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SAMUEL OSCHIN COMPREHENSIVE CANCER INSTITUTE

IIT2016-16-POSADAS-TRC105
A phase 2 study of TRC105 (anti-endoglin antibody) with abiraterone and with enzalutamide in patients progressing on therapy

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Results Reporting *(required)*
NCT No.: ***Pending***

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Signature Page

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

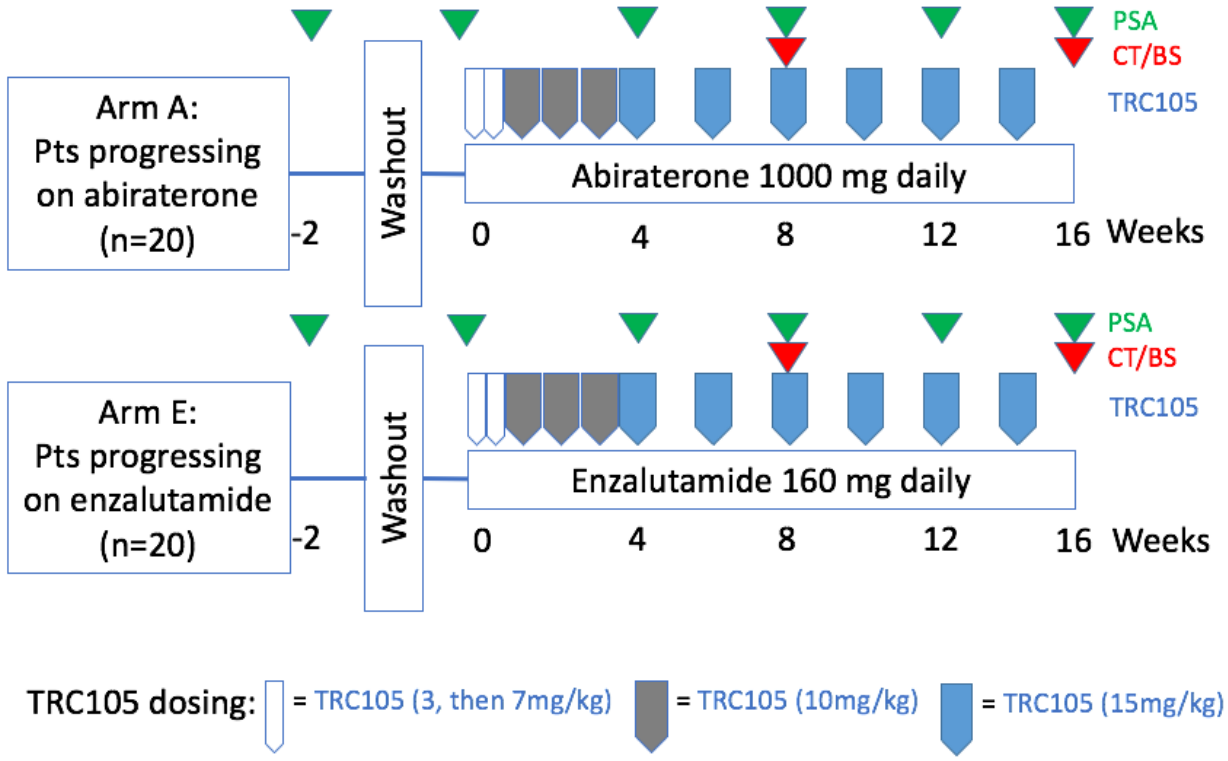
AE	Adverse Event
ALT	Alanine Aminotransferase
ALC	Absolute Lymphocyte Count
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CBR	Clinical benefit rate
CBR2	Clinical benefit rate at 2 months
CBR4	Clinical benefit rate at 4 months
CMP	Comprehensive Metabolic Panel
CR	Complete Response
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
DSMB	Data and Safety Monitoring Board
ECOG	Eastern Cooperative Oncology Group
H&P	History & Physical Exam
HRPP	Human Research Protections Program
IV (or iv)	Intravenously
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
ORR	Overall Response Rate
OS	Overall Survival
PBMCs	Peripheral Blood Mononuclear Cells
PD	Progressive Disease
PFS	Progression Free Survival
p.o.	per os/by mouth/orally
PR	Partial Response
SAE	Serious Adverse Event
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SPGT	Serum Glutamic Pyruvic Transaminase
WBC	White Blood Cells

VERSION HISTORY

STUDY SUMMARY

Title	A prospective study of TRC105 (anti-endoglin antibody) with abiraterone or with enzalutamide
Protocol Number	IIT2016-16-POSADAS-TRC105
Phase	2
Methodology	Parallel Bayesian phase 2
Study Duration	24 months
Study Center(s)	SOCCI and affiliated practices only
Objectives	**Primary Objectives** • To measure clinical benefit of TRC105 with enzalutamide or with abiraterone) in patients who have demonstrated resistance by rising PSA on that particular agent. **Secondary Objectives** • Describe the adverse events associated with TRC105 and abiraterone or enzalutamide. • Measure the impact of the addition of TRC105 to abiraterone or enzalutamide on serum PSA concentration. • Measure the effect of the addition of TRC105 to abiraterone or enzalutamide by radiographic response (RECIST, Prostate Cancer Working Group 3 (PCWG3)[43]) after 2 and 4 months. • Measure the proportion of patients who remain progression free by PSA and/or radiographic measurement at 2 and 4 months after the addition of TRC105 to abiraterone or enzalutamide
Number of Subjects	40 maximum subjects (limited by Bayesian design) Arm A: patients progressing on Abiraterone by PSA Arm E: patients progressing on Enzalutamide by PSA
Diagnosis and Main Inclusion Criteria	Metastatic, castration-resistant prostate cancer actively progressing on abiraterone or enzalutamide by PSA Adequate organ function ECOG PS 0-2
Study Product(s), Dose, Route, Regimen	TRC105 10 mg weekly x4 then 15 mg/kg q2weeks; abiraterone or enzalutamide per standard dosing
Duration of administration	Until radiographic progression by RECIST 1.1 or PCWG3 criteria
Reference therapy	N/A
Statistical Methodology	Bayesian phase 2 per Tighiouart

STUDY SCHEMA



1.0 BACKGROUND AND RATIONALE

1.1 Prostate cancer and androgen receptor (AR) inhibition

Prostate cancer (PCa) is the most common, non-cutaneous cancer affecting men in the United States and the second leading cause of cancer death in American males. For those men who do not have disease amenable to means of local control, systemic agents are employed to control disease in efforts to extend life and improve quality. Androgen deprivation therapy (ADT) has been used as the primary mode of systemic treatment given the dependence of this cancer upon AR-signaling rooted in the embryonic origin of the genitourinary tract. While ADT is typically highly effective initially, most men facing metastatic PCa will progress to a state of castration resistance and require additional treatment. Metastatic, castration-resistant PCa (mCRPC) is universally recognized as a lethal evolution in PCa biology. While there are a number of approaches being explored and approved for men affected by mCRPC, the use of next-generation AR-inhibition strategies have been among the most effective and widely used. Examples of such agents include abiraterone acetate and enzalutamide (see below). While these two FDA-approved and commercially available agents are effective in many cases, even those men who benefit initially will progress to this form of the disease which is no longer well controlled by either (or both) of these drugs. Typically, this manifests as rising serum prostate specific antigen (PSA) concentrations and eventually by the appearance of new or expanding radiographic lesions. Given therapeutic alternatives such as chemotherapy, means of extending the benefit of ADT, generally well tolerated, would represent an important advancement in PCa therapy.

1.2 Endoglin (CD105)

CD105 (endoglin) is a homodimeric cell membrane glycoprotein that was initially identified as a human leukemia-associated antigen [1] and later also found on endothelial cells [2, 3]. CD105 is a TGF-β and BMP co-receptor that is essential for angiogenesis [4, 5] and CD105 is strongly expressed on the proliferating vascular endothelium of solid tumors [3, 6]. All of these properties make CD105 an attractive target for the antiangiogenic therapy of cancer [7]. Vascular targeted therapy may more effectively address large established tumors than conventional antiangiogenic therapy such as anti-VEGF therapy [8]. In animal models, CD105 targeted therapy has demonstrated both vascular targeting effects and antiangiogenic effects by inducing regression of established tumors as well as by preventing new tumor formation and inhibiting the expansion of existing tumors [3, 9-12]. Therefore, CD105 offers a novel alternative target relative to the VEGF inhibitors currently available for antiangiogenesis therapy.

CD105 acts to modulate signaling of multiple kinase receptor complexes of the TGF-β superfamily, including TGF-β receptors, bone morphogenic protein (BMP) and activin receptors [13]. Binding of TGF-β to CD105 complexed with TGF-β receptors results in phosphorylation of SMAD 2/3 proteins that reinforces a quiescent phenotype and inhibits endothelial cell growth. However, activation of CD105 by BMP phosphorylates SMAD1/5, thereby overriding the growth inhibitory effects of TGF-β on endothelium. Not surprisingly, prevention of BMP binding to CD105 by an anti-CD105 antibody acts synergistically with TGF-β to inhibit endothelial cell growth [14].

CD105 expression is required for endothelial cell proliferation, and CD105 is upregulated in the setting of hypoxia through the induction of hypoxia-inducible factor-1-α (HIF-1-α) [15, 16]. CD105 has also been shown to protect hypoxic cells from apoptosis [17]. The expression of CD105 by endothelial cells is essential for the development of new vasculature. Targeted inactivation (knockout) of murine CD105 results in defective vascular development. Mice lacking CD105 die *in utero* from defective vascular development by gestational day 11 [4].

CD105 is critical for normal human blood vessel development [18]. CD105 haplotype insufficiency causes a well-described syndrome known as hereditary hemorrhagic telangiectasia type 1 (HHT-1 or Rendu-Osler-Weber Syndrome). HHT-1 is a rare autosomal dominant genetic disorder characterized by localized angiodysplasia involving the nasal, buccal, gastrointestinal mucosa and skin microvasculature. Angiodysplasia also occurs in vessels from internal organs including the lungs, liver and brain [19]. The

genotype is manifested *in utero*, but the phenotype does not become apparent until many years following birth. Affected patients commonly present with epistaxis in the second decade of life. The phenotype of this disorder is characterized by vascular effects, indicating the specific role of CD105 in the vasculature [20].

