COMPREHENSIVE CANCER CENTER
UNIVERSITY OF ALABAMA AT BIRMINGHAM

Local Protocol #: UAB 1514

A Phase I/II Study of Preoperative (Neoadjuvant) Combination of Letrozole (Femara®), Everolimus (Afinitor®), and TRC105 in Postmenopausal Women with Newly Diagnosed Local or Locally Advanced Potentially Resectable Hormone-Receptor positive and Her2 negative Breast Cancer

Brief Title: Letrozole, everolimus, and TRC105 in postmenopausal HR positive Breast Cancer

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PROTOCOL SYNOPSIS

Title of Study: A Phase I/II Study of Preoperative (Neoadjuvant) Combination of Letrozole (Femara®), Everolimus (Afinitor®), and TRC105 in Postmenopausal Women with Newly Diagnosed Local or Locally Advanced Potentially Resectable Hormone-Receptor positive and Her2 negative Breast Cancer

Brief Title: Letrozole, everolimus, and TRC105 in postmenopausal hormone receptor positive Breast Cancer

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Phase: Pilot	**Type of Investigation:** drug

Study Center(s): UAB, Comprehensive Cancer Center

Rationale: Our group has demonstrated in 2 different and independent studies that the addition of an anti-angiogenic agent to hormonal therapy in the neoadjuvant setting increased not only the proportion of patients with a pathologic complete remission (pCR) and near pCR (invasive residual disease less than 5 mm in the primary tumor) but also the proportion of patients that were down-staged (from stage 2 and 3 to 0 and 1)[1,2] (a prognostic factor in neoadjuvant hormonal therapy). In addition, preclinical and clinical studies have shown that the addition of an mTOR inhibitor to hormonal therapy is able to reverse resistance to hormonal agents; these studies led to the approval of everolimus in combination with exemestane by the FDA for hormone receptor positive metastatic breast cancer[3-6]. However, preclinical studies have shown that mTOR inhibition results in VEGF inhibition which leads to overexpression of CD105 in the endothelial cells as a compensatory mechanism[7,8]. Thus, the addition of an anti-CD105 agent may increase the efficacy observed in the randomized trial of letrozole and everolimus in patients with stage 2 and 3 breast cancer published by Baselga recently[4].

Background: The achievement of a pCR with neoadjuvant therapy in early-stage breast cancer has a favorable impact on long-term outcomes and is an excellent prognostic surrogate marker[9-13]. Responses to neoadjuvant therapy however, are intrinsically dependent on the molecular subtype[14] with the immunophenotypically luminal (hormone-receptor positive and HER2 negative) breast cancers rarely achieving a pCR[12]. Nonetheless, in the latter subgroup, downstaging to at least pathologic stage 1 or 0 is equally important as it has been associated with a significant low risk of recurrence[15]. With chemotherapy yielding similar benefits with hormonal therapy in the neoadjuvant setting[16,17], the latter is generally preferred as it is associated with fewer adverse events and is overall better tolerated among postmenopausal women who frequently have other comorbidities[18]. In the neoadjuvant setting, aromatase inhibitors have been consistently shown to be superior over tamoxifen[19-23], making the former the agents of choice in postmenopausal women with hormone-receptor positive [HR(+)] breast cancer. Despite the high rates of clinical and radiographic response achieved with hormonal therapy[19-23], the rates of downstaging are still low[15]. **Collectively, there is a need to improve the efficacy of aromatase inhibitors in the neoadjuvant setting with minimal additional toxicities as it directly impacts clinical outcomes.**

Targeting angiogenesis in HR(+) breast cancer: Angiogenesis is an integral step in tumor progression[24]. In mouse xenograft models of HR(+) breast cancer, enhanced angiogenesis

accelerated tumor growth[25], and overexpression of the Vascular Endothelial Growth Factor (VEGF) either by the tumor[26] or stromal cells[27] was associated with hormone independent growth and resistance to endocrine therapies.

The concept of targeting angiogenesis to restore or enhance the efficacy of neoadjuvant hormonal therapy in breast cancer has been successfully translated in the clinical arena. The Translational Breast Cancer Research Consortium completed a study comparing preoperative letrozole versus letrozole plus bevacizumab in postmenopausal women with operable HR(+) breast cancer. The study (TBCRC002) showed that bevacizumab "deepened" the responses obtained with letrozole alone. Downstaging from stage 2/3 to 0/1 was achieved in 45% of patients in the letrozole-bevacizumab arm as compared to 25% in the letrozole-alone group. Remarkably, the combination induced a pCR in 11.1% of patients [1] (and 8% near pCR), comparable to the 12% pCR rate reported in a prior pilot single-institution study [2]; no complete or near-complete pathologic remissions were observed in the letrozole-alone group. **Taken together, enhanced angiogenesis is associated with hormone resistance and there is a successful clinical precedence of rationally combining an antiangiogenic agent with hormonal therapy that even led to an unprecedented pCR rate in an intrinsic subgroup where pCRs are rarely observed.**

Endoglin (CD105) as a rational target of malignant angiogenesis: Endoglin is a transmembrane accessory receptor for transforming growth factor-beta (TGF-β) that is predominantly expressed on proliferating endothelial cells in culture and on angiogenic blood vessels in vivo[28]. It diverts TGF-β downstream signaling toward proliferation and migration as well as transcription of pro-angiogenic genes including endoglin itself[29-31]. Preclinical evidence suggests that CD105 is highly and exclusively or mainly expressed in *de novo* tumor vessels[32,33], as opposed to the normal quiescent endothelium which has a very low turnover. In preclinical murine xenograft models of HR(+) breast cancer, anti-endoglin monoclonal antibodies selectively inhibited tumor angiogenesis and delayed the growth of established tumors, while sparing normal vasculature[34]. Endoglin expression has been investigated in multiple malignancies and has been consistently associated with adverse clinical outcomes[31]. This observation is in line with the premise that enhanced tumor vasculature correlates with adverse prognosis and the fact that endoglin is a marker of activated angiogenic endothelium[31]. **Collectively, its exclusive expression in the activated endothelium and its pathophysiologic role in promoting angiogenesis make endoglin a rational target of tumor angiogenesis.**

Pharmacologic targeting of endoglin (CD105) with TRC105: TRC105 is a chimeric IgG1 monoclonal antibody that binds with high avidity to CD105 (endoglin). It inhibits TGF-β mediated pro-angiogenic signaling and induces potent antibody-dependent cell-mediated cytotoxicity. In a phase I study, the maximum tolerated dose was determined at 10 mg/kg weekly; 15 mg/kg every two weeks was equally well tolerated and also resulted in continuous serum concentrations known to saturate CD105 binding sites on proliferating endothelium[35]. The most common adverse events associated with the investigational agent were infusion reactions that were successfully mitigated with pre-medications and prolongation of the infusion time, hemorrhages (mostly grade 1 and 2), headaches, and hypoproliferative anemia[35]. Importantly, the median age in this phase I clinical trial was 63[35], making this study highly informative in terms of adverse events for the patient population intended to be used in our proposed study. **Considering the non-overlapping toxicity profile with aromatase inhibitors**

and the preclinical evidence highlighting malignant angiogenesis being in the core of hormone independence, there is an unprecedented potential of synergism between TRC105 and letrozole that is worth clinical investigation.

Rationale for incorporating everolimus in the neoadjuvant setting: The phosphatidyl inositol-3 kinase (PI3K)/ protein kinase B (AKT)/ mammalian target of rapamycin (mTOR) pathway is an intracellular signal transduction pathway downstream of receptor tyrosine kinases[36]. Class IA PI3K molecules are heterodimers composed of a regulatory subunit (p85) and a catalytic subunit (p110), the α isoform of which (encoded by *PIK3CA*) is widely mutated or amplified in human cancers[37,38], including breast cancer[39,40]. Comprehensive genomic analyses of breast cancer samples in the context of The Cancer Genome Atlas Project also highlighted the high frequency of mutations along the PI3K/AKT/mTOR pathway[41]. In particular, activating mutations in the *PIK3CA,* inactivating mutations or loss of *PTEN,* and loss of *INPP4B* (a negative regulator of the PI3K/AKT pathway) were detected in 49%, 13%, and 9% (luminal A) and 32%, 24%, and 16% (luminal B), respectively. At the preclinical level, breast cancer cells with upregulated PI3K/AKT/mTOR pathway were shown to be resistant to hormonal therapy and this resistance was restored by everolimus and other mTOR inhibitors [42,43]. **Taken together, the PI3K/AKT/mTOR pathway can be activated by various genetic events in HR (+) breast cancer and its inhibition with the FDA-approved agent everolimus has been proven at the clinical level to restore[3] or enhance[4] sensitivity to endocrine therapies.**

Synergism between TRC105 and everolimus. The molecular basis for combining antiangiogenic therapy with everolimus is provided by preclinical studies [44-46]. In animal models, blocking mTOR was shown to reduce the VEGF levels and to inhibit significantly the response of endothelial cells to stimulation by VEGF [45]. mTOR signaling was also shown to play a key role in hypoxia-triggered smooth muscle and endothelial cell proliferation and angiogenesis in vitro; this response (triggered by growth factors other than VEGF, such as FGF and PDGF) was abrogated with rapamycin, the prototypic mTOR inhibitor[46].

Moreover, VEGF inhibition leads to reactive upregulation of endoglin[7] and large established CD105(+) vessels lying in the core of the tumors have been shown to be insensitive to VEGF inhibitors[8]. In those studies, CD105 expression was increased following inhibition of the VEGF pathway. CD105 expression increased more than two-fold in human pancreatic cancers grown in mice treated with an antibody that binds VEGF[7]. 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[8]. Finally, a study of spontaneously occurring neuroendocrine tumors in RIP-Tag2 mice treated with antibody to VEGF indicated that TGF- β was the most highly upregulated factor, as determined by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR)[47]. These studies indicate that the CD105 pathway is a significant mechanism of escape from VEGF inhibitor therapy.

Rationale for ovarian suppression with gonadotropin-releasing hormone analogs in premenopausal women. The IMPACT trial (Immediate Preoperative Anastrozole, Tamoxifen or Combined with Tamoxifen) established the superiority of aromatase inhibition over tamoxifen or tamoxifen + aromatase inhibition in the preoperative setting [48] . In addition, suppression of the proliferation marker Ki67 (which in itself has been shown to be predictive of favorable outcome) was statistically greater with anastrozole as compared with tamoxifen or the

combination [49]. Aromarase inhibitors in premenopausal women are contraindicated as they may increase gonadotropin secretion because of the reduced feedback of estrogen to the hypothalamus and pituitary[50]. To that end, in order for premenopausal women to benefit from aromatase inhibition, they should become postmenopausal through ovarian suppression with gonadotropin-releasing hormone analogs. For this clinical trial goserelin subcutaneously q 4 weeks will be used.

Collectively, considering the non-overlapping toxicity profiles and the preclinical evidence highlighting malignant angiogenesis and the PI3K/AKT/mTOR pathway being in the core of resistance to and failure of endocrine therapies, there is an unprecedented potential of synergism among letrozole, TRC105, and everolimus that is worth clinical investigation.

Primary Objective: Determine the tolerability and feasibility of combining everolimus and TRC105 with letrozole in postmenopausal women with newly diagnosed stage 2 and 3 (T2-T4a-c, N0-2, M0; excluding patients with T4d or inflammatory breast cancer) estrogen and/or progesterone receptor positive, HER2/neu negative breast cancer, treated for 24 weeks in the preoperative (neoadjuvant) setting.

Secondary Objective:

1. Determine the efficacy of the combination of letrozole, everolimus, and TRC105
2. Determine the pharmacokinetic parameters of everolimus and TRC105 when used in combination with letrozole.
3. Determine the pharmacodynamic parameters of everolimus and TRC105 when used in combination with letrozole.

Exploratory objective: Evaluate specific biomarkers of potential prognostic value and biomarkers potentially predictive of response or resistance to the combination of everolimus and TRC105 with letrozole.

Primary Endpoint: Determine the maximum tolerated dose (MTD), the recommended phase 2 dose (RP2D) and rates of adverse events associated with the combination of letrozole with everolimus and TRC105.

Secondary Endpoints:

1. Determine the rates of complete pathologic remission and downstaging achieved with the investigational treatment at the time of surgery.
2. Determine the C_{max}, T_{max}, AUC, T½, clearance of everolimus and TRC105 when used in combination with letrozole.
3. Determine the changes in tumor cell proliferation by means of changes in Ki67 expression and changes in serum concentration of markers of angiogenesis (VEGF-A, soluble VEGFR2, activin A and activin B, angiopoietin-1 and -2, TGF-β, soluble endoglin) before and after the investigational treatment and correlate with clinical, radiographic, and pathologic response.

Exploratory Endpoints:

1. Correlate the levels of PTEN immunostaining with response
2. Correlate levels of phosphorylated AKT (pAKT) immunostaining with response
3. Correlate expression of endothelial (CD105 and CD31/CD34) and pericyte (NG2, and MCAM [CD146]) markers in the initial diagnostic and final surgical specimens with response.

4. Determine translational (mRNA to protein) profiles by means of next-generation whole exome sequencing and sequencing of ribosome-protected mRNA associated with resistance to the investigational combination.

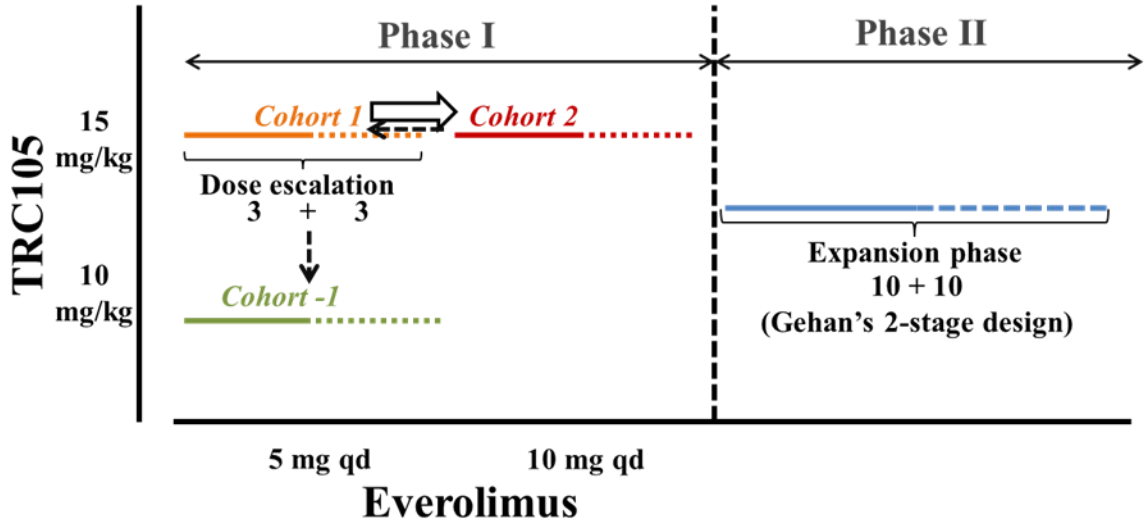
Study Design: the study consists of 2 parts.

The first part of the study will follow the conventional 3+3 dose escalation design. Up to 18 patients will be enrolled. During the first part (phase I, **dose escalation**), the Maximum Tolerated Dose (MTD) and the Recommended Phase 2 Dose (RP2D) of the combination will be determined. The dose of letrozole will be kept the same for all cohorts (2.5 mg PO daily) (Table 1). The dose of everolimus will be escalated from 5 mg PO daily (cohort 1) to 10 mg PO daily (cohort 2, *continuous arrow*). The dose of everolimus will be de-escalated to 5 mg PO daily (cohort 1) if the dose of 10 mg PO daily is not well tolerated (*dashed arrow*). The dose of TRC105 will be 15 mg/kg q 2 weeks (cohorts 1 and 2, *continuous arrow*). The dose of TRC105 will be de-escalated to 10 mg/kg q 2 weeks (cohort -1) if 15 mg/kg q 2 weeks in combination with everolimus 5 mg PO daily are not well tolerated (*dashed arrow*). Different doses of the 3 agents evaluated in this trial have shown to be active against breast cancer in clinical trials.
Premenopausal women at the time of study enrollment will receive goserelin 3.6 mg subcutaneously q 4 weeks to achieve ovarian suppression.

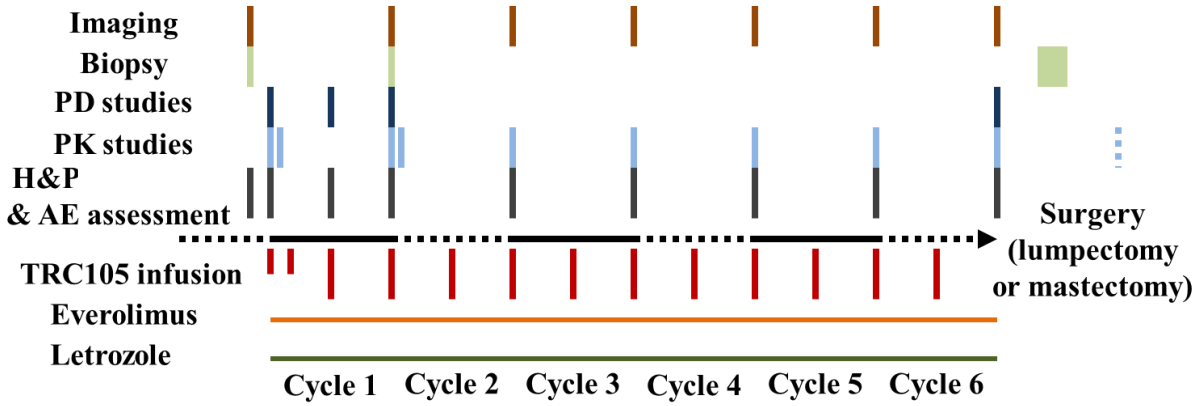
Cohort	TRC105 (mg/kg IV q 2 weeks)	Everolimus (mg PO daily)
1	15	5
2	15	10
-1	10	5

The second part of the study (phase II, **expansion phase**), will ensue once the MTD and RP2D have been determined. In order to minimize exposure of patients to ineffective therapy, a Gehan's two-stage design will be used[51]. The study will enroll 10 patients first, and if no patient has pCR or downstaging, the study will close on the basis that it is unlikely (p=0.1074) that 0/10 responses would occur if the true response rate were >20%. If at least one patient has pCR or downstaging, an additional 10 patients will be treated (total of 20). Accrual will be halted as a result of any grade ≥3 hemorrhage, cerebrovascular and vasovagal adverse events; the FDA will be contacted to discuss further accrual or potential need for protocol amendment.

Study Schema



Study Procedures



Premenopausal women at the time of study enrollment will receive goserelin 3.6 mg subcutaneously q 4 weeks to achieve ovarian suppression

Drug Dose (doses, frequency, duration):
Letrozole 2.5 mg PO daily, continuously until the day prior to surgery. TRC105 15 or 10 mg/kg IV every two weeks until 4 weeks prior to surgery. The first dose of TRC105 will be split into two doses; see Table 7-1 (Study Calendar) for additional details. Everolimus 5 or 10 mg PO daily, continuously until 4 weeks prior to surgery. Premenopausal women at the time of study enrollment will receive goserelin 3.6 mg subcutaneously q 4 weeks to achieve ovarian suppression.

Main Criteria for Inclusion/Exclusion:
Key Inclusion Criteria

- Recent diagnosis of hormone receptor positive and HER2 negative breast cancer.
- Stage 2 and 3 hormone receptor positive and HER2 negative breast cancer (stage T2-4 but not inflammatory, N0-2, M0).

- Histological grade I, II or III according to the modified Bloom Richardson scale[52].
- No prior treatment specific for breast cancer.
- Menopausal status as defined by the National Comprehensive Cancer Network. Premenopausal women are eligible if ovarian suppression is achieved with goserelin
- ECOG performance status ≤ 2 (Karnofsky ≥ 60%).
- Must have signed study-specific informed consent.
- Liver Function Tests ≤ 2.5 times the upper normal limit (UNL).
- Staging workup: medical/surgical/radiation therapy evaluation, CT scans and bone scan.
- Lipid profile (cholesterol and triglycerides).
- ANC ≥ 1,500/mm^3, platelets ≥ 100,000/mm^3, Hemoglobin ≥ 10g%.
- Renal function: serum creatinine ≤ 1.5 institutional UNL or creatinine clearance > 40 cc/min.

Key Exclusion Criteria
- Inflammatory breast cancer.
- Pregnancy.
- Metastatic disease.
- HER2 positive breast cancer by immunohistochemistry or FISH.
- Triple negative breast cancer (hormone receptor and Her2 negative).
- Disease that cannot be followed by imaging studies.

Safety Assessments:	**Efficacy Assessments:**
• Vitals signs • Weight • ECOG performance status • Hematology • Chemistry • Lipid profile • Hepatitis panel • Adverse events • Concomitant medications	• Physical examination • Imaging studies (mammography, breast ultrasound, breast magnetic resonance imaging) • Surgical Pathology

Other Assessments:

a. Pharmacokinetic (PK) studies. To investigate the pharmacokinetic interaction between TRC105 and everolimus, samples will be collected and concentrations of the agents measured on cycle 1 day 1 and cycle 2 day 1 at the following time points: pre-dose, 1, 2, 4, 6, and 24 hours and also cycle 1 day 4 predose. Pharmacokinetic parameters to be estimated include maximum serum concentration (C_{max}), time of maximum serum concentration (T_{max}), area under the serum concentration versus time curve (AUC), terminal half-life (T½), and clearance. Serum for assessment of TRC105 anti-product antibody formation will be collected before dosing on cycle 1 day 1, every 4 weeks thereafter, 4 and 12 weeks following the last dose of TRC105 and concentrations will be determined by a validated ELISA[53].

b. Pharmacodynamic (PD) studies. Markers of angiogenesis (VEGF-A, soluble VEGFR2, activin A and activin B, angiopoietin-1 and -2, TGF-β, soluble endoglin) will be measured on samples collected pre-dose, cycle 1 day 15, cycle 2 day 1, and prior to surgery.

c. Research biopsy. A research biopsy will be obtained at baseline, on cycle 2 day 1, and the time of the surgery. At least 2 core biopsies will be taken, one will be embedded in paraffin and the other one will be immediately frozen. Immunohistochemistry for pAKT and PTEN (markers

of the PI3K/AKT/mTOR pathway), CD105 and CD31/CD34 (pan-endothelial markers), and NG2 and MCAM/CD146 (pericyte markers) will be conducted on the research biopsies, and the final surgical specimen. Next Generation Sequencing. Determine translational (mRNA to protein) profiles by means of next-generation whole exome sequencing and sequencing of ribosome-protected mRNA associated with resistance to the investigational combination.

Statistical Methods: this is a phase I study and primarily descriptive statistics will be used.

(a) Sample size justification: The dose escalation part of the study will follow a conventional 3+3 dose escalation design according to the schema above. Up to 18 patients will be enrolled in this part anticipating that dose level 1 or 2 will be the RP2D. The number of patients to be enrolled ranges from 3 (≥2/3 DLTs in cohort 1) to 18 (enrollment of 6 patients in all cohorts). The sample size of 20 in Phase II part study is NOT determined by the statistical power but the feasibility and relative precision of estimate, e.g. standards error of the estimation is ≤10%. We will estimate the pCR of the combination to generate hypotheses for further clinical investigations. A Gehan's two-stage design will be used[51] which allows for the rapid rejection of an ineffective treatment at the end of the first stage, and provides an estimation of the success rate with a given precision, at the end of the second stage. The study will enroll 10 patients first. If no patient has pCR or downstaging, it is unlikely (p=0.1074) that 0/10 responses would occur if the true response rate were >20% and the phase II portion will be closed. If at least one patient has pCR or downstaging, an additional 10 patients will be treated (total of 20). With an accrual of 20 patients to phase II portion of the study, estimated two-sided 95% confidence intervals (CI) would be 1.2%-31.7%, 5.7% -43.6% and 11.8%-54.3% if pCR is 10%, 20% or 30% respectively. The confidence intervals are calculated using the exact method Clopper-Pearson intervals[54]. Final analysis will include all patients enrolled in the trial.

(b) Analytic plan for primary objective: The rate of adverse events will be estimated at the end of the study along with two-sided 95% exact CIs (Clopper-Pearson intervals).

(c) Analytic plan for secondary objectives:

1. pCR or downstaging rate will be estimated along with two-sided 95% CIs with the exact method of Clopper-Pearson intervals.
2. We will use descriptive statistics (mean, median, geometric mean, coefficient of variation, and 95% confidence intervals) and graphical displays to evaluate the pharmacokinetic parameters of the TRC105 and everolimus.
3. We will use descriptive statistics (mean, median, geometric mean, coefficient of variation, and 95% confidence intervals) and graphical displays to analyze the changes in markers of proliferation and angiogenesis with the investigational treatment.
4. Exploratory analysis will be conducted on the correlations between clinical outcomes and pharmacodynamic biomarkers or translation profiles.

TABLE OF ABBREVIATIONS

4-EBP-1	4E Binding Protein 1
ACE	Angiotensin-Converting Enzyme
AdEERS	Adverse Event Expedited Reporting System
ADR	Adverse Drug Reaction
AE	Adverse events
ANC	Absolute Neutrophil Count
AUC	Area Under the Curve
AKT	Protein Kinase B
ALT	Alanine aminotransferase/glutamic pyruvic transaminase/GPT
APA	Anti-Product (TRC105) Antibody
AST	Aspartate aminotransferase/glutamic oxaloacetic transaminase/GOT
BAL	Bronchoalveolar lavage
BCG	Bacillus Calmette-Guérin
BIRADS	Breast Imaging Reporting and Data System
BUN	Blood Urea Nitrogen
C_{max}	Maximum Serum Concentration
CBC	Complete Blood Count
CC	Craniocaudal
CD	Cluster of Differentiation
CD105	Endoglin
CI	Confidence Intervals
CoA	Coenzyme A
CPK	Creatine Phosphokinase
CR	Complete Response
CRF	Case Report Form
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CYP3A4	Cytochrome P450 3A4
DEXA	Dual-Energy X-ray Absorptiometry
DLCO	Diffusing capacity of the Lung for Carbon Monoxide
DLT	Dose Limiting Toxicity
DM	Diabetes Mellitus
DS&E	Drug Safety and Epidemiology Department
ECOG	Eastern Cooperative Oncology Group
EKG	Electrocardiogram
ELISA	Enzyme-linked Immunosorbent Assay
ER	Estrogen Receptor
FDA	Food and Drug Administration
FGF	Fibroblast Growth Factor
FISH	Fluorescence *in situ* Hybridization
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GERD	Gastroesophageal Reflux Disease
GI	Gastrointestinal

GnRH	Gonadotropin-releasing hormone
HAMA	Human Anti-Murine Antibody
HACA	Human Anti-Chimeric Antibody
HbA1c	Hemoglobin A1c
HBcAb	Hepatitis B core antibody
HbsAb	Hepatitis B surface antibody
HbsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HHT-1	Hereditary Hemorrhagic Telangiectasia T1 (Rendu/Osler/Webber
HMG	syndrome)
HR	3-hydroxy-3-methyl-glutaryl
HUVEC	Hormone Receptor
IB	Human Umbilical Vein Endothelial Cells
ICH	Investigator's Brochure
IgG1	International Conference on Harmonization
IHC	Immunoglobulin G1
IRB	Immunohistochemistry
IUD	Institutional Review Board
IUS	Intrauterine Device
IV	Intrauterine System
kg	Intravenous
LD	kilogram
LH	Longest Diameter
log_{10}	Luteinizing Hormone
MedDRA	Decadic logarithm (common logarithm)
mg	Medical Dictionary for Regulatory Activities
MLO	milligram
MMP-14	Mediolateral Oblique
MRI	Matrix Metalloproteinsae – 14
mRNA	Magnetic Resonance Imaging
MTD	messenger RNA
mTOR	Maximum Tolerated Dose
MUGA	mammalian Target Of Rapamycin
NCI	Multi Gated Acquisition Scan
pAKT	National Cancer Institute
pCR	phosphorylated Protein Kinase B
PCR	Pathologic Complete Remission
PD	Polymerase Chain Reaction
PD	Pharmacodynamic
PDGF	Progressive Disease
PET	Platelet Derived Growth Factor
P-gp	Positron-Emission Tomography
PHI	P-glycoprotein
PJP	Protected Health Information
PNET	Pneumocystis jirovecii Pneumonia
PI3K	Pancreatic Neuroendocrine Tumor

PK	Phosphatidyl Inositol-3 Kinase
PO	Pharmacokinetic
PR	Oral
PTEN	Partial Response
RAD001	Phosphatase and Tensin Homolog
RECIST	everolimus
RP2D	Response Evaluation Criteria for Solid Tumors
RT-PCR	Recommended Phase 2 Dose
SAE	Reverse Transcriptase-Polymerase Chain Reaction
SD	Serious Adverse Event
SEGA	Stable Disease
SUSARs	Subependymal Giant cell Astrocytoma
T½	Suspected Unexpected Serious Adverse Reactions
T_{max}	Half-life
TAC	time of maximum serum concentration
TBCRC	docetaxel 75 mg/m ^{2}, doxorubicin 50 mg/m^2, cyclophosphamide 500 mg/m^2
TGF-β	Translational Breast Cancer Research Consortium
TGF-βR	Transforming Growth Factor-beta
TSC	Transforming Growth Factor-beta Receptor
UA	Tuberous Sclerosis Complex
ULN	Urinalysis
US	Upper Level of Normal
VEGF	Ultrasound
VEGF-A	Vascular Endothelial Growth Factor
VEGFR2	Vascular Endothelial Growth Factor – A
WHO	Vascular Endothelial Growth Factor Receptor 2
	World Health Organization

GLOSSARY OF TERMS

Assessment	A procedure used to generate data required by the study
Baseline	For efficacy evaluations, the baseline assessment will be the last available assessment before or on the date of randomization. For safety evaluations (i.e. laboratory assessments and vital signs), the baseline assessment will be the last available assessment before or on the start date of study treatment. The value obtained at baseline assessments, referred to as "baseline value" will be used as reference for the patient.
Control drug	A study treatment used as a comparator to reduce assessment bias, preserve blinding of investigational drug, assess internal study validity, and/or evaluate comparative effects of the investigational drug
Dose level	The dose of drug given to the patient (total daily or weekly etc.)
Enrollment	Point/time of patient entry into the study; the point at which informed consent must be obtained (i.e. prior to starting any of the procedures described in the protocol)
Investigational drug	The study treatment whose properties are being tested in the study; this definition is consistent with US CFR 21 Section 312.3 and is synonymous with "investigational new drug."
Investigational treatment	Drug whose properties are being tested in the study as well as their associated placebo and active treatment controls (when applicable). This also includes approved drugs used outside of their indication/approved dosage, or that are tested in a fixed combination. Investigational treatment generally does not include other study treatments administered as concomitant background therapy required or allowed by the protocol when used in within approved indication/dosage
Medication number	A unique identifier on the label of each study treatment package which is linked to one of the treatment groups of a study
Other study treatment	Any drug administered to the patient as part of the required study procedures that was not included in the investigational treatment
Patient	A unique identifying number assigned to each patient/subject/healthy volunteer who enrolls in the study
Period	A subdivision of the study timeline; divides stages into smaller functional segments such as screening, baseline, titration, washout, etc.
Premature patient withdrawal	Point/time when the patient exits from the study prior to the planned completion of all study treatment administration and/or assessments; at this time all study treatment administration is discontinued and no further assessments are planned, unless the patient will be followed for progression and/or survival

Stop study participation	Point/time at which the patient came in for a final evaluation visit or when study treatment was discontinued whichever is later
Study treatment	Includes any drug or combination of drugs in any study arm administered to the patient (subject) as part of the required study procedures, including placebo and active drug run-ins. In specific examples, it is important to judge investigational treatment component relationship relative to a study treatment combination; study treatment in this case refers to the investigational and non-investigational treatments in combination.
Study treatment discontinuation	Point/time when patient permanently stops taking study treatment for any reason; may or may not also be the point/time of premature patient withdrawal
Supportive treatment	Refers to any treatment required by the exposure to a study treatment, e.g. premedication of vitamin supplementation and corticosteroid for pemetrexed disodium.
Variable	Identifier used in the data analysis; derived directly or indirectly from data collected using specified assessments at specified time points

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1. OBJECTIVES AND ENDPOINTS

1.1 Objectives

1.1.1 Primary Objective

Determine the tolerability and feasibility of combining everolimus and TRC105 with letrozole administered for 24 weeks in the preoperative (neoadjuvant) setting in postmenopausal women with newly diagnosed potentially resectable local or locally advanced (T2-T4a-c, N0-2, M0; excluding patients with T4d or inflammatory breast cancer) estrogen and/or progesterone receptor positive, Her2/neu negative breast cancer.

