A Phase 2 Study of the Dual mTOR Inhibitor MLN0128 in Patients with Metastatic Castration-Resistant Prostate Cancer (CRPC)

PROTOCOL FACE PAGE FOR
MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

Principal Investigator/Department:	Dana Rathkopf, MD	Medicine/Genitourinary
Co-Principal Investigator(s)/Department:	Howard Scher, MD	Medicine/Genitourinary
Investigator(s)/Department:	Michael Morris, MD Yu Chen, MD, PhD Susan Slovin, MD, PhD David Solit, MD Daniel Danila, MD Lewis J. Kampel, MD Karen Autio, MD Jason Koutcher, MD, PhD James Hsieh, MD, PhD Martin Voss, MD Gopakumar Iyer, MD Jonathan Rosenberg, MD Anthony DeLaCruz, RN, NP Tracy B. Curley, RN Gabrielle Arauz, RN Richard Bambury, MB, BCh, BAO Josef Fox, MD Jonathan Landa, DO Steve Larson, MD Jeremy Durack, MD Joseph Osborne, MD, PhD Glenn Heller, PhD Victor Reuter, MD Martin Fleisher, PhD	Medicine/Genitourinary Radiology/Nuclear Medicine Radiology Epidemiology and Biostatistics Pathology Laboratory Medicine
Consenting Professional(s)/Department:	Dana Rathkopf, MD Howard Scher, MD Yu Chen, MD, PhD Michael Morris, MD	Medicine/Genitourinary

	Susan Slovin, MD, PhD David Solit, MD Daniel Danila, MD Lewis J. Kampel, MD Karen Autio, MD Jason Koutcher, MD, PhD James Hsieh, MD, PhD Martin Voss, MD Gopakumar Iyer, MD Jonathan Rosenberg, MD Richard Bambury, MB, BCh, BAO	

Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York, New York 10065
Table of Contents

1.0 PROTOCOL SUMMARY AND/OR SCHEMA 7

2.0 OBJECTIVES AND SCIENTIFIC AIMS 9

3.0 BACKGROUND AND RATIONALE 10
3.1 Disease Background 10
3.2 MLN0128 11
3.2.1 Preclinical Studies 11
3.2.2 Clinical Studies 14
3.2.3 Potential Risks and Benefits 16
3.3 Correlative Studies 17
3.3.1 Tumor biopsies 17
3.3.2 CTC enumeration and molecular profiling 17
3.3.3 FDG and FDHT PET imaging 18
3.4 Rationale for conducting the study 18

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION 19
4.1 Design 19
4.2 Intervention 19
4.2.1 Treatment Administration 19
4.2.2 Dosing Schedule 20
4.2.3 Treatment Compliance 20
4.2.4 Re-treatment Criteria 20
4.2.5 Dose Modifications 20
4.3 Management of Clinical Events 21
4.3.1 Management of Hyperglycemia 21
4.3.2 Management of Noninfectious Pneumonitis 22
4.3.3 Management of Hyperlipidemia 23
4.3.4 Management of Oral Mucositis 24
4.3.5 Management of Rash 25
4.3.6 Management of Nausea and/or Vomiting 25
4.3.7 Management of Cardiac Events 26
4.3.8 Management of Other Toxicities 27
4.4 Concomitant Medications 29
4.4.1 Supportive care medications 29
4.4.2 Prohibited therapies 30
4.4.3 Restricted therapies 30
4.4.4 Washout periods for prohibited medications 30

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS 31
5.1 Pharmacology 31
5.2 Packaging and Labeling 31
5.3 Storage and Accountability 31
5.3.1 Storage requirements 31
5.3.2 Drug dispensing log and pill diary 31

6.0 CRITERIA FOR SUBJECT ELIGIBILITY 32
6.1 Subject Inclusion Criteria 32
6.2 Subject Exclusion Criteria 33

7.0 RECRUITMENT PLAN 35

8.0 PRETREATMENT EVALUATION 35
8.1 Screening (Day -30 to Day 1) 35
8.2 Baseline Evaluation (Day -14 to Day 1) 35

9.0 TREATMENT/INTERVENTION PLAN 36
9.1 Cycle 1, Week 1 (Day 1) 36
9.2 Cycle 1, Week 2 (Day 8±1 day) 36
9.3 Cycle 1, Week 3 (Day 15±1 day) 36
9.4 Cycle 1, Week 4 (Day 22±1 day) 36
9.5 Cycle 2, Week 1 (Day 1±2 day) 37
9.6 Cycle 2, Week 2 (Day 8±2 day) 37
9.7 Cycle 2, Week 3 (Day 15±2 day) 37
9.8 Cycle 2, Week 4 (Day 22±2 day) 37
9.9 Cycle 3, Week 1 (Day 1±2 day) 38
9.10 Cycle 3, Week 3 (Day 15±2 day) 38
9.11 Cycle 4, Week 1 (Day 1±2 day) 38
9.12 Cycle 4, Week 3 (Day 15±2 day) 38
9.13 Cycle 5 through end of treatment 39
9.14 Every 8 weeks (± 7 days) 39
9.15 End-of-treatment visit 39
9.16 Follow-up (30 days or until death) 39

10.0 EVALUATION DURING TREATMENT/INTERVENTION 39
10.1 Medical History 40
10.2 Physical Examination 40
10.3 Performance Status 40
10.4 Cardiac Function 40
10.5 Clinical Laboratory Tests 41
10.5.1 Laboratory parameters 41
10.5.2 Sample collection, storage and shipping 41
10.6 Efficacy Assessments 41
10.7 Tumor Biopsies 42

10.8 Circulating Tumor Cell Studies 42
10.9 Whole Blood Lymphocyte Assay 43
10.10 Buffy Coat for Germline DNA 43
10.11 FDG and FDHT PET 43

11.0 TOXICITIES/SIDE EFFECTS 43
11.1 Defining Adverse Events 43
11.1.1 Adverse Event (AE) 43
11.1.2 Unexpected Adverse Event 45
11.1.3 Adverse Events of Special Interest 45
11.1.4 Serious Adverse Event (SAE) 45
11.2 Recording Adverse Events 46
11.2.1 Timeframe for Recording Adverse Events and Serious Adverse Events 46
11.2.2 Recording of Adverse Events and Serious Adverse Events 46
11.3 Grading Adverse Events 47
11.3.1 Grading severity 47
11.3.2 Attributing causality 47

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT 47

13.0 CRITERIA FOR REMOVAL FROM STUDY 48
13.1 Definition of Progression 48
13.2 Safety Evaluation 49

14.0 BIOSTATISTICS 49
14.1 Analysis Population 49
14.2 Demographics and Baseline Characteristics 49
14.3 Safety Analysis 49
14.3.1 Adverse events 49
14.3.2 Clinical laboratory tests 49
14.4 Statistical Procedures 49

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES 50
15.1 Research Participant Registration 50
15.2 Randomization 51

16.0 DATA MANAGEMENT 51
16.1 Quality Assurance 51
16.2 Data and Safety Monitoring 51
16.2.1 Data Collection and Review 51
16.2.2 Source documents 52
16.2.3 Record retention 52

17.0 PROTECTION OF HUMAN SUBJECTS 52
17.1 Privacy 52
17.2 Serious Adverse Event (SAE) Reporting 52

17.2.1 Millennium Reporting timelines 52
17.2.2 Millennium Procedures for Reporting Serious Adverse Events 52
17.2.3 Millennium Procedures for Reporting Drug Exposure During Pregnancy 54
17.2.4 MSKCC Procedures for Reporting Serious Adverse Events 56
17.2.5 Adverse events 57
17.2.6 Clinical laboratory tests 57

18.0 INFORMED CONSENT PROCEDURES 57

19.0 REFERENCES 59

20.0 APPENDICES 61

Appendix A: Performance Status Criteria 61
Appendix B: Study Calendar 62
Appendix C: Circulating Tumor Cell (CTC) Laboratory Manual 64
Appendix D: Glossary of Abbreviations and Acronyms 68
Appendix E: Pill Diary 72
Appendix F: Glucose Monitoring Diary 74
Appendix G: New York Heart Association Classification of Cardiac Disease 76
Appendix H: Strong Inhibitors and Inducers of CYP2C9, CPY2C19, and CYP3A4 77

1.0 PROTOCOL SUMMARY AND/OR SCHEMA

Title	A Phase 2 Study of the Dual mTOR Inhibitor MLN0128 in Patients with Metastatic Castration-Resistant Prostate Cancer (CRPC)
Investigational agent	MLN0128, an orally-available, potent and selective TORC1/2 inhibitor
Target population	Men with metastatic CRPC who have no prior exposure to PI3K/mTOR pathway inhibitors.
Expected enrollment	21-42
Rationale	Dysregulated activation of the PI3K signaling pathway is one of the most common alterations in human prostate cancer—both at initial diagnosis and at the time of relapse to castration-resistant disease. Loss of the tumor suppressor PTEN is by far, the most common mechanism of PI3K pathway activation, with PTEN gene copy number loss present in 42% of primary tumors and 100% of metastatic castrate-resistant prostate cancers.[1] Despite the high rate of PI3K pathway dysregulation in CRPC, inhibition of the downstream regulator TORC1 using rapalogs has been disappointing in the clinic.[2-4] With further elucidation of the PI3K signaling pathway, we now realize that the lack of response to treatment with these rapalogs is likely due to the demonstrable role of TORC2 (not inhibited by rapalogs) in models of *PTEN*-driven prostate cancer,[5] and also to negative feedback inhibition on AR and other oncogenic pathways that maintain cell survival.[6-8] MLN0128 is a first in class, dual mTOR inhibitor that has the potential to overcome resistance seen with standard rapalogs through inhibition of both TORC 1 and 2, and inhibitory effects on cancer cell invasion and metastasis through regulation of 4EBP1-eIF4E . We will evaluate MLN0128 in this single agent phase II study for patients with CRPC with the goal of determining anti-tumor activity and dissecting mechanisms of sensitivity and resistance to this agent using correlative studies such as circulating tumor cells, FDG and FDHT PET imaging, and tumor biopsies when possible.

Objectives	**Primary:** To evaluate the antitumor effects of MLN0128 by determining progression-free survival (PFS) at 6 months. **Secondary Endpoints**: PSA kinetics, Radiographic Response **Exploratory Endpoints:** • To explore baseline and changes in biomarker expression (eg, PTEN, AR, TMPRSS2-ERG , pS6K, p4EBP1, pAKT, AR, PHLPP 1 and 2) in pre- and post-therapy tumor biopsies. • To explore pre- and post-therapy changes in circulating tumor cell (CTC) number and biomarker expression (ie: PTEN, AR). • To study the accumulation and biodistribution of fluoro-2-deoxy-D-glucose (FDG) and fluorinated dihydrotestosterone (FDHT) in patients treated with MLN0128.
Study design	Patients will be treated with the established phase II dose of MLN0128 (4 mg po daily continuously; 1 cycle=4 weeks) to assess mechanisms of sensitivity and resistance in men with CRPC who have received either enzalutamide and/or abiraterone.
Criteria for evaluation	**Primary Endpoint**: Progression-free survival (PFS) at 6 months from the start of treatment, as defined by the Prostate Cancer Working Group 2 (PCWG2) guidelines. **Secondary Endpoints**: PSA kinetics, Radiographic Response **Exploratory Endpoints**: Pre and post-treatment tumor biopsies for IHC, circulating tumor cell enumeration and molecular analysis, FDG and FDHT PET imaging.

Statistical method	The primary objective of this study is to determine the efficacy of MLN0128 in patients with metastatic castration- resistant prostate cancer. The primary endpoint of the study is six month progression free survival (6mPFS). A patient that is followed for six months and remains progression free during this time period is defined as a success. Recently there have been two phase III studies demonstrating an overall survival benefit for patients with CRPC. The 6mPFS endpoint has therefore been selected based on these single agent studies using the hormonal intervention abiraterone (6mPFS 44%) and chemotherapy cabazitaxel (6mPFS 23%). In this study, a two-stage design that differentiates between 6mPFS rates of 0.30 and 0.50 will be used to assess treatment efficacy. In the first stage of the study 21 patients will be enrolled. If at most 6 patients remain alive and progression free at 6 months, accrual will be terminated. If at least 7 patients are alive and progression free at 6 months, an additional 21 patients will be accrued in this cohort. At the conclusion of this second stage, if at least 17/42 patients remain alive and progression free at 6 months, then the treatment will be declared sufficiently active. The probability of declaring the treatment effective is 0.10 when the 6mPFS in the population is 0.30 and increases to 0.90 when the 6mPFS is 0.50. It is anticipated that accrual in this trial will be completed in approximately 1-2 years. Circulating tumor cells, FDG PET imaging, and FDHT PET imaging will be recorded over time and their temporal association with progression-free survival time (and overall survival time) will be modeled using a time-dependent Cox model.
Safety analysis	Standard safety summaries will be provided for treatment exposure, patient disposition, adverse events leading to discontinuation, serious adverse events, and all events resulting in death, including those up to 30 days after treatment discontinuation. The incidence of adverse events will be tabulated and reviewed for potential significance and clinical importance.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

Primary Objective

To evaluate the antitumor effects of MLN0128 by determining progression-free survival (PFS) at 6 months.

Secondary Objectives

PSA kinetics and radiographic response

Exploratory Endpoints

To explore changes in biomarker expression in pre- and post-therapy tumor biopsies.

To explore pre- and post-therapy changes in circulating tumor cell (CTC) number and biomarker expression.

To study the accumulation and biodistribution of fluoro-2-deoxy-D-glucose (FDG) and fluorinated dihydrotestosterone (FDHT) in patients treated with MLN0128.

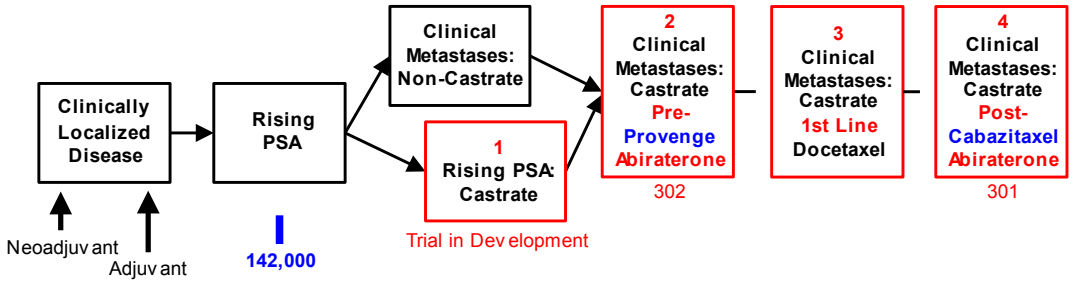
3.0 BACKGROUND AND RATIONALE

3.1 Disease Background

Prostate cancer is the second leading cause of cancer deaths in men. It is estimated that there are 142,000 men in the US in this state who require active treatment (Figure 1).[9]

The course of prostate cancer from diagnosis to death is best categorized as a series of clinical states (Figure 1).[10] These clinical states involve the complex interplay of a network of signaling molecules that collectively promote net cell proliferation relative to cell death. Based on the extent of disease, hormonal status, and absence or presence of detectable metastases on an imaging study, the states are localized disease, rising levels of prostate-specific antigen (PSA) after radiation therapy or surgery with no detectable metastases, and clinical metastases in the non-castrate or castrate state.

Figure 1. Clinical states of prostate cancer



Although surgery, radiation, or a combination of both can be curative for patients with localized disease, a significant proportion of these patients have recurrent disease as evidenced by a rising level of PSA, which, if left untreated, is followed by detectable metastases on imaging studies. For these individuals, the issue is to determine whether the disease is local or systemic, and if the later, the risk of developing metastatic disease – a transition to the lethal phenotype of the illness and in what time frame. Androgen depletion is the standard treatment with a generally predictable outcome: an initial apoptotic response, a period of quiescence in which tumor does not proliferate, followed unfortunately by disease progression. Once the castration-resistant phenotype has become manifest, docetaxel, the standard first-line chemotherapy, is not curative, and most patients succumb to their disease. Second-line or third-line hormonal therapy

can induce post-treatment-PSA declines in 20-30% of patients, but these have never been shown to impact overall survival.

3.2 MLN0128

MLN0128 (formerly INK128) is an orally bioavailable, potent, highly selective, adenosine 5' triphosphate (ATP)-competitive inhibitor of the serine/threonine kinase referred to as the metabolic target of rapamycin (mTOR). The mTOR is a kinase that regulates cell growth, translational control, angiogenesis, and cell survival by integrating nutrient and hormonal signals. mTOR kinase plays a key role in several pathways that are frequently dysregulated in human cancer.[11] The mTOR complex (mTORC) is an important therapeutic target that is stable (does not mutate) and is a key intracellular point of convergence for a number of cellular signaling pathways. Inhibiting mTOR may inhibit abnormal cell proliferation, tumor angiogenesis, and abnormal cellular metabolism, thus providing the rationale for mTOR inhibitors as potential agents as either monotherapy or in combination with other chemotherapeutic agents in the treatment of solid tumor and hematological malignancies.

