruritus and alopecia.

1.2.5 Rationale for combining TRC105 with Sorafenib

By targeting a unique and important angiogenic pathway, TRC105 has the potential to complement existing antiangiogenic therapies. One of the reasons postulated for the limited efficacy of anti-angiogenic agents is that they cause intra-tumoral hypoxia. Intra-tumoral hypoxia causes the induction and up-regulation of hypoxia-inducible factors (HIF) such as HIF-1α which can result in evasive mechanisms of resistance[18]. CD105, the target for TRC105, is expressed at significantly higher levels following VEGF inhibition in animal models of human cancer. The increase in CD105 expression is likely an attempt to compensate for or escape from VEGF inhibition. This escape mechanism could be inhibited with a combination approach targeting both pathways simultaneously.

Our basic concept is based on two hypotheses: 1) angiogenesis plays a particularly crucial role in the pathogenesis of HCC and 2) interrupting the angiogenic pathway at different levels in combination will be more effective than targeting one level.

1.2.6 Summary of Phase I

As of July 2014, 15 patients were enrolled onto the phase 1 portion of this study which combined TRC105 – a monoclonal antibody that binds CD105, a transmembrane receptor overexpressed by proliferating endothelial cells – with standard of care sorafenib. The dose of sorafenib was fixed across all dose levels of the study and was as per standard of care (400mg BID). TRC-105 was administered every two weeks at doses of 3, 6, 10 and 15mg/kg. All patients had HCC with either non-cirrhotic livers or cirrhosis (the majority) with compensated liver dysfunction (Childs Pugh A/B7) and an ECOG of 0/1. A recommended phase II dose was established of TRC-105 15mg/kg in combination with sorafenib 400mg bid. Overall the treatment was well tolerated and the study proceeded as per protocol to testing of the maximum planned dose level, DL4 (15mg/kg). One DLT was encountered at 10mg/kg – transaminase elevation – which necessitated expansion of that cohort. One grade 5 cardiac event was also experienced. The subject had severe three vessel disease, which is the most likely cause of his death. However, the contribution of protocol treatment could not be excluded. The most common side-effects we encountered were hand-foot skin reactions as a result of sorafenib. With regard to efficacy two patients - treated at 10 and 15mg/kg dose level - developed a PR by RECIST and the majority of patients had tumor reduction. Median time on study was 4 months and one patient remained on treatment after 22 months.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have histopathological confirmation of hepatocellular carcinoma (HCC) by the Laboratory of Pathology of the NCI prior to entering this study.

Or

2.1.1.2 histopathological confirmation of carcinoma in the setting of clinical and radiological characteristics which, together with the pathology, are highly suggestive of a diagnosis of HCC.

2.1.1.3 Patients must have disease that is not amenable to potentially curative resection or ablative techniques. In addition, disease must not be amenable to or have progressed on transhepatic arterial chemoembolization (TACE). Patients must not be considered potential candidates for liver transplantation. This determination will be made after hepatobiliary surgical input at the NCI multidisciplinary conference.

2.1.1.4 If liver cirrhosis is present, patient must have a Child-Pugh A or B (7 points) classification (See Section 11.2).

2.1.1.5 Patients with cirrhosis must have had esophagogastric endoscopy within the previous 6 months prior to study entry for the assessment of varices. If the patient has not had this done they must be willing to undergo this procedure prior to study entry.

2.1.1.6 Age $\geq$18 years

2.1.1.7 Life expectancy of greater than 3 months.

2.1.1.8 ECOG performance status 0-2 (see Section 11.1)

2.1.1.9 Patients must have normal organ and marrow function as defined below:

2.1.1.9.1 Absolute neutrophil count $\geq$1,500/mcL

2.1.1.9.2 Platelets $\geq$60,000/mcL without transfusion support within the past 30 days

2.1.1.9.3 Total bilirubin $\leq$ 3 mg/dl.

2.1.1.9.4 AST/ALT $\leq$ 10 × upper limit of normal

2.1.1.9.5 Creatinine $\leq$ 1.5 x upper normal limits OR creatinine clearance $\geq$ 40 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal, as calculated by the Cockcroft Gault formula.

2.1.1.10 Patients must have recovered from any acute toxicity related to prior therapy, including surgery. Toxicity should be $\leq$ grade 1 or returned to baseline.

2.1.1.11 Patients must not have other invasive malignancies within the past 5 years (with the exception of non-melanoma skin cancers or non-invasive bladder cancer).

2.1.1.12 Patient must be able to understand and willing to sign a written informed consent document.

2.1.2 Additional Inclusion Criteria for **PHASE I Portion**

2.1.2.1 Patients may have measurable or evaluable disease only.

2.1.2.2 Prior therapy: prior systemic therapy with sorafenib is allowed.

2.1.3 Additional Inclusion Criteria for **PHASE II Portion**

2.1.3.1 All patients will be required to have measurable disease.

2.1.3.2 Prior therapy: prior systemic therapy with sorafenib is allowed.

2.1.4 Exclusion Criteria

2.1.4.1 Patients who have had chemotherapy (other than sorafenib treatment), large field radiotherapy, or major surgery must wait 4 weeks prior to entering the study.

2.1.4.2 Patients may not be receiving any agents not approved by the FDA within the past 4 weeks.

2.1.4.3 Patients with known brain metastases will be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.

2.1.4.4 Proteinuria, as demonstrated by a 24-hour protein of ≥ 2000 mg. Urine protein will be screened by urine protein-creatinine ratio (UPC). For UPC ratio > 1.0, a 24-hour urine protein will need to be obtained and the level should be < 2000 mg for patient enrollment.

2.1.4.5 Uncontrolled intercurrent illness including, but not limited to, hypertension (systolic BP > 140, diastolic BP > 90), ongoing or active systemic infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia or psychiatric illness/social situations that would limit compliance with study requirements.

2.1.4.6 No anti-coagulation therapy is allowed with the exception of low-dose aspirin.

2.1.4.7 No bleeding diathesis.

2.1.4.8 Patients with a history of bleeding varices in previous 1 year are excluded (unless patient has subsequently had a liver transplant). Those with gastric varices or varices that are deemed as high risk by the endoscopist should be placed on appropriate medical therapy as advised by the gastroenterologist.

2.1.4.9 History of peptic ulcer disease or hemorrhagic gastritis within 6 months of TRC105 administration, unless patient has received adequate treatment for peptic ulcer disease or hemorrhagic gastritis and has evidence of complete resolution documented by EGD. Mild gastritis is allowed.

2.1.4.10 QTc > 500 msec

2.1.4.11 HIV-positive patients receiving anti-retroviral therapy are excluded from this study due to the possibility of pharmacokinetic interactions between antiretroviral medications and sorafenib or TRC105. HIV positive patients not receiving antiretroviral therapy are excluded due to the possibility that sorafenib or TRC105 may worsen their condition and the likelihood that the underlying condition may obscure the attribution of adverse events with respect to sorafenib or TRC105.

2.1.4.12 History of hypersensitivity reaction to human or mouse antibody products

2.1.4.13 Patients with a history of familial bleeding disorders

2.1.4.14 Patients with a history of hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu Syndrome).

2.1.4.15 Pregnancy and breast feeding are exclusion factors. Enrolled patients must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, the duration of study participation and 3 months after the end of the treatment.

2.1.4.16 Patients with unhealed wounds for more than 30 days.

2.1.5 Inclusion of Women and Minorities

2.1.5.1 Men and women of all races and ethnic groups are eligible for this trial.

2.2 SCREENING EVALUATION

This does not include the baseline correlative studies that will only be performed after the patient has signed the consent form.

2.2.1 Imaging studies (baseline – obtained within one month prior to enrollment):

- CT scan of chest, abdomen and pelvis
- DCE-MRI will be obtained within 7 days of cycle 1 day 1 (optional based on availability).
- Ultrasound evaluation of tumor volume

2.2.2 Laboratory evaluation (baseline – obtained within 7 days prior to dosing)

- Hematological profile: CBC with differential and platelet count, PT, aPTT, fibrinogen.
- Biochemical profile: electrolytes, BUN, creatinine, urine protein-creatinine ratio (UPC), AST, ALT, total bilirubin, calcium, phosphorus, albumin, magnesium, uric acid, albumin, amylase.
- Tumor marker profile: αFP (baseline – within 7 days prior to dosing)

2.2.3 Laboratory evaluation (baseline- obtained within 28 days prior to dosing)

- Hepatitis serology

2.2.4 History and physical exam with vital signs within 1 week prior to dosing.

2.2.5 Electrocardiogram (baseline – obtained within 28 days prior to dosing)

2.2.6 Requirements for endoscopic evaluation: All patients with cirrhosis will be required to have had a screening endoscopy for varices within 6 months of study enrollment. If this has not been done within the previous 6 months it will be performed by Dr. Heller and his team at NCI. Varices identified on endoscopy will be treated as per standard of care. All appropriate therapies for this are available here at NCI. A history of prior variceal bleed in previous 1 year will be an exclusion factor.

2.2.7 Detailed cardiac history to be taken including known risk factors and the presence of symptoms such as exercise-induced chest discomfort. Suspicion of elevated cardiac risk will mandate referral and assessment to cardiology, unless patient is already under the care and surveillance of a cardiologist.

2.3 REGISTRATION PROCEDURES

2.3.1 On-Study Registration

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office (HOIS) ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. Please note, it is very important for all registrars to acquire encrypted e-mail from NIH Help Desk, since the verification of registration includes patient's information. A recorder is available during non-working hours.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

For both the phase I and phase II portions of this study TRC105 will be administered as an IV infusion every two weeks. Sorafenib will be self-administered as a daily oral pill. Patients will be reviewed every week in clinic, with labs and routine history and physical performed. Patients will be re-staged every 8 weeks. Please see the Study Calendar for a detailed summary.

3.2 PHASE I (DOSE ESCALATION COMPLETE AS OF AMENDMENT L)

The first part of this study is a standard 3+3 dose escalation phase I study with the primary objective of establishing MTD for the combination. Sorafenib will be taken orally at a dose of 400 mg twice daily. TRC105 will be administered as an infusion every 2 weeks. Patients will be re-staged every 8 weeks. The TRC105 dose will be escalated as tolerated in cohorts of 3 to 6 patients (see table below). Intra-patient dose escalation is not allowed. However, if a patient has had a dose reduction of sorafenib for toxicity and tolerates this dose for 28 days, the sorafenib may be re-escalated by one dose-level only, as per standard clinical practice.

The planned dose-escalation schedule is as follows:

Cohort	Sorafenib (mg PO twice daily)	TRC105 (mg/kg IV every 2 weeks)
0	400	1
1	400	3

Cohort	Sorafenib (mg PO twice daily)	TRC105 (mg/kg IV every 2 weeks)
2	400	6
3	400	10
4	400	15

Each cohort in the phase I portion of the study is planned to have at least 3 patients to evaluate for toxicity. Three patients will be treated at a given dose level and observed for acute toxicity for one course of treatment before any more patients are entered. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting.

If none of the three patients at a given dose level experiences dose-limiting toxicity (DLT), accrual will proceed to the next cohort.

If one of three patients treated at a dose level experiences DLT that is considered to be at least possibly related to TRC105, then three more patients will be enrolled at that same level. If the incidence of DLT among those six patients is one in six, then the next cohort of three patients will be treated at the next higher dose.

In general, if two or more of the six patients treated at a dose level experience DLT, then the MTD is considered to have been exceeded. The MTD is defined as the highest dose studied for which the incidence of DLT was less than 33%.

For MTD determination, dose-limiting toxicities will be evaluated throughout the first 28 days of treatment (Cycle 1). Patients who exit the study for reasons other than drug-related toxicity prior to completion of the 28-day DLT evaluation period will be replaced to ensure an adequate safety assessment of each cohort. If at the first dose level more than one DLT is observed in the first three subjects, or more than two DLTs in six subjects are observed, 3-6 patients will be accrued to Dose Level - 1.

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next dose level
≥ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter at least 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next

	higher dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤1 out of 6 at highest dose level below the maximally administered dose	This is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

If ≥2 patients experience a DLT at any dose level, at any time in phase I, then subsequent patients will be enrolled at the next lower dose level.

3.2.1 Dose Limiting Toxicities

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

3.2.2 Definition of Dose-limiting Toxicities (DLTs):

3.2.2.1 Dose limiting toxicity

- Grade 3 electrolyte toxicities unable to be corrected to Grade 1 or less within 24 hours will be considered dose limiting (proteinuria >3.5 g/24 hour will be defined as a DLT).
- Drug-related Grade 4 hematological toxicity will be considered dose limiting.
- Toxicity requiring a dose reduction or a delay in treatment for >7 days will be considered dose limiting.
- Other Grade 3 or higher toxicity considered related to TRC105 will be considered dose-limiting.

3.2.2.2 Exclusions to Dose limiting toxicities

- Grade 3 hypertension that can be controlled with oral medications and does not require treatment delay for > 7 days or dose reduction will not be considered a DLT. Refer to Section 3.4.4.2 for guidelines on managing drug-induced hypertension.
- Grade 3 diarrhea will only be considered dose-limiting if it is refractory to treatment. Grade 4 diarrhea will be dose limiting.
- Nausea and vomiting Grade 3 will only be considered dose-limiting if it is refractory to anti-emetic therapy and unable to be corrected to Grade 1 or less within 48 hours.

- Grade 3 rise in creatinine, not corrected to Grade 1 or less after 2 liters of intravenous fluids within 24 hours, will be considered dose limiting. All Grade 4 rises in creatinine will be dose limiting.
- Any grade elevation in transaminases that is attributable as being likely related to sorafenib and/or disease alone and will be an exception to DLT.
- Grade 3 rash will not be considered dose limiting unless it does not return to ≤ Grade 2 after 1 week of symptomatic treatment.
- Grade 3 hand-foot syndrome will not constitute DLT.
- Grade 3 and 4 infusion reactions due to TRC105 administration will be considered to be dose-limiting toxicity.

Three patients must complete at least 1 cycle of therapy prior to considering dose escalation in the next cohort of patients. Determination of DLT for the purpose of dose escalation enrollment will be based on toxicities observed in the first 28 days of treatment (cycle 1) and must be considered as at least possibly related to study drug. Evaluation for toxicity will continue throughout the study and should dose limiting toxicities occur beyond the cycle one, consideration will be given to halting the dose escalation and studying lower dose levels.

3.3 PHASE II

The protocol will be reassessed by the IRB before proceeding to the phase II component of the study. Specifically, data from the phase I that supports treatment of larger cohorts in the phase II at a specific dose will be presented to the IRB along with a revised consent document that will summarize updated results and toxicities.

TRC105 and sorafenib will be administered at the 15 mg/kg TRC105 IV every 2 weeks and 400 mg sorafenib PO twice per day (the recommended phase 2 dose defined from the phase I portion). The sample size and interim stopping rule will be determined using a Simon optimal two-stage design.

The first stage will initially enroll 6 evaluable patients, and if 0 of the 6 have a clinical response, then no further patients will be accrued. If 1 or more of the first 6 patients has a clinical response, then accrual would continue until a total of 23 patients have been enrolled. As it may take several weeks to determine if a patient has experienced a response, a temporary pause in the accrual may be necessary to ensure that enrollment to the second stage is warranted. If there are 1 to 2 clinical responses in 23 patients, this would be an uninterestingly low response rate. If there were 3 or more complete responses in 23 patients (13.0%), this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (5% response rate), the probability of early termination is 73.5%.Drug Administration

3.3.1 TRC105 Drug Administration

All treatment will be administered on an outpatient basis.

Thirty minutes to two hours prior to the start of each TRC105 infusion, all patients will receive the following premedications:

- Acetaminophen 650 mg PO x 1

- Dexamethasone 20 mg IV x 1
- Famotidine 50 mg IV x 1 (or similar H2 blocker)
- Cetirizine 10 mg IV or PO x 1 (or similar oral or intravenous antihistamine)
- Granisetron 1mg IV x 1

After completing pre-medications, TRC105 will be administered intravenously utilizing an infusion pump. TRC105 has been demonstrated to be compatible with polyethylene lined, non-DEHP infusion sets and polyvinyl chloride, non-DEHP infusion sets. TRC105 is required to be administered with a 0.2 micron downstream filter. The attachment of the infusion pump administration set to the i.v. bag and transport of the TRC105 study drug to the patient will be performed as per standard study site procedures.

On Cycle 1 Day 1, TRC105 will be infused over a period of four hours. If a patient completes one 4 hour infusion without the development of an infusion reaction, then subsequent TRC105 infusions may be reduced to 2 hours. If a patient completes one 2 hour infusion without the development of an infusion reaction, subsequent TRC105 infusions may be reduced to a minimum of 1 hour (7 mg/mL TRC105 material) or 90 minutes (25 mg/mL TRC105 material).

After the minimum TRC105 infusion duration of 1 hour (7 mg/mL TRC105 material) or 90 minutes (25 mg/mL TRC105 material) has been safely administered, the dexamethasone should be gradually tapered as tolerated with each subsequent infusion and eventually discontinued if possible. The dexamethasone dose should not be reduced unless the prior infusion was well-tolerated (no infusion reaction of any grade).

Recommended Dexamethasone Taper Schedule

Infusion	Dexamethasone Dose and Schedule
First 1 hr infusion	20 mg iv 30 minutes to two hours prior to each infusion
Second 1 hr infusion	10 mg iv 30 minutes to two hours prior to each infusion [a]
Third 1 hr infusion	5 mg iv 30 minutes to two hours prior to each infusion [a]
Subsequent infusions	**No dexamethasone**[a]

[a]The dexamethasone dose should not be reduced unless the prior infusion was well-tolerated (no infusion reaction of any grade).

Patients with infusion–related events of any grade should be managed appropriately (Section 3.3.1.1) and are not permitted to reduce infusion duration or pre-medications on the subsequent infusion. Patients who experience a grade 3 or higher hypersensitivity reaction will be taken off treatment until toxicity resolution. Each infusion of TRC105 must be completed within 8 hours of reconstitution. Once the i.v. bag containing TRC105 is empty, the i.v. line should be flushed with 20 mL of normal saline at the same rate of infusion. The dose level and infusion start and stop times must be recorded in the source documents.

3.3.1.1 Management of TRC105 Infusion Reactions

If a patient experiences a grade 2, 3 or 4 adverse reaction during infusion, the infusion should be stopped and the patient treated accordingly. Antipyretic, antihistamine and other therapies should be administered as indicated. If appropriate, the infusion may be restarted at half of the

previous rate when the patient is able to continue. Infusion reactions will be recorded as AEs in the case report form. Interventions should be documented as concomitant medications or concomitant treatments as appropriate.

3.3.2 Sorafenib Administration

Sorafenib will generally be administered on an outpatient basis except when admission is required for any reason. No investigational or commercial agents or therapies other than those described in the protocol may be administered with the intent to treat the patient's malignancy.

Sorafenib is supplied as 200-mg tablets and is administered orally twice a day. Patients are to swallow the tablets whole with approximately 250 ml (8 oz.) of water, each morning and evening (i.e., 12-hourly). Tablets may be taken with or without food. Sorafenib will be given as self-administered oral doses at 400 mg bid continuously in a 28 days cycle

3.3.2.1 Special Precautions:

Sorafenib tosylate is metabolized by the P450 CYP3A enzyme and has been shown in preclinical studies to inhibit multiple CYP isoforms. The following medications will be excluded prior to and during the study if is indicated: ketoconazole, itraconazole, ritonavir, products containing grapefruit juice, cyclosporine, carbamazepine, phenytoin, phenobarbital and prophylactic use of G-CSF, GM-CSF.

Sorafenib has the ability to inhibit a variety of liver metabolic enzymes *in vitro*. The clinical impact of this inhibition in humans taking drugs metabolized by these enzymes is unknown. Therefore, all patients enrolled onto this trial who are taking concomitant medications that are known to be metabolized by the liver should be closely observed for side effects of these concomitant medications. Furthermore, patients taking narrow therapeutic index medications, (e.g. warfarin, quinidine, or digoxin) should be monitored proactively.