1.1.2. Secondary Objectives

a. Determine the efficacy of the combination of letrozole, everolimus, and TRC105 in postmenopausal women with newly diagnosed potentially resectable local or locally advanced estrogen and/or progesterone receptor positive, Her2/neu negative breast cancer.

b. Determine the pharmacodynamic parameters of everolimus and TRC105 when used in combination with letrozole in the same patient population.

c. Determine the pharmacokinetic parameters of everolimus and TRC105 when used in combination with letrozole in the same patient population.

1.1.3 Exploratory Objective

Evaluate specific biomarkers of potential prognostic value and biomarkers potentially predictive of response or resistance to the combination of everolimus and TRC105 with letrozole.

1.2 Endpoints

1.2.1 Primary Endpoint

Determine the maximum tolerated dose (MTD), the recommended phase 2 dose (RP2D) and rates of adverse events associated with the combination of letrozole with everolimus and TRC105.

1.2.2. Secondary Endpoints

a. Determine the rates of complete pathologic remission and downstaging achieved with the investigational treatment at the time of surgery.

b. Determine the changes in tumor cell proliferation by means of changes in Ki67 expression and changes in serum concentration of markers of angiogenesis (VEGF-A, soluble VEGFR2, activin A and activin B, angiopoietin-1 and -2, TGF-β, soluble endoglin) before and after the investigational treatment and correlate with clinical, radiographic, and pathologic response.

c. Determine the C_{max}, T_{max}, AUC, T½, clearance of everolimus and TRC105 when used in combination with letrozole.

1.2.3 **Exploratory Endpoint**

a. Correlate the levels of PTEN immunostaining with response.

b. Correlate levels of phosphorylated AKT (pAKT) immunostaining with response.

c. Correlate expression of endothelial (CD105 and CD31/CD34) and pericyte (NG2, and MCAM [CD146]) markers in the initial diagnostic and final surgical specimens with response.

d. Determine translational (mRNA to protein) profiles by means of next-generation whole exome sequencing and sequencing of ribosome-protected mRNA associated with resistance to the investigational combination.

2. BACKGROUND

2.1 **Rationale and Background**

2.1.1 **Rationale for improving outcomes with neoadjuvant treatment**

The achievement of a pathologic complete remission (pCR) with neoadjuvant (preoperative) therapy in early-stage breast cancer has an extremely favorable impact on long-term outcomes and is an excellent prognostic surrogate marker[9-13,55,56]. The Food and Drug Administration (FDA) has adopted the achievement of a pCR as an endpoint in neoadjuvant trials leading to accelerated approval of novel agents in breast cancer[13,57].

Responses to neoadjuvant therapy however, depend on the intrinsic molecular subtype[14] with the immunophenotypically luminal (hormone-receptor positive and Her2 negative) breast cancers rarely achieving a pCR[12]. Nonetheless, in this luminal subgroup, downstaging to at least pathologic stage 1 is equally important as it has been associated with a low risk of recurrence[15]. In a preoperative study that compared 4 months of hormonal treatment with letrozole or tamoxifen (P024) in stage 2 or 3 hormone receptor positive breast cancer, women whose disease shrunk to stage 1 or 0 (n=30) had a favorable long term outcome (no relapse with a median follow up of 61.2 months, one death due to unknown causes) as compared to the 30% relapse incidence among women whose disease was not preoperatively downstaged (n=175)[15,19,21]. An approach that intends to enhance or deepen the responses in luminal breast cancer is also supported by 2 large studies with response guided neoadjuvant chemotherapy[58-60]. After 2 cycles of neoadjuvant chemotherapy with docetaxel at 75 mg/m ^{2}, doxorubicin at 50 mg/m^2, and cyclophosphamide at 500 mg/m^2 (TAC), in women whose disease responded, the treatment was extended to a total of 8 cycles (versus standard 6 cycles)[59] or alternatively, in women whose disease did not respond, the treatment was changed to a non-cross resistant regimen (capecitabine and vinorelbine versus continuation of TAC)[60]. In subsequent exploratory analyses, both disease-free and overall survival were better with a response guided approach[58] despite the fact that the rates of pCR were not significantly improved. Moreover, at least in Luminal A breast cancers, the achievement of a pCR per se was not associated improved

outcomes[58]. **Taken together, although the achievement of pCR is not uniformly associated with improved clinical outcomes, a preoperative strategy that intends to enhance the responses in luminal breast cancer holds particular promise in yielding superior long term clinical outcomes.**

In the neoadjuvant setting for hormone receptor positive/Her2 negative breast cancer, chemotherapy and hormonal therapy have been shown to yield similar benefits in postmenopausal women[16,17]. While in one study the clinical response rates were higher (but not statistically significant) with neoadjuvant chemotherapy as opposed to hormonal therapy, by subgroup analysis, there was no difference in the efficacy of either intervention in postmenopausal women (57% vs. 52% clinical response rate with neoadjuvant chemotherapy vs. hormonal therapy, respectively)[16]. Of note, the pCR rate was low with either intervention (1/47 vs. 0/48 achieved a pCR with neoadjuvant chemotherapy vs. hormonal therapy, respectively)[16]. In another study that compared the efficacy of neoadjuvant chemotherapy vs. hormonal therapy specifically in postmenopausal women, the outcomes were highly similar: rates of complete and partial clinical response were 62%, 67%, and 63% for anastrozole, exemestane and chemotherapy, respectively[17]. In fact, neoadjuvant hormonal therapy was found to be associated with an increase in the proportion of patients suitable for breast conserving surgery[17]. Along these lines, hormonal therapy is generally preferred as it is associated with fewer adverse events and is overall better tolerated in this subgroup of patients who frequently have other comorbidities[18].

In the neoadjuvant setting, aromatase inhibitors have been consistently shown to be superior over tamoxifen[19-23], making the former the agents of choice in postmenopausal women with hormone-receptor positive breast cancer. Despite the high rates of clinical and radiographic response achieved with hormonal therapy alone[19-23], the rates of downstaging are still low (approximately 15%[15]). Similarly, the rates of pCR are very low (less than 5%) [4,15,16]. **Collectively, there is a need to improve the efficacy of aromatase inhibitors in the neoadjuvant setting with minimal additional toxicities as it directly impacts clinical outcomes.**

2.1.2 Rationale for incorporating everolimus in the neoadjuvant setting

The phosphatidyl inositol-3 kinase (PI3K)/ protein kinase B (AKT)/ mammalian target of rapamycin (mTOR) pathway is an intracellular signal transduction pathway downstream of receptor tyrosine kinases (**figure A[36]**). Class IA PI3K molecules are heterodimers composed of a regulatory subunit (p85) and a catalytic subunit (p110), the α isoform of which (encoded by *PIK3CA*) is widely mutated or amplified in human cancers[37,38], including breast cancer[39,40]. Comprehensive whole-genome sequencing data indicate that the frequency of *PIK3CA* mutations in hormone receptor positive breast cancer is 41.3%[36] (35.8% in a randomized clinical trial with everolimus where *PIK3CA* mutations were systematically evaluated[4]). Deletions were identified also in *PTEN* (7%), a negative regulator of the PI3K/AKT/mTOR pathway, as well as copy number aberrations and mutations in *AKT* (52% and 2%, respectively)[36]. Comprehensive genomic analyses of breast cancer samples in the context of The Cancer Genome Atlas Project also highlighted the high frequency of mutations along the PI3K/AKT/mTOR pathway[41]. In particular, activating mutations in the *PIK3CA,* inactivating mutations or loss of *PTEN,* and loss of *INPP4B* (a negative regulator of the PI3K/AKT pathway) were detected in 49%, 13%, and 9% (luminal A) and 32%, 24%, and 16% (luminal B), respectively. At the preclinical level, breast cancer cells with upregulated PI3K/AKT/mTOR pathway were shown

to be resistant to hormonal therapy and this resistance was restored by everolimus and other mTOR inhibitors[42,43]. The mechanistic connection between hormone resistance and PI3K/AKT/mTOR pathway activation relies on the ligand-independent activation of the estrogen receptor (ER) by downstream substrates of the mTOR complex[5,6]. **Taken together, the PI3K/AKT/mTOR pathway can be activated by various genetic events in hormone receptor positive breast cancer and its inhibition with the FDA-approved agent everolimus can restore or enhance sensitivity to endocrine therapies.** Moreover, inhibiting mTOR, a downstream signaling node where multiple pathways converge as opposed to blocking upstream mediators or individual surface receptors, has the potential to abrogate pleiotropic primary and escape tumor-promoting signaling cascades that mediate resistance to endocrine therapies.

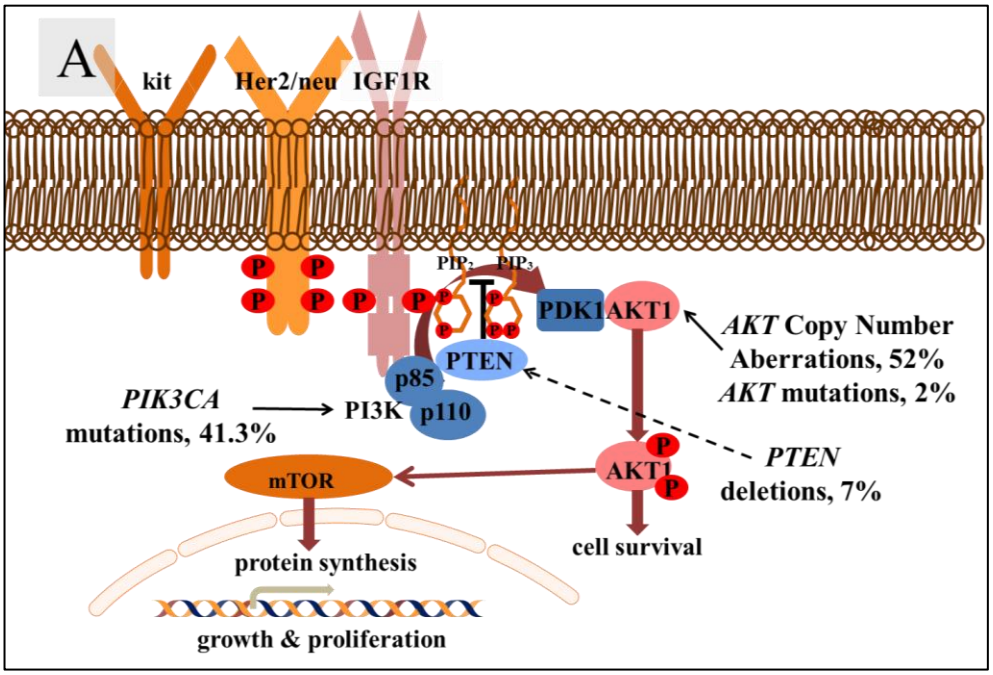


Figure A. Genomic aberrations along the PI3K/AKT/mTOR pathway. Genomic aberrations along the PI3K/AKT/mTOR pathway identified by comprehensive next-generation sequencing in luminal breast cancer[36] leading to constitutive signaling and evasion from hormone blockade.

2.1.3 **Background**: **Clinical studies with everolimus in HR positive breast cancer**

The elucidation of the mechanisms of resistance to endocrine therapies[61] set the stage for many successful clinical trials where responses to endocrine therapies and aromatase inhibitors in particular were enhanced[4] or restored[3] with the addition of everolimus. **Everolimus has been the prototypic agent which, in combination with aromatase inhibitors led to enhancement or restoration of responses to endocrine therapies in luminal breast cancer.** Furthermore, its success in luminal breast cancer demonstrated that the strategy with the highest potential to successfully address the limitations of preoperative endocrine therapy is with rational combinations of novel agents capitalizing on the knowledge garnered in the preclinical arena.

An upfront dual ER and mTOR co-targeting approach to enhance the responses of hormonal therapy

and/or circumvent *de novo* endocrine resistance[62] is supported by a randomized placebo-controlled neoadjuvant clinical trial in postmenopausal women with ER-positive breast cancer, whereby the addition of everolimus to letrozole led to an increase in the clinical response rate (68.1% vs 59.1%)[4]. Although the benefit was greater in patients harboring *PIK3CA* mutations (especially in exon 9), patients with wild type *PIK3CA* still benefitted from the combination[4]. This observation suggests that everolimus inhibits alternative operational mechanisms of hormone resistance either within or outside the PI3K/AKT pathway, consistent with mTOR lying at a downstream signaling node where multiple pathways converge[4].

Two clinical trials evaluated the addition of everolimus to exemestane[3] and tamoxifen[63] in restoring resistance to hormone therapy in hormone-receptor positive, Her2 negative advanced or metastatic breast cancer. In both studies the clinical outcomes were significantly improved with the addition of everolimus. In the former study[3] (which led to the approval of everolimus in postmenopausal women with advanced hormone receptor-positive, Her2-negative breast cancer in combination with exemestane after failure of treatment with letrozole or anastrozole), the addition of everolimus to exemestane led to a 6.5 month incremental improvement in the progression free survival (10.6 months in the combination arm vs 4.1 months in the exemestane alone arm)[3]. There was however an increased incidence of adverse events in the combination arm with stomatitis (8% in the combination arm vs. 1% in the exemestane alone arm), anemia (6% vs <1%), dyspnea (4% vs. 1%), hyperglycemia (4% vs. <1%), fatigue (4% vs. 1%), and pneumonitis (3% vs. 0%) constituting the most frequent adverse events associated with the addition of everolimus[3].

Taken together, these clinical studies support the addition of everolimus to letrozole in enhancing the responses of hormonal therapy and/or circumventing *de novo* or acquired resistance to endocrine therapies.

2.1.4 Rationale for incorporating anti-angiogenic therapy in the neoadjuvant setting

Angiogenesis is an integral step in tumor progression[24]. Specifically in hormone-receptor positive breast cancer, enhanced angiogenesis accelerated tumor growth[25], and overexpression of the Vascular Endothelial Growth Factor (VEGF) either by the tumor[26,64] or stromal cells[27] was associated with hormone-independent growth and resistance to endocrine therapies. An interplay between estrogens and angiogenic activity exists[65,66] and *VEGF* is one of the genes whose expression is modulated by estrogens. Indeed, the presence of functional estrogen response elements in the *VEGF* gene[67-69] underpin the transcriptional activation of *VEGF* by estrogens[70]. In mouse models of luminal breast cancer, overexpression of VEGF by human MCF-7 breast cancers not only enhanced estrogen dependent tumor growth, but also enabled estrogen independent tumor formation *in vivo*[26]. The stimulation of MCF-7 tumor growth by VEGF is through both a paracrine effect on tumor angiogenesis leading to neovascularization and an autocrine effect on tumor cell proliferation[26]. Tumor-released VEGF recruits stromal cells and promotes a desmoplastic microenvironment; stromal cells in turn, provide mitogenic and angiogenic growth factors stimulating both tumor and stromal cell growth[64]. Beside the breast cancer cells themselves, VEGF may be secreted by the activated stroma. In mouse models of xenografted ER-positive tumors, VEGF secreted by the stroma and acting cooperatively with other factors can substitute for estrogens and foster hormone-independent growth of luminal tumors[27]. At the clinical level, in hormone-receptor positive breast cancer, elevated intratumoral levels of VEGF have been associated with suboptimal responses to

hormonal therapies and poorer clinical outcomes[71-73] lending support to the hypothesis that VEGF and angiogenesis lie in the core of intrinsic resistance to endocrine therapies.

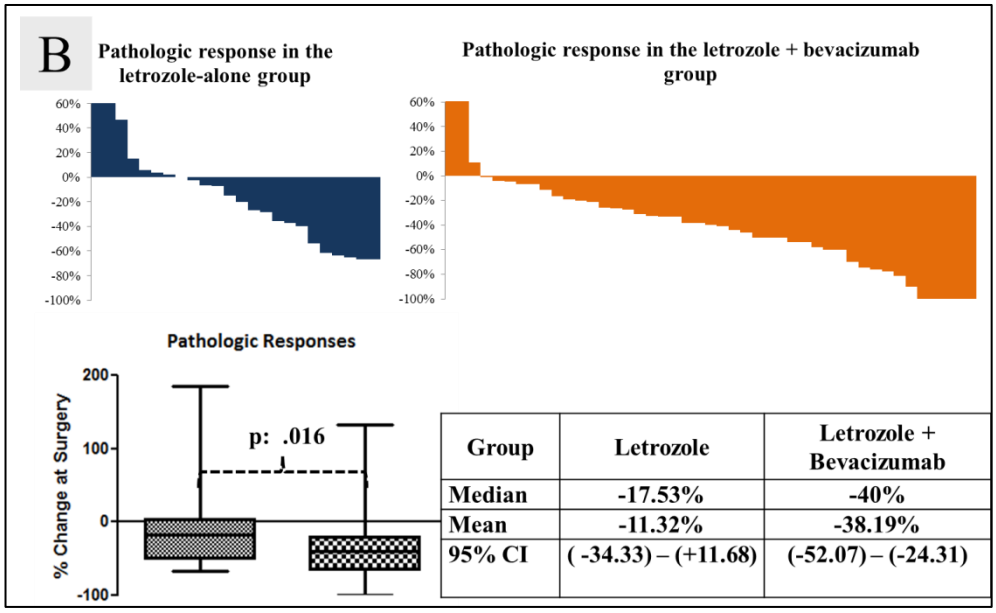
Our studies in the UAB Breast Cancer Spore using the MCF-7 tamoxifen murine xenografts model by Dr. Fran Kern and collaborators indicate that paracrine effects of VEGF over-expression by ERα+ breast cancer cells can reverse the effectiveness of tamoxifen as a cytostatic or cytotoxic agent[64]. In this MCF-7 model, they demonstrated that increased expression of VEGF causes an acquired Tamoxifen resistance and tumor loss of estrogen dependence *in vivo*. MCF-7 cells with VEGF co-expression (stably transfected or regulated expression) had *in vitro* tumor cell proliferation and estrogen dependence identical to non-transfected MCF-7, while MCF-7/VEGF cells *in vivo* displayed increased tumor growth rates, higher rates of metastases, and estrogen independence. These survival and proliferation signals may be an indirect effect of VEGF presumably reflecting the enhanced vascularity of tumors with secondary growth factor and signal pathway modulation. Reversal of VEGF over-expression *in vivo* returned tumors to estrogen dependent growth.

Dr. Zhican Qu, Ph.D., a recipient of a career development award of the UAB-Breast SPORE in our group, conducted preclinical efficacy studies of tamoxifen and bevacizumab in combination for the treatment of breast cancer using a novel mouse model. In her previous studies she showed that elevated levels of VEGF increases tumor growth, and promotes metastasis and tamoxifen resistance; thus, she hypothesized that a combination of agents targeting both ER and VEGF signaling may be a more effective treatment than the use of either drug alone. She tested this hypothesis using a combination of tamoxifen and bevacizumab in a xenograft nude mouse model with regulated-VEGF expression. The efficacy of the treatment was tested in two groups of mice, one with early and one with late stage ER-positive breast cancer. All mice received orthotopic implantation of 10^7 tumor cells at mammary fat pat and a 17β-estradiol pellet supplement on Day 1. Doxycycline was given to all mice to induce expression and secretion of VEGF from tumor cells. Animals in the early stage group started therapy (tamoxifen or bevacizumab or placebo or tamoxifen-bevacizumab in combination) on Day 7 when the tumor size reached about 100 mm^3. The bevacizumab-tamoxifen combined treatment demonstrated better control of primary tumor growth than therapy with tamoxifen or bevacizumab alone. The survival rate was superior in the group of mice treated with combination therapy compared with the groups treated with single agents or placebo. Animals in the late stage group started therapy on Day 32 when the tumor size reached about 1000 mm^3. The combined treatment showed again a better reduction of tumor growth rate and a significant better survival. 83% of mice survived in the combined treatment group, while only about 50% of mice survived in the monotherapy groups. These results demonstrated that the Avastin-tamoxifen combination is more effective on inhibiting tumor growth of ER positive breast cancer and increasing survival rate than either drug treatment alone (2005 AACR Annual Meeting, abstract #1091).

2.1.5 **Background: Clinical studies with antiangiogenic agents in HR positive breast cancer**

These preclinical studies that support the role of angiogenesis in hormone-independent growth of luminal breast cancer set the stage for a pilot, single-institution (UAB) study of preoperative letrozole in combination with bevacizumab (a monoclonal antibody targeting VEGF, the prototypic proangiogenic factor and a crucial regulator of both normal and pathologic angiogenesis[74]) in postmenopausal women with hormone-receptor positive breast cancer[2]. In our study (n=25), preoperative letrozole and bevacizumab for 24 weeks resulted in a pCR rate of 12% (n=3), while the overall objective response rate was 68% (n=17). Additionally, 4% (n=1) achieved pCR in the breast but not in the lymph nodes and the rate of stage 0/1 attainment was 32%[2]. The treatment was overall well tolerated with a rate of discontinuation due to adverse events of 8% (n=2). With a median follow-up of 55 months and 2 responders lost to follow up, none of the other 15 patients with objective response have relapsed. So far, 2 patients experienced a recurrence despite postoperative chemotherapy and hormonal therapy; these patients had achieved stable (SD) and progressive (PD) disease, respectively as their best response while on the investigational preoperative regimen. Our results are consistent with the concept that downstaging to at least pathologic stage 1 is equally important as achievement of a pCR and it has been associated with an extremely low risk of recurrence[15].

We conducted a separate, independent randomized open-label phase II study was conducted by the Translational Breast Cancer Research Consortium (TBCRC) comparing preoperative letrozole with or without bevacizumab in postmenopausal women with operable hormone receptor positive breast cancer[1] (trial TBCRC002). The study showed that bevacizumab "deepened" the responses obtained with letrozole alone (**figure B**). Downstaging from stage 2/3 to 0/1 was achieved in 45% of patients in the letrozole-bevacizumab arm as compared to 25% in the letrozole-alone group. Remarkably, the combination induced a pCR in 11.1% of patients[1], comparable to the 12% pCR rate reported in a prior pilot single-institution study[2]; no complete or near-complete remissions were observed in the letrozole-alone group. Of note, the addition of everolimus to letrozole has not led to such a high pCR rate: of the 246 patients who received letrozole +/- everolimus preoperatively for 4 months with available surgical excision specimens, only 3 achieved a pCR (2 in the combination arm versus 1 in the letrozole alone arm)[4].



Group	Letrozole	Letrozole + Bevacizumab
Median	-17.53%	-40%
Mean	-11.32%	-38.19%
95% CI	(-34.33) – (+11.68)	(-52.07) – (-24.31)

Figure B. **Results of the TBCRC002 study**. Waterfall plots of the pathologic responses (comparison of the tumor as assessed by imaging at the time of enrollment and tumor size at the time of surgery) achieved in the 2 arms (upper panel). Comparison between the pathologic responses in the letrozole arm and letrozole + bevacizumab arm (lower left panel). The table indicates the median and mean pathologic response between groups and the 95% confidence interval (CI) of the mean.

Our study sets a successful clinical precedence of rationally combining an antiangiogenic agent with an aromatase inhibitor in postmenopausal women with hormone receptor-positive and Her2 negative breast cancer; this combination not only enhanced the response rate but even led to an unprecedented pCR rate in an intrinsic subgroup where pCRs are rarely seen.

2.1.6 **Rationale for targeting endoglin in cancer therapeutics**

Endoglin (CD105) is essential for normal vascular development[75], and heterozygous expression of endoglin is associated with hereditary hemorrhagic telangiectasia type 1 (HHT-1,Rendu–Osler–Webber syndrome), a human disease characterized by ectatic blood vessel formation[76]. In adults, endoglin is a marker of activated angiogenesis that is predominantly or exclusively expressed in *de novo* tumor vessels[32,33], as opposed to the minimal expression in the normal quiescent endothelium which has a very low turnover[28]. Endoglin is a transmembrane accessory receptor for transforming growth factor-beta (TGF-β) receptor (TGF-βR) and diverts TGF-β downstream signaling toward proliferation and migration as well as transcription of proangiogenic genes including endoglin itself[29-31]. As TGF-β signaling can mediate diverse and opposing processes in endothelial cells, endoglin inhibits TGF-β-mediated endothelial quiescence (**figure C** [77,78]). Indeed, in the absence of endoglin, TGF-β mediated endothelial cell quiescence is significantly enhanced[28,79]. The clinical implications of this observation is that **targeting endoglin will selectively inhibit tumor angiogenesis and at levels below baseline**.

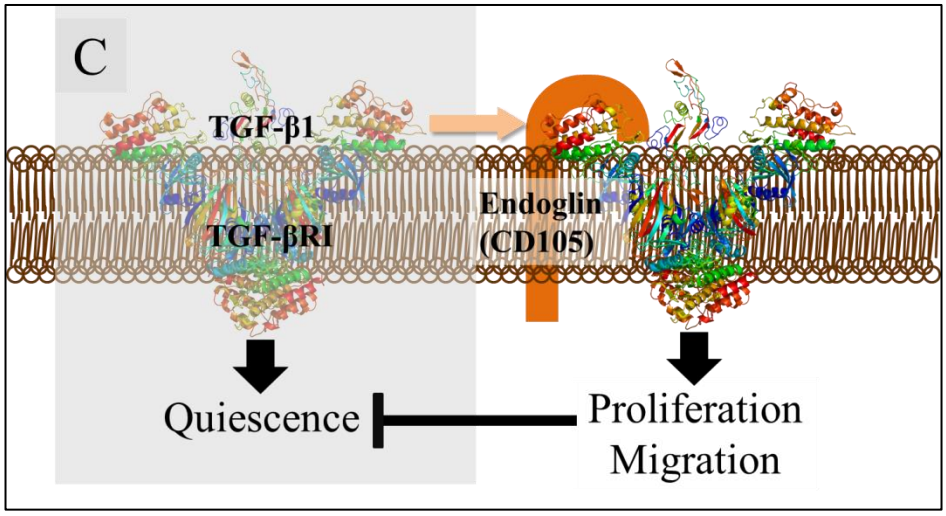


Figure C. Interactions of endoglin with TGF-βR. Endoglin is a transmembrane accessory receptor for TGF-βR and diverts TGF-β downstream signaling toward proliferation, migration, and transcription of proangiogenic genes including endoglin, while in its absence, TGF-βR signaling mediates endothelial quiescence.

In line with the premise that enhanced tumor vasculature correlates with adverse prognosis and the fact that endoglin is a marker of activated angiogenic endothelium, endoglin expression has been consistently associated with adverse clinical outcomes across multiple malignancies[31]. VEGF inhibition leads to reactive upregulation of endoglin[7] and large established CD105(+) vessels lying in the core of the tumors have been shown to be insensitive to VEGF inhibitors[8]. In those studies, CD105 expression was increased following inhibition of the VEGF pathway. CD105 expression increased more than two-fold in human pancreatic cancers grown in mice treated with an antibody that binds VEGF[7]. 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[8]. Finally, a study of spontaneously occurring neuroendocrine tumors in RIP-Tag2 mice treated with antibody to VEGF indicated that TGF- β was the most highly upregulated factor, as determined by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR)[47]. Taken together, these studies indicate that the **CD105 pathway is a significant mechanism of escape from VEGF inhibitor therapy**.

Endoglin is extensively expressed in breast cancer[80] and increased endoglin expression at baseline has been associated with poorer responses to neoadjuvant treatment[81]. In preclinical murine xenograft models of HR(+) breast cancer, anti-endoglin monoclonal antibodies inhibited selectively tumor angiogenesis and delayed the growth of established tumors, while sparing normal vasculature[34]. Of note, in these preclinical "proof-of-concept" studies, the antitumor efficacy of the monoclonal antibody may have been obscured by the fact that almost 60% of the malignant vasculature was murine to which the human investigational antibody had little affinity[34]. **Collectively, endoglin is a rational target of tumor angiogenesis as it is exclusively expressed in the activated endothelium and plays a central pathophysiologic role in promoting angiogenesis.**

Endoglin is cleaved near the plasma membrane by membrane-anchored matrix metalloproteinase

(MMP)-14 to release a soluble form of endoglin (sEng) into the circulation[82]. Although membrane-bound endoglin promotes angiogenesis, sEng antagonizes this process[82] through multiple mechanisms including cell surface receptor downregulation and ligand sequestration[83,84]. The mechanism by which the novel monoclonal antibody intended to be used in the proposed study (TRC105) exerts its antiangiogenic effect is not clear in its details; however one of the proposed mechanisms involves enhanced endoglin shedding by stabilizing preformed endoglin/MMP-14 complexes[83]. Indeed, results from the phase I trial reveal that there is a dramatic dose-dependent increase in sEng levels in patients treated with TRC105[83]. Enhanced shedding not only reduces the overall cell surface level of endoglin, but also the resulting product (sEng) exerts an antiangiogenic effect by itself[83].

2.1.7 **Clinical studies with TRC105**

TRC105 was administered to 50 patients with solid tumors in the context of a phase 1 first-in-human dose escalation study following a 3+3 design[35]. The maximum tolerated dose was determined at 10 mg/kg weekly; 15 mg/kg every two weeks was equally well tolerated and also resulted in continuous serum concentrations known to saturate CD105 binding sites on proliferating endothelium. Hypoproliferative anemia was the dose-limiting toxicity: above the maximum tolerated dose, at 15 mg/kg weekly, all 3 patients that remained on study through cycle 2 developed dose-limiting grade 3 anemia, and one of the three progressed to grade 4 anemia by cycle 3. Infusion reactions, including some that were serious, were observed following TRC105 administration, usually with the initial dose. Other adverse events most commonly observed with TRC105, either alone or in combination, include fatigue, mucocutaneous telangiectasia resulting in epistaxis and gingival bleeding, flushing, and headache. Classic toxicities associated with VEGF inhibition, including hypertension, proteinuria, and thrombosis, have been seen in isolation. In fact, 3 clinical trials combining TRC105 with bevacizumab are currently ongoing (NCT01564914, NCT01648348, and NCT01332721). Multiple other phase Ib and phase II studies of TRC105 in combination with chemotherapy, with VEGF inhibitors, and as a single agent in patients with advanced prostate, sarcoma, renal cell carcinoma, choriocarcinoma, glioblastoma multiforme, and hepatocellular cancer are currently ongoing[35,85].

Collectively, **TRC105 allows for the rational therapeutic targeting of malignant angiogenesis while sparing the cardiovascular toxicities of most contemporaneous antiangiogenic agents to which postmenopausal women with preexistent conditions may be particularly predisposed.**

Although the completed clinical trials with TRC105 were not designed to assess efficacy, several patients benefitted from the investigational drug. In the phase I study where TRC105 was administered as a single agent in patients with advanced metastatic malignancies and having received a median of 4 prior lines of therapy, 6/44 patients remained progression-free for longer than 4 months[35].