The mTOR kinase operates in 2 distinct multi-protein complexes, mTORC1 and mTORC2. mTORC1 is best known as a key regulator of protein translation through phosphorylation of 4EBP1, the eukaryotic translation initiation factor, and S6 kinase. mTORC2 is best known for its ability to fully activate AKT by phosphorylation on the S473 site, which regulates proliferation and survival pathways.[12] Analogs of the natural product rapamycin (known as rapalogs) have demonstrated therapeutic benefit in several cancer types, including renal, non-small cell lung cancer (NSCLC), neuroendocrine and hematologic malignancies. [13,14]

However, rapalogs can be considered partial inhibitors of mTORC1. Additionally, inhibition of only mTORC1 without mTORC2 leads to reactivation (by inhibition of feedback regulation) of AKT, which is involved in cancer cell survival, proliferation, growth, metabolism, angiogenesis, and metastasis. [15] Inhibition of AKT activity through mTORC2 inhibition has been shown to prevent the AKT activation induced by mTORC1 inhibition.

To address the incomplete inhibition of mTOR by rapalogs, MLN0128 was developed. MLN0128 is a potent, selective, orally bioavailable, ATP-competitive inhibitor of mTOR that is currently in phase 1 clinical trials. MLN0128 targets both mTORC1 and mTORC2, and is designed to overcome the shortcomings of the current rapalogs to achieve greater clinical benefit.

3.2.1 Preclinical Studies

Nonclinical studies have been conducted to demonstrate the mechanism of action, efficacy, and safety of MLN0128 in biological models to characterize pharmacodynamics, define the pharmacokinetic (PK) properties, characterize the toxicity profile, and support a safe starting dose in humans for MLN0128 drug product.

Pharmacology

MLN0128 selectively and potently inhibits mTOR kinase (IC50 = 1.1 nM), inhibits mTORC1/2 signaling, and prevents cellular proliferation. The in vitro cellular potency of MLN0128 was not diminished in the presence of human whole blood components.

The pharmacodynamics and antitumor activity of MLN0128 was studied in vivo in murine xenograft models of human glioblastoma, NSCLC, breast cancer, renal cell cancer, endometrial adenocarcinoma, and castration-resistant prostate cancer (CPRC). Consistent with the mode of action, MLN0128 inhibited phosphorylation of downstream modulators of mTORC1 (namely 4EBP1 and S6) and mTORC2 (namely AKT [S473]) in human U87 glioblastoma tumor xenograft models in mice at doses as low as 0.1 mg/kg. Additionally,

MLN0128 showed strong tumor growth inhibition (TGI) in all 8 xenograft models at tolerable oral (PO) doses from 0.15 mg/kg (daily [QD]; tested in MDA-MB-361 breast carcinoma) to 3.0 mg/kg (every other day [Q2D] or once weekly [QW]; tested in all models).

In addition to single-agent activity in these xenograft models, MLN0128 was combined with the standard of care (SOC) agent paclitaxel in the breast and endometrial models. The combination of MLN0128 with paclitaxel resulted in enhanced antitumor activity and reduced tumor burden. When tumors were pretreated with paclitaxel, there was an added benefit in tumor reduction compared to the effects of simultaneous administration of both agents, consistent with the mechanism of action of paclitaxel. Findings from these nonclinical pharmacology studies suggest that MLN0128, alone and in combination with paclitaxel, has therapeutic potential as an orally administered mTORC1/2 inhibitor for the treatment of cancers associated with dysregulated activation of the PI3K/AKT/mTOR pathway, such as renal cell, endometrial, breast, lung, and prostate cancers.

Drug Metabolism and Pharmacokinetics

MLN0128 was rapidly absorbed after oral administration to mice, rats, dogs, and monkeys, with high oral bioavailability. MLN0128 did not inhibit P-glycoprotein (P-gp).

A study of the tissue distribution of [14C]MLN0128 showed that [14C]MLN0128 was rapidly and widely distributed throughout the body in Long-Evans rats; radioactivity was eliminated from most tissues at 48 hours postdose, and from all but the adrenal cortex, adrenal gland, adrenal medulla, eye, liver, and uveal tract at 168 hours. MLN0128 displayed doseproportional plasma exposures and a moderate propensity to cross the blood-brain barrier. MLN0128 was modestly bound to human plasma proteins (approximately 70%). MLN0128 inhibited breast cancer resistance protein (BCRP), organic cation transporter (OCT)1, and OCT2.

M1, the single metabolite (monohydroxylation product) observed in human microsomal incubations, was also observed in rats and monkeys, the species used for the Good Laboratory Practice (GLP) toxicology studies. The main isozymes responsible for phase 1 metabolism appear to be cytochrome P450 (CYP) 2C9, 2C19, and 3A4. MLN0128 displayed low potential (IC50 > 30 µM) for inhibition of CYP1A2, 2C19, 2C8, 2C9, CYP2D6, and 3A4. MLN0128 did not induce CYP1A2, 2B6, and 3A4 activity and expression at concentrations up to 30 µM.

Oral administration of MLN0128 in humans has a low potential for metabolic and transporter-based drug-drug interactions (DDIs), especially given clinical exposures observed to date after administration of the highest single dose (total maximum plasma concentration [Cmax] of 0.64 µM [free Cmax of 0.19 µM] at 40 mg QW).

Toxicology

The MLN0128 toxicology program consisted of single- and repeat-dose studies in rats and monkeys, single-dose studies in dogs, and an Ames genotoxicity study.

The toxicologic profiles obtained in the non-GLP-compliant and GLP-compliant studies were generally consistent. The observed toxicities were consistent between rats and monkeys, with no apparent sex differences.

The toxicity profile of MLN0128 in rats and monkeys, as established in GLP-compliant repeat-dose studies, is consistent with pharmacologic inhibition of mTORC1/2 activity. The dose limiting toxicities (DLTs) of MLN0128 in rats and monkeys were secondary to an exaggerated pharmacologic response and consisted of body weight loss and associated clinical observations that included gastrointestinal (GI) distress and decreased activity, appetite, and body temperature. Adverse effects in rats included body weight loss, decreased activity, increased glucose and insulin levels, alterations in white blood cells (WBCs), bone marrow and lymphoid depletion, thymic necrosis, oligospermia, testes degeneration/atrophy, nonglandular stomach epithelial degeneration/ulceration/hyperplasia, and alveolar histiocytosis. The microscopic findings observed in the testes, epididymides, and nonglandular stomach were not resolved after a 14-day recovery period, while partial to complete resolution was seen in the lungs, thymus, and bone marrow. The adverse effects in monkeys included decreased activity, appetite, and body weight; increased glucose and insulin; lymphoid and bone marrow depletion; adrenal hypertrophy/hyperplasia; pancreatic and salivary gland acinar cell secretory depletion; GI tract erosion and ulceration; and skin ulceration/epidermal hyperplasia. The findings in the pancreas, adrenal glands, and salivary glands may have been related to a stress response or reduced food consumption. The findings were generally reversible after a 14-day recovery period.

The findings in rat and monkey repeat-dose toxicology studies with MLN0128, including bone marrow and lymphoid depletion, GI and skin effects, and effects on glucose and insulin levels, can be monitored in clinical trials. The toxicities seen in the repeat-dose toxicology studies, such as GI effects and glucose and insulin increases, are consistent with the treatment-emergent adverse events (TEAEs), including mucositis and hyperglycemia, observed to date in patients receiving MLN0128.

MLN0128 was negative for mutagenicity in the Ames assay, and shows low potential for phototoxicity.

3.2.2 Clinical Studies

Single-agent MLN0128 is in clinical development in two phase 1 studies in subjects with advanced solid malignancies and hematologic malignancies (multiple myeloma [MM] and Waldenstrom macroglobulinemia [WM]), and in a third study in combination with paclitaxel with or without trastuzumab in subjects with advanced solid tumors.

Study INK128-001

Study INK128-001 is evaluating safety and anti-tumor activity of MLN0128 in subjects with advanced solid malignancies. As of 09 December 2012, 106 subjects have been treated in Study INK128-001. The most common adverse events (AEs) (≥ 20%), regardless of causality were hyperglycemia (64%), nausea (60%), vomiting (49%), decreased appetite (40%), diarrhea (37%), asthenia (35%), fatigue and mucosal inflammation (28% each), rash (27%), and pruritus (24%). Most commonly reported (> 3%) Grade ≥ 3 AEs, regardless of causality, include hyperglycemia (10%), asthenia (7%), anemia (6%), lymphopenia (6%), hypophosphatemia (5%), mucosal inflammation (5%), and rash (5%).

In Study INK128-001, as of 09 December 2012, the maximum tolerated doses (MTD) for all 4 schedules has been determined: for the QD dosing the MTD is 6 mg QD, for the QDx3dQW dosing, the MTD is 16 mg; for the QDx5dQW dosing, the MTD is 10 mg; and for the QW dosing schedule, the MTD is 40 mg. The MTDs for each of the 4 schedules was determined by evaluation of cohorts of 6 evaluable patients. At each MTD, up to 6 additional patients were enrolled to further evaluate safety and tolerability. A significant proportion of patients treated at the MTDs required dose modifications due to drug-related AEs beyond 1 or 2 cycles, and therefore were not representative of a recommended phase 2 dose. The study is currently further evaluating doses at less than the MTD for QDx3dQW and QDx5dQW, to determine a dose(s) and schedule(s) to be studied further in the expansion phase of the study, as well as in future phase 2 studies. The dose escalation portion of the study has evaluated dose regimens ranging from 2 to 7 mg QD, 7 to 40 mg QW, 6 to 20 mg QDx3dQW, and 7 to 13 mg QDx5dQW.

Study INK128-002

Study INK128-002 is evaluating safety and anti-tumor activity of MLN0128 in subjects with hematologic malignancies (MM and WM). As of 09 December 2012, 39 subjects have been treated in Study INK128-002. The most common AEs (≥ 20%), regardless of causality were fatigue and nausea (51% each), hyperglycemia (38%), thrombocytopenia (36%), diarrhea (26%), decreased appetite and vomiting (23% each), and stomatitis/anemia (21% each).

Most commonly reported (at least 2 subjects) Grade ≥ 3 AEs, regardless of causality included thrombocytopenia (18%), fatigue (10%), neutropenia (8%), hypocalcemia (5%), hypophosphatemia (5%), mucosal inflammation (5%), and pneumonia (5%).

In Study INK128-002, dose escalation is completed, with 4 mg determined as the MTD for the QD schedule, and 9 mg determined as the MTD for the QDx3dQW schedule.

Study INK128-003

Study INK128-003 is evaluating safety and anti-tumor activity of MLN0128 in subjects with advanced solid tumors in combination with paclitaxel (and trastuzumab for HER2+ subjects). As of 09 December 2012, 48 subjects have been treated in Study INK128-003; no subject has been treated with trastuzumab. The most common AEs (≥ 20%), regardless of causality were fatigue (67%); nausea (56%); diarrhea (50%); dehydration and hyperglycemia (44% each); anemia (40%); anorexia, mucosal inflammation, and vomiting (38% each); rash (35%); asthenia and neutropenia (31% each); hypokalemia (27%); hypophosphatemia and urinary tract infection (23% each); and constipation (21%). Most commonly reported (at least 2 subjects) Grade ≥ 3 AEs, regardless of causality, include neutropenia (23%); hypophosphatemia (17%); diarrhea, fatigue, and hyperglycemia (15%); and dehydration (10%).

In the dose expansion phase of this study, additional subjects were enrolled once the MTD was determined and evaluated for each of the dosing schedules. These subjects were enrolled into an arm of HER2- subjects receiving MLN0128 in combination with paclitaxel (n = 11) at the MTD or an arm of HER2+ subjects receiving MLN0128 in combination with paclitaxel plus weekly trastuzumab (n = 2) at the MTD. The most common AEs (≥ 20%), regardless of causality, were alopecia, fatigue, and nausea (23% each) and anorexia, asthenia, diarrhea, dyspepsia, mucosal inflammation, neutropenia, and vomiting (15% each). Most commonly reported (at least 2 subjects) Grade ≥ 3 AEs in the dose expansion phase of INK128-003, regardless of causality, include mucosal inflammation, neutropenia, and pneumonia (reported by 1 subject each in the HER2- arm).

In Study INK128-003, dose escalation is completed, with 8 mg of MLN0128 QDx3dQW being selected for the dose expansion phase of the study. The QDx5dQW and QW schedules were abandoned before MTDs were declared, as these schedules were viewed as less convenient relative to the QDx3dQW schedule, from the perspective of administering the paclitaxel and trastuzumab combination. The dose expansion portion of this study is ongoing, with HER2-/unknown patients receiving 8 mg of MLN0128 QDx3dQW in combination with paclitaxel, and HER2+ patients receiving 8 mg of MLN0128 QDx3dQW in combination with paclitaxel and trastuzumab.

PK Summary

Preliminary pharmacokinetic (PK) data from these studies indicate that MLN0128 exhibits fast oral absorption (first time to maximum plasma concentration [Tmax] generally between 1 to 4 hours after dosing) and dose-linear pharmacokinetics with a mean plasma half-life of ~8 hours and does not accumulate meaningfully in plasma on either dosing regimen. The pharmacokinetics of MLN0128 was generally consistent with

no appreciable differences across the three phase 1 studies. Neither paclitaxel nor MLN0128 appeared to alter the PK of the other agent when co-administered.

3.2.3 Potential Risks and Benefits

Currently, 206 subjects have participated in phase 1 studies including 145 subjects in single agent studies INK128-001 and INK128-002 (N = 106 and N = 39, respectively); and 61 subjects in the paclitaxel combination study INK128-003. Toxicities have been mostly Grades 1 and 2, reversible, and manageable with supportive care and/or interruption or dose reduction of study drug. Commonly reported study drug-related AEs have included hyperglycemia, asthenia, fatigue, mucosal inflammation, decreased appetite, rash, nausea, vomiting, and diarrhea. This emerging safety profile is consistent with those of other TORC1/2 and PI3K pathway inhibitors.

As of 2012, there are no FDA-approved TORC1/2 inhibitors. Rapamycin and rapalogs are TORC1 inhibitors with well-described toxicity profiles. Common toxicities include the following: immunosuppression with the potential to increase the risk of both nonserious and serious infections, and/or malignancies; mucositis, stomatitis, and mouth sores with a frequency from 41% to 78%; anorexia (approximately 30%), pneumonitis including interstitial lung disease (5%-36%); diarrhea (25%-56%); skin toxicity (48%-66%) which manifests typically as maculopapular or acneform rash, skin dryness, eczema, skin discoloration, and nail dystrophy; hyperlipidemia (hypercholesteremia and/or hypertriglyceridemia) with incidences from 8% to 44%; hyperglycemia (8%-22%); thrombocytopenia (10%-33%); anemia (27%-94%); leucopenia (27%-38%); hypokalemia (11%-21%); hypophosphatemia (15%-49%); hypertension (4%-7% in renal cancer subjects); elevated serum creatinine (37%-57%); elevated liver function tests (about 20%); arthralgia (25%-30%); asthenia (about 30%); peripheral edema (24%-35%)[16,17,18,19] Serious infections have included sepsis, opportunistic infections, and even death. An increase in the development of lymphomas is also a possibility because of the immunosuppression.

Additionally, hypersensitivity reactions (18%), and fatal bowel perforation (1%) have been reported. Rapidly progressive, and sometimes fatal, acute renal failure not clearly related to renal cancer disease progression, abnormal wound healing, and increased risk of developing intracerebral bleeding (including fatal outcomes) in subjects with central nervous system (CNS) malignancies and/or receiving anticoagulation therapy have been reported in subjects receiving temsirolimus. Because of potential hazard to the developing fetus, women of childbearing potential are advised to avoid becoming pregnant while receiving Rapamycin or rapalogs.[17,18,19]

The toxicities of rapamycin or rapalogs are typically reversible and infrequently serious. MLN0128 targets both TORC1 and TORC2, and thus may prove to have a different risk/benefit profile from the rapalogs. There is no human information available on inhibition of TORC2 alone. The safety profile of MLN0128 continues to be explored in advanced malignancies, including non-Hodgkin lymphoma (NHL), and hematologic malignancies.

3.3 Correlative Studies

3.3.1 Tumor biopsies

Tissue samples will be utilized for organoid growth, morphologic assessment, percent tumor involvement (if applicable), and immunohistochemistry. Samples will be evaluated for downstream indicators of PI3K pathway modulation and additional pathways of interest. Specific markers may include: pS6K, p4EBP1, pAKT, AR and PHLPP 1 and 2. The analysis will be performed under the guidance of Dr. Victor Reuter, and members of the MSKCC genitourinary pathology staff will score both staining intensity and the percentage of positive cells exhibiting immunoreactivity (as a continuous variable).