3.4 DOSING DELAYS AND DOSE MODIFICATIONS

Grade	**Occurrence**	**Immediate action**	**Resumption of therapy**
1	Any	None	No interruption
2	Any	Hold therapy until ≤ G1	No change in dose but initiate appropriate medical therapy.
3	1st	Hold therapy until ≤ G1	Initiate appropriate medical therapy and reduce either sorafenib or TRC105 to next lowest dose level per tables below in

Grade	Occurrence	Immediate action	Resumption of therapy
			sections 3.4.1 and 3.4.2 respectively.
4	1st	Stop therapy	Terminate therapy with experimental agents. Follow patient until resolution/stabilization of toxicity.

#In the event that it is unclear which medication is responsible for the observed toxicity, the investigator should proceed to decrease doses of both study medications to the next lowest level.

For all grade ≥2 non-hematologic toxicity and all grade ≥3 hematologic toxicities (excluding lymphopenia), sorafenib and TRC105 will be held until toxicities resolve to ≤ grade 1. If treatment is delayed for > 7 days due to adverse events, treatment will be discontinued. It will be in the clinical judgment of the investigator – based on an assessment of the adverse event and knowledge of both compounds – which of the agents to dose-reduce, unless specifically stated below for a particular toxicity (e.g., hand-foot syndrome).

3.4.1 Sorafenib

Dose Level	Sorafenib (by mouth)
0	400 mg twice daily
-1	600 mg daily (200 mg AM, 400 mg PM)
-2	400 mg daily
-3	400 mg every other day or 200 mg daily

3.4.2 TRC105

Dose Level	TRC105 (mg/kg IV every two weeks)
0	1

Dose Level	TRC105 (mg/kg IV every two weeks)
1	3
2	6
3	10
4	15

3.4.3 Dosing Delays/Dose Modifications: General Guidelines (See section below for specific toxicities).

3.4.3.1 Grade 1 Toxicity

Treatment with sorafenib and TRC105 need not be interrupted. For symptoms that last more than 7 days and have been found to be intolerable to the patient, the dose of sorafenib and TRC105 may be reduced to the next lower dose level.

3.4.3.2 Grade 2 Non-Hematologic Toxicity

Patients who have a grade 2 event may have a dose interruption. However, re-initiation of sorafenib and TRC105 at the same dose is allowed provided the adverse event has resolved to grade 1 or baseline.

3.4.3.3 Grade 3 Non-Hematologic Toxicity

Hold sorafenib and TRC105 and re-evaluate at least bi-weekly until toxicity improves to ≤ grade 1 or pre-treatment baseline. Reduce dose of either sorafenib or TRC105 by one dose level based on the discretion of the investigator. Treatment will be discontinued in patients who experience grade 3 non-hematologic toxicities that do not resolve to grade 1 or baseline within 4 weeks.

3.4.3.4 Grade 4 Non-Hematologic Toxicity

Patients with clinical grade 4 non-hematologic toxicity (except pulmonary embolism without significant hypoxia and hemodynamic instability) will be taken off treatment permanently. Patients with intolerable or limiting toxicity following two successive dose reductions will be removed from treatment. Unacceptable toxicities that have not resolved at time of "off treatment" must be followed until stabilization or resolution, at which time they will continue in follow up for survival.

3.4.4 Dosing Delays/Dose Modifications for Specific Toxicities:

3.4.4.1 Dermatologic Toxicities:

Rash/dry skin: In patients experiencing grade 2 skin rash or grade 3 dry skin (there is no grade 4 for dry skin), sorafenib will be held for 1-2 weeks (until rash less than grade 2, dry skin less than grade 3) and will be restarted at the same dose after the first occurrence. Sorafenib will be reduced to dose level -1 after a second occurrence and dose level -2 after a third occurrence. Treatment will be discontinued and the patient taken off treatment if the patient does not improve from a previous episode or has a fourth occurrence in spite of dose reductions. In patients experiencing grade ≥3 rash, dose reduction will begin with the first occurrence, and treatment will be discontinued and the patient taken off treatment if the patient does not improve from a previous episode or has a second occurrence.

Hand/foot skin reaction: In patients experiencing grade 2 hand/foot skin reaction, sorafenib will be held for 1-2 weeks (until skin reaction less than grade 2) and will be restarted at the same dose after the first occurrence. The dose of sorafenib will be reduced dose level -1 after the second occurrence and to dose level -2 after the third occurrence. Treatment will be discontinued and the patient taken off treatment if the patient does not improve from a previous episode or has a fourth occurrence. However, if a patient has had a dose reduction of sorafenib for toxicity and tolerates this dose for 28 days, the sorafenib may be re-escalated by one dose-level only, as per standard clinical practice. TRC105 may be continued throughout the episode at the discretion of the PI, and its dose will not be reduced.

3.4.4.2 Hypertension:

Whilst hypertension is a potential side-effect of TRC105 this has not been seen so far in the phase I study. On the other hand, this is a well-established side-effect of sorafenib. Therefore, in the event of hypertension dose modifications will be made for sorafenib.

Treatment and dose modification guidelines for sorafenib- induced hypertension:	**Grade of Event Management/ Next Dose**
grade 1	No change
grade 2 asymptomatic	Treat patient with antihypertensives and continue sorafenib
grade 2 symptomatic/ persistent OR diastolic BP > 110 mm/Hg OR grade 3	Treat patient with antihypertensives and hold BAY 43-9006 until symptoms resolve and diastolic < 100 mmHg.* Resume treatment at 1 dose level lower if further treatment is indicated**
grade 4	Off protocol therapy
* Patients requiring a delay of > 2 weeks should go off protocol therapy.	

Treatment and dose modification guidelines for sorafenib- induced hypertension:	**Grade of Event Management/ Next Dose**
** Patients requiring > 2 dose reductions should go off protocol therapy.	

Grade 1: asymptomatic, transient (< 24 hours) increase by > 20 mmHg (diastolic) or to >150/100 if previously WNL; intervention not indicated.

Grade 2: recurrent or persistent (> 24 hours) or symptomatic increase by > 20 mmHg (diastolic) or to > 150/100 if previously WNL.

Grade 3: requiring more than one drug or more intensive therapy than previously, including increasing dose of antihypertensive medication.

Grade 4: life threatening (e.g. hypertensive crisis)

3.4.4.3 Nausea/Vomiting:

Grade 3 nausea and grade 3 vomiting will only require dose modification if it is refractory to antiemetic therapy. In the case of refractory nausea/vomiting and grade 4 nausea or vomiting, sorafenib and TRC105 will be held until toxicities resolve to ≤grade 2 nausea and/or ≤grade 1 vomiting, and then the dose of sorafenib will be reduced by one dose level.

3.4.4.4 Diarrhea:

Grade 3 diarrhea will only require dose modification if it is refractory to treatment. In the case of grade 3 diarrhea, sorafenib will be held until diarrhea resolves to ≤grade 2 diarrhea. Sorafenib will be reintroduced at the same dose unless the diarrhea is refractory to therapy in which case the dose of sorafenib will be reduced one dose level. TRC105 may be continued throughout the episode, and TRC105 dose will not be reduced. All patients will be carefully monitored for gastrointestinal adverse events.

3.4.4.5 Electrolyte abnormalities:

Grade 3 or greater hypokalemia, hypophosphatemia, or hypomagnesemia will require dose modification only if not correctable with oral or IV replacement therapy within 48 hours. If the abnormality fails to correct to ≤grade 2 after ≤ 48 hours sorafenib and TRC105 will be held until the electrolyte abnormality resolves to ≤grade 2, and then the dose of both medications will be reduced by one dose level.

Grade 3 or greater hyponatremia will require dose modification only if not correctable within 24 hours by the administration of intravenous 0.9% Sodium Chloride Injection (0.9% NaCl Inj.). If the abnormality fails to correct to ≤ grade 2 after ≤ 24 hours of IV 0.9% Sodium Chloride Injection (0.9% NaCl Inj.), sorafenib and TRC105 will be held until the electrolyte abnormality resolves to ≤ grade 2, and then the dose of both medications will be reduced by one dose level.

3.4.4.6 Transaminase abnormalities:

Grade 3 elevation in transaminases will only require a therapy introduction and dose modification if they are also ≥ 1.5 times the baseline level. (This is because patients with Grade 2 transaminase levels are eligible for study entry.)

Patients who experience grade 3 elevation (+ ≥ 1.5x baseline) in transaminases will have them rechecked within 96 hours to ensure that they are trending down following interruption of sorafenib.

3.4.4.7 Uric acid:

No dose modification will be necessary for any grade of hyperuricemia without physiologic consequences.

3.4.4.8 Glucose:

Grade 3 or greater hyperglycemia or hypoglycemia will require dose modification only if believed to be probably related to study medication(s).

3.4.4.9 Hematologic abnormalities:

Grade 3 or higher leucopenia (excluding lymphopenia), neutropenia or thrombocytopenia will require dose modification of sorafenib alone, the dose of TRC105 will be maintained.

3.4.4.10 Proteinuria:

Proteinuria, as demonstrated by a 24 hour protein of ≥ 2000 mg. Urine protein will be screened by urine protein-creatinine ratio (UPC). For UPC ratio > 1.0, a 24-hour urine protein will need to be obtained and the level should be < 2000 mg for patient enrollment.

Proteinuria	[Proteinuria should be monitored by urine analysis for urine protein creatinine (UPC) ratio prior to every other cycle of TRC105]	
	UPC ratio < 3.5	Continue TRC105 and sorafenib.
	UPC ratio ≥ 3.5	Hold TRC105 and sorafenib until it UPC recovers to < 3.5.
	Grade 4 or nephrotic syndrome	Discontinue TRC105 and sorafenib.

3.4.4.11 Bleeding episode:

In the event of a bleeding episode both drugs will be held until resolution. The patient will be closely monitored and the merits of continued participation in the study will be discussed. We will only restart study medication if the source of bleeding has been remedied.

3.4.4.12 Infusion-related toxicities:

A permanent 50% decrease in the infusion rate of TRC105 will be required in patients experiencing a grade 1/2 (mild to moderate) infusion-related reaction. TRC105 will be permanently discontinued in patients experiencing a grade 3/4 infusion-related reaction, and the patient will be taken off treatment. All patients experiencing any grade of infusion reaction should be monitored for at least an hour after completion of infusion.

3.5 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

The correlative studies which we wish to perform are outlined below and summarized in the table. All blood and urine samples will be sent to Dr Figg's laboratory for initial handling and processing, before being forwarded to the appropriate lab as indicated. Tumor tissue will be handled by NCI Tissue Core.

3.5.1 Molecular characterization of HCC using tumor tissue, blood and urine (Xin Wang lab):

3.5.1.1 Description

Investigators at the NCI have played a leading role in the molecular characterization of HCC[36, 37]. The heterogeneous nature of hepatocellular carcinoma (HCC) and the lack of appropriate biomarkers have hampered patient prognosis and treatment stratification. Yamashita et al. have identified that a hepatic stem cell marker, epithelial cell adhesion molecule (EpCAM), which may serve as an early biomarker of HCC because its expression is highly elevated in premalignant hepatic tissues and in a subset of fully developed HCC. The same investigators have also identified novel HCC subtypes that resemble certain stages of liver lineages by searching for EpCAM-coexpressed genes. A unique signature of EpCAM-positive HCCs was identified by cDNA microarray analysis of 40 HCC cases and validated by oligonucleotide microarray analysis of 238 independent HCC cases, which was further confirmed by immunohistochemical analysis of an additional 101 HCC cases. EpCAM-positive HCC was found to display a distinct molecular signature with features of hepatic progenitor cells including the presence of known stem/progenitor markers such as cytokeratin 19, c-Kit, EpCAM, and activated Wnt-beta-catenin signaling, whereas EpCAM-negative HCC displayed genes with features of mature hepatocytes. Moreover, EpCAM-positive and EpCAM-negative HCC could be further subclassified into four groups with prognostic implication by determining the level of alpha-fetoprotein (AFP). These four subtypes displayed distinct gene expression patterns with features resembling certain stages of hepatic lineages.

This information holds the promise of an easy classification system defined by EpCAM status and AFP to reveal HCC subtypes similar to hepatic cell maturation lineages, which may enable prognostic stratification and assessment of HCC patients in addition to providing new insights into the potential cellular origin of HCC and its activated molecular pathways.

In order to perform this molecular analysis we will attempt to perform a baseline tumor biopsy. We will also attempt to perform a post-therapy biopsy at 2 months. This biopsy will be performed to assess CD105 staining by IHC. Molecular analysis will also be performed on this post-therapy sample by the Wang lab to identify any altered gene expression profile. Both biopsies are voluntary.

3.5.1.2 Timing of Tumor Biopsies (Optional):

Biopsies will be performed at the following time points:

- At baseline i.e. after consent, prior to treatment.
- During week 8 of therapy.

3.5.1.3 Biopsy Procedure:

The tumor biopsy will be obtained through Interventional Radiology by a percutaneous approach. Location of the biopsy within tumor will be recorded. A maximum of 2 core biopsies 18-gauge in diameter and at least 1 cm in length will be obtained at each time point. Only percutaneous biopsies will be performed on patients with solid tumors. It is estimated that there will be between 2 to 5 million cells from each biopsy. If a site is deemed appropriate for biopsy with minimal risk to the participant by agreement between the investigators and Interventional Radiology, an attempt at biopsy will be made. The use of imaging to facilitate biopsies will be decided by members of the Interventional Radiology team and may include ultrasound, CT scan, or MRI. Should CT scan be needed for biopsy, the number of scans for each procedure will be limited to the minimum number needed to safely obtain a biopsy. Tumor biopsies will be performed under local anesthesia and conscious sedation only if they are considered to be of low risk to the participant as determined by the investigators and Interventional Radiology.

All cases will be carefully reviewed with the interventional radiologists who have extensive experience in performing such procedures. Only if the procedure is considered to be low risk then we will proceed with tumor biopsy in a given participant. If the attempt is unsuccessful, the patient will still be eligible for treatment. If a patient declines biopsy he/she will still be eligible for study.

3.5.1.4 Sample Analysis:

If two cores are obtained at each biopsy time point, they will be handled as follows:

1) One core will be flash frozen and shipped on dry ice to Dr. Xin Wang's laboratory for molecular characterization:
2) The other core (or half of a core if only one is obtained will be divided in two) will be fixed in 10% formalin and submitted to Surgical Pathology, CCR/NCI (Bldg 10, 2N212) and for routine diagnostics. The specimens will have routine H&E stains (including CD105) made as well as 5 additional unstained sections.

3.5.1.5 Tumor tissue analysis at Dr. Wang's lab: Gene expression analysis:

1) Fresh-frozen tissue will be cut into serial 8μm sections
2) Microdissect tissue into tumor and non-tumor tissue, using laser-captured microdissection (LCM). A pathologist will help with the procedure. The equipment for LCM is provided by the NCI core facility.
3) Isolate, amplify and label RNA, using existing protocols. Currently, our laboratory uses a QIAGEN protocol for the isolation of total RNA from microdissected tissue and the small sample labeling protocol by Affymetrix to produce biotin-labeled cRNA.
4) Hybridize cRNA onto the Affymetrix GeneChip Plus 2 arrays (gene expression)
5) Array quality assessment will include array image inspection and in-house software packages developed by our bioinformatics staff
6) Data analysis will be performed at Dr. Wang's laboratory

3.5.1.6 Collection of non-tumor tissue (blood, urine and mouthwash samples) for analysis in the Wang laboratory:

We will collect blood (or mouth wash), urine and tissue specimens. The collected blood (or mouth wash) will be used for the analysis of genotypes, single nucleotide polymorphisms, methylation status, and the measurement of cytokine and microRNA concentrations in serum and plasma. Urine samples are collected to study the association between metabolites in the liver and risk or progression of the disease. The tissue specimens are collected for whole-genome gene expression analysis and the preparation of tissue arrays for immunohistochemical analysis of protein expression. All non-tumor tissue samples will be initially collected and handled by the Figg laboratory and assigned a study ID number. Personal identifiers will not be used on stored research samples.

Genotyping will be performed from whole blood or buffy coat. The genotyping will be performed at the NCI Core Genotyping Facility using a global standard platform (currently, the HumanOmniExpress chip) which covers over 700K single nucleotude variants or through targeted Taqman assays based on genes of interest found in our gene expression analysis described elsewhere in this protocol. Data will be analyzed and streamlined strictly for the purposes of the research aim(s) and results will not be shared with the patient. Since samples are de-identified, researchers analyzing the data will be blind to the patient identifiers. Thus, any additional findings that are incidental to the study will not be available to, nor benefit the patient whose sample was studied.

3.5.1.6.1 Procedures for collection of blood (or MOUTH WASH) and urine

Protocol for blood collection:

1. Collect about 35cc of blood from each patient. 35cc of blood will be collected and divided into two 7 ml red top clot tubes (serum) and two 10ml green top sodium heparin tubes (plasma). Blood (separation of serum and blood clot; buffy coat; plasma and red blood cells) and urine to be processed within 8-24 hours at the Clinical Center.
2. The date and exact time of each blood draw should be recorded on the blood tubes. Store the red tops, upright, at room temperature for 30-60min and then store at 4°C. Place the green tops on wet ice and store at 4°C in the refrigerator.
3. Please page 102-11964 (Dr. Figg's Lab) for immediate pick-up. Contact Dr. Figg's Blood Processing Core (BPC) in 10/5A09 at 301-402-3622 or 301-594-6131 with any questions.
4. Aliquots (1.5ml) will be frozen and – after initial handling and processing at the Figg laboratory – batch-shipped to Dr. Xin Wang's Laboratory every three to four weeks.
5. If a blood draw is not possible, a mouth wash is obtained.

Protocol for mouthwash collection (optional if blood obtained):

1. Rinse mouth with about 20ml water to wash out any food in mouth. Discard.
2. Rinse mouth with 30ml Blue color Listerine for 30 seconds and spit to a collection container.
3. Cover with lid and send to lab with ID label.
4. Complete Intake Form.

5. Fill out Specimen Collection Form.
6. Please page 102-11964 (Dr. Figg's Lab) for immediate pick-up. Contact Dr. Figg's Blood Processing Core (BPC) in 10/5A09 at 301-402-3622 or 301-594-6131 with any questions.

Protocol for urine collection

1. Collect approximately 50ml urine from each patient in a clean catch container.
2. Store at 4°C in the refrigerator after collection.
3. The date and time of collection should be recorded on the container. Please page 102-11964 (Dr. Figg's Lab) for immediate pick-up.

3.5.2 Genotyping (Xin Wang lab):

1) Isolate DNA from whole blood or buffy coat using a previously tested DNA isolation kit
2) Determine DNA quality with Taqman-assays and $OD_{260/280}$ readings >1.8
3) Perform genotype analysis with quality-controlled Taqman assays, using 384-well plates. Assays will be performed at the NCI Core Genotyping Facility. Each assay will contain negative and positive controls, and 10% blinded duplicates across and within Taqman plates. Greater than 90% concordance among duplicates will be required for inclusion in data analysis. A >90% completion rate, >98% concordance rate and Hardy-Weinberg equilibrium tests will be used for inclusion. Odds ratios for cases and controls will be assessed using logistical regression while association with survival among cases will be assessed by cox hazard regression using STATA. Our survival assessment will be survival after date of study entry.

3.5.3 Stem Cell Marker Analysis (Jane Trepel lab)

1) Fresh blood which has been collected (~10mL) in green top tubes containing sodium heparin will be sent to NCI for immediate processing or stored at 4^{o}c overnight. Cancer stem cells will also be monitored in available fresh frozen tissue by IHC analysis of stem cell markers.
2) Red blood cells will be removed with lysis buffer (25mL lysis buffer/1mL blood) with 5 minute incubation followed by centrifugation at 300g.
3) Circulating tumor cells (CTCs) will be isolated via negative enrichment to remove normal blood cells
4) CTCs will be labeled for stem cell marker expression such as EpCAM, CD133 etc using commercial antibodies and relative amounts will be determined by FACS

3.5.4 Blood Product analysis (Xin Wang lab):

1) Isolate small RNA from serum or plasma samples using commercially available kit (MirVANA, Invitrogen)
2) Perform MicroRNA expression analysis using pre-made Taqman probes for specific microRNA purchased from Applied Biosystems; Each sample will be performed in triplicate and those with greater than 5% error in expression readings will be excluded from further

analyses. Cycle readings under 36 will be considered good. Differences between high and low RNA readings will be based upon the median, tertile or quartile cutoffs.