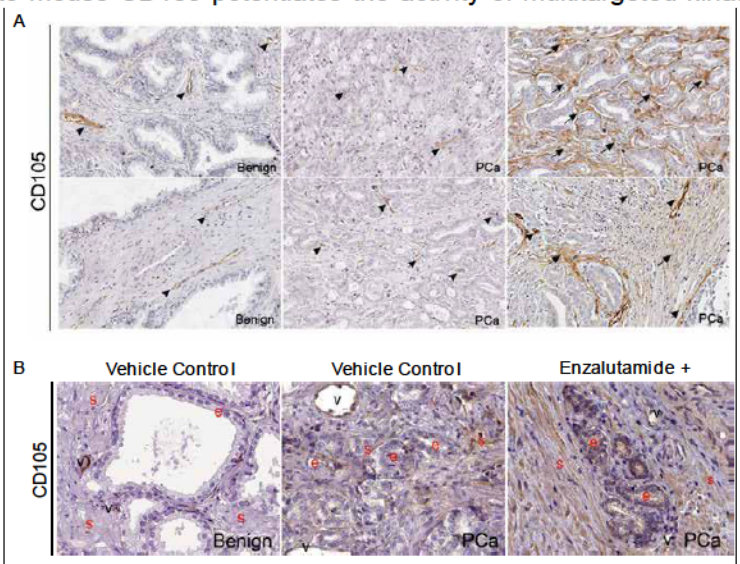
CD105 is highly expressed on the proliferating endothelial cells of tumor vessels including lung, breast, colorectal, gastric, liver, endometrial, renal cell, head and neck, and ovarian cancers. Until recently, CD105 expression in adults had been demonstrated on proliferating endothelial cells and proerythroblasts, a red blood cell precursor [21]. CD105 expression is a prognostic factor in solid tumor patients. High microvessel density of CD105-positive vessels has been correlated with poor prognosis in clinical studies of breast cancer [22, 23], lung cancer [24], prostate cancer [25, 26], colorectal cancer [27, 28], ovarian cancer [29, 30], gastric cancer [31], endometrial cancer [32], astrocytic brain tumors [33], hepatocellular carcinoma [34], esophageal adenocarcinoma [35], and head and neck cancer [36, 37].

Plasma soluble-CD105 (cleaved extracellular domain) is prognostic in retrospective studies of cancer patients. In one study, the mean plasma CD105 concentration in 76 patients with colorectal cancer was 4-fold higher than the mean value in 40 healthy subjects without cancer [28]. In the study, a positive correlation was observed between plasma soluble-CD105 concentration and stage of disease.

Importantly, CD105 expression is up regulated in tumor endothelial cells following inhibition of the VEGF pathway. CD105 expression increased more than 2-fold in human pancreatic cancers grown in mice treated with an antibody that binds VEGF [38]. Similarly, 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 [39]. TRC105 is a novel IgG1 that binds CD105 with high avidity and competitively inhibits BMP binding to CD105. Recent studies at Duke University explored the in vitro effects of dual angiogenesis inhibition using bevacizumab and TRC105 in human umbilical vein endothelial cells (HUVEC). Combination therapy was found to be more potent in decreasing HUVEC proliferation, migration, and tubular network formation than bevacizumab or TRC105 treatment alone [40]. Furthermore, TRC105 induced apoptosis in HUVEC, while promoting SMAD2/3 phosphorylation and inhibiting SMAD1/5/8 signaling, confirming its anti-angiogenic properties. Finally, antibody to mouse CD105 potentiates the activity of multitargeted kinase inhibition that targets the VEGFR-2, in mouse bearing cancer grafts.

1.3 Endoglin and prostate cancer

The Bhowmick lab has found that CD105 is expressed in both prostate cancer epithelia and associated fibroblasts. Furthermore, CD105 expression on prostatic epithelia and associated fibroblasts contributes to castrate resistance. Analysis of CD105 expression by immunohistochemistry on a tumor array showed that in benign prostate tissue, CD105 is restricted to blood vessels (**Figure 1**). In contrast, endothelial cells, epithelial cancer cells, and stromal fibroblasts of PCa tissues expressed CD105. In addition, patient derived tissues, including neoplastic cells and associated fibroblasts, were grafted into the sub-renal capsules in mice to develop patient derived xenograft (PDX) models. A subset of



Immunohistochemical staining for CD105 staining (brown) was counterstained w hematoxylin for the indicated tissue specimens. A. Representative tissue sections fron tumor microarray are shown (n = 48). Arrowheads indicate positive blood vessel stain and arrows indicate positive stromal cell staining. B. Prostate cancer patient deriv xenograft (PDX) tissue staining for CD105 is shown on the indicated specimens for m given vehicle control or enzalutamide treatment.

PDX mice received Enzalutamide treatment. CD105 expression was shown to be elevated following enzalutamide treatment compared to control mice.

Interestingly, antagonizing CD105 has no statistically significant effect on prostate cancer cell lines (PC3, 22Rv1, LNCaP, C42B) and minimal effect on primary prostatic fibroblasts in normoxic conditions *in vitro* (21% O_2). The treatment of TRC105 to these cells did not appreciably change the cell surface expression of CD105, as determined by flow sorting in normoxic conditions. In contrast, under hypoxic conditions (2% O_2), mimicking the oxygen concentration found in solid tumors, CD105 was elevated dramatically in both 22Rv1 epithelia and primary prostate fibroblasts (**Figure 2**). Furthermore, enzalutamide treatment in hypoxia led to elevated CD105 levels, similar to those observed in mice. Combination treatment of TRC105 with enzalutamide decreased these elevated levels. It has been shown that human epithelial 22Rv1 cells xenografted in isolation of their stromal CAF counterpart are sensitive to enzalutamide therapy.

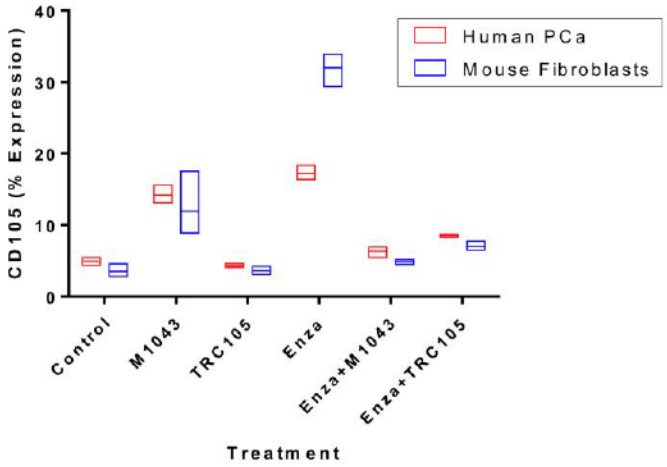
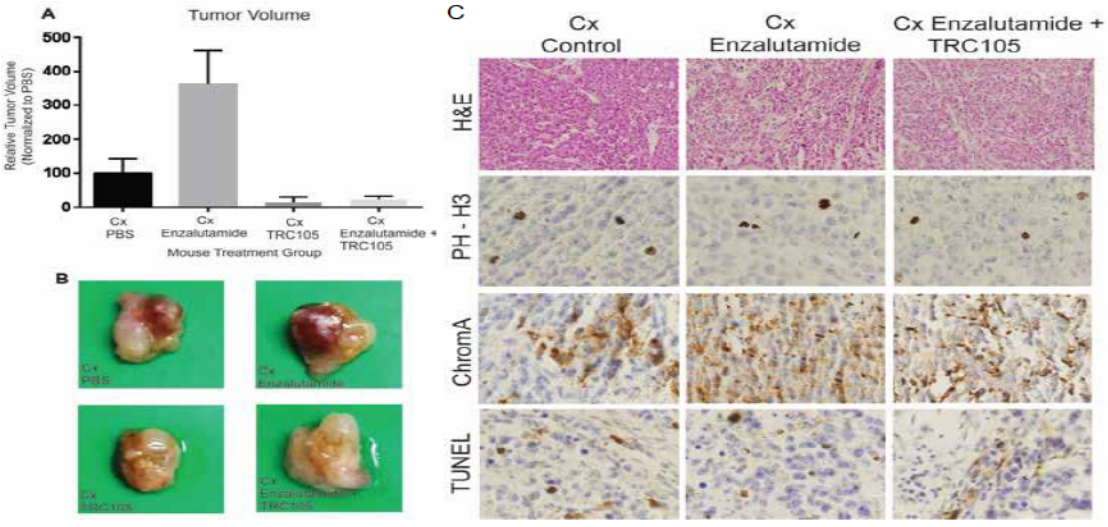


Figure 2. CD105 expression is elevated due to enzalutamide treatment under hypoxic conditions that mimic physiologic conditions. Human prostate cancer (PCa) epithelial cells (red boxes) were grown in 3D co-cultures with mouse fibroblasts (blue boxes) under hypoxia (2% O2) with the indicated treatments. After 72 hours, the cells were dissociated and assessed by FACS for CD105 expression as shown. Antagonizing CD105 by either M1043 or TRC105 down regulated enzalutamide-induced CD105 cell surface expression in both mouse prostatic fibroblasts and human prostate cancer epithelia.