3.3.2 CTC enumeration and molecular profiling

CTC number has been proposed as a marker of prognosis pre-therapy and as a surrogate for treatment efficacy post-therapy in phase III clinical trials powered to detect a difference in survival with treatment. We have also shown that AR amplification and increased AR gene copy number are detected by fluorescence in situ hybridization (FISH) in CTCs of more than 50% of patients with progressive CRPC. Currently, we use the EpCAM-based immunomagnetic enriched CTC sample to analyze the AR, TMPRSS2-ERG and PTEN by FISH, after fixing the cells into the enumeration chambers (Neon, Veridex). We propose to explore pre- and post-therapy changes in circulating tumor cell (CTC) number as a marker of response efficacy while on treatment on this protocol. Genomic alterations in AR, TMPRSS2-ERG and PTEN in CTC isolated at baseline will be prospectively tested as a predictive value of treatment sensitivity. CTC enumeration will be performed in clinical laboratories with the CellSearch FDA-cleared assay. Molecular profiling in CTC will be performed in collaboration with core labs at MSK for isolation of CTC based on EpCAM expression, whole genome amplification of the DNA, and NextGen sequencing for copy number analysis, gene fusion and point mutation detection. In parallel, WBC from same patients are similarly analyzed to determine germline versus somatic mutations. The genomic alterations in prostate cancer specific genes will be confirmed by orthogonal molecular methods from RNA extracted from non-enriched blood samples collected in PAXgene tubes by an analytically validated orthogonal molecular method available in Clinical Chemistry at MSK.[25] We plan to further explore this technology and approach within the context of this MLN0128 trial (See Appendix C: Circulating Tumor Cell (CTC) Laboratory Manual).

Return of Genomic Data: It is possible that some patients whose tumors or blood are analyzed through investigational “next-generation” profiling will be found to have somatic or germline mutations in genes that are known to be associated with an increased risk of cancer or other diseases. It will not be possible to provide results of research tests not performed in a New York State Department of Health approved clinical laboratory. It will be stated in the consent that participants will be told that they will not receive any specific results from potential research tests. The consent will tell patients that if they wish to have genetic testing done for personal reasons than they should make an appointment with the MSKCC Clinical Genetics Service. If in the course of this research a research finding is obtained that may be critical to the preventive care of the participate or their family, as determined by procedures overseen by the IRB, those

participants who have consented to recontact to discuss research findings will be referred to the Clinical Genetics Service for consultation.

3.3.3 FDG and FDHT PET imaging

Developed as an AR-binding radiotracer, FDHT is an analog of DHT, the most prominent androgen at the tumor-tissue level in non-castrate subjects. As therapies have been developed that target AR, we have recognized the clinical need for (i) detecting pharmacodynamic changes caused by treatment with each class of drugs; (ii) developing an early indicator of response; and (iii) developing predictors of survival.

Since securing an IND for FDHT at MSKCC, we have developed a paradigm of study using a combination of FDHT and FDG PET in close temporal proximity. Using this approach, we have studied more than 100 CRPC patients including follow-up after treatment (MSKCC protocol 00-095; PI Larson and Morris). This dual imaging approach has allowed us to classify individual prostate cancer lesions with respect to both metabolic phenotype (FDG PET) and AR status (FDHT), and thus we are beginning to understand how these tumor phenotypes correlate with and or determine therapeutic response. For the purposes of this trial, optional FDHT PET imaging will be performed at MSKCC under a separate PET acquisition protocol (MSKCC protocol 00-095). We propose to study both FDG and FDHT PET imaging at baseline and post-treatment (1 week, 4 weeks, and at the time of progression), and to correlate the changes to test the following hypotheses:

Since Akt activation causes increased transcription and plasma membrane localization of GLUT1, we hypothesize that comparing FDG-PET uptake immediately before and after treatment with MLN0128 will provide an early assessment of whether a given dose inhibits mTOR activity in tumor cells, independent of overall effects on tumor growth that might be evident weeks or months later.

An increase in binding and uptake FDHT may occur when there is an escape through upregulation of AR.[20] This may or may not be accompanied by increased FDHT uptake depending on the mechanism of tumor growth.

3.4 Rationale for conducting the study

Drug development for castration-resistant prostate cancer (CRPC) has shifted focus from cytotoxic agents to the rational development of targeted approaches based on a fundamental understanding of disease biology. We have selected the mTORC1/2 kinase inhibitor MLN0128 because: (i) our published data indicate that mTORC1/2 is the most critical target in the PI3K pathway[6]; (ii) MLN0128 has the potential to overcome resistance seen with standard rapalogs through inhibition of both TORC1 and TORC2; (iii) MLN0128 has been characterized in phase I studies that have revealed encouraging single agent activity.

It is essential that we understand the effects of single agent mTOR inhibitors against different aspects of the malignant phenotype, so that we can comprehensively define the relevant pathways activated in response to MLN0128 and define rational therapeutic combinations on this basis.

We will therefore evaluate MLN0128 in this study with the goal of determining benefit as measured by progression-free survival. We also plan to explore mechanisms of sensitivity and resistance to this agent in men with metastatic CRPC using exploratory markers of response such as tumor biopsies, circulating tumor cells, and FDHT PET imaging.

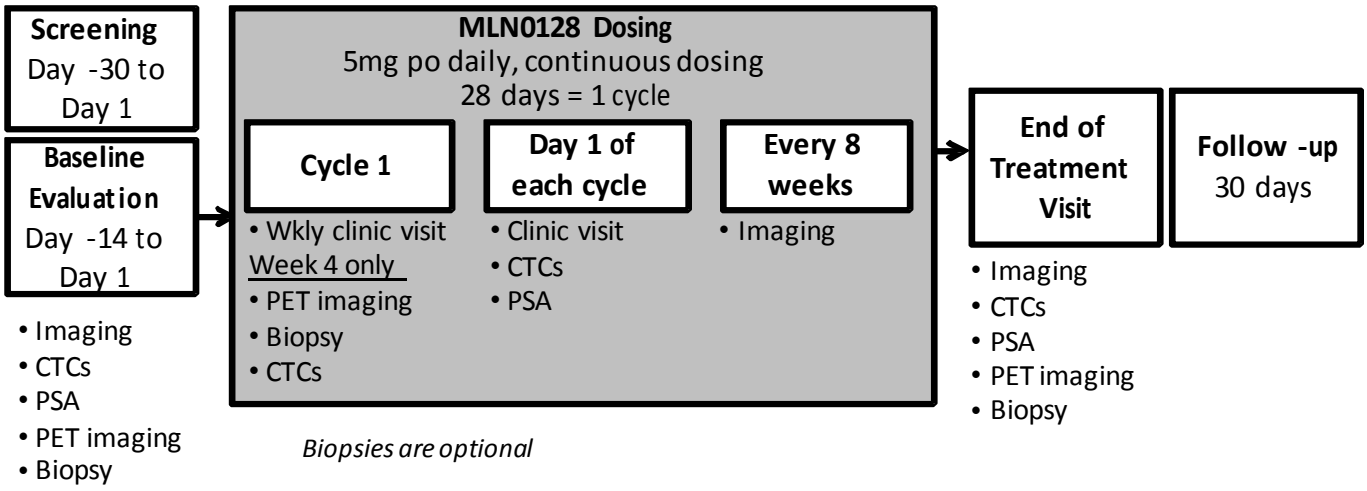
A daily dose of four milligrams (4mg QD) has been selected as the preferred dose and schedule for studies of MLN0128.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

Consented patients undergo a screening evaluation to determine eligibility (Figure 2). Eligible patients begin to receive daily MLN0128 beginning on Day 1 of Cycle 1. It is planned that each patient will receive continuous treatment in a 4-week dosing cycle, which will be discontinued at any time for documented disease progression, or unacceptable toxicity. Details regarding tumor evaluations, safety assessments and correlative studies are outlined in Section 9 and Section 10.

Figure 2. Study schema



4.2 Intervention

4.2.1 Treatment Administration

The Research Staff will provide each patient detailed instructions and training for the handling of study drug and administration to each patient at the beginning of the study. In general, the full prescribed dose of MLN0128 should be taken in the morning at approximately the same time each day. It is recommended that patients take their dose after a light meal and with 8 ounces of water.

4.2.2 Dosing Schedule

MLN0128 will be given at a dose of 4mg daily po, continuously. A cycle will consist of 4 weeks of treatment.

The time of dose administration will be called “0” hour. If a patient vomits after receiving MLN0128, the dose should not be repeated.

All patients who are enrolled in the study and receive a dose of MLN0128 are considered evaluable for toxicity. Additional subjects will be enrolled to replace any subjects who are enrolled, but do not receive treatment.

4.2.3 Treatment Compliance

All doses will be administered on an outpatient basis. Patients should report missed or partial doses of MLN0128 to the study site personnel and this should be recorded in the medical record with a reason for the incomplete dose.

4.2.4 Re-treatment Criteria

Patients will receive treatment until they meet any of the criteria for discontinuation listed in Section 13.

4.2.5 Dose Modifications

Patients will be monitored continuously for AEs while on study therapy. Patients will be instructed to notify their physician immediately for any and all toxicities.

MLN0128 dosing should be withheld for ≥ Grade 2 renal insufficiency, ≥ Grade 3 MLN0128 possibly related hematologic or nonhematologic toxicities, or at the discretion of the investigator. If the event resolves to Grade ≤ 1 or baseline values within 14 days of interrupting therapy, the subject may resume study treatment at a dose reduction.

See table of dose adjustments below according to the schedule applied in this protocol.

If MLN0128 dosing is delayed for > 14 consecutive days for MLN0128-related toxicity despite supportive treatment per standard clinical practice or more than 2 dose reductions of MLN0128 is required in a subject, stop MLN0128 therapy, discontinue the subject from the study, and complete the follow-up visit within 30 days of the last administration of MLN0128.

Table 1. Dose level modifications

Dose Level	Dose
1	4 mg QD
-1	3 mg QD
-2	Discontinue

Level 1 is the starting dose.

Any patient who’s treatment is interrupted for a toxicity that is related to study drug who does not re-start treatment after 14 consecutive days will be withdrawn from study treatment.

4.3 Management of Clinical Events

4.3.1 Management of Hyperglycemia

In addition to obtaining fasting serum glucose (FSG) levels at clinic visits, all subjects will be given a glucometer to monitor their daily pre-dose fasting blood glucose (FBG) levels at home. Subjects will be instructed to notify the study staff immediately with any abnormal readings (ie, ≥ 140 mg/dL) for further instructions on the management of their hyperglycemia. Hyperglycemia observed during home glucose monitoring should be confirmed in the clinic. Investigators will be responsible for reviewing the home glucose monitoring logs for hyperglycemia. If no irregularities in the fasting blood glucose level are observed during a minimum of 6 consecutive months, then the frequency of in-home fasting glucose testing may be reduced to twice weekly if the investigator approves. Subjects will continue to notify the investigator of fasting blood glucose levels that exceed 140 mg/dL and, if blood glucose levels are not well-controlled, or if the subject requires either oral hypoglycemic agents or insulin to control blood glucose levels, then the frequency of in-home testing of fasting blood glucose levels will be reinstated to daily.

Guidance for MLN0128 dose management in the event of hyperglycemia is provided in the table below.

Table 3 Management of Hyperglycemia

Grade	Description	Treatment	MLN0128 Dose Modification
1	Fasting blood sugar > ULN–160 mg/dL	Continue close monitoring of blood sugars. Initiate oral hypoglycemic agent.	None
2	Fasting blood sugar > 160–250 mg/dL	Initiate oral hypoglycemic agent and/or insulin if not well controlled on oral agent.	None
≥3	Fasting blood sugar > 250 mg/dL	Initiate oral hypoglycemic agent and/or insulin.	Hold drug until ≤ Grade 2. Resume MLN0128 based on timing of recovery: ≤ 1 week: resume at same dose and schedule; >1 but ≤ 2 weeks: reduce dose > 2 weeks: stop MLN0128 and discontinue subject from the study.

Prevention/Prophylaxis

- Follow fasting serum glucose levels during clinic visits.
- Monitor home glucometer test results.
- Check HbA1c levels every 3 months during therapy.

- Life-style modifications, as appropriate (balanced diet, limit alcohol consumption, increase physical activity).
- Most episodes of Grade 1 and 2 hyperglycemia respond quickly to oral metformin.
- Early initiation of therapy is recommended to prevent higher grade hyperglycemia.
- Fasting blood glucose levels ≥ 140 mg/dL by glucometer should be followed by closer monitoring of serum glucose and possible intervention.

Abbreviations: dL = deciliters; mg = milligrams; ULN = upper limit of normal.

In the event that any FSG reading performed at the site indicates hyperglycemia (> upper limit of normal [ULN] or ≥ 110 mg/dL), the study staff should first ascertain that the subject was fasting at the time of the blood draw (ie, nothing by mouth for at least 8 hours prior to blood being obtained), had continued to take their concomitant antiglycemic medications should the subject have underlying diabetes mellitus, and repeat the FSG as needed. If the repeat FSG continues to demonstrate hyperglycemia, investigators should initiate steps to aggressively manage the hyperglycemia per standard clinical practice. The following guidelines are provided to aid the investigator in initiating antiglycemic therapies.

Based on the clinical experience from MLN0128 trials, most episodes of hyperglycemia observed have been Grade 1 or 2 that have responded quickly to oral metformin. Hyperglycemia has not been dose-limiting since instituting a standard regimen for early treatment of hyperglycemia. All subjects developing hyperglycemia on the study should have their glucose closely monitored by study staff. The investigator may choose either to continue close monitoring of subjects who develop Grade 1 hyperglycemia (FSG > ULN ≤ 160 mg/dL) or, alternatively, consider initiating treatment with an oral hypoglycemic agent, such as metformin. All subjects with Grade ≥ 2 hyperglycemia (FSG > 160 mg/dL) must be treated aggressively with oral hypoglycemic agents and/or insulin as clinically indicated while continuing on MLN0128. The investigator should consult an endocrinologist if needed to aid in optimizing the subject's hyperglycemia treatment plan.

It is recommended that subjects be treated initially with a fast acting, insulin sensitizer, such as metformin at 500 mg PO QD, and titrate up to a maximum of 1000 mg PO BID as needed. Concurrent addition to metformin of DPP-4 inhibitors (eg, sitagliptin or vildagliptin) and/or insulin should also be considered. Oral sulfonylureas (eg, glipizide or glyburide) should be used with caution due to the higher risk of inducing hypoglycemia in subjects. The dose of oral hypoglycemic agents should be adjusted in subjects with renal insufficiency.

4.3.2 Management of Noninfectious Pneumonitis

Guidance for MLN0128 dose management in the event of noninfectious pneumonitis is shown in Table 4 table below. Noninfectious pneumonitis has not been observed with MLN0128 as of December 2012.

Table 4 Management of Non-infectious Pneumonitis

Grade	Description	Treatment	MLN0128 Dose Modification
1	Asymptomatic: Radiographic findings only	Rule out infection and closely monitor.	None.
2	Symptomatic: Not interfering with ADLs	Rule out infection and consider treatment with corticosteroids until symptoms improve to ≤ Grade 1.	Interrupt MLN0128 treatment: When symptoms ≤ Grade 1, re-initiate MLN0128 treatment at a dose reduction Discontinue MLN0128 treatment if failure to recover within 4 weeks.
3	Symptomatic: Interfering with ADLs; Requires administration of O_2	Rule out infection and consider treatment with corticosteroids until symptoms improve to ≤ Grade 1.	Discontinue MLN0128 treatment.
4	Life-threatening: Ventilatory support indicated	Rule out infection and consider treatment with corticosteroids.	Discontinue MLN0128 treatment.

Abbreviations: ADL = activities of daily living ; O_2 = oxygen gas.

4.3.3 Management of Hyperlipidemia

Guidance for MLN0128 dose management in the event of hyperlipidemia is shown in the table below.

Table 5 Management of Hyperlipidemia

Grade	Description	Treatment	MLN0128 Dose Modification
1	Cholesterol: > ULN - 300 mg/dL Triglycerides: > 150 - 300 mg/dL	None.	None.
2	Cholesterol: > 300 – 400 mg/dL Triglycerides: > 300 - 500 mg/dL	Treat hyperlipidemia according to standarc guidelines. Triglycerides ≥ 500 mg/dl should be treated urgently due to risk of pancreatitis.	Maintain dose if tolerable. If toxicity becomes intolerable, interrupt MLN0128 dosing until recovery to ≤ Grade 1. Reinitiate at same dose.
3	Cholesterol: > 400 - 500 mg/dL Triglycerides:	Same as for Grade 2.	Hold dose until recovery to ≤ Grade 1, then restart at a dose reduction

Table 5 Management of Hyperlipidemia

Grade	Description	Treatment	MLN0128 Dose Modification
	> 500 - 1000 mg/dL		
4	Cholesterol: > 500 mg/dL Triglycerides: > 1000 mg/dL	Same as for Grade 2.	Discontinue treatment.