2.1.8 **Synergism between TRC105 and everolimus**

In animal models, mTOR inhibition was shown to reduce the levels of VEGF and downregulate significantly the response of endothelial cells to stimulation by VEGF[45]. mTOR signaling was also shown to play a key role in hypoxia-triggered smooth muscle and endothelial cell proliferation and

angiogenesis in vitro; this response (triggered by growth factors other than VEGF) was abrogated with mTOR inhibition[46]. On the other hand, VEGF inhibition leads to reactive upregulation of endoglin[7] and large established CD105(+) vessels lying in the core of the tumors have been shown to be insensitive to VEGF inhibitors[8]. Other preclinical studies have also demonstrated that endoglin/CD105 represents a principal escape pathway from VEGF inhibition[7,47]. **Collectively, mTOR inhibition with anti-CD105 targeted therapy is anticipated to represent a more inclusive antiangiogenic approach as opposed to anti-VEGF therapy alone as it inhibits signaling from alternative proangiogenic factors and targets malignant angiogenesis with inherent (preexistent CD105 expression) or acquired (reactive CD105 upregulation) resistance to VEGF inhibition.**

2.1.9 **Duration of neoadjuvant treatment**

Most studies that prospectively evaluated preoperative hormonal therapy have limited the duration of treatment to 3-4 months[14,15,19-21,23]. Several studies however, suggest that extending neoadjuvant hormonal therapy beyond 3-4 months is safe and associated with further and sustained reductions in the tumor size leading to a higher proportion of breast conserving surgeries[14,86,87]. Indeed, in a prospective phase II clinical trial with preoperative letrozole treatment to maximum response, the mean time to first response was 3.9 months while the median time to maximum response was 4.2 months[87]. It is noteworthy that approximately one third of the responders achieved maximum response beyond 6 months; however, very few responders were identified after 6 months and none from 8 to 12 months. The overall clinical response rate with preoperative letrozole was 76.8%[87]. Similarly, in a retrospective study of 182 patients treated preoperatively with letrozole, the rate of clinical complete or partial response rose from 69.8% at 3 months to 83.5% by extending letrozole treatment up to 24 months[86]. Moreover, continuing letrozole beyond 3 months increased the number of women who initially required mastectomy or had locally advanced breast cancer and subsequently became suitable for breast conserving surgery from 60% at 3 months to 72%[86].

Taken together, neoadjuvant endocrine therapy requires longer time to achieve responses as compared to cytotoxic chemotherapy. The majority of responders can be identified within the first 4 months of treatment; however, some patients require longer durations of treatment to achieve their maximum response[14]. In luminal breast cancer, such sustained and increased reductions in the tumor size are associated with better long-term clinical outcomes.

2.1.10 **Rationale for ovarian suppression with gonadotropin-releasing hormone analogs in premenopausal women.**

The IMPACT trial (Immediate Preoperative Anastrozole, Tamoxifen or Combined with Tamoxifen) established the superiority of aromatase inhibition over tamoxifen or tamoxifen + aromatase inhibition in the preoperative setting. Although the overall clinical response rate was not significantly different between the 3 arms, more patients classified as requiring mastectomy were eligible for breast conserving surgery on the anastrozole treatment arm[48] . In addition, suppression of the proliferation marker Ki67 (which in itself has been shown to be predictive of favorable outcome) was statistically greater with anastrozole as compared with tamoxifen or the combination [49]. Aromarase inhibitors in premenopausal women are contraindicated as they may increase gonadotropin secretion because of the reduced feedback of estrogen to the hypothalamus and

pituitary[50]. To that end, in order for premenopausal women to benefit from aromatase inhibition, they should become postmenopausal through ovarian suppression with gonadotropin-releasing hormone analogs. Furthermore, ovarian suppression in combination with aromatase inhibition has been shown to be superior over tamoxifen in the adjuvant setting in premenopausal women [88] For this clinical trial goserelin subcutaneously q 4 weeks will be used to suppress ovarian function.

2.2 Correlative Studies Background

2.2.1 PI3K/AKT/mTOR pathway in breast cancer

As outlined in section 2.1.2 and 2.1.3, the PI3K/AKT/mTOR pathway can be activated by various genetic aberrations at multiple nodes along the pathway leading to resistance to or evasion from hormone blockade. Loss of PTEN increases PI3K activity and increased phosphorylation, activation, or membrane translocation of signal transducers downstream of the PI3K including Akt, mTOR, S6 kinase, and 4-EBP-1[89]. Immunohistochemistry for the phosphorylated AKT (pAKT) is a surrogate for activation of the PI3K/AKT/mTOR pathway irrespective of the underlying genetic aberration; activating point mutations of signal transducers along the pathway can be captured by whole-exome sequencing.

2.2.2 Regulation of gene expression at the translational level

Over the recent years, significant progress has been made in understanding the mechanisms of transcriptional (DNA to RNA) regulation; however, little is known about the regulation of gene expression at the **translational (mRNA to protein)** level. Comprehensive analyses have shown that while mRNA and respective protein levels may correlate better than previously thought (still only at the level of R^2=0.41), the abundance of a protein in a cell is predominantly determined at the level of translation[90,91]. Moreover, changes in translation have been shown to precede and be considerably more dramatic than changes in transcription in response to growth factor signaling modulation[92]. While mass spectrometry allows us to investigate translation at the protein level, it has its limitations (limitations in quantitation, incomplete and biased coverage[93]) and small changes in the expression of critical genes that cast a large shadow at multiple cellular levels may be missed. The advent of ribosomal profiling[94-100] (deep sequencing of the ribosome protected mRNA fragments in parallel with mRNA-sequencing) harnesses the comprehensive and unbiased nature of massively parallel deep sequencing to provide a global genomic view of ribosome occupancy across the entire exome and is a good proxy of active protein production. **There is a strong rationale in investigating ribosome occupancy across the exome as genes exert their functions by the protein products they encode.** Ribosomal profiling provides "snapshots" of which mRNAs are being actively translated and how efficiently. It serves as a proxy of protein synthesis and currently, no other technology can capture which mRNAs are being actively translated as close as ribosomal profiling. Which mRNAs are actively translated and at what efficiency is a layer of complexity in gene expression which has not be captured in the currently completed comprehensive genomic analyses in breast cancer.

3. PATIENT SELECTION

3.1 Study Population

Patients with newly diagnosed, potentially resectable (stage 2 or 3), local or locally advanced estrogen and/or progesterone receptor positive and HER2/neu negative breast cancer will be enrolled in this study

3.2 Eligibility Criteria

Patients must have baseline evaluations performed prior to the first dose of study drug and must meet all inclusion and exclusion criteria. Results of all baseline evaluations, which assure that all inclusion and exclusion criteria have been satisfied must be reviewed by the Principal Investigator or his/her designee prior to enrollment of that patient. In addition, the patient must be thoroughly informed about all aspects of the study, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from the patient prior to enrollment. The following criteria apply to all patients enrolled onto the study unless otherwise specified.

3.2.1 Newly diagnosed, histologically confirmed breast cancer.

3.2.2 hormone receptor positive and HER2 negative breast cancer [12,58]; patients with Her2/neu positive tumors irrespective of their hormone receptor status will be excluded. At least 10% of tumor cell nuclei should be immunoreactive for hormone receptors (ER and/or PR) to be deemed eligible for the study[101]. Her2/neu negative is defined as[102]:

a. Immunohistochemistry (IHC) score of 0 (no staining is observed or membrane staining that is incomplete and is faint/barely perceptible and within ≤ 10% of tumor cells) **OR**
b. IHC score of 1 (incomplete membrane staining that is faint/barely perceptible and within <10% of tumor cells) **OR**
c. Fluorescence *in situ* hybridization (FISH) HER2/CEP17 ratio of <1.8 or average HER2 gene copy number of <4 signals/nucleus for test systems without an internal control probe.
d. Equivocal results for Her2/neu (defined as: IHC 2+ or FISH HER2/CEP17 ratio of 1.8-2.2 or average HER2 gene copy number 4-6 HER2 signals/nucleus for test systems without an internal control probe) should prompt reflex test (same specimen using an alternative method) or order a new test (new specimen if available, using IHC or FISH).

3.2.3 Histological grade I, II or III according to the modified Bloom Richardson scale[52].

3.2.4 Stage at diagnosis T2 through T4a-c, N0 through N2, and M0. Patients with inflammatory breast cancer or metastatic disease at diagnosis will be excluded. Patients with multicentric, multifocal, and/or bilateral disease are allowed to participate so long as all tumors meet the histologic criteria of the study.

3.2.5 Determined menopausal status. Postmenopausal status is defined according to the National Comprehensive Cancer Network[103]

a. age >60 years old **OR**
b. prior bilateral oophorectomy regardless of age
c. if patient <60 years old and amenorrheic for >12 months in the absence of ovarian suppression, FSH and estradiol have to be in the postmenopausal range

women who do not meet these criteria will be determined as non-postmenopausal and will require goserelin subcutaneously q 4 weeks to achieve ovarian suppression.

3.2.6 No prior treatment with therapeutic intent for breast cancer.

3.2.7 No life threatening parenchymal disease or rapidly progressing disease warranting cytotoxic chemotherapy.

3.2.8 Patients must have disease that can be measured and followed by mammogram and/or breast ultrasound (in special cases a dedicated breast MRI may be clinically indicated). The target lesion must not have been previously irradiated.

3.2.9 Resectable/Operable or potentially resectable/operable breast cancer as determined by the treating surgical oncologist.

3.2.10 ECOG performance status ≤2 (Karnofsky ≥60%, see **Appendix A**)

3.2.11 Life expectancy of greater than 6 months

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

- leukocytes	≥3,000/mcL
- absolute neutrophil count	≥1,500/mcL
- hemoglobin	≥10 gr/dL
- platelets	≥100,000/mcL
- total bilirubin	≤2 X institutional ULN (except for known Gilbert's syndrome/familial non-hemolytic jaundice)
- AST(SGOT)/ALT(SGPT)	≤2.5 X institutional upper limit of normal
- creatinine	Up to 1.5 UNL, or
- creatinine clearance	≥40 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal
- INR	≤ 2

3.2.13 Fasting serum cholesterol ≤300 mg/dL OR ≤7.75 mmol/L AND fasting triglycerides ≤2.5x ULN. NOTE: In case one or both of these thresholds are exceeded, the patient can only be included after initiation of appropriate lipid lowering medication;

3.2.14 Age ≥19 years. Because no dosing or adverse event data are currently available on the use of TRC105 in patients <18 years of age[85], children are excluded from this study.

3.2.15 No bleeding from gastrointestinal ulcers or other sites of bleeding.

3.2.16 Ability to understand and willingness to sign a written informed consent document.

3.2.17 Signed informed consent obtained prior to any screening procedures.

3.3 **Exclusion Criteria**

3.3.1 Patients currently receiving anticancer therapies or who have received anticancer therapies within 4 weeks of the start of the investigational therapy (including chemotherapy, radiation therapy, antibody based therapy).

3.3.2 Her2/neu positive[102] or estrogen and progesterone receptor negative[101] breast cancer. Patients with triple-negative breast cancer are also excluded.

3.3.3 Metastatic breast cancer.
3.3.4 Inflammatory (T4d) breast cancer.

3.3.5 Patients may not be receiving any other investigational agents. If patients are currently part of or have participated in any clinical investigation with an investigational drug, the last administration of the investigational study should be at least 1 month prior to dosing.

3.3.6 Inoperable breast cancer even after neoadjuvant treatment as assessed by the treating surgical oncologist.

3.3.7 Undetermined or unknown menopausal status.

3.3.8 Disease that cannot be measured and/or accurately followed by mammogram and/or breast ultrasound and/or dedicated breast MRI.

3.3.9 Target lesion that has been previously irradiated.

3.3.10 History of allergic reactions, intolerance or hypersensitivity attributed to compounds of similar chemical or biologic composition to TRC105 and/or everolimus or other rapamycin analogs (e.g. sirolimus, temsirolimus).

3.3.11 Known impairment of gastrointestinal (GI) function or GI disease that may significantly alter the absorption of oral Everolimus

3.3.12 Patients must have normal organ and marrow function. Patients with any of the following abnormal parameters will be excluded:

- leukocytes <3,000/mcL
- absolute neutrophil count <1,500/mcL
- hemoglobin <10 gr/dL
- platelets <100,000/mcL

- total bilirubin	>2 X institutional ULN (except for known Gilbert's syndrome/familial non-hemolytic jaundice)
- AST(SGOT)/ALT(SGPT)	>2.5 X institutional upper limit of normal
- creatinine	> 1.5 UNL, or
- creatinine clearance	<40 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal.
- INR	≥2

3.3.13 Uncontrolled diabetes mellitus as defined by HbA1c >8% despite adequate therapy. Patients with a known history of impaired fasting glucose or diabetes mellitus (DM) may be included, however blood glucose and antidiabetic treatment must be monitored closely throughout the trial and adjusted as necessary.

3.3.14 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements. Examples of uncontrolled medical conditions include:

a. Unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction ≤ 6 months prior to start of Everolimus, serious uncontrolled cardiac arrhythmia, or any other clinically significant cardiac disease
b. Symptomatic congestive heart failure of New York Heart Association Class III or IV
c. Active (acute or chronic) or uncontrolled severe infection, liver disease such as cirrhosis, decompensated liver disease, and active and chronic hepatitis (i.e. quantifiable HBV-DNA and/or positive HbsAg, quantifiable HCV-RNA),
d. Known severely impaired lung function (spirometry and DLCO 50% or less of normal and O_2 saturation 88% or less at rest on room air),
e. Active, bleeding diathesis.

3.3.15 Chronic treatment with corticosteroids or other immunosuppressive agents. Topical or inhaled corticosteroids are allowed.

3.3.16 Patients who have received live attenuated vaccines within 1 week of start of Everolimus and during the study. Patient should also avoid close contact with others who have received live attenuated vaccines. Examples of live attenuated vaccines include intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, varicella and TY21a typhoid vaccines

3.3.17 ECOG performance status >2 (Karnofsky <60%, see Appendix A)

3.3.18 Pregnancy. Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, (including female pediatric patients who are menarcheal or who become menarcheal during the treatment) must use highly effective contraception during the study and for 8 weeks after stopping treatment. Highly effective contraception is defined as either:

- Total abstinence: When this is in line with the preferred and usual lifestyle of the subject. [Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception]
- Female Sterilization: have had surgical bilateral oophorectomy (with or without hysterectomy), total hysterectomy or tubal ligation at least six weeks before taking study treatment. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment
- Male partner sterilization: The vasectomized male partner should be the sole partner for that subject with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate.
- Use of oral, injected or implanted hormonal methods of contraception or placement of an intrauterine device (IUD) or intrauterine system (IUS) or other forms of hormonal contraception that have comparable efficacy (failure rate <1%), for example, hormone vaginal ring or transdermal hormone contraception. In case of use of oral contraception women should have been stable on the oral agent for a minimum of 3 months before taking everolimus

3.3.19 Known history of HIV seropositivity

3.3.20 Need for concurrent treatment with medications that strongly interact with everolimus (CYP3A4 inducers or inhibitors, see **Appendix B**).

3.3.21 History of another malignancy within the last five years except non-melanoma skin cancer, carcinoma in–situ of uterine cervix, uteri, and breast from which the patient has been disease free for at least 3 years. Second primary breast cancers are allowed regardless of the number of years since they were first diagnosed.

3.3.22 Major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to Day 0, anticipation of need for major surgical procedure during the course of the study. As an example, port placement or core biopsies are not considered major surgical procedures.

3.4 **Inclusion of Women and Minorities**

Both men and women and members of all races and ethnic groups are eligible for this trial.
Minority patients are particularly encouraged to participate.

3.5 **Subjects Who Fail to Meet the Eligibility Criteria**

A subject may fail to meet entrance criteria because of an abnormal laboratory value, vital sign(s), or concurrent medications with strong interactions with the investigational combination. The abnormal screening test(s) may be repeated as soon as the investigator anticipates that it will be within acceptable range to meet eligibility criteria, but must be completed within the 4-week screening phase. Rescreening can be performed only once and the subject is not required to sign a new informed consent form.

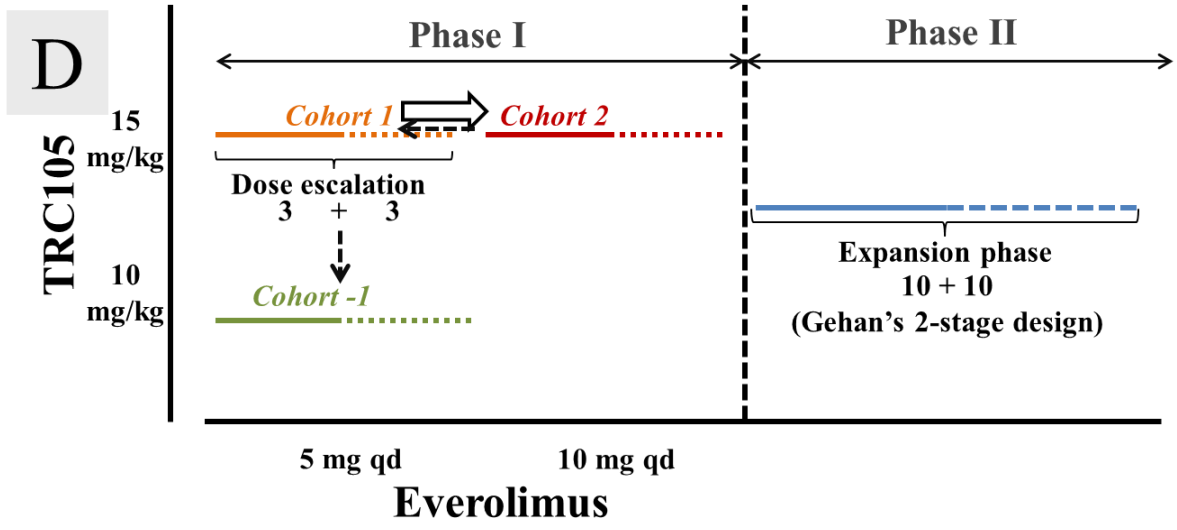
If the rescreening test(s) cannot be performed within the 4-week screening phase or return abnormal not meeting the entrance criteria, or the medical condition of the subject deteriorates so that she no

longer meets the eligibility criteria, the subject will be deemed a screen failure and withdrawn from the study.

4. STUDY DESIGN

The principal investigator will hold the IND for the trial (**Appendix F**). This is a single-institution open-label phase I/II study of preoperative treatment with the combination of letrozole, everolimus, and TRC105 in postmenopausal women with newly diagnosed potentially resectable local or locally advanced (T2-T4a-c, N0-2, M0; excluding patients with T4d or inflammatory breast cancer) estrogen and/or progesterone receptor positive, Her2/neu negative breast cancer. Accrual will be halted as a result of any grade ≥3 hemorrhage, cerebrovascular and vasovagal adverse events; FDA will be contacted to discuss further accrual or potential need for protocol amendment. Premenopausal women at the time of study enrollment will receive goserelin 3.6 mg subcutaneously q 4 weeks to achieve ovarian suppression. The study consists of 2 parts: a phase I and a phase II part (**figure D**).

Figure D. Study design.



4.1 Phase I, dose escalation

The first part of the study will follow the conventional 3+3 dose escalation design. Up to 18 patients will be enrolled. During the first part (phase I, **dose escalation**), the Maximum Tolerated Dose (MTD) of the combination in terms of safety will be determined. The dose of letrozole will be kept the same for all cohorts (2.5 mg PO daily) (**Table 4-1**). The dose of everolimus will be escalated from 5 mg PO daily (cohort 1) to 10 mg PO daily (cohort 2). The dose of everolimus will be de-escalated to 5 mg PO daily (cohort 1) if the dose of 10 mg PO daily is not well tolerated. The dose of TRC105 will be 15 mg/kg q 2 weeks (cohorts 1 and 2). The dose of TRC105 will be de-escalated to 10 mg/kg q 2 weeks (cohort -1) if 15 mg/kg q 2 weeks is not well tolerated. All doses that will be used in this trial have shown to be safe and active in clinical trials. Premenopausal women at the time

of study enrollment will receive goserelin 3.6 mg subcutaneously q 4 weeks to achieve ovarian suppression.

Table 4-1. Cohorts and dose escalations of the phase I part of the study

Cohort	TRC105 (mg/kg IV q 2 weeks)	Everolimus (mg PO daily)
1	15	5
2	15	10
-1	10	5

4.1.1 **Definition of a Dose Limiting Toxicity (DLT)**

A **dose-limiting toxicity (DLT)** is defined as:

a) Any grade 3 or 4 non-hematologic toxicity except anorexia, alopecia, nausea (which is not refractory to antiemetics), fatigue, fever without neutropenia;
b) Failure to recover to baseline (except alopecia) after delaying the next dose by more than 14 days;
c) Grade 3 or 4 neutropenia complicated by fever >38.5°C or infection, or grade 4 neutropenia of ≥7 days duration; or

d) Grade 4 thrombocytopenia, or grade 3 thrombocytopenia complicated by hemorrhage.
In addition, accrual will be halted as a result of any grade ≥3 hemorrhage, cerebrovascular and vasovagal adverse events; FDA will be contacted to discuss further accrual or potential need for protocol amendment.

4.1.2 **Dose escalation rule:**

Dose escalation will follow the decision rules shown in **table 4-2.**

Table 4-2. Dose escalation rules

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next dose level.
1 out of 3	Enter 3 additional patients in the same dose level (for a total of 6 patients at this dose level). If 0 of the 3 additional patients experience DLT, then proceed to the next dose level. If 1 or more of these additional patients experience DLT (i.e., ≥ 2 of 6 total patients), dose escalation will be

	stopped.
≥2 out 3	Dose escalation will be stopped.
If MTD not reached	Cohort 2 will be selected for RP2D

4.1.3 **Definition of Maximum Tolerated Dose (MTD)**

The **maximum tolerated dose** (MTD) is defined as the highest dose at which 0 out of first 3 or 1 out of total of 6 patients experience DLT during the first cycle of therapy; this dose level will be recommended as RP2D in the second portion of the study. If accrual to cohort 2 (highest dose of both everolimus and TRC105) is completed without reaching the MTD, this dose will be selected as the RP2D. In every cohort, patients withdrawn without receiving study treatment will be replaced, as will patients withdrawn before completing the first cycle of treatment for any reason other than DLT.

4.2 **Phase II, expansion phase**

The second part of the study (phase II, **expansion phase**), will ensue once the MTD and RP2D have been determined. The objectives of the dose expansion phase is to estimate the rate of complete pathologic remission (pCR) or downstaging in addition to continue evaluation of the safety. In order to minimize exposure of patients to ineffective therapy, Gehan's two-stage design will be used[51]. The study will enroll 10 patients first, and if no patient has pCR or downstaging, the study will close on the basis that it is unlikely (p=0.1074) that 0/10 responses would occur if the true response rate were >20%. If at least one patient has pCR or downstaging, an additional 10 patients will be treated (total of 20). For patients enrolled in Phase II, dose of everolimus can be de-escalated down to 2.5mg if needed. No further de-escalation is allowed. If 2.5mg not tolerated then the patient will be removed from study.

4.3 **Study accrual and treatment considerations**

It is anticipated that 26 – 38 patients will be enrolled in the study and accrual is expected to be completed in 24 months (see Section **10. STATISTICAL CONSIDERATIONS**). Patients meeting the eligibility criteria and who have signed the consent form will start Letrozole 2.5 mg PO daily, Everolimus 5 or 10 mg PO daily, and TRC105 15 or 10 mg/kg IV every two weeks with the first infusion administered in 2 split doses on days 1 (3 mg/kg) and 4 (12 mg/kg or 7 mg/kg), respectively. Premenopausal women at the time of study enrollment will receive goserelin 3.6 mg subcutaneously q 4 weeks to achieve ovarian suppression. A cycle is defined as 4 weeks of treatment. Breast ultrasound, will be conducted upon completion of each cycle to complement clinical assessments of the size of the tumor(s). The duration of neoadjuvant therapy is 24 weeks or 6 cycles. No extensions of the neoadjuvant therapy beyond 6 months are allowed. After neoadjuvant therapy, patients will undergo surgical treatment and will receive adjuvant therapy (radiation therapy, chemotherapy, hormonal therapy) according to the standard of care or at the discretion of the treating physician. The accrual rate is projected to be approximately 1-2 patients per month. Accrual will be halted as a result of any grade ≥3 hemorrhage, cerebrovascular and vasovagal adverse events; FDA will be contacted to discuss further accrual or potential need for protocol amendment.

4.4 **Rationale for the Study Doses and Design**

The safety and efficacy of letrozole and everolimus for 4 months in the preoperative setting have been well established[4], allowing the use of these agents in combination at their approved therapeutic doses. Nonetheless, the addition of everolimus at 10 mg PO daily was associated with an increase in the adverse events of any grade[4] suggesting that extension of treatment to 6 months without dose reductions, delays, or interruptions may not be possible. To that end, a dose of everolimus at 5 mg PO daily is selected for cohort 1.

The addition of an antiangiogenic agent with a non-overlapping pharmacokinetic and toxicity profile is anticipated to increase the proportion as well as the magnitude of responses achieved without a significant increment in the rate of adverse events. However, TRC105 has not been administered in combination with letrozole and everolimus before. To that end, the phase I portion of the study is designed to determine the MTD and RP2D. Because of a concern that a dose of 10 mg/kg every two weeks will not allow for continuous serum levels of TRC105 placing patients at risk for infusion reactions each time they receive TRC105, the starting dose for the latter was selected at 15 mg/kg q 2 weeks. Provisions are made for a cohort -1 with a dose of TRC105 of 10 mg/kg in case the dose of 15 mg/kg is not well tolerated.

Preoperative treatment with an aromatase inhibitor has been shown to be superior over tamoxifen[48]. Premenopausal women cannot receive aromatase inhibitors[50] and in order to benefit from the latter they should become postmenopausal. GnRH analogs can safely achieve ovarian suppression and in fact ovarian suppression in combination with an aromatase inhibitor has been shown already to be superior to tamoxifen in the adjuvant setting[88].

The primary objective of the study is to establish the safety and tolerability of combining everolimus and TRC105 with letrozole in the preoperative setting for 24 weeks. During the phase II part of the study we will continue to evaluate the safety and tolerability of the investigational combination as well as collect preliminary data about the activity of the regimen.

4.5 **Rationale for the Preoperative Setting**

The preoperative setting is increasingly utilized in the evaluation and development of novel investigational therapies that can potentially provide meaningful clinical benefits over the current standard of care in breast cancer[104]. Although the long term outcomes with adjuvant and neoadjuvant therapy may be similar, the most pertinent advantages of neoadjuvant therapy are summarized as follows:

a. Neoadjuvant therapy allows for the clinical and radiographic evaluation of a regimen real-time. In doing so, an ineffective therapy can be promptly discontinued sparing toxicities and time.
b. The neoadjuvant setting allows for the evaluation of tumor tissue biomarkers predictive of response from the time of biopsy to the time of definitive breast surgery.
c. The neoadjuvant setting allows for the utilization of endpoints that are indicative of clinical benefit and can be readily assessed (rates of pCR and downstaging) as opposed to endpoints

such as progression-free and overall survival which require considerably longer duration of follow-ups for their definitive assessment.

d. Preoperative treatments permit breast conserving surgeries in patients who would otherwise require mastectomy (ies) and, therefore, may be more acceptable among patients potentially eligible for the proposed study.

4.6 **Rationale for the Drug Combination**

The proposed combination relies on preclinical evidence that has placed aberrant angiogenesis in the core of resistance to endocrine therapies and has elucidated the pathways that are operational in luminal breast cancer and can mitigate or completely abrogate the effect of aromatase inhibitors (see section **2. Background**).

In the clinical arena, the concept of targeting angiogenesis in the preoperative setting in luminal breast cancer was successfully translated into 2 independent clinical trials: a single-institution pilot study[2] and a randomized, open-label phase 2 study conducted by the *Translational Breast Cancer Research Consortium* (TBCRC). In both studies, antiangiogenic therapies have been shown to significantly enhance the antitumor response to endocrine therapies[1,2]. Targeting the pathways that mediate *de novo* or acquired resistance to aromatase inhibitors in luminal breast cancer in the preoperative setting with everolimus has also been clinically validated in a large randomized phase II clinical trial[4]. The addition of everolimus to letrozole led to higher clinical response rates irrespective of the *PIK3CA* mutational status[4]. The clinical precedence set by these studies along with the preclinical evidence of potential synergism between everolimus and TRC105 provide the rationale of the proposed investigational therapy.

4.7 Rationale for Tumor Assessments by Both Physical Examination and Imaging

It is anticipated that some tumors may not respond to the investigational therapy. With letrozole alone in the neoadjuvant setting, the rate of progressive disease has ranged between 2.2 and 12%[1,17,86]. In addition, certain tumors may not be well visualized or cannot be followed accurately with successive imaging studies.

It is important to ensure accurate clinical and imaging assessment of the tumor to safeguard against progressive disease. In equivocal cases as well as cases of discrepancy between imaging modalities and physical examination, provisions will be made for the use of MRI. Altogether though, patients whose disease cannot be measured and/or accurately followed by mammogram and/or breast ultrasound and/or exceptionally dedicated breast MRI are not eligible for the study. As most responders to neoadjuvant hormonal therapy can be identified within the first 4 months[14,87], patients with stable disease after 4 cycles or progressive disease at any time point will be promptly diverted to surgery or cytotoxic chemotherapy at the discretion of the treating physician.

4.8 Rationale for the Duration of Preoperative Treatment

The rationale for extending the preoperative investigational therapy beyond 3-4 months relies on 2 prior clinical studies that have shown a sustained and continued benefit by extending neoadjuvant hormonal therapy to 6 months and beyond[14,86,87]. The efficacy and tolerability of letrozole in combination with bevacizumab has been established in 2 prior clinical trials[1,2]. Extension of preoperative treatment beyond 6 months is not allowed by the protocol.

5. TREATMENT PLAN

5.1 Subject Selection and Registration

At the time of study entry, the patient will be evaluated by a multidisciplinary team of medical oncologists, radiation oncologists, and surgical oncologists. Before registration, investigators are required to indicate the type of operation they intend to perform. Patients will be consented by the investigator and the coordinator of the trial. Patients will be registered in the Clinical Trials Network and Monitoring Office; the office will verify the inclusion/exclusion criteria in real time.

5.2 Study Medications

5.2.1 Letrozole

For this trial, patients will receive 2.5 mg PO daily until 1 day before surgery. The hormonal agent is commercially available and will not be provided by the study.

Description of the drug: Letrozole (Femara®) is a nonsteroidal competitive inhibitor of the aromatase enzyme system, which is responsible for the synthesis of estrogens from androgenic substrates (specifically, the synthesis of estrone from the preferred substrate androstenedione and estradiol from testosterone)[50]. It is an oral agent with a half-life approximating 48 hours allowing for a once-daily administration schedule[50].

Pharmacokinetic properties: Letrozole is completely and rapidly absorbed from the gastrointestinal tract[105]. Absorption is not affected by food. Once absorbed, it is rapidly and extensively distributed into tissues (volume of distribution at steady state is ~2 liters/kg). Letrozole is eliminated mainly by metabolism. The major metabolite, which does not inhibit aromatase, is 4′methanobisbenzonitrile. This metabolite is glucuronidated and excreted primarily in the urine. Neither renal impairment (creatinine clearance < 9 ml/min) nor moderate hepatic impairment (Child-Pugh classification A and B) significantly influences letrozole pharmacokinetic parameters. Letrozole pharmacokinetics are not altered by age (range 35 to >80 years); the effects of race have not been studied[105].

Pharmacodynamic properties: In postmenopausal patients with advanced breast cancer, daily letrozole doses of 0.1–5 mg suppress plasma concentrations of estradiol, estrone, and estrone sulfate by 75–95%, with maximal suppression within 2–3 days. Suppression is dose related, with doses of ≥0.5 mg often reducing estrone and estrone sulfate levels to below the limit of detection. Estrogen suppression was maintained throughout treatment in all patients given ≥0.5 mg. At a clinically used dosage (2.5 mg PO daily), letrozole does not impair adrenal synthesis of glucocorticoids or aldosterone[105].

