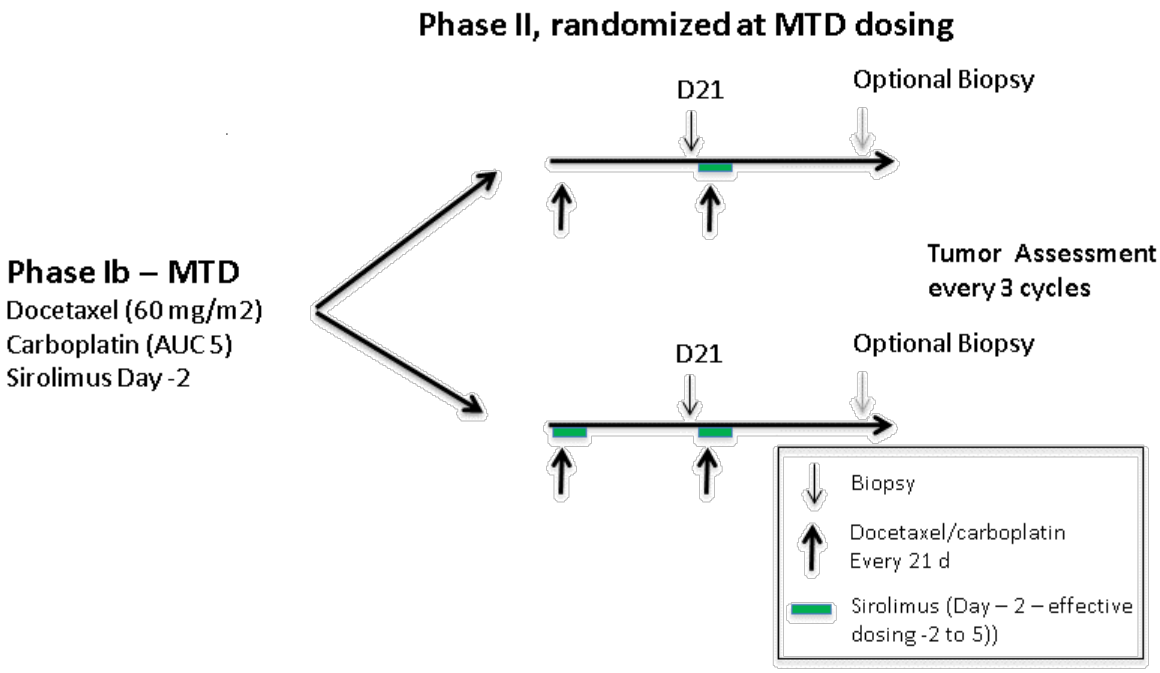
Clinical Study Protocol

Study Title:	A Phase 1-2 Study of Sirolimus, Docetaxel and Carboplatin for Treatment of Patients with Metastatic, Castration Resistant Prostate Cancer: (Rapamycin inhibition of DDSP [RID])
Study Number:	9388
Study Phase:	1-2
Product Name:	Sirolimus administered with Docetaxel and Carboplatin
IND Number:	IND Exempt
Indication:	Treatment of metastatic, castration resistant prostate cancer
Study sites	University of Washington Seattle Cancer Care Alliance
Investigators	**Principal investigator:** Robert Bruce Montgomery, M.D. **Co-Principal investigator:** Peter Nelson, M.D.

Protocol version	6.0; 7/01/2019
STUDY OBJECTIVES:	
Phase 1:	Primary Objective: Phase 1 • To determine the recommended phase 2 dose (RP2D) of Sirolimus combined with docetaxel and carboplatin in the treatment of metastatic castration resistant prostate cancer (CRPC).
	Secondary Objectives: Phase 1 • To assess maximal PSA response to Sirolimus combined with docetaxel and carboplatin • To assess PSA response duration to Sirolimus combined with docetaxel and carboplatin • To assess response of measurable disease • To assess time to progression of bone lesions or measurable disease (RECIST 1.1)
Phase 2:	Primary Objective: Phase 2 • To assess the ability of Sirolimus to suppress induction of DNA damage surrogates by docetaxel and carboplatin in metastatic cancer associated stroma.
	Secondary Objectives: Phase 2 • To assess maximal PSA response to docetaxel and carboplatin with or without Sirolimus • To assess PSA response duration to Sirolimus combined with docetaxel and carboplatin • To assess response of measurable disease • To assess time to progression of bone lesions or measurable disease (RECIST 1.1) • To assess toxicity of the RP2D dose of Sirolimus with docetaxel and carboplatin. • To determine the effect of docetaxel and carboplatin therapy on the DNA damage secretory program (DDSP) in serum. • To determine response to sirolimus, docetaxel and carboplatin in tumors with mutation of DNA repair pathway genes (BRCA2, ATM and other FANC pathway genes involved in DNA repair) • To correlate the presence of DNA repair pathway mutations in circulating tumor cells (CTC) and circulating tumor DNA (ctDNA) with tumor biopsy assessments and correlate changes in CTC number and ctDNA mutation status with PSA and clinical responses.

STUDY SCHEMATIC:



METHODS:

Phase 1

This is a dose finding scheme, where dose groups will be enrolled with a target of up to six (no less than three) patients per dose. Groups will be treated with de-escalating doses of Sirolimus combined with a fixed dose of docetaxel and carboplatin (60 mg/m2 and AUC 5 every 21 days). Sirolimus will be started at 35 mg given on Day -2 every 21 days, with dose de-escalations occurring within cohorts. Patients may continue in their cohort for repeated cycles of dosing; however, dose de-escalation within a patient will not occur. A safety review will be performed by the Principal Investigators for each individual patient at the end of Cycle 1 before the patient is allowed to continue to Cycle 2 in his dose level. Biopsy of suitable bone or soft tissue metastasis will be performed on day 21 to evaluate the DNA damage secretory program response to docetaxel, carboplatin and Sirolimus.

Safety evaluations will be performed by the Principal Investigator for all patients at the end of Cycle 1 in each cohort. The Principal Investigator will decide whether proceeding to phase 2 or if dose de-escalation into the next cohort will be necessary. After the maximally tolerated dose (MTD) is defined (one dose level below observed dose limiting toxicity (DLT) rate of $\geq$ 35%) the recommended phase 2 dose RP2D) will be determined. A DLT will be defined as febrile neutropenia, grade 3 or 4 thrombocytopenia or any Grade 3 or higher non-hematologic adverse event (AE), considered to be possibly, probably, or definitely related to therapy. Throughout Phase 1 of the study, safety and tolerability will be assessed by frequent recording of adverse events, vital signs, and safety laboratory assessments.

Phase 2

One dose will be chosen for study in Phase 2 to be tested in sequential vs. concurrent fashion. Forty (20 per cohort) new patients will be enrolled depending on response rate within each cohort.

Patients in Cohort 1 will initiate docetaxel and carboplatin (60 mg/m^2 and AUC 5 respectively) without sirolimus on Cycle 1 Day 1, followed by CT or ultrasound guided biopsy of suitable bone or soft tissue metastasis on day 21; to evaluate the DNA damage secretory program response (DDSP) to docetaxel and carboplatin. Sirolimus at the RP2D will be then be initiated on day 22, with docetaxel and carboplatin administered 2 days later.

Patients in Cohort 2 will initiate docetaxel and carboplatin 60 mg/m^2 and AUC 5 on Cycle 1 Day 1 with Sirolimus administered on Cycle 1 Day -2 and with CT or ultrasound guided biopsy of suitable bone or soft tissue metastasis on day 21 to evaluate the DDSP response to docetaxel and carboplatin with Sirolimus.

In both cohorts docetaxel and carboplatin will be administered at 60 mg/m^2 and AUC 5 every 21 days with Sirolimus administered on day -2 until progression by Prostate Cancer Working Group 2 (PCWG2) criteria or undue toxicity. Throughout Phase 2 of the study, safety and tolerability will be assessed by frequent recording of adverse events, vital signs and safety laboratory assessments. Progression will be evaluated with bone scan, CT of the abdomen/pelvis and PSA as per PCWG2 criteria.

Number of Patients: Phase 1: 6-18 patients Phase 2: 40 patients

Inclusion Criteria:

Patients who meet the following inclusion criteria will be eligible to participate in this study:

1. Signed informed consent form (ICF) providing agreement to adhere to the dosing schedule, report for all trial visits and authorization, use and release of health and research trial information
2. Age ≥ 18 years
3. Histologically or cytologically confirmed adenocarcinoma of the prostate (excluding neuroendocrine differentiation or small cell histology)
4. Ongoing gonadal androgen deprivation therapy with gonadotropin-releasing hormone (GnRH) analogues, antagonists or orchiectomy. Patients who have not had an orchiectomy must be maintained on effective GnRH analogue/antagonist therapy
5. Castration resistant prostate cancer as defined by serum testosterone < 50 ng/ml and one of the following:
    - PSA level of at least 2 ng/ml that has risen on at least 2 successive occasions at least 1 week apart.
    - Evaluable disease progression by modified Response Evaluation Criteria in Solid Tumors (RECIST 1.1)
    - Progression of metastatic bone disease on bone scan with > 2 new lesions
6. Prior therapy with abiraterone, enzalutamide and/or docetaxel.
7. The presence of metastatic disease amenable to CT or ultrasound guided biopsy. This may include thoracolumbar vertebral bodies, pelvis, femur or humerus, or soft tissue or nodal metastasis amenable to biopsy (excluding lung or pleural lesions).

8. Agree to participate in biopsy of metastatic lesion during the study at Day 21.
9. Eastern Cooperative Oncology Group (ECOG) Performance Status of ≤ 1
10. Life expectancy >12 weeks.
11. No prior malignancy is allowed except:
    - Adequately treated basal cell or squamous cell skin cancer or
    - In situ carcinoma of any site or
    - Other adequately treated malignancy for which the patient is currently disease free for at least one year
12. Patients with any prior chemotherapy regimens are eligible.
13. Patients with disease only in the bone may not have received Xofigo/Radium 223 to avoid ongoing DNA damage in bone marrow.
14. Patients who are or are not receiving bisphosphonates or denosumab are eligible. Bisphosphonates or denosumab should not be initiated after registration and during active treatment.
15. Patients must have adequate organ and marrow function as defined below obtained within 14 days prior to registration:
    - Absolute neutrophil count >1.5 x 10^9 cells/L
    - Hgb > 9.0 g/dL
    - Platelets >100,000 x 109/L
    - Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and bilirubin levels < 1.5 x ULN
    - Total bilirubin < 1.5 x ULN
    - Serum creatinine < 1.5 X institutional ULN mg/DL OR eGFR > 50 mL/min

Exclusion Criteria

Patients must NOT meet any of the following exclusion criteria:

1. Patients currently receiving active therapy for other neoplastic disorders.
2. Patients with histologic evidence of small cell carcinoma of the prostate will not be eligible.
3. Patients with disease only in the bone previously treated with radium-223 will not be eligible
4. Known parenchymal brain metastasis.
5. Active or symptomatic viral hepatitis or chronic liver disease.
6. Estimated creatinine clearance less than 50 ml/minute.
7. Clinically significant heart disease as evidenced by myocardial infarction, or arterial thrombotic events in the past 6 months, severe or unstable angina, or New York Heart Association (NYHA) Class II-IV heart disease.
8. Atrial fibrillation, or other cardiac arrhythmia requiring medical therapy.
9. Administration of an investigational therapeutic within 30 days of Cycle 1, Day -2.
10. Patients with dementia/psychiatric illness/social situations that would limit compliance with study requirements or would prohibit the understanding and/or giving of informed consent.
11. Patients with medical conditions, which, in the opinion of the investigators, would jeopardize either the patient or the integrity of the data obtained will not be eligible.
12. Any condition which, in the opinion of the investigator, would preclude participation in this trial.
13. Patients on anticoagulation therapy which cannot be held for metastatic biopsies.

CRITERIA FOR EVALUATION

Safety: Phases 1 and 2

- The number and percentage of patients who experience a Serious Adverse Event;
- The number and percentage of patients who experience an Adverse Event; and
- The number and percentage of patients who discontinue study drug treatment early due to an adverse event.

Anti-Tumor Effects: Phase 2

- The percentage of patients achieving at least a 50% reduction in PSA according to PCWG2 criteria;
- The percentage of patients achieving at least a 90% reduction in PSA according to the PCWG2 criteria.
- The percentage of patients achieving a 30% reduction in measurable disease by RECIST 1.1.
- Median time to tumor progression by PCWG2 criteria
- Average tissue transcript level of DDSP components WNT16B, IL-6 or SFRP2 at day 21 biopsy

STATISTICAL METHODS:

Safety: Phases 1 and 2

Safety will be assessed through summaries of adverse events, vital signs, physical examinations, ECGs, and clinical laboratory test data (including change from baseline). Safety analyses will include all enrolled patients who receive any amount of study drug (safety population). All adverse events will be captured using National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (NCI CTCAE v. 4.0). Laboratory values will be classified by the NCI CTCAE V. 4.0 severity grade. Laboratory shift tables of the baseline results to each of the subsequent visits will be produced.

Efficacy: Phase 2

The primary endpoint is suppression of DDSP induction following genotoxic chemotherapy. Based on preliminary data, we anticipate that 50% of patients will increase the expression of at least one of WNT16, IL6, or SFRP2 (key members of the DNA damage response) >3-fold over the background level in tissue stroma (microenvironment) following treatment with DNA damaging chemotherapy (carboplatin/docetaxel). We hypothesize that the administration of Sirolimus will suppress this induction in 90% of patients. 20 patients randomized to treatment with Sirolimus and 20 patients randomized to treatment without Sirolimus (for a total of N=40 patients) will provide 81% power to detect a difference in the proportions of patients with < 3-foldinduction of DDSP over background in at least one of the 3 markers at an alpha of 5% based on a 2-sided, 2-sample test of proportions. We will accrue up to 4 additional patients as necessary if patients drop out before the endpoint can be evaluated; final analysis will investigate sensitivity of inference to possible informative missing for any dropouts.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ADR	Adverse drug reaction
AE	Adverse event
ALT	Alanine aminotransferase (SGPT)
AR	Androgen receptor
AST	Aspartate aminotransferase (SGOT)
BUN	Blood urea nitrogen
C	Celsius
CALGB	Cancer and leukemia group B
CBC	Complete blood count
CR	Complete response
CrCl	Creatinine clearance
CPK	Creatinine phosphokinase
CRF	Case Report Form
CRPC	Castration resistant prostate cancer
ctDNA	Circulating tumor DNA
CT	Computed tomography
CTC	Circulating tumor cells
CTCAE	Common Terminology Criteria for Adverse Events
DDSP	DNA damage secretory pathway
DLT	Dose limiting toxicity
ECG	Electrocardiogram
ECOG	Eastern Cooperative Group
F	Fahrenheit
FDA	Food and Drug Administration
GCSF	Granulocyte stimulating factor
GFR	Glomerular filtration rate
GLP	Good Laboratory Practice
HEENT	Head, Eyes, Ears, Nose, Throat
Hct	Hematocrit