Prevention/Prophylaxis

- Life-style modifications, as appropriate (balanced diet, limit consumption of alcoholic beverages, increase physical activity).

Abbreviations: dL = deciliters; mg = milligrams; ULN = upper limit of normal.

4.3.4 Management of Oral Mucositis

Guidance for MLN0128 dose management in the event of oral mucositis is provided in the table below.

Table 6 Management of Oral Mucositis

Grade	Description	Treatment	MLN0128 Dose Modification
1	Asymptomatic or mild symptoms	Non-alcoholic mouth wash or 0.9% salt water rinse; Consider topical corticosteroids at earliest signs of mucositis.	None.
2	Moderate pain, not interfering with oral intake Modified diet indicated	Topical analgesic mouth treatments; Topical corticosteroids; Initiate antiviral or antifungal therapy, if indicated.	Maintain dose if tolerable. If toxicity becomes intolerable, interrupt MLN0128 dosing until recovery to ≤ Grade 1. Reinitiate at same dose.
3	Severe pain, interfering with oral intake	Same as for Grade 2; Consider intra-lesional corticosteroids.	Hold dose until recovery to ≤ Grade 1, then restart at a dose reduction
4	Life-threatening consequences	Same as for Grade 2. Consider intra-lesional corticosteroids.	Discontinue treatment.

Prevention/Prophylaxis

- Consider initiation of a non- alcoholic mouth wash or 0.9% salt water rinses 4-6 times daily with start of therapy before signs of mucositis develop.
- Avoid using agents containing hydrogen peroxide, iodine, and thyme derivatives in management of stomatitis as they may worsen mouth ulcers.

4.3.5 Management of Rash

Guidance for MLN0128 dose adjustment for the event of rash is provided in the table below.

Table 7 Management of Rash

Grade	Description	Treatment	MLN0128 Dose Modification
≤ 2	Macules/papules covering ≤ 30% body surface area with or without symptoms	Consider treatment with topical steroid cream/ointment and/or oral anti-histamines.	None.
3	Macules/papules covering > 30% body surface area with or without symptoms	Consider treatment with topical steroid cream/ointment, oral anti-histamines, and/or pulsed steroids.	Hold until ≤ Grade 2; Resume MLN0128 based on timing of recovery: ≤ 2 weeks: reduce dose ; > 2 weeks: stop MLN0128 and discontinue subject from the study.
≥4			Discontinue MLN0128 treatment.

4.3.6 Management of Nausea and/or Vomiting

Guidance for MLN0128 dose adjustment for the event of nausea and/or vomiting is provided in the table below.

Table 8 Management of Nausea and/or Vomiting

Grade	Description	Treatment	MLN0128 Dose Modification
≤ 2	Loss of appetite with or without decreased oral intake; 1-5 episodes of vomiting within 24 hours	Maximize anti-emetic therapy; Consider IV fluid hydration.	None.
≥ 3	Inadequate oral intake; ≥ 6 episodes of vomiting within 24 hours	Maximize anti-emetic therapy; Initiate tube feeding, IVF, or TPN.	Hold until ≤ Grade 1; Resume MLN0128 without dose modification. If toxicity continues despite optimal anti-emetic therapy for > 3 days: reduce dose.

Prevention/Prophylaxis

Prophylactic use of anti-emetic, anti-nausea, and anti-diarrheal medications are encouraged and may be used before each dose of MLN0128 as needed throughout the study.

Abbreviations: IV = intravenous; IVF = intravenous fluids; TPN = total parenteral nutrition

4.3.7 Management of Cardiac Events

Management of Cardiac Instability

For subjects showing signs of cardiac instability after MLN0128 dosing, additional monitoring onsite before clinic discharge should be considered.

Management of Left Ventricular Dysfunction

Guidance for MLN0128 dose adjustment for the event of left ventricular dysfunction is provided in the table below.

Table 9 Management of Left Ventricular Dysfunction

Grade	Description	MLN0128 Dose Modification
1	Asymptomatic decline in LVEF > 15% from baseline values OR; LVEF > 10%-15% from baseline values and is below institution's LLN	No change; continue MLN0128 at same dose and schedule.
≥ 2	Symptomatic cardiac dysfunction/congestive heart failure	Discontinue treatment.

Abbreviations: LLN = lower limit of normal; LVEF = left ventricular ejection fraction.

Management of QTc Prolongation

Guidance for MLN0128 dose adjustment for the event of QTc prolongation is provided in the table below.

Table 10 Management of QTc Prolongation

Grade	Description	Treatment	MLN0128 Dose Modification
2	480 ms < QTc < 501 ms	Evaluate for other possible causes (eg, electrolyte disturbance, concomitant medication, etc.)	None; continue MLN0128 at the same dose and schedule.
≥ 3	QTc ≥ 501 ms	Evaluate for other possible causes (eg, electrolyte disturbance, concomitant medication)[a]; Consider a formal consult by a cardiologist; Notify the study doctor; Additional ECGs may be performed at intervals that the treating physician deems clinically appropriate until repeated QTc measurements fall or are below the threshold interval that triggered the repeat measurement.	MLN0128 should be interrupted. The decision whether to reinitiate MLN0128 treatment with or without dose reduction and additional monitoring in those subjects who had asymptomatic prolonged QTc ≥ 501 msec (Grade 3) that has reverted to an acceptable interval, have previously tolerated MLN0128, and appear to have benefitted from MLN0128 treatment with either disease control or response, will be agreed to by the

	investigator and the study doctoron a case-by-case basis.

Abbreviations: ECG = electrocardiogram; IV = intravenous; ms = milliseconds; QTc = QT interval corrected for heart rate

a A list of medications known to prolong QTc can be found at www.torsades.org and www.QTdrugs.org.

See Section 10.4 Cardiac Function for instructions regarding ECG acquisition and review.

4.3.8 Management of Other Toxicities

Guidance for MLN0128 dose management in the event of nonhematologic and hematologic toxicities is shown in the tables below.

Table 11 Management of Other Nonhematologic Toxicities (including asthenia/weakness)

Grade	Description	Treatment	MLN0128 Dose Modification
1	Mild: Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated		If tolerable, no adjustment required.
2	Moderate: minimal, local, or non-invasive intervention indicated	Initiate appropriate medical therapy and monitor.	If tolerable, no adjustment required. If toxicity becomes intolerable, interrupt MLN0128 dosing until recovery to ≤ Grade 1. Reinitiate at same dose level. If toxicity recurs at Grade 2, interrupt MLN0128 dosing until recovery to ≤ Grade 1. Reinitiate at a dose reduction.
3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated		Interrupt MLN0128 treatment until symptoms resolve to ≤ Grade 1. Re-initiate MLN0128 treatment at a dose reduction If toxicity recurs at Grade 3, discontinue MLN0128 treatment.
4	Life-threatening consequences – urgent intervention required		Discontinue MLN0128 treatment.

Table 12 Management of Other Hematologic Toxicities (for clinically significant laboratory values only)

Grade	Description	Treatment	MLN0128 Dose Modification
1	Mild	Monitor as necessary	No adjustment required.
2	Moderate: intervention may be indicated	Initiate appropriate medical therapy as necessary and monitor.	No adjustment required. Interrupt MLN0128 dosing at investigator discretion. Reinitiate at same dose level. If toxicity recurs at Grade 2, interrupt MLN0128 dosing until recovery to ≤ Grade 1. Reinitiate at a dose reduction at investigator discretion.
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization may be indicated	Initiate appropriate medical therapy as necessary and monitor.	Interrupt MLN0128 treatment until resolved to ≤ Grade 1 or baseline. Re-initiate MLN0128 treatment at a dose reduction If toxicity recurs at Grade 3, discontinue MLN0128 treatment.
4	Life-threatening consequences: urgent intervention required	Initiate appropriate medical therapy as necessary and monitor.	Discontinue MLN0128 treatment.

4.4 Concomitant Medications

All concomitant medications administered to the patient will be reported from the first day of Study Drug administration through 4 weeks following receipt of the last dose of Study Drug. Drug name, start and stop dates will be recorded. Drug doses, dose changes, frequency, and routes of administration will not be reported. Missed doses will not be considered stop dates unless the drug has been discontinued.

4.4.1 Supportive care medications

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 and soy supplements
- Additional systemic glucocorticoid administration such as “stress dose” glucocorticoid is permitted if clinically indicated for a life threatening medical

condition, and in such cases, the use of steroids will be documented as concomitant drug

4.4.2 Prohibited therapies

No other chemotherapy, hormonal therapy, immunotherapy, or experimental anti-cancer medications will be permitted while the patient is on treatment with MLN0128. Patients who have not undergone surgical orchiectomy must continue on medical therapies (i.e., gonadotropin releasing hormone analogs [GnRH analogs] to maintain castrate levels of serum testosterone). Any disease progression requiring other forms of specific anti-tumor therapy will be cause for discontinuation from the study.

4.4.3 Restricted therapies

Symptomatic antiemetics may be administered at the Investigator's discretion; however, they should not be administered within 2 hours of any scheduled pharmacokinetic sample collection. Palliative and supportive care for disease-related symptoms will be offered to all patients on this trial. Details of interventions (e.g., analgesic use, paracentesis, etc.) will be collected on the case report form.

In certain instances, focal radiation therapy for palliation of bone disease-related symptoms might be allowable after discussion between the Principal Investigator and the Investigator. The need for radiation therapy will generally be considered indicative of progressive disease. Patients on stable doses of bisphosphonates for palliation of bone metastases may continue on this medication. Patients are not allowed to initiate bisphosphonate therapy immediately prior to or during the study.

Colony-stimulating factors (i.e., G-CSF, GM-CSF, erythropoietin-stimulating agents, etc.) should not be administered prophylactically during the study period. However, interventional use of myeloid growth factor may be used in the case of febrile neutropenia, at the discretion of the Investigator. Growth factor use must be consistent with the product label.

Strong CYP3A4, CYP2C9, and CYP2C19 inhibitors and inducers should be avoided. If a patient requires treatment with one or more of the strong inhibitors or inducers, alternatives with a reduced potential to inhibit or induce these enzymes should be considered. If a suitable alternative does not exist or is not appropriate, strong CYP3A4, CYP2C9, and CYP2C19 inhibitors and inducers should be used with caution. Examples of strong CYP3A4, CYP2C9, and CYP2C19 inhibitors and inducers can be found in Appendix H: Strong Inhibitors and Inducers of CYP2C9, CPY2C19, and CYP3A4.

4.4.4 Washout periods for prohibited medications

Prohibited medications, such as bicalutamide, or flutamide, should be discontinued 4 weeks prior to Week 1 Day 1 in accordance with the standard of care suggested in guidelines published by the Prostate Cancer Working Group 2 (PCWG2).[21] The decision to administer a prohibited drug/treatment should be made based on the consideration of

the safety of study participant. Patients who require the use of any of these agents will be discontinued from study treatment.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 Pharmacology

MLN0128 will be supplied in tamper-resistant bottles as capsules containing 1 of 3 dose strengths.

- MLN0128 capsules, 1 mg - white opaque color
- MLN0128 capsules, 3 mg – orange opaque color; and/or
- MLN0128 capsules, 5 mg – grey opaque color

Each 1-, 3-, and 5-mg capsule for oral administration contains 1, 3, and 5 of MLN0128, respectively in addition to the following inactive ingredients: microcrystalline cellulose (solid filler/diluents), magnesium stearate (lubricant), and hard gelatin capsule.

5.2 Packaging and Labeling

MLN0128 drug product is packaged in high-density polyethylene (HDPE), white, opaque, round, tamper- and child-resistant bottles in counts (Ct) of 30 capsules. MLN0128 will be packaged and labeled according to all regulations. Sites must store according to the labeled conditions.

5.3 Storage and Accountability

5.3.1 Storage requirements

MLN0128 should be stored at controlled room temperature 15°C to 30°C (59°F to 86°F). All study supplies must be kept in a restricted access area. Accountability for MLN0128 at all study sites is the responsibility of the sponsor-investigator.

5.3.2 Drug dispensing log and pill diary

Study site personnel will record all study drugs administered during this trial on the drug-dispensing log.

The drug dispensing log will contain the following information:

- patient study identification number
- date(s) of study drug administered
- quantities of study drug administered
- signature of the investigator

Subjects will be provided with a diary in which to record their intake of study drug (Appendix E: Pill Diary). However, the actual number of tablets taken by the subject must be calculated from the number of tablets dispensed and returned.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

Target Population

Men with CRPC who have received either enzalutamide and/or abiraterone but have no prior exposure to PI3K/mTOR pathway inhibitors.

6.1 Subject Inclusion Criteria

To be included in this study, patients should have histologically confirmed castration-resistant metastatic prostate cancer with evidence of disease progression. Patients must have been in a castrate state either by orchiectomy or by GnRH analogues. In detail, they should meet all of the following criteria:

1. Histologically confirmed prostate cancer with progressive metastatic disease based on any of the following: i) a rise in PSA, ii) transaxial imaging, or iii) radionuclide bone scan.
    a. PSA - a minimum of 3 consecutive rising levels, with an interval of ≥ 1 week between each determination. The last determination must have a minimal value of ≥ 2 ng/mL and be determined within two weeks prior to enrollment.
    b. Measurable Disease - patients showing new or progressive soft tissue masses on CT or MRI scans as defined by the PCWG2 criteria[21]
    c. Radionuclide bone scan – at least two new metastatic lesions.
2. Detectable metastases by bone scan, CT-scan or MRI.
3. Ongoing androgen depletion therapy with a Gonadotropin Releasing Hormone (GnRH) analogue or inhibitor, or orchiectomy (i.e., surgical or medical castration). For patients who have not had an orchiectomy, there must be a plan to maintain effective GnRH-analogue therapy for the duration of the trial.
4. Castrate levels of serum testosterone < 50 ng/dL determined within 4 weeks prior to starting treatment.
5. Patients who are receiving an anti-androgen as part of their first-line hormonal therapy must have shown progression of disease off the anti-androgen prior to enrollment.
6. Patients must have received treatment with either enzalutamide and/or abiraterone prior to study entry.
7. At least 4 weeks must have elapsed from the use of androgen receptor antagonists (i.e., flutamide, nilutamide, bicalutamide, enzalutamide); 5-α reductase inhibitors (i.e., finasteride, aminoglutethimide); abiraterone acetate; estrogens; nitrosoureas, mitomycin C, isotype therapy, ketoconazole, chemotherapy and other anti-cancer pharmacologic therapy prior to beginning protocol therapy.

8. At least 8 weeks must have elapsed from the use of Strontium-89, Radium-223, Samarium-153, or immunotherapy (e.g., Provenge) prior to beginning protocol therapy.

9. At least 4 weeks must have elapsed from the use of any investigational agent prior to beginning protocol therapy.
    a. Note: Prior treatment with PI3K/mTOR pathway inhibitors prohibited.

10. At least 4 weeks must have elapsed from major surgery.

11. Toxicities related to prior therapy must either have returned to ≤ Grade 1, baseline or deemed irreversible.

12. Patients with treated, non-progressive epidural disease are eligible.

13. KPS performance status 70-100% (Appendix A: Performance Status Criteria)

14. At least 18 years of age, with a life expectancy at least 3 months.

15. Patient must be willing to comply with study procedures.

16. Physical and laboratory test findings

    a. Adequate hepatic function with serum bilirubin ≤ 1.5 times the upper institutional limits of normal (ULN), ALT and AST ≤ 2.5 x ULN. Patients with a history of Gilbert's syndrome may be enrolled if the total bilirubin is < 3 mg/dL with a predominance of indirect bilirubin

    b. Adequate renal function with serum creatinine ≤ 1.5 x ULN.

    c. Adequate hematologic function with absolute neutrophil counts ≥ 1,500 cell/mm^3 and platelets ≥ 100,000 cells/mm^3 and hemoglobin value ≥ 9 g/dL (Note: patients whose anemia has been corrected to a hemoglobin value ≥ 9 g/dL with blood transfusions are allowed).

    d. Electrolytes (including potassium, sodium, and serum calcium corrected for albumin or ionized calcium) must be within normal limits.