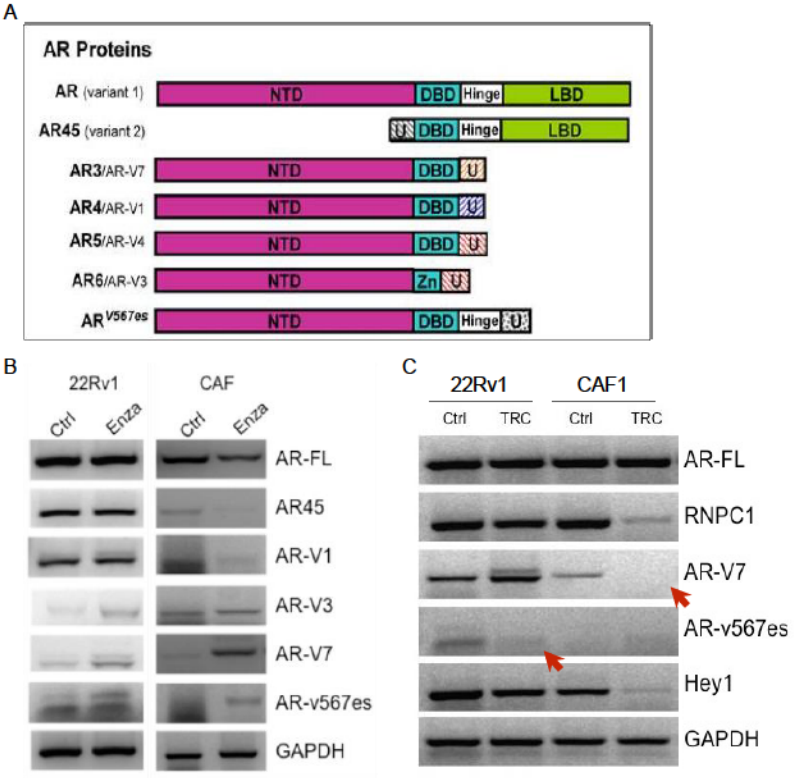


Human prostate cancer CW22Rv1 epithelial cells were orthotopically xenografted with carcinoma associated fibroblasts. A. Relative tumor volume (normalized to castrated (Cx) PBS control tumors) is plotted for the indicated mouse treatment groups. B. Gross pictures of the orthotopic prostate tumors within the prostate are shown for the indicated mouse treatment groups. C. Immunohistochemical staining is shown for the for the indicated mouse treatment groups. H&E staining in the top panels shows the morphology of the prostate tumors. Phosphorylated histone-H3 (PH-H3) (brown) in the second row indicate cells undergoing mitosis, chromagranin A (ChromA) (brown) in the third row indicate neuroendocrine differentiation, and Terminal deoxynucleotidyl transferase dUTP neck end labeling (TUNEL) (brown) is an apoptotic cell marker. All tissues were counterstained with hematoxylin (blue).

carcinoma tumors) is nors within staining is shows the ng mitosis, ell marker.

Notably, the Bhowmick lab has shown that xenografts using 22Rv1 cells combined with human cancer associated fibroblasts (CAFs) derived from patient biopsies led to resistance of 22Rv1 cells following castration or enzalutamide treatment. To further test TRC105 in the context of an *in vivo* system that mimics the patient population proposed in this clinical trial, human epithelial 22Rv1 cells and CAFs were orthotopically xenografted and treated with combinations of castration (similar to abiraterone treatment), with or without enzalutamide, and with or without TRC105. Administering TRC105 in the context of castration or castration plus enzalutamide resulted in a significant decrease in tumor size compared to castration or castration plus enzalutamide (**Figure 3**). Thus, blockade of CD105 in fibroblasts and cancer cells sensitizes the PCa to ADT.

Vascular antagonists have failed in various stages of PCa therapy in patients. To determine the mechanism of action for TRC105 to be effective, we analyzed the resulting 22Rv1/fibroblast xenograft tissues. They were found to express elevated levels of the neuroendocrine differentiation marker chromagranin A when treated with castration or castration plus enzalutamide, which has been shown to promote cancer stem cell survival. However, the addition of TRC105 with castration plus enzalutamide decreased this elevation of neuroendocrine differentiation (tumors with TRC105 plus castration were too small to properly evaluate by immunohistochemistry). This suggests that TRC105 promoted the differentiation of the PCa epithelia, which makes it sensitive to androgen deprivation therapies instead of developing castrate resistance and recurrence. To further address this mechanism, we have shown that TRC105 treatment decreased the expression of androgen receptor splice variants (AR-v7 in fibroblasts and AR-v567 in epithelia) induced by enzalutamide (**Figure 4**).



A. A diagram of the various AR splice variants is shown. B. Human prostate epithelial cell line CW22Rv1 or primary prostate carcinoma associated fibroblasts (CAF) were treated with control (Ctrl) or enzalutamide (Enza) and checked for mRNA expression of various AR splice variants as indicated and GAPDH control expression. C. CW22Rv1 and CAF cells were treated with control or TRC105 (TRC) and assessed for the indicated mRNA expression of AR splice variants and Notch downstream signaling proteins (Hey1 and RNPC1) thought to be responsible for the changes AR variant expression. Red arrows indicate the loss of AR variants with TRC105 treatment.

Bhowmick

indicate the loss of AR variants with TRC105 treatment.

1.4 TRC105

1.4.1 Background

TRC105 is a genetically engineered human/murine chimeric monoclonal antibody directed against human CD105 developed by Tracon pharmaceuticals. The antibody is an IgG1 kappa immunoglobulin containing murine variable region sequences and human constant region sequences [41]. TRC105 has an approximate molecular weight of 148 kDa. TRC105 has a binding avidity for human CD105 of approximately 5 pM. TRC105 is formulated in a histidine buffer at a concentration of 25 mg/mL.

SN6j, the murine parent antibody of TRC105, binds to human umbilical vein endothelial cells (HUVECs) with nearly identical avidity as TRC105. SN6j has been shown to bind the tumor vasculature of malignant tissues including prostate, breast, colon, rectum, kidney and lung cancers and to inhibit the growth of tumor xenografts [10]. Reactivity with tumor tissues is most dense on the tumor endothelium, as CD105 is not generally expressed on epithelial tumor cells [9]. TRC105 induces ADCC on proliferating HUVECs at low concentrations and induces apoptosis and growth inhibition at higher concentrations.

1.4.2 TRC105 Clinical experience

Several studies with TRC105 are underway or have been completed. An open-label, phase 1, multicenter study of TRC105 (Study 105ST101) is complete. Fifty patients were treated until disease progression with TRC105 at 0.01-15 mg/kg/q2wk or 10-15 mg/kg/wk. Completed studies include a phase 1 study of TRC105 monotherapy in prostate cancer (Karzai, 2015), phase 1b study of TRC105 in combination with bevacizumab, phase 2 studies of TRC105 monotherapy in liver cancer and ovarian cancer, and in combination with bevacizumab in glioblastoma multiforme (2 studies) and renal cell carcinoma. Ongoing studies include phase 1b/2 studies of TRC105 in combination with sorafenib, a phase 2 study of TRC105 in combination with axitinib in renal cell carcinoma, and a phase 2 study of TRC105 in combination with pazopanib in soft tissue sarcoma.

In Study 105ST101, intensive PK was collected following TRC105 administration on Cycle 1 Day 1 and Cycle 2 Day 15 and pre-dose concentrations were taken before dosing in subsequent cycles. Volume of distribution was similar to plasma volume, which was consistent with preclinical toxicokinetics and would be expected for an antibody administered intravenously. AUC increased supra-proportionally with dose whereas the C_{max} appeared to be dose proportional. TRC105 clearance was consistent with target mediated disposition and decreased clearance at higher doses suggests beginning of target saturation. Serum concentrations were achieved continuously at doses of 15 mg/kg every 2 weeks (in most but not all patients) and 10 mg/kg weekly (in all patients), and TRC105 accumulated at a dose of 15 mg/kg weekly (Figure 5). Importantly, weekly dosing with 10 mg/kg produced continuous TRC105 serum concentrations above the target concentration of 25 µg/mL shown to produce maximum inhibition of HUVEC activation *in vitro* (i.e., maximal inhibition of SMAD1 phosphorylation in response to BMP binding) (**Figure 5**).

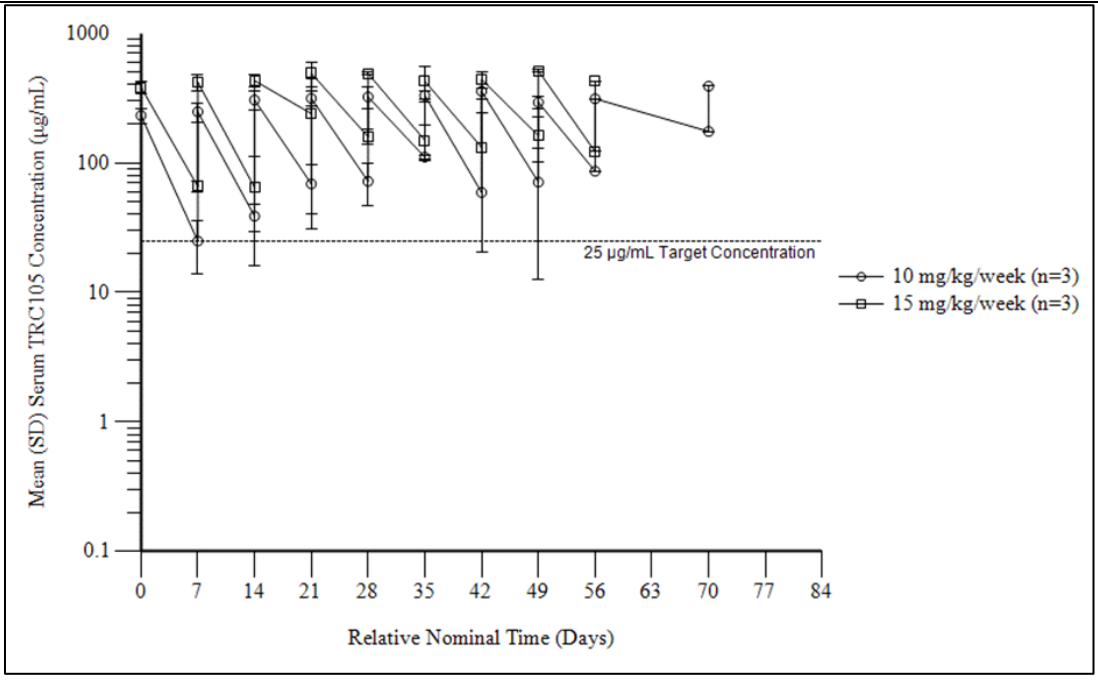


Figure 5. Single-dose and multiple dose pharmacokinetic data from Study 105ST101

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 has been used in all trials since 2007 and will be used in this study.

Further studies of a hybrid dosing scheme by where TRC105 is given weekly at 10 mg/kg for four doses and then every two weeks at 15 mg/kg indicated the dose and schedule were tolerable and produced continuous TRC105 serum levels above target concentrations in all patients. This hybrid dosing scheme has the advantage of allowing every two week dosing after the initial month of treatment, which improves patient convenience.

A total of 50 patients were treated on 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 or 15 mg/kg every two weeks. 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 dexamethasone-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, dexamethasone was 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 nonsteroidal 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.

Potential Risks of TRC105

Grade 3 anemia has occurred with TRC105 therapy at the recommended phase 2 dose. 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 sequellae. 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.