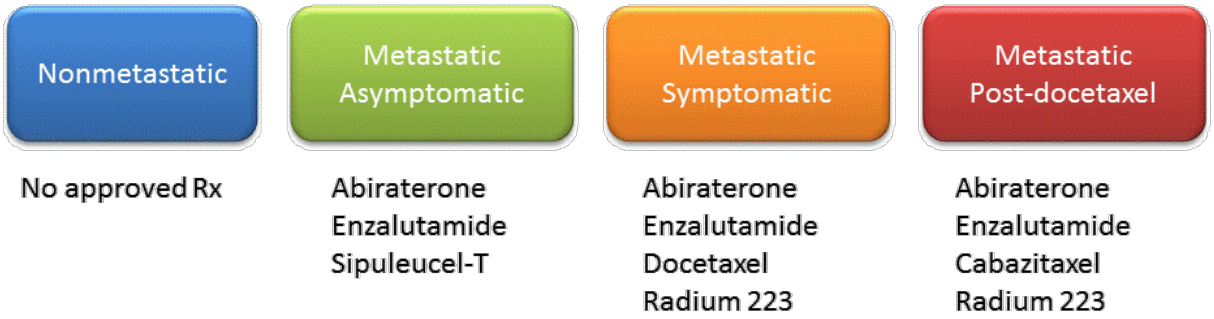
Hgb	Hemoglobin
HIPAA	Health Information Portability and Accountability Act
HUS	Hemolytic uremic syndrome
ICH	International Conference on Harmonisation
IHC	Immunohistochemistry
IND	Investigational New Drug
INR	International normalized ratio
IRB	Institutional Review Board
LDH	Lactic dehydrogenase
LLN	Lower limit of normal
LN	Lymph node
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging
MTD	Maximally tolerated dose
mTOR	Mammalian target of Rapamycin
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
PBMC	Peripheral blood mononuclear cell
pCR	pathologic complete response
PCR	Polymerase chain reaction
PCWG2	Prostate Cancer Working Group 2
PD	Progressive disease
PFS	Progression free survival
PR	Partial response
PSA	Prostate specific antigen
PT	Prothrombin time
RECIST	Response evaluation criteria in solid tumors
RP2D	Recommended Phase 2 dose
SAE	Serious adverse event
SD	Stable disease
SOC	System Organ Class

SRS	Special reporting situations
SU2C	Stand Up to Cancer
SUSAR	Suspected unexpected adverse event reporting
TMA	Thrombotic microangiopathy
TTP	Thrombotic thrombocytopenic purpura
ULN	Upper limit of normal
US	Ultrasound
WBC	White blood cell (count)

1 INTRODUCTION

1.1 Therapy for metastatic, castration resistant prostate cancer (CRPC)

Prostate cancer is the most common cancer among men with approximately 220,000 new cases per year in the United States alone. Roughly 10-20% of patients present with metastatic disease, and 40% of patients relapse after surgery or radiation therapy for presumed localized disease. While androgen-deprivation therapy for advanced disease is usually initially effective, almost all tumors become castration resistant after a median of 18-24 months. The currently FDA approved therapies for metastatic CRPC includes the androgen receptor (AR) targeting agents abiraterone or enzalutamide prior to or after docetaxel, radium 223 for patients declining docetaxel or after docetaxel, Provenge/sipuleucel-T prior to or after docetaxel, and cabazitaxel. In current clinical practice the vast majority of patients receive either abiraterone or enzalutamide, and often radium 223 prior to treatment with docetaxel. Patients who have then received docetaxel have very limited options except for cabazitaxel. The registration studies of cabazitaxel demonstrated a survival advantage compared to the use of mitoxantrone, with progression free survival of 2.8 months and a concerning toxic death rate of 5% [1]



In 2004, two phase III randomized trials demonstrated the survival benefit of docetaxel-based chemotherapy in the treatment of metastatic castration resistant prostate cancer CRPC [2, 3] Although one of these studies utilized estramustine in combination with docetaxel, this drug is now considered toxic and does not add additional benefit to docetaxel. The principal anti-neoplastic mechanism of docetaxel and other taxanes is thought to be inhibition of microtubule depolymerization causing G_2/M cell cycle arrest. Recent data has suggested that part of the mechanism by which docetaxel functions in prostate cancer may be by blocking nuclear translocation of the AR, suggesting the potential for cross resistance with other AR targeting agents [4] It is also important to note that the initial studies of docetaxel were carried out in an era when more effective AR targeting agents (enzalutamide or abiraterone) were not available. Although docetaxel-containing chemotherapy offers a survival advantage, the benefit is modest (median of approximately 2.5 months) and is not durable.

The second generation AR targeting agents, abiraterone (Zytiga) and enzalutamide (Xtandi) are now FDA approved prior to docetaxel use in patients with metastatic

CRPC. No prospective studies have been performed with docetaxel after either agent, and these are unlikely to be carried out given the cost and lack of industry sponsor. Single institution series suggest that treatment of prostate cancer with abiraterone prior to docetaxel reduces PSA response rates and progression free survival (PFS) to docetaxel. In the analysis by Mezynski, PSA decline by 50% (PSA50) was 26% with median PSA PFS of 4.6 months and overall survival of 12.5 months [5]. These results compare to the phase III registration studies of docetaxel (TAX-327) in which PSA50 was 45-50%, PFS 6 months and median OS of 18-19 months [2, 3]. These studies support the concept that docetaxel efficacy in the current treatment environment is significantly less than reported in the registry studies, approximating 50% less likely to provide significant PSA declines and that approaches that improve docetaxel efficacy are needed.

Regimen	Indication	PSA50	PFS	OS	Reference
Docetaxel	mCRPC 1st line	45%	NA	18.9 mos	Tannock
Docetaxel	mCRPC 1st line	50%	6 mos	18 mos	Petrylak
Docetaxel after Abi	mCRPC 1st line	26%	4.6 mos	12.5 mos	Mezynski
Docetaxel after abi	mCRPC 1st line	38 vs. 63%	4.4 mos	NA	Schweizer

1.2 Sensitivity and resistance of CRPC to DNA damaging agents

The addition of other agents to docetaxel might improve response in untreated or abiraterone treated tumors. The first chemotherapeutic agent which was FDA approved for the treatment of CRPC was the anthracycline and DNA damaging agent mitoxantrone [6]. Subsequent studies of the use of Satraplatin, a platinum agent in phase III randomized studies as second line chemotherapy (including docetaxel) demonstrated a significant improvement in progression free survival (HR 0.67, PFS 11 weeks, PSA50 25%) [7]. In that context investigators have considered that combining the DNA damaging agent carboplatin with docetaxel may improve efficacy. A CALGB phase II study of the combination of docetaxel and carboplatin with estramustine induced a PSA50 response rate of 68% with progression free survival of 8 months [8]. Estramustine is no longer considered to improve the clinical efficacy of chemotherapy because it did not enhance response or survival compared to docetaxel alone, while adding significant toxicity. In patients with tumors refractory to docetaxel, the addition of carboplatin induced a PSA50 rate of 18% and PFS of 3 months [9]

There are several potential explanations for the efficacy of platinum compounds in CRPC. First, unpublished results from the SU2C CRPC 500 collaborative study being carried out at UW/FHCRC shows that approximately 20% of metastatic CRPC contains biallelic inactivation of BRCA2 or inactivating mutations of ATM. These genes are critically important to repair of DNA damage and inactivation of these genes is predicted to provide sensitivity to agents such as carboplatin. Secondly, the transdifferentiation of adenocarcinoma of the prostate into a neuroendocrine/small cell phenotype is being recognized more commonly in patients with CRPC [10]. Platinum based chemotherapy is the most effective treatment for patients with small cell histologies from any primary site and carboplatin would be anticipated to be more effective in this subset of patients.

Activity of carboplatin with docetaxel in prostate cancer						
Docetaxel/carboplatin	mCRPC line	2nd	18%	3 mos	12 mos	Ross
Docetaxel/EMP carboplatin	mCRPC line	1st	68%	8 mos	19 mos	Oh

1.3 Inhibition of mTOR in combination with DNA damaging agents can improve response in prostate cancer.

Mitoxantrone, carboplatin and radiation are DNA damaging agents used in the treatment of prostate cancer. Intrinsic and acquired resistance to DNA damage limits the magnitude and duration of response. One potential mechanism of tumor resistance to DNA damage is the tumor microenvironment. This microenvironment for all cancers is comprised of a variety of cell types including epithelial cells, fibroblasts, inflammatory cells, blood vessels and extracellular matrix which surround and infiltrate tumors and all may modulate tumor activity. For example, tumor associated fibroblasts enhance preneoplastic cell growth and tumor invasiveness, [11, 12] This dynamic environment may in part explain drug resistance by tumors against DNA damaging agents (e.g., radiation or carboplatin) commonly used to treat prostate cancer. [13 , 14] The microenvironment responds to DNA damage by secretion of cytokines and growth factors which augment the immune clearance of damaged cells while promoting tissue repair through angiogenesis and recruitment of epithelial and stromal cells. This DNA Damage Secretory Program (DDSP) elaborated by stroma can also promote tumor survival and metastasis [15]. The cytokines and growth factors that comprise the DDSP provide a survival advantage for the tumor itself through suppression of apoptosis and promotion of proliferation while also inducing epithelial-mesenchymal transition and metastasis [16]. Tumor clones which survive the initial chemotherapy cycle will grow to serve as a site of treatment failure and/or relapse. In this way, the DDSP in the tumor microenvironment can drive adverse cancer outcomes. Recent work from the Nelson laboratory at Fred Hutchinson Cancer Research Center (FHCRC) has demonstrated that inhibition of different components of the DDSP can improve

responses to DNA damaging agents in preclinical models [17]. Unpublished results from the same group has demonstrated that the use of the mammalian Target Of Rapamycin (mTOR) inhibitor rapamycin (Sirolimus) can block DDSP induction and augment activity to DNA damaging agents. Blocking mTOR activity may therefore improve prostate cancer responses to DNA damaging agents such as carboplatin based regimens.

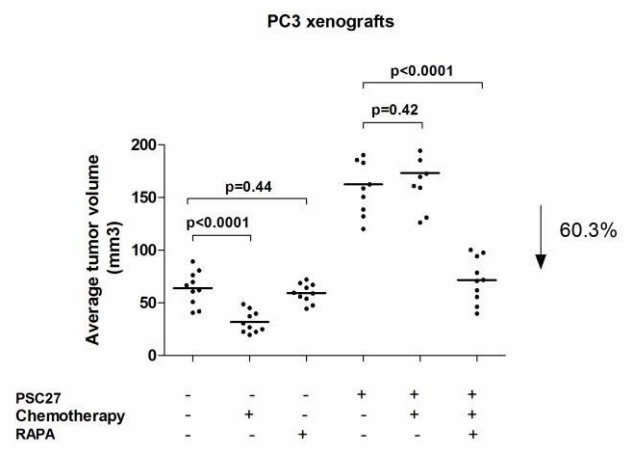


Figure 1 The addition of the mTOR inhibitor sirolimus improves activity of mitoxantrone PC3 prostate cancer xenografts ± benign PSC27 stromal cells were untreated, treated with mitoxantrone with and without rapamycin (sirolimus).

Based on these data we propose to attempt to improve response to docetaxel based chemotherapy through the combination of the DNA damaging agent carboplatin with an mTOR inhibitor, in order to prevent microenvironment induced resistance.

1.4 The addition of the mTOR inhibitor Sirolimus to chemotherapy does not increase toxicity.

The combination of Sirolimus with gemcitabine and cisplatin is currently being used in a phase II neoadjuvant study of chemotherapy for patients with localized bladder cancer (CC-IRB 8027). The phase I portion of that study, utilizing the same mTOR inhibitor regimen proposed for the current study showed no evidence of increased toxicity, with one episode of grade 4 neutropenia seen in a patient who was heavily pretreated with myelotoxic agents. The safety seen in that study provides evidence that mTOR inhibition with chemotherapy is safe. In that study and in the current study we will administer Sirolimus at the time of chemotherapy improve the pharmacokinetics by administering the Sirolimus with grapefruit juice, which provides pharmacokinetics equivalent to once weekly administration of temsirolimus [18].

In summary, docetaxel is a standard therapy for prostate cancer with modest improvements in survival and there is evidence from recent studies that prior therapy with abiraterone or enzalutamide compromises responses to docetaxel. The rationale for the current study is that it is critical to identify approaches to improve taxane response in the current treatment environment which invariably involves treatment with AR targeting agents prior to a taxane. The study will also answer the question of whether inhibition of DDSP can be achieved and whether there is a suggestion of

improved response with mTOR inhibition. The combination of docetaxel with carboplatin is active as an upfront regimen and combination with mTOR inhibition carries the potential of improving responses by inhibiting intrinsic resistance mediated by the tumor microenvironment. In this study we will evaluate the ability to block the DDSP with the mTOR inhibitor Sirolimus while evaluating pilot data regarding objective tumor responses to this combination.

1.5 Sirolimus

1.5.1 Chemistry

Sirolimus (Rapamycin, Rapamune) is a macrolide compound obtained from *Streptomyces hygroscopicus* that acts by selectively blocking the transcriptional activation of cytokines, thereby inhibiting cytokine production. It is bioactive only when bound to immunophilins.

1.5.2 Clinical pharmacology

Mechanism of Action - Sirolimus inhibits T-lymphocyte activation and proliferation that occurs in response to antigenic and cytokine (Interleukin [IL]-2, IL-4, and IL-15) stimulation by a mechanism that is distinct from that of other immunosuppressants. Sirolimus also inhibits antibody production. In cells, Sirolimus binds to the immunophilin, FK Binding Protein-12 (FKBP-12), to generate an immunosuppressive complex. The Sirolimus-FKBP-12 complex has no effect on calcineurin activity. This complex binds to and inhibits the activation of mTOR, a key regulatory kinase. This inhibition suppresses cytokine-driven T-cell proliferation, inhibiting the progression from the G1 to the S phase of the cell cycle, as well as mTOR driven signaling in other tissues.

The mTOR complex functions as a serine/threonine kinase and a key regulator of protein translation via its ability to phosphorylate S6K and 4EBP1. Sirolimus binds intracellularly to FKBP12, inhibiting mTOR and resulting in growth inhibition in preclinical tumor models primarily by inducing cell-cycle arrest or apoptosis. Intermittent dosing schedules in animal models have shown antitumor activity equivalent to continuous administration, but without prolonged immunosuppression. [19].

1.5.3 Human Toxicity

Human toxicity of Sirolimus alone given in a phase 1 study with grapefruit juice as planned in this study was as per section 1.5.6 [25] and consisted of hyperglycemia, hyperlipidemia, lymphopenia, anemia, and diarrhea. Other adverse effects include: bronchial anastomotic dehiscence in lung transplant patients, exfoliative dermatitis, angioedema, renal toxicity, anorexia, vomiting, fluid accumulation, impaired or delayed wound healing, hypertriglyceridemia, hypercholesterolemia, renal insufficiency with long-term combination with cyclosporine, interstitial lung disease, hemolytic uremic syndrome/thrombotic thrombocytopenic purpura/thrombotic microangiopathy. Increased

susceptibility to infection and the possible development of lymphoma and other malignancies may result from immunosuppression.

Hypersensitivity reactions, including anaphylactic reactions, angioedema, exfoliative dermatitis and hypersensitivity vasculitis have been associated with the administration of Sirolimus.

The most common (≥ 30%) adverse reactions observed with Sirolimus in clinical studies are: peripheral edema, hypertriglyceridemia, hypertension, hypercholesterolemia, creatinine increased, constipation, abdominal pain, diarrhea, headache, fever, urinary tract infection, anemia, nausea, arthralgia, pain, and thrombocytopenia.