17. Left ventricular ejection fraction (LVEF) no more than 5 absolute percentage points below the institutional standard of normal as measured by echocardiogram (ECHO) or multiple gated acquisition scan (MUGA) within 4 weeks prior to first study drug administration (ie, if the institutional normal is 50%, subject's LVEF may be as low as 45% to be eligible for the study)

6.2 Subject Exclusion Criteria

Patients that meet any of the criteria listed below will not be eligible for study entry:

1. History of, or current known metastases in the brain or untreated spinal cord compression;

2. History of another malignancy within the previous 2 years except for the following:
    a. Adequately treated basal cell or squamous cell skin cancer, superficial bladder cancer,
    b. Adequately treated Stage I or II cancer currently in complete remission, or any other cancer that has been in complete remission for at least 2 years;
3. Prior treatment with PI3K/mTOR pathway inhibitors;
4. Diabetes mellitus on active treatment, or subjects with either of the following:
    a. Fasting blood glucose (FBG) ≥ 126 mg/dL (7.0 mmol/L), or
    b. HbA1c ≥ 6.5%;
5. Use of herbal products that may decrease PSA levels (i.e., saw palmetto) or systemic corticosteroid greater than the equivalent of 10 mg of prednisone per day during the 4 weeks prior to screening or plans to initiate treatment with the above during the entire duration of the study;
6. Any history of unstable angina, myocardial infarction, New York Heart Association (NYHA) Class III or IV heart failure (See Appendix G: New York Heart Association Classification of Cardiac Disease), and/or pulmonary hypertension;
7. Significant active cardiovascular disease including:
    a. Uncontrolled high blood pressure (ie, systolic blood pressure > 180 mmHg, diastolic blood pressure > 95 mmHg)
    b. Grade 3 or higher valvular disease
    c. Grade 3 or higher atrial fibrillation
    d. Grade 3 or higher bradycardia
    e. Endocarditis
    f. Pulmonary embolism
    g. Recent cerebrovascular accident within 6 months prior to enrollment
8. A requirement for positive inotropic support (excluding digoxin) or serious uncontrolled cardiac arrhythmia (including atrial flutter/fibrillation) within 1 year prior to screening
9. A pacemaker or implantable cardiac defibrillator
10. Known history of infection with human immunodeficiency virus (HIV), based on medical history (screening labs to rule out HIV infection are not required);

11. Any other condition that, in the opinion of the Investigator, would impair the patient's ability to comply with study procedures.

7.0 RECRUITMENT PLAN

Patients will be recruited through outpatient clinics. Potential research subjects will be identified by a member of the patient's treatment team, the protocol investigator, or the research team. Study investigators will discuss the details of the study and enrollment with their patients as part of the recruitment process. Patients will be required to sign a statement of informed consent that meets the requirements of the IRB of this center. The medical record will include a statement that written informed consent was obtained. No women will be included in this study, which addresses treatment of prostate cancer.

Subjects will be enrolled without respect to race or ethnicity. This study will be conducted in accordance with the ethical principles that have their origin in the current Declaration of Helsinki and will be consistent with International Conference on Harmonization Good Clinical Practice (ICH GCP) and applicable regulatory requirements.

8.0 PRETREATMENT EVALUATION

8.1 Screening (Day -30 to Day 1)

The screening visit will determine patient eligibility according to the inclusion and exclusion criteria (Sections 6.1 Subject Inclusion Criteria and 6.2 Subject Exclusion Criteria). The following assessments will be performed at this visit:

- Obtain informed consent and research authorization
- Obtain histologic and radiologic confirmation of disease
- Record medical history (including prior treatment for prostate carcinoma)
- Conduct physical exam (KPS performance status, vital signs, HEENT, height/weight)
- Echocardiogram or MUGA scan
- Toxicity/AE assessment
- Discuss concurrent medications
- Perform 12-lead ECG
- Radionuclide bone scan
- FDG PET with contrast (if feasible)
- FDHT PET (Optional—on protocol 00-095)
- Tumor biopsy (Optional)

8.2 Baseline Evaluation (Day -14 to Day 1)

A visit within 14 days prior to initiation of protocol therapy will further determine patient eligibility and collect baseline patient data. The visit must include the assessments listed below:

- Laboratory testing (All components of Table 13)
- CTCs

9.0 TREATMENT/INTERVENTION PLAN

9.1 Cycle 1, Week 1 (Day 1)

- Interim medical history
- Physical exam and vital signs
- Assess KPS performance status
- Blood samples for laboratory tests: hematology, serum chemistry, PSA (see Table 13) and CTCs
- Toxicity/AE assessment
- Discuss concurrent medications
- Patient to monitor daily fasting blood glucose level at home
- ECG pre dose, 2 hours post dose (+/- 15 minutes), and 4 hours post dose (+/- 15 minutes)
- Urinalysis

9.2 Cycle 1, Week 2 (Day 8±1 day)

- Interim medical history
- Physical exam and vital signs
- Assess KPS performance status
- Blood samples for laboratory tests: hematology and serum chemistry (see Table 13)
- Toxicity/AE assessment
- Discuss concurrent medications
- Patient to monitor daily fasting blood glucose level at home
- ECG 2 hours post dose (+/- 15 minutes)

9.3 Cycle 1, Week 3 (Day 15±1 day)

- Interim medical history
- Physical exam and vital signs
- Assess KPS performance status
- Blood samples for laboratory tests: hematology and serum chemistry (see Table 13)
- PT, PTT, INR
- Toxicity/AE assessment
- Discuss concurrent medications
- Patient to monitor daily fasting blood glucose level at home
- ECG 2 hours post dose (+/- 15 minutes)
- Urinalysis

9.4 Cycle 1, Week 4 (Day 22±1 day)

- Interim medical history
- Physical exam and vital signs
- Assess KPS performance status
- Blood samples for laboratory tests: hematology and serum chemistry (see Table 13)
- Toxicity/AE assessment
- Discuss concurrent medications
- FDG PET (± 7 days)
- FDHT PET (Optional—on protocol 00-095) (± 7 days)

- Tumor Biopsy (Optional) (± 7 days)
- Patient to monitor daily fasting blood glucose level at home
- ECG 2 hours post dose (+/- 15 minutes)

9.5 Cycle 2, Week 1 (Day 1±2 day)

- Interim medical history
- Physical exam and vital signs
- Assess KPS performance status
- Blood samples for laboratory tests: hematology, serum chemistry, PSA (see Table 13) and CTCs
- Toxicity/AE assessment
- Discuss concurrent medications
- Patient to monitor daily fasting blood glucose level at home
- ECG pre dose and 2 hours post dose (+/- 15 minutes)
- Urinalysis

9.6 Cycle 2, Week 2 (Day 8±2 day)

- Interim medical history
- Physical exam and vital signs
- Assess KPS performance status
- Blood samples for laboratory tests: hematology and serum chemistry (see Table 13)
- Toxicity/AE assessment
- Discuss concurrent medications
- Patient to monitor daily fasting blood glucose level at home
- ECG 2 hours post dose (+/- 15 minutes)

9.7 Cycle 2, Week 3 (Day 15±2 day)

- Interim medical history
- Physical exam and vital signs
- Assess KPS performance status
- Blood samples for laboratory tests: hematology and serum chemistry (see Table 13)
- PT, PTT, INR
- Toxicity/AE assessment
- Discuss concurrent medications
- Patient to monitor daily fasting blood glucose level at home
- ECG 2 hours post dose (+/- 15 minutes)
- Urinalysis

9.8 Cycle 2, Week 4 (Day 22±2 day)

- Interim medical history
- Physical exam and vital signs
- Assess KPS performance status
- Blood samples for laboratory tests: hematology and serum chemistry (see Table 13)
- Toxicity/AE assessment
- Discuss concurrent medications
- Patient to monitor daily fasting blood glucose level at home

- ECG 2 hours post dose (+/- 15 minutes)

9.9 Cycle 3, Week 1 (Day 1±2 day)

- Interim medical history
- Physical exam and vital signs
- Assess KPS performance status
- Blood samples for laboratory tests: hematology, serum chemistry, PSA (see Table 13) and CTCs
- Imaging assessments (contrast-enhanced CT [if feasible], radionuclide bone scan) (± 7 days)
- Toxicity/AE assessment
- Discuss concurrent medications
- Patient to monitor daily fasting blood glucose level at home
- Urinalysis
- HbA1c

9.10 Cycle 3, Week 3 (Day 15±2 day)

- Interim medical history
- Physical exam and vital signs
- Assess KPS performance status
- Blood samples for laboratory tests: hematology and serum chemistry (see Table 13)
- PT, PTT, INR
- Toxicity/AE assessment
- Discuss concurrent medications
- Patient to monitor daily fasting blood glucose level at home
- Urinalysis

9.11 Cycle 4, Week 1 (Day 1±2 day)

- Interim medical history
- Physical exam and vital signs
- Assess KPS performance status
- Blood samples for laboratory tests: hematology, serum chemistry, PSA (see Table 13) and CTCs
- Toxicity/AE assessment
- Discuss concurrent medications
- Patient to monitor daily fasting blood glucose level at home
- Urinalysis

9.12 Cycle 4, Week 3 (Day 15±2 day)

- Interim medical history
- Physical exam and vital signs
- Assess KPS performance status
- Blood samples for laboratory tests: hematology and serum chemistry (see Table 13)
- PT, PTT, INR
- Toxicity/AE assessment
- Discuss concurrent medications
- Patient to monitor daily fasting blood glucose level at home

- Urinalysis

9.13 Cycle 5 through end of treatment

The following evaluations will be performed on Day 1 (±2 days) of each 4-week cycle:

- Interim medical history
- Physical exam and vital signs
- Assess KPS performance status
- Blood samples for laboratory tests: hematology, serum chemistry, PSA (see Table 13) and CTCs
- Toxicity/AE assessment
- Discuss concurrent medications
- Urinalysis
- HbA1c (Cycle 6 and every 3 cycles afterwards)
- PT, PTT, INR
- Patient to monitor daily fasting blood glucose level at home for the first 6 months, then 2 times weekly. If changes to metformin dose, then patient should resume daily fasting blood glucose level at home

9.14 Every 8 weeks (± 7 days)

The following evaluations will be performed every 8 weeks:

- Imaging assessments (contrast-enhanced CT [if feasible], radionuclide bone scan) (± 7 days)

9.15 End-of-treatment visit

Within 28 days of the last dose of study medication the following study activities will occur:

- Blood samples for laboratory tests: hematology, serum chemistry, PSA (see Table 3) and CTCs
- Toxicity/AE assessment
- Discuss concurrent medications
- Radionuclide bone scan (± 7 days, unless performed in the 30 days prior)
- FDG PET with contrast (if feasible) (± 7 days)
- FDHT PET (Optional on protocol 00-095) (± 7 days)
- Tumor Biopsy (Optional) (± 7 days)
- ECG

9.16 Follow-up (30 days or until death)

Patients will be followed for at least 30 days after removal from treatment or until death. Patients withdrawn from the study because of AEs will be followed until the AE has either resolved or stabilized. Reasons for premature withdrawal should be determined and noted.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

A tabular schedule of assessments is provided in Appendix B: Study Calendar.

The following assessments and procedures will occur during the study.

10.1 Medical History

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

10.2 Physical Examination

Evaluations should be performed by the same evaluator throughout the study whenever possible.

Physical examination includes HEENT (head, eyes, ears, nose, and throat), chest, cardiac, abdominal, extremities, neurologic, and lymph node examinations. Weight will be recorded at every visit. Height will be recorded at screening visit only.

Vital signs include upright blood pressure, heart rate, respiratory rate, and oral or aural body temperature.

10.3 Performance Status

Performance status will be assessed using KPS performance status criteria as outlined in Appendix A: Performance Status Criteria.

10.4 Cardiac Function

To assess cardiac function at baseline, a 12-lead ECG should be obtained. Hypokalemia should be corrected prior to ECG collection.

Acquisition of 12-Lead Electrocardiograms

All scheduled ECGs should be performed after the patient has rested quietly for at least 5 minutes in a supine position. In some cases, it may be appropriate to repeat an abnormal ECG to rule out improper lead placement as contributing to the ECG abnormality.

Review of 12-Lead Electrocardiograms

To ensure safety, a qualified individual at the site will review any clinically significant ECG abnormalities, including confirmation that the machine-estimates of the QTc are accurate using the appropriate QT correction formula. In the event that a QTc value confirmed by the qualified reader is > 480 msec, an evaluation should be conducted to correct other possible causes (eg, electrolyte disturbance, concomitant medication, etc.). A list of medications known to prolong QTc can be found at www.torsades.org and www.QTdrugs.org. If done prior to protocol enrollment and if a repeat ECG meets eligibility requirements, the patient may enroll to the study upon review and agreement by the study doctor.

Review of Clinically Significant Electrocardiographic Abnormalities

In the event that a QTc value confirmed by the qualified reader is > 500 msec for any ECG, the following will occur:

- MLN0128 should be interrupted and an evaluation should be conducted to correct other possible causes (eg, electrolyte disturbance, concomitant medication).

- A formal consult by a cardiologist should be considered. Additional ECGs may be performed at intervals that the treating physician deems clinically appropriate until repeated QTc measurements fall are below the threshold interval that triggered the repeat measurement.

The decision on whether to reinitiate MLN0128 treatment with or without dose reduction and additional monitoring in those patients who had asymptomatic prolonged QTc > 500 msec (Grade 3) that has reverted to an acceptable interval, have previously tolerated MLN0128, and appear to have benefitted from MLN0128 treatment with either disease control or response, will be determined on a case-by-case basis.

10.5 Clinical Laboratory Tests

10.5.1 Laboratory parameters

Clinical laboratory tests will include the following (**Error! Reference source not found.**13):

Table 13. List of laboratory tests

Hematology:	**Serum Chemistry (Fasting)**:
- Hematocrit (Hct)	- Alkaline phosphatase (ALK-P)
- Hemoglobin (Hgb)	- Alanine aminotransferase (ALT; SGPT)
- Platelet count (PLT)	- Aspartate aminotransferase (AST; SGOT)
- Red blood cell (RBC) count	- Blood urea nitrogen (BUN)
- White blood cell (WBC) count with differential	- Carbon dioxide (CO2)
Coagulation Factors:	- Chloride (Cl)
- Prothrombin Time (PT)	- Creatinine (Cr)
- Partial Thromboplastin Time (PTT)	- Glucose (Glu)
- International Normalized Ratio (INR)	- Lactate Dehydrogenase (LDH)
	- Magnesium (Mg)
	- Potassium (K)
	- Sodium (Na)
	- Total bilirubin
	- Lipid Profile
	Additional laboratory tests:
	- Prostate specific antigen (PSA)
	- Serum testosterone (T),
	- HbA1c

10.5.2 Sample collection, storage and shipping

Local laboratories will analyze all hematology, blood chemistry collected for the study. Samples will be analyzed at a facility meeting Clinical Laboratory Improvement Amendments (CLIA) requirements and/or using methods documented in a methods validation report.

10.6 Efficacy Assessments

Progression-free survival (PFS) at 6 months from the start of treatment, as defined by the Prostate Cancer Working Group 2 (PCWG2) guidelines (primary endpoint measure).

10.7 Tumor Biopsies

With agreement of the investigator and sponsor, and with the patient's consent, paired tumor tissue samples (including bone lesions in subjects with bone metastases) may be collected during Screening , at Week 4 (± 7 days) and at End of Treatment (± 7 days). Tumor samples at Week 4 can be collected either pre- or post-dose. Image-guided biopsies will be performed after the patient's prior imaging is reviewed and target lesions are identified for biopsy. Lesions will be chosen based upon the strength of the evidence suggesting the presence of metastasis so as to minimize patient risk. Sample types may include surgical biopsies, core needle biopsies, fine needle aspirates or punch biopsies, where applicable. Collection of tumor fluid samples (confirmed as malignant by a cytopathologist) may also be considered on a case-by-case basis. The sample type should be kept consistent per patient for paired tissue collection whenever feasible.

Tissue samples may be utilized for organoid growth, morphologic assessment, percent tumor involvement (if applicable), and immunohistochemistry depending on the amount of specimen that can be safely and feasibly collected. Providing adequate tissue is available, samples will be evaluated for downstream indicators of PI3K pathway modulation and additional pathways of interest. Specific markers may include: pS6K, p4EBP1, pAKT, AR and PHLPP 1 and 2. The analysis will be performed under the guidance of Dr. Victor Reuter, and members of the MSKCC genitourinary pathology staff will score both staining intensity and the percentage of positive cells exhibiting immunoreactivity (as a continuous variable). Intensity will be scored in a 4-point scale (0-3). The percentage of cells with weak (1), moderate (2) and strong (3) staining will also be recorded allowing us to establish an H-score. The H-score is the sum of percentages of positive cells in each of 3 intensity categories with a scale ranging from 0-300. In addition, all cases will be digitally scanned and the staining parameters will be evaluated using the Aperio signal quantification software.

10.8 Circulating Tumor Cell Studies

The CTC number will be measured at baseline, Day 1 of each cycle, and at the time of progression (end-of-treatment) using an FDA-cleared analytically validated semi-automated system, CellSearch (Veridex, LLP) in the CLIA certified laboratory of Dr. Martin Fleisher at MSKCC. Currently, we use the EpCAM-based immunomagnetic enriched CTC sample to analyze the AR, TMPRSS2-ERG and PTEN by FISH, after fixing the cells into the enumeration chambers (Neon, Veridex). After hybridization, the chambers are re-interrogated for genomic abnormalities in the previously identified CTC. We are prospectively testing the predictive value of genomic alterations in AR, TMPRSS2-ERG and PTEN by FISH in CTC isolated by CellSearch EpCAM-immunomagnetic enrichment, and sensitivity to treatment with AR targeted agents such as ARN-509 and we plan to further explore this technology and approach within the context of this MLN0128 trial.