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). A grade 2 transient ischemic attack was reported in a study of TRC105 and pazopanib. Transient Grade 3 hepatic encephalopathy occurred in one patient with cirrhosis and hepatocellular carcinoma who received TRC105 in combination with sorafenib. Grade 3 pancreatitis was also observed in this study. 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. A third patient with glioblastoma with a history of recurrent meningitis developed recurrent grade 3 bacterial meningitis while treated with bevacizumab and TRC105.

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 sequellae. 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. Pneumothorax (collapsed lung) has been observed in trials of TRC105 administered with VEGF inhibitors in patients with lung metastases. In addition, pnemothorax was observed in one patient, also with lung metastases, receiving single-agent TRC105.

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. Reversible grade 3 colitis was reported in a patient treated with TRC105 and pazopanib.

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. 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.

1.5 TRC105 and prostate cancer

There has been a limited experience with TRC105 in prostate cancer. During the phase I 105ST101, across all tumors, stable disease was the best radiographic response reported outcome in 21 of 45 patients. 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 has remained on TRC105 treatment for 9 years at a TRC105 dose of 0.01 mg/kg every 2 weeks with an ongoing complete PSA response, and resolution of bone pain and bone scan normalization.

A second phase I study was conducted in prostate cancer by Karzai et al. This disease focused study used a standard 3+3 phase I design and treated a total of 20 patients. No dose limiting toxicity (DLT) was identified. The maximum dose tested of 20 mg/kg every 2 weeks was declared the maximum tolerated dose (MTD). Common adverse effects included infusion-related reaction (90%), low grade headache (67%), anemia (48%), epistaxis (43%) and fever (43%). Ten patients had stable disease on study and eight patients had declines in prostate specific antigen (PSA). Vascular endothelial growth factor (VEGF) was increased after treatment with TRC105.

1.6 Rationale

The Bhowmick laboratory has shown that TRC105 inhibits multiple biochemical changes that promote the onset of resistance to AR-targeted therapies. Models of AR inhibition (blocking androgen synthesis and androgen receptor antagonism) result in CD105 upregulation in prostate cancer epithelia and associated stromal cells. CD105 expression can stimulate tumor vascular and fibroblast proliferation as well as mediate tumor expression of androgen receptor variants resistant to AR-targeted therapies. TRC105, as a single agent, was not inhibitory to PCa expansion in CRPC patients [42]. Accordingly, we anticipate a combination of AR-targeted therapy (enzalutamide or abiraterone) with TRC105 (in patients with rising PSA) will inhibit PCa tumor progression. In tissue culture and tissue recombination studies in mice, CD105 was elevated with enzalutamide treatment and importantly was suppressed with TRC105 treatment. Similarly, human PCa xenografted castrated mice treated with TRC105, demonstrated decreased tumor volume compared to castration alone (similar to abiraterone) or castration plus enzalutamide. In tumor tissues of mice treated with TRC105, tumor epithelial cells showed a decrease in neuroendocrine differentiation- a suspected mode of resistance to AR-targeted therapy. Furthermore, TRC105 treatment decreased the expression of AR therapy-induced splice variant generation (AR-V7) in PCa epithelia and fibroblasts. Such expression of androgen receptor splice variants lacking the ligand binding domain are transcriptionally active in the context of AR-targeted therapies. This data supports the use of TRC105 in combination with AR-targeted therapies (enzalutamide or abiraterone) to suppress factors promoting resistance to AR-targeted therapies in both the PCa epithelia and fibroblasts to effectively treat PCa.

Hypotheses

In light of the above findings, we hypothesize that inhibition of CD105 can overcome resistance to AR targeted therapy inducing clinical benefit (including PSA decline) in patients who have developed early resistance to AR-targeted therapy

To test this hypothesis, we will conduct a clinical trial to test the addition to TRC105 to two existing AR-targeted therapies with differing mechanisms and pharmacokinetics: abiraterone and enzalutamide.

2.0 STUDY OBJECTIVES

2.1 Primary Objectives

- To measure clinical benefit of TRC105 with enzalutamide or with abiraterone in patients who have demonstrated resistance by rising PSA on that particular agent.

2.2 Secondary Objectives

- Describe the adverse events associated with TRC105 and abiraterone or enzalutamide.
- Measure the impact of the addition of TRC105 to abiraterone or enzalutamide on serum PSA concentration.
- Measure the effect of the addition of TRC105 to abiraterone or enzalutamide by radiographic response (RECIST, Prostate Cancer Working Group 3 (PCWG3)[43]) after 2 and 4 months.
- Measure the proportion of patients who remain progression free by PSA and/or radiographic measurement at 2 and 4 months after the addition of TRC105 to abiraterone or enzalutamide

2.3 Exploratory Objectives

- When possible, to obtain archival biopsy tissue specimens on primary tumor and subsequent metastasis biopsies preceding the current trial.
- Measure the impact of the addition of TRC105 to abiraterone on serum DHEA-S and other steroid metabolites and/or intermediates.
- Measure the impact of the addition of TRC105 to enzalutamide on serum testosterone and other steroid metabolites and/or intermediates.
- Measure the impact of the addition of TRC105 to enzalutamide or abiraterone on PFA (platelet function).
- To associate changes in biomarkers with clinical behavior on combination therapy including Nanovelcro and CellSearch CTCs and associated subsets within the CTCs
    - rtPCR for AR status (full length AR and variants AR-V7, AR-v567es, AR-V1, AR45, and AR-V3)
    - Immunofluorescence for RNPC1, Phosphorylated-Smad1/5 and phosphorylated-Smad2/3, Neuropilin, NE differentiation markers (Chromagranin A, Synaptophysin, NSE), EMT markers (E-cadherin, N-cadherin, Snail, Twist), sCD105
- To associate changes in serum biomarkers with clinical behavior on combination therapy including
    - sCD105,
    - Cysteine
    - P1GF, bFGF, sVEGFR1
    - Osteopontin
    - P1NP
    - CTX
    - RANKL
    - PTHrP, PTH
    - OPG
- To associate changes in cells within the buffy coat with clinical behavior on combination therapy including
    - T cells (CD4, CD8, activation markers, and PD-1)
    - Monocytes expanded and differentiated to macrophage (CD68, CD206 (M2), CD62(activation), MHC class II (M1))
- When possible, to associate tissue changes and biomarker expression with clinical outcomes including:
    - Proliferation (by Ki67)
    - Apoptosis (TUNEL, caspase-3)
    - NE differentiation
    - Biochemical markers of stemness

- AR Status
- Phosphorylated-Smad1/5 and phosphorylated-Smad2/3
- Tumor neo-vascularization (CD31, von willbrand factor)
- LOX (measure of hypoxia)
- EMT markers (E-cadherin, N-cadherin, Snail, Twist)

3.0 ENDPOINTS

3.1.1 Primary endpoint

- Clinical benefit will be defined as stabilization of disease for at least 2 months or improvement at any time from the start of combination therapy by radiographic and/or biochemical criteria.
    - Radiographic Improvement will be defined as a PR or CR by RECIST 1.1 or improvement by PCWG3 criteria (see section 7.1).
    - Biochemical response will be defined by PCGW3 criteria (see section 7.3)
    - Stabilization will be defined as the absence of progression by BOTH radiographic and biochemical criteria

3.1.2 Secondary endpoints

- Adverse events from TRC105 and abiraterone or enzalutamide described using CTCAE 4.0
- Measurement of progression free survival by PSA and radiographic criteria (as noted above)

4.0 STUDY DESIGN

This is a single-institution, open-label study of TRC105 in combination with abiraterone or enzalutamide in patients who are taking either abiraterone or enzalutamide and showing signs of biochemical progression without radiographic progression. In essence, a patient who is progression on AR-therapy will continue the same AR-therapy on study with the addition of TRC105. The two arms will accrue in parallel and independently.

There will be a 2-week washout of the active AR-targeted therapy prior to initiation of combination therapy. TRC105 will be administered at 10 mg/kg weekly for the first 4 weeks then every two weeks at 15 mg/kg.

The first TRC105 dose will be split into two doses whereby 3 mg/kg is administered over 4 hours (+/- 15 minutes) on Cycle 1 Day 1 and the balance is administered on Cycle 1 Day 4.

5.0 PATIENT ELIGIBILITY

5.1 Inclusion Criteria

1. History of metastatic, castration-resistant prostate cancer with rising PSA on either abiraterone or enzalutamide
    a. PSA rise will be defined as an increase in PSA of 0.2 ng/mL or higher on at least 2 separate occasions greater than 1 week apart while on either abiraterone or enzalutamide
    b. If there is a drop in serum PSA after the first rise, and the patient has another PSA rise which is greater than the first, the patient will still be considered eligible.
2. ECOG 0-2
3. Resolution of adverse events results from prior cancer therapies to NCI CTCAE grade 1 OR baseline with the exception of the laboratory tests specified below.
4. Adequate organ function defined by:
    - AST and ALT < 2.5 x ULN
    - Total serum bilirubin < 1.5 x ULN
    - Platelets > 100K
    - Hgb > 8.5 g/dL
    - Serum Cr <1.5 x ULN
    - INR 0.8 – 1.2

5.2 Exclusion Criteria

1. Non-PSA producing prostate cancers- such as small cell prostate cancers or those prostate cancers which exhibit radiographic progression without PSA rise
2. Inability to tolerate standard doses of abiraterone (1000 mg daily) or enzalutamide (160 mg daily).
3. Other prior malignancy requiring active anticancer therapy
4. Prior exposure to TRC105 or any CD105 targeted antibody
5. Any major surgical procedure within 2 weeks of starting therapy
6. Uncontrolled chronic hypertension defined by systolic pressure (SBP) >150 mmHg or diastolic pressure (DBP) >90 despite optimal therapy that is present in more than 3 readings taken no less than 10 minutes apart.
7. Active bleeding or pathologic conditions that carries a high bleeding risk
8. Use of thrombolytics within 10 days prior to the first day of TRC105
9. Known hypersensitivity to Chinese hamster ovary products or other recombinant human, chimeric, or humanized antibodies
10. A known diagnosis of Osler-Weber-Rendu syndrome
11. Ascites or pericardial or pleural effusion requiring external drainage procedures
12. History of untreated brain involvement with cancer, spinal cord compression, or carcinomatous meningitis, or new evidence of brain or leptomeningeal disease. Patients with radiated or resected lesions are permitted, provided the lesions are fully treated and inactive, patients are asymptomatic, and no steroids have been administered for at least 28 days. Imaging for CNS disease will not be

required for screening unless there is a history of a neurological finding such as new onset weakness or numbness that cannot be explained by other medical history.

