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TITLE: A Randomized Phase II Study of Androgen Deprivation Therapy with or without Palbociclib in RB-Positive Metastatic Hormone-Sensitive Prostate Cancer.

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Pfizer Supplied Agent	**Palbociclib (Ibrance®)**

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Site Principal Investigator Protocol Signature Page

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

Printed Name: _____

Signature: _____

Date: _____

STUDY SCHEMA

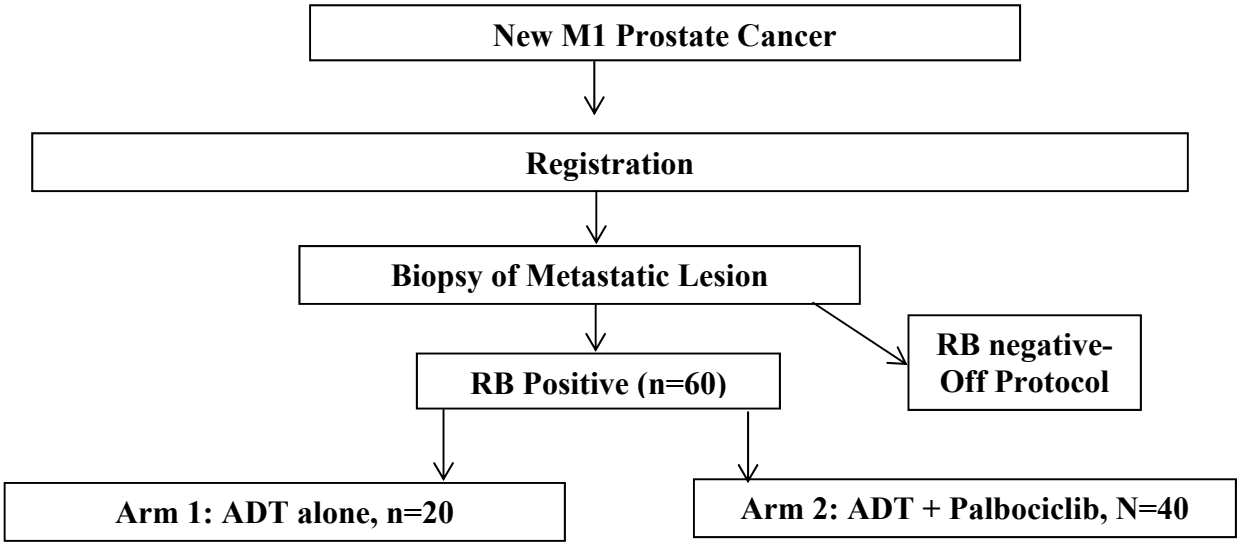

TABLE OF CONTENTS

1. OBJECTIVES

1.1 Primary Objectives

1.1.1 To compare the rate of PSA $\leq$ 4ng/mL after 28weeks of protocol treatment between RB-positive patients with metastatic hormone-sensitive prostate cancer randomized to combined androgen deprivation (CAD: LHRH agonist + bicalutamide) versus those randomized to CAD + palbociclib.

1.2 Secondary Objectives

1.2.1 To assess the safety and tolerability of the combination of CAD + palbociclib.
1.2.2 To assess the rate of undetectable PSA ($\leq$0.2ng/mL).
1.2.3 To assess biochemical and clinical progression-free survival.
1.2.4 To assess PSA and radiographic response rates.

1.3 Correlative Objectives

1.3.1 To determine whether cyclin D1, cyclin D1B, p16, CDK4, E2F1, Cyclin A, MCM7, or Ki67 levels in pretreatment metastatic tumor biopsy tissue predict a subset of tumors responsive to palbociclib or overall response rates.

1.3.2 To evaluate tumor transcriptome and mutational signatures to identify biomarkers which predict response to palbociclib.

1.3.3 To create a tissue repository consisting of hormone-sensitive metastatic prostate cancer specimens with associated RB status and clinical data which can be used to correlate RB status with general clinical outcomes and explore and validate important genes identified in 1.3.2.

1.3.4 To assess if the number of circulating tumor cells (CTCs) is associated with response to therapy when collected at pre-treatment, week 12, after 28 weeks of therapy, and at progression/or coming off protocol treatment.

1.3.5 To assess if CTC heterogeneity, as assessed by the relative distribution of CTC phenotypes, predicts either response to palbociclib or overall response rates.

2. BACKGROUND

2.1 Metastatic Prostate Cancer and Androgen Signaling

Every year in the United States, about 30,000 men die of metastatic prostate cancer (PCa).[1] Since the introduction of orchiectomy as the first androgen deprivation therapy (ADT) over seventy years ago,[2] progress in developing new treatments for patients with metastatic hormone-sensitive prostate cancer (mHSPC) has been limited to introducing different modalities of delivering ADT.[3]

The primary therapeutic maneuver for newly diagnosed metastatic prostate cancer is androgen deprivation, which is accomplished by surgical castration or combined androgen deprivation therapy using LHRH-agonists/antagonists with or without an antiandrogen such as bicalutamide. Although the vast majority of mHSPC will initially respond to ADT, it is well established that the quality and duration of response is variable, and most will progress to castration-resistant disease. The current median survival of mHSPC is about 4 years.[4] At present, all patients with newly diagnosed mHSPC are treated with a "one size fits all" approach with no means by which to select appropriate therapeutic regimens based on tumor profiling.

2.2 Role of RB in Metastatic Prostate Cancer

Androgens drive proliferation of prostate cancer cells both in initial disease and even after progression to castration-resistance. An important mechanism by which androgens drive proliferation is up-regulation of cyclin D which complexes with the cyclin-dependent kinase (CDK) 4/6, resulting in phosphorylation of RB, a cell-cycle brake. Because RB is a transcriptional repressor which binds to the regulatory region of genes whose functions are important for cell cycle progression and DNA replication, phosphorylation of RB relieves this repression and allows the cell to proceed through the cell-cycle and divide. RB is therefore an important tumor suppressor and is lost in early stages in several human malignancies (*e.g.* breast, cervical, and small cell lung cancer).[5,6] Because AR signaling is able to inactivate this cell-cycle brake, most early stage prostate cancers retain wild-type RB function, and perturbations in this pathway (loss of RB, upregulation of cyclin D) are instead felt to promote castration-resistance.[7]

The relationship between AR and RB is complex. While AR signaling results in RB phosphorylation and cell-cycle progression, expression of the gene encoding the androgen receptor (AR) is also under RB control.[8] Loss of RB caused de-repression of the AR locus, excessive AR production, and castration-resistant (ligand-independent) AR activity that proved sufficient to bypass hormone therapy.[8] Therefore, AR signaling is closely linked with RB control.

2.3 Role of CDK4/6 Inhibitors in Prostate Cancer

<u>CDK4/6 inhibition markedly inhibits prostate cancer growth *in vitro* and *in vivo*.</u>

Exposure of hormone-sensitive prostate cancer cell lines to the CDK4/6 inhibitor, palbociclib (PD 0332991) significantly decreases active cell cycle progression (through measurement of BrdU incorporation kinetics) (Figure 1, top left) and production of factors (cyclins A and E1) necessary for cell cycle progression in an RB-dependent manner (Figure 1, top right). These results were recapitulated in studies examining human xenograft cultures (Figure 1, bottom) as well as *ex vivo* human tissue model systems. Such decreases in cell cycle progression were accompanied by significant decreases in the clinical tumor proliferation marker Ki67 (Figure 2).[9]

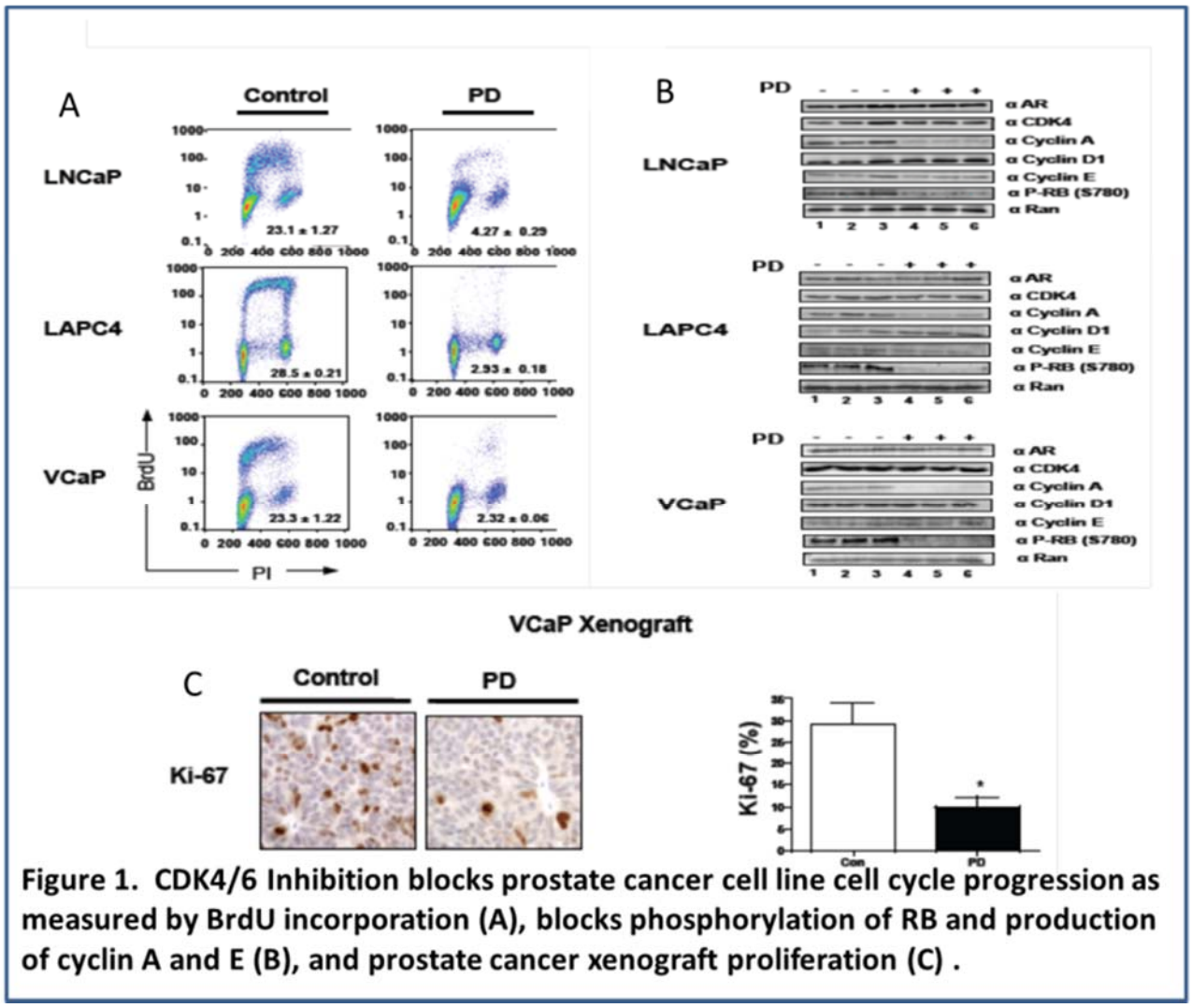

Figure 1. CDK4/6 Inhibition blocks prostate cancer cell line cell cycle progression as measured by BrdU incorporation (A), blocks phosphorylation of RB and production of cyclin A and E (B), and prostate cancer xenograft proliferation (C) .