The following adverse reactions resulted in a rate of discontinuation of > 5% in clinical trials: increased creatinine, hypertriglyceridemia, and thrombotic thrombocytopenic purpura (TTP).

1.5.4 Pharmaceutical data

Formulation: Each Sirolimus Oral Solution carton, NDC 0008-1030-06, contains one 2 oz (60 mL fill) amber glass bottle of Sirolimus (concentration of 1 mg/mL).

Storage: Oral Solution bottles should be stored protected from light and refrigerated at 2°C to 8°C (36°F to 46°F). Once the bottle is opened, the contents should be used within one month. The product may be kept in the dosing syringe for a maximum of 24 hours at room temperatures up to 25°C (77°F) or refrigerated at 2°C to 8°C (36°F to 46°F). After dilution, the preparation should be used immediately. The syringe should be discarded after one use.

1.5.5 Supplier

Commercial supplies of Sirolimus will be used.

1.5.6 Dosing rationale for Sirolimus

The phase 1 component of the study is a dose de-escalation study. The rationale for a dose de-escalation study is that the side effect profile of all the drugs in the study has been defined over many years and through extensive experience in clinical practice. A short duration exposure of a non-cytotoxic compound in the form of Sirolimus during only the first week of therapy with docetaxel and carboplatin is not anticipated to adversely affect the pharmacokinetics of docetaxel and carboplatin and the toxicity profile has limited overlap. Given the expectation of limited toxicity, a dose de- escalation approach is favored in order to define the recommended phase 2 dose (RP2D) for testing in the phase 2 component of study. The planned dose de-escalation strategy is based on a series of phase 1 studies carried out with Sirolimus alone, Sirolimus with ketoconazole or Sirolimus with grapefruit juice.[18] and the safety seen in Sirolimus with gemcitabine/cisplatin in patients with bladder cancer.

Performing the phase 1 study with grapefruit juice was based on the defined ability of grapefruit juice to deactivate intestinal CYP3A4 and block metabolism of sirolimus. Inhibition of intestinal CYP3A4 lasts for at least 4 hours. Based on toxicity and pharmacokinetics, the recommended phase 2 dose of Sirolimus with grapefruit juice is 35 mg weekly [18]. This study also demonstrated inhibition of phosphoS6 kinase levels in circulating PBMC at 48 hours after administration of Sirolimus; demonstrating effective mTOR signaling 2 days after Sirolimus.

Toxicity of Sirolimus with grapefruit juice at all doses once weekly was limited. The most common toxicities attributed to Sirolimus observed in this phase 1 study of Sirolimus were hyperglycemia, hyperlipidemia, lymphopenia, anemia, and diarrhea. The toxicities of the treatment doses alone and in combination with drugs anticipated to improve pharmacokinetics are as shown in Table 1.

Table 1. Toxicities of Sirolimus alone, with ketoconazole, or with grapefruit juice.

	Sirolimus alone							Sirolimus plus ketoconazole								Sirolimus plus grapefruit juice					
Adverse event	10 mg	20 mg	30 mg	60 mg	30 mg (4 h)	30 mg (24 h)	45 mg (24 h)	1 mg	2 mg	3 mg	4 mg	5 mg	6 mg	8 mg	16 mg	15 mg	20 mg	25 mg	30 mg	35 mg	Total ($n = 138$)
Altered taste	1	1							1		1				1						5
Anemia	3	5	3					4 (1)	3	3	2	5 (1)	3	1	4 (2)	4 (1)	1		1	4	46 (5)
Anorexia	1	1	1 (1)	3			1	5	1	2			1	1	3	2	2	3	1	4	32 (1)
Decreased platelets		1	1	1	1			1			1	1	2		2	3	1	3		4	22
Diarrhea	1	5	2	6 (1)	4	2	3 (1)	2	1	1	2	4	1 (1)	2	3	5	4	3	1	6	58 (3)
Mucocutaneous dryness	1		1													1		1		2	6
Dyspnea		1													1	2		1		1 (1)	6 (1)
Edema												1		3	1 (1)						5 (1)
Elevated creatinine												1									1
Elevated transaminases	1	4	2	1	1				1 (1)	1 (1)	1	3	4			2		1	1		23 (2)
Epistaxis															1						1
Fatigue	2	4	1	5	1			4	1	4	1	2		1	3 (1)	6 (1)	2	2	2	5 (1)	46 (3)
Fever			2	1				1													4
Hand and foot syndrome													1		1						2
Hyperlipidemia	2	4	3 (1)	6 (1)				3	1	3	3	4	2	2	6	9 (1)	1	1	2	7	59 (3)
Hyperglycemia		6	6	4	1		1	4 (1)	5	4	2	4	3	2	4 (2)	10 (1)	3 (1)	4 (1)	2	7 (1)	72 (7)
Hypocalcemia												2									2
Hypertension			1										1								2
Hypoglycemia												1 (1)									1 (1)
Hypoxia		2																			2
Infection	1	1 (1)	1 (1)	1		1 (1)		1 (1)	1		1	3				1	1			1	14 (4)
Leukopenia					2			1	2	2	1		2		1		1	2		3	17
Lymphopenia	3 (2)	4 (1)	3 (1)	3				4	2	2 (1)	3 (1)	3 (1)	5 (2)	2	3 (1)	7 (1)	2 (1)	4	2 (1)	4 (1)	56 (14)
Mood alteration							1		1												2
Mucositis	2	1				1				1	2	1		1	2	1	2	1		2	17
Myalgia								2					1								3
Nail changes															1 (1)						1 (1)
Nausea	3	2	4 (1)	6	2	1	2	2	1	2	1	4	2		2	6 (1)		2	2	4	48 (2)
Neutropenia								1		1	1	1	2							1	7
Paresthesia	1													1							2
Pleural effusion												1									1
Pneumonia		2 (2)	1									2 (2)									5 (4)
Pruritis										1	2	1	1		1						6
Rash	1		1	1		1		1		1	1			2	1	2				2	14
Vomiting	2		2	5	2		4	3	1		1 (1)	2	2	1		3		1	1	3	33 (1)
Weight loss				4				2	1	1	1		1		1	1			1	2	15

Table 2. DLTs observed in all 3 studies

Study	Nature of DLT	Dose level (mg sirolimus)
Sirolimus alone	Pneumonia	20
	Dehydration	30
	Unable to complete cycle 1 because of toxicity (anorexia and fatigue ≤grade 2)	60
Sirolimus/ ketoconazole	Pneumonia	5
	Hand/foot syndrome[a]	6
	Diarrhea[a]	6
	Hyperglycemia	16
Sirolimus/ grapefruit juice	Hyperglycemia	25
	Mucositis	25
	Anorexia	30

[a]These occurred concurrently in the same subject.

Table 3. Pharmacokinetic analysis (noncompartmental analysis) of sirolimus with or without ketoconazole or grapefruit juice

Sirolimus dose, mg, *n*	Keto 0 = non 1 = Keto	GFJ 0 = non 1 = GFJ	C_{max} (ng/mL)[a]	C_{max} CV%[a]	AUC_{∞} (ng-h/mL)[a]	AUC_{∞} CV%[a]	$T_{1/2}$ (h)	$T_{1/2}$ CV%	CL/F (l/h)[a]	CL/F CV%[a]
1 (6)	0	—	5.4	31.4	186	102	82	156	10.3	87.2
	1	—	25.4	36.8	935	30	50	47	1.2	43.7
2 (6)	0	—	9.9	59.6	271	74	45	41	9.5	37.8
	1	—	33.2	47.8	1,347	31	52	28	1.8	58.5
3 (6)	0	—	17.4	54.9	400	26	35	36	8.0	27.3
	1	—	49.2	62.2	2,448	73	84	101	1.6	43.8
4 (6)	0	—	21.3	35.7	456	42	36	35	10.0	37.4
	1	—	66.8	71.9	2,897	79	61	50	2.1	53.5
5 (6)	0	—	25.8	43.2	676	46	35	27	9.0	49.9
	1	—	71.2	70.1	3,144	72	57	35	2.8	81.5
6 (3)	0	—	15.0	55.5	510	63	78	76	15.6	62.0
	1	—	74.6	92.7	2,620	38	62	60	2.6	45.3
8 (1)	0	—	25.2	—	420	—	18	—	19.1	—
	1	—	85.2	—	1,624	—	34	—	4.9	—
16 (2)	0	—	47.4	15.8	1,456	17	25	79	11.2	17.1
	1	—	123.0	7.6	5,007	3	32	6	3.2	3.2
15 (5)	—	0	50.3	28.1	858	37	36	71	20.5	52.4
	—	1	127.9	52.0	4,361	52	46	41	5.9	109.4
20 (5)	—	0	45.6	73.0	863	82	26	44	36.9	71.6
	—	1	105.3	65.8	2,797	62	53	52	11.5	79.1
25 (4)	—	0	84.7	68.0	1,449	77	30	47	32.6	102.4
	—	1	173.9	55.3	4,538	80	40	29	9.2	79.5
30 (5)	—	0	68.5	36.1	1,814	48	30	37	18.7	39.9
	—	1	82.1	37.5	2,419	17	35	21	12.6	14.7
35 (8)	—	0	68.1	62.3	1,799	72	43	58	29.4	64.0
	—	1	129.7	65.3	6,655	83	56	103	12.8	135.3

In this study we are proposing to test the hypothesis that mTOR is a master regulator of the DDSP derived from the prostate cancer microenvironment. In this study we will assess whether administration of Sirolimus with grapefruit juice will inhibit mTOR for 7 days, which would be substantially longer than the anticipated duration of DNA damage induced by a single administration of docetaxel and carboplatin and whether co-administration is safe and improves efficacy (as reflected by response in phase 2).

The planned dose de-escalation in phase 1 varies the dose of Sirolimus administered on Day -2 (with grapefruit juice (240 ml) administered on Days -3 through 4 and docetaxel and carboplatin (60 mg/m2 and AUC 5) administered on Day 1 every 21 days).

The Sirolimus dose in dose level 0 is 35 mg once on Day -2. If zero or one dose limiting toxicity (DLT) occurs in the first three patients, an additional 3 patients will be treated in dose level 0. If the observed DLT rate is $\leq$ 33% in six patients, dose level 0 will be the RP2D. Patients may continue in their cohort for repeated cycles of dosing; however, dose de-escalation within a patient will not occur.

If the DLT rate is > 33% in 3 or 6 patients, the dose will be decreased to dose level -1, 25 mg once per 21 day cycle on Day -2. The same cohort algorithm will occur at dose level -1 as above. If the DLT rate is > 33% at dose level -1 in 3 or 6 patients, the dose level will decrease to dose level -2 at 20 mg once per 21 day cycle on Day -2.

If the DLT rate is > 33% at dose level -2, no phase 2 will be carried out. Dose

de-escalation values:

Dose Level	Sirolimus
100% (Dose Level 0)	**35mg**
First dose reduction (Dose Level -1)	**25mg**
Second dose reduction (Dose Level -2)	**20mg**

1.6 Docetaxel and carboplatin

Docetaxel (Taxotere) and carboplatin (Paraplatin) are synthetic antineoplastic agents which have been extensively used in the treatment of solid tumors.

1.6.1 Human toxicity

Human toxicity of docetaxel includes:

- Allergic Reaction: Hypotension, urticaria, and hypersensitivity are reported. Premedication with dexamethasone is required.
- Hematologic: Taxanes, including docetaxel, alone and in combination with other antineoplastic agents, have been associated with neutropenia, anemia and thrombocytopenia.
- Neurologic: Neuropathy is reported in over 10% of patients
- Dermatologic: Alopecia (56% to 76%), dermatological reaction (20% to 48%; severe: ≤5%), nail disease (11% to 41%)
- Endocrine & metabolic: Fluid retention (13% to 60%; severe: 7% to 9%; dose

dependent)

- Gastrointestinal: Stomatitis (19% to 53%; severe 1% to 8%), diarrhea (23% to 43%; severe: 5% to 6%), nausea (34% to 42%), vomiting (22% to 23%)

- Hepatic: Increased serum transaminases (4% to 19%). If bilirubin > ULN, or if AST and/or ALT > 1.5 × ULN concomitant with alkaline phosphatase > 2.5 × ULN docetaxel should not be administered.

- Neuromuscular & skeletal: Weakness, myalgia and arthralgia are reported in greater than 10% of patients.

- Ophthalmic: Epiphora (associated with canalicular stenosis ≤1% with every 3-week administration)

Human toxicity of carboplatin includes:

- Endocrine & metabolic: Hyponatremia (29% to 47%), hypomagnesemia (29% to 43%), hypocalcemia (22% to 31%), hypokalemia (20% to 28%)

- Gastrointestinal: Vomiting (65% to 81%), abdominal pain (17%), nausea (without vomiting: 10% to 15%)

- Hematologic & oncologic: Bone marrow depression (dose related and dose limiting; nadir at ~21 days with single-agent therapy), anemia (3/4: 21%), leukopenia (grades 3/4: 15% to 26%), neutropenia (grades 3/4: 16% to 21%), thrombocytopenia (grades 3/4: 25% to 35%)

- Hypersensitivity: Hypersensitivity (2% to 16%)

- Renal: Decreased creatinine clearance (27%), increased blood urea nitrogen (14% to 22%)

- Central nervous system: Peripheral neuropathy (4% to 6%), neurotoxicity (5%)

- Dermatologic: Alopecia (2% to 3%)

The data regarding tolerance of docetaxel and carboplatin alone or myelotoxic chemotherapy with sirolimus/rapamycin comes from several sources. The tolerance of docetaxel/carboplatin alone comes from references #8 and #9 (Oh and Ross). In those studies there was no safety signal suggesting significantly increased toxicity to patients receiving the combination of docetaxel and carboplatin, with the primary difference between this regimen and docetaxel alone being an increased incidence of Grade 3 neutropenia (56% vs. 32%), but febrile neutropenia was 1% with docetaxel/carboplatin vs. 3% in the phase III study of docetaxel alone (Tannock, NEJM).

We have an ongoing study combining sirolimus with gemcitabine and cisplatin in bladder cancer, with both a phase I and II component. In the phase I study 6 patients were treated with gemcitabine/cisplatin with rapamycin with no DLT's and MTD was full dose gemcitabine and cisplatin with the same dose of sirolimus that is proposed for the current study (9388). Thus far 12 patients in total have been enrolled on the bladder cancer study with no safety signal for increased toxicity with sirolimus (incidence of toxicity 70% grade 3 or 4 neutropenia, 10% febrile neutropenia, 5% other grade 3 or 4 toxicities). This is consistent with toxicity seen in phase III studies of gemcitabine and cisplatin alone. For these reasons the combination proposed in this study appears reasonable given the inclusion of a phase I component to rule out an unforeseen interaction between sirolimus and docetaxel/carboplatin.

1.6.2 Supplier

Commercial supplies of docetaxel and carboplatin will be used.