3) Data analysis will be performed at the Dr. Wang's laboratory.

3.5.5 Cytokine expression analysis (Xin Wang lab)

1) Incubate serum or plasma samples with cytokine probes using commercially available multiplexed human cytokine plate (MesoScale)
2) Each assay will contain negative and positive controls, blanks and 10% blinded duplicates across and within Taqman plates. Greater than 95% concordance among duplicates will be required for inclusion in data analysis. Differences between high and low RNA readings will be based upon the median, tertile or quartile cutoffs.
3) Data analysis will be performed at the Dr. Wang's laboratory

3.5.6 Metabolomics analysis (Xin Wang lab / Metabolon):

Metabolomic profiling will be carried out at Metabolon (North Carolina, USA), using the general protocol outlined in Lawton et al (2008). Liquid chromatography/mass spectrometry in both positive and negative modes (LC+/LC-) and gas chromatography/mass spectrometry (GC/MS) will be used.

1) Urine samples from HCC cases (~150uL) will be prepared with neat HPLC grade acetonitrile (1:1) and centrifuged followed by processing by MS-based mass spectrometry in both positive and negative-ion mode.
2) Quality will be assessed using control samples including pooled samples, blanks, duplicates and endogenous "spike-ins".
3) Data analysis will be performed at the Dr. Wang's lab.

3.5.7 Genotyping for SNPs in angiogenesis-related genes (eg VEGFR) (Figg lab):

3.5.7.1 Description

Of particular interest in studies evaluating anti-angiogenic agents in various cancers has been the finding (albeit often in an exploratory post-hoc analysis) of an improved median survival for those patients who experienced hypertension. For example in a randomized phase II study of axitinib (an inhibitor of VEGFR) in pancreatic cancer those who experienced a single incident of diastolic hypertension (>90mm HG) during treatment visits had an improved median survival compared to those who also received the combination treatment but who didn't experience any recorded hypertension (13.0m [95% CI 8.5 – 16.6] v 5.6m [95% CI 4.8 – 7.2])[38]. This association between the onset of hypertension and improved therapeutic outcomes with anti-angiogenic or anti-VEGF agents has also been found in other diseases and studies[39-41]. It is unclear whether the attainment of hypertension is a surrogate biomarker for those patients who would benefit from anti-VEGF therapy or indeed if it is a marker of efficacy that should be strived for. It appears that the former is more likely to be the case based on an analysis of patient tumor blocks from a study in breast cancer comparing paclitaxel with or without bevacizumab. In this study there was an association found between VEGF genotype and median overall survival as well as grade 3 or 4 hypertension[41, 42]. Although the DNA evaluated in this study was

derived from the primary tumor, it was host variability that was being evaluated, which is an important difference from other biomarkers, for example EGFR or KRAS mutations, which are tumor- related. Because angiogenesis is a host-mediated process, germline genetic variability may be an important contributor to the heterogeneity of response seen and this variability has the potential to be used for better selection of patients for future studies evaluating anti-VEGF agents[43].

3.5.7.2 Procedure:

A single blood test will be performed at baseline (after consent, prior to treatment initiation). One 6ml EDTA tube (BD, Franklin Lakes, NJ) will be collected from patients.

1. Immediately after collection, invert the blood tube 8-10 times. Place the tube on wet ice and then refrigerate. The date and exact time of each blood draw should be recorded on the tube.
2. Please page 102-11964 (Dr. Figg's Lab) for immediate pick-up. Contact Dr. Figg's Blood Processing Core (BPC) in 10/5A09 at 301-402-3622 or 301-594-6131 with any questions.

Genomic DNA will be analyzed for polymorphisms in angiogenesis-related genes. Genotyping will be performed by sequencing analysis, RFLP or gene array analysis.

3.5.8 Circulating endothelial cells (CECs), Circulating endothelial progenitor (CEP) cells, plasma levels of angiogenic factors, soluble CD105 (baseline) and Rat aortic ring bioassay.

3.5.8.1 Brief description of background and rationale for performing this test:

1. Both TRC105 and sorafenib inhibit angiogenesis and tumor growth. However, there are no current human clinical data to support such biological activity in TRC105.
2. Consistent fluctuations in plasma VEGF and PlGF (placenta-derived growth factor), as well as in CEC's and CEP's have been detected following an array of anti-angiogenic therapies, including the anti-VEGF antibody bevacizumab, VEGFR2 inhibitors, and their combinations in clinical studies.
3. Such analysis in this study would provide the necessary support for the putative mechanism of action of the agent under investigation.
4. Given the mechanism of TRC105 it is possible that efficacy is related to baseline soluble CD105.

3.5.8.2 Implementation:

3.5.8.2.1 CEPs and CECs (Jane Trepel lab):

Two 7cc EDTA lavender tubes will be drawn for analysis of circulating endothelial progenitor cells (CEP) and mature circulating endothelial cells (CEC). Samples will be collected from patients at 30 min prior to dose on cycle 1 day 1, cycle 1 day 15 and cycle 2 day 15.

3.5.8.2.2 VEGF, PlGF, bFGF and sVEGFR1 (soluble VEGF receptor 1) (Liang Cao lab):

One 4 ml EDTA plasma tube (BD, Franklin Lakes, NJ) is collected from each patient prior to drug administration at Day 1 of Cycle 1 (baseline), Day 15 of Cycle 1, at Day 1 of Cycle 2, and at the end of the study.

1. The date and exact time of each blood draw should be recorded on the blood tubes. Place the tubes on wet ice and store at 4°C in the refrigerator.
2. Please page 102-11964 (Dr. Figg's Lab) for immediate pick-up. Contact Dr. Figg's Blood Processing Core (BPC) in 10/5A09 at 301-402-3622 or 301-594-6131 with any questions.

The plasma will be transferred to Dr. Cao's lab at Bldg 37, Rm. 6134 in batches (tel: 301-435-9039). Plasma levels of angiogenic factors will be performed for VEGF, PlGF, bFGF and sVEGFR1 (soluble VEGF receptor 1). The analysis will be done with assays developed on electrochemiluminescence platform that provides ultra-high sensitivity and very large signal dynamic range. Purified protein standard will be used for generating standard curves for concentration determination. Data analysis will be performed with Prism (GraphPad, San Diego, CA) to determine the medium value, interquartile range, and *P* value in paired t test. If the induction of VEGF and PlGF can be observed, correlative studies will be performed between the induced levels of angiogenic factors and the dose levels of TRC105 in the phase I combination segment of the trial. In the phase II study, when the responses can be established, correlative studies will be performed between the initial levels of the angiogenic factors, or the degree of their induction with the clinical responses.

3.5.8.2.3 Soluble CD105 (Seon Lab):

A 5mL K_2EDTA lavender top tube will be collected at Day 1 of Cycle 1 (baseline), Day 15 of Cycle 1, at Day 1 of Cycle 2, and at the end of the study.

1) Record the patient number on the collection and storage labels for each collection time point using a permanent marker. Affix the completed collection and storage labels vertically on the 5mL K_2EDTA (lavender top) blood collection tube and two 2mL clear polypropylene storage cryovials with lavender top, respectively, prior to specimen collection.
2) Collect 5mL of blood into the K_2EDTA (lavender top) blood collection tube.
3) Gently invert the 5mL K_2EDTA (lavender top) blood collection tube (~15 times) to completely mix the blood and anticoagulant.
4) Please page 102-11964 (Dr. Figg's Lab) for immediate pick-up. Contact Dr. Figg's Blood Processing Core (BPC) in 10/5A09 at 301-402-3622 or 301-594-6131 with any questions.

At the completion of the study samples will be shipped to Dr Seon's lab at Roswell Park at the following address:

Jill Duzen
Seon Laboratory
CGP bldg. Room L5-126
Roswell Park Cancer Institute

Elm and Carlton Streets
Buffalo, NY 14263
Phone: 716-845-4482

3.5.9 Rat aortic ring bioassay (Figg lab):

At least 7 ml of blood in a red top clot tube for Dr. Figg's lab will be collected at baseline and C3D1.

1) The date and exact time of each blood draw should be recorded on the blood tubes. Store the red tops, upright, at room temperature for 30-60min and then store at 4°C.
2) Please page 102-11964 (Dr. Figg's Lab) for immediate pick-up. Contact Dr. Figg's Blood Processing Core (BPC) in 10/5A09 at 301-402-3622 or 301-594-6131 with any questions.

This assay as described by Ng et al utilizes a Matrigel system for optimal neovessel sprouts from implants of rat aortic sections[44]. After a 5-day incubation period, image analysis following photo microscopy allows for the quantization of sprout density. The assay conditions are able to use human serum as the stimulus source. The serum to be utilized will be allotted from the quantity sent to the Clinical Pharmacology Program.

3.5.10 Fc receptor polymorphisms (Jane Trepel lab)

Genotyping of CD16a will be performed using a TaqMan SNP probe assay. The assay contains two primers for amplifying the sequence of interest and two TaqMan MGB (minor groove binding) probes for detecting specific alleles. The genotyping assay determines the presence or absence of a SNP based on the change in fluorescence of the dyes associated with the probes. Pre-designed TaqMan SNP genotyping assays from the Applied Biosystems database (http://www.appliedbiosystems.com) will be used for rs396991, the relevant SNP of interest on CD16a (amino acid 158, V/F).

Peripheral blood will be collected in one lavender top tube on C1D1 pre-therapy. DNA will be isolated using the Qiagen Blood DNA isolation kit, and quantified using NanoDrop instrumentation. For each DNA sample PCR amplification will be performed using the TaqMan SNP probe assay reagents. After PCR amplification an endpoint plate read on an ABI 7500 instrument will be performed and analyzed using SDS software provided by Applied Biosystems. The results of the allelic discrimination assay will be displayed as a scatter plot of the fluorescence intensity of allele 1 versus allele 2.

3.5.11 Immunogenicity Testing (Tracon):

At each timepoint outlined in the schedule of assessments, a 5 mL blood sample will be collected to assess immunogenicity (Human Anti-Murine Antibodies/HAMA and Human Anti-Chimeric Antibodies/HACA). Immunogenicity studies (both HAMA and HACA) will be collected at baseline and then 28 days following the end of the study treatment.

The date and exact time of each blood draw should be recorded on the blood tubes. Store the red tops, upright, at room temperature for 30 min and then store at 4°C. Please page 102-11964 (Dr. Figg's Lab) for immediate pick-up. Contact Dr. Figg's Blood Processing Core (BPC) in 10/5A09 at 301-402-3622 or 301-594-6131 with any questions.

Sera will be separated and stored at -70°C. Samples will be batch shipped every 3 to 6 months to 3rd party laboratory for analysis. HAMA and HACA concentrations will be measured using validated ELISA methods and evaluated in the context of pharmacokinetic parameters and adverse event profiles.

3.5.11.1 HAMA/HACA Serum Collection, Processing and Storage Instructions

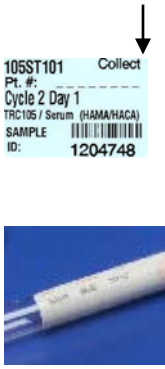
Step 1: Record the patient number on the collection and storage labels for each collection time point using a permanent marker. Affix the completed collection and storage labels vertically on the 5mL red top blood collection tube and four 0.5mL clear polypropylene storage cryovials, respectively, prior to specimen collection.

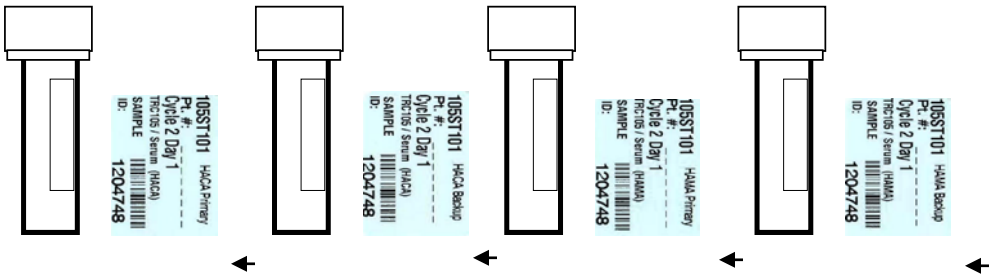
Label application instructions:

"HAMA Primary" & **"HAMA Backup"** labels – affix to 0.5mL clear polypropylene storage cryovials (vertically)

"HACA Primary" & **"HACA Backup"** labels – affix to 0.5mL clear polypropylene storage cryovials (vertically)

"HACA Extra" & **"HAMA Extra"** labels – to be used as replacement for damaged or lost label with corresponding assessment, collection time point and sample ID





5mL red top blood collection tube

4 x 0.5mL clear polypropylene storage cryovial

Step 2: Collect 5mL of blood into the red top blood collection tube.

Step 3: Keep the blood specimen at room temperature for 30 minutes until clotted**.**

Step 4: Centrifuge the 5mL red top blood collection tube at 1700 g (~3,000 to 3,500 rpm) for 10 to 15 minutes at 4°C in a refrigerated centrifuge or an unrefrigerated centrifuge with a centrifuge rotor/cups that have been pre-chilled to 4°C for 2 hours**.**

Step 5: Transfer the serum equally into four pre-labeled 0.5mL clear polypropylene storage cryovials. Use a fresh transfer pipette for each time point**.**

4 x 0.5mL clear polypropylene storage cryovial

Step 6: Place the 0.5mL clear polypropylene storage cryovials in the cryovial storage box provided and freeze immediately. Store the specimens in a freezer at -70°C. Once frozen, do not allow the specimens to thaw, including during shipment

3.5.12 Immunomonitoring (Greten lab)

3.5.12.1 Description

Non-immune based therapies have been shown to have significant effects on tumor-specific immune responses. While it was always a general believe that chemotherapy impairs immune responses in patients with cancer, there is increasing evidence demonstrating that under certain circumstances chemotherapy might have potential beneficiary effects on tumor specific immune responses[45]. Until today the mechanism of how chemotherapy might support immune responses remains unclear. While it has been shown in different mouse models that chemotherapy induced cell death supports T cell priming through cross-priming[46] others have been able to demonstrate an effect of targeted therapies on different immune suppressor mechanisms including regulatory T cells as wells as MDSCs. Sunitinib has been shown to impair both, the function of regulatory T cells as well as MDSCs[47, 48]. Although the direct mechanism remains unclear how sunitinib impairs MDSCs and Treg function. Previous studies have shown that VEGF plays a major role in abnormal dendritic cell differentiation and function in cancer. It has been proposed that inhibition of VEGF may result in improved immune responses and that anti-VEGF based therapies improve immune responses in patients with cancer.

In this study we plan to monitor immune effector function in detail. For this PBMC will be analyzed from patients prior to first treatment and 4 and 10 weeks after initiation. Blood will be collected and processed by the Figg lab and frozen until later analysis. Frequency, phenotype and function of different immune cells (including B cells, CD8+ T cells, CD4+ T cells, NK cells, NKT cells, myeloid DCs, Macrophages, Monocytes, Tregs and MDSCs) will be analyzed in Dr. Greten's laboratory.

Peripheral blood will be collected in one 60cc heparinized syringe and stored at room temperature. The date and exact time of each blood draw should be recorded on the blood tubes. Please page 102-11964 (Dr. Figg's Lab) for immediate pick-up. Contact Dr. Figg's Blood Processing Core (BPC) in 10/5A09 at 301-402-3622 or 301-594-6131 with any questions.

3.5.13 TRC105 Pharmacokinetics

3.5.13.1 TRC105 Pharmacokinetics Trough Concentration:

A 5 mL blood sample to be collected C1D1, C1D15, C2D1 and C2D15, immediately prior to starting the TRC105 infusion. Samples will be stored at approximately -70°C for shipment every 3 months. See separate laboratory guide for further collection and shipment information. Additional PK samples may also be collected at the time of unexpected clinical events.

3.7.13.2 TRC105 Pharmacokinetics Peak Concentrations:
A 5 mL blood sample to be collected within 5 minutes prior to the completion of the TRC105 infusion on c C1D1, C1D15, C2D1, C2D15 and the end of the study. Samples will be stored at approximately -70°C for shipment every 3 months. See separate laboratory guide for further collection and shipment information. Additional PK samples may also be collected at the time of unexpected clinical events.

3.6 IMAGING STUDIES

An attempt will be made to perform all correlative imaging studies in accordance with the schedule; however, given scheduling issues and unforeseen circumstances which may result in treatment delays and patient inconvenience, these tests will be optional.

3.6.1 Dynamic Contrast Enhanced – Magnetic Resonance Imaging (DCE-MRI)

3.6.1.1 Description

Dynamic Contrast Enhanced Magnetic Resonance Imaging (DCE-MRI) has been used extensively with novel antiangiogenic agents in phase I and II trials to monitor their effect on tumor vasculature through parameters reflecting both tumor perfusion and permeability. This protocol is limited to DCE-MRI using T1 weighted studies with low molecular weight gadolinium (Gd) chelates. Other MRI techniques (which are not covered by this protocol) include the following: DCE-MRI using T2* weighted images; contrast-enhanced MR with high molecular weight contrast agents; and non-contrast enhanced MRI techniques such as diffusion-weighted MRI, T2* weighted MRI or BOLD.

DCE-MRI studies will be interpreted by Dr. Peter Choyke, MD, Chief, NCI Molecular Imaging Department, 301.435.4046, 10/1B40. Other members of the clinical imaging program may contact Dr. Choyke for information purposes regarding the imaging protocol and data capture methodology.

3.6.1.2 DCE-MRI End Points

- The primary end points of these DCE-MRI studies are the Initial Area Under the Gd Curve measured over 60 seconds (IAUC60) and the Transport Constant (K^{trans}).
- IAUC (initial area under the gadolinium concentration time curve).
- Transport Constant (K^{trans}).
- Both K^{trans} and IAUC60 require calculation of instantaneous tumor Gd concentration based on the change in relaxivity due to contrast uptake $\Delta R1$. The basic requirements for these calculations are:
    - An estimate of contrast agent relaxivity in tumor vasculature and tissue

- Measurement of tumor T1 immediately prior to contrast uptake
- An accurate T1 measurement method verified for all spatial locations, coils and scanners used
- Cardiac output (or arterial input function) for K^{trans} (population based estimates may be appropriate)
- Reproducible injection (ideally power injector)

- All data including ROI definition and analysis should be recorded and traceable to support external review.
- Additional end points may be generated at a study / protocol level in addition to these primary end points. The production of the primary DCE-MRI end points for comparison between studies does not preclude other end points within studies such as parametric maps of IAUC/K^{trans}, blood volume (Vb), extravascular space (Ve), Kep (a function of K^{trans} and Ve), etc.

3.6.1.3 Tumor Type and Location

- Lesions within the **lung** are excluded from DCE-MRI studies due to "breathing" artifacts which make image data difficult to analyze reliably.
- Recommendations for lesion selection:
  - Exclude lung lesions.
  - If possible, exclude lesions in close proximity to the heart or major vascular structures
  - If possible, choose lesions towards the center of the image volume.
  - If possible, choose lesions towards the center of the image plane.
  - Lesions should be at least 1.5 cm and preferably > 2 cm in longest in plane diameter
  - Lesions preferably should be < 7 cm in the z plane [the entire lesion(s) should be within the volume scanned].
  - Lesions should be well defined on standard CT or MR imaging.

3.6.1.4 Schedule of DCE-MRI Assessments

- The use of DCE-MRI to assess tumor vascular factor parameters is exploratory is nature. When possible, patients enrolled into the phase II portion of the study will be selected for enrollment based on appropriate lesions for pharmacodynamic imaging with planned DCE-MRI scans.
- DCE-MRI scans should be obtained:
  - Prior to treatment (preferably within 7 days of treatment start)
  - 24-36 hours after Cycle 1 Day 1
  - 28 +/- 2 days after Cycle 1 Day 1
  - Further follow-up scans, depending on the study design

3.6.1.5 Data to be collected

- The following information on a per patient basis should be supplied to the NCI with the final study manuscript.
  - Study identifier (NCI protocol number)
  - Subject identifier (patient I.D.)