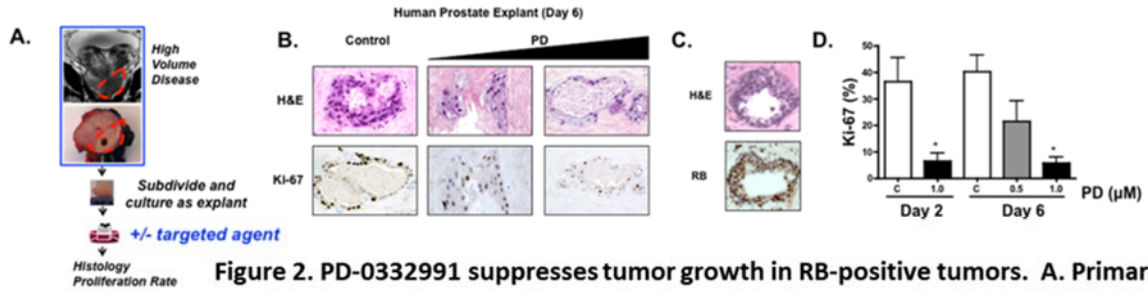

Figure 2. PD-0332991 suppresses tumor growth in RB-positive tumors. A. Primary prostate tumors harvested from radical prostatectomy were treated with PD-0332991 in a novel explant system. B. PD-0332991 suppresses Ki67 indices in (C) Rb-positive tumors. (D) Quantification of Ki67 positivity in 7 individual prostate tumors treated with PD-0332991 or control demonstrates inhibition of proliferation.

RB-proficient prostate cancer cells are preferentially sensitive to CDK4/6 inhibition.

Studies utilizing matched RB-knockdown and control populations have demonstrated a clear requirement for the retention of functional RB protein in the response to CDK4/6 inhibition. Among populations deficient for RB protein, inhibition of CDK4/6 activity was not able to decrease the rate of cells progressing through active cell cycle.[9]

CDK4/6 inhibitors suppress AR function.

Previous studies have demonstrated that CDK4/6 directly associates with the androgen receptor and enhances transcriptional activity in prostate cancer cells.[10] AR activity is coupled to the cell cycle machinery primarily through its ability to up-regulate cyclin D protein level (reviewed in[8]). Once produced, cyclin D partners with CDKs 4 and 6, and phosphorylates RB protein, functionally inactivating the protein and ultimately relieving the transcriptional repression elicited by RB. This results in increased E2F-mediated gene transcription and production of factors that are required for cells to progress through the cell cycle and in to S-phase. In this context, the effect of palbociclib administration is stabilization of cyclin D protein levels.[11] Results from the Knudsen laboratory and others have shown that cyclin D protein is capable of negatively regulating AR activity at AR target genes.[12] Thus, increases in intracellular cyclin D protein levels are known to negatively regulate AR function, and would be hypothesized to decrease AR-driven prostate cancer cell growth. This postulate is supported by recent, unpublished microarray analyses of AR-positive prostate cancer cells in the presence and absence of PD 0332991 (Karen Knudsen, personal communication). palbociclib selectively suppressed expression of a subset of AR target genes known to be critical for castrate-resistant AR activity (eg UBE2C, Rad51, Fen1), while having no effect on early-stage AR targets (e.g. PSA, not shown), and little impact on others such as Nkx3.1. Therefore, because palbociclib suppressed AR-dependent gene expression of some targets, it may act in concert with standard-of-care (androgen deprivation) to further suppress expression of target genes known to promote disease progression/therapeutic response.

2.4 Palbociclib

Palbociclib is a highly selective reversible inhibitor of CDK 4 and 6 currently being evaluated as an anti-cancer therapy and approved for the treatment of metastatic breast cancer.[13] Palbociclib specifically inhibits CDK4-catalyzed phosphorylation of retinoblastoma protein. It has previously been shown to inhibit growth of human xenograft tumors including prostate cancers grown in mice.[9]

Mechanism of Action

Selectivity: Palbociclib is a highly selective inhibitor of CDK4/cyclinD1 kinase activity (IC50 = 11 nM; Ki = 2 nM). Palbociclib has selectivity for CDK4/6, with little or no activity against a large panel of 34 other protein kinases including other CDKs and a wide variety of tyrosine and serine/threonine kinases. CDK6, another enzyme that also complexes with cyclin-D subunits, is also commonly expressed in mammalian cells and tumors. CDK6 is highly homologous to CDK4 and can perform the same function by phosphorylating RB, thus potentially creating a redundant mechanism to promote cell cycle progression. Consequently, inhibition of both enzymes is necessary to ensure complete suppression of RB phosphorylation and the greatest possible spectrum of antitumor activity. Results indicate that palbociclib inhibits CDK6 with equivalent potency to CDK4.

Inhibition of RB phosphorylation: The only known natural substrate for CDK4/cyclinD1 is the retinoblastoma gene product, RB. Specific CDK4 phosphorylation sites on RB include serine-780 and serine-795. Therefore, the phosphorylation status of RB at these specific sites in treated tumors can serve as an appropriate biomarker for target modulation by palbociclib. The IC50 for reduction of RB phosphorylation at serine-780 in the MDA-MB-435 breast carcinoma cell line was 0.066 μM. Palbociclib was equally effective at reducing RB phosphorylation at serine-795 in this tumor cell line with an IC50 of 0.063 μM. Similar effects on serine-780 and serine-795 phosphorylation were obtained in the hormone sensitive prostate cancer cell line VCaP.[9]

Anti-Proliferative Effects: Palbociclib inhibits cellular proliferation and prevents cellular DNA synthesis by preventing cells from entering S phase of the cell cycle. Palbociclib inhibited thymidine incorporation into the DNA of a panel of RB-positive human breast, colon, lung and prostate cancers. Palbociclib was also effective in preventing cell cycle progression in human leukemias and in nontransformed human epithelial cells and fibroblasts and was equally effective in suppressing cell division in human tumor cell lines.

A selective CDK4/cyclinD inhibitor should cause a specific accumulation of cells in G1, but have no effect on other phases of the cell cycle, in which cells should continue to progress and eventually decline in number. MDA-MB-453 breast carcinoma cells that were exposed to various concentrations of palbociclib for 24 hours show a significant increase in the percentage of cells in G1 in the presence of as little as 0.04 μM palbociclib with a concomitant decline in other phases of the cell cycle. Similar results were obtained in panels of prostate cancer cell lines.[9]

Finally, to provide further evidence of the selectivity of palbociclib, the compound was tested against RB-negative tumor cells, which should not be sensitive to a specific CDK4 inhibitor. Palbociclib was tested against the MDA-MB-468 human breast carcinoma and the H2009 human non-small cell lung carcinoma, both of which have deleted RB. The compound had no anti-proliferative activity on these cells when assayed at 3 μM (highest concentration tested), which is 1 to 2 orders of magnitude higher than the concentration necessary to inhibit RB-positive tumor cells.

Nonclinical Activity

The MTD in SCID mice was 150 mg/kg/day when administered orally, once a day, for 14 days. The MTD was defined as the highest dose that was nonlethal (<LD10). At the MTD on this regimen, palbociclib has significant antitumor efficacy against multiple human tumor xenograft models (including the PC-3 prostate cancer cell line). The Colo-205 model is exquisitely sensitive to palbociclib. At doses as low as 12.5 mg/kg, a 13-day growth delay was obtained, indicating a 90% inhibition of tumor growth rate. Further evidence that the anti-tumor activity observed in RB-positive tumors is due to inhibition of CDK4/CDK6 protein kinase activity was obtained by testing palbociclib in the MDA-MB-468 breast carcinoma and the DU-145 prostate tumor models. These are RB-negative tumors; and neither responded to this compound. The lack of efficacy in RB-negative tumors is consistent with the lack of anti-proliferative activity observed in vitro. Taken together, these results support the proposed mechanism of palbociclib (inhibition of CDK4/6-mediated RB phosphorylation) and the specificity of the compound demonstrated in enzyme activity tests.

Nonclinical Pharmacology and Toxicology

Dosing Schedules: Further studies investigated whether continuous daily dosing of palbociclib was needed for optimal efficacy. Four dosing schedules were employed against the MDA-MB-435 breast carcinoma model over 14 days of treatment, including continuous daily, every other day, every third day, and 3 courses of 3 days dosing followed by 4-day drug holidays. The design of this experiment was such that the total compound administered over the 2-week period was identical for each treatment schedule. The results show that a similar degree of efficacy was attained with all schedules, implying that an intermittent regimen is feasible without compromising activity.

Inhibition of RB Phosphorylation: To determine if palbociclib prevented RB phosphorylation, efficacious and non-efficacious doses of palbociclib were given to mice bearing the MDA-MB-435 breast carcinoma and the phosphorylation status of serine-780 on RB in tumor tissue was monitored over time. The results show that while all doses caused a reduction in the biomarker shortly after drug administration, phosphorylation returned at the non-efficacious doses (12.5 and 37.5 mg/kg) over the 24-hour interval before the next dose. However, the highly efficacious dose of 150 mg/kg suppressed RB serine-780 phosphorylation during the full 24-hour period. These data suggest that complete suppression of RB phosphorylation needs to be maintained between drug doses to achieve significant efficacy against this particular tumor model.

Similar experiments with the Colo-205 colon carcinoma xenografts, which are exquisitely sensitive to palbociclib, shows that complete suppression of RB phosphorylation between doses was found to be unnecessary for producing growth inhibition. However, for maximal effects against Colo-205 tumors (i.e., regression), total inhibition had to be maintained.

Comparing results of the efficacy experiments between the highly sensitive colon tumor and the moderately sensitive breast tumor reveals a 7 to 8-fold difference in the dose necessary to produce comparable efficacy. The pharmacodynamic results show a similar difference between the 2 tumors in dose requirements necessary to suppress serine-780 phosphorylation on RB, indicating that efficacy correlates with modulation of this biomarker. Ki-67, a common marker of cell proliferation, was monitored in xenografts by immunohistochemical staining with the MIB-1 antibody. MDA-MB-435 and Colo-205 xenografts were treated with an efficacious but nontoxic dose of palbociclib (130 mg/kg) daily for 7 and 4 days, respectively. Tumor samples were harvested at various times after the last dose and analyzed for RB phosphorylation and Ki-67 expression. Relative to vehicle treated controls, RB phosphorylation and Ki-67 expression were both strongly inhibited from 1 to 24 hours after dosing. At 48 and 72 hours after the last dose, RB phosphorylation and Ki-67 returned to control levels in MDA-MB-435. In Colo-205, RB phosphorylation and Ki-67 expression remained partly suppressed through 72 hours. These results are consistent with the anti-proliferative mechanism expected for a specific Cdk4/6 inhibitor. They also provide support for the use of Ki-67 as a biomarker of cell proliferation in conjunction with palbociclib.

Clinical Investigations

As of 01 Sep 2015, palbociclib has been investigated in 18 clinical trials and at least 7 ongoing trials in humans; 19 phase 1 trials (solid tumors and hematologic malignancy) , three phase 1/2

trials (multiple myeloma and breast cancer) in combination with bortezimib or letrozole and two phase 3 trials in breast cancer in combination with letrozole or fulvestrant.

Phase 1 Experience and MTD Determination. 74 patients with a variety of solid tumors were treated as part of the Phase 1 trial. 41 patients received palbociclib on a 3-week on, 1-week off treatment schedule (3/1) with doses ranging from 25 to 150 mg one daily. An additional 33 patients received daily treatment with 2-week on/1-week off schedule (2/1) at a similar dose range. The MTD doses for Phase 2 investigations was determined to be 125mg daily for the 3/1 schedule and 200mg daily for the 2/1 schedule. Common adverse events related to treatment are listed in Section 3.1 and included fatigue, neutropenia, nausea, diarrhea, and anemia.