Molecular profiling in CTC will be performed in collaboration with core labs at MSK for isolation of CTC based on EpCAM expression, whole genome amplification of the DNA,

and NextGen sequencing for copy number analysis, gene fusion and point mutation detection. In parallel, WBC from same patients are similarly analyzed to determine germline versus somatic mutations. The genomic alterations in prostate cancer specific genes will be confirmed by orthogonal molecular methods from RNA extracted from non-enriched blood samples collected in PAXgene tubes by an analytically validated orthogonal molecular method available in Clinical Chemistry at MSK.[25]

The CTC blood samples will be drawn in kits provided for the study, will be labeled and delivered to the central laboratory at MSKCC, and the blood will be processed and analyzed as described in the Laboratory Manual (Appendix C: Circulating Tumor Cell (CTC) Laboratory Manual) outlining collection and analysis procedures for CTC (all done at MSKCC).

10.9 Whole Blood Lymphocyte Assay

A blood sample will be collected at baseline and Day 8 of Cycle 1to determine target inhibition through lymphocyte analysis.

10.10 Buffy Coat for Germline DNA

A blood sample will be collected at baseline for isolation of buffy coat and germline sequencing.

10.11 FDG and FDHT PET

We propose to study both FDG and optional FDHT PET imaging at baseline, 4 weeks after treatment initiation and at the time of progression (end-of-treatment), and to correlate the changes with treatment response. For the purposes of this trial, optional FDHT PET imaging will be performed at MSKCC under a separate PET acquisition protocol (MSKCC protocol 00-095). *Image Analysis:* The linking of molecular images of lesions (on a lesion by lesion basis) to drug response profiles is an important concept. We can now assess all the lesions in the body using whole-body PET and monitor their activity over time using a data analysis tool, PET-VCAR (volume computer assisted reading), which Dr. Steve Larson's group has co-developed with GE Healthcare Systems. This application runs from within a PACS-integrated Advantage Workstation and contains software to load and segment PET/CT image sets. The PET-VCAR allows for registration of post-therapy PET/CT scans based on co-registration of the corresponding whole-body CT image sets. This co-registration allows lesions to be matched, ie, identified and followed over time. In this way, important data on individual lesion response can be acquired. These measured data, obtained by metabolic FDG PET imaging and AR expression FDHT PET imaging, will be compared with the primary endpoint of PFS.

11.0 TOXICITIES/SIDE EFFECTS

11.1 Defining Adverse Events

11.1.1 Adverse Event (AE)

An AE is defined as any adverse event associated with the use of a drug in humans, whether or not considered drug related, including the following:

- An adverse event occurring in the course of the use of the medication in professional practice: an adverse event occurring from medication overdose, whether accidental or intentional;
- An adverse event occurring from drug abuse/misuse
- An adverse event occurring from drug withdrawal
- Inadvertent or accidental exposure to a drug product

- Medication error; suspected transmission of any infectious agent via a drug product, unexpected therapeutic or clinical benefit from use of a drug product; and any patient or subject who becomes pregnant while on a drug product.

Currently, 206 subjects have participated in phase 1 studies including 145 subjects in single agent studies INK128-001 and INK128-002 (N = 106 and N = 39, respectively); and 61 subjects in the paclitaxel combination study INK128-003. Toxicities have been mostly Grades 1 and 2, reversible, and manageable with supportive care and/or interruption or dose reduction of study drug. Commonly reported study drug-related AEs have included hyperglycemia, asthenia, fatigue, mucosal inflammation, decreased appetite, rash, nausea, vomiting, and diarrhea. This emerging safety profile is consistent with those of other TORC1/2 and PI3K pathway inhibitors.

As of 2012, there are no FDA-approved TORC1/2 inhibitors. Rapamycin and rapalogs are TORC1 inhibitors with well-described toxicity profiles. Common toxicities include the following: immunosuppression with the potential to increase the risk of both nonserious and serious infections, and/or malignancies; mucositis, stomatitis, and mouth sores with a frequency from 41% to 78%; anorexia (approximately 30%), pneumonitis including interstitial lung disease (5%-36%); diarrhea (25%-56%); skin toxicity (48%-66%) which manifests typically as maculopapular or acneform rash, skin dryness, eczema, skin discoloration, and nail dystrophy; hyperlipidemia (hypercholesteremia and/or hypertriglyceridemia) with incidences from 8% to 44%; hyperglycemia (8%-22%); thrombocytopenia (10%-33%); anemia (27%-94%); leucopenia (27%-38%); hypokalemia (11%-21%); hypophosphatemia (15%-49%); hypertension (4%-7% in renal cancer subjects); elevated serum creatinine (37%-57%); elevated liver function tests (about 20%); arthralgia (25%-30%); asthenia (about 30%); peripheral edema (24%-35%). Serious infections have included sepsis, opportunistic infections, and even death. An increase in the development of lymphomas is also a possibility because of the immunosuppression.

Additionally, hypersensitivity reactions (18%), and fatal bowel perforation (1%) have been reported. Rapidly progressive, and sometimes fatal, acute renal failure not clearly related to renal cancer disease progression, abnormal wound healing, and increased risk of developing intracerebral bleeding (including fatal outcomes) in subjects with central nervous system (CNS) malignancies and/or receiving anticoagulation therapy have been reported in subjects receiving temsirolimus. The toxicities of rapamycin or rapalogs are typically reversible and infrequently serious. MLN0128 targets both TORC1 and TORC2, and thus may prove to have a different risk/benefit profile from the

rapalogs. There is no human information available on inhibition of TORC2 alone. The safety profile of MLN0128 continues to be explored in advanced malignancies, including non-Hodgkin lymphoma (NHL), and hematologic malignancies.

11.1.2 Unexpected Adverse Event

An unexpected AE is any event not associated by nature or intensity with the Investigational agent under study. The Agent Specific Adverse Event List (ASAEL) contains events that are considered expected for expedited reporting purposes only. A listing of expected and unexpected events for the agents under investigation in this study may be found in the Investigator's Brochure.

11.1.3 Adverse Events of Special Interest

Any newly identified malignancy or case of active pulmonary tuberculosis (TB) occurring after first administration of MLN0128 in subjects participating in this clinical study must be reported. These events are to be considered serious only if they meet the definition of an SAE.

11.1.4 Serious Adverse Event (SAE)

An SAE is defined as any untoward medical occurrence that at any dose:

- Results in **death.**
- Is **life-threatening** (refers to an AE in which the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe).
- Requires inpatient **hospitalization or prolongation of an existing hospitalization** (see clarification in the paragraph below on planned hospitalizations).
- Results in **persistent or significant disability or incapacity**. (Disability is defined as a substantial disruption of a person's ability to conduct normal life functions).
- Is a **congenital anomaly/birth defect**.
- Is a **medically important event**.

This refers to an AE that may not result in death, be immediately life threatening, or require hospitalization, but may be considered serious when, based on appropriate medical judgment, may jeopardize the patient, require medical or surgical intervention to prevent 1 of the outcomes listed above, or involves suspected transmission via a medicinal product of an infectious agent. 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; any organism, virus, or infectious particle (eg, prion protein transmitting Transmissible Spongiform Encephalopathy), pathogenic or nonpathogenic, is considered an infectious agent.

Clarification should be made between a serious AE (SAE) and an AE that is considered severe in intensity (Grade 3 or 4), because the terms serious and severe are NOT synonymous. The general term *severe* is often used to describe the intensity (severity)

of a specific event; the event itself, however, may be of relatively minor medical significance (such as a Grade 3 headache). This is NOT the same as *serious*, which is based on patient/event outcome or action criteria described above, and is usually associated with events that pose a threat to a patient's life or ability to function. A severe AE (Grade 3 or 4) does not necessarily need to be considered serious. For example, a white blood cell count of 1000/mm^3 to less than 2000 is considered Grade 3 (severe) but may not be considered serious. Seriousness (not intensity) serves as a guide for defining regulatory reporting obligations.

11.2 Recording Adverse Events

11.2.1 Timeframe for Recording Adverse Events and Serious Adverse Events

For each subject, AEs and SAEs occurring after informed consent is obtained should be recorded until the subject has completed his participation in the study.

An SAE must be reported if it occurs during a subject's participation in the study (whether receiving study drug or not) and within 30 days of receiving the last dose of Study Product in a clinical trial, whichever is longer.

Any SAE that is ongoing when a subject completes his participation in the Study must be followed until any of the following occurs:

- The event resolves or stabilizes;
- The event returns to baseline condition or value (if a baseline value is available);
- The event can be attributed to agents(s) other than the Study Product, or to factors unrelated to Study conduct.

Any subsequent AE felt to be possibly related to the use of the Study Product should be reported.

11.2.2 Recording of Adverse Events and Serious Adverse Events

Recording should be done in a concise manner using standard, acceptable medical terms.

The AE recorded should not be a procedure or a clinical measurement (i.e., a laboratory value or vital sign) but should reflect the reason for the procedure or the diagnosis based on the abnormal measurement.

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 AE).

Further, a procedure or surgery is not an AE; rather, the event leading to the procedure or surgery is considered an AE. Any event requiring in-patient hospitalization that occurs during the course of a subject's participation in a trial must be reported as an SAE. Surgeries or procedures planned prior to entry into the study for a pre-existing condition that has not worsened do not meet the criteria for SAE reporting.

If a clinical significant worsening from baseline is observed in any laboratory or other test parameter (e.g., ECG, angiogram), physical exam finding, or vital sign, a corresponding clinical AE should be recorded.

If a specific medical diagnosis has been made, that diagnosis or syndrome should be recorded as the AE 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 AE, using appropriate medical terminology (e.g., thrombocytopenia, peripheral edema, QT prolongation).

11.3 Grading Adverse Events

11.3.1 Grading severity

All AEs will be graded for intensity on a scale of 0 to 5. Severity grades will be recorded and based on the CTCAE v4.0.

11.3.2 Attributing causality

The investigator must evaluate all clinical AEs and clinically significant abnormal laboratory values for possible causal relationship to MLN0128. Causality attribution will be decided using the criteria outlined in Table .

Table 14. Relationship of adverse event to study drug

Relationship	Description
Unrelated	AE is clearly not related to study drug
Unlikely	AE is doubtfully related to study drug
Possible	AE may be related to study drug
Probable	AE is likely related to study drug
Definite	AE is clearly related to study drug

Abnormal laboratory values of clinical significance that were present at baseline and did not change in severity or frequency during experimental therapy or intervention and those that can obviously be attributed to underlying disease will be recorded as unrelated.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Response and progression will be evaluated in this study using a combination of the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee and modified for prostate cancer [23, 24] and the guidelines for prostate cancer endpoints developed by the Prostate Cancer Clinical Trials Working Group (PCWG2).[21]

Traditional measures of response reflect when a treatment is working and measures of progression indicate when a drug should be stopped. Because assessing response in

bone (the most common site of prostate cancer spread) is uncertain and the clinical significance of PSA changes in response to therapy is not a reliable predictor of response, measures of response have been expanded to include measures of progression.Patients will need to be reevaluated for response every 8 weeks or more frequently (if necessary) according to the guidelines above.

13.0 CRITERIA FOR REMOVAL FROM STUDY

In the absence of treatment delays because of AEs, treatment will continue until one of the following criteria applies:

- Patient decides to withdraw from the study
- Disease progression
    - symptomatic disease progression at any time
    - objective clinical disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable AE(s) that may or may not be directly related to treatment but that, in the judgment of the treating physician, makes it dangerous for the patient to be retreated
- General or specific changes in the patient's condition that render the patient unacceptable for further treatment, in the judgment of the investigator

Because an excessive rate of withdrawals can render the study uninterpretable, unnecessary withdrawal of patients should be avoided. When a patient discontinues treatment early, the investigator should make every effort to contact the patient and to perform a final evaluation. The reason(s) for withdrawal should be recorded.

13.1 Definition of Progression

When evaluating tumors, the following definitions will apply:

- Disease progression will be defined radiographically, by bone scan and CT scan, by at least one of the following:
    - Progression on bone scans with ≥ 2 new lesions not consistent with tumor flare, confirmed on a second bone scan ≥ 6 weeks later that shows ≥ 2 additional new lesions.
    - Soft tissue disease progression by RECIST 1.1 criteria
- Preclinical data have suggested that endocrine manipulations of prostate cancer cells may result in short-term changes in PSA mRNA expression and circulating PSA levels that do not necessarily reflect changes in tumor cell number. Instead, they represent a direct result of modulation of AR activity. Therefore, PSA is not a reliable marker of tumor response, especially when the treatment instituted involves endocrine manipulations. As a result, other, AR-independent, markers are needed. Consequently, for the purposes of this study, a rise in PSA will not be considered disease progression. In addition, PSA has not been shown to correlate with survival or progression for patients with metastatic castration-resistant prostate cancer and has not been used as a marker for disease progression in any of the phase 3 trials

resulting in approval of treatments such as abiraterone (de Bono et al., *N Engl J Med*, 2011), enzalutamide (Scher et al., *N Engl J Med*, 2012), or alpharadin (Parker et al,. *N Engl J Med*, 2013). Accordingly, PSA will not be used as a marker of disease progression for this study.

- CTC count will be monitored during the study, but a rise in CTC will not be part of the definition of disease progression.

13.2 Safety Evaluation

Safety will be evaluated according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.0. Safety assessments will be based on medical review of AE reports and the results of vital sign measurements, physical examinations, and clinical laboratory tests throughout the conduct of the study. The incidence of AEs will be tabulated and reviewed for potential significance and clinical importance.

14.0 BIOSTATISTICS

14.1 Analysis Population

The analysis population will include all subjects who receive at least 1 dose of study drug.

14.2 Demographics and Baseline Characteristics

Demographic variables will include age, race, ethnicity, height, and weight. Baseline disease characteristics will include time from diagnosis, time from radical prostatectomy to PSA progression and time from radical prostatectomy to initiation of study drug.

14.3 Safety Analysis

14.3.1 Adverse events

Safety analysis will be summarized using the Safety Population defined as any patient receiving any part of study treatment.

Extent of exposure to study treatment will be summarized and details will be provided. Treatment emergent AEs are those events that occur or worsen on or after first dose of study drug up through 30 days post last dose. AEs will be coded using the MedDRA coding system and all AEs will be graded according to the most current National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE).

14.3.2 Clinical laboratory tests

All Grade 3 and 4 abnormal laboratory test results will be reported according to the NCI-CTCAE Version 4.0 criteria.

14.4 Statistical Procedures

The primary objective of this study is to determine the efficacy of MLN0128 in patients with metastatic castration-resistant prostate cancer. The primary endpoint of the study is six month progression free survival (6mPFS). A patient that is followed for six months and remains progression free during this time period is defined as a success. A patient

that is lost to follow-up prior to the six month landmark time is counted as a failure for the 6mPFS endpoint.

Recently there have been two phase III studies demonstrating an overall survival benefit for patients with CRPC. The 6mPFS endpoint has therefore been selected based on these single agent studies using the hormonal intervention abiraterone (6mPFS 44%) and chemotherapy cabazitaxel (6mPFS 23%). In this study, a two-stage design that differentiates between 6mPFS rates of 0.30 and 0.50 will be used to assess treatment efficacy. In the first stage of the study 21 patients will be enrolled (Cohort 1). If at most 6 patients remain alive and progression free at 6 months, accrual will be terminated. If at least 7 patients are alive and progression free at 6 months, an additional 21 patients will be accrued in this cohort (Cohort 2). At the conclusion of this second stage, if at least 17/42 patients remain alive and progression free at 6 months, then the treatment will be declared sufficiently active. The probability of declaring the treatment effective is 0.10 when the 6mPFS in the population is 0.30 and increases to 0.90 when the 6mPFS is 0.50. It is anticipated that accrual in this trial will be completed in approximately 1-2 years.

Secondary Anti-tumor Endpoints

PSA. Summary tables and waterfall plots describing change in PSA relative to baseline will be reported at 8 weeks (or earlier for those who discontinue therapy), and separately, the maximal change at any time on study will also be reported for each patient using summary tables and waterfall plots.

Soft Tissue. Summary tables and waterfall plots describing change in target lesions relative to baseline will be reported every 8 weeks using RECIST version 1.1 (Response Criteria in Solid Tumors) criteria. Separately, the maximal change at any time on study will also be reported using summary tables and waterfall plots.

Bone Disease. Summary tables describing the change in radionuclide bone scans relative to baseline will be reported every 8 weeks. Since there are no validated criteria for response on radionuclide bone scan, results will be recorded as “no new lesions” or “new lesions”. The appearance of ≥ 2 new lesions on confirmatory bone scan is considered disease progression.

Exploratory Endpoints

Circulating tumor cells, FDG PET imaging, and FDHT PET imaging will be recorded over time and their temporal association with progression-free survival time (and overall survival time) will be modeled using a time-dependent Cox model.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

15.2 Randomization

This study will not require randomization procedures.