- TRC105 dosing information
- Date of first dose of TRC105
    - Date of last dose of TRC105
    - Dose of TRC105
    - Any relevant dosing information, e.g., drug holidays

- Tumor type
- For each DCE-MRI scan
    - Location of lesion studied, e.g., liver metastases
    - Date of DCE-MRI scan
    - Tumor IAUC60
    - Tumor K^{trans}
- For each RECIST response assessment
    - Date of each RECIST assessment
    - Response assessment (if progression is recorded, then it should be specified whether the progression is in the lesion assessed by DCE-MRI)

3.6.2 Doppler U/S of tumor vasculature.

3.6.2.1 Ultrasound Evaluation Description

Ultrasound evaluation of the patient's liver tumor volume and tumor blood flow will be performed at the same time points as DCE-MRI

3.6.2.2 Schedule:

- Prior to treatment (preferably within 7 days of treatment start)
- 24-36 hours after Cycle 1 Day 1
- 28 +/- 2 days after Cycle 1 Day 1
- 56 +/- 2 days after Cycle 1, Day 1
- Further follow-up scans, depending on the study design

3.7 SUMMARY OF CORRELATIVE TESTS

Tissue sample type	Test		Quantity	Tube and storage	Timing
Blood	Genotyping (Wang) Circulating Tumor cells, cytokines (Wang)		35 mls	2- 7mL red 2- 10mL green (on ice)	C1D1
	SNP for angiogenesis- related genes (Figg)		6 mls	EDTA (on ice)	C1D1
	Serum PD (Trepel/Cao)	CEP/CECs	16 mls	2-7 mL (on ice) Lavender	C1D1, C1D15, C2D15
		VEGF,FGF,PIGF	5 mls	EDTA (on ice)	C1D1, C1D15, C1, D1 and end of study
		Soluble CD105	5 mls	Lavender top tubes	C1D1, C1D15, C2D1, and end of study
		Rat aortic ring assay	7 mls	Red (room temp)	C1D1, C3D1
	HAMA/HACA (Figg)		7mL	Red top (room temp)	C1D1, 28 days post end of study
	TRC 105 Pharmacokinetics		5ml	Red top (room temp)	C1D1, C1D15, C2D1, C2D15 and end of study (trough only) pretreatment and 5 minutes post completion of TRC 105 infusion

Tissue sample type	Test	Quantity	Tube and storage	Timing
	Fc Receptor polymorphisms (Trepel)	4 mls	Lavender top tubes	C1D1
	Immunomonitoring (Greten)	60 mL	1- 60cc Sodium Heparin Syringes (room temp)	C1D1, 4wks, 10 wks
Tumor tissue	Molecular profiling (Wang) Histology for confirmatory diagnostics and CD105 (Histopath.)	Optional 1-2 cores	N/A	Optional C1D1 and after 2 cycles
Urine	Metabolomics (Wang)	50 mLs	13 ml plastic tubes (on ice)	C1D1
Mouthwash	Genotyping (Wang)	30mLs	50 ml centrifuge tube	C1D1
Radiology	DCE-MRI (Choyke)	3	N/A	1) Prior to treatment (preferably within 7 days of treatment start) 2) 24-36 hours after Cycle 1 Day 1 3) 28 +/- 2 days after Cycle 1 Day 1 4) Doppler Only 56 +/- 2 after Cycle 1 Day 1
	Doppler U/S (Wood)	4		

3.8 SAMPLE STORAGE, TRACKING, AND DISPOSITION

See Section **11.5**

3.9 ON-STUDY AND FOLLOW UP EVALUATIONS

3.9.1 See study Calendar (Section **11.4**)

3.9.2 All study subjects will be followed for overall survival only once active treatment has been completed.

3.9.3 If toxicities cause discontinuation of therapy, patients will be followed until resolution of toxicity to at least Grade 1. This may be done by phone if appropriate.

3.9.4 Follow-up will be annual telephone contact to assess survival status. Every attempt will be made to contact patient/subject including: contacting referring physician, contacting emergency contact patient identified on admission, checking SSDI (Social Security Death Index)

3.10 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

3.10.1 Criteria for removal from protocol therapy

In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

3.10.1.1 Progressive disease as defined in Section **5.2**

3.10.1.2 Intercurrent illness that prevents further administration of treatment,

3.10.1.3 Unacceptable adverse event(s)

3.10.1.4 Patient decides to withdraw from treatment

3.10.1.5 General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

3.10.2 Off-Study Criteria

3.10.2.1 Voluntary withdrawal of consent to participate in the study or the follow up period.

3.10.2.2 Patient noncompliance with the study.

3.10.2.3 Patient death

3.10.3 Off Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off-study. An off-study form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office (HOIS) ncicentralregistration-l@mail.nih.gov.

3.11 EARLY STOPPING RULES FOR SAFETY/ACCRUAL

The following stopping rules for safety and accrual/futility will be implemented in the protocol:

i. In the event of more than 1 variceal bleeding event during the phase I part of the study or more than 2 variceal bleeding events in the phase II part the study, the study will be suspended pending full investigation of the bleeding cause.

ii. In the event of 1 other major bleeding complication (CTC grade 3), apart from variceal hemorrhage, during the phase I part or more than 2 bleeding events in the phase II part the study, the study will be suspended pending full investigation of the bleeding cause.

iii. A screening log will be initiated to identify those in/exclusion criteria, which are most likely hindering patients to enroll into the studies. This log will not only help us for the design of other future trials, but we can also use it to evaluate if we need to amend the protocol to allow for a better (but still safe, accrual). Should we notice that not enough patients are referred to NCI, we will open a second center at a location outside of the Washington/Baltimore area, possibly at Memorial Sloan-Kettering Cancer Center or Harvard.

4 SUPPORTIVE CARE

Patients will receive appropriate supportive care medications at the investigator's discretion. See section **3.4** for management of specific toxicities.

5 DATA COLLECTION AND EVALUATION

5.1 DATA COLLECTION

Data will be prospectively collected and entered into the NCI C3D database. Medical records will be maintained to include but not limited to:

- Signed, Dated Consent Form
- Completed Eligibility Checklist
- Source documents verifying eligibility criteria
- Pre-study lab, radiology, pathology reports, histopathological results
- Interim monitoring test results
- Physician notes/progress notes documenting physical evaluations, PS, history, prior therapy
- Physician/Nursing notes documenting vital signs, adverse event assessment,
- Treatment administration forms-In-patient/Out-patient
- Response evaluation- results/tumor measurements
- Research DCE-MRI Scan/biologic correlate sample collection/analysis
- Off study summary

See also the CCR Standard Operating Procedure: Conducting and Documenting Drug Accountability for Oral Investigational Agents that are Self-Administered by Patients (http://ccrintra.cancer.gov/clin_ops/policies/SOPCLIN1.pdf).

All data will be kept secure. The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All human subjects personally

identifiable information (PII) as defined in accordance to the Health Insurance Portability and Accountability Act, eligibility and consent verification will be recorded. Primary data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

End of study procedures: Data will be stored according to HHS and FDA regulations as applicable.

Loss or destruction of data: Should we become aware that a major breech in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

5.2 RESPONSE CRITERIA

Re-staging CT scan of chest, abdomen, and pelvis will be required every two months of treatment. Confirmatory scans should also be obtained 4 weeks following initial documentation of objective response (CR or PR)

Objective response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee [Eisenhauer EA, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1) 2009; Eur J Ca 45:228-247].

Note: Lesions are either measurable or non-measurable using the criteria provided below. The term "evaluable" in reference to measurability will not be used because it does not provide additional meaning or accuracy.

5.2.1 Response Criteria for Radiographic Studies

5.2.1.1 Measuring of Soft Tissue Disease

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009] as well as – in an exploratory fashion as a secondary endpoint – the EASL-modified RECIST criteria (Lencioni and LLovet Semin Liv dis 2010). Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

a. Evaluation of Target Lesions

Complete Response (CR)

Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm

Partial Response (PR)

At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum of diameters

Progressive Disease (PD)

At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions)

Stable Disease (SD)

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of diameters while on study

b. Evaluation of Non-Target Lesions

Complete Response (CR)

Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis)

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD (Stable Disease, SD)

Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD)

Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

c. Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**

CR	Non-CR Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR Non-PD Not evaluated	No	PR	
SD	Non-CR Non-PD Not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	No prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

*See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
**Only for non-randomized trials with response as primary endpoint.
***In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression

d. Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

e. In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesions be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

5.2.1.2 Confirmatory Measurement/Duration of Response

a. Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed at least 4 weeks after the criteria for response are first met.

b. Duration of Overall Response

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

5.2.1.3 Measurable Disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray, as ≥10 mm with CT scan, or ≥10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. *If the investigator thinks it appropriate to include them, the conditions under which such lesions should be considered must be defined in the protocol*.

5.2.1.4 Malignant Lymph Nodes

To be considered pathologically enlarged and measurable, a lymph node must be ≥20 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

5.2.1.5 Non-Measurable Disease

All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

5.2.1.6 Target Lesions

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A

sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Progressive disease by RECIST criteria [1] noted after the first re-staging scan may represent disease that was not detected on the pre-study scan, and a confirmatory scan will be required at the next scheduled re-staging evaluation unless clinically not indicated. If confirmed, progression should be dated by the initial time when the lesions are first detected. If progressive disease by RECIST criteria is seen after cycle 2, but not confirmed on subsequent restaging scan, the scans from after cycle 2 would serve as the baseline scan to evaluate for disease progression, (ref: Scher, HI et al. J Clin Onc, 26 (7), 2008)

5.2.1.7 Non-Target Lesions

All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

5.2.1.8 Metastatic Bone Lesions

Disease progression is considered if a minimum of two new lesions is observed on bone scan. New lesions seen by the end of cycle 2 or before cycle 3 (with the first re-staging bone scan) may represent disease that was not detected on the pre-study scan, and a confirmatory scan will be required at the next scheduled re-staging bone scan unless clinically not indicated. If confirmed, progression should be dated by the initial time when the lesions are first detected. If new lesions are seen after cycle 2, but no additional lesions are seen on confirmatory scans, the scans from post-cycle 2 would serve as the baseline scan to evaluate for disease progression, (ref: Scher, HI et al. J Clin Onc, 26 (7), 2008)

5.2.1.9 Clinical Lesions

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

5.2.1.10 Guidelines for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

5.2.1.11 Methods of Measurement

Chest X-ray - Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

CT and MRI - CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. For this study helical Multi-detector CT will be performed with cuts of 5 mm in slice thickness for chest, abdomen and pelvis lesions and 2-3 mm thickness for head and neck lesions.

5.2.2 EASL-modified RECIST criteria.

5.2.2.1 Background

The Amercian Association for the Study of Liver Disease (AASLD) convened an expert panel to reach consensus on issues relating to trial design in HCC, a particular challenge given the almost invariable existence of underlying cirrhosis[49]. A number of recomendations were made including:

- The Barcelona Clinic Liver Cancer Staging System (BCLCS) was recommeded for selection of the target population in an effort to standardise inclusion criteria and make data interpretation easier.
- For phase II studies the panel recommended a randomized design with a time-to-event endpoint, such as time to tumor progression (TTP). This is in place of a composite endpoint, such as progression-free survival, which may be influenced by the underlying cirrhosis and mask any potential anti-cancer efficacy of the drug.
- The use of smarter correlative endpoints which better reflect the mechanistic effect of the investigational agent can also be useful, especially given the lack of randomized studies in this disease.
- The AASLD panel also recommended that new agents be tested in patients with well-preserved liver function (Child-Pugh Class A).

In addition, the panel addressed the deficiencies in the standard RECIST criteria as they apply to HCC, particularly in the era of molecular therapies where changes in tumor viability, as determined by contrast-enhanced techniques, are important. This is especially relevant given the vascularity of HCC. Certain amendments to the RECIST criteria were therefore proposed which are centred on the concept of measuring viable tumor, defined as the uptake of contrast agent in the arterial phase of dynamic CT or MRI. These EASL-modified RECIST criteria will used to evaluate response as a secondary endpoint of this study.

The mRECIST for HCC has introduced the following amendments to RECIST in the determination of tumor response for target lesions:

- Complete response: the disappearance of any intratumoral arterial enhancement in all target lesions
- Partial response: at least a 30% decrease in the sum of diameters of viable (contrast enhancement in the arterial phase) target lesions, taking as reference the baseline sum of the diameters of target lesions

- Progressive disease: an increase of at least 20% in the sum of the diameters of viable (enhancing) target lesions, taking as reference the smallest sum of the diameters of viable (enhancing) target lesions recorded since the treatment started
- Stable disease: any cases that do not qualify for either partial response or progressive disease

The measurement of the longest diameter of the viable tumor may be challenging in lesions showing partial internal necrosis. The following points should be taken into account in such cases:

- The measurement of the viable tumor should be performed on CT or MRI obtained in the arterial phase, when the contrast between viable vascularized tumor tissue and nonenhancing necrotic tissue is the highest.
- The longest diameter of the viable tumor is not necessarily located in the same scan plane in which the baseline diameter was measured: a thorough careful evaluation of the CT or MRI scans is required.
- The measurement of the viable tumor diameter should not include any major intervening areas of necrosis.

5.2.2.2 NONTARGET LESIONS RESPONSE

The RECIST guideline provides the following definitions of the criteria used to determine the objective tumor response for nontarget lesions: complete response is the disappearance of all nontarget lesions; incomplete response/stable disease is the persistence of one or more nontarget lesions; and progressive disease is the appearance of one or more new lesions and/or unequivocal progression of existing nontarget lesions.

According to mRECIST for HCC, tumor necrosis should be taken into account when assessing the response of nontarget lesions. The disappearance of intratumoral arterial enhancement in nontarget lesions should be considered equivalent to the disappearance of nontarget lesions, and therefore, should declare complete response of nontarget lesions. The persistence of intratumoral arterial enhancement in one or more nontarget lesions should be considered equivalent to persistence of one or more nontarget lesions, and therefore, should declare incomplete response / stable disease. The appearance of one or more new lesions and/or unequivocal progression of existing nontarget lesions should declare progressive disease.

Special recommendations for the assessment of tumor response in nontarget lesions in patients with HCC and cirrhosis can be made regarding the following points:

1. Portal vein thrombosis. Malignant portal vein thrombosis should be considered a nonmeasurable lesion due to the difficulty in performing consistent measurements of the malignant thrombus during the course of the treatment. Measurements of the extent of the malignant thrombus may be impaired by the possible presence of a bland component of the thrombosis.

2. Porta hepatis lymph node. Lymph nodes detected at the portal hepatis can be considered as malignant if the lymph node short axis is at least 20 mm. Evidence of reactive lymph nodes at the porta hepatis, in fact, is a common finding in patients with cirrhosis regardless of the presence of an HCC. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumor.
3. Pleural effusion and ascites. The original RECIST publication specifies that cytologic confirmation of the neoplastic nature of any effusion that appears or worsens during treatment is required when the measurable tumor has met criteria for response or stable disease. Under such circumstances, the cytologic examination of the fluid collected will permit differentiation between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease (if the neoplastic origin of the fluid is confirmed). The mRECIST for HCC panel of experts considered this issue to be of high importance in the setting of HCC in cirrhosis. The emergence or the increase in ascites is a common event during the course of treatment in a cirrhotic patient, which may be due to worsening of the underlying chronic liver disease and be unrelated to cancer progression. Other effusions, such as pleural effusion, may also be unrelated to cancer progression and be caused by the liver insufficiency. Thus, the mRECIST for HCC emphasizes that cytopathologic confirmation of the neoplastic nature of any effusion (particularly ascites) that appears or worsens during treatment is required when the measurable tumor has met criteria for response or stable disease. It has to be underlined that peritoneal carcinomatosis is a very rare event in HCC.

5.2.2.3 NEW LESIONS

Characterization of a newly detected focal liver lesion as true HCC is a challenging issue in the setting of cirrhosis because pathologic abnormalities related to cirrhosis changes—such as large regenerative nodules and dysplastic nodules—may be indistinguishable from a small tumor. Moreover, the clear-cut separation of the hepatic phases of liver enhancement routinely achieved by state-of-the-art CT or MRI creates additional problems in a cirrhotic liver, mostly related to the presence of perfusion abnormalities resulting in areas of abnormal liver enhancement. In most cases, such perfusion abnormalities are detected as arterially hyperenhancing areas caused by a selective impairment of the portal venous feeding. Such perfusion abnormalities may ultimately mimic or conceal focal liver lesions; hence, they represent an additional major source for interpretation errors.

The AASLD practice guideline for the clinical management of HCC has recommended strict criteria for the imaging diagnosis of HCC in cirrhosis. Noninvasive diagnostic criteria of HCC can be made without histology in lesions of at least 1 cm in diameter showing characteristic vascular features of HCC—arterial hypervascularization with washout in the portal venous or the late phase—at dynamic imaging studies. For diagnostic purposes, two imaging techniques—CT and MRI—are required for such a confirmation in tumors of 1 to 2 cm in diameter, and one imaging technique in tumors beyond 2 cm in cirrhotic patients.

In the assessment of tumor progression, these concepts have been adopted by the mRECIST assessment proposal, considering some specificities for the frame of progression mode:

- A newly detected hepatic nodule will be classified as HCC—and therefore will be declared as evidence of progression—when its longest diameter is at least 1 cm and the nodule shows the typical vascular pattern of HCC on dynamic imaging, that is, hypervascularization in the arterial phase with washout in the portal venous or late venous phase.
- Liver lesions larger than 1 cm that do not show a typical vascular pattern can be diagnosed as HCC by evidence of at least 1-cm-interval growth in subsequent scans.
- An individual radiologic event will be adjudicated in retrospect as progression at the time it was first detected by imaging techniques, even if strict criteria were fulfilled only on subsequent radiologic testing.

5.2.2.4 Overall Response Assessment

In mRECIST for HCC, identical to conventional RECIST, overall patient response is a result of the combined assessment of target lesions, nontarget lesions, and new lesions. It is important to point out that appearance of one or more new lesions declares progression whatever the response of target and nontarget lesions. Overcalling of equivocal lesions as new HCC, therefore, has a major impact on the outcome of studies with a radiologic endpoint, such as tumor response or time to progression. Hence, any newly detected focal liver lesion that does not meet the criteria reported above should be considered equivocal and not conclusive for disease progression.

5.3 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov). All appropriate treatment areas should have access to a copy of the CTEP Active Version of CTCAE.

5.4 SAMPLE STORAGE, TRACKING AND DISPOSITION

See Section 11.5

6 STATISTICAL CONSIDERATIONS

The primary purpose of this study is to evaluate the ability of a combination of sorafenib and TRC105 to improve upon the time to progression of patients with HCC.

The study will be conducted with both a phase I trial and then a phase II trial.

6.1 PHASE I PORTION

In the phase I trial, a conventional 3+3 design was used and required a maximum of 6 patients per dose level.

As of July 24, 2014, we completed accrual to the phase I portion of this study and established a recommended phase II dose of TRC-105 15mg/kg every two weeks in combination with sorafenib 400mg bid.

6.2 PHASE II PORTION

Based upon the largest reported trial of sorafenib in an identical patient population, the median time to progression in patients with this disease was shown to be 5.5 months. Patients enrolled on this study will receive scans every 8 weeks. Thus, the third scan would take place at approximately 6 months. If the median time to progression were 5.5 months, this would be equivalent to having 47% without progression at 6 months. Thus, the primary endpoint of this study will be the proportion of patients who are progression-free after 6 months on-study.

The sample size and interim stopping rule will be determined using a Simon optimal two-stage design. A median 5.5 month time to progression, translating into a 6 month progression free survival rate of 47%, would be considered not promising (p0=0.47), while a 67% 6-month PFS rate (corresponding to a median time to progression of 10.5 months) would be considered promising (p1=0.67). With alpha=0.10 and beta=0.10, 21 patients will be accrued to the study in the first stage.