Adverse events: Adverse events associated with these medications are provided in the package insert (http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/020726s027lbl.pdf). The most frequent adverse events associated with letrozole include hot flashes, arthralgia/arthritis, night sweats, nausea, fatigue, myalgias, and edemas. Hypercholesterolemia grade 1 and 2 is frequent but grade 3 and 4 is rare (0.4%). Also, all aromatase inhibitors have been associated with osteoporosis. Formal assessment of bone density by means of DEXA scan relies at the discretion of the treating physician, as treatment for 6 months alone is not anticipated to incur clinically significant decreases in bone density. Following surgery, if patients proceed to adjuvant hormonal therapy with an aromatase inhibitor for 5 years, a DEXA scan is recommended as well as bone-strengthening medication(s) per institutional standards.

5.2.2 **Everolimus (**Further details can be found in the Everolimus Investigator's Brochure**)**

For this trial, the dose for everolimus is 5 (cohort 1) or 10 (cohort 2) mg PO daily and it will be continued until 4 weeks prior to surgery.

Everolimus is a novel derivative of rapamycin. It has been in clinical development since 1996 as an immunosuppressant in solid organ transplantation. Everolimus is approved in Europe and other global markets (trade name: Certican®) for cardiac and renal transplantation, and in the United States (trade name: Zortress®) for the prevention of organ rejection of kidney transplantation.

Afinitor® was approved for adults with advanced renal cell carcinoma (RCC) after failure of treatment with sunitinib or sorafinib in 2009. In 2010, Afinitor® received United States (US) approval for patients with subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis complex (TSC). Everolimus is also available as Votubia® in the European Union (EU) for patients with SEGA associated with TSC who require therapeutic intervention but are not candidates for curative surgical resection. Afinitor® was approved for "progressive pancreatic neuroendocrine tumor (PNET) in patients with unresectable, locally advanced, or metastatic disease" in 2011 in various countries, including the US and Europe. In 2012 Afinitor® received approval for the

treatment of postmenopausal women with advanced hormone receptor-positive, HER2- negative breast cancer in combination with exemestane, after failure of treatment with letrozole or anastrozole. Furthermore in 2012, Afinitor® received approval for the treatment of patients with TSC who have renal angiomyolipoma not requiring immediate surgery. Approximately 35,982 cancer patients have been treated with everolimus as of 31-Mar-2014 (19,668 patients in Novartis-sponsored clinical trials, 2,394 patients in the individual patient supply program, and more than 13,930 patients in investigator-sponsored studies). In addition, healthy volunteer subjects and non-oncology hepatically impaired subjects have participated in the clinical pharmacology studies. The following is a brief summary of the main characteristics of Everolimus. More complete information can be obtained from the Everolimus Investigator's Brochure (IB).

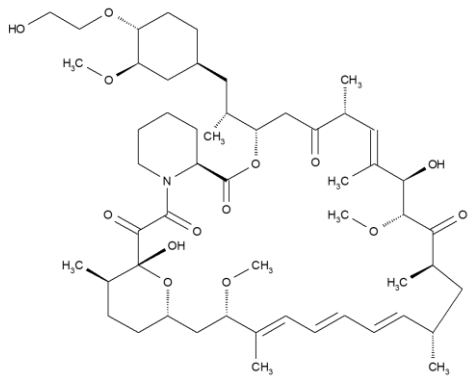
Description of the drug: Everolimus is an orally bioavailable derivative of rapamycin that acts as a signal transduction inhibitor (**Table 5-1**, **Figure E)**. Everolimus selectively inhibits mTOR (mammalian target of rapamycin), specifically targeting the mTOR-raptor signal transduction complex. mTOR is a key serine-threonine kinase in the PI3K/AKT signaling cascade, which is known to be dysregulated in a wide spectrum of human cancers[106].

Everolimus is being investigated as an anticancer agent based on its potential to act directly on the tumor cells by inhibiting tumor cell growth and proliferation, and indirectly by inhibiting angiogenesis leading to reduced tumor vascularity (via potent inhibition of tumor cell VEGF (vascular endothelial growth factor) production and VEGF-induced proliferation of endothelial cells).

Table 5-1: Everolimus – Drug substance

Chemical name	(1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28E,30S,32S,35R)-1,18-dihydroxy-12-{(1R)-2-[(1S,3R,4R)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]-1-methylethyl}-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-aza-tricyclo[30.3.1.$0^{4,9}$]hexatriaconta-16,24, 26,28-tetraene-2,3,10,14,20-pentaone
International non-proprietary name	Everolimus

Figure E: Chemical structure of Everolimus



mTOR pathway and cancer: At the cellular and molecular level, Everolimus acts as a signal transduction inhibitor. It selectively inhibits mTOR (mammalian target of rapamycin), a key protein kinase which regulates cell growth, proliferation and survival. The mTOR kinase is mainly activated via the phosphatidylinositol 3-kinase (PI3-Kinase) pathway through AKT/PKB and the tuberous sclerosis complex (TSC1/2). Mutations in these components or in PTEN, a negative regulator of PI3-kinase, may result in their dysregulation. Abnormal functioning of various components of the signaling pathways contributes to the pathophysiology of numerous human cancers. Various preclinical models have confirmed the role of this pathway in tumor development[107]. The main known functions of mTOR include the following[44]:

- mTOR functions as a sensor of mitogens, growth factors and energy and nutrient levels;
- Facilitating cell-cycle progression from G1-S phase in appropriate growth conditions;
- The PI3K/mTOR pathway itself is frequently dysregulated in many human cancers, and oncogenic transformation may sensitize tumor cells to mTOR inhibitors;
- PI3-kinase mutations have been reported in the primary tumor in 10-20% of human colorectal cancers[108,109];
- The loss of PTEN protein, either through gene deletion or functional silencing (promoter hypermethylation), is reported in approximately 60% of primary human colorectal cancers[110];
- The mTOR pathway is involved in the production of pro-angiogenic factors (i.e., VEGF) and inhibition of endothelial cell growth and proliferation;
- Through inactivating eukaryotic initiation factor 4E binding proteins and activating the 40S ribosomal S6 kinases (i.e., p70S6K1), mTOR regulates protein translation, including the HIF-1 proteins. Inhibition of mTOR is expected to lead to decreased expression of HIF-1.

Non-clinical experience: Everolimus inhibits the proliferation of a range of human tumor cell lines in vitro including lines originating from lung, breast, prostate, colon, melanoma and glioblastoma. IC50s range from sub/low nM to µM. Everolimus also inhibits the proliferation of human umbilical vein endothelial cells (HUVECs) in vitro, with particular potency against VEGF-induced proliferation suggesting that Everolimus may also act as an anti-angiogenic agent. The anti-

angiogenic activity of Everolimus was confirmed in vivo. Everolimus selectively inhibited VEGF-dependent angiogenic response at well tolerated doses. Mice with primary and metastatic tumors treated with Everolimus showed a significant reduction in blood vessel density when compared to controls.

The potential of Everolimus as an anti-cancer agent was shown in rodent models. Everolimus is orally bioavailable, residing longer in tumor tissue than in plasma in a subcutaneous mouse xenograft model, and demonstrating high tumor penetration in a rat pancreatic tumor model. The pharmacokinetic profile of Everolimus indicates sufficient tumor penetration, above that needed to inhibit the proliferation of endothelial cells and tumor cell lines deemed sensitive to Everolimus in vitro.

Everolimus administered orally daily was a potent inhibitor of tumor growth, at well tolerated doses, in 11 different mouse xenograft models (including breast, pancreatic, colon, epidermoid, lung and melanoma) and two syngeneic models (rat pancreatic, mouse orthotopic melanoma). These models included tumor lines considered sensitive and "relatively resistant" in vitro. In general, Everolimus was better tolerated in mouse xenograft models than standard cytotoxic agents (i.e., doxorubicin and 5-fluorouracil), while possessing similar anti-tumor activity. Additionally, activity in a VEGF-impregnated subcutaneous implant model of angiogenesis and reduced vascularity (vessel density) of Everolimus-treated tumors (murine melanoma) provided evidence of in vivo effects of angiogenesis.

It is not clear which molecular determinants predict responsiveness of tumor cells to Everolimus. Molecular analysis has revealed that relative sensitivity to Everolimus in vitro correlates with the degree of phosphorylation (activation) of the AKT/PKB protein kinase and the S6 ribosomal protein; in some cases (i.e., glioblastoma) there is also a correlation with PTEN status. In vivo studies investigating the anti-tumor activity of Everolimus in experimental animal tumor models showed that Everolimus monotherapy typically reduced tumor cell growth rates rather than produced regressions. These effects occurred within the dose range of 2.5 mg to 10 mg/kg, orally once a day. In preclinical models, the administration of Everolimus is associated with reduction of protein phosphorylation in target proteins downstream of mTOR, notably phosphorylated S6 (p-S6) and p-4E-BP1, and occasionally with an increase in phosphorylated AKT, a protein upstream of mTOR signaling pathway.

All significant adverse events observed in toxicology studies with Everolimus in mice, rats, monkeys and mini-pigs were consistent with its anticipated pharmacological action as an anti-proliferative and immunosuppressant and at least in part reversible after a 2 or 4-week recovery period with the exception of the changes in male reproductive organs, most notably testes. In vitro genotoxicity studies covering relevant genotoxicity end-points showed no evidence of clastogenic or mutagenic activity.

In male fertility studies in rats, testicular morphology was affected at 0.5 mg/kg and above, and sperm motility, sperm head count and plasma testosterone levels were diminished at 5 mg/kg which corresponded to 0.7 times the estimated clinical exposure at 10 mg/day, and caused a decrease in male fertility. There was evidence of reversibility. Female fertility was not affected, but everolimus caused an increase of pre-implantation loss in female rats at doses > 0.1 mg/kg, suggesting it could

also potentially impact fertility in females. Everolimus crossed the placenta and was toxic to the conceptus. In rats, everolimus caused embryo/fetotoxicity at systemic exposure below the planned therapeutic level comprising mortality and reduced fetal weight. The incidence of skeletal variations and malformations at 0.3 and 0.9 mg/kg (e.g. sternal cleft) was increased. In rabbits, embryo toxicity was evident by an increase in late resorptions. Effects of everolimus on the pre- and postnatal development of rats were limited to slightly affected body weight and survival in the F1-generation at ≥0.1 mg/kg, and did not indicate a specific toxic potential.

The potential reproductive risk for humans is unknown. However, due to the observed malformations in rats, everolimus should be considered potentially teratogenic. Everolimus should not be given to pregnant women unless the potential benefit outweighs the potential risk for the fetus. Women of childbearing potential should be advised to use highly effective contraception methods while they are receiving everolimus and up to 8 weeks after treatment has been stopped. It is not known whether everolimus is excreted in human milk. In animal studies, everolimus and/or its metabolites were readily transferred into the milk of lactating rats. Therefore women who are taking everolimus should not breastfeed.

Pharmacokinetic properties: Everolimus is rapidly absorbed after oral administration, with a median time to peak blood levels (tmax) of 1.3-1.8 hours post dose[111]. The extent of absorption is estimated at above 11%. The area under the blood concentration-time curve (AUC) is dose-proportional over the dose range tested while maximum blood concentration (Cmax) appears to plateau at dose levels higher than 20 mg. The elimination half-life in cancer patients averaged 30 hours, which is similar to that in healthy subjects. Inter-patient variability is moderate with the coefficient of variation of approximately 50%. In healthy subjects, high fat meals reduced systemic exposure to everolimus 10 mg (as measured by AUC0-∞) by 22% and the peak plasma concentration Cmax by 54%. Light fat meals reduced AUC0-∞ by 32% and Cmax by 42%. Food, however, had no apparent effect on the post absorption phase concentration-time profile. Steady-state trough levels are highly predictive of AUC, with a coefficient of determination of 0.96, as has been reported in renal transplantation patients. Everolimus is mainly metabolized by the cytochrome P450 isoenzyme 3A4 (CYP3A4) in the liver and to some extent in the intestinal wall[111]. Everolimus is also a substrate of P-glycoprotein (P-gp). Therefore, absorption and subsequent elimination of systematically absorbed everolimus may be influenced by medicinal products that interact with CYP3A4 and/or P-gp. Strong CYP3A inhibitors (such as ketoconazole, itraconazole, ritonavir; **Appendix B**) and inducers (such as rifampicin, rifabutin; **Appendix B**) should be avoided.

Everolimus, at a daily dose of either 5 mg or 10 mg, was added to the daily letrozole regimen of 2.5 mg in breast cancer patients[4]. Letrozole is mainly metabolized by CYP3A4 and CYP2A6 with minor contribution of renal clearance. Due to the low affinity of letrozole to CYP3A4, the potential for pharmacokinetic interaction is remote. Pharmacokinetic profiles of letrozole were investigated before addition of everolimus and after everolimus reached steady state (day 15). Data suggested that, indeed, co-administration of everolimus with 2.5 mg/day letrozole did not influence the pharmacokinetics of letrozole. Pharmacokinetic characteristics are similar for Caucasian and Japanese subjects. Pharmacokinetic studies in Black transplant patients have shown an average 20% higher clearance. **No dose adjustments are required for renal impairment or in geriatric populations but are required in hepatic impairment** (see section 5.3.2).

Pharmacodynamic properties: The pharmacodynamic properties of everolimus have been investigated in a phase I study in patients with advanced solid tumors (including breast cancer) in peripheral blood mononuclear cells[112]. S6 kinase 1 activity (a substrate of mTOR) in peripheral-blood mononuclear cells was inhibited for at least 7 days at doses ≥20 mg/week. Duration of suppression lengthened with increasing dosage. 20 mg was presumed to be the minimum dose ensuring target inhibition over 7 days[112].

Adverse events. The addition of everolimus to letrozole has been associated with a higher rate of dose reductions or interruptions due to adverse events (52.9%) as well as discontinuations (18.8%) compared with letrozole alone (7.6% and 9.1%, respectively) in the neoadjuvant setting[4]. Grade 1 and 2 adverse events were also more frequent in the combination arm (89.8% vs. 63.6% in the letrozole alone arm)[4]. The most frequent adverse events seen in higher frequency in the combination arm as compared to the letrozole alone group include stomatitis (36.5% vs. 6.1%), rash (20.4% vs. 7.6%), asthenia (17.5% vs. 9.8%), fatigue (12.4% vs. 4.5%), anorexia (12.4% vs. 3.8%), headache 10.9% vs. 5.3%), pruritus (13.1% vs. none), dyspnea (7.3% vs. 1.5%), pneumonitis (2.9% vs. none). Laboratory abnormalities seen in higher frequency in the combination arm as compared with the control arm include hypercholesterolemia (16.1% vs. 6.1%), thrombocytopenia (18.2% vs. 0.8%), hyperglycemia (13.1% vs. 3%), increased ALT (11.7% vs. 3.8%), anemia (11.7% vs 0.8%), and neutropenia (9.5% vs. 1.5%).

Grade 3 and 4 adverse events were also more frequent in the combination arm (22.6%) as opposed to the letrozole alone arm (3.8%). Stomatitis (2.2%), fatigue (1.5%), and pneumonitis (2.2%) were the most frequent grade 3 and 4 adverse events seen in more than 1 patients (n=137) associated with the addition of everolimus, while hyperglycemia (5.1%), increased ALT (1.5%), hypokalemia (1.5%), thrombocytopenia (1.5%) constituted the most frequent biochemical grade 3 and 4 adverse events. Serious adverse events associated with everolimus occurred in 7/137 patients: pneumonitis in three patients; pneumonia and mouth ulcers/stomatitis each in two patients; and dyspnea, neutropenia, and thrombocytopenia in one patient each. All three patient cases of pneumonitis resolved within 15 days of everolimus discontinuation. Among the placebo-treated patients, there was one serious adverse event of myocardial ischemia[4].

Overall, safety data available from completed, controlled and uncontrolled studies indicate that everolimus is generally well tolerated at weekly or daily dose schedules. The safety profile is characterized by manageable adverse events (AEs). These AEs are generally reversible and non-cumulative.

Adverse events most frequently observed with everolimus are stomatitis, rash, diarrhea, fatigue, infections, asthenia, nausea, peripheral edema, decreased appetite, headache, dysgeusia, epistaxis, mucosal inflammation, pneumonitis, weight decreased, vomiting, pruritus, cough, dyspnea, dry skin, nail disorder, and pyrexia. Overall, the most frequently observed laboratory abnormalities include: decreased hematology parameters including hemoglobin, lymphocytes, platelets, and neutrophils (or collectively as pancytopenia).; increased clinical chemistry parameters including cholesterol, triglycerides, glucose, aspartate transaminases, creatinine, alanine transaminases, and bilirubin; and decreased clinical chemistry parameters including phosphate and potassium. The majority of these AEs have been of mild to moderate severity. See section 8.6 **Adverse reactions with everolimus** for details regarding the reported adverse events associated with everolimus so far. The proceeding

sections summarize recommendations for dose interruption, reduction, or discontinuation of Afinitor in the management of adverse drug reactions (**Table 5-2** and **Table 5-3**). General management recommendations are also provided as applicable. Clinical judgment of the treating physician should guide the management plan of each patient based on individual benefit/risk assessment.

Table 5-2: Dosing guidelines for Everolimus-related non-hematologic toxicities(see 4.2)

Toxicity	Action
Non-Infectious Pneumonitis	Please refer to **Table 5-9**.
Reactivation of HBV or HCV flare	Please refer to **Table 5-7** and **Table 5-8**.
AST or ALT elevation Grade 1 (> ULN - 3.0 x ULN) Grade 2 (> 3.0 - 5.0 x ULN)	Maintain current dose level
AST or ALT elevation Grade 3 (> 5.0 - 20.0 ULN)*	- Interrupt Everolimus administration until resolution to ≤ grade 1 (or ≤ grade 2 if baseline values were within the range of grade 2). If resolution occurs ≤ 7 days, Everolimus should be re-started at the dose level prior to interruption. - If resolution takes > 7 days, or if event recurs within 28 days, hold Everolimus until recovery to ≤ grade 1 or baseline grade / value and reintroduce Everolimus at one dose level lower, if available (**for this trial if it was 2.5 mgs, then therapy will be discontinued**).
AST or ALT elevation Grade 4 (> 20 x ULN)*	Interrupt Everolimus administration until resolution to ≤ grade 1 (or ≤ grade 2 if baseline values were within the range of grade 2). If resolution occurs ≤ 7 days, Everolimus should be re-started at one dose level lower. If resolution takes > 7 days, discontinue Everolimus.
Recurrence of grade 4 after dose reduction or toxicity requiring Everolimus interruption for > 28 days	Discontinue Everolimus.
Intolerable grade 2 mucositis, or grade 3 AE, except hyperglycemia or hypertriglyceridemia or hypercholesterolemia (see Section5.2.2.1)	- Interrupt Everolimus administration until resolution to ≤ grade 1 or baseline grade / value. If resolution occurs within ≤ 7 days, Everolimus should be re-started at the dose level prior to interruption. - If resolution takes > 7 days, or if event recurs within 28 days, hold Everolimus until recovery to ≤ grade 1 or baseline grade / value and reintroduce Everolimus at one dose level lower, if available (**for this trial if it was 2.5 mgs, then therapy will be discontinued**). - Patients will be withdrawn from the study if they fail to recover to ≤ grade 1 or baseline grade / value within 28 days.
Any other grade 4	Hold Everolimus until recovery to grade ≤ 1 or baseline value Reintroduce Everolimus at one dose level lower, if available (**for this trial if it was 2.5 mgs, then therapy will be discontinued**).
Grade 3 or 4 clinical liver failure (asterixis or encephalopathy/coma)	Discontinue Everolimus
Recurrence of intolerable grade 2 mucositis or grade 3 event after dose reduction	Reduce dose to the next lower dose level, if available. The lowest possible dose level of Everolimus is 2.5 mg daily. Below this level, Everolimus must be discontinued. If toxicity recurs at Grade 3, consider discontinuation
Recurrence of grade 4 after dose reduction	Discontinue Everolimus
Any non-hematologic toxicity requiring Everolimus interruption for > 28 days	Discontinue Everolimus
* Should HCV flare be confirmed, the guidelines for flare must take precedence (please refer to **Tables 5-7 and 5-8**)	

Table 5-3: Dosing guidelines for Everolimus-related hematologic toxicities (see 4.2)

Toxicity	Action
Grade 2 thrombocytopenia (platelets <75, ≥ 50x109/L)	No action
Grade 3 thrombocytopenia (platelets <50, ≥ 25 x109/L)	- Interrupt Everolimus until resolution to grade ≤1 - If resolution occurs ≤ 7 days, reintroduce Everolimus at the dose level prior to interruption. - If resolution occurs > 7 days, or event occurs within 28 days, reintroduce Everolimus at one dose level lower, if available (**for this trial if it was 2.5mgs, then therapy will be discontinued**).
Grade 4 thrombocytopenia (platelets < 25 x109/L)	Interrupt Everolimus until recovery to grade ≤ 1. Then reintroduce Everolimus at one dose level lower, if available (**for this trial if it was 2.5mgs, then therapy will be discontinued**).
Grade 3 neutropenia or anemia (neutrophil <1, ≥0.5 x109/L)	- Interrupt Everolimus until resolution to grade ≤1 or baseline value - If AE resolution occurs ≤ 7 days, reintroduce Everolimus at the same dose level. - If AE resolution occurs > 7 days, or event occurs within 28 days, reintroduce Everolimus at one dose level lower, if available (**for this trial if it was 2.5mgs, then therapy will be discontinued**).
Grade 4 neutropenia or anemia	Interrupt Everolimus until recovery to grade ≤ 1 or baseline value. Reintroduce Everolimus at one dose level lower, if available (**for this trial if it was 2.5mgs, then therapy will be discontinued**).*
Febrile neutropenia	Interrupt Everolimus until resolution to grade ≤ 1 (or baseline value) and no fever. Reintroduce Everolimus at one dose level lower, if available (**for this trial if it was 2.5mgs, then therapy will be discontinued**).*
Recurrence of grade 3 toxicity after dose reduction	Reduce dose to the next lower dose level, if available. The lowest possible dose level of Everolimus is 2.5 mg every day. Below this level, Everolimus must be discontinued.
***Recurrence of grade 4 toxicity (including febrile neutropenia) after dose reduction**	**Discontinue Everolimus**
***Any hematologic toxicity requiring Everolimus interruption for > 28 days**	**Discontinue Everolimus**

5.2.2.1 **Management of stomatitis, oral mucositis, and mouth ulcers**

Patients with a clinical history of stomatitis/mucositis/mouth ulcers and those with gastrointestinal morbidity associated with mouth/dental infections, irritation of esophageal mucosa e.g. gastroesophageal reflux disease (GERD) and pre-existing stomatitis/mucositis must be monitored even more closely. Patients should be instructed to report the first onset of buccal mucosa irritation/reddening to their study physician immediately.

Mouth ulcers, stomatitis and oral mucositis have been seen in patients treated with everolimus. Stomatitis/oral mucositis/mouth ulcers due to everolimus should be treated using local supportive care. If examination reveals mouth ulcers rather than a more general inflammation of the mouth,

please classify the adverse event as such. Please follow the paradigm below for treatment of stomatitis/oral mucositis/mouth ulcers (**Table 5-4**):

1. For mild toxicity (grade 1) no dose adjustment required; use conservative measures such as **non-alcoholic mouth wash or salt water (0.9%) mouth wash** several times a day until resolution.

2. For more severe toxicity (grade 2 in which case patients have pain but are able to maintain adequate oral alimentation, or grade 3 in which case patients cannot maintain adequate oral alimentation), the suggested treatments are **topical analgesic mouth treatments (i.e., local anesthetics such as benzocaine, butyl aminobenzoate, tetracaine hydrochloride, menthol, or phenol)** with or without **topical corticosteroids**, such as triamcinolone oral paste 0.1% (Kenalog in Orabase®).

3. Agents containing hydrogen peroxide, iodine, and thyme derivatives may tend to worsen mouth ulcers. It is preferable to avoid these agents.

4. Antifungal agents must be avoided unless a fungal infection is diagnosed. In particular, systemic imidazole antifungal agents (ketoconazole, fluconazole, itraconazole, etc.) should be avoided in all patients due to their strong inhibition of everolimus metabolism, therefore leading to higher everolimus exposures. Therefore, topical antifungal agents are preferred if an infection is diagnosed. Similarly, antiviral agents such as acyclovir should be avoided unless a viral infection is diagnosed.

Table 5-4: Management of oral mucositis (stomatitis) associated with everolimus.

Severity	**Everolimus Dose Adjustment and Management Recommendations (see 4.2)**
Grade 1 (Minimal symptoms, normal diet)	No dose adjustment required. Manage with non-alcoholic or salt water (0.9%) mouth wash several times a day.
Grade 2 (Symptomatic but can eat and swallow modified diet)	- Temporary dose interruption until recovery to grade ≤1. Re-initiate everolimus at the same dose. - If stomatitis recurs at grade 2, interrupt dose until recovery to grade ≤1. Re-initiate everolimus at a lower dose (**for this trial if it was 2.5mgs, then therapy will be discontinued**). Manage with topical analgesic mouth treatments (e.g. benzocaine,butyl aminobenzoate, tetracaine hydrochloride, menthol or phenol)with or without topical corticosteroids (i.e. triamcinolone oral paste).
Grade 3 (Symptomatic and unable to adequately eat or hydrate orally)	Temporary dose interruption until recovery to grade ≤1. Re-initiate everolimus at lower dose (**for this trial if it was 2.5mgs, then therapy will be discontinued**). Manage with topical analgesic mouth treatments (i.e. benzocaine,butyl aminobenzoate, tetracaine hydrochloride, menthol or phenol) with or without topical corticosteroids (i.e. triamcinolone oral paste)*
Grade 4 (Symptoms associated with life-threatening consequences)	Discontinue everolimus and treat with appropriate medical therapy.

5.2.2.2 **Management of Hyperlipidemia and Hyperglycemia**

Treatment of hyperlipidemia should take into account the pre-treatment status and dietary habits. Blood tests to monitor hyperlipidemia must be taken in the fasting state. Grade 2 or higher hypercholesterolemia (>300 mg/dL or 7.75 mmol/L) or grade 2 hypertriglyceridemia or higher (>2.5x upper normal limit) should be treated with a 3-hydroxy-3-methyl-glutaryl (HMG)-CoA reductase inhibitor (e.g. atorvastatin, pravastatin, fluvastatin) or appropriate triglyceride-lowering medication, in addition to diet. Note: Concomitant therapy with fibrates and an HMG-CoA reductase inhibitor is associated with an increased risk of a rare but serious skeletal muscle toxicity manifested by rhabdomyolysis, markedly elevated creatine phosphokinase (CPK) levels and myoglobinuria, acute renal failure and sometimes death. The risk versus benefit of using this therapy should be determined for individual patients based on their risk of cardiovascular complications of hyperlipidemia.

Dyslipidemia (including hypercholesterolemia and hypertriglyceridemia) has been reported in patients taking everolimus. Monitoring of blood cholesterol and triglycerides prior to the start of everolimus therapy and periodically thereafter as well as management with appropriate medical therapy is recommended. Hyperglycemia has been reported in patients taking everolimus. Monitoring of fasting serum glucose is recommended prior to the start of Everolimus and periodically thereafter. More frequent monitoring is recommended when everolimus is co-administered with other drugs that may induce hyperglycemia. Optimal glycemic control should be achieved before starting a patient on Everolimus. Hyperlipidemia and hypertriglyceridemia should be treated according to local best clinical practice (**Table 5-5**). Patients should be monitored clinically and through serum biochemistry for the development of rhabdomyolysis and other adverse events as required in the product label/data sheets for HMG-CoA reductase inhibitors (statins).

Table 5-5. Management of hyperlipidemia/hypertriglycemia associated with everolimus

Severity	Everolimus Dose Adjustment and Management Recommendations
Grade 1	No dose adjustment required. Initiate appropriate medical therapy and monitor.
Grade 2	No dose adjustment required. Manage with appropriate medical therapy and monitor.
Grade 3	Temporary dose interruption. Re-initiate everolimus at lower dose (**for this trial if it was 2.5mgs, then therapy will be discontinued**). Manage with appropriate medical therapy and monitor.
Grade 4	Discontinue everolimus and treat with appropriate medical therapy.

5.2.2.3 **Management of Hepatitis reactivation**

Reactivation of Hepatitis B (HBV) has been observed in patients with cancer receiving chemotherapy[113]. Sporadic cases of Hepatitis B reactivation have also been seen in this setting with everolimus. Use of antivirals during anti-cancer therapy has been shown to reduce the risk of Hepatitis B virus reactivation and associated morbidity and mortality[114]. A detailed assessment of Hepatitis B/C medical history and risk factors must be done for all patients at screening, with testing performed prior to the first dose of everolimus. **Hepatitis B. Table 5-6** provides details of monitoring and prophylactic therapy according to the baseline results of viral load and serologic markers testing.

Table 5-6. Recommendations for positive baseline results for hepatitis B

Test	Result	Result	Result	Result	Result
HBV-DNA	+	+ or -	-	-	-
HBsAg	+ or -	+	-	-	-
HBsAb	+ or -	+ or -	+ and no prior HBV vaccination	+ or -	- or + with prior HBV vaccination
HBcAb	+ or -	+ or -	+ or -	+	-
Recommendation	Prophylaxis treatment should be started 1-2 weeks prior to first dose of Everolimus. Monitor HBV-DNA approximately every 4-8 weeks		No prophylaxis. Monitor HBV-DNA approximately every 3-4 weeks		No specific action

Antiviral prophylaxis therapy should continue for at least 4 weeks after last dose of study drug. **Table 5-7** provides guidelines for definition and management of hepatitis B reactivation.

Table 5-7: Guidelines for management of hepatitis B

HBV reactivation (with or without clinical signs and symptoms): All reactivations of HBV are to be recorded as grade 3 (e.g. CTCAE Version 3.0 - Investigations/Other: Viral Reactivation), unless considered life threatening by the investigator, in which case they should be recorded as grade 4. Date of viral reactivation is the date on which the rise or reappearance of HBV-DNA was recorded.	
For patients with baseline results: positive HBV-DNA, OR positive HBsAg	**Treat**: Start a second antiviral AND interrupt everolimus administration until resolution: • ≤ grade 1 ALT (or baseline ALT, if > grade 1) and • ≤ baseline HBV-DNA levels - **If resolution occurs within ≤ 28 days** everolimus should be restarted at one dose lower, if available. (see 5.2.5.2) If the patient is already receiving the lowest dose of everolimus according to the protocol, the patient should restart at the same dose after resolution. Both antiviral therapies should continue at least 4 weeks after last dose of study drug. **- If resolution occurs > 28 days** patients should discontinue everolimus but continue both antiviral therapies at least 4 weeks after last dose of study drug.
Reactivation is defined as: Increase of 1 log in HBV-DNA relative to baseline, HBV-DNA value, OR new appearance of measurable HBV-DNA	
For patients with baseline results: Negative HBV-DNA and HBsAg AND Positive HBs Ab (with no prior history of vaccination against HBV), OR Positive HBc Ab	**Treat** : Start first antiviral medication AND interrupt study drug administration until resolution: ≤ baseline HBV-DNA levels **- If resolution occurs within ≤ 28 days** everolimus should be restarted at one dose lower, if available. (see 5.2.5.2) If the patient is already receiving the lowest dose of everolimus according to the protocol, the patient should restart at the same dose after resolution. Antiviral therapy should continue at least 4 weeks after last dose of study drug. **- If resolution occurs > 28 days** Patients should discontinue study drug but continue antiviral therapy at least 4 weeks after last dose of everolimus.
Reactivation is defined as: New appearance of measurable HBV-DNA	

Hepatitis C. The following two categories of patients should be monitored every 6 weeks for HCV reactivation: patients with detectable HCV RNA-PCR test at baseline, and patients known to have a history of HCV infection, despite a negative viral load test at baseline (including those that were treated and are considered 'cured'). **Table 5-8** provides definition and guidelines for the management of hepatitis C.