Phase 1/2 Experience with Palbociclib in Combination with Letrozole in Women with Breast Cancer: Data is available for a Phase 1/2 trial assessing the safety and efficacy of palbociclib 125 mg QD on Schedule 3/1 in combination with letrozole 2.5 mg QD on a continuous regimen for the first-line treatment of female patients with ER-positive, HER2-negative advanced breast cancer.[14] The Phase 1 portion of the study has completed accrual with 12 patients. In the Phase 2 portion of the study, patients were randomized to receive either letrozole 2.5 mg QD alone or palbociclib 125 mg QD on Schedule 3/1 + letrozole 2.5 mg QD. Safety data are available for 95 patients who received the combination, which includes 12 patients from Phase 1 and 83 patients from the Phase 2 portion of the study.

Phase 3 Experience with Palbociclib in Combination with Letrozole in Women with Breast Cancer. In this ongoing study 666 patients were randomized in a double-blind, placebo-controlled trial to receive either palbociclib 125mg QD (Schedule 3/1) + letrozole or letrozole alone. The most common treatment related adverse events were neutropenia (41.6%), fatigue (28.5%), nausea (26.3%), arthralgia (22.5%), and hot flush (21.3%).

Phase 3 Experience with Palbociclib in Combination with Fulvestrant in Women with Breast Cancer Whose Disease Progressed after Prior Endocrine Therapy. This is an ongoing, multicenter, randomized, double-blind, placebo-controlled trial comparing palbociclib to placebo in combination with fluvestrant with or without goserelin for women whose disease has progressed after prior endocrine therapy. The most common adverse events were neutropenia (39.5%), fatigue (33.5%) and nausea (27.5%).

Efficacy: **Results are available from 4 completed studies.** In the Phase 1 trial, stable disease was observed for patients with liposarcoma, testicular, kidney, ovarian, breast, appendiceal, peritoneal, melanoma, thymoma and lung. One patient had a partial response (testicular).

Final efficacy results from the Phase 2 portion of the palbociclib/letrozole study demonstrated that PFS was significantly longer in the palbociclib plus letrozole arm than in the letrozole alone arm (20.2 months vs 10.2 months, respectively with a hazard ratio of 0.488 (95% CI: 0.319-0.748) in favor of palbociclib plus letrozole (p=0.0004)). Palbociclib plus letrozole was also associated with higher objective response rates and higher clinical benefit. The efficacy data from palbociclib trials led to its FDA approval for use in metastatic breast cancer with letrozole.

2.5 Rationale for Combination of Palbociclib and ADT in Patients with RB-intact Hormone-Sensitive Metastatic Prostate Cancer

Androgens drive proliferation of prostate cancer cells via up regulation of cyclin D which complexes with the kinase CDK4/6, resulting in phosphorylation of RB which in turn drives G1/S progression (Fig 3). Although most early stage prostate cancers retain wild-type RB function, perturbations in this pathway (loss of RB, upregulation of cyclin D) are felt to promote castration-resistance.

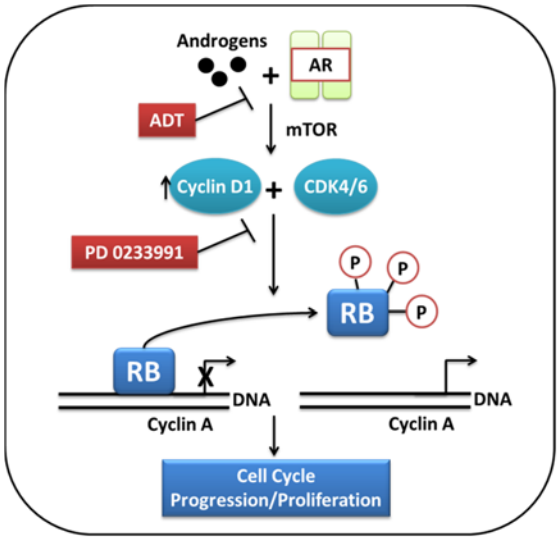

Figure 3. Dual targeting of AR and Rb phosphorylation may act synergistically to block prostate cancer proliferation.

Preclinical data demonstrated that PD 0332991 (Palbociclib) inhibited proliferation and promoted G1 arrest in an RB and Cyclin D-dependent manner in prostate cancer cell lines and xenografts.[9] Thus, this laboratory data support the rationale for treating prostate cancers with intact RB function with palbociclib. Currently, the rates of RB loss or mutation (as measured by array CGH or exome capture) ranges between roughly 1-20% in localized prostate cancer (1% for 2 copy deletion versus 10-20% for 1 copy deletion)[15] to 30-40% in heavily treated metastatic castration-resistant prostate cancer[16]. The reported rates of loss of RB protein expression, by immunohistochemistry, range from 0% in prostatectomy series[17] to 40% in primary (TURP) tumor specimens in CRPC patients (unpublished data, Karen Knudsen, Thomas Jefferson University) to 70% in metastases in CRPC patients.[7] Although, there is little data examining RB loss in the hormone sensitive metastatic population we aim to study here, by extrapolation of existing data, we hypothesize the rate to be between 10-20%.

We hypothesize that the addition of palbociclib to initial ADT in patients with newly metastatic RB-positive prostate cancer may significantly increase the efficacy of ADT. We postulate that similar to the Phase II trial of palbociclib in breast cancer, responses and survival could be predicated on retention of wild-type RB function. To assess this, we are conducting this randomized phase II study of palbociclib in which patients with newly diagnosed mHSPC and RB-expressing tumors based on metastatic disease biopsy will be randomized (1:2) to ADT or ADT plus palbociclib. We propose to use confirmed PSA response ($\leq$ 4 ng/mL) after 28 weeks of therapy as the primary endpoint because it is an intermediate endpoint that correlates significantly with overall survival in hormone-sensitive patients.[18,19]

2.6 Correlative Studies Background and Rationale

Targeted Analysis of Potential Predictive Biomarkers.
As part of the patient selection for this trial, patients will undergo a metastatic disease biopsy which will be tested by immunohistochemistry (IHC) for total RB status in a CLIA certified laboratory.[7] Because the RB pathway can be disrupted in human cancers via distinct mechanisms with distinct effects, additional correlative studies will be done to assess for potential biomarkers along and downstream of the RB pathway.[5] For example, the RB protein can be inactivated by posttranslational modifications that are the subject of oncogenic (e.g., cyclin D1 or cyclin D1B overexpression/amplification) or tumor suppressive (e.g., p16ink4a silencing) alterations. As previous studies from our group have defined a signature of RB loss, key RB target genes (such as Cyclin A and MCM7) will be assessed as potential biomarkers of response.[7] Given that RB interactors may also modulate the therapeutic response, the key RB interactor, E2F1, will be investigated as a predictive biomarker. Finally, as RB also plays a critical role in the response to many antiproliferative stresses that are engaged by therapeutic agents, the status of Ki-67, a common marker of cell proliferation, will be determined.[5] Immunohistochemical approaches for quantifying these potential biomarkers are described in Section 9. In total, these correlative studies will test the hypothesis that distinct alterations in the RB pathway could modulate the response to commonly used therapeutic regimens.

While the potential biomarkers to be assessed in the correlative studies (i.e., p16, cyclin D1B, E2F1, etc) may modulate, interact with, or be an effector of the RB pathway, each of these biomarkers is not completely specific for the RB pathway, and therefore will be assessed as in secondary (correlative studies) instead of being included in the primary eligibility criteria. For example, in the case of p16, while RB deficiency causes p16 overexpression, many other biological processes (aside from RB deficiency) also cause p16 overexpression. In general, it appears that p16 overexpression is twice as common as RB deficiency. In triple negative breast cancers, there is a 25% chance of RB deficiency, but a 50% chance of p16 overexpression.[20] In prostate cancers, the rate of RB deficiency is estimated to be <10% in the mHSPC population. However, a study using thousands of localized prostate cancer specimens (primarily including Gleason 6/7 disease) showed that there was a 20% chance of p16 overexpression in this population,[21] suggesting that the frequency of p16 overexpression will be at least 20% or higher in the newly metastatic population. Therefore, given the fact that p16 is regulated by processes other than RB, and p16 overexpression is approximately twice as common as RB loss, the status of p16 and other RB modulators/interactors/effectors will be assessed in the correlative studies but will not be used to exclude patients up front.

Analysis for Additional Predictive Biomarkers using Genomic Methods. To further uncover predictive markers, we will also perform targeted sequencing and expression profiling on biopsy tissues. Genes commonly mutated in prostate cancer, those defining basic molecular subtypes, and those potentially related to RB will be assessed for point mutations, short insertions/deletions [indels] as well as copy number alterations. Transcriptome sequencing and/or qPCR will also be performed to assess expression of AR signaling, proliferation, molecular subtyping and RB-related genes. Given the challenges of routinely assessing metastatic sites, we will also perform the same sequencing on diagnostic specimens to determine concordance between transcriptome/genome alterations in primary and metastatic samples. We hypothesize that there may be many additional molecular events which determine whether prostate cancers will respond to palbociclib. In

addition, we will also perform exome capture and comprehensive next-generation sequencing on biopsy tissues if qualitatively and quantitatively sufficient RNA and DNA are recoverable. Our team has previously initiated one of the first biomarker-stratified randomized trials for patients with metastatic castration-resistant disease (NCI 9012), entitled "A Randomized Gene Fusion Stratified Phase 2 Trial Of Abiraterone With Or Without ABT888 For Patients With Metastatic Castration-Resistant Prostate Cancer." In the context of this trial, >70 biopsies (stored frozen) of metastatic disease were performed over the past 1.5 years, with an 80% success rate of determining the status of the biomarker of interest.

While comprehensive sequencing studies have been performed in the context of locally advanced prostate cancer[22] as well as metastatic end-stage castration-resistant prostate cancer (CRPC),[16] there have been, to our knowledge, no such studies in the context of metastatic hormone-sensitive prostate cancer. Thus, by characterizing the genome and transcriptome in this unstudied space, this project will provide a significant contribution to our knowledge regarding the genomic landscape of prostate cancer, by providing an extensive look at metastatic, but hormone-sensitive, prostate cancer. This data would represent an extremely valuable resource, which could serve as a "baseline" for comparison to subsequent sequencing studies in the CRPC space, as well as an opportunity to identify targetable biological concepts that may drive prostate cancer progression.

Analysis of blood specimens for circulating, cell-free tumor DNA
Recently, it has been demonstrated that patients with a variety of advanced solid tumors shed cell-free DNA (cfDNA) into the circulation and that mutations present in tumor tissue can be detected by high-throughput sequencing of cfDNA isolated from blood specimens.[23] This rapidly developing research area has the potential of enabling the so-called "liquid biopsy", in which relevant genomic changes which currently need to be sought by biopsy of metastatic sites could instead be assayed from a blood specimen. In the current study we will collect the matched blood specimens needed to evaluate the efficacy of this approach as a surrogate for molecular analysis of tissue biopsy specimens. Once key predictive genomic biomarkers are identified from the tissue biopsy analyses above, they can be assayed in blood as well to determine concordance between blood and metastatic tissue biopsy. Given that optimal methods for cfDNA analysis are rapidly evolving, we plan to use the most current, robust technologies available when the time comes for analysis of the collected cfDNA specimens.

Analysis for Additional Predictive Biomarkers in Circulating Tumor Cells
Assessment of circulating tumor cells potentially represents a “liquid biopsy” approach with the potential for identifying biomarkers of treatment response. To enumerate and characterize circulating tumor cells in this study, we will utilize a platform developed by Epic Sciences for CTC isolation and identification, which has previously been described.[24-28] This platform allows for quantification of CTC number, as well as analysis of potential predictive biomarkers. One potential biomarker of response is the heterogeneity of CTCs, as measured by distribution of previously characterized CTC subtypes, such as nucleoli CTCs, CTC clusters, CK- CTCs, CK speckled CTCs, small CTCs, AR nuclear CTCs, and AR cytoplasmic CTCs.[29] The extent of CTC heterogeneity has been previously demonstrated to predict for resistance to androgen targeting therapeutics.[30] Thus, we hypothesize that CTC count and heterogeneity may predict for longer-term PSA responses, and will explore this hypothesis in this study.