If 10 or fewer patients demonstrate lack of progression on imaging after 6 months (three scans) on-study, the accrual to the study will be terminated early and the trial declared to have a negative result. A pause in the accrual may be necessary to ensure that enrollment may continue to the second stage. If 11 or more patients are progression-free at 6 months, enrollment will be extended to accrue a total of 45 patients. If 11-25 have not progressed by 6 months, this will be considered insufficient to warrant further study, while if 26 or more of 45 patients are progression-free after their third scan (at 6 months) the treatment will be declared effective and worthy of further testing. Under the null hypothesis (47% without progression by 6 months), the probability of early termination is 61%. A Kaplan-Meier curve of progression free survival and of survival will also be constructed in addition to evaluating the proportions without progression by the third scan.

Amendment L, version dated July 31, 2014:

Starting with Amendment L version dated July 31, 2014, patients will be enrolled on the phase II portion and will be evaluated according to the language below.

Results from previous studies suggest that an overall response rate for sorafenib alone in this patient population was between 2 and 8%. The primary objective of this portion of the trial is to establish if the combination of sorafenib and TRC105 is able to be associated with an improved overall response rate. This trial will be conducted using an optimal two-stage phase II trial design (Simon R, Controlled Clinical Trials 10:1-10, 1989) in order to rule out an unacceptably low PR+ 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 first stage will initially enroll 6 evaluable patients, and if 0 of the 6 have a clinical response, then no further patients will be accrued. If 1 or more of the first 6 patients has a clinical response, then accrual would continue until a total of 23 patients have been enrolled. As it may take several weeks to determine if a patient has experienced a response, a temporary pause in the accrual may be necessary to ensure that enrollment to the second stage

is warranted. If there are 1 to 2 clinical responses in 23 patients, this would be an uninterestingly low response rate. If there were 3 or more complete responses in 23 patients (13.0%), this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (5% response rate), the probability of early termination is 73.5%.

6.3 ACCRUAL AND STUDY DURATION

This trial has accrued 19 patients in phase I and no additional patients will be accrued as accrual for the phase I portion is complete. An additional 23 patients may be required for the phase II portion of the study. Thus, a total of 19+23 patients may be needed to complete the accrual for this study. In order to allow for the possibility of a small number of inevaluable patients, the accrual ceiling will be set at 44 patients. If 1-2 patients are accrued onto this trial per month, accrual for the phase II portion is expected to be completed within 1 to 2 years.

7 HUMAN SUBJECTS PROTECTIONS

7.1 RATIONALE FOR SUBJECT SELECTION

Subjects treated on this study, will be individuals with advanced hepatocellular carcinoma (HCC), which has recurred (or persisted) after appropriate standard treatment. Individuals of any race or ethnic group will be eligible for this study. Eligibility assessment will be based solely on the patient's medical status. Recruitment of patients onto this study will be through standard CCR mechanisms. No special recruitment efforts will be conducted.

7.2 PARTICIPATION OF CHILDREN

Individuals under the age of 18 will not be eligible to participate in this study because they are unlikely to have hepatocellular carcinoma, and because of unknown toxicities in pediatric patient.

7.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The potential benefit to a patient that goes onto study is a reduction in the bulk of their tumor which may or may not have favorable impact on symptoms and/or survival. Potential risks include the possible occurrence of any of a range of side effects which are listed in the Consent Document. The procedure for protecting against or minimizing risks will be to medically evaluate patients on a regular basis as described.

7.4 RISKS/BENEFITS ANALYSIS

For patients with hepatocellular carcinoma cancer, median survival is in the range of 6 months. Potential risks include the possible occurrence of any of a range of side effects listed. Risk of baseline biopsy: All care will be taken to minimize risks that may be incurred by tumor sampling. However, there are procedure-related risks (such as bleeding, infection and visceral injury) that will be explained fully during informed consent. If patients suffer any physical injury as a result of the biopsies, immediate medical treatment is available at the NIH's Clinical Center in Bethesda, Maryland. Although no compensation is available, any injury will be fully

evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

7.5 CONSENT PROCESS AND DOCUMENTATION

Patients will meet with an associate or principal investigator on the trial in the GI oncology Clinic, during the initial evaluation for this study. During that meeting, the investigator will inform patients of the purpose, alternatives, treatment plan, research objectives and follow-up of this trial. The investigator will then provide a copy of the IRB-approved informed consent document that is included in this protocol. The patient will be allowed to take as much time as he wishes, in deciding whether or not he wishes to participate. If a prolonged period of time expires during the decision making process (several weeks, as an example), it may be necessary to reassess the patient for protocol eligibility. The original signed consent goes to Medical Records; copy placed in research record (NIH policy).

All patients must have a signed informed consent form and an on-study (confirmation of eligibility) form filled out and signed by a participating investigator before entering on the study.

7.5.1 Reconsent via Telephone

The informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signaturewill sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records.

The informed consent process will be documented on a progress note by the consenting investigator and a copy of the informed consent document and note will be kept in the subject's research record.

7.5.2 Informed Consent of non-English Speaking Subjects

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OSHRP SOP 12, 45 CFR 46.117 (b) (2), and 21 CFR 50.27 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation (using either the long translated form or the short form). Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of

the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process for non-English speaking subjects and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

8 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

8.1 DEFINITIONS

8.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. All AEs must be recorded on the AE case report form.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported as per sections **8.2** and **8.3.**

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

8.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

8.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

8.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

8.1.4.1 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.5 Disability

A substantial disruption of a person's ability to conduct normal life functions.

8.1.6 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

8.1.7 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB approved research protocol.

8.1.8 Non-Compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

8.1.9 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and

  (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.2 NCI-IRB REPORTING

8.2.1 NCI-IRB Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report to the NCI-IRB:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received by the NCI-IRB within 7 working days of PI awareness via iRIS.

8.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
   - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
   - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
   - All Grade 5 events regardless of attribution;

- All Serious Events regardless of attribution.

NOTE: Grade 1 events are not required to be reported.

8.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

8.3 IND SPONSOR REPORTING CRITERIA

An investigator must immediately report to the sponsor, using the mandatory MedWatch form 3500a, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

Study endpoints that are serious adverse events (e.g. all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the drug and the event (e.g. death from anaphylaxis). In that case, the investigator must immediately report the death to the sponsor.

Events will be submitted to Dr. William Dahut, authorized representative for the IND Sponsor (CCR) at:

William Dahut, M.D.
Bldg 10, Room 3-2571 MSC 1206
10 Center Drive
Bethesda, MD 20892
Telephone: 301-496-4251
William.Dahut@nih.gov

Copy all MedWatch forms to: nciprotocolsupportoffice@mail.nih.gov

8.4 FDA REPORTING CRITERIA

8.4.1 IND Safety Reports to the FDA (Refer to 21 CRF 312.32)

8.4.1.1 Expedited reporting to the FDA

The Sponsor will notify the FDA of any unexpected fatal or life-threatening suspected adverse reactions as soon as possible but no later than 7 calendar days of initial receipt of the information.

The Sponsor is also responsible for reporting any:

- suspected adverse reaction that is both serious and unexpected
- any findings from clinical, epidemiological, or pooled analysis of multiple studies or any findings from animal or in vitro testing that suggest a significant risk in humans exposed to the drug

- clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure

to the FDA and to all investigators no later than 15 calendar days after determining that the information qualifies for reporting using the MedWatch Form 3500a. If FDA requests any additional data or information, the sponsor must submit it to the FDA as soon as possible, but no later than 15 calendars days after receiving the request.FDA Annual Reports (Refer to 21 CRF 312.32)

8.4.2 FDA Annual Reports (Refer to 21 CFR 312.33)

The study Sponsor will submit a brief report annually of the progress of the trial within 60 days of the anniversary date that the IND went into effect as indicated in 21CFR 312.33 and any associated FDA correspondences regarding the IND annual report.

8.4.3 Expedited Adverse Event Reporting Criteria to the IND Manufacturer (Tracon Pharmaceuticals, Inc.)

The initial telephone/facsimile report should contain as much information as is available concerning the event in order to permit Tracon Pharmaceuticals, Inc. to file a report, which satisfies regulatory requirements. Initial telephone report of SAE must be followed-up by a completed SAE report (Medwatch form 3500a signed by the investigator) faxed to the Medical Monitor at Tracon Pharmaceuticals, Inc. or designee. The event should also be entered into the source documents and case report forms, as appropriate.

If only limited information is initially available, follow-up reports will be required. A completed SAE form report should follow the initial phone call or incomplete fax report within 5 working days for all SAEs.

When additional information is available, a follow-up SAE report form should be faxed to the Medical Monitor at Tracon Pharmaceuticals, Inc. or designee.

Any adverse events that are initially ‘non-serious’ but become ‘serious’ shall be reportable according to the time of the most recent classification.

One serious adverse event report (SAER) form will be completed per serious adverse event (SAE). The SAE term should match with the term captured on the AE page of the CRF. In order to avoid vague, ambiguous, or colloquial expressions, the AE term should be recorded in standard medical terminology.

For all serious adverse events, the Principal Investigator or an Associate Investigator will be responsible for completing the SAE Report Form and will fax the SAE Form **within 24 hours** of their knowledge of the event to the Medical Monitor at Tracon Pharmaceuticals, Inc:

Charles Theuer, MD, PhD
Tracon Pharmaceuticals, Inc.
8910 University Center Lane, Suite 700
San Diego, CA 92122
Direct Phone: (858) 550-0780 x233
Cell Phone: (858) 344-9400

Facsimile: (858) 550-0786

8.5 RECORDING OF ADVERSE EVENTS

Adverse events will be assessed from the time the patient receives the first dose of TRC105 through completion of the final end-of-treatment assessment. All new events, as well as those that worsen in intensity or frequency relative to baseline, must be captured. All adverse events will be recorded on case report forms. The Investigator or his staff should elicit information regarding the occurrence of adverse events through information volunteered by the patient, open-ended questioning of the patient, physical examination results and review of laboratory results.

Information to be recorded for each adverse event includes:

- CTC AE Term
- The date and time of onset of the event
- The date and time of resolution of the event
- Assessments of severity, causal relationship to study drug, and seriousness of the adverse event.
- Action(s) taken (if any) for management of the adverse event, including but not limited to change in the study drug administration (e.g., temporary interruption in dosing, dose reduction); drug treatment; non-drug treatment; diagnostic procedures performed
- Outcome of the adverse event: patient recovered without sequelae; patient recovered with sequelae; event ongoing; patient died

If a laboratory abnormality is one component of a diagnosis or syndrome, then only the diagnosis or syndrome should be records on the AE page of the CRF. If the abnormality was not a part of a diagnosis or syndrome, then the abnormality should be recorded as the AE.

For causality assessment, the Investigator must assess whether the event was related to the study medications, concurrent drug therapy, underlying cancer, concurrent illness, a combination of these factors, or if it is unknown.

Adverse events characterized as intermittent require documentation of onset and duration of each episode.

Events resulting from concurrent illnesses, reactions to concurrent illnesses, reactions to concurrent medications, or progression of disease states must be reported as adverse events.

Any medical condition that is present at the time that the patient is screened but does not deteriorate should not be reported as an adverse event. However, if it deteriorates at any time during the study, it should be recorded as an adverse event.

All adverse events will be followed to adequate resolution/stabilization. An adverse event will be considered to have resolved if either of the following events has occurred:

- The principal investigator has documented that all abnormal symptoms, physical signs, and laboratory abnormalities associated with the adverse event have returned to normal (normal is defined as the baseline conditions for that individual established at the screening visit) or satisfactory stabilization has been reached.

- In cases where resolution/stabilization is not obvious, the Investigator should obtain written confirmation from the sponsor that the event can be considered resolved and that further follow-up is unnecessary.

8.5.1 Reported Adverse Events Related to TRC105

See Section **1.2.3.5**

8.6 DATA AND SAFETY MONITORING PLAN

8.6.1 Principal Investigator/Research Team

A formal DSMB will not be required for this study; the principal investigator, research nurse, and research team will monitor the study for AEs on an ongoing basis for determining DLTs and MTD. All data will be collected in a timely manner and reviewed by the principal investigator or an associate investigator for adverse events. In addition, stopping rules are in place for ensuring safe conduct of the trial. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8.6.2 Sponsor Monitoring Plan

This trial will be monitored by personnel employed by Harris Technical Services on contract to the NCI, NIH, who are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

At least 25% of enrolled patients' records will be randomly selected and monitored biannually or as needed, based on accrual rate. The patients selected will have 100% source document verification done. Additional monitoring activities will include: adherence to protocol specified study eligibility, treatment plans, data collection for safety and efficacy, reporting and time frames of adverse events to the NCI IRB and FDA, and informed consent requirements. Written reports will be generated in response to the monitoring activities and submitted to the Principal Investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

9 PHARMACEUTICAL INFORMATION

9.1 TRC105 (NSC #754227)

TRC105 is an IgG1, kappa immunoglobulin containing murine light- and heavy-chain variable region sequences and human constant region sequences. TRC105 has an approximate molecular weight of 148 kDa.

9.1.1 TRC105 Packaging and Labeling

TRC105 may be provided in one or more of the following presentations.

Phosphate Buffered Saline Formulation (7 mg TRC105/mL)

210 mg TRC105/30 mL single-use vial

20 mM L-Histidine/L-Histidine Monohydrochloride, 240 mM Trehalose, 0.01% Polysorbate 20 Formulation (25 mg TRC105/mL)

100 mg TRC105/4mL single-use vial

400 mg TRC105/16 mL single-use vial

Vials of TRC105 are labeled with one of the following:

TRC105
NSC# 754227
210 mg per vial (7 mg/mL, 30 mL vial)
Store refrigerated at 2-8°C.
For Intravenous Use Only. Single-use vial.
Lot: XXXXXXX Mfg Date: XX/XX/XXXX
Caution: New Drug Limited by
Federal (or United States) law to investigational use.
TRACON Pharmaceuticals Inc., San Diego, CA 92122 USA

TRC105
NSC# 754227
100 mg per vial (25 mg/mL, 4 mL vial)
Store refrigerated at 2-8°C.
For Intravenous Use Only. Single-use vial.
Lot: XXXXXXX Mfg Date: XX/XX/XXXX
Caution: New Drug Limited by
Federal (or United States) law to investigational use.
TRACON Pharmaceuticals Inc., San Diego, CA 92122 USA

TRC105
NSC# 754227
400 mg per vial (25 mg/mL, 16 mL vial)
Store refrigerated at 2-8°C.
For Intravenous Use Only. Single-use vial.
Lot: XXXXXXX Mfg Date: XX/XX/XXXX
Caution: New Drug Limited by
Federal (or United States) law to investigational use.
TRACON Pharmaceuticals Inc., San Diego, CA 92122 USA

For information regarding current formulation, please refer to Section **1.2.3.6**, TRC105 Physical, Chemical, and Pharmaceutical Properties and Formulations.

9.1.2 TRC105 Storage and Shipping

TRC105 must be stored upright between 2 °C and 8 °C (36 °F to 46 °F).

9.1.3 TRC105 Preparation

TRC105 will be prepared in the pharmacy and diluted into normal saline using appropriate aseptic technique. TRC105 will be administered using an in-line 0.2 micron filter. No incompatibilities between TRC105 and polyvinyl chloride or polyolefin bags have been observed. Multiple vials will be required for a single dose. The following formulae should be used to calculate the volume of TRC105 to be added to normal saline:

- Patient weight (kg) × dose level (mg/kg) divided by TRC105 concentration (mg/mL) = volume of TRC105 (mL) to be administered.

The volume of TRC105 that is to be administered can be rounded up or down to the nearest 1.0 mL; in the case of an increment of 0.5 mL the volume should be rounded up. The maximum weight that should be used for dose calculation in this study is 85 kg for women and 100 kg for men (note: there is not a weight restriction for enrollment purposes). If the patient's weight changes by > 10% during the study, the dose of TRC105 will be recalculated. At that time a new baseline weight will be established such that subsequent weight changes by >10% from the new baseline weight would require further recalculation of the TRC105 dose. The calculated volume of TRC105 will be diluted with normal saline. Appropriate judgment should be exercised in withdrawing an adequate amount of saline necessary to permit injection of the appropriate volume of antibody into a normal saline bag in accordance with the dose needed. The final TRC105 concentration must be between 0.3 mg/mL and 10 mg/mL. The prepared TRC105 must be gently inverted several times in order to ensure a homogeneous solution. The diluted infusion solution of TRC105 should be used within 8 hours of preparation if stored at room temperature, or within 24 hours of dilution if stored at 2° to 8°C (36º to 46ºF). The expiration time should be labeled on the bag. If the diluted infusion solution of TRC105 cannot be infused within 8 hours of preparation (i.e.: the prepared infusion is at room temperature for more than 8 hours), a second bag will be prepared that contains the balance of the planned dose that was not already delivered. The prepared solution should not be frozen.

9.1.4 TRC105 Handling and Disposal

The Investigator should not return clinical study materials to TRACON unless specifically instructed to do so by TRACON. All used or expired vials of TRC105 should be retained. A TRACON representative will periodically conduct an accountability of the used or expired vials and authorize their destruction.

If the participating pharmacy is prohibited by institutional policy from retaining open or expired vials, the Site Pharmacist will then be responsible for documenting the destruction of the vials and completing an Investigational Drug Product Destruction Form.

9.1.5 Adverse Events and potential safety risks based on ongoing and completed studies of TRC105

Grade 3 anemia has occurred with TRC105 therapy at the recommended phase 2 dose of 10 mg/kg weekly. All patients treated with TRC105 should be monitored closely for anemia and treated appropriately, including the possibility of TRC105 dose reductions. Anemia may be caused by correctable mineral or vitamin deficiency. The anemia related to TRC105 is

hypoproductive in nature and is reversible with interruption of treatment, transfusion, erythropoietin, and other interventions as appropriate.

Gastrointestinal hemorrhage has occurred with TRC105 therapy. Patients with active ulcer disease or risk factors for ulcer disease are excluded from this study.

Grade 1 and 2 cutaneous telangiectasia related to TRC105 occur early in the course of therapy and have been the source of gingival bleeding and epistaxis. Telangiectasia are also seen in patients with hereditary hemorrhagic telangiectasia (HHT), a disease of CD105 haplotype insufficiency. Patients with HHT are at risk of hemorrhage from abnormal blood vessels and this could be exacerbated by treatment with TRC105. Other contraindications to TRC105 therapy include a history of significant hemorrhage or tumors located in the central chest or another location where bleeding is associated with high morbidity. All patients treated with TRC105 should be monitored for signs of hemorrhage and the risks and benefits of drug treatment reevaluated in any patient with hemorrhage.

Premedication including the use of glucocorticoids is required prior to infusion of TRC105 to reduce the frequency and severity of infusion reactions. Infusion reactions following TRC105 dosing generally occur with the first TRC105 dose and include a grade 4 vasovagal reaction that resolved without sequelae. Signs and symptoms of TRC105 infusion reactions include hypertension, hypotension, dyspnea, bronchospasm, chills/rigors, chills, sweats, fever, nausea, tachycardia, bradycardia, EKG changes, flushing, urticaria, pruritus, and headache, generally of grade 1 and 2 severity. Potential infusion reactions seen with other therapeutic antibodies include angioedema, asthenia, throat irritation, rhinitis, vomiting, joint pain, fatigue and neurologic disorders including inflammation of the spine and/or brain.

Hypersensitivity reactions with infusions are a potential risk for sensitized patients, and TRC105 should be used with caution in patients with known hypersensitivity to any component of the drug product. Host anti-TRC105 antibodies to the murine or human portions of CHO-produced TRC105 are rare. In general, the risk of immunogenicity to therapeutic chimeric antibodies is small (<10%) and the clinical significance of immunogenicity is not well defined. The current trial will collect serial blood samples for anti-product antibody concentrations to further characterize the immunogenicity of TRC105 and potential clinical implications.