Table 5-8: Definitions and Guidelines for the management of Hepatitis C

Baseline results	HCV flare definition*	HCV flare management
Detectable HCV-RNA	> 2 $\log_{10}$ IU/mL increase in HCV-RNA **AND** ALT elevation > 5 x ULN or 3 x baseline level, whichever is higher.	Discontinue Everolimus
Knowledge of past hepatitis C infection with no detectable HCV-RNA	New appearance of detectable HCV-RNA **AND** ALT elevation > 5 x ULN or 3 x baseline level, whichever is higher.	Discontinue Everolimus

* All flares of HCV are to be recorded as grade 3 (e.g. CTCAE Version 3.0 - Investigations - Other: Viral Flare), unless considered life threatening by the investigator; in which case they should be recorded as grade 4. Date of viral flare is the date on which both the clinical criteria described above were met. (e.g., for a patient whose HCV-RNA increased by 2 logs on 01 JAN 2011 and whose ALT reached > 5 x ULN on 22 JAN 2011, the date of viral flare is 22 JAN 2011).

All reactivations of hepatitis B or C are considered grade 3 adverse events according to the NCI CTCAE version 4.03 unless associated with decompensated liver dysfunction in which case they are deemed grade 4.
(http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf)

5.2.2.4 **Management of Non-infectious Pneumonitis**

Non-infectious pneumonitis is a class effect of rapamycin derivatives. Cases of non-infectious pneumonitis (including interstitial lung disease) have also been described in patients taking everolimus. Some of these have been severe and on rare occasions, a fatal outcome was observed.

A diagnosis of non-infectious pneumonitis should be considered in patients presenting with non-specific respiratory signs and symptoms such as hypoxia, pleural effusion, cough or dyspnea, and in whom infectious, neoplastic and other non-medicinal causes have been excluded by means of appropriate investigations. Opportunistic infections such as PJP should be ruled out in the differential diagnosis of non-infectious pneumonitis. Patients should be advised to report promptly any new or worsening respiratory symptoms. Diagnosis is generally suspected in individuals receiving mTOR inhibitors who develop these symptoms or in asymptomatic individuals in whom a routine chest CT scan reveals a new ground glass or alveolar infiltrate.

The frequency of symptomatic pulmonary toxicity (all grades) was approximately 13% in a phase III study of everolimus in patients with metastatic renal cell carcinoma[115]. Severe (CTCAE grade 3) pneumonitis occurred in 4% of patients, and an occasional fatality was reported. Similarly, in the

phase III study of everolimus in patients with advanced pancreatic neuroendocrine tumors symptomatic pulmonary toxicity was seen in 17% of patients with grade 3 or 4 pneumonitis seen in only 2%[116]. Lastly, in the clinical study that compared letrozole +/- everolimus in the neoadjuvant setting, grade 3 or 4 pneumonitis was seen in 2.2% of the patients in the everolimus (vs. none in the placebo) arm[4]. The lung toxicity was partly or completely reversible in the majority of cases with interventions including drug interruption, discontinuation and the use of corticosteroids.

Patients who develop radiological changes suggestive of non-infectious pneumonitis and have few or no symptoms may continue Afinitor therapy without dose alteration. If symptoms are moderate (grade 2), consideration should be given to interruption of therapy until symptoms improve. The use of corticosteroids may be indicated. Afinitor may be reintroduced at a daily dose approximately 50% lower than the dose previously administered. For cases of grade 3 non-infectious pneumonitis, interrupt Afinitor until resolution to less than or equal to grade 1. Afinitor may be re-initiated at a daily dose approximately 50% lower than the dose previously administered depending on the individual clinical circumstances. If toxicity recurs at grade 3, consider discontinuation of Afinitor. For cases of grade 4 non-infectious pneumonitis, Afinitor therapy should be discontinued. Corticosteroids may be indicated until clinical symptoms resolve. For patients who require use of corticosteroids for treatment of non-infectious pneumonitis, prophylaxis for pneumocystis jirovecii pneumonia (PJP) may be considered. The two compounds studied most extensively for prophylaxis against PJP have been trimethoprim-sulfamethoxazole, given orally, and pentamidine, given as an aerosol. Individuals participating in this trial will be routinely questioned as to the presence of new or changed pulmonary symptoms consistent with lung toxicity. CT scans and pulmonary function test should be done, as clinically indicated, if there are symptoms that indicate that the patient has developed non-infectious pneumonitis. If non-infectious pneumonitis develops, the guidelines in **Table 5-9** should be followed. Dose modification should be done as outlined in 5.2.5.2. Consultation with a pulmonologist is recommended for any case of pneumonitis that develops during the study.

Table 5-9. Guidelines for the management of treatment-related non-infectious pneumonitis

Worst grade pneumonitis	Suggested investigations	Management of pneumonitis	Everolimus dose adjustment
Grade 1 (Asymptomatic, radiographic findings only)	CT scans with lung windows.	No specific therapy is required	No dose adjustment required. Initiate appropriate monitoring.
Grade 2 (Symptomatic, not interfering with Activities of Daily Living)	CT scan with lung windows. Consider pulmonary function testing includes: spirometry, DLCO, and room air O_2 saturation at rest. Consider a bronchoscopy with biopsy and/or BAL. Monitoring at each visit until return to ≤ grade 1. Return to initial monitoring frequency if no recurrence.	Symptomatic only. Consider corticosteroids and/or other supportive therapy if symptoms are troublesome.	Rule out infection and consider interruption of Everolimus until symptoms improve to Grade ≤ 1. Re-initiate Everolimus at one dose level lower (**for this trial if it was 2.5 mgs, then therapy will be discontinued**). Discontinue Everolimus if failure to recover within ≤ 28 days.
Grade 3 (Symptomatic, Interfering with Activities of Daily Living. O_2 indicated	CT scan with lung windows and pulmonary function testing includes: spirometry, DLCO, and room air O2 saturation at rest. Monitoring at each visit until return to ≤ grade 1. Return to initial monitoring frequency if no recurrence. Bronchoscopy with biopsy and/or BAL is recommended.	Consider corticosteroids if infective origin is ruled out. Taper as medically indicated.	Rule out infection and interrupt Everolimus until symptoms improve to Grade ≤ 1. Consider re-initiating Everolimus at one dose level lower (approximately 50% lower than the dose previously administered depending on individual clinical circumstances) (**for this trial if it was 2.5 mgs, then therapy will be discontinued**). Discontinue Everolimus if failure to recover within ≤ 28 days. If toxicity recurs at Grade 3, consider discontinuation
Grade 4 (Life-threatening, ventlatory support indicated)	CT scan with lung windows and required pulmonary function testing, if possible, includes: spirometry, DLCO, and room air O2 saturation at rest. Monitoring at each visit until return to ≤ grade 1. Return to initial monitoring frequency if no recurrence. Bronchoscopy with biopsy and/or BAL is recommended if possible.	Consider corticosteroids if infective origin is ruled out. Taper as medically indicated.	Rule out infection and discontinue Everolimus.

5.2.2.3 **Management of Infections**

From a historical perspective, everolimus has been in clinical development since 1996 initially in solid organ transplantation as a medication to prevent rejection. It has immunosuppressive properties and may predispose patients to bacterial, fungal, viral or protozoan infections, including infections with opportunistic pathogens. Localized and systemic infections, including pneumonia, other bacterial infections, invasive fungal infections, such as aspergillosis or candidiasis and viral infections including reactivation of hepatitis B virus, have been described in patients taking everolimus. Some of these infections have been severe (e.g. leading to respiratory or hepatic failure) and occasionally have had a fatal outcome. In the phase III study of everolimus in patients with advanced pancreatic neuroendocrine tumors, infectious complications of any grade were seen in 23% (predominantly in the upper respiratory tract; grade 3 or 4, 2%) in the investigational arm vs. 6% (grade 3 or 4, <1%) in the placebo arm[116]. On the other hand though, no infectious adverse events were reported in the clinical study that compared letrozole +/- everolimus in the neoadjuvant setting[4].

Physicians and patients should be aware of the increased risk of infection with everolimus. Pre-existing infections should be appropriately treated prior to starting the investigational therapy. While taking everolimus, the patient and the investigator should be vigilant for symptoms and signs of infection. If a diagnosis of infection is made, appropriate treatment should be instituted promptly and, if the infection is severe enough, interruption or discontinuation of everolimus should be considered. If a diagnosis of invasive systemic fungal infection is made, everolimus will be discontinued; the patient will be taken off study and treated with appropriate antifungal therapy. Cases of pneumocystis jirovecii pneumonia (PJP), some with a fatal outcome, have been reported in patients who received everolimus. PJP may be associated with concomitant use of corticosteroids or other immunosuppressive agents. Prophylaxis for PJP should be considered when concomitant use of corticosteroids or other immunosuppressive agents are required.

5.2.2.4 **Management of skin toxicity**

For patients with grade 1 toxicity, no specific supportive care is usually needed or indicated. Rash must be reported as an AE. Patients with grade 2 or higher toxicity may be treated with the following suggested supportive measures at the discretion of the investigator: oral minocycline, topical tetracycline, topical clindamycin, topical silver sulfadiazine, diphenhydramine, oral prednisolone (short course), topical corticosteroids, or pimecrolimus.

5.2.2.5 **Management of Hypersensitivity reactions**

Hypersensitivity reactions manifested by symptoms including, but not limited to, anaphylaxis, dyspnea, flushing, chest pain or angioedema (e.g. swelling of the airways or tongue, with or without respiratory impairment) have been observed with everolimus.

5.2.2.6 **Angioedema with concomitant use of angiotensin-converting enzyme inhibitors**

Patients taking concomitant ACE inhibitor therapy may be at increased risk for angioedema (e.g. swelling of the airways or tongue, with or without respiratory impairment).

5.2.2.7 **Renal Failure Events**

Cases of renal failure (including acute renal failure), some with fatal outcome, occurred in patients treated with everolimus. Renal function of patients should be monitored particularly where patients have additional risk factors that may further impair renal function. Elevations of serum creatinine, usually mild, and proteinuria have been reported in patients taking everolimus. Monitoring of renal function, including measurement of blood urea nitrogen (BUN), urinary protein, or serum creatinine, is recommended prior to the start of everolimus therapy and periodically thereafter.

5.2.2.8 **Management of diarrhea**

Appearance of grade 1-2 diarrhea attributed to study drug toxicity may be treated with supportive care such as loperamide, initiated at the earliest onset (for example 4 mg orally followed by 2 mg orally every 2 hours until resolution of diarrhea).

5.2.2.9 **Cytochrome P450 and P-glycoprotein inhibitors/inducers/substrates**
Co-administration with strong inhibitors of CYP3A4 or P-gp should be avoided; and may cause increased everolimus concentrations. For a current table of Substrates, Inhibitors and Inducers please access the following website:

http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm.

Everolimus is metabolized by CYP3A4 in the liver and to some extent in the intestinal wall. Therefore, the following are recommended:

- Co-administration with strong inhibitors of CYP3A4 (e.g., ketoconazole, itraconazole, ritonavir) or P-glycoprotein (P-gp) inhibitor should be avoided.

- Co-administration with moderate CYP3A4 inhibitors (e.g., erythromycin, fluconazole) or P-gp inhibitors should be used with caution. If a patient requires co-administration of moderate CYP3A4 inhibitors or P-gp inhibitors, reduce the dose of everolimus by approximately 50%. Additional dose reductions to every other day may be required to manage toxicities. If the inhibitor is discontinued, the Everolimus dose should be returned to the dose used prior to initiation of the moderate CYP3A4/P-gp inhibitor after a washout period of 2 to 3 days.

- Grapefruit, Seville oranges, and star fruit affect P450 and P-gp activity. Concomitant use should be avoided.

- If patients require co-administration of a strong CYP3A4 inducer, consider doubling the daily dose of Afinitor (based on pharmacokinetic data), using increments of 5 mg or less. This dose of Afinitor is predicted to adjust the AUC to the range observed without inducers. However, there are no clinical data with this dose adjustment in patients receiving strong CYP3A4 inducers. If the strong inducer is discontinued, consider a washout period of at least 3 to 5 days (reasonable time for significant enzyme de-induction), before the Afinitor dose is returned to the dose used prior to initiation of the strong CYP3A4 inducer.

- This dose adjustment of Everolimus is intended to achieve similar AUC to the range observed without inducers. However, there are no clinical data with this dose adjustment in patients receiving strong CYP3A4 inducers. If the strong inducer is discontinued the Everolimus dose should be returned to the dose used prior to initiation of the strong CYP3A4/P-gp inducer.

Please refer to **Table 5-11** listing relevant inducers and inhibitors of CYP3A and **Table 5-12** for a list of relevant substrates, inducers, and inhibitors of P-gp.

Everolimus and drugs influencing CYP3A4 enzyme

Everolimus is a substrate of CYP3A4, and a substrate and moderate inhibitor of the multidrug efflux pump, P-gp (P-gp, MDR1, and ABCB1). Therefore, extent of absorption and subsequent elimination of systemically absorbed everolimus may be influenced by products that are substrates, inhibitors, or inducers of CYP3A4 and/or P-gp. Concurrent treatment with strong CYP3A4-inhibitors should be avoided. Refer to **Table 5-8** in section 6 for a comprehensive list of inducers and inhibitors of CYP3A4 and **Table 5-9** for a list of relevant substrates, inducers and inhibitors of P-gp. Inhibitors of P-gp may decrease the efflux of everolimus from brain or tumor and therefore increase everolimus concentrations in these tissues. In vitro studies showed that everolimus is a competitive inhibitor of CYP3A4 and of CYP2D6, potentially increasing the concentrations of products eliminated by these enzymes. Thus, caution should be exercised when co-administering everolimus with CYP3A4 and CYP2D6 substrates with a narrow therapeutic index. Clinical studies have been conducted in healthy subjects to assess pharmacokinetic drug interactions between everolimus and potential CYP3A modifiers (ketoconazole, verapamil, erythromycin, rifampin, midazolam, and HMGCoA reductase inhibitors (statins).

Table 5-11: Clinically relevant drug interactions: inducers, and inhibitors of isoenzyme CYP3A

Inducers
Strong inducers: avasimibe, carbamazepine, mitotane, phenobarbital, phenytoin, rifabutin, rifampin (rifampicin), St. John's Wort (hypericum perforatum) **Moderate inducers:** bosentan, efavirenz, etravirine, genistein, modafinil, nafcillin, ritonavir, [talviraline], thioridazine, tipranavir **Weak inducers:** amprenavir, aprepitant, armodafinil (R-modafinil), bexarotene, clobazam, danshen, dexamethasone, Echinacea, garlic (allium sativum), gingko (ginkgo biloba), glycyrrhizin, methylprednisolone, nevirapine, oxcarbazepine, pioglitazone, prednisone, [pleconaril], primidone, raltegravir, rufinamide, sorafenib, telaprevir, terbinafine, topiramate, [troglitazone] , vinblastine
Inhibitors
Strong inhibitors: boceprevir, clarithromycin, cobicistat, conivaptan, elvitegravir, indinavir, itraconazole, ketoconazole, lopinavir, mibefradil, nefazodone, nelfinavir, posaconazole[117], ritonavir, saquinavir, telaprevir, telithromycin, tipranavir, troleandamycin, voriconazole **Moderate inhibitors:** Amprenavir, aprepitant, atazanavir, casopitant, cimetidine, ciprofloxacin, cyclosporine, darunavir, diltiazem, dronedarone, erythromycin, fluconazole, fosamprenavir, grapefruit juice (citrus parasidi fruit juice), imatinib, schisandra sphenanthera, tofisopam, verapamil

Table 5-12: Clinically relevant drug interactions: substrates, inducers, inhibitors of P-gp and P-gp/CYP3A dual inhibitors

Substrates
colchicine, digoxin, fexofenadine, indinavir, paclitaxel, talinolol, topotecan, vincristine, everolimus
Inducers
rifampin, St John's Wort
P-gp Inhibitors and P-gp/CYP3A Dual Inhibitors
amiodarone, azithromycin, captopril, carvedilol, clarithromycin, conivaptan, diltiazem, dronedarone, elacridar, erythromycin, felodipine, fexofenadine, fluvoxamine, ginkgo (ginkgo biloba), indinavir, itraconazole, lopinavir, mibefradil, milk thistle (silybum marianum), nelfinavir, nifedipine, nitrendipine, paroxetine, quercetin, quinidine, ranolazine, rifampin, ritonavir, saquinavir, Schisandra chinensis, St John's Wort (hypericum perforatum), talinolol, Telaprevir, telmisartan, ticagrelor, tipranavir, tolvaptan, valspodar, verapamil
Reference: Internal Clinical Pharmacology Drug-drug interaction (DDI) memo, updated Oct. 2, 2011,29-Oct-2012 which summarizes DDI data from three sources including the FDA's "Guidance for Industry, Drug Interaction Studies", the University of Washington's Drug Interaction Database, and Indiana University School of Medicine's Drug Interaction Table.

5.2.2.10 **Vaccinations**

Immunosuppressants may affect the response to vaccination and vaccination during treatment with Everolimus may therefore be less effective. The use of live vaccines should be avoided during treatment with Everolimus. For pediatric patients with SEGA that do not require immediate treatment, complete the recommended childhood series of live virus vaccinations prior to the start of therapy according to local treatment guidelines. Examples of live vaccines are: intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, varicella, and TY21a typhoid vaccines.

5.2.3 **TRC105**

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

Manufacturing. A high producing CHO cell line using media that is free of animal derived components has been developed for production of TRC105. The TRC105 currently in use in all clinical studies is CHO-produced. The manufacturing process uses conventional purification and filtration steps and concludes with the formulation of TRC105 in 20 mM L-Histidine/L-Histidine Monohydrochloride, 240 mM Trehalose, 0.01% Polysorbate 20 Formulation (25 mg TRC105/mL)[85].

Pharmacokinetic properties. In the phase 1 first-in-human dose escalation study of TRC105, the medication was detectable at all dose levels immediately after intravenous infusion[35]. In patients enrolled at doses below 0.3 mg/kg every 2 weeks, circulating TRC105 was detectable but not measurable above the lower limit of quantitation of the assay (78 ng/mL). On an every 2-week

schedule, TRC105 was measurable above the target concentration that saturates CD105 receptors (200 ng/mL) for a duration of 4 hours at a TRC105 dose of 0.3 mg/kg, for 24 hours at 1 mg/kg, 120 hours at 3 mg/kg, and 188 hours at 10 mg/kg (Fig. 1). The observed terminal T½ was considerably prolonged following a single dose of 15 mg/kg compared with 3 and 10 mg/kg. Serum concentrations expected to saturate CD105 binding sites (≥200 ng/mL) were achieved continuously at 15 mg/kg dosed every 2 weeks and 10 mg/kg dosed weekly. At doses above 1 mg/kg, AUC increased supraproportionally with dose, whereas the Cmax seemed to be dose proportional[35]. TRC105 clearance was consistent with target mediated disposition, and decreased clearance at higher doses was related to target saturation[85].

Immunogenicity.. Neither Human anti-murine antibody (HAMA) nor Human anti-chimeric antibody (HACA) were detected in patients treated with CHO-produced TRC105 which was administered at the 0.3, 1, 3, 10 and 15 mg/kg dose levels[35,85] and will be used in the proposed study.

Pharmacodynamic properties. Please refer to the investigational brochure[85] for nonclinical pharmacodynamics studies with TRC105.

The dose for TRC105 is 15 (cohorts 1 and 2) or 10 (cohort -1) mg/kg IV every two weeks and it will be continued until **4 weeks prior to surgery**.

Adverse events. The most common adverse events associated with TRC105 are infusion reactions that are successfully mitigated with premedications and prolongation of the infusion time, hemorrhages (mostly grade 1 and 2), headaches, and hypoproliferative anemia[35]. See section 8.3 for additional information regarding TRC105 adverse events.

5.2.3.1 **Management of Infusion Reactions**

Infusion reactions have been observed following TRC105 administration, usually with the initial dose (including a grade 4 vasovagal reaction that resolved without sequelae), and were commonly associated with one or more of the following signs or symptoms: 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. 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%). The presence of HAMA of HACA neither correlated nor predicted the development of infusion reactions.

By extending the duration of the initial infusion from one to four hours and adding a glucocorticoid-based premedication regimen prior to each dose, the frequency and severity of infusion reactions were reduced. At dose levels where continuous TRC105 serum levels were achieved, with subsequent infusions dexamethasone was safely discontinued and the infusion duration reduced to one hour. **Table 5-10** provides guidelines for the definition and management of infusion related reactions.

To mitigate or avoid infusion reactions associated with TRC105, the following actions will be undertaken:

a. The first dose of TRC105 will be split in two separate infusions to be infused over 4 hours each on days 1 (3 mg/kg) and 4 (12 mg/kg or 7 mg/kg if dose de-escalation occurs). If well tolerated, all subsequent infusions will be administered as a whole (i.e., 15 mg/kg or 10 mg/kg if dose de-escalation occurs) in their scheduled times and infusion time will gradually be reduced to 2 hours and then to a minimum of 90 minutes.
b. The following TRC105 premedications should be administered 2 hours to 30 minutes prior to the start of each infusion:
    1. Acetaminophen 650 mg p.o. x 1
    2. Methylprednisolone 100 mg i.v.. In the absence of any infusion reaction with the prior dose, methylprednisolone may be discontinued beginning with cycle 3. In addition, methylprednisolone will be given in the case of a delay of ≥ 18 days between any two doses or if the patient develops an infusion reaction during the immediate prior infusion.
    3. Famotidine 20 mg i.v. or p.o. (or similar H2 blocker) x 1. Famotidine (or similar H2 blocker) may be discontinued starting with Cycle 2 in the absence of infusion reactions with the prior dose.
    4. Cetirizine 10 mg i.v. or p.o. x 1 (or similar oral or intravenous antihistamine). Cetirizine (or similar oral or intravenous antihistimine) may be discontinued starting with Cycle 2 in the absence of infusion reactions with the prior dose.
c. During the first (day 1 and 4) infusion, vital signs should be checked every 30 minutes and methylprednisolone 100 mg IV should be readily available for infusion.

Table 5-10: Guidelines for the management of infusion related reactions

Grade	Signs or Symptoms	Recommended Action	Dose Adjustment or Modification
Grade 1 Mild transient reaction	Mild increase in heart rate but <100 bpm Mild increase in respiratory rate but <25 breaths per minute	Interruption not indicated Intervention not indicated	None
Grade 2 Infusion interruption indicated but responds promptly to symptomatic treatment	Increase in heart rate but<120 bpm Increase in respiratory rate but <30 breaths per minute Skin rash or mild pruritus	Withhold infusion until resolution of symptoms but no less than 30 minutes. Administer methylprednisolone 100 mg IV ± diphenhydramine 50 mg IV.	None Premedications as per cycle 1. Do not shorten subsequent infusions until no infusion reactions are seen

		Resume infusion once symptoms resolve	
Grade 3 Prolonged recurrence of symptoms following initial improvement; hospitalization indicated	Increase in heart rate >120 bpm, palpitations and sense of "impending doom" Diffuse skin rash and pruritus Tachypnea >30 breaths per minute and mild wheezing	Interrupt infusion and do not resume. Administer methylprednisolone 250 mg IV and diphenhydramine 50 mg IV. Admit to the hospital for observation	None Escalate premedications to dexamethasone 8 mg PO BID in the day before and day of chemotherapy. Do not shorten subsequent infusions until no infusion reactions are seen. If the reaction recurs the patient will be taken off study.
Grade 4 Life-threatening consequences	Palpitations, tachycardia, dyspnea with stridor or wheezing, sense of "impending doom", chest pain, diffuse rash with wheals and pruritus	Interrupt infusion and admit to the MICU Administer methylprednisolone 250 mg IV and diphenhydramine 50 mg IV. Administer pressors as indicated.	Do not rechallenge with TRC105. The patient will be taken off study.

5.2.3.2 **Management of Hemorrhages**

Grade 3 or 4 hemorrhage with TRC105 is rare. In the phase I study, 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[35]. TRC105 was withheld, the patient was transfused 2 units of packed red blood cells and the bleeding resolved with nonsurgical management by the time of upper endoscopy. Patients with bleeding gastrointestinal ulcers or other sources of bleeding will be excluded from the study unless there is documentation of healing.

Subsequent bleeding associated with TRC105 has been limited to low grades including superficial gingival bleeding, epistaxis, and intermittent post-coital vaginal bleeding in a patient with uterine cancer recurrent at the vaginal cuff. Gingival bleeding and epistaxis are related to the development of mucocutaneous telangiectasia and represent on-target effects of TRC105 therapy. Patients with epistaxis may benefit from saline nasal gel. 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.

Cerebrovascular hemorrhage (grade 3) resulting in hemiparesis occurred in one patient with hepatocellular cancer who was thrombocytopenic (platelet count of 60,000/uL) in a study of TRC105 with sorafenib. Patients in TRC105 protocols are required to have platelet counts of 100,000/μL.

Accrual will be halted as a result of any grade ≥3 hemorrhage, cerebrovascular and vasovagal adverse events; FDA will be contacted to discuss further accrual or potential need for protocol amendment.

5.2.3.3 **Management of Anemia**

Hypoproliferative anemia was the dose-limiting toxicity in the phase I study[35] and may be related to the action of TRC105 on proerythroblasts, the only cells in the bone marrow known to express high levels of CD105. When hemoglobin drops in a patient on the study, it is important to investigate for any ongoing blood loss. A reticulocyte count should be obtained to document the hypoproliferative nature of the anemia associated with TRC105. Anemia should be managed with standard supportive measures including transfusions of packed red blood cells if deemed necessary. If grade 3 or higher treatment related anemia develops, the dose of TRC105 should be reduced from 15 mg/kg to 10 mg/kg or held if the assigned dose is 10 mg/kg until resolution of the anemia.

5.2.3.4 **Management of Headache**

Headaches (grade 1 to 3) have been observed following TRC105 treatment, generally within hours following completion of the initial infusion. Headaches are throbbing in nature, have not been associated with radiographic abnormalities, and have responded to treatment with non-steroidal anti-inflammatory agents (including ketorolac) and to triptans. Extending the infusion to 4 hours or interrupting the infusion may also by tried to mitigate the headaches Nasal congestion and periorbital edema have been observed with TRC105. The edema has been transient in nature and treated with corticosteroids. Antihistamines are useful for nasal congestion.

5.2.4 Goserelin

Description of the drug: Goserelin (Zoladex®) is a synthetic decapeptide analog of endogenous gonadotropin-releasing hormone. GnRH regulates follicle-stimulating hormone (FSH) and luteinizing hormone (LH) synthesis and secretion by the anterior pituitary gland, which in turn stimulates the production of estrogens by the ovary. In response to GnRH, FSH and LH synthesis initially increases, causing a transient increase in circulating levels of sex hormones. These hormones are however, regulated by feedback loops, so further hormone release is suppressed. Chronic administration of goserelin leads to sustained suppression of pituitary gonadotropins FSH and LH as the pituitary gland down-regulates and desensitizes GnRH receptors.
Pharmacodynamic properties: Within 3 weeks following initial administration of goserelin, serum estradiol is suppressed to levels similar to those observed in postmenopausal women. However, after suppression was attained, isolated elevations of estradiol were seen in up to 10% of the patients enrolled in clinical trials. Serum LH and FSH are suppressed to follicular phase levels within four weeks after initial administration of drug and are usually maintained at that range with continued use of goserelin. In 5% or less of women treated with goserelin, FSH and LH levels may not be suppressed to follicular phase levels on day 28 post treatment with a single 3.6 mg depot injection.
Pharmacokinetic properties: Goserelin in its depot formulation is released slowly initially for the first 8

days, and then there is more rapid and continuous release for the remainder of the 28-day dosing period. Despite the change in the releasing rate of goserelin, administration of goserelin every 28 days resulted in testosterone levels that were suppressed to and maintained in the range normally seen in surgically castrated men. When goserelin 3.6 mg depot was used for treating male and female patients with normal renal and hepatic function, there was no significant evidence of drug accumulation. However, in clinical trials the minimum serum levels of a few patients were increased. These levels can be attributed to interpatient variation. The apparent volumes of distribution determined after subcutaneous administration of 250 μg aqueous solution of goserelin was 20.3 liters for females. The plasma protein binding of goserelin obtained from one sample is 27.3%.
Metabolism of goserelin, by hydrolysis of the C-terminal amino acids, is the major clearance mechanism. All metabolites found in humans have also been found in toxicology species. Clearance of goserelin following subcutaneous administration of the solution formulation of goserelin is very rapid and occurs via a combination of hepatic metabolism and urinary excretion. More than 90% of a subcutaneous radiolabeled solution formulation dose of goserelin is excreted in urine. Approximately 20% of the dose in urine is accounted for by unchanged goserelin. (AstraZeneca, Package Insert).

Goserelin is generally safe to administer with other medications. Goserelin should be administered subcutaneously every 28 days into the anterior abdominal wall below the navel line using an aseptic technique under the supervision of a physician.

5.2.5 **Concomitant Medications**

The reason(s) for treatment dosage, and dates of treatment should be recorded in the source documents. Concomitant medications should be reported to the investigator and will be recorded as instructed in the study specific case report forms. Patients who experience TRC105-related temperature elevations or other infusion-related symptoms may be treated symptomatically with acetaminophen, dyphenhydramine, meperidine, or other medications as clinically indicated, including < 48 hours of treatment with corticosteroids. Patients should receive full supportive care, including transfusions, antibiotics, anti-emetics, etc. when appropriate. Pre-trial supportive care will continue. The following therapies are excluded during active protocol therapy: radiotherapy, hormonal therapy different from the agent prescribed in the protocol, cytotoxic chemotherapy, herbal medicines and cancer immunotherapy or other biologic agents.

If the patient is on low dose aspirin at baseline he/she may continue it if medically indicated. If the patient is on NSAIDs on study should also receive peptic ulcer disease (PUD) prophylaxis with an H2 or proton pump blocker.

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

5.3 **Dose Modifications and Delays**

Dose modifications and delays are allowed. Determinations to reduce the dose, withhold, or delay the administration of a drug rely on the treating physician. The choice of the drug(s) (letrozole and/or everolimus and/or TRC105, or all three components) that need(s) to be withheld or dose reduced (everolimus or TRC105; delays but no dose reductions are allowed for letrozole) relies on the treating

physician and should be based on the nature of the observed adverse events. **The justification for dose modifications and delays should be clearly documented.** The maximum duration that a patient is allowed to withhold up to all three medications of the regimen is 7 days. Treatment can be resumed after 7 days at a lower dose level as outlined below for each individual component of the regimen. If treatment cannot be resumed within 7 days, the patient will be taken off study.

5.3.1 **Dose modifications and delays with letrozole**

Modifications. The dose of letrozole will not be modified (increased or decreased) in this protocol. Also letrozole cannot be substituted for another hormonal agent (e.g. anastrozole). Delays. The maximum duration that a patient is allowed to withhold letrozole due to adverse events is 7 days. If a patient cannot resume letrozole after 7 days or it is deemed necessary to withhold the medication again, the patient will be taken off study.

5.3.2 **Dose modifications and delays with everolimus in phase II portion**

Modifications. The dose for everolimus is 5 or 10 mg PO daily. In patients receiving 10 mg PO daily, dose reduction to 5 mg PO daily is allowed for adverse events or intolerance at the discretion of the treating physician. The reason for dose reduction must be clearly documented. Dose reductions at levels below 5 mg PO daily are allowed to 2.5 mg PO. No further reductions are allowed. Dose adjustments should be made if a patient's hepatic (Child-Pugh) status changes during treatment.

- Mild hepatic impairment (Child-Pugh A) – if the assigned dose was 5 or 10 mg PO daily, the recommended dose is 2.5 and 7.5 mg PO daily, respectively.
- Moderate hepatic impairment (Child-Pugh B) – if the assigned dose was 10 mg PO daily, the recommended dose is 5 mg daily. If the assigned dose was 5 mg PO daily, withhold everolimus until resolution of hepatic impairment and resume at 5 mg PO daily.
- Severe hepatic impairment (Child-Pugh C) – not recommended. If the desired benefit outweighs the risk, a dose of 2.5 mg daily must not be exceeded.