16.0 DATA MANAGEMENT

16.1 Quality Assurance

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of the activities of the protocol study team.

16.2 Data and Safety Monitoring

16.2.1 Data Collection and Review

Data for each subject will be recorded by authorized site personnel on eCRFs (an electronic case report form), which must be completed for every subject who signs an informed consent form. The eCRF will be signed by the Investigator to affirm the accuracy of information recorded.

Data will be reviewed routinely by the Study Coordinator assigned at the study site and by the Data Management Department of the Sponsor (or designee) to assess missing data and inconsistencies. Accrual rates, extent and accuracy of evaluations, and follow-up will be monitored periodically throughout the study period; potential problems will be brought to the attention of the study team for discussion and action. The study team will conduct random audits of data quality and protocol compliance.

The responsibilities of the Investigator include protocol compliance, problem resolution and prioritization, regulatory monitoring, and ensuring the accuracy, completeness, legibility, and timeliness of the data reported to the Sponsor on the case report form. The responsibilities of the Study Coordinator include data collection and entry on the case report form, verification, abstraction, and reporting; and coordination of activities of the study team.

Direct access to source data and documents will be permitted for representatives of the Quality Assurance Department of the Sponsor (or designee), the Institutional Review

Board/Ethics Committee, and regulatory agencies. All information that may specifically identify the subject will be protected prior to provision of this access.

16.2.2 Source documents

Study personnel will record clinical data in each patient's source documents (i.e., the patient's medical record). Source documentation will be made available to support the patient research record. Study monitors will review entries on the CRFs at regular intervals, comparing the content with source documents.

16.2.3 Record retention

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. After study closure, the investigator will maintain all source documents, study-related documents, and the CRFs. The original of all such Reports will be maintained by the Participating Member(s) until the later of: (a) two (2) years following the date a New Drug Application is approved for the Study Drug that is the subject of the Clinical Trial; or (b) two (2) years after the Investigational New Drug Application for such Study Drug is terminated or withdrawn, or such longer period of time as may be required by Participating Member policies, applicable laws, rules or regulations.

17.0 PROTECTION OF HUMAN SUBJECTS

17.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

It is also stated in the consent and Research Authorization that research data (e.g. genomic sequence) may be placed into databases monitored by the National Institutes of Health, and may be made accessible to investigators approved by the U.S. government. It is difficult to identify genotype/phenotype specifics at this time and therefore, the requirements for submission of genotype/phenotype data into the NIH GWAS Repository (or any other public database) will be followed as per the MSKCC IRB GWAS SOP-503.

17.2 Serious Adverse Event (SAE) Reporting

17.2.1 Millennium Reporting timelines

All SAEs, AEs of Special Interest, and Pregnancy/Paternal Exposure Reports should be reported to Millennium within 24-hours of becoming aware of the event(s).

17.2.2 Millennium Procedures for Reporting Serious Adverse Events

AEs may be spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or

other diagnostic procedures. Any clinically relevant deterioration in laboratory assessments or other clinical finding is considered an AE. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event. AEs which are serious must be reported to Millennium Pharmacovigilance (or designee) from the first dose of MLN0128 up to and including 30 days after administration of the last dose of MLN0128. Any SAE that occurs at any time after completion of MLN0128 treatment or after the designated follow-up period that the sponsor-investigator and/or sub-investigator considers to be related to any study drug must be reported to Millennium Pharmacovigilance (or designee). Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was enrolled in the trial are not to be considered AEs unless the condition deteriorated in an unexpected manner during the trial (e.g., surgery was performed earlier or later than planned). All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

Since this is an investigator-initiated study, the principal investigator Dr. Rathkopf, also referred to as the sponsor-investigator, is responsible for reporting serious adverse events (SAEs) to any regulatory agency and to the sponsor- investigator's EC or IRB. Regardless of expectedness or causality, all SAEs must also be reported to Millennium Pharmacovigilance or designee as soon as possible, but no later than 24 hours of the sponsor-investigator's observation or awareness of the event. See below for contact information for the reporting of SAEs to Millennium Pharmacovigilance.

The sponsor-investigator should fax the SAE Form within five calendar days after becoming aware of the event. A sample of an SAE Form will be provided. Follow-up information on the SAE may be requested by Millennium. The SAE report must include event term(s), serious criteria, and the sponsor-investigator's or sub-investigator's determination of both the intensity of the event(s) and the relationship of the event(s) to study drug administration. Intensity for each SAE, including any lab abnormalities, will be determined by using the NCI CTCAE v. 4 as a guideline, whenever possible. The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html.

Relationship to all study drugs for each SAE will be determined by the investigator or sub-investigator by responding yes or no to the question: Is there a reasonable possibility that the AE is associated with the study drug(s)?

Sponsor-investigator must also provide Millennium Pharmacovigilance with a copy of all communications with applicable regulatory authorities related to the study or study drug(s), including, but not limited to, telephone conversation logs, as soon as possible but no later than 5 calendar days of such communication.

SAE and Pregnancy Reporting Contact Information:

Millennium Pharmacovigilance **SAE and Pregnancy Reporting Contact Information** **Cognizant Contacts:**

Fax: 1-800-963-6290

Email: TakedaOncoCases@cognizant.com

Suggested Reporting Form:

- SAE Report Form (a sample will be provided)
- US FDA MedWatch 3500A: http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm
- Any other form deemed appropriate by the sponsor-investigator

Product Complaint Information:

A product complaint is a verbal, written, or electronic expression that implies dissatisfaction regarding the identity, strength, purity, quality, or stability of a drug product. Individuals who identify a potential product complaint situation should immediately contact MedComm Solutions (see below) and report the event. Whenever possible, the associated product should be maintained in accordance with the label instructions pending further guidance from a Millennium Quality representative.

For Product Complaints,

call MedComm Solutions at

877-674-3784 (877 MPI DRUG)

(US and International)

Product complaints in and of themselves are not AEs. If a product complaint results in an SAE, an SAE form should be completed and sent to PPD.

17.2.3 Millennium Procedures for Reporting Drug Exposure During Pregnancy and Birth Events

If a female partner of a male patient becomes pregnant during the male patient's participation in this study, the sponsor-investigator must also immediately fax a completed Pregnancy Form to the Millennium Department of Pharmacovigilance or designee. Every effort should be made to follow the pregnancy for the final pregnancy outcome.

Suggested Pregnancy Reporting Form:

Pregnancy Report Form (a sample will be provided)

The investigator must assess each event to determine if it meets the criteria for classification as a Serious Adverse Event (SAE). An SAE as defined in the Code of Federal Regulations (21CFR312.32) is any event that:

- Results in subject death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity (i.e., a substantial disruption in a person's ability to conduct normal activities of daily living)
- Is a congenital anomaly/birth defect.
- Is a suspected transmission of infectious agents by a medicinal product

In addition, an important medical event that may not result in death, be life-threatening, or require/prolong hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the subject and/or may require medical or surgical intervention to prevent one of the outcomes listed above.

Examples of such 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.

Progression of malignancy

Progression of a patient's malignancy should not be considered an AE, unless in the investigator's opinion, study treatment resulted in an exacerbation of the patient's condition. If disease progression results in death or hospitalization while on study or within 30 days of the last dose, progressive disease will be considered an SAE.

Life-threatening events

A life-threatening event is any AE that places the patient at immediate risk of death from the reaction as it occurs. It is not a reaction that, had it occurred in a more severe form, might have caused death.

Hospitalization or prolongation of hospitalization

Hospitalization encompasses any inpatient admission (even for less than 24 hours) resulting from a precipitating, treatment-emergent AE. For chronic or long-term patients, inpatient admission also includes transfer within the hospital to an acute or intensive care inpatient unit. Hospitalizations for administrative reasons or a non-worsening preexisting condition should not be considered AEs (e.g., admission for workup of a persistent pretreatment laboratory abnormality, yearly physical exam, protocol-specified admission, elective surgery). Pre-planned treatments or surgical procedures should be noted in the baseline documentation. Hospitalization because of an unplanned event will be deemed an SAE.

Prolongation of hospitalization is any extension of an inpatient hospitalization beyond the stay anticipated or required for the original reason for admission.

Significant disability

Disability is a substantial disruption of the patient's ability to conduct normal life functions.

Congenital anomaly

If the female partner of a male patient becomes pregnant during the course of the study, the treating physician must be notified immediately. All pregnancies will be followed until resolution (i.e., voluntary or spontaneous termination or birth) and assessed for congenital anomalies and birth defects.

Medical significance

An event that is not fatal or life-threatening and that does not necessitate hospitalization may be considered serious if, in the opinion of the investigator, it jeopardizes the patient's status and might lead to medical or surgical intervention to prevent any of the above outcomes. Such medically significant events could include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias that do not result in inpatient hospitalization, or the development of drug dependency or abuse.

17.2.4 MSKCC Procedures for Reporting Serious Adverse Events

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org. The report should contain the following information:

Fields populated from CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
    - If an amendment will need to be made to the protocol and/or consent form.

The PI's signature and the date it was signed are required on the completed report.

For IND/IDE protocols:
The CRDB AE report should be completed as above and the FDA assigned IND/IDE number written at the top of the report. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

Sponsor-investigator must also provide Millennium Pharmacovigilance with a copy of all communications with applicable regulatory authorities related to the study or study drug(s), including, but not limited to, telephone conversation logs, as soon as possible but no later than 5 calendar days of such communication.

17.2.5 Adverse events

Safety analysis will be summarized using the Safety Population defined as any patient receiving any part of study treatment.

Extent of exposure to study treatment will be summarized and details will be provided. Treatment emergent AEs are those events that occur or worsen on or after first dose of study drug up through 30 days post last dose. AEs will be coded using the MedDRA coding system and all AEs will be graded according to the most current National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE).

17.2.6 Clinical laboratory tests

All Grade 3 and 4 abnormal laboratory test results will be reported according to the NCI-CTCAE Version 4.0 criteria.

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information.

In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

19.0 REFERENCES

1. Taylor BS, Schultz N, Hieronymus H, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell 2010;18:11-22.
2. George DJ, Armstrong AJ, Creel P, et al. A phase II study of RAD001 in men with hormone-refractory metastatic prostate cancer (HRPC). 2008 ASCO Genitourinary Cancers Symposium; February 14-16, 2008; San Francisco, California;Abstract 181.
3. Rathkopf DE, Danila DC, Chudow JJ, et al. Anti-insulin-like growth factor-1 receptor (IGF-IR) monoclonal antibody cixutumumab plus mammalian target of rapamycin (mTOR) inhibitor temsirolimus in metastatic castration-resistant prostate cancer (CRPC). 2010 ASCO Annual Meeting; June 4-8, 2010; Chicago, IL;Abstract TPS242.
4. Rathkopf DE, Danila DC, Morris MJ, et al. Anti-insulin-like growth factor-1 receptor (IGF-1R) monoclonal antibody cixutumumab (cix) plus mTOR inhibitor temsirolimus (tem) in metastatic castration-resistant prostate cancer (mCRPC): Results of a phase I pilot study. 2011 ASCO Annual Meeting; June 3-7, 2011; Chicago, IL;Abstract e15081.
5. Guertin DA, Stevens DM, Saitoh M, et al. mTOR complex 2 is required for the development of prostate cancer induced by Pten loss in mice. Cancer Cell 2009;15:148-59.
6. Carver BS, Chapinski C, Wongvipat J, et al. Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. Cancer Cell 2011;19:575-86.
7. Nardella C, Carracedo A, Alimonti A, et al. Differential requirement of mTOR in postmitotic tissues and tumorigenesis. Sci Signal 2009;2:ra2.
8. O'Reilly KE, Rojo F, She QB, et al. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res 2006;66:1500-8.
9. Jemal A, Siegel R, Xu J, et al. Cancer statistics, 2010. CA Cancer J Clin 2010;60:277-300.
10. Scher HI, Heller G. Clinical states in prostate cancer: toward a dynamic model of disease progression. Urology 2000;55:323-7.
11. Chiang GG, Abraham RT. Targeting the mTOR signaling network in cancer. Trends in Molecular Medicine 2007;13(10):433-42.
12. Sabatini DM. mTOR and cancer: insights into a complex relationship. Nature Reviews. Cancer 2006;6(9):729-34.
13. Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo controlled phase III trial. Lancet 2008;372(9637):449-56.
14. Fasolo A, Sessa C. Current and future directions in mammalian target of rapamycin inhibitors development. Expert Opinion on Investigational Drugs 2011;20(3):381-94.
15. Benjamin D, Colombi M, Moroni C, Hall MN. Rapamycin passes the torch: a new generation of mTOR inhibitors. Nature Reviews. Drug Discovery 2011;10(11):868-80.
16. Sankhala K, Mita A, Kelly K, Mahalingam D, Giles F, Mita M. The emerging safety profile of mTOR inhibitors, a novel class of anticancer agents. Targeted Oncology 2009;4(2):135-42.
17. RAPAMUNE (sirolimus) Oral Solution and Tablets [package insert]. Philadelphia, PA: Pfizer; 2010.
18. AFINITOR (everolimus) tablets for oral administration [package insert]. East Hanover, NJ: Novartis; 2011.
19. TORISEL Kit (temsirolimus) injection, for intravenous infusion only [package insert]. Philadelphia, PA: Wyeth Pharmaceuticals Inc (Pfizer); 2011.
20. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228-47.
21. Scher HI, Halabi S, Tannock I, 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;26:1148-59.

22. Danila DC, Fleisher M, Scher HI. Circulating tumor cells as biomarkers in prostate cancer. Clin Cancer Res 2011;17:3903-12.
23. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205-16.
24. Scher HI, Morris MJ, Kelly WK, et al. Prostate cancer clinical trial end points: "RECIST"ing a step backwards. Clin Cancer Res 2005;11:5223-32.
25. Danila DC, Anand A, Schultz N, et al. Analytic and clinical validation of a prostate cancer-enhanced messenger RNA detection assay in whole blood as a prognostic biomarker for survival. Euro Urology 2013.

20.0 APPENDICES

Appendix A: Performance Status Criteria

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

MSKCC

Appendix B: Study Calendar

	Screening		Cycle 1				Cycle 2				Cycle 3 and 4		Subsequent cycles		
Assessments[1]	Within 30 days prior to initiation of protocol therapy	Within 14 days prior to initiation of protocol therapy	C1 D1	C1 D8	C1 D15	C1 D22	C2 D1	C2 D8	C2 D15	C2 D22	D1	D15	D1	Every 8 Weeks	End of Treatment (Within 28 days of last dose of study medication)
Informed consent	X														
Medical history, demographics	X														
Interim medical history, Physical Exam[2]	X		X	X	X	X	X	X	X	X	X	X	X		
Hematology & Chemistry[3]		X	X	X	X	X	X	X	X	X	X	X	X		X
Coag, PT, PTT, INR		X			X				X			X	X		
HbA1c		X	X								C3D1		C6 & q3 cycles		
Serum Testosterone		X													
PSA		X	X				X				X		X		X
Blood for CTC studies		X	X				X				X		X		X
Whole blood lymphocyte[4]		X		X											
Buffy coat for germline DNA		X													
Daily Fasting glucose[5]			X	X	X	X	X	X	X	X	X	X	X		
Urinalysis			X		X		X		X		X	X	X		
ECG[6]	X		X	X	X	X	X	X	X	X					X
ECHO/MUGA	X														
Contrast enhanced (if feasible) CT & Radionuclide bone scan	X										C3D1			X[7]	X[8]
FDG PET scans[9]	X					X									X
Optional FDHT PET scans[10]	X					X									X
Optional tumor biopsy[11]	X					X									X
Treatment administration			X----------												----X
AE assessment			X----------												----X
Concomitant medications	X----------														----X

[1]With the exception of ECGs, assessments may be done within 24-48 hours of dosing with MLN0128 administration

[2]PE symptom directed post D1. Physical examination includes vital signs, HEENT (head, eyes, ears, nose, and throat), chest, cardiac, abdominal, extremities, neurologic, and lymph node examinations. Weight will be recorded at every visit. Height will be recorded at screening visit only. KPS will be recorded at every visit. Vital signs include upright blood pressure, heart rate, respiratory rate, and oral or aural body temperature.

[3]Fasting, including electrolytes and lipid profile

[4]Whole blood lymphocytes will be collected 0 to 1hr post treatment with MLN0128 on MLN0128 treatment days.

[5]Daily at home with glucometers for first 6 months then twice weekly. If applicable, if changes to metformin dose, resume daily monitoring

[6]C1D1 ECG pre dose, 2 hr post dose and 4 h post dose (+/- 15 minutes); C2D1 ECG pre dose and 2 h post dose (+/- 15 minutes); all other ECGs 2 h post dose (+/- 15 minutes)

[7]CT and bone scan can be performed ± 7 days during treatment period.