Grade 3 cerebrovascular hemorrhage resulting in hemiparesis occurred in one patient with hepatocellular cancer who was thrombocytopenic (who entered the study with a platelet count of 60,000/uL) in a study of TRC105 with sorafenib. Patients must have a platelet count of > 100,000/uL to enter this study (see inclusion criteria). Transient Grade 3 hepatic encephalopathy occurred in one patient with cirrhosis and hepatocellular carcinoma who received TRC105 in combination with sorafenib. Additionally, Grade 5 intracranial hemorrhage occurred in one glioblastoma patient with markedly abnormal blood clotting parameters in a study of TRC105 with bevacizumab. A patient with glioblastoma developed temporary confusion and slurred speech following treatment with TRC105 and bevacizumab that required hospitalization for observation. Another patient with glioblastoma, who underwent resection and had a history of an abnormal collection of cerebral spinal fluid, developed a grade 2 cerebral spinal fluid leak.

Grade 3 myocardial infarction (non-Q wave infarct associated with hypertension following an infusion reaction) was observed in a patient with hepatocellular cancer following treatment with

TRC105 that resolved without sequelae. In addition, a Grade 5 myocardial infarction occurred in a patient with coronary artery disease who received TRC105 in combination with sorafenib. Patients with evidence of active coronary artery disease are excluded from participation in this trial (see exclusion criteria).

Adult respiratory distress syndrome that required temporary intubation occurred in one patient who received TRC105 with pazopanib, from which the patient recovered. Of note, interstitial lung disease has been added as an adverse drug reaction and warning/precaution to the core safety information for pazopanib.

Infections have been observed rarely. Grade 3 infected lipoma/cyst was observed in a Phase 2 study of TRC105 as a single agent in patients with metastatic bladder cancer. Grade 3 orbital cellulitis and grade 3 brain abscess were observed in patients treated with TRC105 and bevacizumab and considered possibly related to TRC105. Grade 1 and 2 gingivitis including infection and ulceration has also been observed. Overall, infections have been observed in fewer than 5% of patients and have largely been considered unrelated to treatment with TRC105.

Grade 1-3 headaches have been observed following TRC105 treatment, generally within hours following completion of the initial infusion. Headaches are throbbing in nature, are not associated with radiographic abnormalities, and have responded to treatment with non-steroidal anti-inflammatory agents and to triptans. Headaches were particularly common when TRC105 and bevacizumab were initially dosed on the same day and were ameliorated when TRC105 was dosed one week following bevacizumab dosing and given over two days during the initial week of dosing.

Nasal congestion and periorbital edema have been observed with TRC105 dosing, particularly when dosed in combination with bevacizumab. The edema has been transient in nature and treated with corticosteroids.

9.1.6 Fatigue of grade 1- 3 severity has been reported following dosing with TRC105. Maculopapular rash and skin flushing of grade 1 and grade 2 severity have also been reported. A patient receiving treatment with TRC105 and sorafenib developed self-limited pancreatitis of grade 2 severity.TRC105 Drug Accountability

The Investigator must maintain an accurate accounting of TRC105 supplied by TRACON. During the study, the following information must be recorded:

- Date of receipt, quantity and identification of the study drug received from TRACON
- ID number of the patient to whom the product is dispensed
- The date(s) and quantity of the product dispensed
- Dates and quantity of product returned, lost or accidentally or deliberately destroyed

Investigational Drug Accountability Logs should be maintained by the site and must be readily available for inspection.

9.1.7 Agent Ordering

TRC105 will be supplied by Tracon Pharmaceuticals.

9.2 SORAFENIB (NEXAVAR ®; BAY 43-9006; NSC 724772)

9.2.1 Product Description

Chemical Name: 4–{4-[3-(4-chloro-3-trifluoromethyl-phenyl) ureido]-phenoxy}-pyridine-2carboxylic acid methylamide-4-methylbenzensulfonate.

Other Names: BAY 54-9085 is the tosylate salt of BAY 43-9006; Nexavar®.

Classification: Kinase inhibitor (Raf, VEGF-R, and PDGF-R)

Mechanism of Action: The ras/raf signaling pathway is an important mediator of responses to growth signals and angiogenic factors. This pathway is often aberrantly activated in human tumors due to presence of activated ras, mutant b-raf, or over expression of growth factor receptors.

BAY 43-9006 is a potent inhibitor of c-raf, and wild-type and mutant b-raf in vitro. Additionally, further characterization of BAY 43-9006 tosylate revealed that this agent inhibits several receptor tyrosine kinases (RTKs) that are involved in tumor progression (VEGF-R, PDGF-R, Flt3, and c-KIT) and p38α, a member of the MAPK family.

Molecular Formula: $C_{12}H_{16}ClF_3N_4O_3$ X $C_7H_8O_3S$

M.W.: BAY 43-9006 tosylate: 637 Daltons; BAY 43-9006 (free base): 465 Daltons

Approximate Solubility: 0.19 mg/100 mL in 0.1 N HCl, 453 mg/100 mL in Ethanol, and 2971 mg/100 mL in PEG 400.

9.2.2 Storage Requirements

Store at controlled room temperature (15ºC – 25ºC). Storage conditions should not exceed 25ºC.

9.2.3 Stability

Stability studies with the 200 mg dosage form are ongoing. The current shelf life is 24 months when stored at controlled room temperature.

9.2.4 Administration

Oral sorafenib tosylate should be taken with at least 250 mL of water and can be given without regards to meals. Food does not appear to have a clear effect on sorafenib tosylate pharmacokinetics.

Sorafenib tosylate is supplied as an immediate-release film-coated, round, and salmon color tablet containing 200 mg of the free base, BAY 43-9006, and the excipients croscarmellose sodium, microcrystalline cellulose, hydroxypropylmethylcellulose, sodium lauryl sulfate, and magnesium stearate. The film-coat consists of hydroxypropylmethyl cellulose, polyethylene glycol, titanium dioxide and red iron oxide. The film coating has no effect on the rate of release of the active sorafenib tosylate.

Sorafenib tosylate 200 mg tablets are supplied in bottles of 140 tablets.

9.2.5 Reported Adverse Events and Potential Risks:

Comprehensive Adverse Events and Potential Risks list (CAEPR) for Sorafenib (BAY 43-9006, NSC 724772)

Adverse Events with Possible Relationship to Sorafenib (BAY 43-9006) (CTCAE 4.0 Term) [n= 2157]			**EXPECTED AEs FOR ADEERS REPORTING Agent Specific Adverse Event List (ASAEL)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	**Expected**
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia***
	Febrile neutropenia		
CARDIAC DISORDERS			
		Acute coronary syndrome	
		Left ventricular systolic dysfunction	
		Myocardial infarction	
GASTROINTESTINAL DISORDERS			
Abdominal pain			***Abdominal pain***
	Anal mucositis		
	Ascites		
	Constipation		***Constipation***
Diarrhea			***Diarrhea***
	Gastrointestinal hemorrhage[2]		***Gastrointestinal hemorrhage[2]***
		Gastrointestinal perforation[3]	
	Mucositis oral		
Nausea			***Nausea***
	Rectal mucositis		
	Small intestinal mucositis		
	Vomiting		***Vomiting***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Edema limbs		
Fatigue			***Fatigue***
	Fever		***Fever***
	Non-cardiac chest pain		
IMMUNE SYSTEM DISORDERS			
		Anaphylaxis	
INFECTIONS AND INFESTATIONS			
	Infection[4]		
INVESTIGATIONS			
	Activated partial thromboplastin time prolonged		***Activated partial thromboplastin time prolonged***
Alanine aminotransferase increased			***Alanine aminotransferase increased***
Alkaline phosphatase increased			***Alkaline phosphatase increased***
Aspartate aminotransferase increased			***Aspartate aminotransferase increased***
Blood bilirubin increased			***Blood bilirubin increased***
	Cholesterol high		
Creatinine increased			***Creatinine increased***
	GGT increased		

INR increased			***INR increased***
	Investigations - Other (bicarbonate, serum-low)		
Lipase increased			***Lipase increased***
Lymphocyte count decreased			***Lymphocyte count decreased***
	Neutrophil count decreased		***Neutrophil count decreased***
Platelet count decreased			***Platelet count decreased***
Serum amylase increased			***Serum amylase increased***
Weight loss			***Weight loss***
White blood cell decreased			***White blood cell decreased***
METABOLISM AND NUTRITION DISORDERS			
Anorexia			***Anorexia***
	Hypercalcemia		
Hyperglycemia			***Hyperglycemia***
	Hyperkalemia		***Hyperkalemia***
	Hypernatremia		
	Hyperuricemia		
Hypoalbuminemia			***Hypoalbuminemia***
Hypocalcemia			***Hypocalcemia***
	Hypoglycemia		***Hypoglycemia***
	Hypokalemia		***Hypokalemia***
Hyponatremia			***Hyponatremia***
Hypophosphatemia			***Hypophosphatemia***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		***Arthralgia***
	Back pain		***Back pain***
	Bone pain		
	Musculoskeletal and connective tissue disorder - Other (muscle spasms)		
	Myalgia		
	Pain in extremity		***Pain in extremity***
NERVOUS SYSTEM DISORDERS			
	Dizziness		
	Headache		***Headache***
		Intracranial hemorrhage	
	Peripheral sensory neuropathy		
		Reversible posterior leukoencephalopathy syndrome	
PSYCHIATRIC DISORDERS			
	Insomnia		
RENAL AND URINARY DISORDERS			
	Acute kidney injury		
	Hematuria		
	Renal hemorrhage		
REPRODUCTIVE SYSTEM AND BREAST DISORDERS			
	Hematosalpinx		
	Ovarian hemorrhage		
	Prostatic hemorrhage		
	Spermatic cord hemorrhage		
	Testicular hemorrhage		

	Uterine hemorrhage		
	Vaginal hemorrhage		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Bronchopulmonary hemorrhage		
	Cough		***Cough***
	Dyspnea		***Dyspnea***
	Epistaxis		
	Laryngeal mucositis		
	Pharyngeal mucositis		
	Tracheal mucositis		
	Voice alteration		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
Alopecia			***Alopecia***
	Dry skin		***Dry skin***
		Erythema multiforme	
Palmar-plantar erythrodysesthesia syndrome			***Palmar-plantar erythrodysesthesia syndrome***
	Pruritus		***Pruritus***
Rash maculo-papular			***Rash maculo-papular***
		Stevens-Johnson syndrome	
VASCULAR DISORDERS			
	Hypertension		***Hypertension***
	Thromboembolic event		

[1]This table will be updated as the toxicity profile of the agent is revised.
[2]Gastrointestinal hemorrhage includes Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal hemorrhage, and Upper gastrointestinal hemorrhage under the GASTROINTESTINAL DISORDERS SOC.

[3]Gastrointestinal perforation includes Colonic perforation, Duodenal perforation, Esophageal perforation, Gastric perforation, Ileal perforation, Jejunal perforation, Rectal perforation, and Small intestinal perforation under the GASTROINTESTINAL DISORDERS SOC.

[4]Includes all 75 infection sites under the INFECTIONS AND INFESTATIONS SOC.
Also reported on sorafenib (BAY 43-9006) trials but with the relationship to sorafenib (BAY 43-9006) still undetermined:

CARDIAC DISORDERS - Atrial fibrillation; Atrial flutter; Chest pain - cardiac; Sinus bradycardia; Sinus tachycardia; Supraventricular tachycardia
EAR AND LABYRINTH DISORDERS - Tinnitus
ENDOCRINE DISORDERS - Hyperthyroidism; Hypothyroidism
EYE DISORDERS - Blurred vision; Cataract; Extraocular muscle paresis
GASTROINTESTINAL DISORDERS - Abdominal distension; Dyspepsia; Dysphagia; Flatulence; Ileus; Pancreatitis; Rectal fistula; Small intestinal obstruction
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Edema face; Flu like symptoms; Pain
IMMUNE SYSTEM DISORDERS - Allergic reaction
INVESTIGATIONS - Fibrinogen decreased
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypermagnesemia; Hypertriglyceridemia; Hypomagnesemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis; Generalized muscle weakness
NERVOUS SYSTEM DISORDERS - Dysgeusia; Encephalopathy; Ischemia cerebrovascular; Memory impairment; Syncope
PSYCHIATRIC DISORDERS - Confusion; Depression
RENAL AND URINARY DISORDERS - Proteinuria
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Erectile dysfunction

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Hypoxia; Pleural effusion; Pneumonitis; Pneumothorax
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Hyperhidrosis; Purpura; Rash acneiform; Skin and subcutaneous tissue disorders - Other (non-life threatening squamous cell carcinoma of skin: keratoacanthoma type); Skin hypopigmentation
VASCULAR DISORDERS - Flushing; Hypotension; Vasculitis

Note: Sorafenib (BAY 43-9006) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

Body as a whole:	Fatigue, flu-like syndromes, fever, arthralgia
Gastrointestinal:	Diarrhea, pancreatitis, elevated amylase/lipase, abdominal pain/cramping, nausea, flatulence, dyspepsia
Hepatic:	Increased bilirubin, ALT, AST, GGT, LDH, and alkaline phosphatase
Metabolic and Nutritional:	Anorexia
Skin:	Hand-foot syndrome, characterized by palmar and plantar erythema; rash/desquamation, hypersensitivity reactions, dry skin, alopecia, nail changes, vitiligo
Cardiovascular:	Hypertension.

The following adverse events have been reported on trials but the relationship to Sorafenib still undetermined: Arthritis, brain stem stroke, dyspnea, increase PT/PTT, and weight loss.

9.2.6 Drug interactions

Sorafenib is metabolized by the P450 CYP3A enzyme and has been shown in preclinical studies to inhibit multiple CYP isoforms. Therefore, it is possible that BAY43-9006 tosylate may interact with drugs that are metabolized by the P450 CYP isoenzymes or with drugs that inhibit CYP 3A. Close monitoring is recommended for patients taking agents with narrow therapeutic indices and metabolized by the liver, such as warfarin, quinidine and digoxin. Additionally, Sorafenib is 97% to 99% protein bound; however, no drug interactions have been reported in studies, thus far.

9.2.7 Availability

Sorafenib is FDA approved for the indication used in this study, and will be purchased by the Clinical Center Pharmacy from commercial sources.

9.2.8 Agent Accountability

Sorafenib, an oral self-administered investigational agent, will be accounted for, handled, and disposed of in accordance with CCR Policy # Clin-1, “Policy on Documenting, Handling and

Disposing of Oral Investigational Agents that are Self-Administered by NCI CCR patients." The Standard Operating Procedure # Clin-1, "Standard Operating Procedure for Conducting and Documenting Drug Accountability for Oral Investigational Agents that are Self-Administered by Patients at the CCR" identifies activities associated with drug accountability and compliance monitoring for orally self-administered investigational agents. Study drug will be dispensed in bottles of 140 tablets. Each patient will be instructed to document in a study diary the daily intake of sorafenib, including the time the dose is taken and whether or not any doses are missed, and to date and sign the entry. They will bring the study diary and any unused drug to their next scheduled clinic appointment. Clinic staff will (1) collect all "old" [i.e., empty bottle(s), partial bottle(s) or full bottle(s)] of study drug; (2) perform a capsule count and record the results on the approved CCR Pill Count Case Report Form which is to be maintained in the research record; and (3) dispense the new partial and full bottle(s) of sorafenib to the patient. Unused study drugs are to be returned to the research nurse who will dispose of them according to the SOP.

10 REFERENCES

1. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. Int J Cancer 2001;94:153-6.
2. Yoo HY, Patt CH, Geschwind JF, Thuluvath PJ. The outcome of liver transplantation in patients with hepatocellular carcinoma in the United States between 1988 and 2001: 5-year survival has improved significantly with time. J Clin Oncol 2003;21:4329-35.
3. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378-90.
4. Gish RG, Porta C, Lazar L, et al. Phase III randomized controlled trial comparing the survival of patients with unresectable hepatocellular carcinoma treated with nolatrexed or doxorubicin. J Clin Oncol 2007;25:3069-75.
5. Leung TW, Patt YZ, Lau WY, et al. Complete pathological remission is possible with systemic combination chemotherapy for inoperable hepatocellular carcinoma. Clin Cancer Res 1999;5:1676-81.
6. Yeo W, Mok TS, Zee B, et al. A randomized phase III study of doxorubicin versus cisplatin/interferon alpha-2b/doxorubicin/fluorouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma. J Natl Cancer Inst 2005;97:1532-8.
7. Abou-Alfa GK, Huitzil-Melendez FD, O'Reilly EM, Saltz LB. Current management of advanced hepatocellular carcinoma. Gastrointest Cancer Res 2008;2:64-70.
8. Louafi S, Boige V, Ducreux M, et al. Gemcitabine plus oxaliplatin (GEMOX) in patients with advanced hepatocellular carcinoma (HCC): results of a phase II study. Cancer 2007;109:1384-90.
9. Cheng AL, Kang YK, Chen Z, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol 2009;10:25-34.
10. G. K. Abou-Alfa PJ, J. Knox, I. Davidenko, J. Lacava, T. Leung, A. Mori, M. Le Berre, D. Voliotis, L. Saltz Final results from a phase II (PhII), randomized, double-blind study of sorafenib plus doxorubicin (S+D) versus placebo plus doxorubicin (P+D) in patients (pts) with advanced hepatocellular carcinoma (AHCC). In: GI ASCO; 2008; 2008.
11. Shen Y, Hsu C, Lin Z, et al. A phase II study of sorafenib in combination with tegafur/uracil (UFT) for Asian patients with advanced hepatocellular carcinoma (HCC). J Clin Oncol (Meeting Abstracts) 2009;27:4589-.
12. Petrini I, Lencioni M, Ricasoli M, et al. A phase II (PhII) trial of sorafenib (S) in combination with 5-fluorouracil (5FU) continuous infusion (c.i.) in patients (pts) with advanced hepatocellular carcinoma (HCC): Preliminary data. J Clin Oncol (Meeting Abstracts) 2009;27:4592-.

13. Azad NS, Posadas EM, Kwitkowski VE, et al. Combination Targeted Therapy With Sorafenib and Bevacizumab Results in Enhanced Toxicity and Antitumor Activity. J Clin Oncol 2008;26:3709-14.

14. Saltz LB, Clarke S, Diaz-Rubio E, et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. J Clin Oncol 2008;26:2013-9.

15. Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer 2008;8:592-603.

16. Huynh H, Ngo VC, Fargnoli J, et al. Brivanib alaninate, a dual inhibitor of vascular endothelial growth factor receptor and fibroblast growth factor receptor tyrosine kinases, induces growth inhibition in mouse models of human hepatocellular carcinoma. Clin Cancer Res 2008;14:6146-53.

17. Raoul JL, Finn RS, Kang YK, et al. An open-label phase II study of first- and second-line treatment with brivanib in patients with hepatocellular carcinoma (HCC). J Clin Oncol (Meeting Abstracts) 2009;27:4577-.

18. Melillo G. Targeting hypoxia cell signaling for cancer therapy. Cancer Metastasis Rev 2007;26:341-52.

19. Rapisarda A, Hollingshead M, Uranchimeg B, et al. Increased antitumor activity of bevacizumab in combination with hypoxia inducible factor-1 inhibition. Mol Cancer Ther 2009;8:1867-77.

20. Rapisarda A, Uranchimeg B, Sordet O, Pommier Y, Shoemaker RH, Melillo G. Topoisomerase I-mediated inhibition of hypoxia-inducible factor 1: mechanism and therapeutic implications. Cancer Res 2004;64:1475-82.

21. Yang LY, Lu WQ, Huang GW, Wang W. Correlation between CD105 expression and postoperative recurrence and metastasis of hepatocellular carcinoma. BMC Cancer 2006;6:110.

22. Li DY, Sorensen LK, Brooke BS, et al. Defective angiogenesis in mice lacking endoglin. Science 1999;284:1534-7.

23. Barbara NP, Wrana JL, Letarte M. Endoglin is an accessory protein that interacts with the signaling receptor complex of multiple members of the transforming growth factor-beta superfamily. J Biol Chem 1999;274:584-94.

24. Lebrin F, Goumans MJ, Jonker L, et al. Endoglin promotes endothelial cell proliferation and TGF-beta/ALK1 signal transduction. EMBO J 2004;23:4018-28.

25. Li C, Gardy R, Seon BK, et al. Both high intratumoral microvessel density determined using CD105 antibody and elevated plasma levels of CD105 in colorectal cancer patients correlate with poor prognosis. Br J Cancer 2003;88:1424-31.