1.6.3 Rationale for study design

The combination of docetaxel and carboplatin has activity as first and second line therapy for patients with CRPC. The recognition that the tumor microenvironment may play a critical role in tumor resistance to DNA damaging agents provides a potential point of therapeutic leverage. The mTOR pathway is a master regulator of the DDSP cytokine and growth factor signaling pathway which supports tumor resistance to DNA damaging agents. This study proposes to use a short duration of mTOR inhibition only during the period of DNA damage to tumor and tumor microenvironment in an attempt to attenuate tumor resistance via suppression of the microenvironment secretory response. We are excluding prior therapy with the agent Radium-223 as it is anticipated that this agent may induce long lasting DNA damage responses in bone given the half-life of the agent and we would be unable to interrogate the effect of Sirolimus on DNA damage induced by carboplatin.

After a RP2D dose of the mTOR inhibitor Sirolimus with docetaxel and carboplatin has been defined, the ability to suppress the relevant DDSP targets will be evaluated. In addition, if an improvement in response is demonstrated concurrent with suppression of DDSP, there would be strong rationale for additional studies with mTOR inhibition with docetaxel and carboplatin in patients with metastatic and localized prostate cancer. Successful implementation of mTOR inhibition with DNA damaging agents could then increase improvement in survival and palliative benefits for all patients with prostate cancer.

2 STUDY OBJECTIVES

2.1 Primary Objective (Phase 1)

The primary objective of the Phase 1 study is:

- To define the RP2D of Sirolimus combined with docetaxel and carboplatin in the treatment of metastatic CRPC.

2.2 Exploratory Objectives (Phase 1)

The exploratory objectives of the Phase 1 study are:

- To assess maximal PSA response to Sirolimus combined with docetaxel and carboplatin.
- To assess PSA response duration to Sirolimus combined with docetaxel and carboplatin.
- To assess response of measurable disease.
- To assess time to progression of bone lesions or measurable disease (RECIST 1.1).
- To assess effect of Sirolimus with docetaxel and carboplatin on DNA damage surrogates in cancer associated stroma compared to untreated and docetaxel and carboplatin treated stroma.

2.3 Primary Objective (Phase 2)

The primary objective of the Phase 2 study is:

- To assess the effectiveness of Sirolimus in suppressing the induction of the DNA damage secretory component WNT16B in tumor stroma following docetaxel and carboplatin.

2.4 Exploratory Objectives (Phase 2)

The exploratory objectives of the Phase 2 study are:

- To assess maximal PSA response to Sirolimus combined with docetaxel and carboplatin.
- To assess PSA response duration to Sirolimus combined with docetaxel and carboplatin
- To assess response of measurable disease (RECIST 1.1)
- To assess time to progression of bone lesions or measurable disease (RECIST 1.1)
- To assess toxicity of the RP2D dose of Sirolimus with docetaxel and carboplatin.

- To determine the effect of docetaxel and carboplatin therapy on the DDSP in serum.
- To determine response to Sirolimus, docetaxel and carboplatin in tumors with mutation of DNA repair pathway genes (BRCA2, ATM and other FANC pathway members)
- To correlate the presence of DNA repair pathway mutations in circulating tumor cells (CTC) and circulating tumor DNA (ctDNA) with tumor biopsy and correlate changes in CTC number and ctDNA mutation levels with clinical responses.

3 INVESTIGATIONAL PLAN

3.1 Overall study design and plan

This is an open-label study with separate phase 1 and phase 2 components.

Phase 1:
6-18 patients will be treated in a dose de-escalation study testing 3 dose levels of Sirolimus with fixed dose docetaxel (60 mg/m^2 Day 1) and carboplatin (AUC 5 Day 1) every 21 days.

Sirolimus will be administered on Day -2 every 21 days, at least two hours before or after grapefruit juice.

The defined product of grapefruit juice will be ingested once daily for 7 consecutive days, on Days -3 through 4. The grapefruit juice dosage will be 8oz (240ml) daily.

Docetaxel and carboplatin will be administered at 60 mg/m^2 and AUC 5 on Day 1of a 21 day cycle. Day -2 will occur two days prior to chemotherapy Day 1(there is no Day 0).

Dose Level	Sirolimus
100% (Dose Level 0)	**35mg**
First dose reduction (Dose Level -1)	**25mg**
Second dose reduction (Dose Level -2)	**20mg**

Patients will remain on treatment until any of the following occur:

- intolerable toxicity
- documented progression by imaging per RECIST 1.1 criteria
- achieving complete response (CR) per RECIST 1.1 criteria
- completion of 10 cycles of therapy – consideration for additional cycles at the investigator discretion if patients are responding and there is no evidence of cardiomyopathy.

Adverse events for determination of maximally tolerated dose (MTD)/ RP2D and DLT will be evaluated within the first 21 days of treatment.

Response in Phase 1 and 2 will be defined per Response Evaluation Criteria in Solid Tumors (RECIST 1.1) and Prostate Cancer Working Group 2 Criteria (22)

RECIST (1.1) – Appendix 5. For the purposes of this study, participants should be re-evaluated every 3 cycles or as clinically indicated with CT or MRI of the abdomen/pelvis and chest film or chest CT.

Bone scan - Baseline bone scan will be performed during screening and repeated every 3 cycles (9 weeks) or as clinically indicated.

Biopsy at on Day 21 will be optional during phase I. Patients may opt for biopsy to determine status of DNA repair genes in tumor.

Phase 2:

Patients will be randomized to either Cohort 1 or 2. The planned number of patients in each arm is 20, for a total of 40 patients enrolled in the Phase 2 portion of the study.

Patients will be randomized by permuted blocks and randomization assigned by the Clinical Trials Service to either Cohort 1 or Cohort 2.

Patients in Cohort 1 will receive only docetaxel and carboplatin during Cycle 1 (Cycle 1 Day 1), followed by CT or US guided biopsy on Cycle 1 Day 21. The first dose of Sirolimus at RP2D, will be administered on Cycle 2 Day -2 with docetaxel and carboplatin administered on Cycle 2 Day 1; this dosing scheduled will continue for all subsequent cycles.

Patients in Cohort 2 will receive Sirolimus at RP2D on Day -2 with docetaxel and carboplatin on Day 1 for all cycles. A CT or US guided biopsy will occur on Cycle 1 Day 21.

RP2D of Sirolimus will be administered on Day -2 every 21 days, at least two hours before or after grapefruit juice.

The defined product of grapefruit juice will be ingested once daily for 7 consecutive days, on days -3 through 4. The grapefruit juice dosage will be 8oz (240ml) daily.

Docetaxel and carboplatin will be administered at 60 mg/m2 and AUC 5 on Day 1of a 21 day cycle. Day -2 will occur two days prior to chemotherapy Day 1(there is no Day 0).

Patients will remain on treatment until any of the following occur:

- intolerable toxicity
- documented progression by imaging per RECIST 1.1 criteria
- achieving CR per RECIST 1.1 criteria
- Completion of 10 cycles of therapy. If patients are continuing to respond, additional cycles can be administered at the investigator's discretion.

Response will be defined per Response Evaluation Criteria in Solid Tumors (RECIST 1.1).

RECIST (1.1) – Appendix 5. For the purposes of this study, participants should be re-evaluated every 3 cycles or as clinically indicated with CT or MRI of the abdomen/pelvis and chest film or chest CT.

Bone Scan- Baseline bone scan will be performed during screening and repeated every 3 cycles or as clinically indicated.

4 STUDY POPULATION SELECTION

4.1 Study Population

Patients for the study will be recruited from within the Seattle Cancer Care Alliance (SCCA) and the University of Washington, which are separate practice sites within the same practice and share a single Institutional Review Board (IRB).

Patients will be identified by their oncologist as eligible for the study and the study will be discussed with the patient by the provider. If patients are interested in participating, they will be counseled, and informed consent will be obtained by one of the investigators. A total of 6-18 subjects will be enrolled into the study in Phase 1, and up to 20 patients per cohort will be enrolled in Phase 2.

4.2 Inclusion Criteria

Patients who meet the following inclusion criteria will be eligible to participate in this study:

1. Signed informed consent form (ICF) providing agreement to adhere to the dosing schedule, report for all trial visits and authorization, use and release of health and research trial information.
2. Age ≥18 years.
3. Histologically or cytologically confirmed carcinoma of the prostate (excluding neuroendocrine differentiation or squamous cell histology).
4. Ongoing gonadal androgen deprivation therapy with gonadotropin-releasing hormone (GnRH) analogues, antagonists or orchiectomy. Patients who have not had an orchiectomy must be maintained on effective GnRH analogue/antagonist therapy.
5. Castration resistant prostate cancer as defined by serum testosterone <50ng/ml and at least one of the following:
   - PSA level of at least 2 ng/ml that has risen on at least 2 successive occasions at least 1 week apart.
   - Evaluable disease progression by modified RECIST 1.1.
   - Progression of metastatic bone disease on bone scan with > 2 new lesions.
6. Prior therapy with abiraterone, enzalutamide and/or docetaxel. If a patient has not received docetaxel or cabazitaxel chemotherapy, the patient must be informed of this treatment choice as an alternative. If the patient has received docetaxel or cabazitaxel chemotherapy or refuses one of both of these therapies, this rationale must be documented, and the patient is then eligible. Patient must be offered and made aware of all FDA-approved treatment options. Patients with bone only disease may not have received radium-223.
7. The presence of metastatic disease amenable to CT or ultrasound guided biopsy. This may include thoracolumbar vertebral bodies, pelvis, femur or humerus or soft tissue or nodal metastasis amenable to biopsy (excluding lung or pleural lesions).
8. Agree to participate in biopsy of metastatic lesion during the study at Day 21.
9. Eastern Cooperative Oncology Group (ECOG) Performance Status of ≤ 1.
10. Life expectancy ≥12 weeks.
11. No prior malignancy is allowed except:
    - Adequately treated basal cell or squamous cell skin cancer or
    - In situ carcinoma of any site or
    - Other adequately treated malignancy for which the patient is currently disease

free for at least one year

12. Patients with any prior chemotherapy regimens are eligible.
13. Patients with disease only in the bone may not have received Xofigo/Radium 223 to avoid ongoing DNA damage in bone marrow.
14. Patients who are or are not receiving bisphosphonates or denosumab are eligible. Bisphosphonates or denosumab should not be initiated after registration and during active treatment.
15. Patients must have adequate organ and marrow function as defined below obtained within 14 days prior to registration:
    - Absolute neutrophil count $\geq$1.5 x 10^9 cells/L
    - Hgb $\geq$ 9.0 g/dL
    - Platelets $\geq$100,000 x 10^9/L
    - Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and bilirubin levels $\leq$ 1.5 x ULN
    - Total bilirubin $\leq$ 1.5 x ULN
    - Serum creatinine < 1.5 X institutional ULN mg/DL **OR** eGFR $\geq$ 50 mL/min

All pre-study labs required for determination of eligibility are to **be completed within 14 days prior to Day -2** (or the next business day if falls on a weekend or holiday)**.**

X-rays and/or scans to assess all disease sites **are to be completed within 30 days prior to Day -2** (or the next business day if falls on a weekend or holiday)**.**

4.3 Exclusion Criteria

Patients who meet any of the following criteria will be excluded from the study:

1. Patients currently receiving active therapy for other neoplastic disorders.
2. Patients with histologic evidence of small cell carcinoma of the prostate will not be eligible.
3. Patients with disease only in the bone previously treated with radium-223 will not be eligible
4. Known parenchymal brain metastasis.
5. Active or symptomatic viral hepatitis or chronic liver disease.
6. Estimated creatinine clearance less than 50 ml/minute.
7. Clinically significant heart disease as evidenced by myocardial infarction, or arterial thrombotic events in the past 6 months, severe or unstable angina, or New York Heart Association (NYHA) Class II-IV heart disease.
8. Atrial fibrillation, or other cardiac arrhythmia requiring medical therapy.
9. Administration of an investigational therapeutic within 30 days of Cycle 1, Day -2.
10. Patients with dementia/psychiatric illness/social situations that would limit compliance with study requirements or would prohibit the understanding and/or giving of informed consent.
11. Patients with medical conditions, which, in the opinion of the investigators, would jeopardize either the patient or the integrity of the data obtained will not be eligible.
12. Any condition which, in the opinion of the investigator, would preclude participation in this trial.
13. Patients on anticoagulation therapy which cannot be held for metastatic biopsies.

5 STUDY TREATMENT(S)

5.1 Treatments Administered

5.1.1 Sirolimus

Patients will be instructed to take Sirolimus Oral Solution on study Day -2, two days prior to chemotherapy Day 1 (there is no Day 0), of each 21 day cycle. Sirolimus is to be administered orally in combination with water or orange juice, and can be taken with or without food.

On Day -2 of every cycle a single dose of Sirolimus Oral Solution will be provided by the Institutional Investigational Pharmacy in a dosing syringe. The dosing syringe may be dispensed by the Institutional Investigational Pharmacy up to 24 hours prior to scheduled dosing of Sirolimus. At the time of administration, the Sirolimus Oral Solution will be emptied into only a glass or plastic container and mixed with up to 120ml (4oz) of water or orange juice. This will be stirred vigorously and the patient will drink at once. This will be repeated until the entire contents or Sirolimus Oral Solution has been consumed.

5.1.1.1 Sirolimus risks and precautions

Please refer to the Package Insert for all risks and precautions.

Increased susceptibility to infection and the possible development of lymphoma and other malignancies, particularly of the skin, may result from myelosuppression. Over suppression of the immune system can also increase susceptibility to infection, including opportunistic infections such as tuberculosis, fatal infections, and sepsis.

Hypersensitivity reactions, including anaphylactic/anaphalactoid reactions, angioedema, exfoliative dermatitis, and hypersensitivity vasculitis have been associated with Sirolimus.

Angioedema has been associated with Sirolimus. The concomitant use of Sirolimus with other drugs known to cause angioedema, such as ACE inhibitors, may increase the risk of developing angioedema.

Fluid accumulation and wound healing: There have been reports of impaired or delayed wound healing in patients receiving Sirolimus, including lymphocele and wound dehiscence. Patients with a body mass index greater than 30kg/m^2 may be at increased risk of abnormal wound healing based on data from medical literature. There have also been reports of fluid accumulation, including peripheral edema, lymphedema, pleural effusion, ascites, and pericardial effusions in patients receiving Sirolimus.

Hyperlipidemia: There were increased incidences of hypercholesterolemia (43-46%) and/or hypertriglyceridemia (45-57%) in patients receiving Sirolimus compared with placebo (each 23%).

Renal Function: Renal function should be closely monitored with administration of Sirolimus because long term administration with Sirolimus with cyclosporine has been associated with deterioration of renal function.

Interstitial Lung Disease: Cases of interstitial lung disease, some fatal, with no identified infectious etiology have occurred in patients receiving immunosuppressive regimens including Sirolimus.

Increased risk of Calcineurin Inhibitor-Induced Hemolytic Uremic Syndrome/ Thrombotic Thrombocytopenic Purpura/ Thrombotic Microangiopathy (HUS/TTP/TMA): The concomitant use of Sirolimus with a calcineurin inhibitor may increase the risk of HUS, TTP or TMA.

Skin Cancer Events: Patients on immunosuppressive therapy are at increased risk for skin cancer, including squamous cell carcinoma, basal cell carcinoma, and malignant melanoma of the skin. Exposure to sunlight and ultraviolet light should be limited by wearing protective clothing and using sunscreen with a high protection factor.