3. DRUG INFORMATION

3.1 IND Agent: Palbociclib (Ibrance®) (PD 0332991) (unless otherwise specified, information is derived from Investigator's Brochure).

Chemical Name: 6-acetyl-8-cyclopentyl-5-methyl-2-((5-(piperazin-1-yl)pyridin-2-yl)amino)pyrido[2,3-d]pyrimidin-7(8H)-one

Other Names: Palbociclib, PD-0332991

Classification: CDK4/6 Kinase Inhibitor

Molecular Formula: $C_{24}H_{29}N_7O2$ **M.W.:** 447.53

Mode of Action: Palbociclib is a highly selective inhibitor of CDK 4/6 which blocks phosphorylation of the retinoblastoma protein.

How Supplied: Pfizer will supply Ibrance® for this study. Ibrance® is supplied as capsules containing 75mg, 100mg or 125mg equivalents of palbociclib. The capsules will be packaged in bottles containing 75mg, 100mg or 125mg capsules.

Note: Capsules should not be repackaged after they are dispensed. Capsules should not be opened or emptied into another vehicle for oral ingestion. Capsules should be swallowed intact.

Storage: Store at controlled room temperature (15-30C, 59-86F) in their original container.

Route(s) of Administration: Oral

Method of Administration: Capsules should be swallowed whole without chewing. Patients should take doses at approximately the same times each day and record this information in the patient diary. Patients should take palbociclib with food. Patients will take medication daily for 21 days of a 28 day cycle.

Metabolism: The ability of cytochrome P450 enzymes to metabolize palbociclib was evaluated by incubation of palbociclib (6 μM) with the 5 major cytochrome P450 (CYP) enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4). CYP3A4 was the only enzyme that metabolized palbociclib. Concordantly, metabolism of palbociclib (6 μM) in human liver microsomes was significantly reduced (>80% decrease) in the presence of the CYP3A4 enzyme-specific inhibitor ketoconazole (1 μM). These data indicate that CYP3A enzymes are the primary enzymes responsible for the metabolism of palbociclib.

Potential Drug Interactions: Palbociclib is primarily metabolized by CYP3A4. Coadministration with strong CYP3A4 inhibitors or inducers may significantly change plasma concentrations in humans. **Therefore the concurrent use of strong inhibitors such as amprenavir, atazanavir, boceprevir, clarithromycin, conivaptan, delavirdine, diltiazem, erythromycin, fosamprenavir, indinavir, itraconazole, ketoconazole, lopinavir, mibefradil, miconazole, nefazodone, nelfinavir, posaconazole, ritonavir,**

saquinavir, telaprevir, telithromycin, verapamil, voriconazole, and grapefruit or strong inducers such as carbamazepine, felbamate, nevirapine, phenobarbital, phenytoin, primidone, rifabutin, rifampin, rifapentin and St. John's wort are not allowed.

Excretion: The excretion balance of radioactivity and radioactivity concentration in the collected plasma and blood samples were evaluated following oral administration of [14C] palbociclib (ethanesulfonic salt) to intact and bile-duct cannulated (BDC) male and female Sprague-Dawley rats at the dose level of 50 mg/kg as salt. The radioactive dose was mainly eliminated via the feces (>82%) with minor contribution of urinary excretion (7.8%). The percentage of dose eliminated in bile within 72 hours after dosing was 53% in male and 82% in female BDC rats, suggesting that biliary excretion is the major route of elimination in rats.

Toxicology: The primary palbociclib toxicities in preclinical studies are to the bone marrow, lymphoid tissues, and testes. These toxicities occurred in both rats and dogs, and are consistent with cell cycle inhibition produced by the intended pharmacology of the drug. Bone marrow pancytopenia resulted in decreases in various hematology parameters; however, the changes were reversible following cessation of dosing. Reversible myelosuppression is anticipated in clinical studies and may be dose-limiting. Palbociclib demonstrated a potential for aneugenicity in the in vitro and in vivo micronucleus assays. Acute IV administration of palbociclib to anesthetized dogs resulted in significant pulmonary effects, including apnea, which were reversible. Effects were transient, appeared related to peak plasma concentrations (>2040 ng/mL), and consistent with centrally-mediated respiratory depression. No changes in pulmonary function occurred at plasma drug concentrations <414 ng/mL. Pulmonary changes were observed in rats to which palbociclib was administered orally, which included rales, dyspnea, and atrophy of tracheal epithelium. The clinical relevance of these effects is unknown. Results of the Purkinje fiber and hERG in vitro assays and cardiovascular study in dogs have indicated a potential for prolongation of the QT interval

Additional routine studies in rats revealed that palbociclib could alter glucose metabolism and affect the pancreas, eye, teeth, kidney and adipose tissue of some rats. Hyperglycemia, glycosuria and pancreatic islet cell changes were observed. Some rats developed cataracts (clouding of the eye lens) after being given palbociclib for 6 months. It is currently unknown what these findings mean for patients treated with palbociclib over time.

Pharmacokinetics in Humans:

As of 10 December 2014, twenty-one clinical studies have evaluated the PK of palbociclib. Four of these trials were conducted in patients with advanced malignant disease. Seventeen Phase 1 clinical pharmacology and biopharmaceutic studies of palbociclib were conducted in healthy subjects. Ten of these 17 clinical trials were clinical pharmacology studies conducted to investigate the absorption, distribution, metabolism, and excretion of palbociclib as well as examine the potential for a drug-drug interaction (DDI) with palbociclib. The remaining 7 of the 17 clinical trials were biopharmaceutic studies conducted to examine the bioavailability, bioequivalence, and food effect of the palbociclib formulations.

Pharmacokinetic (PK) data from patients with advanced cancer from Study A5481001 indicate that the plasma pharmacokinetics of palbociclib are low to moderately variable with generally dose proportional exposures over the dose range evaluated (25 mg to 225 mg) following single and multiple doses. PK data from Studies A5481001, A5481003, and A5481010 indicate that palbociclib is slowly absorbed with a median time of maximum concentration (T_{max}) between 4 and 8 hours post-dose, and is slowly eliminated with an elimination half-life ($t_{1/2}$) ranging from 23.2 hours to 28.8 hours. Palbociclib accumulates after repeated daily dosing (median Rac ranged from 1.9 to 2.4), which was consistent with its terminal $t_{1/2}$. In Study A5481010, the median R_{ss} (the predicted accumulation to estimate linearity) was 1.1, indicating that palbociclib clearance does not change over time. In Study A5481003, palbociclib was shown to achieve steady-state concentrations following 8 days of QD dosing. The palbociclib geometric mean volume of distribution (V_z/F) was 2583 L in women with advanced breast cancer (Study A5481003), which is significantly greater than total body water (42 L), indicating that palbociclib extensively distributes to peripheral tissues.

In humans, metabolism is the major route of elimination of palbociclib. Following a single oral administration of [^{14}C]palbociclib to healthy subjects (Study A5481011), the overall median recovery of the administered radioactivity in the excreta was 91.6% with a median of 17.5% recovered in urine and a median of 74.1% recovered in feces. Excretion of unchanged palbociclib in the feces and urine was 2.3% and 6.9% of dose, respectively, indicating that excretion plays a minor role in elimination of palbociclib. A study in healthy volunteers (A5481015) indicated that the absolute oral bioavailability of palbociclib was approximately 46%.

In vitro data indicate that CYP3A and SULT enzyme SULT2A1 are mainly involved in the metabolism of palbociclib. Palbociclib is a weak time-dependent inhibitor of CYP3A following daily 125 mg dosing to steady state in humans. In vitro, palbociclib is not an inhibitor of CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, and 2D6, and is not an inducer of CYP1A2, 2B6, 2C8, and 3A4 at clinically relevant concentrations.

In vitro evaluations indicated that palbociclib has a low potential to inhibit the activities of drug transporters P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), organic anion transporter (OAT)1, OAT3, organic cation transporter (OCT)2, organic anion transporting polypeptide (OATP)1B1, OATP1B3, and bile salt export pump (BSEP) at clinically relevant concentrations.

An itraconazole DDI study in healthy volunteers (Study A5481016) and a rifampin DDI study in healthy volunteers (Study A5481017) were conducted to evaluate the potential for strong CYP3A inhibitors and inducers, respectively, to alter the PK of palbociclib. Coadministration of itraconazole and palbociclib increased palbociclib AUC_{inf} and C_{max} by approximately 87% and 34%, respectively, relative to those when palbociclib dose was given alone. Coadministration of rifampin and palbociclib decreased palbociclib AUC_{inf} and C_{max} by approximately 85% and 70%, respectively, relative to palbociclib given alone. Based on this data, the concurrent administration of strong CYP3A inhibitors and inducers with palbociclib should be avoided.

A midazolam DDI study in healthy volunteers (Study A5481012) was conducted to evaluate the potential for palbociclib to act as a time-dependent inhibitor of CYP3A4/5 at steady-state. Plasma midazolam C_{max} and AUC_{inf} values increased 37% and 61%, respectively, when single oral doses of midazolam were co-administered with multiple doses of palbociclib as compared to its administration alone. This is consistent with weak time-dependent CYP3A4/5 inhibition mediated by palbociclib at steady-state following daily 125 mg dosing.

PK data from the Phase 1 portion of Study A5481003 was analyzed to evaluate the potential for a DDI between palbociclib and letrozole at steady-state. These data indicate a lack of a potential for DDIs between palbociclib and letrozole when administered in combination.

Data from a DDI study in healthy male subjects indicated that palbociclib exposures were comparable when a single dose of palbociclib was coadministered with multiple doses of tamoxifen and when palbociclib was given alone.

The effect of food on the exposure of palbociclib when administered as the commercial free base capsule was evaluated in healthy subjects (A5481021). Compared to palbociclib given under overnight fasted conditions, the AUC_{inf} and C_{max} of palbociclib increased by 21% and 38% when given with high-fat food, by 12% and 27% when given with low-fat food, and by 13% and 24% when moderate-fat food was given 1 hour before and 2 hours after palbociclib dosing. In addition, food intake significantly reduced the intersubject and intrasubject variability of palbociclib exposure. Based on these results, palbociclib commercial free base capsules should be taken with food.

The solubility of the palbociclib free base is pH dependent—palbociclib is water soluble at low pH (2.1-4.5), while the solubility dramatically decreases as pH rises above 4.5. Concomitant administration of agents which increase gastric pH can alter the solubility and absorption of palbociclib free base formulations.

In a drug interaction trial in healthy subjects (A5481038), coadministration of a single 125 mg dose of commercial free base capsule with multiple doses of the proton pump inhibitors (PPI) rabeprazole under fed conditions decreased palbociclib C_{max} by 41%, but had limited impact on AUC_{inf} (13% decrease), when compared to a single dose of palbociclib administered alone. Given the reduced effect on gastric pH of H2-receptor antagonists and local antacids compared to PPIs, the effect of these classes of acid-reducing agents on palbociclib exposure under fed conditions is expected to be minimal. Under fed conditions there is no clinically relevant effect of PPIs, H2-receptor antagonists, or local antacids on palbociclib exposure. In another healthy subject study, coadministration of a single dose of commercial free base capsule with multiple doses of the PPI rabeprazole under fasted conditions decreased palbociclib AUC_{inf} and C_{max} by 62% and 80%, respectively, when compared to a single dose of palbociclib administered alone. Collectively, these antacid DDI data further support the requirement that the free base capsule of palbociclib should be taken with food.