13. Acute cardiovascular event within the past 6 months. An acute cardiovascular event will be defined as a myocardial infraction, NYHA Class II or worse congestive heart failure, cerebrovascular accident, transient ischemic attack, arterial embolism, pulmonary embolism, percutaneous transluminal coronary angioplasty (PTCA), or CABG. Deep venous thrombosis within 6 months, unless the patient is anti-coagulated without the use of warfarin for at least 2 weeks. In this situation, low molecular weight heparin is preferred.
14. Patients must be surgically sterile or must agree to use effective contraception during the study and for 3 months following last dose of TRC105. The definition of effective contraception will be based on the judgment of the Principal Investigator or a designated associate. Abstinence from intercourse is an acceptable form of contraception.

6.0 TREATMENT PLAN

6.1 Washout for AR-targeted agents

To maximally "reset" AR-driven signaling, patients will be required to have a washout of the AR-targeted agent prior to starting study treatment. Pre-clinical studies demonstrated an induction of CD105 following androgen targeted therapy given to naïve tumors. This suggests that TRC105 effects may be maximized following a washout period. This washout period will consist of at least 2 weeks prior to starting combination therapy.

6.2 Dosing plan

Table 1. Dosing of AR agent and TRC105

AR Agent at entry	AR agent dose	TRC105 dose (weeks 1-4)	TRC105 dose (weeks 5 +)
Abiraterone	Abiraterone 1000 mg daily	10 mg/kg weekly	15 mg/kg every two weeks
Enzalutamide	Enzalutamide 160 mg daily	10 mg/kg weekly	15 mg/kg every two weeks

6.3 Investigational compound

Composition of TRC105

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.

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 TRC105/4 mL single-use vial
200 mg TRC105/8 mL single-use vial
400 mg TRC105/16 mL single-use vial

TRC105 Storage and Shipping

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

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 for men in this study is 100 kg (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 0.6 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.

TRC105 Administration
Patients should be encouraged to drink abundant fluid (e.g., two eight ounce glasses of water or juice) prior to the first treatment. Intravenous hydration prior to and during therapy is left to the discretion of the Investigator, but should be considered for patients that may be volume depleted.

The following TRC105 premedications should be administered 2 hours to 30 minutes prior to the start of each infusion:

- Acetaminophen 650 mg p.o. x 1
- Methylprednisolone 100 mg IV will be given prior to the cycle 1 day 1 and cycle 1 day 4 infusions. In addition, methylprednisolone will be given in the case of a delay of ≥ 10 days between any two scheduled weekly doses or in the case of a delay of ≥ 17 days between any two scheduled every other week doses, or if the patient develops an infusion reaction ≥ grade 2 during the immediate prior infusion.
- Famotidine 20 mg IV or p.o. (or similar H2 blocker) x 1. Famotidine (or similar H2 blocker) may be discontinued starting with Cycle 3, in the absence of infusion reactions with the prior dose.
- Cetirizine 10 mg IV or p.o. x 1 (or similar oral or intravenous antihistamine). Cetirizine (or similar oral or intravenous antihistamine) may be discontinued starting Cycle 3, in the absence of infusion reactions with the prior dose.

TRC105 infusions will begin 2 hours to 30 minutes following the completion of the TRC105 premedication, including the methylprednisolone infusion.

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 IV bag and transport of the TRC105 study drug to the patient will be performed as per standard study site procedures.

Following the appropriate premedication regimen, the first weekly TRC105 dose will be split into two doses whereby 3 mg/kg is administered over 4 hours (+/- 15 minutes) on Cycle 1 Day 1 and the balance is administered on Cycle 1 Day 4.

Patients who complete one 4-hour infusion without the development of any infusion reactions, will reduce the subsequent TRC105 infusion to 2 hours (+/- 15 minutes) and patients who complete a 2-hour infusion without the development of any infusion reactions will reduce subsequent TRC105 infusions to 1 hour (+/- 15 minutes). See Table 2 for ideal dosing schemas. Patients with infusion reactions of any kind should be managed appropriately and are not permitted to reduce the duration of the next planned infusion.

The rate of TRC105 infusion must not exceed 25 mg/min. When the IV. bag containing TRC105 is empty, flush the IV line with a 20 mL normal saline. The dose level, time of transfer to IV bag, and the infusion start and stop times must be recorded in the source documents.

If a patient misses a scheduled weekly TRC105 dose during the first month of Qweekly dosing and dosing is resumed ≥ 10 days after the last dose or if a patient misses a scheduled every 2-week dose after Week 5, and dosing is resumed ≥ 17 days after the last dose, premedication (including methylprednisolone) are to be administered as was done with the initial TRC105 dose. Split dosing is not required. However, it is recommended that if the patient experienced a severe headache with a previous infusion, the first TRC105 dose upon resumption should be administered over two days as was done for the initial dose.

Table 2. TRC105 doses and schedules for Dose Level A1/E1

	C1D1	C1D4	C1D8	C1D15	C1D22	C2D1	C2D15	C3D1+
TRC105 Dose (mg/kg)	3	7	10	10	10	15	15	15
Infusion Duration (hours)	4	2	1	1	1	1	1	1
Premedication								
Methylprednisolone (mg)	100	100	0	0	0	0	0	0
Famotidine (mg)	20	20	20	20	20	20	20	0*
Cetirizine (mg)	10	10	10	10	10	10	10	0*
Acetaminophen (mg)	650	650	650	650	650	650	650	650

* in the absence of infusion reactions with the prior dose, cetirizine and famotidine may be discontinued starting at cycle 3

Management of TRC105 Infusion Reactions

If a patient experiences a grade 2 or high adverse reaction during infusion, the infusion should be interrupted and the patient treated accordingly. Antipyretic, antihistamine, antiemetic, anti-inflammatory or other symptomatic medications may be administered as indicated. For grade 2 and certain grade 3 infusion reactions, the infusion may be restarted (the same day) at half of the previous rate after the infusion reaction has resolved, then increased per patient tolerance to a maximum of 25 mg/min. For grade 4 infusion reactions, the infusion should not be restarted and the patient should be discontinued from study treatment. Infusion reactions will be recorded as AEs in the case report form.

Infusion reaction severity	Recommended management
Grade 1	No intervention. Continue infusion unless symptoms worsen
Grade 2	1. Interrupt infusion 2. Treat with symptomatic medications 3. Resume infusion at half the previous rate when infusion-related symptoms improve to grade 1 or less
Grade 3	1. Interrupt infusion 2. Treat with symptomatic medications 3. Monitor patient until infusion-related symptoms resolve, including hospitalization if necessary 4. Withdraw patient from study unless other factors that contributed to the infusion reaction are identified and corrected
Grade 4	1. Discontinue infusion 2. Treat with symptomatic medications 3. Hospitalize patient 4. Withdraw from study

Symptomatic medications may include but are not limited to

- diphenhydramine 50 mg i.v. and/or hydrocortisone 100 mg i.v. (for fever, rash, hypoxia, or other hypersensitivity reactions)
- meperidine 50-100 mg i.v. (for shaking chills/rigors),
- oxygen by mask or nasal cannula (for hypoxia),
- epinephrine 0.5 mg i.m. (for hypotension or bronchospasm),
- albuterol inhaler or nebulizer (for bronchospasm),
- i.v. fluids (for hypotension),
- ondansetron 0.15 mg/kg i.v. (for nausea).

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 lot number of the TRC105 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

6.4 AR-targeted therapy

Abiraterone and enzalutamide will not be provided by the study site. These agents should be prescribed as part of standard of care therapy.

Investigational pharmacy will not be responsible for drug accountability of these agents. Patients will be asked to maintain a pill diary to verify dosing per protocol.

6.5 Toxicities and Dosing Delays/Dose Modifications

6.5.1 AR Therapy

Dose modifications to AR-therapy will not be allowed.

6.5.2 TRC105 Dose Reduction/Dose Interruptions

TRC105 dose reductions and interruptions should be avoided in cycle 1. In cycle 2 and beyond, TRC105 dose reductions are allowed using the guidelines below. TRC105 should be held for two weeks prior and for two weeks following surgical procedures. However, resumption of study treatment can be shorter (but no less than 7 days) or longer than two weeks based on clinical judgement of the treating investigator of

adequate wound healing and recovery from the procedure. For minor procedures (e.g., port placement), TRC105 should be held for at least 1 week prior and for at least 1 week after (or until adequate healing).

Toxicity Attributed to TRC105	Dose Adjustment for Next Dose of TRC105 (% of Starting Dose)
Grade 1	Maintain dose
Grade 2	At the discretion of the treating investigator, dose may be maintained or decreased to 80%
Grade 3 or 4	
- first appearance	Decrease to 80%
- second appearance	Decrease to 60%
- third appearance	Discontinue TRC105 permanently

6.6 Concomitant Medications/Treatments

No other approved or investigational anticancer treatment will be permitted during the study period with the exception of primary medical castration therapy (i.e. LHRH analogs). No other investigational drug may be used during treatment on this protocol, and concurrent participation in another clinical trial is not allowed.

Patients who are on abiraterone as part of this study should continue to use prednisone 5 mg twice daily or an equivalent adrenal androgen supplement at the treating investigator's discretion. This dose should be stable from the patient's previous utilization of steroid while on abiraterone.

Patients who receive NSAIDs on study may also receive peptic ulcer disease (PUD) prophylaxis with an H2 or proton pump blocker.

Narcotic analgesics, nonsteroidal anti-inflammatory drugs, and triptans (e.g. sumatriptan) may be offered as needed for relief of pain or headaches. Antihistamines and decongestants may be offered for the treatment of sinus congestion.

Packed red blood cell, colony stimulating factors, and platelet transfusions should be administered as clinically indicated.

Patients with arterial thrombosis or grade 3 or 4 venous thrombosis should be removed from the study. Patients with grade 1 or 2 venous thrombosis who require anticoagulation will have their TRC105 therapy interrupted. TRC105 therapy may resume once the following criteria are met:

- The patient is on a stable dose of heparin or low molecular weight heparin or Factor X inhibitor.
- The patient has a platelet count > 100,000.
- The patient has not had a hemorrhagic event of grade 2 or higher while on study.
- The patient does not have a pathological condition that carries a high risk of bleeding (e.g., tumor involving major vessels).
- The patient is benefiting from TRC105 therapy (no evidence of disease progression).