Delays. The maximum duration that a patient is allowed to withhold everolimus due to adverse events is 7 days. If a patient cannot resume everolimus after 7 days or it is deemed necessary to withhold the medication again, the patient will be taken off study.

5.3.3 **Dose modifications and delays with TRC105**

Modifications. The dose for TRC105 is 10 or 15 mg/kg IV every two weeks and it will be continued until 4 weeks prior to surgery. Dose reductions to 10 mg/kg due to adverse events are allowed and the justification(s) should be documented. Dose reductions below 10 mg/kg are not allowed and if deemed necessary, the patient will be taken off study.

Delays. All infusions of TRC105 should be administered within 3 days of the protocol-specified schedule. The maximum duration that a patient is allowed to withhold TRC105 due to adverse events is 14 days. If TRC105 is delayed > 3 days the first dose of TRC105 following the break needs to be split into two doses; 3 mg/kg should be given on the first day and the remainder of the dose (i.e., 12 mg/kg, or 7 mg/kg in the case of a dose de-escalation) will be given 3 days later and the

methylprednisolone dose should be reinstituted. If a patient cannot resume TRC105 after 14 days or it is deemed necessary to withhold the medication again, the patient will be taken off study. Resumption at 10 mg/kg if the subject was receiving 15 mg/kg are allowed with the reinstitution of full premedication, as specified in Section 5.2.3.1.

5.3.4 **Dose modifications and delays with goserelin**

Modifications. No dose modifications are allowed with goserelin

Delays: delays up to 3 days are allowed to accommodate for scheduling issues, inclement weather, or other extraneous circumstances.

5.4 **Study Procedures**

5.4.1 **Screening phase**

Informed consent will be obtained before study-specific screening evaluations are performed. Screening evaluations must be performed within 4 weeks prior to day 0. Results of tests or examinations done as standard of care prior to obtaining informed consent and within 56 days prior to day 0 may be used rather than repeating tests.

The following evaluations and procedures will be performed during the screening phase:

- Signed informed consent.

- Clinical evaluations: medical history, demographics, weight and height, complete physical examination, vital signs and performance status.

- Determination of the surgical procedure to be performed at the time of diagnosis without the neoadjuvant treatment by the surgical oncologist in the multidisciplinary team. Determinations include unresectable (for locally advanced disease that cannot be adequately resected unless tumor shrinkage is achieved), mastectomy, and lumpectomy.

- Laboratory assessment: UA, CBC with differential and platelet count, serum chemistries (glucose, BUN, creatinine, total protein, albumin, bilirubin, calcium, alkaline phosphatase, AST, and ALT), serum electrolytes (Na, K, Cl), ferritin, and a lipid panel (cholesterol, triglycerides, HDL, and LDL) in a fasting state (at least 8 hours).

- Determination of menopausal status: FSH, LH, and estradiol levels.

- Pregnancy test in women with childbearing potential.

- Research biopsy (core needle biopsy of the tumor) (required).

- Sentinel lymph node biopsy if required.

- Estrogen and Progesterone receptor status.

- Her-2-neu by immunohistochemistry and or FISH (Negative: IHC 0, 1 or 2+ or non-amplified by FISH).

- Tumor assessments: Mammogram and breast ultrasound are required. PET/CT scan or CT scan of chest, abdomen, pelvis, with bone scan will be obtained. If the scan shows disease outside the breast and/or the axilla, the patient will not be enrolled in the trial.

- 12-lead EKG. Evaluation of the ejection fraction with an echocardiogram or MUGA, if clinically indicated.

- Concomitant medication assessment.

- Patients who have satisfied basic eligibility criteria will be assigned with a study patient number and the patient can proceed to be consented.

- Hepatitis B and C panels (see 5.2.2.3, table 5-2 and table 5-4 for Hepatitis B and C interpretation).

5.4.1.1 **Screening for hepatitis B**

Prior to initiation of Everolimus, the following three categories of patients should be tested for hepatitis B viral load and serologic markers, that is, HBV-DNA, HBsAg, HBs Ab, and HBc Ab:

- All patients who currently live in (or have lived in) Asia, Africa, Central and South America, Eastern Europe, Spain, Portugal and Greece. [http://wwwnc.cdc.gov/travel/yellowbook/2012/chapter-3-infectious-diseases-related-to-travel/hepatitis-b.htm]

- Patients with any of the following risk factors:
  - known or suspected past hepatitis B infection,
  - blood transfusion(s) prior to 1990,
  - current or prior IV drug users,
  - current or prior dialysis,
  - household contact with hepatitis B infected patient(s),
  - current or prior high-risk sexual activity,
  - body piercing or tattoos,
  - mother known to have hepatitis B
  - history suggestive of hepatitis B infection, e.g., dark urine, jaundice, right upper quadrant pain
  - Additional patients at the discretion of the investigator.

The management guidelines, in 5.2.2.3, are provided according to the results of the baseline assessment of viral load and serological markers for hepatitis B.

5.4.1.2 **Screening for hepatitis C**

Patients with any of the following risk factors for hepatitis C should be tested using quantitative RNA-PCR:

- known or suspected past hepatitis C infection (including patients with past interferon 'curative' treatment),
- blood transfusions prior to 1990,
- current or prior IV drug users,
- current or prior dialysis,
- household contact of hepatitis C infected patient(s),
- current or prior high-risk sexual activity,
- body piercing or tattoos

At the discretion of the investigator, additional patients may also be tested for hepatitis C. The management guidelines, in 5.2.2.3, are provided according to the results of the baseline assessment of hepatitis C viral load.

5.4.1.3 **Pregnancy and assessments of fertility**

There are no adequate data from the use of everolimus and TRC105 in pregnant women. The safety of women of childbearing capacity cannot be implied for the existing data with TRC105. Studies with everolimus in animals have shown reproductive toxicity effects including embryo-toxicity and feto-toxicity. The potential risk for humans is unknown. Everolimus should not be given to pregnant women unless the potential benefit outweighs the potential risk to the fetus. If a pregnancy occurs while on study treatment, the newborn will be followed for at least 12 months. It is not known whether everolimus is excreted in breast milk. However, in animal studies everolimus and/or its metabolites readily passed into the milk of lactating rats. Women taking everolimus should therefore not breast-feed.

Women of childbearing potential
Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, must use highly effective contraception during the study and for 8 weeks after stopping treatment. Highly effective contraception is defined as either:

- Total abstinence: When this is in line with the preferred and usual lifestyle of the subject. [Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception]
- Sterilization: have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks before taking study treatment. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment

- Male partner sterilization (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate). [For female subjects on the study, the vasectomised male partner should be the sole partner for that subject].
- Use of a combination of any two of the following (a+b or a+c or b+c):
  a. Use of oral, injected, implanted or other hormonal methods of contraception
  b. Placement of an intrauterine device (IUD) or intrauterine system (IUS)
  c. Barrier methods of contraception: Condom or Occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/vaginal suppository
- In case of use of oral contraception, women should have been stable on the oral agent before taking study treatment.

Male Contraception

Sexually active males must use a condom during intercourse while taking the drug and for 8 weeks after stopping treatment and should not father a child in this period. A condom is required to be used also by vasectomized men in order to prevent delivery of the drug via seminal fluid. Female partners of male patients must also be advised to use one of the following contraception methods: Use of (1) oral, injected, implanted or other hormonal methods of contraception, or (2) intrauterine device (IUD) or intrauterine system (IUS), or (3) prior male/female sterilization.

Fertility

The potential for everolimus and TRC105 to cause infertility in male and female patients is unknown. However, menstrual irregularities, secondary amenorrhea and associated luteinizing hormone (LH)/follicle stimulating hormone (FSH) imbalance has been observed with everolimus. Based on non-clinical findings, male and female fertility may be compromised by treatment with everolimus and TRC105.

5.4.2 **Treatment Phase**

The following evaluations and procedures will be performed during the **treatment phase**: (All assessments should be performed within 5 working days of the protocol-specified date).

- Physical examination, vital signs, weight, and performance status on weeks 4, 8, 12, 16, 20 and 24.

- Laboratory assessments: CBC with differential and platelet count, serum chemistries (glucose, BUN, creatinine, total protein, albumin, bilirubin, calcium, alkaline phosphatase, AST, and ALT), and serum electrolytes (Na, K, Cl) on week 1 and at the beginning of each cycle until week 24.

- Lipid panel (cholesterol, triglycerides, HDL, and LDL) in a fasting state (at least 8 hours) should be obtained at least on cycle 2 and, if elevated, repeated on each cycle until recovery to baseline value or grade 1 (see 5.2.2.2 for management of hyperlipidemia and hyperglycemia).

Serum estradiol levels on weeks 4, 12, and 20 in women receiving goserelin.

- Hepatitis panel as clinically indicated.

- Research biopsy (core needle biopsy of the tumor) at week 4 (required).

- Tumor assessments: Ultrasound of the breast will be obtained on week 4, 8, 12, 16, 20 and 24. Mammogram will be obtained on week 24. Additional PET scans or CT of the chest, abdomen, and pelvis, or bone scans are not required during treatment phase except as clinically indicated (see section **7.2 Measurement of Effect**). MRI breast will be obtained at baseline, week 12 and 24.

- Evaluation of the ejection fraction with an echocardiogram/MUGA only as clinically indicated.

- Surgical evaluation at the end of neoadjuvant therapy. **There must be at least 4 weeks between the last dose of everolimus and TRC105 and surgery**. Patients will continue letrozole until the day before surgery.

- Concomitant medication assessment.

- Assessment of adverse events.

5.4.3 **Safety Follow-up Phase**

Patients will complete participation in the protocol at the time of surgery and will not be followed unless the patient requires follow up of adverse events. The following evaluations and procedures will be performed at **early termination.** Every effort should be undertaken for the following procedures to be completed within 10 days.

- Clinical evaluations: Weight, physical examination (complete), vital signs and performance status. There must be a complete and clear documentation of objective response or disease progression using the RECIST criteria. Reason(s) for discontinuation must be documented.

- Laboratory assessment: UA, CBC with differential and platelet count, serum chemistries (glucose, BUN, creatinine, total protein, albumin, bilirubin, calcium, alkaline phosphatase, AST, and ALT), serum electrolytes (Na, K, Cl), and lipid panel (cholesterol, triglycerides, HDL, and LDL) in a fasting state (at least 8 hours).

- Tumor assessments: Mammogram, breast ultrasound, and breast MRI. Spiral CT scans of chest, abdomen, and pelvis, bone scan, and any other procedure are not required per protocol but will be obtained if clinically indicated.

- Concomitant medication assessment.

- Assessment of adverse events. If the patient has an ongoing toxicity, the patient should be followed until resolution or stabilization. Serious and non-serious adverse events occurring

within 30 days of day 0 of the last treatment should be reported on the appropriate adverse event CRF.

5.5 **Study Termination**

The principal investigator retains the right to terminate study participation in consultation with the other investigators in the study. The principal investigator will notify the institutional review board and FDA in writing of the early termination or completion of the study and keep a copy of the notification in the regulatory file. Circumstances that may raise consideration for early termination include but are not limited to: inordinate toxicity of the proposed combination that is not amenable to protocol amendment, and slow accrual (rate of accrual of less than 1 patient in 6 months).

5.6 **Subject Discontinuation**

Patients may withdraw from this study at any time. Any patient who withdraws will be encouraged to return to the study center for the post-dose evaluations. The termination visit consists of all evaluations scheduled for the termination visit. The primary reason(s) for discontinuation must be recorded on the appropriate CRF page.

The PI may discontinue a patient from treatment. Reasons may include, but are not limited to, the following: progressive disease, failure to obtain postmenopausal status (defined as serum estradiol levels any time during treatment in the premenopausal range) clinically significant deterioration, noncompliance, persistent grade 3 or 4 adverse event or any significant adverse event that compromises the patient's ability to participate in the study, requirement of a significant surgical procedure or radiation therapy during the treatment period of the study, investigator's determination that it is not in the patient's best interest to continue participation, anemia greater than grade 4, persistent grade 3 mucositis despite maximal supportive care, development of brain metastases, pregnancy.

Patients who have an ongoing grade 4 or serious adverse event at the time of discontinuation from study treatment will continue to be followed at least weekly for 4 weeks and at least monthly thereafter at the discretion of the investigator until resolution of the event or until the event is considered irreversible.

6. STUDY DRUG PREPARATION AND DISPENSATION

6.1 **Dosing Regimen and Schedule**

Table 6-1: Dosing regimen and schedule

Drug	Formulation	Dose and Route	Frequency
Letrozole	Tablet for oral use	2.5 mg PO	Daily
Everolimus	Tablet for oral use	5 or 10 mg PO	Daily
TRC105	Solution for intravenous infusion	10 or 15 mg/kg IV	q 2 weeks*
Goserelin**	Depot for subcutaneous injection	3.6 mg subcutaneously	q 4 weeks

***** The first infusion of TRC105 will be split in 2 separate infusions to be administered on cycle 1 day 1 (3 mg/kg) and cycle 1 day 4 (12 or 7 mg/kg).
** when applicable

6.1.2 TRC105 Preparation

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

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

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

6.1.3 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 if the patient is thought to 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 i.v. In the absence of any infusion reaction with the prior dose, methylprednisolone may be discontinued beginning with **cycle 3.** In addition, methylprednisolone will be given in the case of a delay of ≥ 18 days between any two doses or if the patient develops an infusion reaction during the immediate prior infusion.
- Famotidine 20 mg i.v. or p.o. (or similar H2 blocker) x 1. Famotidine (or similar H2 blocker) may be discontinued starting with Cycle 2 in the absence of infusion reactions with the prior dose.
- Cetirizine 10 mg i.v. or p.o. x 1 (or similar oral or intravenous antihistamine). Cetirizine (or similar oral or intravenous antihistimine) may be discontinued starting with Cycle 2 in the absence of infusion reactions with the prior dose.

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

Following the appropriate premedication regimen, the patient will receive TRC105 on day 1 and day 15 of every cycle. The first TRC105 dose will be split with 3 mg/kg administered on cycle 1 day 1 and infused over 4 hours (+/- 15 minutes) and 12 mg/kg (or 7 mg/kg if dose is de-escalated) administered on cycle 1 day 4 and infused over 2 hours (+/- 15 minutes), and then the full dose of 15 mg/kg (or 10 mg/kg if dose is de-escalated) given on cycle 1 day 15 and every 2 weeks thereafter and will be administered over a minimum of 90 minutes. The patient must complete at least one 4 hour infusion (+/- 15 minutes) without the development of any infusion reactions, in order to reduce the subsequent TRC105 infusion to 2 hours (+/- 15 minutes) and complete a 2 hour infusion (+/- 15 minutes) without the development of any infusion reactions in order to reduce subsequent TRC105 infusions to a **minimum of 90 minutes**. If the patient develops infusion reactions of any kind they should be managed appropriately (see Section 5.3.2.1) and the patient is not permitted to reduce the duration of the next planned infusion.

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

If the patient misses a TRC105 dose (i.e., ≥ 18 days between doses), the full premedication (including methylprednisolone) should be reinstituted as per the initial infusion and TRC105 dose should be administered over two days as was done for the initial dose.

6.2 **Drug Formulations**

All dosages prescribed and dispensed to the patient and all dose changes during the study must be recorded. Medication labels will comply with US legal requirements and be printed in English. They will supply no information about the patient.

6.2.1 **Letrozole Formulation**

Commercially available tablets of letrozole will be used in the study. Complete guidelines for the management and administration of letrozole can be found in the package insert (http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/020726s027lbl.pdf). Letrozole will not be provided from the study

6.2.2 **Everolimus Formulation**

Everolimus is formulated as tablets for oral administration of 2.5mg, 5mg, and 10mg strength. Tablets are blister-packed under aluminum foil, which should be opened only at the time of administration as drug is both hygroscopic and light-sensitive. Everolimus should be administered orally once daily at the same time every day, either consistently with or consistently without food. Refer to label for expiration date and storage conditions.

The extent of absorption of everolimus through topical exposure is not known. Therefore, caregivers are advised to avoid contact with suspensions of everolimus Tablets. Hands should be washed thoroughly before and after preparation of either suspension. The blisters for everolimus tablets should be opened only at the time of administration as the formulation is both hygroscopic and light-sensitive. Storage conditions for everolimus are described on the medication label (http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/022334s021s023s024,203985s002s004s005lbl.pdf). The study drugs should be stored in a secure, locked area while under the responsibility of the principal investigator. Receipt and dispensing of study drug must be recorded by an authorized person at the investigator's site.

The tablets should be swallowed whole with a glass of water and should not be chewed or crushed. For patients unable to swallow tablets, the tablet(s) should be dispersed completely in a glass of water (containing approximately 30 mL) by gently stirring until the tablet(s) is fully disintegrated (approximately 7 minutes), immediately prior to drinking. The glass should be rinsed with the same volume of water and the rinse completely swallowed to ensure the entire dose is administered. If vomiting occurs, no attempt should be made to replace the vomited dose. Patients should be instructed that if they miss a dose on one day, they must not take any extra dose the next day, but instead to immediately contact the study center as soon as possible to ask for advice. Everolimus will be provided from the study.

6.2.3 **TRC105 Formulation**

TRC105 is a sterile, clear colorless to slightly yellow opalescent solution for intravenous (IV) infusion. The solution may contain small amounts of visible particulates. TRC105 will be filtered through a 0.2 µm low protein binding filter at the clinical site prior to administration. TRC105 will be supplied in 100 and 400 mg single-use vials in 20 mM L-Histidine/L-Histidine Monohydrochloride, 240 mM Trehalose, 0.01% Polysorbate 20 (25 mg TRC105/mL). Vials will be labeled with the following:

TRC105 NSC# 754227 100 mg per vial (25 mg/mL, 4 mL per vial) Store refrigerated at 2-8˚C. For Intravenous Use Only. Single-use vial. Lot: XXXXXXX Mfg Date: XX/XX/XXXX Caution: New Drug Limited by Federal (or United States) law to investigational use. TRACON Pharmaceuticals Inc., San Diego, CA 92122 USA	TRC105 NSC# 754227 400 mg per vial (25 mg/mL, 16 mL per vial) Store refrigerated at 2-8˚C. For Intravenous Use Only. Single-use vial. Lot: XXXXXXX Mfg Date: XX/XX/XXXX Caution: New Drug Limited by Federal (or United States) law to investigational use. TRACON Pharmaceuticals Inc., San Diego, CA 92122 US

TRC105 will be provided by TRACON Pharmaceuticals, Inc.

6.2.4 Goserelin Formulation

Goserelin is supplied as a sterile, biodegradable product containing goserelin acetate equivalent to 3.6 mg of goserelin. Goserelin is designed for subcutaneous injection with continuous release over a 28-day period. Goserelin acetate is dispersed in a matrix of D,L-lactic and glycolic acids copolymer (13.3-14.3 mg/dose) containing less than 2.5% acetic acid and up to 12% goserelin-related substances and presented as a sterile, white to cream colored 1-mm diameter cylinder, preloaded in a special single use syringe with a 16-gauge x 36 +/- 0.5 mm siliconized needle with protective needle sleeve (SafeSystem™ Syringe) in a sealed, light and moisture proof, aluminum foil laminate pouch containing a desiccant capsule. Studies of the D,L-lactic and glycolic acids copolymer have indicated that it is completely biodegradable and has no demonstrable antigenic potential (AstraZeneca, Package Insert).

6.3 **Study Drug Storage and Handling**

Study treatments must be received by designated personnel at the study site, handled and stored safely and properly, and kept in a secured location to which only the investigator and designated site personnel have access. Upon receipt, the study treatment should be stored according to the instructions outlined in section 6.2.

6.4 **Study Drug Compliance**

The investigator should promote compliance by instructing the patient to take the study drug exactly as prescribed and by stating that compliance is necessary for the patient's safety and the validity of

the study. The patient should be instructed to contact the investigator if he/she is unable for any reason to take the study drug as prescribed.

Compliance will be assessed by the investigator and/or study personnel at each patient visit and information provided by the patient and/or caregiver. Records of study medication used, dosages administered, and intervals between visits and the completion of the study will be captured in the Drug Accountability Form. This information must be captured in the source document at each patient visit.

On PK sampling days, compliance will be assured by administration of the study treatment under the supervision of investigator or his/her designee, and will be verified by determination of letrozole, everolimus, and TRC105 in serum.

6.5 **Study Drug Accountability**

The investigator or designee must maintain an accurate record of the shipment and dispensing of study treatment in a drug accountability log. Patients will be asked to return all unused study treatment and packaging at the end of the study or at the time of study treatment discontinuation.

At study termination, and, as appropriate during the course of the study, the investigator will return all used and unused study treatment, packaging, drug labels, and a copy of the completed drug accountability log to the Novartis and TRACON monitor or to the Novartis and TRACON address provided in the investigator folder at each site.

7. VISIT SCHEDULE AND ASSESSMENTS

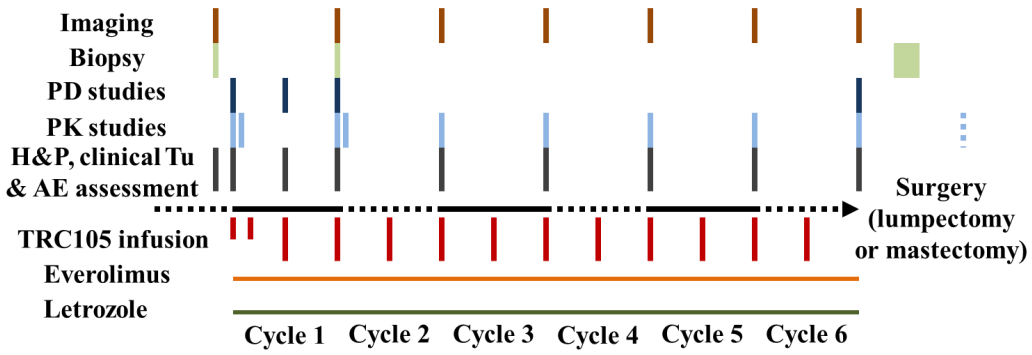
7.1 **Study Flow and Visit Schedule**

Figure F and **table 7-1** (following page) list all the assessments and indicate with an "x" the times they need to be performed.

Baseline evaluations are to be conducted within 1week prior to start of protocol therapy. Imaging studies must be done within 4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. For all visits except day 1, there is a temporal window of ±3 days to account for holidays, inclement weather or other extraneous circumstances. The temporal window does not apply for PK sampling, which needs to be undertaken at the specified time frames. Every effort must be undertaken to follow this schedule.

In patients who discontinue their treatment early, all procedures outlined in 5.4.3 (5.4.3 **Safety Follow-up Phase**) must occur within 10 days. Patients who complete the protocol treatment and proceed to surgery as planned do not need to have an end of treatment visit.

Figure F: Study Schema



Premenopausal women at the time of study enrollment will receive goserelin 3.6 mg subcutaneously q 4 weeks to achieve ovarian suppression.

7.2 **Measurement of Effect**

Although response is not the primary endpoint of this trial, patients with measurable disease will be assessed by standard criteria. **Patients must have disease that can be measured and followed by mammogram and/or breast ultrasound. Dedicated breast MRI evaluation will be done baseline, week 12 and 24.** For the purposes of this study, patients should be re-evaluated every 4 weeks.

7.2.1 **Antitumor Effect**

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST)[118] Committee (http://ctep.cancer.gov/protocolDevelopment/docs/recist_guideline.pdf)[118]. Changes in only the largest diameter (one-dimensional measurement) of the tumor lesions are used in the RECIST criteria.

Table 7-1: Study Calendar (see **5.4 Study Procedures**)

	SC	Treatment Phase[11, 13]													End of treatment[7]	Follow up
		C1D1	C1D4	C1D15	C2D1	C2D15	C3D1	C3D15	C4D1	C4D15	C5D1	C5D15	C6D1	C6D15		
Informed consent	X															
Demographics	X															
Inclusion/Exclusion	X															
Past Medical History	X															
History Present Illness	X															
Concurrent Medications			X----------												----------X	
Prior therapy	X															
Physician Visits	X	X			X		X		X		X		X		X	X
Physical Examination	X	X			X		X		X		X		X		X	X
Performance status	X	X			X		X		X		X		X		X	X
Vital Signs	X	X			X		X		X		X		X		X	X
Height/Weight	X	X			X		X		X		X		X		X	X
CBC and differential	X		X		X		X		X		X		X		X	X
Chemistries/Electrolytes	X		X		X		X		X		X		X		X	X
FSH, LH,estradiol levels	X															
Estradiol levels[15]					X				X				X			
Pregnancy test[14]	X															
Lipid panel[1]	X				X											
Hepatitis panel[2]	X															
Urinalysis[3]	X															
12-lead EKG	X															
Serum ferritin level	X															
Everolimus serum level		X[10]			X[10]											
TRC105 serum level		X[10]			X[10]											
Anti-TRC105 antibodies (APA)[11]		X			X		X		X		X		X			X[11]
VEGF-A serum[12]	X			X	X										X	
sEng / TGf-β serum[12]	X			X	X										X	
Angiopoietin-1/2 serum[12]	X			X	X										X	
Research Biopsy[4]	X				X										X	
Adverse Events			X----------												----------X	

Mammography	X														X	
Breast ultrasound	X				X		X		X		X		X		X	
Breast MRI[5]	X								X						X	
Body CT/MRI/PET[6]	(X)															
Letrozole[8]	X- ---------- ---------- X															
Everolimus[9]	X---------- ---------- X															
TRC105[9]		X	X	X	X	X	X	X	X	X	X	X	X	X		
Goserelin[13]		x			x		x		x		x		x			
Surgery															X	

Notes:

1. Cholesterol, triglycerides, HDL, and LDL in a fasting state (8 hours) should be obtained at least on cycle 2 and, if elevated, repeat on each cycle until recovery to baseline value or grade 1.
2. Hepatitis panel should be obtained on screening and repeated as clinically indicated (HBV-DNA, HBsAg, HBs Ab, and HBc Ab, and RNA-PCR).
3. Urinalysis should be obtained on screening and repeated as clinically indicated.
4. Research biopsy (core needle biopsy of the tumor).
5. Breast MRI is required by the protocol.
6. As clinically indicated after baseline diagnosis.
7. Within a week of surgery.
8. Continuation until the day prior to surgery.
9. Continuation no later than 4 weeks prior to surgery.
10. Samples will be collected and concentrations of the agents measured on cycles 1 and 2 at the following time points: pre-dose, 1, 2, 4, 6, and 24, hours, and cycle 1 day 4 pre-dose.
11. TRC105 anti-product antibodies (APA) will be collected before dosing on cycle 1 day 1, every 4 weeks thereafter, 4 and 12 weeks following the last dose of TRC105.
12. Markers of angiogenesis (VEGF-A, soluble VEGFR2, activin A and activin B, angiopoietin-1 and -2, TGF-β, soluble endoglin) will be measured on samples collected pre-dose, cycle 1 day 15, cycle 2 day 1, and prior to surgery.
13. Administered only if FSH, LH, and estradiol NOT within postmenopausal range at the time of enrollment.
14. In women with childbearing potential
15. Only in women receiving goserelin

7.2.2 **Definitions**

Evaluable for toxicity. All patients who receive at least one dose of any or all of the drugs of the investigational regimen will be evaluable for toxicity from the time of their first treatment until discontinuation or completion of the investigational therapy.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one 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.

7.2.3 **Disease Parameters**

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm with conventional techniques (mammogram, breast ultrasound, exceptionally MRI). All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters). Note: Tumor lesions that are situated in a previously irradiated area should not be considered measurable.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <20 mm with conventional techniques), are considered non-measurable disease. For this protocol it will be satellite lesions in the affected breast.

Target lesions (not applicable for this trial). All measurable lesions up to a maximum of 5 lesions per organ and 10 lesions in total, representative of breast(s) involved, 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 longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

Non-target lesions (not applicable for this trial. All other lesions including any measurable lesions over and above the 10 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.

7.2.4 **Evaluation of Response Reporting**

Studies in the neoadjuvant setting in breast cancer have used various endpoints in the assessment of response including rates of pCR[12,59,60,119], radiographic response[1], and clinical response[4,14,17]. Although these endpoints may have their limitations, the rates of pCR is the outcome measure least subject to serious errors of measurement and interpretation. All three outcome measures will the recorded and reported. The following definitions will be used:

a. Clinical response: the target lesion will be clinically assessed and measured using calipers on each visit. At least 2 dimensions will be measured and recorded. The maximum reduction in the maximum dimension of the tumor from baseline will be recorded as the best clinical response.

b. Radiographic response: the target lesion(s) will be measured and recorded at least in 2 dimensions on mammograms, breast ultrasound, and MRI. RECIST will be used to assess the best radiographic response (see below 7.2.5 **Methods for Evaluation of Measurable Disease**).

c. Pathologic response: the 2-dimensional size of the surgically excised residual tumor will be measured and compared to the radiographic size of the tumor at baseline. The maximum dimension will be used for comparison.

Definition of pathologic complete remission (pCR): the definition adopted by the Center for Drug Evaluation and Research of the FDA[104] will be used to define and report pCRs. According to the Center for Drug Evaluation and Research of the FDA, a pCR is defined as the absence of any residual invasive cancer on hematoxylin and eosin evaluation of the resected breast specimen and all sampled ipsilateral lymph nodes following completion of neoadjuvant systemic therapy (i.e., ypT0 ypN0 in the current AJCC staging system)[104]. For this protocol, near pCR will be considered if the breast residual disease is less or equal to 0.5 cm.

7.2.5 **Methods for Evaluation of Measurable Disease**

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

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Evaluation by clinical examination will always be complemented by imaging evaluation. See **Appendix C** for guidelines issued by the American College of Radiology for imaging studies of the breast. In cases of discrepancy between clinical and radiographic evaluation by >1 cm, a complementary imaging technique (breast ultrasound when the mammograms are inconclusive and breast MRI when both mammograms and breast ultrasound are inconclusive) will be used for the initial and subsequent assessments of the target lesion(s).

7.2.5.1 **Clinical Assessments**

Target lesion(s) in the breast or target ipsilateral lymph nodes will be measured using calipers and the size in two dimensions will be recorded on each clinical visit.

7.2.5.2 **Mammography**

Standard diagnostic mammograms will be used to identify and follow the target lesion(s) during the course of the protocol treatment. All mammograms will be obtained at the Kirklin Clinic which meets the requirements set forth by the Mammography Quality Standards Act (MQSA) final rule published by the Federal Drug Administration (FDA).

Technical considerations of diagnostic mammography (The American College of Radiology Practice Guideline for the Performance of Screening and Diagnostic Mammography, http://www.acr.org/~/media/3484ca30845348359bad4684779d492d.pdf):

1. A diagnostic mammogram will include mediolateral oblique (MLO), craniocaudal (CC), and/or supplemental views to evaluate an area of clinical or radiographic concern.

2. Supplemental mammographic views might include spot compression, spot compression with magnification, tangential views, or other special views.

3. When selecting a view, the proximity of the area of concern to the image receptor should be considered.

4. Diagnostic mammography should be performed under the immediate supervision of the interpreting physician.

7.2.5.3 **Breast Ultrasound**

Breast ultrasound (US) is particularly helpful in further delineating findings on mammography. All breast ultrasounds will be obtained at the Kirklin Clinic to complement diagnostic mammograms.

Technical considerations of Breast Ultrasonography (The American College of Radiology Practice Guideline for the Performance of a Breast Ultrasound Examination, http://www.acr.org/~/media/52D58307E93E45898B09D4C4D407DD76.pdf)

1. The breast sonogram should be correlated with clinical signs and/or symptoms and with mammographic and other appropriate breast imaging studies. If sonography has been performed previously, the current examination should be compared with prior sonograms. A lesion or any area of the breast being studied should be viewed in 2 perpendicular projections, and real-time scanning by the interpreter is encouraged.