The most common (≥ 20%) adverse reactions observed with Sirolimus in clinical studies are:

- peripheral edema (54-58%)
- edema (18-20%)
- hypertriglyceridemia (45-57%)
- hypertension (45-49%)
- hypercholesterolemia (43-46%)
- creatinine increased (39-40%)
- constipation (36-38%)
- abdominal pain (29-36%)
- diarrhea (25-36%)
- headache (34%)
- fever (23-34%)
- urinary tract infection (26-33%)
- anemia (23-33%)
- nausea (25-31%)
- arthralgia (25-31%)
- pain (29-33%)
- thrombocytopenia (14-30%)
- acne (22%)
- rash (10-11%)

The following adverse reactions resulted in a rate of discontinuation of > 5% in clinical trials:

- creatinine increased
- hypertriglyceridemia
- thrombotic thrombocytopenic purpura (TTP)

5.1.2 Docetaxel and carboplatin

Docetaxel and carboplatin will be administered per Standard Practice Guidelines.

- Docetaxel 60 mg/m^2 will be administered on Day 1 of a 21 day cycle.
- Carboplatin AUC 5 will be administered on Day 1 of a 21 day cycle.

5.1.2.1 Docetaxel and carboplatin risks and precautions

Please refer to the Package Insert for docetaxel and carboplatin for all risks and precautions. The following are risks when docetaxel and carboplatin have been used in combination.

	Grade 3	Grade 4
Anemia	**6%**	**0**
Neutropenia	**56%**	**6%**
Thrombocytopenia	**6%**	**0**
Febrile neutropenia	**3%**	**0**
Infection	**6%**	**0**
Hyperglycemia	**6%**	**0**
Pain	**6%**	**0**
Renal insufficiency	**3%**	**0**

5.1.3 Grapefruit Juice

The grapefruit juice will be provided to the patient by the study. Eight ounces (240ml) of grapefruit juice will be ingested once daily for 7 consecutive days, on Days -3 through 4 of every 21 day cycle.

5.1.4 Antiemetics

Dose modifications for nausea and vomiting should not be made until patients are on adequate doses of antiemetics. Chemotherapy regimens which include docetaxel and carboplatin are categorize as moderately emetogenic, with a frequency of emesis 30-60%. Institutional guidelines for moderately emetogenic chemotherapy should be followed.

5.1.5 Growth Factors

Filgrastim (GCSF), pegfilgrastim, sargramostim (GM-CSF), and other growth factors may be utilized at the discretion of the investigator. All growth factors must be recorded as concomitant medication. The National Comprehensive Cancer Network (NCCN) guidelines for use of growth factors and prophylactic antibiotics will be followed based on patient risks of neutropenia and infection [20].

5.1.6 Concomitant Therapy

The use of any concurrent drug from screening and while on study, prescription or over-the-counter, is to be included in the clinic note from study visits.

Concurrent enrollment in another clinical investigational drug or device study is prohibited. Supportive care medications are permitted with their use following institutional guidelines.

The following supportive care medications are considered permissible during the study:

- Conventional multivitamins
- Selenium
- Soy supplements

If the permissibility of a specific drug/treatment is in question, please contact the study investigator-sponsor.

5.1.7 Restrictions

The concurrent administration of other anticancer therapy, including cytotoxic or immunotherapy, is prohibited therapy. Use of other investigational drug therapy for any reason is prohibited. The decision to administer a prohibited drug/treatment will be made by the investigator based on the consideration of the safety of study participant.

5.1.8 Potential for Drug-Drug Interactions

Sirolimus is known to be a substrate for both cytochrome P-450 3A4 (CYP3A4) and p-glycoprotein (P-gp). Inducers of CYP3A4 and P-gp may decrease Sirolimus concentrations whereas inhibitors of CYP3A4 and P-gp may increase Sirolimus concentrations.

Co-administration of Sirolimus with strong inhibitors of CYP3A4 (such as ketoconazole, voriconazole, itraconazole, erythromycin, telithromycin, or clarithromycin) or strong inducers of CYP3A4 (such as rifampin or rifabutin) should be avoided.

Additional inducers and inhibitors of CYP3A4 and P-gp:

- Drugs that could increase Sirolimus blood concentrations: Bromocriptione, cimetidine, cisapride, clomitrazole, danazol, diltiazem, fluconazole, protease inhibitors, nicardipine, troleandomycin, verapamil

- Drugs that could decrease Sirolimus blood concentrations: Carbamazepine, phenobarbital, phenytoin, rifapetine, St. John's Wort

- Drugs with concentrations that could increase when given with Sirolimus: Verapamil

Immunosuppressants may affect response to vaccination. Therefore, during treatment with Sirolimus vaccination may be less effective. The use of live vaccines should be avoided.

5.1.9 Potential Drug Interactions with Grapefruit Juice

Grapefruit interacts with medications via several mechanisms, most notably by inhibition of CYP3A enzymes in the small bowel wall. Co-administration of grapefruit juice with medications known to be metabolized by the CYP3A pathway will be reviewed by the study PI. Medications deemed by the study PI to not be safely administered with grapefruit juice will be discontinued prior to starting therapy on study.

5.1.10 Contraception

Participants must agree to the use of an effective method of contraception. This should be started from the signing of the informed consent and continue throughout period of study treatment until at least 30 days after the last dose of sirolimus, docetaxel and carboplatin.

5.2 Supply, Packaging and Storage

5.2.1 Sirolimus Pharmacy Storage Requirements

Sirolimus Oral Solution will be provided to the Institutional Investigational Pharmacy, packaged for dispensing on a per patient basis. Patients will be provided with a single dose of Sirolimus in a dosing syringe, which may be dispensed up to 24 hours prior to scheduled dosing of Sirolimus on Day -2.

Storage: Oral Solution bottles should be stored protected from light and refrigerated at 2°C to 8°C (36°F to 46°F). If necessary, bottles may be stored at room temperatures upto 25°C (77°F) for up to 15 days. Once the bottle is opened, the contents should be used within one month.

The product may be kept in the dosing syringe for a maximum of 24 hours at room temperatures up to 25°C (77°F) or refrigerated at 2°C to 8°C (36°F to 46°F).

5.2.2 Docetaxel and Carboplatin Supply, Packaging, Storage

Docetaxel and carboplatin are stored and administered per institutional protocol.

5.2.3 Grapefruit Juice Supply, Packaging, Storage

Grapefruit juice will be supplied in the form of frozen juice concentrate. Individual cans of frozen grapefruit juice concentrate will be stored in an onsite freezer at or below 0º C. Patients will be instructed to maintain concentrate in a frozen state until instructed to drink the grapefruit juice.

5.3 Dose modifications for toxicity

On day 1 of a cycle	Dose
ANC ≥1.5 x10^9 cells /L and platelet count ≥100 x 10^9 /L	100%
ANC <1.5 x10^9 cells /L and/or platelet count <100 x 10^9 /L	Delay Docetaxel and carboplatin. Repeat CBC weekly. If ANC resolves to ≥1.5 x10^9 cells /L and platelet count to ≥100 x 10^9/L, resume docetaxel and carboplatin at 100% of present dose. If treatment is delayed by more than 4 weeks, the patient should be removed from chemotherapy treatment.
Alkaline phosphatase 2.5-5X ULN (and no bone metastases) or AST/ALT 1.5-5X ULN	Decrease docetaxel dose to 75%
Alkaline phosphatase >-5X ULN (and no bone metastases) or AST/ALT > 5X ULN or total bilirubin > ULN	Hold therapy until less than grade 1 and restart at previously administered dose
At any time during a cycle	
Grade 3 febrile neutropenia (defined as an ANC <1.0 x 10^9 cells/L) and a single Temperature of >38.3°C or a sustained temperature of ≥38°C for more than one hour) **Documented infection with Grade 3 neutropenia (defined as an ANC <1.0 x 10^9 cells/L)**	Delay treatment until improvement of symptoms and resolution of the ANC to ≥1.5 x10^9 cells /L and platelet count to ≥100 x 10^9/L. Decrease docetaxel and carboplatin to 75% starting dose for all subsequent cycles.
Grade 4 neutropenia (defined as an ANC <0.5 x 10^9 cells/L lasting more than 5 days	With recurrence of any of these toxicities, the patient should be removed from chemotherapy

Treatment may be delayed for other hematological and non-hematological reasons at the discretion of the treating physician and/or PI. Treatment may also be delayed for situations other than treatment-related adverse events such as medical/surgical events not related to therapy.

5.3.1 Dose modifications for gastrointestinal toxicity

For Grade 4 nausea and vomiting **despite maximal antiemetic therapy**, delay docetaxel and carboplatin until symptoms have resolved to ≤ Grade 2. When symptoms have resolved to ≤ Grade 2, continue docetaxel and carboplatin at 100% of the present dose. If Grade 4 nausea and vomiting recur despite maximal antiemetic therapy, the patient should be removed from chemotherapy.

5.3.2 Dose modifications for Sirolimus

There will be no dose modification of Sirolimus in phase 2. In chronic dosing of Sirolimus dose adjustment is performed to maintain blood levels within defined parameters. This is not necessary with single dose administration.

Any non-hematological toxicity, grade 4 lasting > 5 days and considered related to Sirolimus, will result in dose reduction of Sirolimus by one level at next dosing cycle and all subsequent cycles. If toxicity does not resolve to grade ≥2 or subsequent non-hematological toxicity grade 4 lasting > 5 days considered related to Sirolimus occurs a second dose reduction of Sirolimus will occur.

6 STUDY PROCEDURES

6.1 Informed Consent

Written Informed Consent and Authorization must be obtained from the patient in accordance with local practice and regulations. The study will be discussed with the patient, and a patient wishing to participate must give written informed consent and authorization for use and release of health and research study information prior to any study-related procedures or change in treatment.

A signed, Institutional Review Board/Ethics Committee (IRB) approved, informed consent must be obtained from patients before any study specific procedures can occur. Confirmation of the patient's informed consent and the informed consent process must also be documented in the patient's medical record.

Explanation of Genomic Results:

Recent work from the Stand Up to Cancer (SU2C) Prostate Cancer Metastasis sequencing project and currently in press show that 20% of CRPC tumors contain biallelic inactivation of DNA damage repair genes, including BRCA1, BRCA2, ATM, ATR, FANC and RAD51. Of these tumors, half have an associated deleterious germline mutation as the cause of inactivation of one allele, with copy loss of the other allele as the cause of homozygous inactivation. Therefore 10% of men with CRPC may have a deleterious germline mutation in BRCA1, BRCA2 or other DNA repair gene which would

put them or other family members at risk for malignancy. A prospective objective of the current study is to determine status of these genes as a predictor of response to docetaxel/carboplatin and Sirolimus.

We consider that patients and family members, who are dealing with the difficult situation of advanced cancer, might prefer to focus on care of their disease rather than receive extraneous information that could be perceived as overwhelming and distracting. We do not have a proven, evidence-based way to implement the informed consent regarding patients' disclosure option preferences yet, we must adopt a reasonable practice in order to conduct this study.

The proposed solution has been to adopt a "provisional" model, pioneered at the University of Michigan and which is currently a component of the SU2C protocol in place at UWMC/FHCRC that is based on the following considerations (Table 4). First, from the patient's perspective, it makes sense to distinguish between results that inform management of the patient's specific cancer ("Cancer of interest") and those incidental results which may affect the patient's and/or their family member's risks of other conditions ("Conditions other than cancer of interest"). Patients should be offered results based on best clinical judgment (i.e., offered a default option), but their preferences regarding incidental findings ought to be respected when possible (i.e., the default can be changed). This is called the Flexible-Default Model of Informed Consent.

Table 4: Provisional Informed Consent: Flexible Default Model

Disease Domain	Impact/Significance	Default	Decline Results?	Description
Cancer of Interest	Direct impact on care of current cancer	Disclose	Not flexible	Marketed treatment available Targeted clinical trial available
	Significance for biological family	Disclose	Flexible	Increased risk of cancer for biological family
	Significance is unknown	Not disclose	Not flexible	Mutation function or role unknown
Conditions other than cancer of interest	Potential medical impact	Disclose	Flexible	Clinically significant relative risk of disease or outcomes
	Significance for biological family	Disclose	Flexible	Significant implications for biological family decisions
	Significance is unknown	Not disclose	Not flexible	Mutation function or role unknown

Flexible-Default Model of Informed Consent:

Patients will be given the option to decline certain results. Ultimately, patients should be offered results about their cancer based on best clinical judgment (i.e., default), but their preferences regarding incidental findings ought to be respected when possible (i.e.,

flexible). Therefore, the default consent is to disclose results to patients and clinicians for results related to "Cancer of interest", which we designate as "not flexible," since it is anticipated that patients who consent will expect these results. The default consent for "Conditions other than cancer of interest" is to disclose results. For the remaining categories of results that considered "flexible", patients will be given an option to share their preferences and say "Yes" or "No" to these other results before they begin the study. The default for these categories is disclosure. However, if patients prefer, they may decline such results at the time of consent. They will be asked for their preference for two simplified categories: A) "Results that may have significance for biological family members" and B) "Results that are not related to your cancer, but may have potential medical impact for you".

A study investigator will assist the patient in completing this section of the informed consent. Categorization of findings is performed by the current Precision Tumor Board at UWMC/SCCA which meets monthly to review Oncoplex results and SU2C sequencing results.

6.2 Medical History

Medical history, such as previous treatments, procedures, and conditions will be collected during the screening period.

6.3 Physical Examination

Evaluations should be performed as below whenever possible.

Physical examination to include HEENT (head, eyes, ears, nose, and throat), chest, cardiac, abdominal, extremities, neurologic, and lymph node examinations.

Vital signs include blood pressure, heart rate and temperature on Day 1 of each cycle. Weight will be determined on Day 1 of each cycle. Height will be assessed at screening visit only.

6.4 Laboratory Tests

6.4.1 Clinical Safety Laboratory Tests

Phase 1: During the Phase 1 portion of the study clinical safety labs will be collected at the following time points:

- Screening
- Day -2 (± 2 days) prior to Sirolimus dosing
- Days 8 & 15 (± 4 days)
- Termination Visit

Phase 2: During the Phase 2 portion of the study clinical safety labs will be collected at the following time points:

- Screening
- Day -2 (± 2 days) prior to Sirolimus dosing
- Termination Visit

If clinical safety labs are drawn within 2 days prior to Day 1 (i.e., Day -2), and patient labs qualify patient to safely receive chemotherapy at standard dosing, clinical safety labs do not have to be repeated on Day 1. However, clinical safety labs may be repeated on Day 1 at the PI or provider's discretion or if treatment dose reduction is necessary.

Clinical safety labs will include the following:

Table 9. List of Clinical Safety Laboratory Tests

Hematology:

Hematocrit (Hct)
Hemoglobin (Hgb)
Platelet count
Red blood cell (RBC) count
White blood cell (WBC) count with differential

Serum Chemistry:

Albumin (ALB)
Blood urea nitrogen (BUN)
Calcium (Ca)
Carbon dioxide (CO_2)
Chloride (Cl)
Creatinine
Glucose
Potassium (K)
Sodium (Na)

Liver Functions:

Alanine aminotransferase (ALT; SGPT)
Aspartate aminotransferase (AST; SGOT)
Alkaline phosphatase (ALK-P)
Total bilirubin

6.4.2 Safety Labs Prior to Metastatic Biopsy

Coagulation tests or any additional laboratory tests will be collected per institutional policy prior to metastatic biopsy on Day 21.

6.4.3 Tumor Markers

PSA level will be assessed during the screening period, on Day -2 of every cycle and at the Termination Visit.