6.7 Duration of Therapy

Therapy will continue until one of the following:

- Disease progression by radiograph or PSA (verified by second rise at least 4 weeks later). A treating physician may continue TRC105 therapy if it is felt that there is clinical benefit despite rising PSA.
- Patient withdrawal
- Excessive toxicity
- Death

6.8 Duration of study participation

Patients will have a screening period of 28 days prior to Cycle 1 Day 1. One cycle of therapy consists of 28 days with any additional time for resolution of adverse events as described. Patients will be treated until

disease progression as defined in the protocol or for other reasons of discontinuation as defined in the protocol, Section 6.13 . A 30-day follow-up visit will occur after the last dose of study medication, with the allowance for those reasons noted in protocol Section 6.11.3

6.9 Removal of Patients from Protocol

Patients will be removed from the study when any of the criteria listed in Section 6.13 apply. Notify the Principal Investigator, and document the reason for study removal and the date the patient was removed in the Case Report Form. The patient should be followed-up per protocol.

6.10 Patient Replacement

Patients will not be considered assessable (and hence can be replaced), if they are unable to be assessed for clinical benefit at 8 weeks by PSA and/or scans.

6.11 Study Procedures

6.11.1 Screening Phase

All subjects must sign an informed consent form prior to the conduct of any study-related procedures. During this phase the following will be completed as specified in the Time and Event Table:

- Informed Consent
- Investigator's Confirmation of Eligibility
- Demographics
- Medical/Surgical History, including treatment history for PCa
- Baseline Physical Exam including Performance Status (ECOG)
- Vital Signs
- PSA
- CTC
- Serum Testosterone
- Urinalysis (w/microscopic examination)
- Baseline CT/MRI (Chest CAP or CT Chest/MRI AP)
- Bone Scan (Tc-99 or NaF)
- Chemistry
- Hematology
- Concomitant Medications (within 30 days of C1D1)

6.11.2 Treatment Phase

The Treatment Phase will begin at Cycle 1 Day 1 of treatment and will continue until study drugs are discontinued as per criteria in section 6.13

6.11.3 EOT Visit

An EOT Visit must be scheduled within 30 days after the last dose of study drug for all subjects, except the following:

- Patient is lost to follow up
- Patient is deceased
- Patient has withdrawn consent for study participation.
- Patient is unable to return to study site

6.12 Time and Events Table

	Screening	C1D1	C1D4	C1D15	Day 1 each subsequent cycle (+/- 7)	Day 15 each subsequent cycle (+/- 7)	EOT 30 days after treatment d/c (+/- 7)
Informed Consent	X						
Medical History & Demographics	X						
History & Physical[1]	X	X		X	X	X	X
Vital signs[2]	X	X	X	X	X	X	X
Adverse events		X		X	X	X	
Concomitant Medications	X	X		X	X	X	
CT or MRI and Bone[3] Scan	X	*Every 8 weeks from C1D1*					
TRC105 dosing[6]		X	X	X	X	X	
AR Inhibitor therapy		*Continuous daily dosing starting on Cycle 1 Day 1*					
CBC w/diff	X	X		X	X	X	X
Serum Chemistry[4]	X	X		X	X		X
Testosterone, CTC, PSA	X	X		X	X		X
Urinalysis with microscopic exam[5]	X	X			X		
Research blood collection	X	X			X		X

[1] Documentation should include performance status using ECOG scoring system
[2] Vital signs to include heart rate, temperature, blood pressure, respiratory rate (Height will be assessed during screening only)
[3] CT or MRI scans of chest, abdomen, and pelvis to be performed time points outlined in the time and events schedule of assessments.
[4] Serum chemistry to include, CMPL, LDH (no LDH on C1D15)
[5] Additional urine studies such as protein and/or creatinine should be performed as clinically indicated
[6] See Table 2 for first cycle dosing schedule.

6.13 Removal of Subjects from Study

Patients can be taken off the study treatment and/or study at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation will be documented and may include:

- Patient voluntarily withdraws (follow-up permitted);
- Patient withdraws consent (termination of treatment and follow-up);
- Patient is unable to comply with protocol requirements;
- Patient demonstrates disease progression (unless continued treatment with study drug is deemed appropriate at the discretion of the investigator);
- Patient experiences toxicity that makes continuation in the protocol unsafe;
- Treating physician judges continuation on the study would not be in the patient's best interest;
- Development of second malignancy (except for basal cell carcinoma or squamous cell carcinoma of the skin) that requires treatment, which would interfere with this study;
- Lost to follow-up.

7.0 MEASUREMENT OF EFFECT

7.1 Tumor Assessment

Tumor response should be assessed at a frequency of 8 weeks by standard Tc-99 bone scan and CT of the chest, abdomen, and pelvis after the initial assessment, which occurs after the first 8 weeks of study treatment. MRI is an acceptable substitution for CT scans provided that the investigator and subject agree to using this as the imaging modality for all soft-tissue tumor assessments for all protocol required imaging studies.

7.1.1 Bone scan evaluation

Prostate cancer working group 3 (PCWG3) criteria will be used to evaluate bone scans. In brief, progressive disease will be defined by at least 2 new lesions from a previous scan verified on a subsequent scan at least 3 weeks after the original scan. Increased intensity of lesions on bone scan will not constitute disease progression.

7.1.2 CT/MRI Assessments: RECIST 1.1

Response and progression will be evaluated in this study using new response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1) [44].

7.2 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with study drug.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least 8 weeks cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below.

7.2.1 Disease Parameters

Measurable disease.
Lesions must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of 10mm by CT scan or MRI. To be measurable a lymph node must be ≥15mm in short axis when assessed by CT scan or MRI.
All tumor measurements must be recorded in millimeters.

Note: Previously irradiated lesions are non-measurable except in cases of documented progression of the lesion since the completion of radiation therapy.

Non-measurable disease.
All other lesions, including small lesions (longest diameter<10mm or pathological lymph nodes with ≥10 to <15mm short axis) as well as truly non-measurable lesions are considered non-measurable disease.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ system 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) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). 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.

The baseline sum diameters will be used as reference by which to characterize the objective tumor response.

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 or absence of each should be noted throughout follow-up.

7.2.2 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric units. 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 when both methods have been used to assess the antitumor effect of a treatment.

7.2.3 Response Criteria

Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions, determined by two separate observations conducted not less than 4 weeks apart. There can be no appearance of new lesions.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum. There can be no appearance of new lesions.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions plus an absolute increase of at least 5 mm, taking as reference the smallest sum recorded since the start of studys.

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 since the treatment started.

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.

Duration of Response

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 recurrent disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

Progression-Free Survival

Progression-free survival (PFS) is defined as the duration of time from start of treatment to time of progression.

7.3 PSA response criteria

While the PCWG3 criteria recommend against decision making based on PSA changes, given the unique hypothesis, PSA will be tracked and considered actionable.

- Responses will be considered any PSA value >50% below baseline.
- Progression will be defined as a rise in serum PSA that is ≥ 25% and 2 ng/mL above nadir which is confirmed by a second value ≥ 3 weeks later.
- Stabilization of disease refers to PSA values that do not meet criteria for progression

7.4 Safety/tolerability

Analyses will be performed for all patients having received at least one dose of study drug. The study will use the CTCAE version 4.0 for reporting of adverse events (**http://ctep.cancer.gov/reporting/ctc.html**) and modified criteria for hematologic adverse events

8.0 ADVERSE EVENTS

8.1 Risks of participation

The potential risks of TRC105 therapy are listed in section 1.4. In addition to the risk of TRC105, during the brief washout period, there is a risk of disease progression that is equivalent to the risk of progression from remaining on their previous AR-therapy.

1.2 Adverse Event Monitoring

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of Subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. Additionally, certain adverse events must be reported in an expedited manner to allow for optimal monitoring of patient safety and care.

AEs (serious and nonserious) will be collected and recorded beginning with the administration of Cycle 1 Day 1 dosing through 30 days after the last administration of study treatment. If a patient begins a new anticancer therapy, the AE reporting period for nonserious AEs ends at the time the new treatment is started.

All patients experiencing an adverse event, regardless of its relationship to study drug, will be monitored until:

- the adverse event resolves or the symptoms or signs that constitute the adverse event return to grade 1 or baseline
- any clinically significant abnormal laboratory values have returned to grade 1 or baseline
- there is a satisfactory explanation other than the study drug for the changes observed
- death

8.2 Definitions

8.2.1 Definition of Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention, whether or not related to the intervention.

Abnormal Test Findings

Laboratory test and diagnostic studies (e.g. cardiac testing) which meet the following criteria must be reported as an AE:

- Test result is determined to be clinically significant by the treating investigator or PI
- Test result requires medical/surgical intervention

8.2.2 Severity of Adverse Events

All adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The CTCAE v4 is available at http://ctep.cancer.gov/reporting/ctc.html

If no CTCAE grading is available, the severity of an AE is graded as follows:

- Mild (grade 1): the event causes discomfort without disruption of normal daily activities.
- Moderate (grade 2): the event causes discomfort that affects normal daily activities.
- Severe (grade 3): the event makes the patient unable to perform normal daily activities or significantly affects his/her clinical status.
- Life-threatening (grade 4): the patient was at risk of death at the time of the event.
- Fatal (grade 5): the event caused death.

8.2.3 Serious Adverse Events

A “serious” adverse event is any untoward medical occurrence that:

- Results in death: If death results from (progression of) the disease, the disease should not be reported as event (SAE) itself.
- Is life-threatening: (the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe).
- Requires in-patient hospitalization or prolongation of existing hospitalization for ≥ 24 hours.
- Results in persistent or significant disability or incapacity.
- Is a congenital anomaly/birth defect
- Is an important medical event
-

Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient may be considered for reporting as a serious adverse event. Steps to Determine If an Adverse Event Requires Expedited Reporting

Step 1: Identify the type of adverse event using the NCI Common Terminology Criteria for Adverse Events (CTCAE v4).

Step 2: Grade the adverse event using the NCI CTCAE v4.

Step 3: Determine whether the adverse event is related to the protocol therapy
Attribution categories are as follows:

- Definite – The AE *is clearly related* to the study treatment.
- Probable – The AE *is likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unlikely – The AE is *doubtfully related* to the study treatment.
- Unrelated – The AE *is clearly NOT related* to the study treatment.

Note: This includes all events that occur within 30 days of the last dose of protocol treatment. Any event that occurs more than 30 days after the last dose of treatment and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly.