26. Guo B, Slevin M, Li C, et al. CD105 inhibits transforming growth factor-beta-Smad3 signalling. Anticancer Res 2004;24:1337-45.

27. Bockhorn M, Tsuzuki Y, Xu L, Frilling A, Broelsch CE, Fukumura D. Differential vascular and transcriptional responses to anti-vascular endothelial growth factor antibody in orthotopic human pancreatic cancer xenografts. Clin Cancer Res 2003;9:4221-6.

28. Tan GH, Huang FY, Wang H, Huang YH, Lin YY, Li YN. Immunotherapy of hepatoma with a monoclonal antibody against murine endoglin. World J Gastroenterol 2007;13:2479-83.

29. Davis DW, Inoue K, Dinney CP, Hicklin DJ, Abbruzzese JL, McConkey DJ. Regional effects of an antivascular endothelial growth factor receptor monoclonal antibody on receptor phosphorylation and apoptosis in human 253J B-V bladder cancer xenografts. Cancer Res 2004;64:4601-10.

30. Ferrara N. VEGF and the quest for tumour angiogenesis factors. Nat Rev Cancer 2002;2:795-803.

31. Bergers G, Song S, Meyer-Morse N, Bergsland E, Hanahan D. Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. J Clin Invest 2003;111:1287-95.

32. Apperley JF, Gardembas M, Melo JV, et al. Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. N Engl J Med 2002;347:481-7.

33. Sawyers CL. Finding the next Gleevec: FLT3 targeted kinase inhibitor therapy for acute myeloid leukemia. Cancer Cell 2002;1:413-5.

34. Heinrich MC, Blanke CD, Druker BJ, Corless CL. Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies. J Clin Oncol 2002;20:1692-703.

35. Alavi A, Hood JD, Frausto R, Stupack DG, Cheresh DA. Role of Raf in vascular protection from distinct apoptotic stimuli. Science 2003;301:94-6.

36. Yamashita T, Forgues M, Wang W, et al. EpCAM and alpha-fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma. Cancer Res 2008;68:1451-61.

37. Yamashita T, Budhu A, Forgues M, Wang XW. Activation of hepatic stem cell marker EpCAM by Wnt-beta-catenin signaling in hepatocellular carcinoma. Cancer Res 2007;67:10831-9.

38. Spano JP, Chodkiewicz C, Maurel J, et al. Efficacy of gemcitabine plus axitinib compared with gemcitabine alone in patients with advanced pancreatic cancer: an open-label randomised phase II study. Lancet 2008;371:2101-8.

39. Dahlberg SE, Sandler AB, Brahmer JR, Schiller JH, Johnson DH. Clinical Course of Advanced Non-Small-Cell Lung Cancer Patients Experiencing Hypertension During Treatment With Bevacizumab in Combination With Carboplatin and Paclitaxel on ECOG 4599. J Clin Oncol.

40. Ryanne Wu R, Lindenberg PA, Slack R, Noone AM, Marshall JL, He AR. Evaluation of hypertension as a marker of bevacizumab efficacy. J Gastrointest Cancer 2009;40:101-8.

41. Schneider BP, Wang M, Radovich M, et al. Association of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 genetic polymorphisms with outcome in a trial of paclitaxel compared with paclitaxel plus bevacizumab in advanced breast cancer: ECOG 2100. J Clin Oncol 2008;26:4672-8.

42. Miller K, Wang M, Gralow J, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 2007;357:2666-76.

43. Schneider BP, Radovich M, Miller KD. The role of vascular endothelial growth factor genetic variability in cancer. Clin Cancer Res 2009;15:5297-302.

44. Ng SS, Gutschow M, Weiss M, et al. Antiangiogenic activity of N-substituted and tetrafluorinated thalidomide analogues. Cancer Res 2003;63:3189-94.

45. Lake RA, Robinson BW. Immunotherapy and chemotherapy--a practical partnership. Nat Rev Cancer 2005;5:397-405.

46. Nowak AK, Robinson BW, Lake RA. Synergy between chemotherapy and immunotherapy in the treatment of established murine solid tumors. Cancer Res 2003;63:4490-6.

47. Finke JH, Rini B, Ireland J, et al. Sunitinib reverses type-1 immune suppression and decreases T-regulatory cells in renal cell carcinoma patients. Clin Cancer Res 2008;14:6674-82.

48. Ko JS, Zea AH, Rini BI, et al. Sunitinib mediates reversal of myeloid-derived suppressor cell accumulation in renal cell carcinoma patients. Clin Cancer Res 2009;15:2148-57.

49. Llovet JM, Di Bisceglie AM, Bruix J, et al. Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst 2008;100:698-711.

11 APPENDICES

11.1 APPENDIX 1: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

11.2 APPENDIX 2: CHILD-PUGH CLASSIFICATION SYSTEM

Parameter	Points assigned		
	1	2	3
Ascites	Absent	Slight	Moderate
Bilirubin	<2 mg/dL (<34.2 micromol/liter)	2-3 mg/dL (34.2 to 51.3 micromol/liter)	>3 mg/dL (>51.3 micromol/liter)
Albumin	>3.5 g/dL (35 g/liter)	2.8-3.5 g/dL (28 to 35 g/liter)	<2.8 g/dL (<28 g/liter)
Prothrombin time			
Seconds over control	<4	4-6	>6
INR	<1.7	1.7-2.3	>2.3
Encephalopathy	None	Grade 1-2	Grade 3-4

Modified Child-Pugh classification of the severity of liver disease according to the degree of ascites, the plasma concentrations of bilirubin and albumin, the prothrombin time, and the degree of encephalopathy. A total score of 5-6 is considered grade A (well-compensated disease); 7-9 is grade B (significant functional compromise); and 10-15 is grade C (decompensated disease). These grades correlate with one- and two-year patient survival: grade A - 100 and 85 percent; grade B - 80 and 60 percent; and grade C - 45 and 35 percent.

11.3 APPENDIX 3: CORRELATIVE STUDY SPECIMEN PROCESSING PROCEDURES

11.3.1 Molecular characterization of HCC using tumor tissue, blood and urine (Xin Wang lab):

Processing of Blood Samples:

1) Centrifuge green top tube(s) and red top tube(s), approximately 850 g (2000 rpm on Sorval T 6000) for 10 minutes to separate components.
2) Withdraw plasma (green tops) and aliquot into sterile cryovials at 1.5 ml per vial; withdraw serum (red tops) and aliquot into sterile cryovials at 0.5 ml per vial.
3) Label each vial appropriately with printed bar code label (i.e., plasma normal [from green top tube], serum normal [from red top tube]).
4) From the green top tubes, remove the buffy coat from the red blood cell pellet; this appears as a white/pink layer on top of the RBCs, and can be done with a serological pipette or a transfer pipette. Place buffy coat in a cryovial, wash once with Phosphate Buffered Saline (PBS), and centrifuge as above to precipitate cells into a pellet. Aspirate supernatant, label vial appropriately (as buffy coat normal) with printed bar code label.
5) From the red top tube, pour the RBC/clot into a 15 ml plastic tube, label appropriately with a bar code label, and place in cryobox in -80 C freezer.
6) For collection of RBCs, add PBS to green top tubes to resuspend the RBC pellet, and centrifuge for 5 min. at 850 g (2000 rpm). Aspirate PBS and repeat this wash step. After the second wash, re-suspend the cells in an equal volume of PBS, aliquot 1.5 ml each into up to 10 cryovials, and centrifuge as above. Aspirate PBS, and label vials appropriately (as RBC normal).
7) Place all plasma, serum, buffy coat, and RBC vials in cryobox in -80 C freezer.

Processing of mouthwash:

1) Transfer specimen from the container to a 50 ml centrifuge tube.
2) Rinse container with about 10 ml of Listerine or PBS (Phosphate Buffered Saline) and add to 50 ml tube.
3) Centrifuge tube at 2700 rpm for 15 minutes to pellet cells.
4) Discard the supernatant carefully so as not to disturb pellet.
5) Wash the cell pellet in 25 ml of 1 X TE buffer and again centrifuge at 2700 rpm for 15 minutes to pellet cells.
6) Discard the supernatant and re-suspend the pellet in 100 ul 1 X TE
7) buffer facilitated by vortexing.
8) Aliquot the sample into two 1.8 ml labeled NUNC Cryovials and store samples in cryobox in -80 C freezer.

Processing of urine:

1) Using a 25 ml serological pipette, aliquot urine into 13 ml plastic tubes, with up to10 ml into each tube, using up to 5 tubes.
2) Label tubes with printed bar code labels and place in cryobox in -80C freezer.

11.3.2 Genotyping for SNPs in angiogenesis-related genes (eg VEGFR)(Figg lab):

1. Samples will be stored at 4C until time of extraction.
2. A Qiagen Maxi Prep Kit will be used for extraction.
3. Resulting DNA will be stored at -80C until time of analysis.

11.3.3 Circulating endothelial cells (CECs), Circulating endothelial progenitor (CEP) cells, soluble CD105 (baseline) and Rat aortic ring bioassay.

11.3.3.1 Processing of CECs and CEPs:

11.3.3.2 Processing of VEGF samples:

1. Centrifuge at 1200*xg* for 5 minutes at 4°C.
2. Transfer plasma to 2X 1ml tubes and immediately freeze on dry ice
3. Store at -80°C.

11.3.3.3 Processing of soluble CD105 samples:

1. Centrifuge the 5mL K_2EDTA (lavender top) blood collection tube at 2500 x g for 15 minutes.
2. Transfer the plasma into a 5mL plastic test tube and repeat centrifuge at 2500 x g for 15 minutes. Use a fresh plastic test tube for each time point.
3. Transfer the plasma equally into two pre-labeled 2mL lavender top clear polypropylene storage cryovials. Use a fresh transfer pipette for each time point.
4. Place the 2mL clear polypropylene storage cryovials with lavender top in the cryovial storage box provided and freeze immediately. Store the specimens in a freezer at -80°C. Once frozen, do not allow the specimens to thaw, including during shipment.

11.3.3.4 Processing of Rat aortic ring assay samples:

1. Centrifuge at 1200*xg* for 5 minutes at 4°C.
2. Transfer serum to 2X 1ml tubes
3. Store at -80°C.

11.3.4 Immunomonitoring (Greten lab).

1)Pour the heparinized blood from the blood sample tubes into 50 ml Falcon tubes and spin at 1800 rpm, 10', 21°C

2)Transfer the plasma (SN) of the centrifugated Falcon tubes into a fresh Falcon tube make aliquots in 1,8 ml cryotubes, store at -80°C

3)Resuspend pellet with RPMI to a total volume of 35 ml and overlay the Ficoll slowly with the diluted blood. Spin at 2100 rpm, 17', 21°C without brake.

4)Remove the lymphocyte ring and also the media and Ficoll with a 10 ml shorty-pipette and transfer to a new 50 ml Falcon tube (discard only the pellet) fill up to 50 ml with RPMI. Spin at 1600 rpm, 13', 21°C, discard the SN transfer the cells (of the same individual,

from multiple Falcon tubes) to a single Falcon in 25 ml RPMI. Spin at 1600 rpm, 13', 21°C, discard the SN resuspend the pellet in 25 ml RPMI and spin at 1300 rpm, 5', 21°C, discard the SN.

5) Resuspend the pellet in 10 ml or 20 ml RPMI (according to the pellet) count the cells in a counting chamber (1/2 or 1/5 in Trypan Blue) separate the cells according to the aliquots that you want to freeze. Spin 1500 rpm, 5', 21°C, discard the SN. Prepare fresh freezing media use 1ml freezing media/cryotube store the cryotubes in ""Mr. Frosty"" at -80°C for one day, then store in liquid N2.

11.4 APPENDIX 4: STUDY CALENDAR

All baseline and follow-up evaluations can be performed during the last week of the prior cycle (+/- 48 hours).

	Pre-study	**Cycle 1**		**Cycle 2 +**		**Cycle 3 Day 1**	**Off Treatment**
Day		**1**	**15**	**1**	**15**	**1**	
TRC105[a]		X	X	X	X	X	
Sorafenib		Daily continuous self-administration					
Premedications[b]		X	X	X	X	X	
Informed consent	X						
Demographics	X						
Medical history	X	X	X	X	X	X	X
Adverse event evaluation		X	X	X	X	X	X
Physical exam	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X
Height	X						
Weight	X	X	X	X	X	X	
Performance Status	X	X	X	X	X	X	X
CBC w/differential, Platelets	X	X	X	X	X	X	X
PT, PTT, Fibrinogen	X	X	X	X	X	X	X
Serum chemistry [c]	X	X	X	X	X	X	X
Serum AFP	X			X		X	
ECG	X						
Urine Protein-creatinine Ratio (UPC)[d]	X			X		X	
Restaging radiologic Evaluation[e]	X					X	
DCE-MRI[h,i]	X	X		X			
Ultrasound[i]	X	X		X		X	
Angiogenic biomarkers[f]	X		X		X		
Tumor biopsy		X				X	
HAMA/HACA[g]	X						X

	Pre-study	**Cycle 1**		**Cycle 2 +**		**Cycle 3 Day 1**	**Off Treatment**
TRC105 Pharmacokinetic		X	X[j]				X[k]
Urine for Metabolomic Analysis		X					

a: TRC105: Dose as assigned; administered I.V. every two week without planned rest/break. One cycle = 28 days

b: Premedications: Thirty minutes to two hours prior to the start of each TRC105 infusion, all patients will receive the following pre-medications: Acetaminophen 650 mg PO x 1; Dexamethasone 20 mg IV x 1; Famotidine 20 mg IV x 1 (or similar H2 blocker); cetirizine 10 mg IV x 1 For details please see section 3.5.

c: Sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, amylase, albumin, total protein, calcium, magnesium, phosphorous, AST, ALT, alkaline phosphatase, total bilirubin, uric acid

d: For UPC ratio > 1.0, a 24-hour urine protein will need to be obtained and the level should be < 2000 mg for patient enrollment . UPC will be performed every other cycle.

e: CT chest, abdomen, pelvis every 8 weeks.

f: see section 3.5 for details as to pharmacodynamic timepoints/angiogenic biomarkers to be drawn.

g: see section 3.5 for details; draw prestudy, pre-dose every 3rd cycle, at end of study, and at month 3 and 6 after end of study. If still positive, draw at month 9 and 12 after end of study.

h. DCE-MRI will be performed at the following timepoints: 1) Prior to treatment (preferably within 7 days of treatment start; 2) 24-36 hours after Cycle 1 Day 1 and 3) 28 +/- 2 days after Cycle 1 Day 1

i. Optional Studies

j. Pretreatment and 5 minutes post completion of TRC 105 infusion

k. Trough Only

11.5 APPENDIX 5: CORRELATIVE STUDY SPECIMEN AND DATA STORAGE PROCEDURES

11.5.1 Sample Data Collection

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in the LABrador (aka LabSamples) utilized by the BPC. This is a secure program, with access to LABrador limited to defined Figg lab personnel, who are issued individual user accounts. Installation of LABrador is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen. All Figg lab personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.

LABrador creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without LABrador access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

11.5.2 Sample Storage and Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BCP and offsite at NCI Frederick Central Repository Services (Fisher BioServices) in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in LABrador. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Samples will remain in storage only for those patients who have provided consent for their samples to be collected for future research. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested), and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the LABrador. It is critical that the sample remains linked to patient information such as race, age,

dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

Any new uses of human subject samples collected during the course of this trial must be reviewed and approved by the NCI IRB. Any loss or unintentional destruction of the samples will be reported to the IRB.

INSTITUTE: National Cancer Institute

STUDY NUMBER: 11-C-0102 PRINCIPAL INVESTIGATOR: Tim Greten, M.D.

STUDY TITLE: A Phase I/II Study of TRC105 in Combination with Sorafenib in Hepatocellular Carcinoma (HCC)

Continuing Review Approved by the IRB on 09/14/15
Amendment Approved by the IRB on 07/07/16 (N) Date posted to web: 07/13/16

Standard

INTRODUCTION

We invite you to take part in a research study at the National Institutes of Health (NIH).

First, we want you to know that:

Taking part in NIH research is entirely voluntary**.**

You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled. However, to receive care at the NIH, you must be taking part in a study or be under evaluation for study participation.

You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with your NIH doctors or research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone at NIH, or with family, friends or your personal physician or other health professional.

Why is this study being done?

This study is divided into two phases. In the phase 1 portion, we determined the highest safe dose of an experimental cancer drug called TRC105, when given with another cancer drug, sorafenib (also called Nexavar®). You are being asked to participate in Phase 2 of this study. To our knowledge, this is the first time TRC105 has been given with sorafenib in humans.

Sorafenib has been approved by the FDA for treatment of kidney and liver cancer (hepatocellular carcinoma - HCC). It has been shown to prolong survival in patients with HCC. Unfortunately, we know that after a period of time the cancer tends to start growing again. This study will try to improve the cancer fighting ability of sorafenib by giving it in combination with TRC105. This study is being done in collaboration with Tracon Pharmaceuticals, Inc., the makers of TRC105.

TRC105 is not approved by the U.S. Food and Drug Administration (FDA) and is experimental. TRC105 is a man-made antibody (a special immune system protein) that blocks the body's steps for making new blood vessels grow, a process called angiogenesis. All solid tumors require new blood vessels to grow. Sorafenib is in a class of medications call 'multikinase inhibitors' and works by slowing the spread of cancer cells. It is thought therefore, that giving patients with HCC tumors TRC105 in combination with sorafenib, we will stop the growth of their tumors or cause tumors to shrink, better than with sorafenib alone.

Why are you being asked to take part in this study?

You are being asked to participate because you have hepatocellular carcinoma (HCC) that has not responded to other types of therapy, and your doctor has determined that you are not a candidate for liver transplantation.

How many people will take part in this study?

To date, the phase 1 portion of this study has enrolled 19 patients. The phase 2 portion of this study will enroll up to a maximum of 23 patients.

Description of Research Study

What will happen if you take part in this research study?

Before you begin the study

All research studies have specific criteria for entry to allow for valid interpretation of the study results and safety of participants, known as eligibility criteria. Before you begin this study you will need to have the following exams and tests to make sure you are eligible for this study. The exams and tests are part of regular cancer care and may be done even if you do not join the study. If you recently had some of the tests, they may not need to be repeated.

- History and physical examination, including vital signs (height, weight, blood pressure, heart rate, temperature, breathing rate)

- Standard blood tests to measure your liver and kidney function, white blood cells, red blood cells and platelets, your blood sugar and blood electrolytes. If you are a woman able to get pregnant, you will also have a pregnancy test done.

- Scans, x-rays and an ultrasound of your tumors to measure your disease.

- Electrocardiogram within 16 days prior to your first dose of TRC105.

- HIV Testing: As part of this study, we will test you for infection with the human immunodeficiency virus (HIV), the virus that causes AIDS. If you are infected with HIV you will not be able to participate in this study. We will tell you what the results mean, how to find care, how to avoid infecting others, how we report newly diagnosed HIV infection, and the importance of informing your partners at possible risk because of your HIV infection.

- Endoscope exam of your esophagus to look for varices (swollen or enlarged blood vessels). Because bleeding is a possible side effect of all cancer drugs that limit blood vessel growth, patients with varices will not be eligible to participate.

If you meet the eligibility criteria for this study you will be offered the option of participating.

During the study

Everyone enrolled in the study will take the same dose of sorafenib (two pills) two times every day, in the morning and at night. The TRC 105 will be given through a vein (intravenously) once every two weeks on days 1 and 15 of a 28 day cycle. In the phase 1 portion of the study we found the highest dose that will be given in the phase 2 portion. Everyone enrolled in phase 2 portion of the study will receive the same dose of sorafenib and TRC 105. The TRC 105 dose was determined in the phase 1 part of this study.

The TRC105 infusion will be given to you in the outpatient clinic, while the nurses and doctors watch you closely for any side effects. The sorafenib pills should be swallowed whole with a full glass (8 ounces) of water in the morning and night every day for the 28 days of the cycle. You can take it with or without food.