2. The size of a lesion should be determined by recording its maximal dimensions in at least 2 planes; orthogonal planes are recommended. At least 1 set of images of a lesion should be obtained without calipers.

3. The images should be labeled as to right or left breast, location of lesions, and the orientation of the transducer with respect to the breast (e.g., transverse or longitudinal, radial or antiradial). The location of the lesion should be recorded using clock face notation and distance from the nipple, and/or shown on a diagram of the breast. The length of the transducer face (footprint), usually between 3.5 cm and 5 cm, can be used to estimate the distance from the nipple. Measurements should not be made from the edge of the areola, as areolar width is widely variable.

4. Sonographic features are helpful in characterizing breast masses. These feature categories and their descriptors are listed and exemplified in the ACR Breast Imaging Reporting and Data System® (BIRADS®). The BI-RADS sonographic categories include size, shape, orientation, margin,

echogenicity, lesion boundary, attenuation (e.g., shadowing or enhancement), special cases, vascularity, and surrounding tissue[120].

5. Elastography, or tissue stiffness assessment, is among the new feature categories applicable to sonographic analysis of masses, to be included in the Associated Findings section in BI-RADS Ultrasound, edition 2. To minimize errors in communication or interpretation, if elastography is performed, the color scales should be annotated to denote hardness or softness.

6. Mass characterization with ultrasonography is highly dependent on technical factors. Breast ultrasound should be performed with a high-resolution scanner. Gain settings, focal zone selections**,** and fields of view should be optimized to obtain high-quality images. The patient should be positioned to minimize the thickness of the portion of the breast being evaluated. For evaluation of lesions in, on, or just beneath the skin, a stand-off device or thick layer of gel may be helpful

7.2.5.4 **Breast Magnetic Resonance Imaging**

Breast Magnetic Resonance Imaging (MRI) is required by the protocol.

Technical considerations of Breast MRI (The American College of Radiology Practice Guideline for the Performance of Contrast-Enhanced Magnetic Resonance Imaging [MRI] of the Breast, http://www.acr.org/~/media/2A0EB28EB59041E2825179AFB72EF624.pdf)

1. Resolution, contrast, and field strength: Although a 1.5T magnet has traditionally been considered a minimum technical requirement because of the relationship between field strength and resolution, improvements in other components of the scanning process have resulted in improved scan quality at lower field strengths. High spatial and temporal resolutions are needed to detect and characterize small abnormalities on MRI. The slice thickness should be 3 mm or less, and in-plane pixel resolution should be 1 mm or less to minimize the problem of volume averaging effects. Optimized contrast between tumor and surrounding tissue is important. When high-resolution images are being obtained, chemical fat suppression is helpful as a method to reduce fat signal while preserving the signal-to-noise ratio. Sole reliance on subtraction imaging for assessment of enhancement may result in misregistration due to patient motion; use of fat suppression is recommended on sequences used to assess contrast enhancement. Protocols may incorporate both fat suppression and subtraction. Motion correction may be helpful in reducing artifacts encountered with image subtraction.

2. Contrast: Gadolinium contrast enhancement is generally needed in the evaluation of breast cancer. Gadolinium contrast should be administered as a bolus with a standard dose of 0.1 mmol/kg followed by a saline flush of at least 10 ml.

3. Scan time: A pre-contrast scan should be obtained. Scan time in relation to contrast injection is extremely important for lesion characterization. Kinetic information should be reported, based on enhancement data determined at specified intervals separated by 4 minutes or less. Imaging **s**ites should have adequately short temporal resolution for accurate capture of lesion kinetics.

4. Examinations should be performed with a dedicated bilateral breast MRI coil.

Tumor markers. Tumor markers alone cannot be used to assess response and will not be routinely obtained in the course of the study. Patients with stage III breast cancer will undergo imaging studies including imaging studies dedicated to identify skeletal metastases (bone scan and/or bone surveys) to confirm absence of metastatic disease.

7.2.6 **Response Criteria**

7.2.6.1 **Evaluation of Target Lesions**

Complete Response (CR): Disappearance of all target lesions

Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD

Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

7.2.6.2 **Evaluation of Non-Target Lesions**

Complete Response (CR): Disappearance of all non-target lesions.

Incomplete Response/Stable Disease (SD): Persistence of one or more non-target lesion(s).

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

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

7.2.6.3 **Evaluation of Best Overall Response**

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Response for this Category Also Requires:
CR	CR	No	CR*	NA for this trial

CR	Non-CR/Non-PD	No	PR**	NA for this trial
PR	Non-PD	No	PR	
SD	Non-PD	No	SD	Documented at least once ≥ 4 wk from baseline
PD	Any	Yes or No	PD	No prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* For the definition of a pCR please refer to section 7.3.1.3. **Definition of pathologic complete remission (pCR)**. No subsequent confirmation is required for pCR. ** Residual disease persistent in pathology specimen (near pCR if less than 0.5 cm residual disease in the breast)

***In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

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

7.2.7 **Duration of Response**

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for clinical CR or PR (whichever is first recorded) until completion of protocol treatment or early termination or 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).

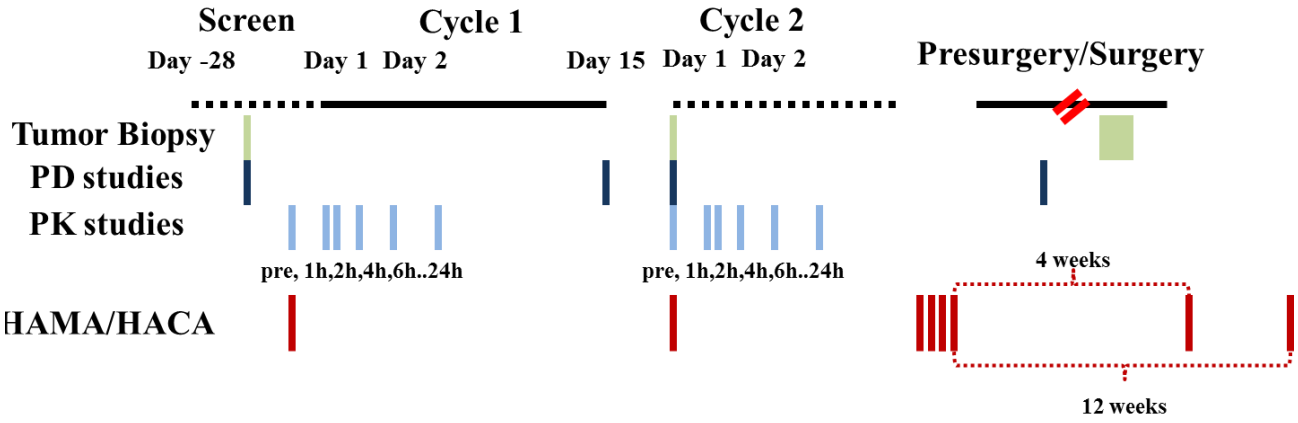
The duration of overall clinical CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

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

7.3 **Pharmacokinetic and Pharmacodynamic Studies and Research Biopsy**

Figure G provides an outline of the planned pharmacokinetic and pharmacodynamics studies and the time or research biopsy.

Figure G



7.3.1 **Pharmacokinetic (PK) and Anti-Product Antibody (APA) studies**

To investigate the pharmacokinetic interaction between TRC105 and everolimus, samples will be collected and concentrations of the agents measured on cycle 1 day 1 and cycle 2 day 1 at the following time points: predose, 1, 2, 4, 6, 24 hours, and cycle 1 day 4 pre-dose. Pharmacokinetic parameters to be estimated include maximum serum concentration (C_{max}), time of maximum serum concentration (T_{max}), area under the serum concentration versus time curve (AUC), terminal half-life ($T_{½}$), and clearance.

Serum for assessment of TRC105 anti-product antibody (APA) formation will be collected before dosing on cycle 1 day 1, every 4 weeks thereafter, 4 and 12 weeks following the last dose of TRC105 and concentrations will be determined by a validated ELISA[53].

7.3.2 **Pharmacodynamic (PD) studies**

Markers of angiogenesis (VEGF-A, soluble VEGFR2, activin A and activin B, angiopoietin-1 and -2, TGF-β, soluble endoglin) will be measured on serum samples collected predose, cycle 1 day 15, cycle 2 day 1, and prior to surgery.

7.3.3 **Research biopsies**

Formalin-fixed paraffin embedded tissue blocks from the patient's primary tumor collected for diagnostic purposes will be requested for all patients enrolled in the study at the time of registration. In addition, a research biopsy will be obtained at baseline, on cycle 2 day 1, and at the time of surgery if residual tumor. The goal is for 2-4 core biopsy specimens obtained using standard institutional guidelines for a diagnostic core biopsy of a breast mass. However, biopsy samples will preferably be obtained using a 14-18 gauge core needle. At least one core will be use to prepare a paraffin block (paraffin-embedded blocks or formalin fixed tissue) and at least one core will be immediately freeze (snap frozen individually for gene expression).

Snap frozen research biopsies will be sent to UAB Tissue Procurement where biopsies will be stored for batching. The batched biopsies will then be forwarded to HudsonAlpha Institute for Biotechnology. All samples will be indelibly labeled with the appropriate study number, and date of

acquisition; these data will be correlated with clinical efficacy including complete response and objective response. All samples sent to HudsonAlpha Institute for Biotechnology for comprehensive genomic analysis will be labeled with the appropriate study number only. Frozen research biopsy will be used for comprehensive genomic analysis such as Next-GEN genomic analysis. "Next-GEN genomic analysis will be done at HudsonAlpha Institute for Biotechnology in Huntsville, Alabama (Shared Facility of the UAB Comprehensive Cancer Center) by Dr. Richard Myers' group. No funding is necessary to conduct the evaluation in the laboratory at this time or macro-dissection; funds are needed only for shipping.

Researchers at HudsonAlpha Institute for Biotechnology led by Dr. Richard M. Myers will obtain tissues from Drs. Erica M. Stringer- Reasor and Christos Vaklavas at UAB from patients enrolled in the pilot Study. HudsonAlpha Institute for Biotechnology will analyze the tissues for several functional genomics features on a genome-wide scale, as well as whole exome DNA sequencing, to identify genetic variants. The functional genomics readout and genetic variation measurements will be assessed to help identify differences associated subtypes and/or responses to drug treatments. The researchers at Hudson will utilize their expertise in applying six different genome-wide functional genomics measurements, as well as whole exome sequencing, whole genome sequencing and specialized array-based genotyping to measure genomic and genetic variants for cancers, brain diseases, autoimmune diseases and drug responses. The functional genomics measurements will include the use of ultrahigh throughput sequencing to measure mRNAs and long non-coding RNAs (RNA-seq), microRNAs (miRNA-seq), DNA methylation (a combination of two approaches, RRBS and Methyl1450 arrays), protein:DNA interations (ChIP-seq), chromatin markers (also ChIP-seq) and chromatin accessibility measurements (DNase-seq).

To measure DNA sequence variation, two types of ultrahigh throughput DNA sequencing will be performed: whole exome sequencing, where all the exons and exon:intron boundaries in the human genome are sequenced deeply; and whole genome sequencing, to be used on a modest number of samples due to expense. A combination of exome and whole genome sequencing with inexpensive genotyping of 1 million or more polymorphic DNA markers around the genome may be used to supplement the DNA sequencing and to improve the ability to identify large DNA sequence variants known as copy number variants.

Fifteen slides will be prepared from the paraffin blocks or formalin fixed tissue by Dr. Grizzle at UAB for analysis by conventional IHC of ER, PR, Her-2, EGFR, Ki67, apoptotic markers, and biomarkers of DNA repair such as H2AX and BRCA localization. All samples will be indelibly labeled with the appropriate study number, and date of acquisition; these data will be correlated with clinical efficacy including complete response and objective response. In addition, immunohistochemistry for pAKT and PTEN (markers of the PI3K/AKT/mTOR pathway), CD105 and CD31/CD34 (pan-endothelial markers), and NG2 and MCAM/CD146 (pericyte markers) will be conducted on the diagnostic research tissue, the research biopsy, and the final surgical specimen.

Any leftover study blood and tissue samples may be stored for future research studies. The subjects will consent to the future use of samples in the consent form for the study. Any samples will only be released for use in future studies after approval by the Principal Investigator and other regulatory bodies, as appropriate.

7.3.4 **Ribosomal profiling on the final surgical specimen**

Fresh frozen, macrodissected surgical specimens collected and stored in RNA*later* with the assistance of Tissue Procurement that meet criteria for expanded ribosomal profiling will undergo next-generation sequencing. We anticipate that small size of the residual tumor that is surgically excised will be the principal limiting factor in the conduction of this correlative study. Based on our previous experience[1,2], we project that in the course of the study approximately 8 surgical specimens will undergo expanded ribosomal profiling. Three samples of previously untreated hormone receptor positive and Her2 negative breast cancer matched for clinical and demographic characteristics with the study samples and 3 samples of normal breast tissue resected for indications other than cancer will undergo expanded ribosomal profiling following the same protocol. Both samples of untreated luminal breast cancer and normal breast tissue will be obtained from patients who will not participate in the study.

8. ASSESSMENT OF SAFETY

8.1 Adverse Events Reporting and Definitions

In the event of an adverse event the first concern will be for the safety of the subject. Toxicities will be graded using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (see http://ctep.cancer.gov/reporting/ctc.html - see Appendix D).Adverse events that begin or worsen after informed consent should be recorded. Conditions that were already present at the time of informed consent should be recorded in the Medical History page of the patient's CRF. Adverse event monitoring should be continued for at least 30 days (or 5 half-lives, whichever is longer) following the last dose of study treatment. Adverse events (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate Adverse Event.

8.1.1 **Adverse Events (AEs)**

Adverse events should be followed to resolution or stabilization, and reported as SAEs if they become serious. This also applies to patients experiencing AEs that cause interruption or discontinuation of investigational product or those experiencing AEs that are present at the end of their participation in the study. Such patients should receive post-treatment follow-up as appropriate as it has been described throughout the protocol. If an ongoing AE changes in its severity or in its perceived relationship to study drug, a new AE should be completed.

The FDA has mandated that Novartis and TRACON be notified, at least one time per year, or at the agreed upon timeframe, of all non-serious adverse events that: (a) are grade 3-4 toxicity and (b) result in the subject's premature discontinuation of the study. In addition, accrual will be halted as a result of "any grade ≥3 hemorrhage, cerebrovascular and vasovagal adverse events; FDA will be contacted to discuss further accrual or potential need for protocol amendment

8.1.2 **Serious Adverse Event (SAEs)**

SAEs require expeditious handling and reporting to UAB to comply with regulatory requirements. Investigators are required to report within 24 hours of investigator's knowledge (MedWatch Form 3500A) to the principal investigators (UAB – Erica M. Striger- Reasor), who will report to the FDA, UAB IRB, Novartis, and TRACON in accordance with regulatory requirements or contractual agreement ANY serious treatment emergent (occurring at any time during treatment, not present at baseline and related to study drug) adverse event (SAE) as soon as possible. SAEs must be reported to local IRB according to their institutional guidelines. All SAEs must be collected and reported until 30 days following patient discontinuation of dosing; if only limited information is initially available, follow-up reports are required. The original SAE form must be kept on file at the study site.

Any SAEs experienced after this 30 days period should only be reported to Novartis or TRACON pharmaceutical if the investigator suspects a causal relationship to the study drug. Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. A SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event. The end date of the first event must be provided.

Follow-up information is sent to the same fax number as the original SAE Report Form was sent, using a new fax cover sheet, stating that this is a follow-up to the previously reported SAE, and giving the date of the original report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not (if applicable), and whether the patient continued or withdrew from study participation.

A SAE is any sign, symptom or medical condition that emerges during treatment (with everolimus and TRC105) or during a post-treatment follow-up period that (1) was not present at the start of treatment and it is not a chronic condition that is part of the patient's medical history, OR (2) was present at the start of everolimus/TRC105 treatment or as part of the patient's medical history but worsened in severity and/or frequency during therapy, AND that meets any of the following regulatory serious criteria:

- Results in death
- Is life-threatening
- Requires or prolongs inpatient hospitalization
- Is disabling
- Is a congenital anomaly/birth defect
- Is medically significant or requires medical or surgical intervention to prevent one of the outcomes listed above.

8.1.3 Reporting of Serious Treatment Emergent Adverse Events

All SAEs should be recorded on a MedWatch 3500A Form (can be accessed at: https://www.accessdata.fda.gov/scripts/MedWatch - see Appendix E) and faxed to:

Pam Dixon Phone: 205-975-5387 Fax: 205-975-9875	and	Erica M. Sringer- Reasor Phone: Fax: 205-995-9996

Once forms are reviewed, UAB will report to:

MedWatch 5600 Fishers Lane Rockville, MD 20852-9787 Fax: 1-800-FDA-0178 (1-800-332-0178)	and	Novartis Drug Safety Fax: 877-778-9739 usdrugsafety.operations@novartis.com

And

UAB IRB

and

TRACON
Drug safety
Fax: (XXX) XXX-XXXX
XXXX@XXXXXX

E-mail:
MedWatch 3500A Reporting Guidelines:
In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description of the MedWatch 3500A form:

- Treatment regimen (dosing frequency, combination therapy)
- Protocol description (and number, if assigned)
- **Description of event, severity, treatment, and outcome, if known**
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-up information:
Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up

- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- **Summarizing new information and faxing it with a cover letter including subject identifiers (i.e. D.O.B., initials, subject number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The subject identifiers are important so that the new information is added to the correct initial report)**

Occasionally the principal investigator and/or UAB may contact the reporter for additional information, clarification, or current status of the subject for whom an adverse event was reported.

Assessing Causality:
Investigators are required to assess whether there is a reasonable possibility that everolimus/TRC105 caused or contributed to an adverse event. The following general guidelines may be used.
Yes: if the temporal relationship of the clinical event to everolimus/TRC105 administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

No: if the temporal relationship of the clinical event to everolimus/TRC105 administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

Attribution of the AE:

- 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.

8.2 Adverse reactions with everolimus

8.2.1 Clinical adverse reactions with everolimus

The most common ADRs (incidence ≥1/10 and suspected to be related to treatment by the investigator) from the pooled safety data were (in decreasing order): stomatitis, rash, fatigue, diarrhea, infections, nausea, decreased appetite, anemia, dysgeusia, pneumonitis, hyperglycemia, weight decreased, pruritus, asthenia peripheral edema, hypercholesterolemia, epistaxis, and headache. The most common grade 3/4 ADRs (incidence ≥1/100 to <1/10 and suspected to be related to treatment by the investigator) were stomatitis, anemia, hyperglycemia, fatigue, infections, pneumonitis, diarrhea, asthenia, thrombocytopenia, neutropenia, dyspnea, lymphopenia, proteinuria, hemorrhage, hypophosphatemia, rash, hypertension, aspartate aminotransferase (AST) increased, alanine aminotransferase (ALT) increased, and pneumonia. **Table 8-1** presents the frequency category of ADRs reported in the pooled safety analysis.

Table 8-1: Adverse Drug Reactions associated with everolimus

Adverse Drug Reactions are listed according to MedDRA system organ class. Within each system organ class, the adverse reactions are ranked by frequency, with the most frequent reactions first. In addition, the corresponding frequency category using the following convention (CIOMS III) is also provided for each adverse reaction: very common (≥1/10); common (≥1/100 to <1/10); uncommon (≥1/1,000 to <1/100); rare (≥1/10,000 to <1/1,000); very rare (<1/10,000)	
Infections and infestations	
Very common	Infections[a]
Blood and lymphatic system disorders	
Very common Common Uncommon Rare	Anemia, Thrombocytopenia, neutropenia, leukopenia, lymphopenia Pancytopenia Pure red cell aplasia
Immune system disorders	
Uncommon	Hypersensitivity
Metabolism and nutrition disorders	
Very common	Decreased appetite, hyperglycemia, hypercholesterolemia
Common	Hypertriglyceridemia, hypophosphatemia, diabetes mellitus, hyperlipidemia, hypokalemia, dehydration
Psychiatric disorders	
Common	Insomnia
Nervous system disorders	
Very common	Dysgeusia, headache
Uncommon	Ageusia
Cardiac disorders	
Uncommon	Congestive cardiac failure
Vascular disorders	
Common	Hemorrhage[b], hypertension,
Uncommon	Deep vein thrombosis
Respiratory, thoracic and mediastinal disorders	
Very common Common Uncommon Rare	Pneumonitis[c], epistaxis, cough, dyspnea Hemoptysis, pulmonary embolism acute respiratory distress syndrome
Gastrointestinal disorders	
Very common Common	Stomatitis[d], diarrhea, nausea Vomiting, dry mouth, abdominal pain, oral pain, dyspepsia, dysphagia
Skin and subcutaneous tissue disorders	
Very common Common Rare	Rash, pruritus Dry skin, nail disorder, acne, erythema, hand-foot syndrome Angioedema
Musculoskeletal and connective tissue disorders	
Common	Arthralgia
Renal and urinary disorders	
Common Uncommon	Proteinuria, Renal failure Increased daytime urination, acute renal failure
Reproductive system and breast disorders	
Common Uncommon	Menstruation irregular [f] Amenorrhea
General disorders and administration site conditions	
Very common Common Uncommon Rare	Fatigue, asthenia, peripheral edema Pyrexia, mucosal inflammation Non-cardiac chest pain impaired wound healing
Investigations	
Very common Common	Weight decreased aspartate aminotransferase increased, Alanine aminotransferase increased, blood creatinine increased
[a] Includes all reactions within the 'infections and infestations' system organ class including common: pneumonia	

and uncommon: herpes zoster, sepsis and isolated cases of opportunistic infections (e.g. aspergillosis, candidiasis and hepatitis B)
[b] Includes different bleeding events not listed individually
[c] Includes common: pneumonitis: interstitial lung disease, lung infiltration, and rare - alveolitis, pulmonary alveolar hemorrhage, and pulmonary toxicity
[d] Includes very common: stomatitis; common: aphthous stomatitis, mouth and tongue ulceration; uncommon: glossitis, glossodynia
[e] reported as palmar-plantar erythrodysaesthesia syndrome
[f] frequency is based upon number of women age 10 to 55 years of age in the safety pool

8.2.2 **Clinically relevant laboratory abnormalities seen with everolimus**

In the pooled double-blind phase III safety database, the following new or worsening clinically relevant laboratory abnormalities were reported with an incidence of ≥1/10 (very common, listed in decreasing frequency).

- Hematology: hemoglobin decreased, lymphocytes decreased, white blood cells decreased, platelets decreased, and neutrophils decreased (or collectively as pancytopenia);

- Clinical chemistry: glucose (fasting) increased, cholesterol increased, triglycerides increased, AST increased, phosphate decreased, ALT increased, creatinine increased, and potassium decreased.

Most of the observed abnormalities (≥ 1/100) were mild (grade 1) or moderate (grade 2). Grade 3/4 hematology and chemistry abnormalities include:

- Hematology: lymphocytes decreased, hemoglobin decreased, (very common); neutrophils decreased, platelet count decreased, white blood cells decreased (all common).

- Clinical chemistry: glucose (fasting) increased, phosphate decreased, potassium decreased, AST increased, ALT increased, creatinine increased cholesterol (total) increased, triglycerides increased (all common).

8.2.3 **Serious adverse events associated with everolimus**

Table 8-2 lists all fatal adverse events reported by investigators across all RAD001 studies as having a suspected causal relationship to everolimus and occurring on or before the cut-off date of 31-Mar-2014. In clinical trials and post-marketing spontaneous reports, everolimus has been associated with serious cases of the following:

- Hepatitis B reactivation, including fatal outcome. Reactivation of infections is an expected event during periods of immunosuppression.
- Renal failure events (including fatal ones) and proteinuria. Monitoring of renal function is recommended.
- Amenorrhea (including secondary amenorrhea).
- Pneumocystis jirovecii pneumonia (PJP) some with a fatal outcome.

- Angioedema has been reported with and without concomitant use of everolimus and ACE inhibitors

Table 8-2

MedDRA system organ class	Event
Blood and lymphatic system disorders	Thrombocytopenia
Cardiac disorders	Acute cardiopulmonary event, cardiogenic shock, cardio-respiratory arrest, myocardial infarction
Gastrointestinal disorders	Diarrhea, duodenal ulcer, GI hemorrhage, intestinal perforation, esophageal perforation, internal hernia
General disorders/administration site conditions	Cold sweat, concomitant disease progression, general physical health deterioration, multi-organ failure, sudden death
Infections and infestations	Candidal sepsis, EBV pneumonia, neutropenic sepsis, pneumocystis jiroveci pneumonia, reactivation of Hepatitis B, septic shock, septicemia with massive Epstein-Barr viremia
Injury, poisoning and procedural complications	Bone fissure
Metabolism and nutrition disorders	Dehydration, ketoacidosis, **hyperglycemia**
Neoplasms benign, malignant and unspecified (including cysts and polyps)	Squamous cell carcinoma of skin
Nervous system disorders	Cerebral hemorrhage, embolic stroke, restlessness
Renal and urinary disorders	Renal tubular necrosis
Respiratory thoracic and mediastinal disorders	Acute cardiopulmonary event, acute respiratory distress syndrome, cardio-respiratory arrest, dyspnea, chronic obstructive pulmonary disease, interstitial pneumonia, pulmonary embolism, pulmonary edema, pneumonitis
Vascular Disorders	Cerebral hemorrhage, disseminated intravascular coagulation, embolic stroke, hemorrhage, hypovolemic shock, myocardial infarction, pulmonary hypertension

Table 8-3 lists all serious, unexpected, life-threatening adverse events considered related to everolimus, and reported on at least one occasion up to the cut-off date of 31-Mar-2014. **It should be stressed that many of these SAE reports have been reported only on a single occasion making an accurate assessment of causality difficult, if not impossible. Due to the imprecision of causality, the causality assessments should not be assumed that all of these events are indeed the result of therapy with everolimus.** Moreover, the assessment of causality is particularly difficult in critically ill patients where confounding factors are present relating mainly to complications of the underlying disease and to the use of prior therapy and/or concomitant medications. In addition to the SAE reports presented below, disease progression has on occasions been reported somewhat paradoxically as an SAE with a suspected causal relationship to everolimus and reported on at least one occasion. Individual events are presented for each MedDRA system organ class.

Table 8-3

MedDRA system organ class	Event
Blood and lymphatic system disorders	Acute leukemia, anemia, bone marrow failure, disseminated intravascular coagulation, febrile neutropenia, hemorrhagic diathesis, leukemoid reaction, leukocytosis, leukopenia, lymphadenopathy, lymphedema, lymphopenia, neutropenia, pancytopenia, platelet disorder, retroperitoneal lymphadenopathy, thrombocytopenia, white blood cell disorder, hemolytic uremic syndrome
Cardiac disorders	atrial fibrillation, atrioventricular block, **cardiorespiratory arrest**, cardiopulmonary arrest, congestive cardiomyopathy, cyanosis, diastolic dysfunction, dilatation left atrial and left ventricular, hypertrophy, left ventricular hypertrophy, **left ventricular dysfunction**, myocardial infarction, myocardial ischemia, hypertensive heart disease, palpitations, pericardial effusion, right ventricular failure, sinus tachycardia, stress cardiopathy, supraventricular tachycardia, tachycardia, ventricular dysfunction Valve incompetence: mitral/pulmonary/tricuspid
Ear and labyrinth disorders	Vertigo, **sudden hearing loss, deafness unilateral**
Endocrine disorders	Hyperthyroidism , hyperglycemic non-ketotic syndrome, hypercalcemia, hypothyroidism
Eye disorders	Conjunctivitis, eyelid edema, scleral discoloration, ocular discomfort, ocular surface disease, phthalmoplegia_cranial nerve paralysis, papilloedema, photophobia, retinal artery thrombosis, retinal detachment, retinal hemorrhage, steroid Induced cataract, **senile cataract, visual acuity reduced**.
Gastrointestinal disorders	Abdominal abscess, abdominal adhesions, abdominal: discomfort/distension/pain/tenderness, anorectal discomfort, anal fissure, anal fistula, anastomotic leak, ascites, colitis, constipation, diarrhea, diverticulum, duodenal ulcer, dyspepsia, dysphagia, esophageal perforation, feces discolored, gastric perforation, gastric ulcer hemorrhage, gastric ulceration, gastrointestinal angiodysplasia, gastrointestinal ischemia, gastrointestinal oedema, **gastroenteritis**, hemorrhoids,hematemesis, hematochezia, hiccups, ileus, intestinal perforation, melena, mesenteric vein thrombosis, mouth ulceration, nausea, pancreatitis, proctalgia, proctitis, recall phenomenon radiation enteritis, rectal discharge/hemorrhage, small intestinal obstruction, stomatitis, swollen tongue, vomiting, oral pain, enterovesical fistula, radiation esophagitis, necrotizing esophagitis, salivary gland calculus, small bowel thickness, thickened bowel Wall Gastritis: erosive/ hemorrhagic Lip: edema/swelling/ulceration Gastrointestinal: disorder/hemorrhage/sounds Mucosal: hemorrhage/inflammation
General disorders and administration site conditions	Application site erythema, asthenia, bloody discharge, calcinosis, chest discomfort/pain, chills, concomitant disease progression, condition aggravated, crepitations, death, discomfort, disease progression, drug ineffective, and drug withdrawal syndrome (asthenia and flushing) edema peripheral, face edema, facial pain, fatigue, feeling of body temperature change, general physical health deterioration, generalized edema, **goiter**, granuloma, hypertrophy, hypothermia, impaired healing, inflammation, influenza like illness, irritability, local swelling, malaise, mass, multi-organ failure, necrosis, non-cardiac chest pain, pain, performance status decreased, pitting edema, pyrexia, sudden death, swelling, tenderness, thirst
Hepatobiliary disorders	Cholecystitis, cholelithiasis, cholestasis, cytolytic hepatitis, hepatic cirrhosis**, hepatic failure**, hepatic function abnormal, hepatic necrosis, **hepatorenal failure**, hepatitis B and C reactivation, hepatomegaly, hyperbilirubinemia, jaundice, portal vein thrombosis
Immune system disorders	Acute disseminated encephalomyelitis, autoimmune disorder, cytokine release syndrome, Guillian Barre syndrome, hypersensitivity, immunosuppression, Stevens- Johnson syndrome
Infections and infestations	Abdominal abscess, aspergillosis, bacteremia, bronchitis, bronchitis viral, bronchopneumonia, bronchopulmonary aspergillosis, candida sepsis, candidiasis, cellulitis, citrobacter sepsis, clostridium bacteremia, endocarditis, escherichia bacteremia, folliculitis, gastroenteritis, gastroenteritis norovirus, hepatitis fulminant, herpes zoster, infected lymphocele, klebsiella sepsis, liver abscess, meningitis, herpes, nasopharyngitis, neutropenic sepsis, oral candidiasis, oral fungal infection, perirectal/anal abscess, pharyngitis, pneumococcal sepsis, pseudomonal bacteremia, purulent discharge, pyelonephritis, rectal abscess, respiratory moniliasis, sepsis, sepsis syndrome, septic shock, sinusitis, staphylococcal