Based on a population pharmacokinetic analysis in 183 patients with cancer (50 male and 133 female patients, age range from 22 to 89 years, and body weight range from 37.9 to 123 kg), gender had no effect on the exposure of palbociclib, and age and body weight had no clinically important effect on the exposure of palbociclib.

Based on a population pharmacokinetic analysis that included 183 patients, where 40 patients had mild hepatic impairment (total bilirubin ≤ ULN and AST > ULN, or total bilirubin >1.0 to 1.5 × ULN and any AST), mild hepatic impairment had no effect on the exposure of palbociclib. The pharmacokinetics of palbociclib have not been studied in patients with moderate or severe hepatic impairment (total bilirubin >1.5 × ULN and any AST).

Based on a population pharmacokinetic analysis that included 183 patients, where 73 patients had mild renal impairment (60 mL/min ≤ CrCl <90 mL/min) and 29 patients had moderate renal impairment (30 mL/min ≤ CrCl <60 mL/min), mild and moderate renal impairment had no effect on the exposure of palbociclib. The pharmacokinetics of palbociclib have not been studied in patients with severe renal impairment.

A pharmacokinetic/pharmacodynamic analysis to evaluate the relationship between palbociclib exposure and ECG endpoints (RR and QTc intervals) were developed using pooled data from 3 clinical trials in patients with advanced malignant disease (Studies A5481001, A5481002, and A5481003). The study population consisted of 48 men and 136 women with a median (range) body weight of 73.0 (37.9-123) kg and age of 61.5 (22-89) years old. Palbociclib doses ranged from 25 mg to 225 mg QD. The data collected from 184 patients consisted of 569 ECG-palbociclib concentration-matched pairs; the observed plasma concentrations had a median (range) of 55.2 (2.51-329) ng/mL. The average heart rate, RR, QT, QT corrected for heart rate according to Bazett (QTcB), QT corrected for heart rate according to Fridericia (QTcF), and QTcS (QT interval corrected for heart rate according to a study-specific correction factor) at baseline for ECG-palbociclib concentration matched data were 76.8 beats per minute, 808 msec, 380 msec, 425 msec, 409 msec, and 412 msec, respectively.

The results of the analysis indicate that palbociclib does not appear to have a concentration-dependent effect on heart rate. A slight positive linear relationship between palbociclib concentration and QTcS was observed; however, at the mean or median steady-state palbociclib C_{max} following administration of the recommended clinical dose of palbociclib (125 mg QD) in patients with cancer, the upper bound of the one-sided 95% CI for the increase in QTcS fell below the threshold of 10 msec, suggesting that QT prolongation is not a safety concern for palbociclib at the recommended clinical dose according to the criteria described in the ICH guidance for Industry E14. Similar results were obtained when QTcF and QTcB were used.

Metabolism in Humans: Assessment of the plasma samples on Day 14 of Cycle 1 indicated that the glucuronide conjugate of palbociclib and the lactam of palbociclib (PF-05089326) were the main metabolites present in plasma. Other metabolites observed were the glucuronide conjugates of hydroxylated palbociclib and the glucuronide conjugate of

reduced palbociclib. PF-05089326 was also observed in the circulation of rats following repeated daily oral administration of palbociclib at the dose levels of 50 and 100 mg/kg/day.

Safety and Adverse Events:

Phase 1 Trials:[13,31]
The most frequently reported treatment-emergent adverse events (TEAEs) (>20% of patients) of any grade, regardless of causality were fatigue (52.7% of patients); neutropenia (48.4%); nausea (35.2%); diarrhea and anemia (30.8% each); constipation (26.4%); decreased appetite (22.0%); and vomiting and thrombocytopenia (20.9% each). The most frequently reported TEAEs (>20% of patients) of any grade that were considered to be related to the study treatment were neutropenia (46.2% of patients); fatigue (45.1%); diarrhea and anemia (25.3% each); and nausea (24.2%).

The most frequently reported Grade > or = 3 TEAEs, all causality, were neutropenia (30.8% of patients); anemia (9.9%); leukopenia (7.7%); fatigue (6.6%); and dyspnea and disease progression (5.5% each). Grade 3 neutropenia was considered to be related to the study treatment for 22.0% of patients. Grade 3 leukopenia and thrombocytopenia were each considered related to treatment in 5.5% of patients.

The most frequently reported Grade 4 TEAEs were neutropenia (6 patients; all considered to be related to the study treatment) and thrombocytopenia (3 patients; considered to be related to study treatment for 2 patients). Grade 4 leukopenia was reported for 2 patients and Grade 4 anemia, pulmonary embolism, increased blood uric acid, decreased hemoglobin level and hyperglycemia for 1 patient each. Grade 5 events were reported for 8 patients and included disease progression (5 patients), cardiac arrest (2 patients) and failure to thrive (1 patient). No Grade 5 event was considered to be related to palbociclib.

Phase 1/2 Trial:[14]
In the Phase 1/2 trial, the most frequently reported TEAEs (>20% of patients) of any grade, regardless of causality were neutropenia (71.6% of the patients); leukopenia (41.1%); fatigue (40.0%); nausea (27.4%); anemia (26.3%); diarrhea (22.1%); and arthralgia and hot flush (20.0% each). All reports of neutropenia and leukopenia were considered to be related to the study treatment. Fatigue and anemia were considered to be related to the study treatment for 25.3% of patients each, nausea for 18.9%, hot flush for 17.9%, and arthralgia for 16.8%. The most frequently reported Grade 3 TEAEs were neutropenia (53.7% of patients) and leukopenia (17.9% of patients). All reports of neutropenia and leukopenia were considered to be related to the study treatment. The most frequently reported Grade 4 TEAEs were neutropenia (6.3% of patients; all considered to be related to study treatment), fatigue and pulmonary embolism (2.1% of patients each; all considered to be unrelated to treatment). As of the 01 September 2012 cutoff, one Grade 5 event (disease progression considered unrelated to study treatment) was reported.

Duration of Toxicity:
An exploratory analysis assessing the duration of neutropenia and thrombocytopenia during the first 2 cycles of treatment with palbociclib showed that neutropenia and

thrombocytopenia were dose dependent, with both nadirs observed at the end of the dose period. In most patients, both cell counts recover during the off-treatment period at the end of the cycle. No significant differences appeared when nadir values during Cycle 1 and Cycle 2 were compared, suggesting that myelotoxicity was not cumulative. 11 first cycle dose-limiting toxicities (DLTs) were noted, 5 for patients on Schedule 3/1 and 6 for patients on Schedule 2/1. All DLTs consisted of neutropenia and/or thrombocytopenia and most were categorized as a DLT due to the need for a treatment interruption of longer than 7 days' duration. Febrile neutropenia was not reported as a DLT.

Effect on QT interval:
The results of analysis of 3 clinical trials demonstrates that palbociclib does not appear to have a concentration dependent effect on the heart rate. There was a possible relationship between palbociclib and QTc although the increase fell below the threshold of 10 msec suggesting that QT prolongation is not a major safety concern at the recommended doses.

Adverse Events with Possible Relationship to Palbociclib		
Likely (>20%)	Less Likely (<20%)	Rare but Serious (<3%)
BLOOD AND LYMPHATIC SYSTEM DISORDERS		
Neutropenia	Lymphopenia	Febrile Neutropenia
Anemia		Bone Marrow Failure
Thrombocytopenia		Anemia
Leukopenia		
CARDIAC DISORDERS		
	Palpitations	Unstable Angina Pericarditis
EYE DISORDERS		
	Vision Blurred	Retinal Hemorrhage
	Conjunctivitis	Bilateral Cataracts
	Increased Tear Production	
GASTROINTESTINAL DISORDERS		
Nausea	Constipation	Ischemic Colitis
Diarrhea	Vomiting	Diarrhea
	Abdominal Pain	
	Flatulence	
	Abdominal Distension	
	Abdominal Discomfort	
	Dry Mouth	
	Dyspepsia	
	Stomatitis	
	Gum Pain	
	Mouth/Throat Pain	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS		
Fatigue	Peripheral Edema	Pyrexia
	Pain	Sudden Death
	Pyrexia	Otitis Media Acute

	Chills	
	Mucosal Inflammation	
	Chest Pain	
	Asthenia	
	Pain During Urination	
INFECTIONS		
	Upper Respiratory Tract Infection	Bronchopneumonia
	Nasopharyngitis	Pneumonia
	Pneumonia	Urinary Tract Infection
	Bronchitis	Disseminated Herpes Zoster
	Viral Infection	Sepsis
	Urinary Tract Infection	Pyelonephritis
METABOLISM and NUTRITIONAL DISORDERS		
	Decreased Appetite	Dehydration
	Hypokalemia	Metabolic Acidosis
	Hyperglycemia	Elevated Liver Enzymes
		Hepatic Failure
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS		
	Arthralgia	
	Back Pain	
	Muscle Spasms	
	Flank Pain	
	Muscular Weakness	
	Myalgia	
	Pain in Extremity	
	Musculoskeletal Chest Pain	
	Tooth ache	
	Bone Pain	
NERVOUS SYSTEM DISORDERS		
	Neuropathy peripheral	
	Headache	
	Dizziness	
	Dysgeusia	
	Hypoesthesia	
	Falling Down	
PSYCHIATRIC DISORDERS		
	Anxiety	Suicide Attempt
	Depression	
	Insomnia	
	Altered Mood	
RESPIRATORY, THORACIC and MEDIASTINAL DISORDERS		
	Dyspnea	Allergic Alveolitis
	Cough	Respiratory Failure
	Epistaxis	

	Rhinorrhea	
	Oropharyngeal Pain	
	Exertional Dyspnea	
SKIN and SUBCUTANEOUS TISSUE DISORDERS		
	Rash	
	Alopecia	
	Pruritus	
	Night Sweats	
	Nail Disorder	
	Dry Skin	
VASCULAR DISORDERS		
Hot flush	Hypertension	Deep Vein Thrombosis
	Hypotension	
INVESTIGATIONS		
	Blood Creatinine Increased	
	Hemoglobin Decreased	
	Neutrophil Count Decreased	
	White Blood Cell Count Decreased	
	Blood Potassium Decreased	
	Weight Decreased	
	Weight Increased	

NOTE: Palbociclib in combination with ADT could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

Special Handling: Handle palbociclib according to institutional guideline for hazardous drugs.

Availability: Palbociclib is an experimental agent supplied by Pfizer**.**

Drug Accountability: The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the investigational drug, palbobciclib. The drug accountability records will capture drug receipt, drug dispensing, drug return and final disposition.

***Commercial Agents:** For this study, bicalutamide, goserelin acetate and leuprolide acetate or equivalent LHRH agonists are commercially available; therefore, Investigator Brochures are not applicable to these drugs. Information about commercial drugs is publicly available in the Physician's Desk Reference (PDR), prescribing information and other resources.

3.2 Bicalutamide (Casodex®) (NSC-722665)

General: Bicalutamide is an active non-steriodal antiandrogen and its antiandrogen activity resides exclusively in the (-) or (R) enantiomer. Unlike flutamide, it is peripherally selective and does not cause a rise in serum LH or Testosterone in male rats and dogs. This peripheral selectivity may be because it penetrates poorly the CNS and Hypothalamus (the site of negative feedback of androgens). In humans, rises in LH, Testosterone and Estradiol concentrations were seen. These rises were not dose related. In 90%, testosterone levels remained within normal limits. There was no significant rise in mean serum FSH.

Chemistry: Bicalutamide is a racemic mixture containing two enantiomers, (2RS)-4'-Cyano-3(4-fluorophenylsulphonyl)-2-hydroxy-2-methyl-3'-trifluoromethyl) propionanilide.