[8]CT and bone scan can be performed ± 7 days at the time of progression, unless performed in the 30 days prior.

[9]FDG PET scans are performed at baseline, week 4 (±7 days) and progression (within 28 days of last dose). Baseline and End of treatment PET scans will be performed with contrast (if feasible).

[10]Optional FDHT PET (patients on protocol 00-095) are performed at baseline, week 4 (±7 days) and progression (within 28 days of last dose).

[11]Biopsies are optional but requested of all patients. Biopsy is done at baseline, 4 weeks (±7 days) and at the time of progression when feasible (within 28 days of last dose).

Appendix C: Circulating Tumor Cell (CTC) Laboratory Manual

1. BACKGROUND

A critical area of unmet medical need in prostate cancer management is the development of tumor specific markers to select targeted therapies and to reliably assess patient clinical outcome. Changes in PSA associate poorly with survival post therapy, and conventional imaging is limited in the assessment of distant metastases. We and others have demonstrated that it is feasible to isolate and characterize circulating tumor cells (CTCs) from a blood sample in real time. CTC number has been proposed as a marker of prognosis pre-therapy and as a surrogate for treatment efficacy post-therapy in phase III clinical trials powered to detect a difference in survival with treatment. In addition, we have shown that a significant proportion of patients with CRPC have genomic amplification of the androgen receptor (AR) gene, or an increased AR gene copy number.

The measurement of CTC number is performed with a semiautomated system, CellSearch (Veridex), which uses an EpCAM (epithelial common antigen molecule) antibody-based immunomagnetic antibody-capture technology. The approach combines an enriching step from blood of epithelial cells with an EpCAM antibody conjugated to immunomagnetic beads, and a negative selection by differentiating CTCs from CD45-expressing cells in order to reduce the mononuclear cell fraction.

In addition to providing pre-treatment prognostic and post-treatment efficacy information, the isolated CTCs can be evaluated at the DNA, RNA, and protein level for the expression of specific biological determinants. Current methodology using immunomagnetic isolation as well as FACS sorting has allowed MSKCC to develop assays to study specific molecular profiles in enriched CTC specimens.

Patients will be drawn for correlative studies related to CTC at the following time-points: Screening, Day 1 of each cycle, and at the end of treatment (time of treatment discontinuation). Specimens are to be collected using kits provided for the study, and shipped same day to MSKCC. A +/-2 day window around each time point is allowed if necessary for scheduling patient visits. CTC blood samples should not be drawn on Fridays.

The collection of blood is summarized below:

Circulating Tumor Cell Sampling	Screening	Day 1 of each cycle	End of Treatment (time of treatment discontinuation)
CellSave (2 tubes)	**X**	**X**	**X**
EDTA (1 tube)	**X**	**X**	**X**
PAXgene (2 tubes)	**X**	**X**	**X**

2. DESCRIPTION OF COLLECTION TUBES

CellSave tube: Evacuated blood collection tube containing a proprietary fixative for the preservation of CTCs.

EDTA tube: Evacuated blood collection tube for plasma collection and genotyping analysis.

PAXgene tube: Evacuated blood collection tube containing a proprietary reagent which stabilizes RNA integrity for gene expression analyses.

3. COLLECTION OF BLOOD SAMPLES

At each time point, blood samples will be drawn in the following order: 2 Cell Save tubes, EDTA tube, and 2 PAXgene tubes last. Samples will then be processed as described below, labeled, and shipped to MSKCC Chemical Chemistry on the same day of collection. All samples must be kept at room temperature.

CellSave Tube (for CTC Collection)
In each tube, 7.5 ml of peripheral blood will be collected. It is essential that the tubes are filled completely in order to be processed for CTC analysis. After collection, the tube must be inverted eight times to prevent clotting and then can be stored at room temperature until same day shipment.

EDTA Tube (for CTC Collection)
Ten mL of peripheral blood will be collected in a lavender top tube provided in the drawing kits. It is essential that the tube is filled completely in order to collect enough genetic material for analysis.

PAXgene Tubes (for mRNA Collection)
In each tube, 2.5 ml of peripheral blood will be collected. The tubes will be held below the patient's arm. After collection of the blood, invert the tube 8 to 10 times and ship same day. It is essential that the tubes are filled completely in order to be processed for mRNA extractions.

4. LABELING AND TRANSPORTING OF BLOOD SAMPLES

Patient identification must be anonymous. Submitted documents and samples will have a unique anonymous identifier for each patient, consisting of a study identifier derived from the study-sponsor's database. This identifier will be used for the purposes of the study only, and will be distinct from the patient identification (medical record) number used at each site.

The blood samples must be shipped on the day of collection to the MSKCC Clinical Chemistry Laboratory for processing, using the shipping boxes that will be provided. Fill out the CTC requisition form completely, which will be included in the kit provided. Include the original requisition form in the shipment to MSKCC. Retain the copy for your records.

Samples should be kept at room temperature at all times. Shipping reservations must be made to allow delivery within 24 hr of specimen collection, and prior to 2:00 PM the next day. **If at all feasible, CTC samples should not be drawn on a Friday.** A +/-2 day window around each time point is allowed if necessary for scheduling patient visits around this restriction.

The shipping labels should be addressed to:

MSKCC Clinical Chemistry Laboratory
Memorial Hospital, Schwartz 359
Rashmi Kamath
Attn. CTC samples for MLN0128
1275 York Avenue
New York, NY 10065
tel (212) 639-5969

5. PROCESSING AND ANALYSIS OF BLOOD SAMPLES

Samples received at the reference MSKCC Clinical Chemistry laboratory will be processed for CTC enumeration and profiling studies. Upon arrival at the laboratory, personnel will ensure that the minimum volume of blood has been collected for these studies.

CTC Enumeration and Interpretation

Blood collected in CellSave tubes will be processed on the CellTracks AutoPrep System with the CellSearch Circulating Tumor Cell Kit which is intended for the enumeration of CTCs of epithelial origin (CD45-, EpCAM+, and cytokeratins 8+, 18+, and/or 19+) in whole blood.

The methodology involves semiautomatic immunomagnetic selection of CTCs based on capture with an anti-EpCAM antibody and immunofluorescence analysis. In short, samples drawn in CellSave tubes containing cell preservatives, maintained at room temperature and shipped to MSKCC, will be processed on the CellSearch Epithelial Cell system (Veridex) after EpCAM antibody-covered ferroparticles are added and incubated at room temperature. A magnetic field is used to collect cells of interest without centrifugation. After unbound supernatant has been removed, the enriched samples are processed for nucleic acid staining with DAPI (4',6-diamidino-2-phenylindole), for markers for epithelial cells with anti-cytokeratin CK-PE. Leukocytes are excluded with an anti-CD45 immunofluorescent antibody. Stained cells are

analyzed on a fluorescence microscope using the CellTrack Analyzer II and CTCs are defined as cytokeratin positive, DAPI nucleated cells lacking CD45 markers. Automatically selected images are reviewed by the operator for identification of tumor cells. Androgen receptor (AR) protein analysis employing a fluorescein conjugate antiandrogen receptor antibody may be tested in order to explore the overexpression of AR in CTCs, and (if performed) will be scored based on the number of cells expressing AR relative to the total number of CTCs. Quality control will be maintained using standard procedures. Using CellSearch technology for metastatic prostate cancer, a value of ≥ 5 CTCs (per 7.5 ml tube) is considered abnormal, with ≥ 50 being the suggested minimum for molecular profiling studies.

Molecular Profiling of CTC

Genomic DNA: Flow cytometry has yielded CTC events almost 100 times as high as those from CellSearch, but more importantly has increased the proportion of patients with CTC number sufficient for profiling. Blood collected in the second CellSave tube would be processed for CTC enrichment by FACS sorting.

Mononucleated cells are obtained through Ficoll-Hypaque density gradient centrifugation and then double-stained with EpCAM-PE antibody to identify CTC, and with CD45-APC antibody to identify white blood cells; DAPI staining is used to exclude dead cells. EpCAM-positive cells are counted and isolated using FACS on MoFlo cell sorter (DAKO), with the exclusion of cells that are CD45 and DAPI positive. The EpCAM positive cells are sorted by FACS in parallel with CD45 positive WBC, deposited in sequential wells in equal quantity onto plates that are stored at -80C for batch processing. These cells will be used to obtain genomic DNA for whole genome amplification (WGA).

Whole genome amplification (WGA) will be performed, followed by quality control PCR, and direct sequencing for AR and other relevant mutations. Sequencing results will be analyzed in comparison to the known AR mutations and available SNP data, and any AR mutations found in CTC will be confirmed in a second sample. Identified AR mutations in CTC will be compared to AR sequencing in WBC obtained from same patients to differentiate somatic mutations from germline SNPs. In addition to AR gene, we propose to sequence other highly relevant genes for prostate cancer previously described or identified in our Oncogenomics project.

Gene Analysis: Isolation of mRNA from CTC enriched by flow cytometry will also be performed from blood drawn into EDTA anticoagulant blood tubes at baseline and with treatment. Primer directed reverse transcription will be performed immediately, and samples can be frozen and stored for gene analysis in batches. Expression of prostate-specific mRNAs, such as AR, PSA (KLK3), and KLK2, as well as for fusion genes specific to prostate cancer, such as TMPRSS-ERG, will be tested in CTC.

Point mutations or prostate cancer specific gene products will be confirmed by PCR from RNA extracted from non-enriched blood samples collected in PAXgene tubes. This will allow us to determine the limits of detection based on the presence of contaminating normal cells, considering the dilution effects of non-malignant mononuclear cell populations on a prostate-specific as opposed to prostate cancer specific gene or gene mutation.

Protein Analysis: Peripheral blood mononuclear cells (PBMC) obtained through Ficoll-Hypaque density gradient centrifugation may also be stained and plated onto glass slides for the purpose of immunocytochemistry for AR protein expression and cellular localization in CTC before and after treatment.

Appendix D: Glossary of Abbreviations and Acronyms

ADR	adverse drug reaction
ADT	androgen-deprivation therapy
AE	adverse event
AI	accumulation index
ALT	alanine aminotransferase
ANC	absolute neutrophil count
ANOVA	analysis of variance
APTT	activated partial thromboplastin time
AR	androgen receptor
ASAEL	Agent Specific Adverse Event List
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
AUC(INF)	area under the concentration-time curve from time zero extrapolated to infinite time
AUC(0-T)	area under the concentration-time curve from time zero to the time of the last quantifiable concentration
AUC(TAU)	area under the concentration-time curve in one dosing interval
AUMC(INF)	area under the moment concentration time curve extrapolated to infinity
A-V	atrioventricular
β-HCG	beta-human chorionic gonadotrophin
BMI	body mass index
BP	blood pressure
BSA	Body Surface Area
BUN	blood urea nitrogen
Ca++	calcium
CBC	complete blood count

CFR	Code of Federal Regulations
CI	confidence interval
Cl-	chloride
Clcr	creatinine clearance
CLNR	nonrenal clearance
CLR	renal clearance
CLT	total body clearance
Cmax	maximum plasma concentration
Cmin	trough observed concentration
CNS	central nervous system
CRF	case report form
CRPC	castration resistant prostate cancer
CT	computerized tomography
CTC	circulating tumor cell
CTCAE	Common Terminology Criteria for Adverse Events
DEV	deviation from the nominal value
DHEA	dehydroepiandrosterone
DHEA-S	dehydroepiandrosterone sulfate
DHT	dihydrotestosterone
DLT	dose-limiting toxicity
ECG	electrocardiogram
EEG	electroencephalogram
EORTC	European Organisation for Research and Treatment of Cancer
FDA	Food and Drug Administration
FDG-PET	2-[18F]fluoro-2-deoxyglucose positron emitting tomography
FDHT	18-fluoro-dehydrotestosterone
GnRH	gonadotropin-releasing hormone
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HR	heart rate

ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IHC	immunochemical
IND	investigational new drug
IRB	Institutional Review Board
ITT	intent-to-treat population
IV	intravenous
K+	potassium
K3EDTA	potassium ethylenediaminetetraacetic acid
KLK1	kallikrein 1
LD	longest diameter
LDH	lactate dehydrogenase
MAD	maximum administered dose
MRI	magnetic resonance imaging
MSKCC	Memorial Sloan-Kettering Cancer Center
MTD	maximum tolerated dose
N	number of subjects or observations
NCI	National Cancer Institute
NIH	National Institutes of Health
NSAID	nonsteroidal anti-inflammatory drug
PCCTC	Prostate Cancer Clinical Trials Consortium
PCRP	Department of Defense Prostate Cancer Research Program
PD	progressive disease
PET	positron emission tomography
PFS	progression-free survival
PI	principal investigator
PK	pharmacokinetics
PSA	prostate-specific antigen
PSADT	prostate-specific antigen doubling time
PT	prothrombin time

PTT	partial thromboplastin time
RBC	red blood cell
RECIST	Response Evaluation Criteria in Solid Tumors
SUV	standardized uptake value
t	temperature
t1/2	terminal half-life
T	time
TAUC(TAU)	trapezoidal area under the concentration-time curve in one dosing interval
TAUC(0-T)	trapezoidal area under the concentration-time curve from time zero to the time of the last quantifiable concentration
TDP	time to disease progression
TGP	prostate-specific transglutaminase
Tmax	time of maximum observed concentration
Vss	volume of distribution at steady-state
WBC	white blood cell

Appendix E: Pill Diary

Pill Diary for Protocol #13-143

Patient ID Number:_____	Bottle or Lot#:_____
Number of Pills Given:_____	Pill Bottle(s) returned: Circle **Yes** or **No**
Total Daily Dose: _____	Number of Pills **returned**: _____

(To be completed by RN)

PLEASE FILL OUT AND BRING THIS SHEET AT YOUR NEXT VISIT.

SPECIAL INSTRUCTIONS for MLN0128: Take the 4mg daily dose by mouth in the morning at approximately the same time each day. It is recommended that you take your dose after a light meal and with 8 ounces of water. DO NOT make up vomited doses.

CYCLE #: _____

# of WEEKS:_____

MLN0128

DAY	DATE	Time MLN0128 taken:	# of MLN0128 tablets taken:	If dose not taken or if full dose not taken, please provide an explanation:
Example	01/01/12	9:00 AM	1	
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				

12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				

Patient Signature: _____ **Date:** _____

Physician/Research RN Signature: _____ **Date:** _____

Physician/Research RN Comments: _____

Appendix F: Glucose Monitoring Diary

Glucose Monitoring Diary for Protocol #13-143

Patient ID Number:_____

SPECIAL INSTRUCTIONS for MLN0128 Glucose Monitoring: ***You must have your glucose level checked first thing in the morning before eating anything and the result will be written in the space provided in this diary. If your Doctor requires additional testing, the results must also be recorded.***

CYCLE #: _____ **# of WEEKS: _____**

DAY	DATE	Blood Glucose Time:		Blood Glucose Result:	Are additional blood glucose results indicated below? (circle Y/N)
Example	01/01/12	9:00	AM		Y/N
1			AM		Y/N
2			AM		Y/N
3			AM		Y/N
4			AM		Y/N
5			AM		Y/N
6			AM		Y/N
7			AM		Y/N
8			AM		Y/N
9			AM		Y/N
10			AM		Y/N
11			AM		Y/N
12			AM		Y/N
13			AM		Y/N
14			AM		Y/N
15			AM		Y/N

16			AM		Y/N
17			AM		Y/N
18			AM		Y/N
19			AM		Y/N
20			AM		Y/N
21			AM		Y/N
22			AM		Y/N
23			AM		Y/N
24			AM		Y/N
25			AM		Y/N
26			AM		Y/N
27			AM		Y/N
28			AM		Y/N
29			AM		Y/N
30			AM		Y/N

Additional blood glucose results (if needed):

Date	Time	Result	Date	Time	Result	Date	Time	Result

Patient Signature: _____ **Date:** _____

Physician/Research RN Signature: _____ **Date:** _____

Physician/Research RN Comments: _____

Appendix G: New York Heart Association Classification of Cardiac Disease

Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

Source: The Criteria Committee of New York Heart Association. Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. 9th Ed. Boston, MA: Little, Brown & Co; 1994:253-256.

Appendix H: Strong Inhibitors and Inducers of CYP2C9, CPY2C19, and CYP3A4

Strong Inhibitors	**Strong Inducers**
Indinavir	Carbamazepine
Nelfinavir	Phenobarbital
Ritonavir	Phenytoin
Clarithromycin	Rifabutin
Itraconazole	St. John's wort
Ketoconazole	Troglitazone
Nefazodone	Secobarbital
Fluconazole	Rifampin
Telithromycin	
Fluvoxamine	
Mibefradil	
Omeprazole	
Ticlopidine	
Fruit and juice:	
Star fruit	
Pomegranate	
Grapefruit	
Seville oranges	
Papaya	

Source: http://www.ganfyd.org/index.php?title=Inhibitors_of_CYP3A4 and http://medicine.iupui.edu/clinpharm/ddis/