6.4.4 Research Labs

Please refer to the laboratory manual for details.

6.4.5 Sample Collection, Storage, and Shipping

The institutional laboratories at the SCCA and University of Washington (UW) will analyze all hematology, blood chemistry samples collected for the study. Samples will be analyzed at a facility meeting Good Laboratory Practice (GLP) requirements and/or using methods documented in a methods validation report.

6.4.6 Biopsy acquisition and tissue handling

Patients will undergo a CT guided or US guided biopsy of a metastatic lesion on Day 21(±4 days, but prior to start of next cycle). An optional repeat biopsy may be performed if the patient consents in order to assess whether suppression of DDSP is lost after initial mTOR inhibition or to identify additional potential mechanisms of resistance to mTOR targeting. If the patient undergoes second optional biopsy (any time after initial biopsy), the biopsy site does not have to be the same location at each time point. Issues that would cause biopsy delay must be discussed with study PI. Biopsy tissue acquisition procedure should be followed per the laboratory manual.

A formalin section of biopsy is evaluated under H and E for tumor content. Less than 20% of tumor content makes the sample inevaluable. This is the standard used to assess the suitability of a tumor biopsy in the ongoing SU2C/AACR from which we recently published the results of the first 150 patients (Robinson, Cell, 2015; 161 (5): 1215). The UWMC site contributed more biopsies than any other site. Based on our experience, we anticipate that 80-90% of biopsies will be informative. The statistics include the potential that 10% of biopsies will not be informative (4 additional patients).

Please refer to the laboratory manual for detailed instructions regarding acquisition and handling of tissue specimens.

6.5 Criteria for Evaluation

6.5.1 Safety: Phases 1 and 2

- The number and percentage of patients who experience a Serious Adverse Event;
- The number and percentage of patients who experience an Adverse Event; and
- The number and percentage of patients who discontinue study drug treatment early due to an adverse event.

Safety will be assessed through summaries of adverse events, vital signs, physical examinations, and clinical laboratory test data (including change from baseline).

Safety analyses will include all enrolled patients who receive any amount of study drug (safety population).

Grade 4 hematological laboratory values and grade 3-4 non-hematological AEs will be collected and classified by the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE v. 4.0).

6.5.2 Anti-Tumor Effects: Phase 2

- The percentage of patients achieving at least a 50% reduction in PSA according to PCWG2 criteria;
- The percentage of patients achieving at least a 90% reduction in PSA according to the PCWG2 criteria.
- The percentage of patients achieving a 30% reduction in measurable disease by RECIST 1.1.

6.5.3 Tissue Endpoints: Phase 2

Immunohistochemistry (IHC) and quantitative polymerase chain reaction (PCR) will be used to evaluate microenvironment damage response gene products and proteins in the resected tumor/tissue. IHC will be performed and evaluated using antibodies to IL6, WNT16B and SFRP2 and will assess mTOR blockade using antibodies to S6. Immunohistochemical assays of tumor and microenvironment damage will quantitate 53BP1 and gammaH2AX expression.

Quantitative reverse transcription PCR will quantitate microenvironment damage-responsive transcripts, and attendant responses within tumor cells. Panels of microenvironment transcripts will be quantitated including WNT16B, SPINK1, IL6, MMPs, and Amphiregulin. Tumor cell transcripts will include Ki67, p16, p27, Myc and EMT markers including vimentin and Snail.

Immune cell infiltration in the tumor microenvironment will be assessed by IHC or flow

cytometry of white blood cell types: CD45 as a general marker of leukocytes; CD68 for monocyte/macrophages; CD3, CD4, CD8 for T cells.

6.6 Safety Assessments

All participants receiving investigational agents will be evaluated for safety. The safety parameters include laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported to the investigator by participants. Grade 4 hematological laboratory values and grade 3-4 non-hematological AEs encountered during the study will be evaluated according to the NCI criteria specified in the protocol and recorded prior to each course of therapy and reported in accordance with local reporting requirements. Life-threatening toxicities should be reported immediately to the Protocol Chair and Institutional Review Board (IRB).

Safety assessments will include:

- Adverse events including laboratory adverse events will be graded according to the NCI CTCAE, version 4.0.
- Laboratory tests (CBC with differential, platelets, LFT's, chemistry)
- Vital Signs (blood pressure, heart rate, temperature and weight)
- Physical exam
- ECOG performance status

Please see section 8.4 concerning safety evaluation information.

6.7 Safety Data Collection, Recording and Reporting

The PI of the study will have primary responsibility for ensuring that the protocol is conducted as approved by the FHCRC/UW Cancer Consortium Scientific Review Committee and Institutional Review Board. The PI will ensure that the monitoring plan is followed, that all data required for oversight of monitoring are accurately reported to the FHCRC/UW Cancer Consortium Data and Safety Monitoring Committee (DSMC), that all adverse events are reported according to the protocol guidelines, and that any adverse reactions reflecting patient safety concerns are appropriately reported.

Under the provisions of the FHCRC/UW Cancer Consortium Data and Safety Monitoring Plan (DSMP), Cancer Consortium Clinical Research Support (CRS) provides monitoring for quality process and compliance by qualified monitors unaffiliated with the conduct of the study. Monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of the previous visit as described in the Cancer Consortium DSMP."

All observed or volunteered Grade 3 and 4 adverse events regardless of causal relationship to study drug will be recorded on the adverse event page(s) of the case report form (CRF).

6.7.1 Definition of Adverse Event (AE)

An adverse event is defined in the International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice as "Any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product and which does not necessarily have to have a causal relationship with this treatment" (ICH E6:1.2). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, diagnosis or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product. For the purposes of this study, temporal association is defined as the time between the subject's informed consent signature through 28 days after the final dose of study medication.

AEs further include worsening of a pre-existing medical condition (e.g., diabetes, migraine headaches, gout, hypertension, etc.) which has increased in severity, frequency or duration, or is associated with significantly worsened outcomes.

The investigator or a medically licensed designee must pursue and obtain information adequate to determine the following: Grade (CTCAE v 4.0), Causality (relationship to docetaxel and carboplatin and/or Sirolimus) and Outcome. The investigator's assessment of Grade, any Intervention (medication, procedure, etc.), Causality and Outcome will be indicated by signature of the PI or designated physician on the adverse event CRF. For adverse events with a causal relationship to the investigational product, follow-up by the investigator is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator, or his/her designated representative.

Attribution is defined as the determination of whether an adverse event is related to a medical treatment or procedure. Attribution categories are as follows:

- *Unrelated* The adverse event is clearly NOT related to therapy
- *Unlikely* The adverse event is doubtfully related to therapy
- *Possible* The adverse event may be related to therapy
- *Probable* The adverse event is likely related to therapy
- *Definite* The adverse event is clearly related to therapy

6.7.2 Adverse Drug Reaction (ADR)

A noxious and unintended response to any dose of the drug (or biological) product for which there is a reasonable possibility that the product caused the response. ''Reasonable possibility'' means there is evidence to suggest a causal relationship between the drug and the adverse event.

Suspected adverse reaction is defined as any adverse event for which there is a

reasonable possibility that the drug caused the adverse event (21 CFR 312.23).

Suspected Unexpected Adverse Event Reaction (SUSAR) is defined as an AE that is not consistent in nature, severity, or frequency with the product information documented in the current package insert or in the protocol, consent form, and/or prior reports.

If the investigator or designee determines that an AE meets the criteria for classification as a Serious Adverse Event (SAE) or Suspected Unexpected Adverse Event Reaction (SUSAR), s/he will immediately notify the UW/FHCRC Cancer Consortium IRB, and FDA. See Section 6.7.3 for definition of SAEs and SUSARs, and 6.7.7 for reporting guidelines.

Interventions for pretreatment conditions (e.g., elective cosmetic surgery) or medical procedures that were planned before study enrollment are not considered adverse events.

6.7.3 Definition of a Serious Adverse Event

An adverse event or suspected adverse reaction is considered ‘‘serious’’ if, in the view of the investigator or a medically licensed designee, it results in any of the following outcomes:

- Death;
- a life-threatening adverse event;
  Life-threatening adverse event or life-threatening suspected adverse reaction.
  An adverse event or suspected adverse reaction is considered ‘‘life-threatening’’ if its occurrence places the patient or subject at immediate risk of death. This definition does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death;
- inpatient hospitalization or prolongation of existing hospitalization;
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- a congenital anomaly/birth defect;
- is a suspected transmission of infectious agents by a medicinal product.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

A hospitalization meeting the definition for “serious” is any in-patient hospital admission that includes a minimum of an overnight stay in a health care facility. Interventions for pretreatment conditions (e.g., elective cosmetic surgery) or medical procedures

that were planned before study enrollment are not considered SAE/SUSARs for the purposes of this study. Inpatient admission does not include admissions to rehabilitation facilities, hospice facilities, skilled nursing facilities, nursing homes, routine emergency room admissions, same day surgery (as outpatient/same day/ambulatory procedures) or social admission (e.g., subject has no place to sleep).

6.7.4 Dose Limiting Toxicity (DLT)

DLT is defined as drug-related febrile neutropenia, grade ≥ 3 non-hematological toxicity or grade 4 hematologic toxicity lasting > 5 days, occurring during the phase 1 component of the study. Only those events which occur in cycle 1 will be considered a DLT.

6.7.5 Dose De-escalation

The planned dose de-escalation in phase 1 varies the dose of Sirolimus administered on day -2 with docetaxel and carboplatin 60 mg/m2 and AUC 5 on day 1every 21 days.

The Sirolimus dose in dose level 0 is 35 mg once on Day -2. If zero or one DLT's occur in the first three patients, an additional 3 patients will be treated in dose level 0. If the observed DLT rate is ≤ 33% in six patients, dose level 0 will be the RP2D. Patients may continue in their cohort for repeated cycles of dosing; however, dose de-escalation within a patient will not occur. If the DLT rate is > 33% in 3 or 6 patients, the dose will be decreased to dose level -1, 25 mg once per 21 day cycle on Day -2. The same cohort algorithm will occur at dose level -1 as above. If DLT rate is > 33% is seen at dose level
-1 at 3 or 6 patients, the dose level will decrease to dose level -2 at 20 mg once per 21 day cycle on Day -2. If DLT rate is > 33% at dose level -2, no phase 2 will be carried out.

Dose de-escalation values:

Dose Level	**Sirolimus**
100% (Dose Level 0)	**35mg**
First dose reduction (Dose Level - 1)	**25mg**
Second dose reduction (Dose Level -2)	**20mg**

6.7.6 Reporting Procedures for Adverse Events

The investigator is responsible for ensuring that all non-serious adverse events (as defined in Section 6.7.1 and as further specified below) observed by the investigator or reported by subjects are collected and recorded in the CRF. Source documents may include the subjects' medical records, patient diaries or study-specific worksheets. Recording for all events should be done in a concise manner using standard,

acceptable medical terms.

Preexisting conditions that worsen in severity or frequency during the study should also be recorded (a preexisting condition that does not worsen is not an adverse event). Further, a procedure or surgery is not an adverse event; rather, the event leading to the procedure or surgery is considered an adverse event. Any event requiring unplanned in- patient hospitalization that occurs during the course of a subject's participation in a trial must be reported as an SAE, as previously stated.

If a specific medical diagnosis has been made, that diagnosis or syndrome should be recorded as the adverse event, whenever possible. However, a complete description of the signs, symptoms and investigations which led to the diagnosis should be provided. For example, if clinically significant elevations of liver function tests are known to be secondary to hepatitis, "hepatitis" and not "elevated liver function tests" should be recorded. If the cause is not known, the abnormal test or finding should be recorded as an adverse event, using appropriate medical terminology (e.g., thrombocytopenia, peripheral edema, QT prolongation).

For all adverse events, sufficient information should be obtained by the investigator to determine the causality of the adverse event (e.g., study drug, other illness, progressive malignancy, etc.). The relationship of the adverse event to the investigational product will be assessed by means of the question, "Is there a reasonable possibility that the event may have been caused by the investigational product?" The investigator should respond to this question with either Yes or No.

6.7.7 Reporting Procedures for Serious Adverse Event (on-site SAEs)

New SAEs will be collected and recorded throughout the study period, from the signing of the informed consent through 28 days after the final dose of study treatment. Ongoing SAE/SUSARs with a causal relationship to the investigational product will be followed until the event or its sequelae resolve or stabilize at a level acceptable to the investigator or designee.

6.7.8 Reporting to IRB

All serious adverse events that occur after the subject has signed the informed consent form or during the study at the University of Washington or Seattle Cancer Care Alliance must be reported to the Cancer Consortium IRB. AEs that are unexpected, possibly related to the study drug, and serious or suggest a risk of greater harm from the research then previously known will be reported to the IRB within 10 calendar days of the Investigator's awareness of the event.

SUSARs will be reported on the Expedited Reporting Form for Unanticipated Problems or Noncompliance.

Reports of SAEs should be signed and dated by the principal investigator. In the absence of the PI, reports should be signed and dated by the individual reporting the event. If s/he is not medically licensed, the report should also be signed by a licensed medical practitioner, preferably a sub-investigator for this protocol. The PI will review and sign the report at the next opportunity.

Each report should contain the following information:

- Protocol number
- Subject number
- Disease/histology, if applicable
- Date the event occurred
- Description of the SAE
- Relationship of the event to treatment or other causality
- Whether the event was “expected’
- Severity of the event
- Intervention
- Outcome of the event
- Detailed text that includes the following information:
  - An explanation of how the SAE was handled
  - A description of the patient’s condition
  - Indication whether the subject remains on study
  - Recommendation whether an amendment will need to be made to the protocol and/or the consent form.

Relevant, redacted medical records should be provided as soon as they become available; autopsy reports should be provided for deaths if available. Determination of expectedness will be based on the contents of the current package insert.

If a subject is permanently withdrawn from the study because of a serious adverse event, this information must be included in the End of Study Case Report Form as well as all SAE/SUSAR reports.

6.7.9 Annual Safety Reports

In addition to the expedited reporting, PI shall submit, once a year throughout the clinical trial or on request a safety report to the competent authority and InstitutionalReview Board, taking into account all new available safety information received during the reporting period.

6.8 Criteria for Discontinuation of Study

- The patient develops an unacceptable toxicity, refer to Sections 6.7.3 & 6.7.4.
- Disease progression per RECIST 1.1 criteria, refer to Appendix 5.
- The patient develops a concurrent illness that is a contraindication to receiving further treatment.
- The patient elects to discontinue treatment or withdraws consent.
- At the discretion of the Investigator.

6.9 Withdrawal from Study

The investigator may withdraw a patient from any phase of the study for any of the following reasons.

- Discontinuation of treatment criteria as defined in Section 6.8

- Sustained Side Effects: Patients who have sustained toxicities, such as hyperglycemia or hypertension that do not return to NCI CTCAE (version 4.0) Grade 1 or less with appropriate medical management, should be discontinued from the study treatment.

- Administration of prohibited medications: The patient will be discontinued from the protocol treatment when prohibited drug is administered. Supportive care medications are permitted with their use following institutional guidelines. The concurrent administration of other anticancer therapy, or immunotherapy is prohibited during study treatment Phase. Use of other investigational drug therapy for any reason is prohibited.