Step 4: Determine the prior experience of the adverse event.
Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in:

- the current known adverse events listed in section 1.4
- the drug package insert
- the current Investigator’s Brochure

8.3 Reporting Requirements for Adverse Events

8.3.1 Expedited Reporting

- The Principal Investigator must be notified within 24 hours of learning of any serious adverse events, regardless of attribution, occurring during the study or within 30 days of the last administration of the study drug

  Phone Number for Expedited Reporting: Edwin Posadas, MD 310-423-7600

- The CSMC IRB must be notified within 10 business days of “any unanticipated problems involving risk to subjects or others” (UPR/UPIRSO)

The following events meet the definition of UPR:

- Any serious event (injuries, side effects, deaths or other problems), which in the opinion of the Principal Investigator was unanticipated, involved risk to subjects or others, and was possibly related to the research procedures.
- Any serious accidental or unintentional change to the IRB-approved protocol that alters the level of risk.
- Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject.
- Any new information (e.g., publication, safety monitoring report, updated sponsor safety report), interim result or other finding that indicates an unexpected change to the risk/benefit ratio for the research.
- Any breach in confidentiality that may involve risk to the subject or others.
- Any complaint of a subject that indicates an unanticipated risk or that cannot be resolved by the Principal Investigator.

- **Reporting to the Food and Drug Administration**

  The investigator or his designee must submit SAEs on FDA Form 3500A (MedWatch) according to the following reporting criteria:

  - Reporting any unexpected fatal or life-threatening suspected adverse reactions no later than 7 calendar days after initial receipt of the information.

  - Reporting any (1) serious, unexpected suspected adverse reactions, (2) findings from other clinical, animal, or in-vitro studies that suggest significant human risk, and (3) a clinically important increase in the rate of a serious suspected adverse reaction no later than 15 calendar days after determining that the information qualifies for reporting.

8.3.2 Reporting to TRACON

- **Pregnancy**

Although not itself an SAE, pregnancy will be reported to TRACON and followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects or congenital abnormalities.

- **Serious Adverse Events**

SAEs will be reported to TRACON no later than 7 business days after initial receipt of the information.

8.4 Stopping Rules

For the phase 2 portion of the study stopping rules for toxicity and futility will be utilized. For safety, we will use a Bayesian continuous monitoring design testing for evidence of a high toxicity rate. Specifically, using a uniform prior for the probability of toxicity p_T, we stop the trial if $P(p_T > 0.4 \mid \text{data}) > 0.7$, that is if the posterior probability that the probability of toxicity is greater than 40% given the data is greater than 0.7. Stopping rules for futility are described in the appendix.

9.0 CORRELATIVES/SPECIAL STUDIES

Details of analyses are listed in the laboratory manual.

9.1 Correlative Studies

The following exploratory correlatives will be examined as part of this study (listed in order of prioritization):

Tissue	Study/biomarker
CTCs	PCR: AR, AR variants IHC: CD105, phosphorylated-SMAD 1/5, NE differentiation markers (Chromagranin A, Synaptophysin, NSE), RNPC1, phosphorylated-Smad2/3, total/phosphorylated-STAT-3, YAP, PD-L1, HEY, HES, EMT markers (E-cadherin, N-cadherin, Snail, Twist),
Plasma	soluble CD105, soluble Jag, cysteine, VEGF, IL-6, P1GF, bFGF, sVEGFR1, osteopontin, P1NP, CTX, RANKL, PTHrP, PTH, OPG
WBC (Buffy Coat)	phosphorylated-SMAD 1/5, HEY/HES, TAK1
T cells (Buffy Coat)	CD3, CD4, CD8, CD25, CD28, CD69, PD1
Monocytes (Buffy Coat)	CD68, CD206 (M2), CD62(activation), MHC class II (M1)
Tissue biopsy (when available and feasible)	PCR: AR variants IHC: CD31, CD105, phosphorylated-SMAD 1/5, NE differentiation markers (Chromagranin A, Synaptophysin, NSE), RNPC1, total/phosphorylated-STAT-3, YAP, HEY, HES, phosphorylated-Smad2/3, Ki67, TUNEL, stemness markers, LOX, EMT markers (E-cadherin, N-cadherin, Snail, Twist), caspase-3, vWF, sCD105.

9.2 Research Specimen collection

9.2.1 Blood collection

At specified time points, patients will have research kits collected consisting of the following:

- 3 yellow top (ACD) tubes- 8.5 mL (25.5 mL total)
- 1 lavender top tube (EDTA)- 6 mL

Details of collection and processing and analysis are included in the laboratory manual.

9.2.2 Tissue biopsy

Patients who are undergoing tissue biopsy as part of their standard clinical care may be asked to provide remnant tissue for molecular studies during the course of treatment. Procurement of the sample will be handled as described in the laboratory manual.

10.0 STATISTICAL CONSIDERATIONS

10.1 Study design and statistical approach

The overall hypothesis of this study is that inhibition of CD105 via TRC105 will re-sensitize patients progressing on an AR-inhibiting therapy to the same AR inhibitor.

It is unclear which of the two currently used next-generation AR-inhibitors is an optimal partner. Moreover, the preclinical data suggests that the effect should be similar for both agents. As such, the clinical translation of this hypothesis will involve two separate but parallel phase 2 studies. The two arms (A and E) will be merged for the testing of the translational hypothesis. The two arms will be studied in the clinic separately to avoid confusion due to the differences in the AR-inhibitor strategy that may not impact effect but may impact other clinically relevant parameters such as toxicity. The two arms will not be compared for effect in the context of this clinical investigation.

In discussion with Tracon, it was decided that the optimal doses for TRC105 is 15 mg/kg administered every 2 week following the 4-week lead-in consisting of weekly dosing at 10 mg/kg. Currently Tracon has been using the 15 mg/kg q2week dosing in a number of studies. Specifically, in prostate cancer, 20 mg/kg q2weeks has been tested and was found to be well tolerated. As it is expected that there will be no significant interactions based on the pharmacology of agents in question as well as the preclinical models from the Bhowmick laboratory, it was decided that there was no advantage to reduction of the AR-targeted agent doses.

To optimize toxicity and effectiveness, a Bayesian design was proposed with continuous reassessment of both variables over the 2 combinations.

Benefit is judged by proportion of patients experiencing clinical benefit at 2 months [45, 46]. As all patients entering this study are progressing on the AR agent already, the null hypothesis will be the proportion of patients who benefit will be 0%. To be conservative, we will set the null hypothesis at benefit rate of 5%. Conversely, the combination will be declared active if at 2 months of treatment, the proportion of patients who benefit is ≥ 50%.

As a matter of contrast, in a reported series, patients previously treated with abiraterone went forward to receive subsequent enzalutamide [47]. This group experienced at 63% benefit rate by PSA stabilization or response. Median time to progression (clinical and radiographic) was 5.3 months with a median duration of treatment of 4.1 months. A complementary series was reported of men who received enzalutamide that went forward to receive abiraterone [48]. In this group, 70% had clinical benefit by PSA of either stabilization or decrease. Median duration of treatment was 3 months (13 weeks). No radiographic responses were reported.

Additionally, if re-sensitization to AR therapy has occurred, we would expect to see stabilization or decreases in serum PSA concentrations. If at 2 months, the proportion of patients with no meaningful rise in PSA is ≥50%, a combination will also be considered worthy of further study.

10.2 Measurement of efficacy

The primary endpoint of these phase 2 trials is to show that the clinical benefit (CB) rate for these patients is at least 40% with a 5% response rate or lower being uninteresting. Let θ be the probability of CB of the new treatment combination. We will use a Bayesian continuous monitoring approach for testing the null hypothesis H_0: $\theta = 0.05$ versus the alternative hypothesis H_1: $\theta > 0.05$. Specifically, we will adapt the approach of Johnson and Cook (2009) by placing a point mass prior distribution on θ to represent the null hypothesis and an inverse moment prior density with mode at $\theta = 0.4$ to represent the alternative hypothesis.

Johnson and Cook (2009) [49] showed that this design has better operating characteristic than previous Bayesian designs which use posterior credible intervals to define stopping boundaries for inferiority and superiority. When compared to Simon's (1989) two-stage design [50], the probabilities of rejecting the experimental treatment were similar but this design uses fewer patients to reject treatments that fall below the target probability of response relative to Simon's design.

A maximum of 20 patients per arm will be enrolled in the trial. Assuming that H_0 and H_1 are equally likely *a priori*, the trial will be terminated after the *n*th patient for inferiority if the posterior probability of the alternative given the data $P(H_1 |\text{data}) < 0.1$. At the end of the trial, the alternative hypothesis is accepted if $P(H_1 |\text{data}) > 0.9$.

Operating Characteristics:

When the true probability of clinical benefit is 0.4, there is at least an 88.6% chance of concluding that the treatment is superior which is equivalent to the frequentist statistical power.

Table 5 gives some operating characteristics for testing the above hypothesis under five scenarios for the "true" probability of CB θ based on 5,000 trial replications. When the true θ is 0.4, there is a 0.048 probability of stopping the trial early for lack of efficacy and this probability is even smaller with smaller values of θ. The futility boundaries for stopping the trial early are listed in Table 6. These simulations were carried out using the software BFDesigner available at http://biostatistics.mdanderson.org/softwaredownload/.

Scenario	True θ	Pr(Stopping for inferiority)	Average # patients treated (10%, 25%, 50%, 75%, 90%)
1	0.05	0.953	10.9 (10,10,10,10,10)
2	0.15	0.593	14.61 (10,10,10,20,20)
3	0.25	0.261	17.58 (10,20,20,20,20)
4	0.4	0.048	19.53 (20,20,20,20,20)
5	0.5	0.013	19.87 (20,20,20,20,20)

Table 5. Operating Characteristics

# Patients	Stop the trial for inferiority if there are this many successes
10	0-1
15	0-1
20	0-2

Table 6. Full Stopping Boundaries

10.3 Safety

Stopping rules for safety

Let P_{tox} be the true probability that a patient experiences a grade 3 or higher toxicity related to the treatment combination. The trial will stop if there is statistical evidence that P_{tox} exceeds 40%. We will use a Bayesian sequential design by checking whether P_{tox} exceeds this threshold value after 5, 10, 15, and 20 patients are evaluable for toxicity as defined above. The decision rule is to stop the trial if the posterior probability that P_{tox} exceeds 0.40 is 0.95; $P(P_{tox} > 0.4 \mid \text{data}) > 0.95$. A noninformative prior distribution for P_{tox} will be used. Table 7 gives the stopping rules for the design at each look and column 2 gives the maximum number of patients with toxic events in order for the trial to proceed. For example, if 10 or more toxic related events are observed after enrolling 15 patients, the trial stops. The third column gives the probability of stopping

the trial when in fact, the true P_{tox} = 0.40. This is the equivalent of the Bayesian type I error probability. The target type I error probability was set at 0.05.