	bacteremia, staphylococcal sepsis, streptococcal bacteremia, tonsillitis, tuberculosis, urosepsis Infection(s): alpha hemolytic streptococcal/atypical mycobacterial/bacterial/clostridial/enterobacter/enterococcal/epstein-barr virus/ escherichia/escherichia urinary tract/gastrointestinal/haemophilus/herpesvirus/fungal/general/klebsiella/localized/low er respiratory tract lung/ morganella/pseudomonas/proteus/respiratory tract/skin/streptococcal/staphylococcal/urinary tract/wound/ wound staphylococcal Pneumonia: Pneumocystis jirovecii/general/bacterial/ streptococcal
Injury, poisoning and Procedural complications	Abdominal adhesion, anastomotic leak, bone fissure, collapse of lung, contusion, eschar, fall, head injury, lung injury, medical device complication, medication error, post procedural swelling, recall phenomenon radiation enteritis, surgical procedure repeated, vena cava injury, wound dehiscence/secretion
Investigations	Aspiration pleural cavity, antinuclear antibody positive, bleeding time prolonged, breath sounds abnormal, cardiac function test abnormal, chest X-ray abnormal, clostridium difficile toxin test positive, computerised tomogram abnormal, ejection fraction decreased, endoscopy upper gastrointestinal tract, general physical condition abnormal, granulocyte count decreased, hematocrit/hemoglobin decreased, hypophosphatemia, liver function test abnormal, neutrophil count abnormal/decreased, occult blood positive, oxygen saturation abnormal/decreased, peak expiratory flow rate decreased, protein urine present, QRS axis abnormal, urine output decreased, X-ray abnormal; Increased: alanine aminotransferase, ammonia, aspartate aminotransferase, aspartate aminotransferase, body temperature, C-reactive protein, eosinophil count, gamma-glutamyltransferase, international normalised ratio, lipase, red blood cell count, sedimentation rate, transaminases, troponin T, troponin Platelet count: abnormal/decreased/increased White blood cell(s): count abnormal/decreased/in urine Lymphocyte count: decreased/increased Blood: amylase increased/albumin decreased/alkaline phosphatase increased/bilirubin increased/creatine phosphokinase increased/creatinine increased/magnesium decreased/glucose increased/lactate dehydrogenase increased/potassium decreased/pressure diastolic decreased/pressure orthostatic abnormal/pressure systolic decreased/triglycerides increased/urea increased/urine present Electrocardiogram: T wave abnormal/T wave amplitude decreased/poor R-wave progression Weight: decreased/ increased
Metabolism and nutrition disorders	Acidosis, anorexia, appetite disorder, cachexia**, cushing's syndrome**, decreased appetite, dehydration, diabetes mellitus, diabetic ketoacidosis, electrolyte imbalance, failure to thrive, fluid overload/retention, food intolerance, glucose tolerance impaired, gout, hypothyroidism, malnutrition, metabolic acidosis, oral intake reduced, polydipsia, type 2 diabetes mellitus Hyper: cholesterolemia/glycemia/kalemia/lipidemia/calcaemia uricemia/ triglyceridemia, glycemic non-ketotic syndrome Hypo: calcemia/glycemia/kalemia/magnesemia/natremia/ proteinemia/ Hypophosphatemia
Musculoskeletal and connective tissue disorders	Arthritis, bone fissure, fistula, gout mobility decreased, joint effusion, rhabdomyolysis Pain: back/flank/musculoskeletal/musculoskeletal chest/extremity/neck Muscle: spasms/weakness; myalgia, arthralgia Osteoarthritis; Osteonecrosis, Osteoreadionecrosis
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	Acute leukaemia, adenocarcinoma of pancreas, Burkitt's lymphoma, carcinoid syndrome, colon neoplasm, lung neoplasm, lymphoproliferative disorder, malignant neoplasm progression, **malignant melanoma**, malignant pleural effusion, **pancreatic carcinoma**, squamous cell carcinoma of skin, uterine leiomyoma Metastases: central nervous system/lung/lymph nodes/neoplasm; Tumor: hemorrhage/necrosis
Nervous system disorders	Acute disseminated encephalomyelitis, asterixis, ataxia, cerebrovascular accident, cognitive disorder, complex partial seizures, complex regional pain syndrome, convulsion, depressed level of consciousness, dizziness, dyslalia, encephalitis, encephalopathy, facial palsy, headache, hemiparesis, hemiplegia, hyperglycemic non-ketotic syndrome, hypersomnia, hypoglycemic coma, lethargy, loss of consciousness, ophthalmoplegia_cranial nerve paralysis, neuralgia, neuropathy peripheral, presyncope, sinus headache, somnolence, speech disorder, tremor, vertigo Cerebral: hemorrhage/ infarction/ischemia; transient ischemic attack
Psychiatric disorders	Agitated depression, anxiety, confusional state, delirium, delusional disorder,

	disorientation, drug withdrawal syndrome, libido decreased insomnia, mental disorder/status changes, mood altered, persecutory type, **personality disorder**, neurosis, staring
Renal and urinary disorders	Acute renal failure, anuria, bladder tamponade, dysuria, hematuria, hemolytic uremic syndrome, hydronephrosis, leukocyturia, nephrolithiasis, oliguria, pollakiuria, polyuria, postrenal failure, renal failure/acute/impairment, urinary bladder hemorrhage, urinary incontinence, renal atrophy, renal colic, renal tubular necrosis
Reproductive/breast disorders	Azospermia, **endometrial hyperplasia**, libido decreased, **menorrhagia**, pelvic pain, ovarian cyst, scrotal edema
Respiratory, thoracic and mediastinal disorders	Alveolitis, alveolitis allergic, asthma, atelectasis, bronchomalacia, bronchospasm, cough, cryptogenic organizing pneumonia, diffuse alveolar damage, dysphonia, dyspnea/dyspnea exertional, emphysema, epistaxis, hemoptysis, hydrothorax, hypercapnia, hypoxia, laryngeal edema, interstitial lung disease, laryngeal inflammation, pneumothorax, rales, sinus congestion, stridor, tachypnea, wheezing Acute: respiratory distress syndrome/respiratory failure Lung: consolidation/disorder/infiltration, obstructive airways disorder, orthopnoea, pharyngolaryngeal pain, pleural disorder/effusion/pleurisy/pain; pneumonia aspiration, pneumonitis, pneumothorax, productive cough, rhinorrhoea Pulmonary: congestion/alveolar/hemorrhage/artery dilatation/cavitation/embolism/fibrosis/haemorrhage/hypertension/edema/toxicity Respiratory: arrest/disorder/distress/failure/gas exchange disorder
Skin and subcutaneous tissue disorders	Acute febrile neutrophilic dermatosis, Angioedema, blister, dermatitis, dermatitis acneiform, erythema, hyperhidrosis, leukocytoclastic vasculitis, night sweats, petechiae, photosensitivity reaction, pruritus, Stevens-Johnson syndrome Rash: erythematous/generalized/ maculo-papular/pruritic/skin disorder/exfoliation/lesion/reaction/ulcer/palmar plantar erythrodysesthesia/hand foot syndrome
Surgical and medical procedures	Abscess drainage, biliary drainage, bladder irrigation, catheter removal, chest tube insertion, cholecystectomy, debridement, endotracheal intubation, fistula repair, gastric ulcer surgery, gastrointestinal tube insertion, ileojejunal bypass, incisional drainage, laparotomy, mechanical ventilation, pleurodesis, sinus operation, surgery, thoracic cavity drainage, tracheostomy Pericardial: drainage/excision/operation Wound: closure/drainage/treatment
Vascular disorders	Capillary leak syndrome, circulatory collapse, embolism, flushing, gastrointestinal angiodysplasia, gastrointestinal ischaemia, haemorrhoids, hematoma, hemodynamic instability, hemorrhage, hypertension, hypertensive angiopathy, hypertensive crisis, hypotension, lymphangiopathy, lymphedema, pallor, shock; transient ischemic attack, retinal haemorrhage, Thrombosis: deep vein/jugular vein

*New events added to IB Editions 12 and 13 are noted in bold

8.3 Adverse reactions with TRC105

The most common adverse events associated with TRC105 are infusion reactions that are successfully mitigated with premedications and prolongation of the infusion time, hemorrhages (mostly grade 1 and 2), headaches, and hypoproliferative anemia.

8.3.1 Hypoproliferative Anemia

TRC105 was administered to 50 patients as part of a phase 1 first-in-human study of solid tumors. Dose escalation proceeded stepwise, and the majority of adverse events were grade 1 or 2. The MTD was 10 mg/kg weekly, and 15 mg/kg every two weeks was also well tolerated. At 15 mg/kg weekly, all 3 patients that remained on study through cycle 2 developed dose-limiting grade 3 anemia, and one of the three progressed to grade 4 anemia by cycle 3. In all three patients, the anemia resolved following discontinuation of treatment. Anemia has been observed in other trials of TRC105.

The dose-limiting anemia at 15 mg/kg weekly 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. Bone marrow aspiration attempts in the patient with grade 4 anemia yielded insufficient material to permit evaluation of the cause of anemia.

The hypoproliferative anemia was believed to result from TRC105-mediated suppression of proerythroblasts, the only cells in the marrow known to express substantial levels of CD105. Anemia was manageable with standard supportive measures including erythropoietin and packed red blood cell transfusion. One patient dosed in study 105ST101 required dose reduction from 10 mg/kg to 7.5 mg/kg weekly for grade 3 anemia at month 12 and further dose reduced to 6 mg/kg and continued treatment with a minor radiographic response for 20 months. All patients receiving TRC105 should be monitored for anemia and treated appropriately (including possible TRC105 dose reduction).

8.3.2. **Infusion Reactions**

Infusion reactions were observed following TRC105 administration, usually with the initial dose, and included one or more of the following signs or symptoms: rigors, bronchospasm, urticaria, change in blood pressure, and change in heart rate. Grade 2 infusion reactions (requiring temporary interruption of the infusion) and grade 3 infusion reactions (requiring discontinuation of the infusion) were initially reported at the 0.3 and 1 mg/kg every 2 weeks in the absence of premedication. By extending the duration of the initial infusion from one to four hours and adding a dexamethasone-based premedication regimen prior to each dose, the frequency and severity of infusion reactions were reduced and dose escalation continued to 15 mg/kg weekly.

Infusion reactions were rare in patients re-dosed at the recommended phase 2 dose of 10 mg/kg weekly or 15 mg/kg every two weeks, where TRC105 serum levels known to saturate CD105 binding sites were present at the time of re-dosing. At dose levels where continuous TRC105 serum levels were achieved, dexamethasone was safely discontinued and the infusion duration reduced to one hour. All patients treated with TRC105 should receive appropriate premedication, be monitored during the TRC105 infusion, and be treated appropriately for infusion reactions (including possible TRC105 dose interruption).

In a phase 2 study of TRC105 monotherapy in patients with hepatocellular carcinoma who progressed on sorafenib, one patient with coronary artery disease and prior bypass surgery dosed at 15 mg/kg TRC105 who received the required pre-medications, experienced an infusion reaction (Grade 3) with elevated heart rate, elevated blood pressure, shortness of breath and chest pain, and developed a non-Q wave myocardial infarction, from which he recovered without sequellae.

One patient participating in a phase 1/2 study of TRC105 for castrate-resistant prostate cancer in a NCI sponsored trial developed a serious adverse event of grade 4 vasovagal reaction. The patient was being treated with TRC105 at 15 mg/kg every 2 weeks and, after the appropriate premedication regimen, the patient fainted 17 mL into the initial infusion. He was treated with oxygen and i.v. fluids and completely recovered within 5 minutes. Vasovagal reaction, generally associated with volume

depletion, is a known adverse event that occurs in <1% of patients receiving therapeutic proteins like monoclonal antibodies. The risk of syncope can be minimized by encouraging patient hydration prior to the initial treatment with TRC105 and avoiding the treatment of patients who are volume depleted.

Hypersensitivity reactions to therapeutic protein infusions are a potential risk for sensitized patients, and TRC105 should be avoided or used with caution in patients with known hypersensitivity to any component of the drug product.

8.3.3. **Hemorrhage**

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 in the phase 1 monotherapy study 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 blood loss was not observed following protocol amendment to exclude patients with a history of peptic ulcer disease (unless healing was documented). Subsequent bleeding associated with TRC105 has been limited to low grades including superficial gingival bleeding, epistaxis, and intermittent post-coital vaginal bleeding in a patient with uterine cancer recurrent at the vaginal cuff. Gingival bleeding and epistaxis are related to the development of mucocutaneous telangiectasia that occur on TRC105 therapy. Patients with epistaxis may benefit from saline nasal gel. 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.

Cerebrovascular hemorrhage (grade 3) resulting in hemiparesis occurred in one patient with hepatocellular cancer who was thrombocytopenic (platelet count of 60,000/uL) in a study of TRC105 with sorafenib. Patients in TRC105 protocols are required to have platelet counts of 100,000/uL, with the exception of the hepatocellular cancer protocol, where patients with platelet counts as low as 60,000/uL (without transfusion support in the past 30 days) are permitted due to the underlying cirrhotic liver disease that results in secondary thrombocytopenia.

Mucocutaneous telangiectasia is observed routinely in patients treated with TRC105 as early as 2 weeks after starting treatment. Telangiectasia is universally 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 that could be exacerbated by treatment with TRC105, and are excluded from TRC105 trials.

Additionally, Grade 5 intracranial hemorrhage occurred in one glioblastoma patient with markedly abnormal blood clotting parameters in a study of TRC105 with bevacizumab.

8.3.4. **Other Potential Risks**

Low grade fatigue is a commonly reported adverse event attributable to TRC105 although its cause is unclear and appears not to be caused by thyroid dysfunction. Headaches (grade 1 to 3) have been observed following TRC105 treatment, generally within hours following completion of the initial infusion. Headaches are throbbing in nature, have not been associated with radiographic abnormalities, and have responded to treatment with non-steroidal anti-inflammatory agents and to

triptans. Headaches were particularly prominent in patients treated with TRC105 in combination with bevacizumab. Headaches were more common and most severe when TRC105 and bevacizumab were initially dosed on the same day and were ameliorated when the first dose of TRC105 was dosed one week following bevacizumab and was given over two days. 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. Antihistamines are useful for nasal congestion.

Flushing and rash (typically maculo-papular and erythematous) occasionally develop following infusion of TRC105. Low grade anorexia, nausea, and vomiting are seen infrequently. Isolated serious adverse reactions include grade 2 pancreatitis in a hepatocellular carcinoma patient in treated with TRC105 in combination with sorafenib and grade 3 infected skin lipoma/cyst observed in a phase 2 study of TRC105 as a single agent in patients with metastatic bladder cancer. Both resolved without sequellae.

While thrombosis has not been frequent on TRC105 treatment, patients who develop arterial thrombosis or grade 3 or 4 venous thrombosis should discontinue TRC105 therapy. Those with uncomplicated grade 1 or 2 venous thrombosis and with normal platelet count and platelet function, who appear to be benefiting from TRC105 therapy, may be anticoagulated and continue on TRC105 therapy at the discretion of the investigator. Patients with coagulation parameters above standard therapeutic levels (e.g., INR > 3) are at increased risk of hemorrhage and should therefore not receive TRC105.

Transient Grade 3 hepatic encephalopathy occurred in one patient with cirrhosis and hepatocellular carcinoma who received TRC105 in combination with sorafenib.

A patient with glioblastoma developed temporary confusion and slurred speech following treatment with TRC105 and bevacizumab that required hospitalization for observation. Another patient with glioblastoma, who underwent resection and had a history of an abnormal collection of cerebral spinal fluid, developed a grade 2 cerebral spinal fluid leak.

Grade 3 myocardial infarction (non-Q wave infarct associated with hypertension following an infusion reaction) was observed in a patient with hepatocellular cancer following treatment with TRC105 that resolved without sequelae. In addition, a Grade 5 myocardial infarction occurred in a patient with coronary artery disease who received TRC105 in combination with sorafenib. Patients with evidence of active coronary artery disease are excluded from participation in this trial (see exclusion criteria).

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

Infections have been observed rarely. Grade 3 infected lipoma/cyst was observed in a Phase 2 study of TRC105 as a single agent in patients with metastatic bladder cancer. Grade 3 orbital cellulitis and grade 3 brain abscess were observed in patients treated with TRC105 and bevacizumab and

considered possibly related to TRC105. Grade 1 and 2 gingivitis including infection and ulceration has also been observed. Overall, infections have been observed in fewer than 5% of patients and have largely been considered unrelated to treatment with TRC105.

9. DATA COLLECTION AND STUDY MANAGEMENT

The study must be conducted as described in this approved protocol. Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator at UAB. The written amendment will be sent to the IRB at the investigator's site for approval. All revisions to the protocol will be provided to Novartis and TRACON by UAB. The Investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB of an Amendment, except where necessary to eliminate an immediate hazard(s) to study patients. Documentation of approval signed by the chairperson or designee of the IRB(s) must be sent to the UAB Clinical Protocols and Data manager Shared Facility Regulatory Office (Alfreda Lewis fax 205-). If the revision is an Administrative Letter, Investigator must inform their IRB(s)/IEC(s).

Principal investigator will hold the IND for the trial. All patients must be registered with the Clinical Trials Network and Monitoring Office of the UAB Comprehensive Cancer Center (Pam Dixon, O.C.N.) before enrollment in study. Prior to initiation of therapy eligibility criteria must be confirmed. At the time of registration, a study identification number will be generated. All subsequent case report forms will use this study identification number.

9.1 **Data Collection**

The study will have electronic CRF in OnCore for each subject (UAB CCC). Entries made in the CRF must be verifiable against source documents; any discrepancies should be explained and documented. The investigator will be responsible for reviewing all data and CRF entries and will sign and date the designated pages in each subject's CRF, verifying that the information is true and correct. The investigator is responsible for the review and approval of all responses. Patients will be registered in the Clinical trial Network and Monitoring office of the UAB Comprehensive Cancer Center. 9.2 **Data Management**

Data management will be performed from CRFs. All CRF data will be entered into a validated database. All data entry, verification, and validation will be performed in accordance with the current standard operating procedures of the Clinical Trials and Data Management Shared Facility of the UAB CCC. The database will be authorized for lock once all defined procedures are completed.

9.3 **Study Management**

9.3.1 **Institutional Review Board Approval and Informed Consent**

Before study initiation, the Investigator must have written and dated approval/favorable opinion from the IRB for the protocol, consent form, patient recruitment materials/process (e.g., advertisements), and any other written information to be provided to patients. The Investigator should provide the IRB with reports, updates, and other information (e.g., Safety Updates, Amendments, Administrative

Letters) according to regulatory requirements or Institution procedures. Copies of the initial IRB approval as well as annual re-approvals must be submitted to UAB. UAB will provide copies of IRB approval to Novartis and TRACON.

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. 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 or the patient's legally acceptable representative, and by the person who conducted the informed consent discussion. Documentation of IRB approval must be on file before any patient can be registered. Registration begins with signed informed consent.

9.3.2 **Data confidentiality**

Information about study subjects will be kept confidential and managed under applicable laws and regulations. Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect follow-up safety information (e.g. has the subject experienced any new or worsened AEs) at the end of their scheduled study period. The data collection system for this study uses built-in security features to encrypt all data for transmission in both directions, preventing unauthorized access to confidential participant information. Access to the system will be controlled by a sequence of individually assigned user identification codes and passwords, made available only to authorized personnel who have completed prerequisite training.

9.3.3 **Study Monitoring**

Data will be captured on electronic CRFs, and supporting documents will be faxed to the UAB Clinical Trials Network and Monitoring Office. Personnel from the Clinical Trials Network and Monitoring office of the University of Alabama at Birmingham will monitor the trial in real time and will periodically visit the investigative site to ensure proper conduct of the trial and proper collection

of the data.

The principal investigator will check the completeness of patient records, the accuracy of entries on the CRFs, the adherence to the protocol to Good Clinical Practice, the progress of enrollment, and to ensure that study treatment is being stored, dispensed, and accounted for according to specifications. Key study personnel must be available to assist the principal investigator in these periodic checks.

The investigator must maintain source documents for each patient in the study, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data, and the results of any other tests or assessments. All information recorded on CRFs must be traceable to source documents in the patient's file. The investigator must also keep the original signed informed consent form (a signed copy is given to the patient).
Trial will be followed by the Clinical Trials Monitoring Committee and audited by the UAB CCC according to the institutional guidelines.

9.3.4 **Record Retention**

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approved 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 conduction of the clinical research study. Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug pursuing regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.
If the Investigator withdraws from the study (e.g., relocation, retirement), the records shall be transferred to a mutually agreed upon designee (e.g., another Investigator, IRB). Notice of such transfer will be given in writing to NOVARTIS and TRACON Pharmaceuticals. Records of the patient's participation in this study will be kept confidential so far as permitted by law. However, the patient's doctor and his/her staff, representatives of NOVARTIS and TRACON Pharmaceuticals, representatives of the U.S. Food and Drug Administration, and the IRB will be able to inspect patient records and have access to confidential information which identifies the patient by name. Any publication of data will not identify the patient by name. Should the patient's medical record need to be reviewed by a foreign regulatory agency, a member of the IRB staff will observe the review so that the record is not removed, copied, or identifiable information recorded in any manner.

9.3.5 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 ensure 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 ensuring 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 data entry. 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.

10. STATISTICAL CONSIDERATIONS

This is a phase I/II study with the primary objective to characterize the tolerability and feasibility of a novel combination of agents. The Primary Endpoint is defined as the determination of MTD and RP2D and rates of treatment-related toxicities. The secondary efficacy endpoints are rates of pCR or down-staging. The principal investigator will hold the IND for the trial (Appendix F).

10.1 Sample Size and Power Justification

A. The dose escalation part of the study will follow a conventional 3+3 dose escalation design according to the schema above. We anticipate that up to 18 patients will be enrolled in this part anticipating that dose level 1 or 2 will be the RP2D. The number of patients to be enrolled ranges from 3 (if ≥2/3 DLTs in cohort 1) to 18 (if enrollment of 6 patients in all cohorts).
B. The sample size of 20 in Phase II part study is NOT determined by the statistical power but the feasibility and relative precision of estimate, e.g. standards error of the estimation is ≤10%. We will estimate the pCR of the combination to generate hypotheses for further clinical investigations. In order to minimize exposure of patients to ineffective therapy, Gehan's two-stage design will be used[51] which allows for the rapid rejection of an ineffective treatment at the end of the first stage, and provides an estimation of the success rate with a given precision, at the end of the second stage. The study will enroll 10 patients first, and if no patient has pCR or downstaging, it is unlikely (p=0.1074) that 0/10 responses would occur if the true response rate were >20%. If responses were 0/10, the phase II portion will be closed. If at least one patient has pCR or downstaging, an additional 10 patients will be treated (total of 20). With an accrual of 20 patients to phase II portion of the study, estimated two-sided 95% confidence intervals (CI) would be 1.2%-31.7%, 5.7% -43.6% and 11.8%-54.3% if pCR is 10%, 20% or 30% respectively. The confidence intervals are calculated using the exact method Clopper-Pearson intervals[54].

Table 10-1 shows the probability of early termination and observing one response or at least one response at the first stage (N_1=10).

Table 10.1: Probability of early termination and observing one response or at least one response at the first stage (N_1=10)

True ORR	Probability of having zero response (x=0)	Probability of having one response (x=1)	Probability of having at least one response (x≥1)

0.1	0.3486	0.3874	0.6513
0.2	0.1074	0.2684	0.8926
0.30	0.0282	0.1211	0.9718

10.2 **Statistical Analysis**

The primary analysis is of safety and will include any patients who receive at least one dose of the investigational regimen. The rate of adverse events will be estimated at the end of the study along with two-sided 95% exact CIs (Clopper-Pearson intervals). At the completion of the study, the secondary endpoint of pCR or downstaging rate will be estimated along with two-sided 95% CIs with the exact method of Clopper-Pearson intervals. Secondary endpoint analysis will be descriptive. Mean, median, geometric mean, range and coefficient of variation will be used to describe continuous demographic variables. Frequency and proportion will be used to describe categorical variables.

We will use descriptive statistics (mean, median, geometric mean, coefficient of variation, and 95% confidence intervals) and graphical displays (mean concentration-time graphs, scatter plots, box plots or spaghetti plots) to evaluate the pharmacokinetic parameters of the TRC105 and everolimus. We will use descriptive statistics (mean, median, geometric mean, coefficient of variation, and 95% confidence intervals) and graphical displays (scatter plots, box plots, plots of PD parameters against PK data) to analyze the changes in markers of proliferation and angiogenesis with the investigational treatment.

Exploratory analysis will be conducted on the correlations between clinical outcomes and pharmacodynamic biomarkers or translation profiles. Translational profiles will be analyzed between responders and non-responders and the differences between responders and non-responders will be presented in scatter plots along with corresponding 95% CI. Quartile normalization and logarithmic transformation will be conducted before analysis. Logistic regression analysis will be used to predict response with translational profiles or biomarkers with or without adjustment for covariates, e.g. age. Multivariate Cox proportional hazards model will be used to identify a set of biomarkers for prediction of response. Odds ratios with 95% CIs will be calculated for each significant biomarker in terms of predicting response. However, it is recognized that sample size may be too small for such analyses and these analyses are conducted only to generate hypotheses that will require further validation.

10.3 **Stratification Factors**

No *a priori* stratification of patients will be undertaken in this study. Subgroup analyses will be conducted *a posteriori* according to grade (grade II vs. III), stage (stage IIA and IIB vs. IIIA and IIIB) lymph node involvement (N0 vs. N1-2), age (as a continuous variable). It is recognized that sample size may be too small for such analyses and these analyses are exploratory to generate hypotheses and will require further validation.

11. PROTOCOL ADHERENCE

Investigators ascertain they will apply due diligence to avoid protocol deviations. No authorized deviations are permitted. If an investigator feels a protocol deviation would improve the conduct of the study this must be considered a protocol amendment, and unless such an amendment is agreed upon by the principal investigator and approved by the IRB it cannot be implemented. All significant protocol deviations will be recorded and reported in the CSR.

11.1 Amendments to the Protocol

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved by the IRB. Only amendments that are required for patient safety may be implemented prior to IRB approval. Notwithstanding the need for approval of formal protocol amendments, the investigator is expected to take any immediate action required for the safety of any patient included in this study, even if this action represents a deviation from the protocol. In such cases, the principal investigator should be notified of this action and the IRB should be informed no later than 10 working days. Novartis requests to review any significant changes, excluding administrative protocol amendments

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APPENDIX A
Performance Status Criteria

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

APPENDIX B
Drugs with potential interactions with everolimus

CYP3A4 inhibitors	CYP3A4 inducers
Alosetron Aprepitant Atorvastatin boceprevir Bosentan buprenorphine Buspirone carbamazepine clarithromycin conivaptan Coumadin cyclosporine Diazepam Valdecoxib dihydroergotamine mesylate diltiazem docetaxel dofetilide efavirenz eplerenone estazolam fentanyl fosamprenavir galantamine gefitinib grapefruit juice imatinib indinavir itraconazole ketoconazole lopinavir/ritonavir lovastatin methadone mibefradil, mifepristone nefazodone, nelfinavir paclitaxel pimozide propafenone posaconazole quinidine ranolazine rifampin rifabutin ritonavir saquinavir simvastatin sirolimus solifenacin succinate tacrolimus telaprevir, telithromycin tinidazole valdecoxib valdenafil vinorelbine voriconazole zonisamide	Alosetron Aprepitant Bexarotene uprenorphine Buspirone cyclosporine diltiazel docetaxel efavirenze erlotinib estazolam exemestane fosamprenavir galantamine gefitinib imatinib irinotecan itraconazole lopinavir/ritonavir methadone mifepristone modafinil nisoldipine paclitaxel pimozide quinidine sirolimus solifenacin succinate sunitinib tacrolimus tramadol tinidazole valdecoxib voriconazole zonisamide

APPENDIX C

American College of Radiology (ACR) Practice Guidelines for Imaging Studies of the Breast

The ACR Practice Guideline for the Performance of Screening and Diagnostic Mammography, http://www.acr.org/~/media/3484ca30845348359bad4684779d492d.pdf

The ACR Practice Guideline for the Performance of a Breast Ultrasound Examination, http://www.acr.org/~/media/52D58307E93E45898B09D4C4D407DD76.pdf

The ACR Practice Guideline for the Performance of Contrast-Enhanced MRI of the Breast, http://www.acr.org/~/media/2A0EB28EB59041E2825179AFB72EF624.pdf

APPENDIX D

NCI Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.03
http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

APPENDIX E

FDA MedWatch 3500A Form

http://www.accesdata.fda.gov/scripts/MedWatch

APPENDIX F

SOP for Investigator INDs Held by Physician Members of the UAB CCC (Available upon request; Pam Dixon 205- 975-5387)

APPENDIX G

Blood and Biopsy Procedures

UAB 1514 - Blood Draw and Biopsy Schedule

Paraffin blocks to UAB
Frozen biopsies to UAB
Blood for Serum Collection (PK, PD, APA)

A. UAB - Paraffin blocks (all patients)

Pre-therapy paraffin blocks and core research biopsies (all patients) will be obtained and prepared. Time points include baseline, cycle 2 day 1 and at the time of surgery. Formalin-fixed paraffin embedded tissue blocks from the patient's primary tumor collected for diagnostic purposes will be requested for all patients enrolled in the study at the time of registration. For the research cores, the goal is for 2-4 core biopsy specimens obtained using standard institutional guidelines for a diagnostic core biopsy of a breast mass. However, biopsy samples will preferably be obtained using a 14-18 gauge core needle. At least one core will be used to prepare a paraffin block (paraffin-embedded blocks or formalin fixed tissue) and at least one core will be immediately frozen (snap frozen individually for gene expression).

Snap frozen research biopsies will be sent to UAB Tissue Procurement where biopsies will be stored for batching. The batched biopsies will then be forwarded to HudsonAlpha Institute for Biotechnology. These biopsies should be labeled with an indelible marker to include:

1. The clinical trial identification number (UAB 1514)
2. The subject's study number
3. The date of acquisition

Fifteen slides will be prepared from the paraffin blocks or formalin fixed tissue by Dr. Grizzle at UAB for analysis by conventional IHC of ER, PR, Her-2, EGFR, Ki67, apoptotic markers, and biomarkers of DNA repair such as H2AX and BRCA localization.

B. UAB – Snap-Frozen research biopsies (all patients)

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All cryovials (NALGENE® Cryoware™ 5000-0000) will be labeled with an indelible marker to include:

1. The study identification number (UAB 1514);
2. The subject's study number;
3. The date of acquisition

Ed Acosta's Lab
Lab Contact: Kevin J. Ryan
University of Alabama at Birmingham
CCC PK/PD Core Laboratory
1670 University Blvd, Volker Hall, Lab 175

Preparing Tissue in Frozen Cryovials

Snap Freezing: Do not Cut the tissue and simply ship as is. Place each core of tissue into a labeled individual 2.0 ml NALGENE® Cryoware™ cryovials. The label should contain the UAB clinical trial number (UAB 1514) followed by the subject's study number, as well as the date the specimen was collected. Do not place the patient's name or medical record number on the label. The container will become very cold. Do not handle with unprotected hands. Safety goggles should be use throughout the procedure. Snap freeze samples in liquid nitrogen. If not available, you may use an alcohol/dry ice bath. Fill pan about 1-2 inches deep with methanol. The depth should be enough to cover the height of the vial. Slowly add crushed dry ice until the boiling stops. The bath is now ready for use. Drop the sealed vials directly into the liquid nitrogen (LN_2) carefully. Leave the vials in the liquid nitrogen for at least one minute. Once frozen, transfer the samples using tongs or a large spoon to remove the vials from the liquid nitrogen and transfer to a –80 °C freezer until shipped.

C. Serum (PK, PD, APA)

1. Blood samples for the assays will be taken at the times described in the protocol. Serum will be obtained in red top serum separator tubes and will be sent to Ed Acosta's Lab. **Dr. Acosta's lab should be notified of these samples. The laboratory address is:**

   Ed Acosta's Lab
   Lab Contact: Kevin J. Ryan
   University of Alabama at Birmingham
   CCC PK/PD Core Laboratory
   1670 University Blvd, Volker Hall, Lab 175

2. **PK: A 5 mL blood sample will be collected pre-dose and 1, 2, 4, 6 and 24 hours post-dose on the days indicated within Table 7-1: Study Calendar. Samples will be separated and stored at approximately -70 °C.**

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   APA: Anti-Product Antibody (APA) will be collected will be collected before dosing on cycle 1 day 1, every 4 weeks thereafter, 4 and 12 weeks following the last dose of TRC105

(Table 7-1: Study Calendar). Concentrations will be measured using validated ELISA methods. APA concentrations will be evaluated in the context of pharmacokinetic parameters and AE profiles. Samples will be separated and stored at approximately -70 °C.

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