Toxicology: In rats, besides antiandrogenic changes, there was evidence of hepatocyte hypertrophy and basophilia. In dogs treated for 6 months, there was increased heart rate with decreased PR interval, transient decrease in circulating PMNs and increased plasma cholesterol. No cardiac pathology was found. In a mouse oncogenicity study, an increased incidence of hepatocellular carcinoma was observed in the top dose male group (75 mg/kg/day). The no effect dose level for hepatocellular carcinoma in this study was 15 mg/kg/day with steady state blood levels in excess of 10 μg/ml. The mechanism for this tumor formation is a non-genotoxic, phenobarbitone-type MFO induction and is not considered to represent a risk for humans. A two-year study in rats and female mice at similar doses did not show an increased incidence of hepatic tumors. Bicalutamide has been given to over 3,500 men in 35 different clinical studies worldwide, in doses up to 600 mg daily. When bicalutamide is given in combination with an LHRH analog, the pharmacologic adverse event profile is dominated by the LHRH analog and includes hot flashes (53%), gynecomastia (9%) and breast pain (6%). Other adverse events reported regardless of causality included diarrhea (12%), constipation (22%), nausea (15%) and abdominal pain (11%). Other adverse were reported, such as fatigue (22%), pain (35%), back pain (25%), pelvic pain (21%), infection (18%), peripheral edema (13%), dyspnea (13%), nocturia (12%), hematuria (12%), anemia (11%), dizziness (10%). Bicalutamide has been associated with changes in liver function, although these are infrequent (7%) and rarely occur with jaundice. Many of these changes improved or resolved despite continuation of bicalutamide therapy. There have been no reports of fatal hepatotoxicity associated with bicalutamide therapy.

PHARMACOLOGY:

- Pharmacokinetics: Animal studies: After oral single dose administration, absorption of the compound was slow with peak concentration occurring 3 - 12 hours and plateau between 2 and 48 hours. There was non-proportional increase in plasma levels with increasing doses. Elimination half-life ranges from 17 - 28 hours in male rats, 21 - 29 hours in female rats and 5 - 7.5 days in dogs. 91 - 96% of bicalutamide is bound to plasma protein. Human studies: After single doses, mean time for peak plasma concentration was 6 hours at 10 and 30 mg, but at 50 mg, it was 16 hours. Mean plasma elimination half-lives after 12 weeks of 10, 30, 50, 100 mg/day was 7 - 10 days. This finding was consistent with single dose data. In patients given daily doses of 50 mg, mean plasma concentration was 10 μg/mL at 12 weeks. After single doses, there was linear increase with doses between 10 and 50 mg, but became non-linear at doses of 50 - 100 mg. At 100 mg, the oral bioavailability is reduced by 30% but plasma elimination half-life is unchanged. Bicalutamide is extensively metabolized and metabolites are excreted by both the biliary and urinary system.

- Formulation: Bicalutamide is prepared as round, film-coated green or white tablets containing standard recipients and 50 mg of the drug. Storage and stability: All packages of bicalutamide should be stored securely in a dry place at room temperature.

- Route of Administration: Bicalutamide is to be administered in tablet form as a once-daily oral dose. Patients should be instructed to take one tablet once daily.

- Supplier: Commercial supply of Bicalutamide will be used. Please refer to the Physician Desk Reference and package insert for complete information.

3.3 Goserelin acetate implant (Zoladex®) (NSC-606864)

PHARMACOLOGY

- Mechanism of Action: Following initial administration in males, goserelin causes an initial increase in serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels with subsequent increases in serum levels of testosterone. Chronic administration of goserelin leads to sustained suppression of pituitary gonadotropins, and serum levels of testosterone consequently fall into the range normally seen in surgically castrated men approximately 2-4 weeks after initiation of therapy.
- Absorption: Goserelin 3.6 mg is released slowly in first 8 days, and then rapid and continuous release for the remainder of the 28 day dosing period. Time to peak concentration for goserelin 3.6 mg is 12-15 days in males and 8-22 days in females. Goserelin 10.8 mg exhibits an initial rapid release resulting in a peak concentration at 2 hours after dosing. From Day 4 until the end of the 12-week dosing interval, the sustained release of goserelin produces a reasonably stable systemic exposure.
- Distribution: Apparent volumes of distribution determined after subcutaneous administration of 250 mcg aqueous solution of goserelin were 44.1 and 20.3 liters for males and females, respectively. Goserelin is approximately 27% protein bound.
- Metabolism: Metabolism of goserelin by hydrolysis of the C-terminal amino acids is the major clearance mechanism. The half-life elimination (t1/2)) is approximately 4 hours in males and 2 hours in females.
- Elimination: Clearance of goserelin is very rapid and occurs primarily via urinary excretion (>90%; 20% as unchanged drug).
- Drug Interactions: Luteinizing hormone-releasing hormone analogs may diminish the therapeutic effect of antidiabetic agents. No formal drug-drug interaction studies have been performed. Please refer to the current FDA-approved package insert for additional information.

ADVERSE EVENTS- Please refer to package insert or company website for up to date information.

Adverse Events with Possible Relationship to Goserelin		
Likely (>20%)	Less Likely (<20%)	Rare but Serious (<3%)
BLOOD AND LYMPHATIC SYSTEM DISORDERS		

	Anemia	
CARDIAC DISORDERS		
	Congestive Heart Failure	Cerebrovascular Accident
	Hypertension	Myocardial Infarction
	Palpitation	
	Vasodilatation	
	Tachycardia	
EYE DISORDERS		
	Amblyopia	
	Dry Eyes	
GASTROINTESTINAL DISORDERS		
	Anorexia	
	Appetite increase	
	Nausea	
	Abdominal Pain	
	Constipation	
	Diarrhea	
	Dyspepsia	
	Flatulence	
	Ulcer	
	Vomiting	
	Xerostomia	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS		
Sweating	Injection site reaction	
Tumor Flare	Voice Alterations	
IMMUNE SYSTEM DISORDERS		
	Fever	
INFECTIONS		
	Infection	
	Flu Syndrome	
METABOLISM and NUTRITIONAL DISORDERS		
	Weight gain/loss	
	Hyperglycemia	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS		
Bone mineral density decrease	Weakness	
	Arthralgia	
	Back pain	
	Hypertonia	
	Bone/Joint Pain	
	Leg Cramps	
	Myalgia	
	Paresthesia	
NERVOUS SYSTEM DISORDERS		
Headache	Dizziness	

	Pain	
PSYCHIATRIC DISORDERS		
	Anxiety	
	Depression	
	Insomnia	
	Emotional lability	
RENAL and URINARY DISORDERS		
	Urinary Frequency	
	Urinary Obstruction	
	Urinary Tract Infection	
REPRODUCTIVE SYSTEM and BREAST DISORDERS		
Hot flashes/sweats	Pelvic Symptoms	
Libido Decreased	Breast Enlargement	
Sexual Dysfunction	Erections Decreased	
Breast Atrophy	Libido Increased	
	Breast Pain/Swelling	
RESPIRATORY, THORACIC and MEDIASTINAL DISORDERS		
	Pharyngitis	
	Upper Respiratory Infection	
	COPD	
	Cough	
	Bronchitis	
	Sinusitis	
	Epistaxis	
	Rhinitis	
SKIN and SUBCUTANEOUS TISSUE DISORDERS		
Acne	Hair Disorders	
Seborrhea	Hirsutism	
	Pruritus	
	Rash	
	Skin Discoloration/Bruising	
VASCULAR DISORDERS		
Peripheral Edema	Hemorrhage	Thromboembolism

Adverse effects occurring in <1%, post marketing, and/or case reports: ALT increased, anaphylaxis, AST increased, diabetes, glucose tolerance decreased, hypercalcemia, hypercholesterolemia, hyperlipidemia, hypersensitivity reactions, hypotension, ovarian cyst, pituitary apoplexy, psychotic disorders, urticarial.

The FDA issued a safety communication in October 2010 based on their ongoing safety review of LHRH agonists. The safety communication discusses the potential for an increased risk of diabetes and cardiovascular disease (myocardial infarction, sudden cardiac death, stroke) associated with these agents. The risk is thought to be low in men receiving LHRH agonists for prostate cancer. In this trial, LHRH agonists are being administered for a short period of time. FDA recommendations include management of cardiovascular risk factors according to current standards of practice.

DOSING & ADMINISTRATION

1. Dosing – See Treatment Plan
2. Goserelin is administered subcutaneously

STORAGE & STABILITY: Refer to the current FDA-approved package insert for storage, stability and special handling information.

HOW SUPPLIED

1. Goserelin acetate implant is available in a 3.6 mg or 10.8 mg disposable syringe device. The unit is sterile and comes in a sealed, light- and moisture-proof, aluminum foil laminate pouch containing a desiccant capsule.
2. Commercial supply of Goserelin will be used. Refer to the current FDA-approved package insert for additional information.

3.4 Leuprolide (Lupron Depot®) (NSC-377526)

PHARMACOLOGY

- Mechanism of Action: Leuprolide inhibits gonadotropin secretion by acting as an luteinizing hormone-releasing hormone (LHRH) agonist. Continuous administration results in suppression of ovarian and testicular steroidogenesis due to decreased levels of LH and FSH with subsequent decrease in testosterone (male) and estrogen (female) levels. In males, testosterone levels are reduced to below castrate levels. Leuprolide may also act directly on the testes as well as act by a different mechanism not directly related to reduction in serum testosterone.
- Absorption: After the initial increase of leuprolide following each injection, mean serum concentrations remain relatively constant.
- Distribution: The mean steady-state volume of distribution of leuprolide following intravenous bolus administration to healthy male volunteers was 27 L. In vitro binding to human plasma proteins ranged from 43% to 49%.
- Metabolism: Upon administration with different leuprolide acetate formulations, the major metabolite of leuprolide acetate is a pentapeptide (M-I) metabolite.
- Elimination: Less than 5% of the leuprolide dose was recovered as parent and M-I metabolite in the urine following the 3.5 mg depot injection.
- Drug Interactions: Luteinizing hormone-releasing hormone analogs may diminish the therapeutic effect of antidiabetic agents. No pharmacokinetic-based drug-drug interaction studies have been performed. Because leuprolide is a peptide that is primarily degraded by peptidase and not by Cytochrome P-450 enzymes and the drug is only about 46% protein bound, drug interactions would not be expected to occur.

ADVERSE EVENTS- Please refer to package insert or company website for up to date information.