To help prevent known side effects of the TRC105 you will be given a steroid, Dexamethasone, through your vein 30 minutes to 2 hours before you receive TRC105. Infusion of any antibody has the potential to cause an allergic-type of infusion reaction, and giving the steroid helps prevent this. In addition, 30 minutes before you receive the TRC105 you will be given cetirizine (or similar drug) to help prevent infusion reactions, in addition to acetaminophen (Tylenol) to prevent chills and fever and famotidine (or similar drug) to prevent stomach upset. The first

dose of TRC105 will be given over a period of 4 hours, and if you do not have side effects, the next dose may be given over two hours. If the second dose is tolerated, subsequent doses can be given over at least one hour.

If you experience side effects (described later in this consent) during the TRC105 infusion, the infusion may be slowed or stopped, or treated with additional medicines, if necessary. If the side effects are too severe, the doctor may decide to stop all further doses of TRC105. If the side effects are not too severe, subsequent doses may be delayed until you have time to recover. The dosages of both TRC105 and sorafenib may be adjusted after the first cycle to reduce the severity of your side effects, if the doctor determines your side effects are not too severe.

At each visit during the first cycle you will have a physical examination and blood tests, which are the same as what you would have done with your regular cancer care, including blood tests for blood counts and chemistries. We will also do a urine test every cycle to evaluate your kidney functions. We will ask you questions about how you are feeling and any medications you may have taken to treat your symptoms. Every third cycle (about every three months) you will have standard scans and x-rays to evaluate your tumor(s). After the first cycle the physical examination and blood tests will be repeated at the beginning of each new cycle (each month).

Research Tests

An important part of this study is testing the effects of TRC105 in combination with sorafenib on your body and your cancer. We would also like to do research studies on the characteristics and genetic analysis of your type of cancer (HCC). These studies will study the DNA or genes that are important in your cancer and may be causing it to grow. We will not be studying your 'normal' genes however. In other words, we will only be studying the genes or DNA in your cancer and not those in normal parts of your body. We therefore will not be able to look at hereditary genes, or ones that cause conditions to 'run in families'. To do these studies we will collect blood, mouth wash, urine, and tissue specimens (optional). We will also look at the blood levels of various markers of angiogenesis (blood vessel growth). These tests are being done for research purposes only, and therefore the results will be preliminary and will not be reported back to you or your referring physician.

To do these tests, we would like to take blood samples before your first infusion, on Day 15 of your first cycle, Day 1 and 15 of your second cycle, Day 1 of your third cycle, and after your last treatment. We may ask you to give us a mouth wash sample before starting therapy.

In addition, we will take 1 teaspoon of blood before your first dose and then on Day 1 of every 3rd cycle, and 3 and 6 months after you stop TRC105 to see if your body is making immune cells against the antibody.

In total we would draw about 31 tablespoons of blood over the course of this study. The amount of blood taken for these research tests is within the safe guidelines for research blood volumes set by the NIH. In adults that limit is 37 tablespoons in an 8-week period.

If the doctors think that we can obtain a piece of your tumor (biopsy) through a needle (percutaneously) with minimal risk to you, we will take a biopsy when you start the study and during week 8 of therapy. You will be given the chance to decide if you want to participate at the time of the procedure. If you decide to have the biopsy, you will be given local anesthesia (numbing medicine) and a sedative prior to the biopsy. The biopsy will be taken through a needle put through the skin into your liver tumor. After the procedure you will have to lay on your right side for 2-4 hours, and the nurses will watch your blood pressure and other vital signs.

Research Scans

DCE-MRI: To study the changes in the tumor vessels you will be asked to undergo an MRI, called a dynamic contrast enhanced magnetic resonance imaging (or DCE-MRI) before starting treatment, 24 to 36 hours after your first dose of TRC105 and then at the end of the first cycle. MRI is a scan that uses a powerful magnetic field, radio frequency pulses and computer to take pictures of your bones, organs and tumor inside the body. DCE is a special test that uses contrast fluid to better see these body structures, including blood vessels. The radiologist will make sure it is safe for you to have this test, as you may not have any metal objects on or in your body.

Ultrasound of tumor blood vessels: Ultrasound imaging, also called ultrasound scanning or sonography, uses high-frequency sound waves to produce pictures of your tumor blood vessels inside of the body. Ultrasound exams do not use ionizing radiation (as used in x-rays). This test will be done before starting treatment, 24 to 36 hours after your first dose of TRC105 and then at the end of the first cycle.

When you are finished taking the drugs (treatment)

After completing treatment with TRC105, a nurse or doctor will contact you or your physician to get an update of your condition, at least every year.

Study Chart

Each Cycle

Day	**What to do and what will happen to you**
Before starting TRC105 and	Get routine blood tests. Provide a history of how you feel and undergo a physical examination by the

sorafenib	research team's Health Care Provider. Research blood, urine and mouthwash samples will be taken, and a tumor biopsy (optional). DCE-MRI and ultrasound may be done.
Day 1	Routine blood tests will be drawn; urine test will be done. 30 minutes to two hours before TRC105, take the premedications (acetaminophen, dexamethasone, cetirizine, famotidine and granisetron) Receive 1st dose of TRC105 in the vein (IV) in the Day Hospital over 4 hours Report any side effects to the research team. Research blood samples will be taken. Start sorafenib twice a day, with a full glass of water (8 oz).
24-36 hours after first dose of TRC105	DCE-MRI and ultrasound may be done. Continue taking sorafenib twice a day, with a full glass of water (8 oz).
Day 15	Return to the NIH clinic to see your doctor. Provide a history of how you feel and have a physical exam by your Health Care Provider. Blood will be taken for routine cancer care tests. If you are tolerating the TRC105, the next dose of the cycle will be given. Research blood samples will be taken. Continue taking sorafenib twice a day, with a full glass of water (8 oz).
Day 28 or 29 (also Day 1 of the next Cycle)	Return to the NIH clinic to see your doctor. Provide a history of how you feel and have a physical exam by your Health Care Provider. Blood will be taken for routine cancer care tests. If you are tolerating the drug well, a new treatment cycle will begin. Research blood samples will be taken. Continue taking sorafenib twice a day, with a full glass of water (8 oz). DCE-MRI and ultrasound may be done.
After every 2-3 Cycles	CT scan of the chest, abdomen and pelvis.
After stopping TRC105 and sorafenib	Return to the NIH clinic to see your doctor. Provide a history of how you feel and have a physical exam by your Health Care Provider. Blood will be taken for routine cancer care tests.

	Research blood samples will be taken.
Follow Up	At least yearly, a member of the research team will contact you or your referring physician to ask about your condition.

Birth Control

If you are a woman who is breast feeding or pregnant, you may not take part in the study because we don't know how this medicine would affect your baby or your unborn child. If you are a woman who can become pregnant, or are the partner of a woman who can become pregnant, you will need to practice an effective form of birth control before starting study treatment, during study treatment, and for 3 months after you finish study treatment. If you think that you or your partner is pregnant, you should tell your study doctor or nurse at once.

Effective forms of birth control include:

- abstinence
- intrauterine device (IUD)
- hormonal [birth control pills, injections, or implants]
- tubal ligation
- vasectomy

Risks or Discomforts of Participation

What side effects or risks can I expect from being in this study?

You may have side effects while you are taking the study drugs. Everyone taking part in the study will be watched carefully for any side effects. However, doctors don't know all the side effects that may happen, so it is important to report any changes that you may notice, even if your study team does not ask specifically about them. Side effects may be mild or severe. Your study team will give you medicines to help lessen side effects. Many side effects go away with those medicines and others may go away soon after you stop the study drugs. In some cases, side effects can be serious, long lasting, or may never go away. There are no known long-lasting

side effects from TRC105 or sorafenib at this time. If new information about side effects becomes available, we will make you aware of this new information.

During the phase I portion of the study one patient died related to a cardiac event. This patient had a preexisting heart condition and this is the suspected cause of death. We cannot exclude 100% that the protocol therapy could have contributed to the death.

TRC105

There have been about 50 patients treated with TRC105 so far in other studies. Generally, the side effects have been mild. The most common side effects that patients have mentioned were fatigue, chills, and diarrhea. The table below lists both the common and uncommon side-effects that have been seen with TRC105.

Risks and side effects related to TRC105 are detailed in the table below:

SIDE EFFECT	Mild	Severe, but not life threatening	Life Threatening
Common	• Fatigue • Back Pain* • Lip Pain • Anemia (low red blood cell count) • Abdominal pain* • Abdominal distension • Constipation • Diarrhea • Nausea • Infusion related reaction (chills, fever, flushing during the TRC105 infusion)		
Uncommon	• Loss of Appetite* • Vomiting • Joint pain • Headache • Abnormal blood tests related to liver function • Chills • Wheezing* • Fever	• Infusion related reaction (chills, fever, flushing during the TRC105 infusion) • Sinusitis • Cellulitis	

	• Heartburn* • Changes in taste • Flushing • Protein in the urine • Swelling of the legs* • Low potassium in the blood* • Difficulty sleeping* • Cough* • Coughing up blood* • Vaginal bleeding • Anal discomfort • Dry mouth • Gas and bloating • Chest pain • Thirst • Eye infection • Herpes zoster (viral infection which can affect nerves and cause pain, i.e. shingles) • infection and sores on the gums		
Rare		• Gastric ulcer • Small intestine blockage • Perforation of the intestine requiring surgery • Collapsed lung • Spinal fluid leak in a patient who underwent resection of a brain tumor and had a history of abnormal collection of spinal fluid	• Liver failure • Kidney failure • Mycardioal Infarction*

*These side effects are events that have occurred during clinical studies using TRC105, but it has not yet been determined whether the events were caused by TRC105.

Sorafenib

SIDE EFFECT	Mild	Severe, but not life threatening	Life Threatening
Common	• Hair loss • Inflammation of the skin on the palms of the hands or soles of the feet • Rash / flaking or shedding of outer layer of skin* • Fatigue or tiredness • Loss of appetite • Diarrhea • Nausea • Low levels of blood protein called albumin • Low blood phosphate level • Stomach ache • Laboratory test-related: increased liver enzymes or abnormal liver enzyme levels		
Uncommon	• Low blood counts: decrease in a red blood cell protein that carries oxygen in the body, decreased total number of white blood cells, decreased number of a type of white blood cell, decreased number of blood cells that help clot blood. • Stomach / Intestine Related: constipation, painful inflammation and ulceration of the lining of the mouth and digestive tract, vomiting. • Laboratory test-related: abnormal bone enzyme levels, abnormal digestive enzyme level, high	• Bleeding: Bleeding in the digestive tract; bleeding in the reproductive organs or urinary system (such as the bladder or kidney) • Breathing-related: shortness of breath, cough, fluid accumulation around the lungs (pleural	

	blood levels of a liver pigment indicative of abnormal liver function, low blood calcium level, high blood sugar, low blood sugar, abnormal level of fat-digesting enzyme, high blood potassium level, low blood potassium level, low blood sodium level. • Voice changes (for example hoarseness, loss or change in voice) • Dry skin, itching. • General: dizziness, fever (with or without a dangerously low white blood cell count), weight loss, infection (with or without a low white blood cell count), pain throughout the body (such as back pain, chest pain, leg pain, headache, joint pain, muscle pain), difficulty sleeping or falling asleep. • Swelling of the arms and legs	effusion), bleeding of the respiratory tract or lungs. • High blood pressure* • Nerve damage causing numbness, tingling, or burning • Allergic reaction	
Rare			• Kidney failure • Formation or presence of a blood clot inside a blood vessel, i.e. heart, lung • Heart failure • Hole in the bowel with Gastrointestinal (GI) bleeding • Bleeding into the brain • Neurological problem: leukoencephalop

			athy syndrome including reversible posterior leukoencephalopathy syndrome [RPLS]

*The skin rash, fatigue, diarrhea, and high blood pressure are reversible, and can usually be alleviated by reducing the dose of sorafenib. We will monitor your blood pressure carefully on the study, and if found to be elevated, a medication to lower your blood pressure can be used. If you have uncontrolled high blood pressure, this elevated pressure can cause damage to other organs including the heart, kidneys, or brain. Having uncontrolled high blood pressure increases the risk of heart attack and stroke in adults. Usually there are no symptoms of high blood pressure; however, dizziness, headache, or nose bleed can occur.

We will monitor your blood pressure carefully on the study, and if found to be elevated, a medication to lower your blood pressure can be used. If you have uncontrolled high blood pressure, this elevated pressure can cause damage to other organs including the heart, kidneys, or brain. Having uncontrolled high blood pressure increases the risk of heart attack and stroke in adults. Usually there are no symptoms of high blood pressure; however, dizziness, headache, or nose bleed can occur.

Dexamethasone:

Common	**Occasional**	**Rare and Serious**
• High blood pressure which may cause headaches, dizziness • Skin changes, rash, acne • Swelling of the body, tiredness, bruising • Weight gain in belly, face, back and shoulders • Pain in belly • Infection • Damage to the bone	• Cloudiness of the eye, visual disturbances • Non-healing wound • Heartburn • Kidney stones	• Blurred vision • Bleeding from sores in stomach • Broken bones

Biopsy of Liver Tumor:

The most common side effect of a liver biopsy is pain. Other rare complications include bleeding, which may rarely be severe enough to require a transfusion, puncturing a nearby organ, or very rarely (less than 1%) death. We will monitor you for 24 hours after your liver biopsy to rule-out bleeding.

DCE-MRI:

Most MRI exams are painless, however, some patients find it uncomfortable to remain still during MR imaging. Others experience a sense of being closed-in (claustrophobia). Therefore, sedation can be arranged for those patients who anticipate anxiety, but fewer than one in 20 require it. It is normal for the area of your body being imaged to feel slightly warm, but if it bothers you, notify the radiologist or technologist. It is important that you remain perfectly still while the images are being recorded, which is typically only a few seconds to a few minutes at a time. For some types of exams, you may be asked to hold your breath. When the contrast material is injected, it is normal to feel coolness and a flushing sensation for a minute or two. The intravenous needle may cause you some discomfort when it is inserted and once it is removed, you may experience some bruising. There is also a very small chance of irritation of your skin at the site of the IV tube insertion.

Ultrasound of tumor blood vessels:

This procedure is painless and requires no preparation.

Potential Benefits of Participation

Are there benefits to taking part in this study?

The aim of the study is to determine the highest safe dose of TRC105 when given in combination with sorafenib. We do not know if you will receive personal, medical benefit from taking part in this study. The potential benefits could include shrinking of your tumor or lessening of your symptoms, such as pain, that are caused by the cancer. Because there is not much information about the effects of this combination on HCC, we do not know if you will benefit from taking part in this study, although the knowledge gained from this study may help others in the future who have cancer.

Alternative Approaches or Treatments

What other choices do I have if I do not take part in this study?

Instead of being in this study, you have these options:

- Getting treatment or care for your cancer without being in a study
- Taking part in another study
- Getting comfort care, also called palliative care. This type of care helps reduce pain, tiredness, appetite problems and other problems caused by the cancer. It does not treat the cancer directly. Instead, it tries to improve how you feel. Comfort care tries to keep you as active and comfortable as possible.

Please talk to your doctor about these and other options.

Research Subject's Rights

What are the costs of taking part in this study?

If you choose to take part in the study, the following will apply, in keeping with the NIH policy:

- You will receive study treatment at no charge to you. This may include surgery, medicines, laboratory testing, x-rays or scans done at the Clinical Center, National Institutes of Health (NIH), or arranged for you by the research team to be done outside the Clinical Center, NIH if the study related treatment is not available at the NIH.
- There are limited funds available to cover the cost of some tests and procedures performed outside the Clinical Center, NIH. You may have to pay for these costs if they are not covered by your insurance company.
- Medicines that are not part of the study treatment will not be provided or paid for by the Clinical Center, NIH.
- Once you have completed taking part in the study, medical care will no longer be provided by the Clinical Center, NIH.

Will your medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- The National Cancer Institute (NCI) and other government agencies, like the Food and Drug Administration (FDA), which are involved in keeping research safe for people.
- National Cancer Institute Institutional Review Board
- The study Sponsor (Center for Cancer Research) or their agent(s).
- Qualified representatives from TRACON Pharmaceuticals, Inc., the pharmaceutical company who produces TRC105.

A description of this clinical trial will be available on http://www.Clinicaltrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most the Web site will include a summary of the results. You can search this Web site at any time.

Stopping Therapy

Your doctor may decide to stop your therapy for the following reasons:

- if he/she believes that it is in your best interest
- if your disease comes back during treatment
- if you have side effects from the treatment that your doctor thinks are too severe
- if new information shows that another treatment would be better for you

In this case, you will be informed of the reason therapy is being stopped.

You can stop taking part in the study at any time. However, if you decide to stop taking part in the study, we would like you to talk to the study doctor and your regular doctor first.

If you decide at any time to withdraw your consent to participate in the trial, we will not collect any additional medical information about you. However, according to FDA guidelines, information collected on you up to that point may still be provided to Tracon Pharmaceticals, Inc. or designated representatives. If you withdraw your consent and leave the trial, any samples of yours that have been obtained for the study and stored at the NCI can be destroyed upon request. However, any samples and data generated from the samples that have already been distributed to other researchers or placed in the research databases cannot be recalled and destroyed.

Conflict of Interest

The National Institutes of Health (NIH) reviews NIH staff researchers at least yearly for conflicts of interest. This process is detailed in a Protocol Review Guide. You may ask your research team for a copy of the Protocol Review Guide or for more information. Members of the research team who do not work for NIH are expected to follow these guidelines but they do not need to report their personal finances to the NIH.

Members of the research team working on this study may have up to $15,000 of stock in the companies that make products used in this study. This is allowed under federal rules and is not a conflict of interest.
The National Institutes of Health and the research team for this study are using a drug developed by Tracon Pharmaceuticals through a joint study with your researchers and the company. The company also provides financial support for this study.

Use of Specimens and Data for Future Research

To advance science, it is helpful for researchers to share information they get from studying human samples. They do this by putting it into one or more scientific databases, where it is stored along with information from other studies. A researcher who wants to study the information must apply to the database and be approved. Researchers use specimens and data stored in scientific databases to advance science and learn about health and disease.

We plan to keep some of your specimens and data that we collect and use them for future research and share them with other researchers. We will not contact you to ask about each of these future uses. These specimens and data will be stripped of identifiers such as name, address or account number, so that they may be used for future research on any topic and shared broadly for research purposes. Your specimens and data will be used for research purposes only and will not benefit you. It is also possible that the stored specimens and data may never be used. Results of research done on your specimens and data will not be available to you or your doctor. It might help people who have cancer and other diseases in the future.

If you do not want your stored specimens and data used for future research, please contact us in writing and let us know that you do not want us to use your specimens and/or data. Then any specimens that have not already been used or shared will be destroyed and your data will not be used for future research. However, it may not be possible to withdraw or delete materials or data once they have been shared with other researchers.

OTHER PERTINENT INFORMATION

1. Confidentiality. When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or authorized hospital accreditation organizations.

2. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. Payments. The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, patients are not paid for taking part in research studies at the National Institutes of Health. Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.

4. Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, Tim Greten, M.D., Building 10, Room 12N226 Telephone: 301-451-4723. You may also call the Clinical Center Patient Representative at (301) 496-2626. If you have any questions about the use of your specimens or data for future research studies, you may also contact the Office of the Clinical Director, Telephone: 301-496-4251.

5. Consent Document. Please keep a copy of this document in case you want to read it again.

COMPLETE APPROPRIATE ITEM(S) BELOW:

A. Adult Patient's Consent

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient/ Legal Representative _____ Date _____

Print Name _____

B. Parent's Permission for Minor Patient.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.

(Attach NIH 2514-2, Minor's Assent, if applicable.)

Signature of Parent(s)/ Guardian _____ Date _____

Print Name _____

C. Child's Verbal Assent (If Applicable)

The information in the above consent was described to my child and my child agrees to participate in the study.

Signature of Parent(s)/Guardian _____ Date _____ Print Name _____

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE FROM SEPTEMBER 14, 2015 THROUGH SEPTEMBER 13, 2016.

Signature of Investigator _____ Date _____

Print Name _____

Signature of Witness _____ Date _____

Print Name _____