- Patient withdraws consent. In this event, the reason(s) for withdrawal must be documented and clarification if withdrawal of consent includes follow-up phase for progression data collection. A patient's decision to take part in the study is voluntary and he may choose not to take part in the study or to stop taking part at any time. If he chooses not to take part or to stop at any time, it will not affect his future medical care or medical benefits.

If a subject terminates the study early, an Early Termination visit will be performed.

7 STUDY ACTIVITIES

7.1 Study Visit Overview

Expected toxicities and potential risks as well as dose modifications for Sirolimus, docetaxel and carboplatin are described in Section 5 (Risks and Precautions and Dose Reductions). No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

7.2 Screening Period

Patients for the study will be recruited from sites from within the SCCA and UW. Patients will be identified as eligible for the study and the study will be discussed with the patient by their provider. If patients are interested in participating, they will be counseled and informed consent will be obtained by one of the investigators.

Screening procedures to be completed within 30 days prior to the start of study treatment, Day -2:

- Informed consent
- Medical history and demographics
- Concurrent illness
- Physical examination, including weight and height
- Vital signs including blood pressure, heart rate, temperature.
- Assessment of ECOG Performance Status
- Clinical laboratory tests:
    - CBC (WBC with differential count, RBC, hemoglobin, hematocrit, platelets)
    - Liver function tests (AST, ALT, alkaline phosphatase, total bilirubin),
    - Serum chemistries (albumin, sodium, potassium, chloride, carbon dioxide, creatinine, BUN, glucose, calcium)
    - PSA
    - Testosterone
- Bone scan
- CT or MRI of the abdomen/pelvis
- Chest film or chest CT
- Concomitant medications listing:
  Obtain a complete and thorough listing of all prescription and nonprescription (over the counter) medications currently taken including pain medications. This also includes any nutritional supplements and/or herbal preparations.

7.3 Treatment Period

7.3.1 Day -2

Patients will complete all Day -2 tests and assessments prior to Sirolimus Oral Solution dosing. Day -2 occurs two days prior to Day 1 chemotherapy infusion (there is no Day 0).
Grapefruit juice will be consumed on Days -3 through 4, as described in Section 5.1.3. for each cycle administered with Sirolimus.

The following procedures should be completed on Day -2 (± 2 days):

- Physical exam
- Vital signs (blood pressure, heart rate, temperature, and weight)
- ECOG performance status

- PSA
- CBC (WBC with differential count, RBC, hemoglobin, hematocrit, platelets)
- Liver function tests (AST, ALT, alkaline phosphatase, total bilirubin)
- Serum chemistries (albumin, sodium, potassium, chloride, carbon dioxide, creatinine, BUN, calcium, glucose)
- Research blood draw (Refer to lab manual for details)
- Concomitant Medications
- Adverse Events
- Administer Sirolimus Oral Solution*

*Patients enrolled in Phase 2 portion of the study will be randomized to either Cohort 1 or Cohort 2. Sirolimus dosing on Day -2 for each cohort is described below:

Cohort 1: Patients randomized to Cohort 1 will receive first dose of Sirolimus Oral Solution on Cycle 2 Day -2 after the metastatic biopsy is complete, and will receive Sirolimus on Day -2 for all subsequent cycles. Patients will not receive Sirolimus during Cycle 1.

Cohort 2: Patients randomized to Cohort 2 will receive first dose of Sirolimus Oral Solution on Cycle 1 Day -2 prior to metastatic biopsy, and will receive Sirolimus on Day -2 for all subsequent cycles. Patients will receive Sirolimus for all cycles.

Dosing and administration of Sirolimus is discussed in Section 5.1.1.

7.3.2 Day 1

The following procedures should be completed on Day 1 (± 2 days):

- Patients will be evaluated for compliance
- Vital signs (blood pressure, heart rate, temperature)
- CBC (WBC with differential count, RBC, hemoglobin, hematocrit, platelets)**
- Liver function tests (AST, ALT, alkaline phosphatase, total bilirubin)**
- Serum chemistries (albumin, sodium, potassium, chloride, carbon dioxide, creatinine, BUN, calcium, glucose)**
- Concomitant Medications
- Adverse Events
- Administer docetaxel and carboplatin

** If clinical safety labs are drawn within 2 days prior to Day 1 (i.e., Day -2), and patient labs qualify patient to safely receive chemotherapy at standard dosing, clinical safety labs do not have to be repeated on Day 1. However, clinical safety labs may be repeated on Day 1 at the PI or provider's discretion or if treatment dose reduction is necessary.

Dosing and administration of docetaxel and carboplatin is discussed in Section 5.1.2.

7.3.3 Days 8 & 15 (Phase 1 only)

During Cycle 1 of the Phase 1 portion of the study, patients will get weekly safety labs while receiving treatment. Additional safety labs will be drawn on Days 8 and 15 (± 4 days). Additionally weekly safety labs during subsequent cycles may be drawn per the discretion of the PI and/or sub-investigators.
The following procedures should be completed on Days 8 & 15 (± 4 days):

- CBC (WBC with differential count, RBC, hemoglobin, hematocrit, platelets)
- Liver function tests (AST, ALT, alkaline phosphatase, total bilirubin)
- Serum chemistries (albumin, sodium, potassium, chloride, carbon dioxide, creatinine, BUN, calcium, glucose)
- Concomitant Medications
- Adverse Events

7.3.4 Additional visits

A CT or US guided metastatic biopsy will occur on Day 21. In the Phase 1 portion of the study the biopsy on Day 21 is optional. In the Phase 2 portion of the study the biopsy on Day 21 is mandatory. There is an opportunity for an additional biopsy during the Phase 2 study if the patient opts for this (in addition to the required biopsy on Day 21).

Research labs are optional for the Phase 1. Research labs will be drawn during the Phase 2 portion of the study. Please refer to lab manual and for details.

CT or MRI of the abdomen/pelvis and Chest film or chest CT will be performed as outlined below, or as clinically indicated.

- Phase 1: CT or MRI imaging will be repeated every 3 cycles
- Phase 2: CT or MRI imaging will be repeated every 3 cycles

A bone scan will be performed as outlined below or as clinically indicated.

- Phase 1: Bone scan will be repeated every 3 cycles
- Phase 2: Bone scan will be repeated every 3 cycles

7.3.5 Termination Visit (28 days, ± 7 days, from withdrawal of protocol)

All patients who receive study drug must have a termination visit within 28 days of the last dose of study medication unless unable to return to the institution due to illness or ongoing disease treatment. All serious adverse events and grade 3 or higher adverse events must be followed until the event resolves or is assessed as chronic.

The following study procedures will be performed at the Termination Visit:

- Physical exam
- Vital signs (blood pressure, heart rate, temperature and weight)

- ECOG performance status
- CBC (WBC with differential count, RBC, hemoglobin, hematocrit, platelets)
- Liver function tests (AST, ALT, alkaline phosphatase, total bilirubin)

- Serum chemistries (albumin, sodium, potassium, chloride, carbon dioxide, creatinine, BUN, calcium, glucose)
- Concomitant Medications
- Adverse Events

8 PLANNED STATISTICAL METHODS

8.1 Determination of Sample Size

8.1.1 Safety: Phases 1 and 2

Safety will be assessed through summaries of adverse events, vital signs, physical examinations, and clinical laboratory test data. Safety analyses will include all enrolled patients who receive any amount of study drug.

Grade 4 hematological laboratory values and grade 3-4 non-hematological AEs will be collected and classified by the NCI CTCAE v. 4.0.

The study design is a dose de-escalation with a minimum of 6 patients at the anticipated RP2D dose. If the first six patients do not suffer an unacceptable DLT rate (> 33%), the study would then proceed to phase 2. Sample size 6-18 patients.

8.1.2 Efficacy: Suppression of DNA damage pathway, Phase 2

The primary endpoint is suppression of DDSP induction following genotoxic chemotherapy. Based on preliminary data, we anticipate that 50% of patients will increase the expression of at least one of WNT16, IL6, or SFRP2 (key members of the DNA damage response) >3-fold over the background level in tissue stroma (microenvironment) following treatment with DNA damaging chemotherapy (carboplatin/docetaxel). We hypothesize that the administration of Sirolimus will suppress this induction in 90% of patients.

We will measure the expression of transcripts and encoded proteins that are upregulated following DNA damage and constitute a component of the DDSP. We will focus on 3 genes that are the most robustly induced: WNT16B, IL6, and SFRP2. We will measure each individually at the transcript level by qRT-PCR and also by immunohistochemistry. As qRT-PCR is quantitative, this will be the primary metric of induction, but in the event of RNA degradation in sample processing, the IHC measure will be the alternative which is semi-quantitative (0-2 scale of expression). We will also include a more expansive assessment of a signature of the DDSP derived from microarray profiling of gene expression. We will measure the levels of these transcripts: WNT16B, IL6, SFRP2 (and protein where necessary) after chemotherapy

and compare the levels against chemotherapy plus rapamycin. The background/baseline measurement for the comparison comprises a cohort of samples from untreated patients (n=10) where the expression of these genes is very low (qRT-PCR cycle numbers >30). We expect that WNT16B, IL6 or SFRP2 will be elevated >3-fold following chemotherapy compared to these baseline measurements (untreated patients) and that rapamycin treatment will attenuate this response so that levels will remain <3- fold over the baseline/reference cohort. For this study, feasibility prevented obtaining pretreatment tissue biopsies for study subjects followed by the additional post-treatment biopsy.

20 patients randomized to treatment with Sirolimus and 20 patients randomized to treatment without Sirolimus (for a total of N=40 patients) will provide 81% power to detect a difference in the proportions of patients with < 3-foldinduction of DDSP over background in at least one of the 3 markers at an alpha of 5% based on a 2-sided, 2- sample test of proportions. We will accrue up to 4 additional patients as necessary if patients drop out before the endpoint can be evaluated; final analysis will investigate sensitivity of inference to possible informative missing for any dropouts.

8.2 Analysis Populations

All patients who receive at least one dose of study drug will be included in the analysis of safety (Safety Population).

8.3 Demographics and Baseline Characteristics

Demographic variables will include age, race, ethnicity, height, and weight. Baseline disease characteristics will include clinical TNM stage, date of diagnosis, histology.

8.4 Safety Evaluations

Treatment emergent AEs directly related to Sirolimus are those events that occur or worsen on or after first dose of study drug up through 28 days post last dose. Grade 4 hematological laboratory values and grade 3-4 non-hematological AEs will be collected and classified by the NCI CTCAE v 4.0.

All adverse events resulting in discontinuation, dose modification, dosing interruption, and/or treatment delay of study drug will also be listed and tabulated by preferred term.

Clinical laboratory test results will be collected pretreatment and through 28 days post last dose of study treatment. All laboratory test results will be classified according to the NCI CTCAE v. 4.0 criteria. Standard reference ranges will be used for missing or discrepant normal ranges. Baseline laboratory test values are the results from the last blood samples drawn on or prior to the first day of study treatment. On-study laboratory test values are those results from blood samples drawn a day after the first study treatment up until 28 days after the last dose of study drug administration.

9 REFERENCE LIST

1. *de Bono JS, Oudard S, Ozguroglu M, Hansen S, Machiels JP, Kocak I, Gravis G, Bodrogi I, Mackenzie MJ, Shen L et al:* ***Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial****. Lancet 2010,* ***376****(9747):1147-1154.*
2. *Petrylak DP, Tangen CM, Hussain MH, Lara PN, Jr., Jones JA, Taplin ME, Burch PA, Berry D, Moinpour C, Kohli M et al:* ***Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer****. N Engl J Med 2004,* ***351****(15):1513-1520.*
3. *Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, Oudard S, Theodore C, James ND, Turesson I et al:* ***Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer****. N Engl J Med 2004,* ***351****(15):1502-1512.*
4. *Darshan MS, Loftus MS, Thadani-Mulero M, Levy BP, Escuin D, Zhou XK, Gjyrezi A, Chanel-Vos C, Shen R, Tagawa ST et al:* ***Taxane-induced blockade to nuclear accumulation of the androgen receptor predicts clinical responses in metastatic prostate cancer****. Cancer Res 2011,* ***71****(18):6019- 6029.*
5. *Mezynski J, Pezaro C, Bianchini D, Zivi A, Sandhu S, Thompson E, Hunt J, Sheridan E, Baikady B, Sarvadikar A et al:* ***Antitumour activity of docetaxel following treatment with the CYP17A1 inhibitor abiraterone: clinical evidence for cross-resistance?*** *Ann Oncol 2012,* ***23****(11):2943-2947.*
6. *Tannock IF, Osoba D, Stockler MR, Ernst DS, Neville AJ, Moore MJ, Armitage GR, Wilson JJ, Venner PM, Coppin CM et al:* ***Chemotherapy with mitoxantrone plus prednisone or prednisone alone for symptomatic hormone-resistant prostate cancer: a Canadian randomized trial with palliative end points****. J Clin Oncol 1996,* ***14****(6):1756-1764.*
7. *Sternberg CN, Petrylak DP, Sartor O, Witjes JA, Demkow T, Ferrero JM, Eymard JC, Falcon S, Calabro F, James N et al:* ***Multinational, double-blind, phase III study of prednisone and either satraplatin or placebo in patients with castrate-refractory prostate cancer progressing after prior chemotherapy: the SPARC trial****. J Clin Oncol 2009,* ***27****(32):5431-5438.*
8. *Oh WK, Halabi S, Kelly WK, Werner C, Godley PA, Vogelzang NJ, Small EJ:* ***A phase II study of estramustine, docetaxel, and carboplatin with granulocyte-colony-stimulating factor support in patients with hormone- refractory prostate carcinoma: Cancer and Leukemia Group B 99813****. Cancer 2003,* ***98****(12):2592-2598.*
9. *Ross RW, Beer TM, Jacobus S, Bubley GJ, Taplin ME, Ryan CW, Huang J, Oh WK:* ***A phase 2 study of carboplatin plus docetaxel in men with metastatic hormone-refractory prostate cancer who are refractory to docetaxel****. Cancer 2008,* ***112****(3):521-526.*