Number of Patients	Number to Continue	Probability to Stop	Cumulative Probability to Stop
5	4	0.01024	0.01024
10	7	0.00904	0.01928
15	9	0.02388	0.04316
20	12	0.00609	0.04925

Table 7. Stopping rules based on four interim looks. Number to continue is the maximum number of toxic events for not stopping the trial.

Table 8 gives the design operating characteristics under selected values of the true probability P_{tox}. It gives the probability of stopping the trial under the alternative hypothesis, the expected sample size, and the average sample size given that the trial stopped. For example, if the true value of P_{tox} is 0.6, then there is an 50% chance that the trial is stopped early and the average sample size is about 16. On the other hand, there is a small chance of stopping the trial if P_{tox} is small; 0.67% chance of stopping the trial if in fact P_{tox} = 0.30.

True Value of P_{tox}	Probability to Stop	Expected N	Expected N given that we Stopped
0.30	0.0067	19.94	10.81
0.40	0.0493	19.64	12.62
0.60	0.5022	16.53	13.09
0.80	0.9763	10.16	9.93

Table 8. Design operating characteristics under different scenarios for the true probability of toxicity P_{tox}.

11.0 STUDY MANAGEMENT

11.1 Conflict of Interest

Any reportable conflict of interest will be disclosed to the local IRB and will be outlined in the Informed Consent Form.

11.2 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

11.3 Registration Procedures

All subjects that sign informed consent and are deemed eligible will be assigned a subject number sequentially at the time of registration. Those subjects that do not pass the screening phase will be listed as screen failures on the master list of consented subjects. Eligible subjects, as determined by screening procedures and verified by a treating investigator, will be registered on study at Cedars-Sinai Medical Center by the Study Coordinator.

Issues that would cause treatment delays after registration should be discussed with the Principal Investigator (PI). If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled.

Subjects found to be ineligible will be recorded as screen failures. Subjects found to be eligible will be registered.

11.3.1 Eligibility Verification

Prior to registration, all subjects must undergo a secondary eligibility verification by the SOCCI Clinical Research Office (CRO). The following documents will be completed and provided for review:

- Applicable source documents
- Eligibility checklist (signed by investigator)
- Signed patient consent form and Subject's Bill of Rights
- HIPAA authorization form

11.3.2 Registration

After eligibility is verified, registration is completed as follows:

- Assign a patient study number
- Enter the patient in OnCore

Oversight by the principal investigator is required throughout the entire registration process

11.4 Data Management and Monitoring/Auditing

The data will be entered into a HIPAA-compliant database. The Study Staff will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

11.5 Data and Safety Monitoring

11.5.1 Data Monitoring and Quality Assurance

Adherence to the protocol, Good Clinical Practices (GCP), and institutional policy will be monitored by the PI during the course of the study through routine Disease Research Group (DRG) meetings or equivalent. In addition, the SOCCI CRO Quality Management Core (QMC) will conduct the following:

- Audit preparations (AP) prior to audit conducted by any external auditing agency (i.e. NCI or FDA). The purpose an AP is to ensure adequate source documentation to support protocol compliance and data integrity are present and organized and to identify and correct any major findings prior to the external audit
- A thorough review of selected subject cases, regulatory files, and IP accountability records (if applicable) within 2-3 months after the first subject is enrolled and annually thereafter while subjects are receiving investigational intervention.
- Central eligibility verification for all subjects enrolled as described in protocol section 11.3.1.
- Central review of all eligibility waiver requests by a SOCCI Medical Reviewer to assess appropriateness and risk to ensure quality data and ensure subject safety protections for investigator-initiated research

For any protocol, QMC has the authority to request more frequent reviews or closer safety monitoring if it is deemed appropriate for any reason.

11.5.2 Safety Monitoring

Oversight of the progress and safety of the study will be provided by the PI. The PI will maintain continuous safety monitoring for the duration of the study by reviewing subject/study data. Adverse events and unanticipated problems are not expected, but if they occur they will be documented and reported according to CSMC IRB policies and procedures. If the PI becomes aware of any new safety information that may place subjects at increased risk than what was previously known the IRB will be promptly notified and if warranted, enrollment may be held until the PI determines whether a modification to the study is necessary and/or the informed consent documents are updated accordingly.

In addition, this protocol will utilize oversight by a **S**afety **Co**mmittee **O**n **E**arly **P**hase **S**tudies (SCOEPS). Committee membership includes experts in the field of oncology and early phase studies and biostatistics. SCOEPS' responsibilities are governed by the committee charter, or equivalent.

The SCOEPS will provide routine monitoring of safety and enrollment for all early phase investigator-initiated trials (IITs). The committee meets routinely and is responsible for reviewing and adjudicating all dose-limiting toxicities, dose escalations and appropriateness of the escalation, cohort expansion, subject replacements, select AEs, SAEs, and confirmation of attainment of maximum tolerated dose.

The SCOEPS findings and recommendations will be reported in writing to the Principal Investigator. A summary report will be forwarded by the Principal Investigator or his/her designee to the Cedars-Sinai Medical Center IRB.

11.6 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, monitoring/auditing logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study. Government agency regulations and directives require that the study investigator must retain all study documentation pertaining to the conduct of a clinical trial. Study documents should be kept on file per local guidelines.

11.7 Adherence to Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, or a protocol exception request approved by the SOCCI Medical Director and CSMC IRB, the study shall be conducted exactly as described in the approved protocol.

11.7.1 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval. For any such emergency modification implemented, the IRB must be notified as soon as possible, but no more than 10 days from the investigator's awareness of the event.

11.7.2 Protocol Exceptions and Eligibility Waivers

An exception is an anticipated or planned deviation from the IRB-approved research protocol, as described in the IRB Policy, Reporting Possible Unanticipated Problems Involving Risks to Subject or Others (UPIRSO) Policy: Institutional Review Board/Research Compliance and Quality Improvement.

A protocol exception most often involves a single subject and is not a permanent revision to the research protocol. Protocol exceptions that extend beyond a single subject should result in a protocol amendment to avoid serial violations.

All exception requests must be reviewed by the SOCCI CRO Medical Director and the Institutional Review Board prior to implementation. The PI or her/his designee is responsible for submitting a protocol exception and its supporting documents to the SOCCI Medical Director for review. Planned exceptions to the protocol that are more than logistical and/or have the potential to affect the subject's safety and/or study integrity may not be implemented without prior approval from the SOCCI Medical Director and IRB.

- Special considerations for Eligibility Waivers (EW)

In general, subjects who do not meet the eligibility requirements should not be enrolled. In the rare event that it is appropriate for subject inclusion, the rationale/justification and subject case history should be forwarded to the SOCCI CRO Medical Director for assessment **prior** to submission to the IRB for approval.

The CRO Medical Director will review the case and contact the investigator if additional information is needed or further discussion is warranted. The CRO Medical Director will provide a written assessment/recommended course of action. The CRO Medical Director's assessment must be uploaded into Webridge with the waiver request for IRB review and consideration. The CRO Medical Director may recommend future protocol changes.

- Eligibility Waiver Submission Process

The PI and/or treating physician should provide written request for waiver which includes case history and justification for prospective deviation from the study design to the SOCCI CRO Medical Director.

11.7.3 Other Protocol Deviations

Logistical deviations from the protocol (e.g., minor changes to the study schedule for an individual subject) do not require prior IRB approval unless the deviation has the potential to affect the subject's safety. Such planned deviations that do meet this definition and do not affect the subject's safety should be noted in the subject's research record or deviation log as described in the SOCCI Clinical Research Office's Working Instruction 11: Deviation and Noncompliance Reporting.

Unintentional deviations from the protocol that might affect subject safety or study integrity should be reported to the IRB within 10 days from when the investigator becomes aware that such a deviation has occurred, as outlined in the SOCCI Clinical Research Office's Working Instruction 11: *Deviation and Noncompliance Reporting*. In this case, a Protocol Deviation report must be submitted in Webridge, per IRB policy, *Reporting Possible Unanticipated Problems Involving Risks to Subject or Others (UPIRSO) Policy: Institutional Review Board/Research Compliance and Quality Improvement*. All submissions should include a description of the plan to avoid similar deviations or exceptions in the future.

11.7.4 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

The written amendment, and if required the amended consent form, must be sent to the IRB for approval prior to implementation. Repeat exceptions or deviations to the protocol may suggest a protocol amendment is needed.

11.8 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

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13.0 APPENDICES

List of CYP3A4 active agents (substrates & inhibitors)

13.1 Appendix 1: Inhibitors of CYP3A4

Amiodarone	Fluconazole	Paroxetine (weak)
Anastrozole	Fluoxetine	Propoxyphene
Azithromzcin	Fluvoaxamine	Quinidine
Cannabinoids	Gestodene	Quinine
Cimetidine	Grapefruit, Grapefruit juice	Quinupristine and dalfopristin
Clarithromycin	Indinavir	Ranitidine
Clotrimazole	Isoniazid	Ritonavir
Cyclosporine	Ketoconazole	Saquinavir
Danazol	Metronidazole	Sertindole
Delavirdine	Mibefradil	Sertraline
Dexamethasone	Miconazole	Troglitazone
Diethyldithiocarbamate	Nefazodone	Troleandomycin
Diltiazem	Nelfinavir	Valproic acid
Dirithyromycin	Nevirapine	
Disulfiram	Norfloxacin	
Entacapone (high dose)	Norfluoxetine	
Erythromycin	Omeprazole	
Ethinyl estradiol	Oxiconazole	

13.2 Appendix 2: Inducers of CYP3A4

Barbiturates	Mitotane	Pioglitazone
Bosentan	Nafcillin	Rifabutin
Carbamazepine	Nevirapine	Rifampin
Efavirenz	Oxcarbazepine	St. John’s wort
Glucocorticoids	Phenobarbital	Tipranavir/Ritonavir
Modafinil	Phenytoin	Troglitazone