Adverse Events with Possible Relationship to Leuprolide

Likely (>20%)	Less Likely (<20%)	Rare but Serious (<3%)
BLOOD AND LYMPHATIC SYSTEM DISORDERS		
	Edema	
CARDIAC DISORDERS		
	Hyper/Hypotension	Arrhythmia
	Tachycardia	Atrial Fibrillation
	Bradycardia	Congestive Heart Failure
	Angina	Syncope
	Palpitation	
GASTROINTESTINAL DISORDERS		
Nausea	Altered Bowel Function	Gastrointestinal Hemorrhage
Vomiting	Ulcer	
	Intestinal Obstruction	
	Constipation	
	Diarrhea	
	Gastroenteritis/Colitis	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS		
Local Injection site burning/stinging	Skin reaction	
IMMUNE SYSTEM DISORDERS		
	Flu-like syndrome	Allergic Reaction
INFECTIONS		
	Urinary Tract Infection	
	Infection	
INVESTIGATIONS		
	BUN increase	
	Creatinine increase	
	Bicarbonate decrease	
	Hyperphosphatemia	
	Hyperuricemia	
	Hypoalbuminemia	
	Hypoproteinemia	
METABOLISM and NUTRITIONAL DISORDERS		
	Dehydration	
	Hyperlipidemia	
	Weight gain/loss	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS		
	Weakness	
	Bone Pain	
	Joint Disorder	
	Myalgia	
	Paresthesia	
NERVOUS SYSTEM DISORDERS		
Headache	Nervousness	Seizure
Pain	Anxiety	

Insomnia	Confusion	
	Fatigue	
	Dizziness/vertigo	
PSYCHIATRIC DISORDERS		
Depression		
RENAL and URINARY DISORDERS		
	Urinary disorders	
	Bladder spasm	
	Urinary retention	
REPRODUCTIVE SYSTEM and BREAST DISORDERS		
Hot flashes/sweats	Gynecomastia	
Testicular atrophy	Breast Tenderness	
	Lactation	
	Testicular Pain	
	Impotence	
	Libido decreased	
	Nocturia	
RESPIRATORY, THORACIC and MEDIASTINAL DISORDERS		
	Emphysema	
	Epistaxis	
	Pleural Effusion	
	Pulmonary Edema	
	Dyspnea	
	Cough	
SKIN and SUBCUTANEOUS TISSUE DISORDERS		
	Acne	
	Alopecia	
	Bruising	
	Cellulitis	
	Pruritus	
	Rash	
	Hirsutism	
VASCULAR DISORDERS		
	Varicose Veins	
	Deep Thrombophlebitis	

<1%, post marketing, and/or case reports: Abdominal pain, anaphylactic/anaphylactoid reactions, asthmatic reactions, bone density decreased, coronary artery disease, diabetes; fibromyalgia-like symptoms; flushing, hemoptysis, hepatic dysfunction, hypokalemia, hypoproteinemia, injection site induration/abscess, liver injury, myocardial infarction, pelvic fibrosis, penile swelling, peripheral neuropathy, photosensitivity; pituitary apoplexy; prostate pain, pulmonary embolism, pulmonary infiltrate, seizure, spinal fracture/paralysis, stroke suicidal ideation/attempt (rare), tenosynovitis-like symptoms, thrombocytopenia, transient ischemic attack, uric acid increased, urticaria, WBC decreased/increased.

The FDA issued a safety communication in October 2010 based on their ongoing safety review

of LHRH agonists. The safety communication discusses the potential for an increased risk of diabetes and cardiovascular disease (myocardial infarction, sudden cardiac death, stroke) associated with these agents. The risk is thought to be low in men receiving LHRH agonists for prostate cancer. In this trial, LHRH agonists are being administered for a short period of time. FDA recommendations include management of cardiovascular risk factors according to current standards of practice.

DOSING & ADMINISTRATION

1. Dosing – See Treatment Plan
2. Leuprolide is administered intramuscular (Lupron Depot®). Injection sites should be varied periodically.

STORAGE & STABILITY: Refer to the current FDA-approved package insert for storage, stability and special handling information.

HOW SUPPLIED

1. Leuprolide acetate, 7.5 mg (1 month), 22.5 mg (3 months), 30 mg (4 months) or 45 mg (6 months) depot formulation kit with accompanying diluent. The prefilled dual-chamber syringe contains lyophilized microspheres of leuprolide acetate incorporated in a biodegradable lactic acid polymer.
2. Commercial supply of Leuprolide will be used. Refer to the current FDA-approved package insert for additional information.

4. PATIENT SELECTION

In calculating days of tests and measurements, the day a test or measurement is done is considered Day 0. Therefore, if a test is done on a Monday, the Monday 4 weeks later would be considered Day 28. This allows for efficient patient scheduling without exceeding the guidelines. If Day 28, 42, or 56 falls on a weekend or holiday, the limit may be extended to the next working day.

4.1 Inclusion Criteria

At registration all patients must:

- Have pathologic diagnosis of prostate cancer.

- Have hormone-sensitive metastatic disease (M1) as evidenced by soft tissue and/or bony metastases.

- Patients may either be untreated for their newly diagnosed metastatic disease (preferred as much as possible) or have started androgen deprivation therapy. Patients who have started androgen deprivation therapy for the treatment of their newly diagnosed metastatic disease are eligible as long as the duration of treatment is less than or equal to 2 weeks (14 days) prior to registration. The start date of androgen deprivation is considered the day the patient first received an injection of a LHRH agonist/antagonist (or orchiectomy), not the date when an oral antiandrogen started.

- Patients must have a minimum PSA ≥ 5 ng/mL within 60 days of registration or prior to the initiation of androgen deprivation for patients who have started androgen deprivation therapy.

- Agree to undergo a biopsy of at least one metastatic site for RB status evaluation (*Please refer to Appendices B and C for specific procedures*). Adequate metastatic tissue from prior biopsy/resection can be used if available in lieu of a biopsy.

- ECOG performance status of 0-2.

- Age ≥ 18 years.

- Patients may have received prior neoadjuvant and/or adjuvant hormonal therapy for non-metastatic disease including in combination with salvage radiation therapy for PSA relapse, but it must not have lasted for more than 36 months. At least 12 months must have elapsed since completion of androgen deprivation therapy in the neoadjuvant and/or adjuvant setting.

Patients must have adequate organ and marrow function as defined below obtained within 14 days prior to registration:

WBC	≥ 3,000/µl
ANC	≥ 1,500/µl
Platelet count	≥ 100,000/µl
Serum Creatinine	≤1.5 x the institutional upper limits of normal or corrected creatinine clearance of ≥ 50 mg/ml/hr/1.73 m^2 BSA
Bilirubin	within the institutional limits of normal
SGOT (AST) SGPT (ALT)	≤ 2 x upper limits of normal ≤ 2 x upper limits of normal

- Patients must be able to take oral medication without crushing, dissolving or chewing tablets.

- Patients may have received prior radiation therapy or surgery. However, at least 14 days must have elapsed since completion of radiation therapy or surgery and patient must have only grade 2 or less adverse effects at the time of registration.

- Patients must agree to use highly effective contraception (details outlined in the consent form) during treatment and for a period of 90 days after ending treatment with palbociclib.

- Ability to understand and the willingness to sign a written informed consent document that is approved by an institutional review board.

4.2 Exclusion Criteria

- Patients who have received androgen deprivation therapy for greater than 14 days (LHRH-agonist or antagonist) for the treatment of their newly diagnosed metastatic disease prior to enrollment are not eligible for this study.

- Patients who are currently being treated with strong CYP3A4 inhibitors (e.g., amprenavir, fosamprenavir, indinavir, itraconazole, ketoconazole, lopinavir, mibefradil, miconazole, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, verapamil, voriconazole, and grapefruit) or strong CYP3A inducers (e.g., carbamazepine, felbamate, nevirapine, phenobarbital, phenytoin, primidone, rifabutin, rifampin, rifapentin and St. John's wort) must either discontinue these drugs or are ineligible.

- Grade 3 QTc prolongation (QTc >500 msec on two separate ECGs).

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

- Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure (New York Heart Association Class III and IV heart failure), unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

- Patients with a "currently active" second malignancy other than non-melanoma skin cancers are not eligible. Patients are not considered to have a "currently active" malignancy if they have completed all therapy and are now considered without evidence of disease for 1 year.

- HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with palbociclib. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy.

4.3 Inclusion of Women and Minorities

This study applies only to men. All races and ethnic groups are eligible for this trial. The anticipated accrual in the ethnicity/race categories is shown in the table below.

Ethnic Category	**Males**	**Total**
Hispanic or Latino	6	6
Not Hispanic or Latino	54	54
Total Ethnic	60	60
Racial Category		
American Indian or Alaskan Native	1	1
Asian	2	2
Black or African American	10	10
Native Hawaiian or other Pacific Islander	0	0
White	47	47
Racial Category: Total of all Subjects	60	60

5. REGISTRATION

5.1 Pretreatment Evaluation

Clinical: (completed within 14 working days prior to registration)

- Complete history to include all past or current medical conditions, current medications, date of diagnosis, and pathological confirmation of prostate carcinoma.
- Physical Examination including height, weight, ECOG Performance Status and EKG.

Laboratory: (completed within 14 working days prior to registration)

- Hematology: Complete blood count, differential and platelet count.
- Comprehensive Panel: sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, AST, ALT, alkaline phosphatase, phosphorous, total bilirubin, albumin, total protein and magnesium.
- PSA.

Imaging and Diagnostic Studies: (completed within 6 weeks prior to registration or prior to initiation of androgen deprivation for patients who have already started ADT prior to registration)

- Chest radiograph (CT or MRI thorax also acceptable)
- CT or MRI of the abdomen/pelvis
- Radionuclide Bone Scan

5.2 Subject Screening and Registration Procedure

Patient registration and randomization for this trial will be centrally managed by the Clinical Trials Office of The University of Michigan Comprehensive Cancer Center (UMCC) as described below:

- A potential study subject who has been screened for the trial and who has signed

the Informed Consent document will be initially documented by the participating site on the Screening and Enrollment Log provided by the Clinical Trials Office.

- It is the responsibility of the local site investigator to determine patient eligibility prior to submitting patient registration request to the multi-site Coordinator of the UMCC Clinical Trials Office. After patient eligibility has been determined, a copy of the completed Eligibility Worksheet together with all the pertinent de-identified source documents will be submitted by the requesting site to the Clinical Trials Office, either by fax or by email to :

  Ben Wright, CAPM CCRP
  Cancer Center Clinical Trials Office
  North Campus Research Complex (NCRC)
  Building 300 Main
  Ann Arbor, MI 48109-2800
  Phone: 734-763-0722
  Fax: 734-232-0744
  Email: bdwright@med.umich.edu

  Kathleen Granlund, CCRP
  Cancer Center Clinical Trials Office
  North Campus Research Complex (NCRC)
  Building 300 Main
  Ann Arbor, MI 48109-2800
  Telephone: 734-936-0563
  Fax: 734-232-0744
  Email: kemarsh@med.umich.edu

- The Multi-Site Coordinator of the Clinical Trials Office, who acts as the registrar, will review the submitted documents and process the registration. Sites should inform the Multi-Site Coordinator of a potential registration by 5 p.m. on the day prior to registration. Same day registrations cannot be guaranteed.

- An email will be sent by the registrar to the requesting site registrar to confirm patient registration and randomization and to provide the study identification number and randomization number that has been assigned to the patient. In addition, a copy of the completed Eligibility Worksheet signed and dated by the registrar, will be emailed back to the requesting site registrar.

- Patients found to be ineligible for participation after being consented will be considered screen failures, and documented as such in the Screening and Enrollment Log. These patients will not have study identification number assigned to them, and will not receive study treatment.

5.3 Biopsy and Randomization

5.3.1 Following registration, patient biopsies must be performed within 10 working days (unless archival biopsy tissue is already available from a metastatic site in which case it must be provided within 10 working days).

5.3.2 Issues that would cause treatment delays should be discussed with the Principal Investigator Dr. Hussain. If a patient is eligible for treatment, protocol therapy must start within 10 working days from the biopsy date or receipt of archival tissue.

5.3.3 Biopsy tissue should be processed and shipped to the Michigan Center for Translational Pathology. The coordinator for the Michigan Center for Translational Pathology will inform the registrar as well as the participating site whether the patient has adequate tissue for RB analysis.

5.3.4 If the patient has adequate tissue which stains positively for RB, the study pathologist will also inform the statistician, Stephanie Daignault-Newton at 734-647-3271 or sfaruzzi@umich.edu and randomization will be performed. (See Section 6, Treatment Plan). Randomized patients should begin protocol treatment within 7 working days from randomization. Issues that would cause treatment delays should be discussed with the Principal Investigator.