10. *Beltran H, Tomlins S, Aparicio A, Arora V, Rickman D, Ayala G, Huang J, True L, Gleave ME, Soule H et al:* ***Aggressive variants of castration-resistant prostate cancer****. Clin Cancer Res 2014,* ***20****(11):2846-2850.*
11. *Allinen M, Beroukhim R, Cai L, Brennan C, Lahti-Domenici J, Huang H, Porter D, Hu M, Chin L, Richardson A et al:* ***Molecular characterization of the tumor microenvironment in breast cancer****. Cancer Cell 2004,* ***6****(1):17-32.*
12. *Olumi AF, Dazin P, Tlsty TD:* ***A novel coculture technique demonstrates that normal human prostatic fibroblasts contribute to tumor formation of LNCaP cells by retarding cell death****. Cancer Res 1998,* ***58****(20):4525-4530.*
13. *Meads MB, Hazlehurst LA, Dalton WS:* ***The bone marrow microenvironment as a tumor sanctuary and contributor to drug resistance****. Clin Cancer Res 2008,* ***14****(9):2519-2526.*
14. *Tredan O, Galmarini CM, Patel K, Tannock IF:* ***Drug resistance and the solid tumor microenvironment****. J Natl Cancer Inst 2007,* ***99****(19):1441-1454.*
15. *Sun Y, Nelson PS:* ***Molecular pathways: involving microenvironment damage responses in cancer therapy resistance****. Clin Cancer Res 2012,* ***18****(15):4019-4025.*
16. *Krtolica A, Parrinello S, Lockett S, Desprez PY, Campisi J:* ***Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging****. Proc Natl Acad Sci U S A 2001,* ***98****(21):12072- 12077.*
17. *Sun Y, Campisi J, Higano C, Beer TM, Porter P, Coleman I, True L, Nelson PS:* ***Treatment-induced damage to the tumor microenvironment promotes prostate cancer therapy resistance through WNT16B****. Nat Med 2012,* ***18****(9):1359-1368.*
18. *Cohen EE, Wu K, Hartford C, Kocherginsky M, Eaton KN, Zha Y, Nallari A, Maitland ML, Fox-Kay K, Moshier K et al:* ***Phase I studies of sirolimus alone or in combination with pharmacokinetic modulators in advanced cancer patients****. Clin Cancer Res 2012,* ***18****(17):4785-4793.*
19. *Bjornsti MA, Houghton PJ:* ***The TOR pathway: a target for cancer therapy****. Nat Rev Cancer 2004,* ***4****(5):335-348.*
20. *Crawford J, Armitage J, Balducci L, Becker PS, Blayney DW, Cataland SR, Heaney ML, Hudock S, Kloth DD, Kuter DJ et al:* ***Myeloid growth factors****. Journal of the National Comprehensive Cancer Network : JNCCN 2013,* ***11****(10):1266-1290.*
21. *Schweizer MT, Zhou XC, Wang H, Bassi S, Carducci MA, Eisenberger MA, Antonarakis ES The influence of prior abiraterone treatment on the clinical activity of docetaxel in men with metastatic castration-resistant prostate cancer.Eur Urol. 2014 Oct;66(4):646-52.*
22. *Scher HI et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. J Clin Oncol. 2008 Mar 1;26(7):1148-59.*

10 APPENDICES

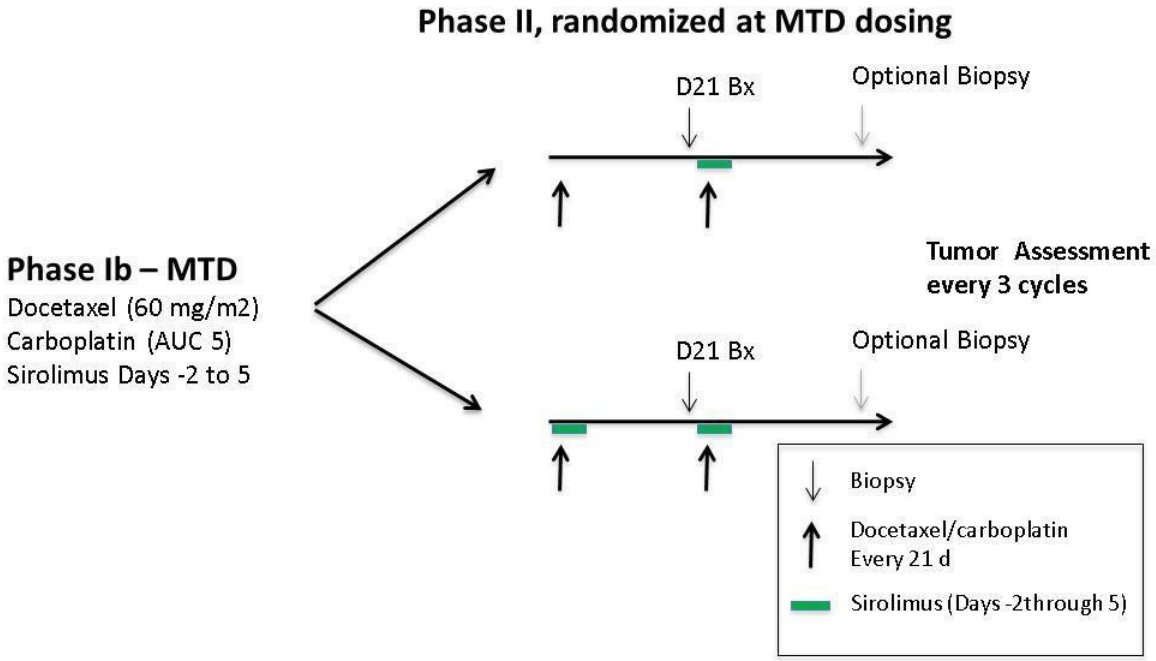
APPENDIX 1 - SCHEDULE OF EVENTS

			Treatment Period					
Procedure	Screening (within 30 days prior to Day -2)[1]	Days -3 through 4 of Every Cycle	Day -2 of Every Cycle[5,14]	Day 1 of Every Cycle[6]	Day 8 of Every Cycle[2]	Day 15 of Every Cycle[2]	Day 21	Termination visit[17]
Informed Consent	X							
Registration	X							
Medical History	X							
Physical exam	X		X					X
Vital Signs[3]	X		X	X				X
ECOG performance status	X		X					X
CBC (w/ platelets & differential)	X		X	(X)[13]	X[9]	X[9]		X
PT/INR[3]	X[3]						X[3]	
Serum chemistry & electrolytes[16]	X		X	(X)[13]	X[9]	X[9]		X
Hepatic function[8]	X		X	(X)[13]	X[9]	X[9]		X
PSA	X		X					X
Testosterone	X							
Research serum[12]			X[19, 20]				X[20]	
Circulating tumor cells and cell free DNA collection			X[19]					
CT or MRI of pelvis/abdomen[10]	X							
Bone scan[11]	X							

Chest film or chest CT[10]	X							
Grapefruit Juice[7]		X[21]	X[21]					
Sirolimus Oral Solution			X[21]					
Docetaxel and carboplatin				X				
Adverse Events			X	X	X	X		X
Concomitant Medications	X		X	X	X	X		X
Tumor Biopsy[4]							X	

[1] Baseline evaluations will be done within 30 days prior to start of therapy. With the exception of laboratory tests, which should be completed within 14 days prior to registration. All screening should be complete prior to registration.
[2] All study assessments and medications should be administered ±4 days of the protocol-specified date, unless otherwise noted.
[3] For patients taking anti-coagulation medication at baseline, a PT/INR test should be done at screening. Repeat PT/INR prior to biopsy per institutional guidelines.
[4] Tumor biopsy to be completed prior to starting Cycle 2.
[5] Vital Signs will include blood pressure, heart rate, temperature and weight. Vital signs should also be documented with any adverse signs or symptoms during or immediately after an infusion. Height will be measured at screening visit only.
[6] Day -2 and Day 1 visit to occur ± 2 days.
[7] Study coordinator will provide patient with grapefruit juice. Patient will drink grapefruit juice at home on the specified days (Days -3 through 4).
[8] Hepatic function includes AST, ALT, alkaline phosphatase and total bilirubin.
[9] Days 8 & 15 Safety Labs will only be drawn during the Phase 1 portion of the study.
[10] CT or MRI of abdomen/pelvis and chest film of chest CT will be repeated as detailed in Section 7.3.4 or as clinically indicated.
[11] A bone scan will be repeated as detailed in Section 7.3.4 or as clinically indicated.
[12] Include plasma for DDSP components. Blood specimens should be optimally drawn before 10:00am, Monday – Friday. *Saturday and Sunday research blood draws are not permissible.* Please refer to laboratory manual for specific instructions regarding obtaining research labs.
[13] If safety labs drawn within 2 day prior to Day 1 (i.e. Day -2), and patient labs qualify patient to safely receive chemotherapy at standard dosing, labs do not have to be re-drawn on Day 1.
[14] Day -2 dosing of Sirolimus will occur two days prior to chemotherapy Day 1. There is no Day 0.
[15] CBC includes: hematocrit, hemoglobin, platelet, RBC, WBC with differential.
[16] Chemistry & electrolyte labs include: albumin, BUN, calcium, carbon dioxide, chloride, creatinine, glucose, potassium, sodium
[17] Termination visit to occur within 28 days (± 7 days) of last dose of study medication.
[18] Day -2 visit to occur ± 2 days.
[19] Research labs will be drawn in the Phase 2 study during Cycle 1. Research labs are not required for subsequent cycles.
[20] Research labs will be drawn for the Phase 1 study at baseline and day 21. The research blood collection is optional for Phase 1 of the study.
[21] Patient in Phase 2 Cohort 1 will not start grapefruit juice and sirolimus until Cycle 2. Patient in Phase 2 Cohort 2 will start grapefruit juice and sirolimus at Cycle 1.

APPENDIX 2 - TREATMENT SCHEMATIC



APPENDIX 3 - ADVERSE EVENTS

Definitions

Adverse Event (AE)

Any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Adverse Drug Reaction (ADR)

A noxious and unintended response to any dose of the drug (or biological) product for which there is a reasonable possibility that the product cause the response. ‘‘Reasonable possibility’’ means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Serious Adverse Event (SAE)

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

- Death,
- a life-threatening adverse event,
  Life-threatening adverse event or life-threatening suspected adverse reaction.
  An adverse event or suspected adverse reaction is considered ‘‘life-threatening’’ if, in the view of the investigator, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.
- is a suspected transmission of infectious agents by a medicinal product

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

APPENDIX 4 – PROGRESSION AND RESPONSE CRITERIA

1. Antitumor Effect– Solid Tumors

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST 1.1 criteria.

2. Definitions

Evaluable for toxicity. All participants who receive at least one dose of study treatment will be evaluable for toxicity from the time of their first treatment.

Evaluable for objective response. Only those participants 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 participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression or die prior to the end of cycle 1 will also be considered evaluable.

3. Disease Parameters

Measurable disease (Target Lesions)

Lesions that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 10 mm (this requirement based on a CT slice thickness of ≤ 5 mm; for slice thicknesses > 5 mm, measurable lesions must have a longest diameter ≥ 2 times the slice thickness).

A lymph node will be considered pathologically enlarged and measurable if its short axis is ≥ 15 mm; the short axis should be measured and followed throughout. Nodes with a short axis ≥ 10 mm and < 15 mm will be considered pathologically enlarged but nonmeasurable (see below).

Lytic bone lesions or mixed lytic-blastic lesions with identifiable soft-tissue components will be considered measurable if the soft-tissue component can be evaluated by cross- sectional imaging (i.e., CT scan) and meets the general definition of measurability.

Simple cysts will not be considered malignant lesions, and will be neither measurable nor
nonmeasurable. Cystic lesions believed to be metastases may be considered measurable if they meet the general definition of measurability, but noncystic lesions are preferred as target lesions.

A lesion located in a previously irradiated area, or in an area previously subjected to any locoregional therapy, will be considered measurable only if there has been a documented increase in lesion size subsequent to prior treatment but prior to study entry.

Non-measurable disease (Non-target Lesions)

All other lesions including small lesions (longest diameter < 10 mm or pathological lymph nodes with a short axis of ≥ 10 mm and < 15 mm) and truly nonmeasurable lesions.

Lesions considered to be truly nonmeasurable include the following: bone lesions; ascites; pleural/pericardial effusion; inflammatory breast disease; lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical examination that is not measurable by reproducible imaging techniques. Blastic bone lesions are nonmeasurable.

4. Methods for Evaluation of Measurable Disease

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

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the anti-tumor effect of a treatment.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

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

Conventional CT and MRI. These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

5. Response Criteria

Overall tumor response, as defined in Table 11, will be based on an integration of the evaluation of target, non-target, and new lesions, as described below:

5.1. Evaluation of Target

Lesions Complete Response (CR):

The disappearance of all non-nodal target lesions, with the short axes of any target lymph nodes reduced to < 10 mm.

Partial Response (PR):

At least a 30% decrease in the sum of the diameters of target lesions (including the short axes of any target lymph nodes), taking as reference the baseline sum diameter.

Stable Disease (SD):

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameter since the treatment started

Progressive Disease (PD):

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

Not Evaluable (NE):

A target lesion present at baseline which is subsequently not measured or which is unable to be evaluated, leading to an inability to determine the status of that particular tumor for the time point in question. This category also includes scans that are not performed at this time point to evaluate the target lesion(s). The reason(s) explaining the absence of the evaluation or nonevaluable nature of the lesion(s) should be specified at the time of the assessment (eg, early death due to malignant disease; early death due to toxicity; tumor assessments not repeated or incomplete; other [specify]).

Note: If tumor response data is missing, an overall assessment cannot be done. However, if there is missing or unevaluable data for non-target lesions, but data is available for all target lesions, the overall response for that time point will be assigned based on the sum LD of all target lesions. Additionally, the assessment of CR cannot be made if there is missing or unevaluable data for non-target lesions. In this case, the overall assessment would be PR.

5.2.Evaluation of Non-Target Lesions

Complete Response (CR):
The disappearance of all non-target lesions, the normalization of the tumor marker level (if tumor markers are measured and are initially above the upper limit of normal, those must normalize for a patient to be considered in complete clinical response). All lymph nodes must be < 10 mm (short axis).

Incomplete Response/Stable Disease (SD):
The persistence of one or more non-target lesions and/or the maintenance of the tumor marker level above normal limits.

Progressive Disease (PD):
The appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. PD may be declared on the basis of "unequivocal progression" in cases where the overall tumor burden increases significantly enough to require a change in therapy; in most cases, a modest increase in the size of one or more non- target lesions is not sufficient to qualify (especially in the presence of SD or PR in target disease).

Unknown (UN):
A nontarget lesion present at baseline which is subsequently not measured or which is unable to be evaluated, leading to an inability to determine the status of that particular tumor for the time point in question.

This category also includes scans that are not performed at this time point to evaluate the nontarget lesion(s). The reason(s) explaining the absence of the evaluation or nonevaluable nature of the lesion(s) should be specified at the time of the assessment (e.g., early death, malignant disease; early death, toxicity; tumor assessments not repeated or incomplete; other.)

Note: Although a clear progression of "non-target" lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed at a later time by review of the Principal Investigator (or Protocol Chair). Additionally, the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment is mandatory to differentiate between stable or progressive disease status.

6. Evaluation of Best Overall Response

Each response parameter (target, non-target, and new lesions) will be reported independently at each radiologic read. The investigator will make a determination of overall response based on the evaluation of target, non-target, and new lesions, as shown in Table 10 and Table 11.

Table 10. Time Point Response: Patients with Target (± Non-target) Disease

Target Lesions	Non-target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Not Evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated[a]	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

[a] In general, if only a subset of lesion measurements are taken at a given assessment time point, the patient as a whole is considered not evaluable for that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response.

Abbreviations: CR = complete response; NE = nonevaluable or inevaluable; PD = progressive disease; PR = partial response; SD = stable disease

Table 11. Time Point Response: Patients with Non-target Disease Only

Non-target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/Non-PD	No	Non-CR/Non-PD[a]
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

[a] Non-CR/non-PD is preferred over stable disease for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials. Thus, it is not advised to assign the category of SD when no lesions can be measured.

Abbreviations: CR = complete response; NE = nonevaluable or inevaluable; PD = progressive disease

7. Duration of Response

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

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

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

8. Progression-Free Survival

Progression-Free Survival (PFS) is defined as the duration of time from start of treatment to time of objective disease progression.

APPENDIX 5 – ECOG GRADING SCALE

ECOG Performance Status Scale

GRADE	SCALE
0	Fully active, able to carry out all predisease performance without restriction
1	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
2	Ambulatory and capable of all self-care but unable to carry out work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.