5.3.5 If the patient does not have adequate tissue for RB analysis, he will be removed from protocol unless the patient is willing to undergo another biopsy. Repeat biopsy will need to be done within 10 working days of initial biopsy as long as patient still fulfills other eligibility criteria. No study labs are needed but safety labs can be done as deemed necessary by the managing oncologist. Patients will be randomized once adequate tissue is evaluated and positive for RB staining.

5.3.6 If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The registrar should be notified of cancellations as soon as possible.

5.3.7 If available, diagnostic tissue from prostatectomy or prior biopsy will also be requested for correlative studies.

- If primary prostatectomy is available (preferred): The block with the highest volume of representative tumor histology from the index focus should be sent. Up to 12 x 5 micron sections will be taken from the block as needed. If blocks cannot be sent, a total of 16 x 4-5 micron unstained, unbaked sections on glass slides (charged or uncharged) from the block with the highest volume of representative tumor histology should be sent.
- If primary biopsy is available: Three blocks with the highest volume of representative tumor histology should be sent. A total of 9 x 10um sections will be taken from one or more blocks as needed without exhausting the blocks. If less than three blocks have cancer, then all blocks with cancer should be sent. If blocks cannot be sent, a total of 15 x 4-5 micron unstained sections on glass slides from one or more blocks with the highest volume of representative tumor histology should be sent.

- If unstained sections are sent, they should be labeled with the block number/letter. If less than three blocks have cancer, then as many unstained 4-5 micron sections (up to 15) as can be cut from blocks with cancer without exhaustion should be sent.

5.4 Data Management

All information will be recorded locally and entered into Case Report Forms (CRFs) on the web-based Velos data management system of the University of Michigan. Online access will be provided to each site by the Coordinating Center.

CRFs will be reviewed and source verified by the MSC during annual monitoring visits and prior to and between visits. Discrepant, unusual and incomplete data will be queried by the MSC. The investigator or study coordinator will be responsible for providing resolutions to the data queries, as appropriate. The investigator must ensure that all data queries are dealt with promptly.

The data submission schedule is as follows:

- At the time of registration
  - Subject entry into Velos
  - Subject Status
  - Demographics
- During study participation
  - All data should be entered online within 10 working days of data acquisition. Information on dose limiting toxicity events must be entered within one working day. Information on Serious Adverse Events must be entered within the reporting timeframe specified in Section 8 of the protocol.

Long term data will be collected periodically either by chart review or by contacting the patients.
All study information should be recorded in an appropriate source document (e.g. clinic chart).

6. STRATIFICATION, RANDOMIZATION AND TREATMENT PLAN

6.1 Stratification and Randomization

Stratification: All patients will be stratified by ADT treatment pre-registration for M1 disease (yes versus no) and disease extent defined as limited (disease confined to the spine, pelvic bones and or lymph nodes) or extensive (ribs, long bones, or visceral organs with or without other sites).

Randomization: All eligible patients with RB-positive tumor will be randomly assigned (1:2) to receive ADT (LHRH agonist + biclutamide) alone (ARM 1) or ADT plus palbociclib (ARM 2).

6.2 Agent Administration

- Patients will only be eligible for protocol therapy if the metastatic disease biopsy or prior metastatic disease biopsy/resection tissue has adequate tumor tissue for RB analysis and the tissue is RB positive.

- Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 3. Appropriate dose modifications are described in Section 7. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

- In both Arms androgen deprivation therapy should be administered after the biopsy is done, unless androgen deprivation was started prior to registration.

- In Arm 2 palbociclib should be administered post randomization as soon as agent is available (within 7 working days from randomization in case time for drug shipment is needed)

Arm 1 Treatment				
Agent	**Dose**	**Route**	**Schedule**	**Cycle Length**
LHRH-analogue*(any formulation)			Continuously	
Bicalutamide	50 mg	Oral	Daily Continuously	4 weeks

Arm 2 Treatment				
Agent	**Dose**	**Route**	**Schedule**	**Cycle Length**
LHRH-analogue*(any formulation)			Continuously	
Bicalutamide	50 mg	Oral	Daily Continuously	4 weeks
Palbociclib**	125 mg	Oral	Daily, Days 1-21 of a 28 day cycle.	28 days (4 weeks: 3 weeks of therapy + 1 week off)

***LHRH-analogue: Either goserelin or leuprolide dosing** to be determined at discretion of treating physician.
** **Palbociclib** : 125 mg to be taken daily for 21 days with 7 days off during a 28 day cycle. Drug should be taken with food.

- For patients who have not started ADT D1 is the day palbociclib + LHRH-a or LHRH-a therapy (based on randomization) is started.
- For patients who have started ADT D1 will be the day of randomization. "D1 exam, labs and correlative studies" must be done within 7 working days from randomization. All subsequent labs, imaging and endpoints will be counted from this day.
- For subsequent cycles, the formal "Day 1" of each cycle is defined as the day that Palbociclib dosing resumes.
- Patients will continue treatment until disease progression or other reason for discontinuation of protocol treatment.
- Patient Diary: Bicalutamide and palbociclib compliance will be recorded on the Patient Diary (see Appendix G and H). Institutional staff will review and ascertain patient adherence with protocol treatment. If a dose of either agent is missed, patients are to take the normal dose on the following day. If more than one daily dose is missed, the study doctor or study team must be informed.
- General rules for palbociclib administration:
    - Palbociclib should be taken with food.
    - Palbociclib capsules should be swallowed whole (do NOT chew, crush or open them prior to swallowing).
    - No capsule should be ingested if it is broken, cracked, or otherwise not intact.
    - Patients should be encouraged to take their dose at approximately the same time each day.
    - Patients who miss a day's dose entirely must be instructed NOT to "make it up" the next day.
    - Patients who vomit any time after taking a dose must be instructed NOT to "make it up," and to resume treatment the next day as prescribed.
    - Patients who inadvertently take 1 extra dose during a day must be instructed to skip the next day's dose.

6.3 General Concomitant Medication, Treatment and Supportive Care Guidelines

- Patients may continue on a daily Multi-Vitamin, calcium and Vitamin D, but all other herbal, alternative and food supplements (i.e. PC-Spes, Saw Palmetto, St John's Wort, etc.) must be discontinued before registration.

- Patients may not receive any other investigational anticancer agents (besides study drug and ADT) for this stage of disease. Patients may not undergo concurrent radiation therapy.

- Other excluded treatments include chemotherapy, immunotherapy, and radiopharmaceuticals or any other therapy intended to treat prostate cancer.

- In case participants develop nausea/vomiting/diarrhea or myelosuppression, supportive medications will be allowed according to institutional standards.

- Because there is a potential for interaction of palbociclib with other concomitantly administered drugs through the cytochrome P450 system, the medical record must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator (Dr. Hussain) should be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes and the agent should be discontinued or situation discussed with her.

- **Drugs that Inhibit or Induce CYP3A4 Enzymes (non-comprehensive list):**

  Patients are not eligible if they are taking one of the agents below. Similarly if any of these agents has to be started for patients randomized to the palbociclib arm then patients must come off treatment. Patients will be followed for progression.

  Strong CYP3A4 inhibitors: amprenavir, fosamprenavir, indinavir, itraconazole, ketoconazole, lopinavir, mibefradil, miconazole, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, verapamil, voriconazole, and grapefruit

  Strong CYP3A4 inducers: carbamazepine, felbamate, nevirapine, phenobarbital, phenytoin, primidone, rifabutin, rifampin, rifapentin and St. John's wort.

- Transfusions and hematologic growth factors are allowed in accordance with institutional guidelines.

6.4 Duration of Therapy

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

- Disease progression (defined in section 11),
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s) (see sections 3 and 8),
- Therapy delay for more than 4 weeks,
- Patient decides to withdraw from the study,
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

6.5 Duration of Follow Up

- Patients removed from study treatment for progression will be followed for 4 weeks or resolution/stabilization of any therapy related adverse events.

- Once off study treatment for any reason other than progression, follow-up visits will be done every 12 weeks. These visits will include H&P and laboratory assessment (CBCPD, Chemistry, PSA). Imaging will be done q 24 weeks. Clinical, Laboratory and imaging evaluations will be done for 2 years or until progression, whichever comes first.

- Patients removed from study treatment for unacceptable adverse event(s) may be followed more frequently than every 12 weeks per the investigator's discretion until resolution or stabilization of the adverse event.

7. DOSING DELAYS/DOSE MODIFICATIONS

- This study will utilize the CTCAE (NCI Common Terminology Criteria for Adverse Events) Version 4.0 for toxicity and Serious Adverse Event reporting. A copy of the CTCAE Version 4.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov). All appropriate treatment areas should have access to a copy of the CTCAE Version 4.0.

- Toxicities (including suspected reactions) that meet the serious reporting criteria as outlined in section 8.0 of the protocol must be reported to the Coordinating Center Lead PI and Multi-site Project Manager and to the IRB per local IRB requirements.

- For treatment or dose modification related questions, please contact Dr. Maha Hussain at 312-695-6180 or maha.hussain@northwestern.edu or cto-multisite@med.umich.edu.

- If a patient experiences several adverse events and there are conflicting recommendations, the investigator should use the recommended dose adjustment that reduces the dose to the lowest level.

- Combined ADT will be required throughout duration of the study.

    - If patient desires to discontinue ADT, he will be removed from study treatment.

    - If patient develops serious adverse event felt to be related to bicalutamide (e.g., liver toxicity) then bicalutamide can be discontinued while continuing LHRH agonist/antagonist plus or minus palbociclib.

7.1 Palbociclib Dose Reductions:

In the event of significant treatment-related toxicity, palbociclib dosing may be interrupted or delayed and/or reduced as described below. In the event of multiple toxicities, dose modification should be based on the worst toxicity observed.
Patients are to be instructed to notify Investigators at the first occurrence of any adverse sign or symptom.

Dose levels are defined below:

Dose Level	Palbociclib
0	125 mg daily
-1	100 mg daily
-2	75 mg daily

- Dose modifications may occur in three ways:
  - Within a cycle: dosing interruption until adequate recovery followed by dose reduction, if required.
  - Between cycles: next cycle administration may be delayed due to persisting toxicity when a new cycle is due to start.
  - At start of the new cycle: dose reduction may be required in a subsequent cycle based on toxicity experienced in the previous cycle.

7.1.1 Palbociclib Dose Modification and Management:

Table 1. Palbociclib Dose Modification and Management – Hematologic Toxicities[a]

CTCAE Grade	Dose Modifications
Grade 1 or 2	No dose adjustment is required.
Grade 3	Day 1 of cycle: Withhold palbociclib, repeat complete blood count monitoring within 1 week. • If recovered to Grade ≤2, resume at the *same dose*. • If Grade 3, hold initiation of next cycle until recovery to Grade ≤2. Resume at the *same dose*. • If Grade 4, hold initiation of next cycle until recovery to Grade ≤2. Resume at the *next lower dose*. Day 14 of first 2 cycles: Continue palbociclib at current dose. Repeat complete blood count on Day 21. • If Grade 3 on Day 21, start subsequent cycles at the *same dose*. • If Grade 4 on Day 21, start subsequent cycles at the *next lower dose*. Consider dose reduction in cases of prolonged (>1 week) recovery from

Table 1. Palbociclib Dose Modification and Management – Hematologic Toxicities[a]

CTCAE Grade	Dose Modifications
	Grade 3 neutropenia or recurrent Grade 3 neutropenia in subsequent cycles.
Grade 3 neutropenia with fever ≥38.5 ºC and/or infection	Withhold palbociclib until recovery to Grade ≤2. Resume at the *next lower dose*.
Grade 4	Withhold palbociclib until recovery to Grade ≤2. Resume at the *next lower dose*.

Grading according to CTCAE 4.0 (Grade 1: ANC < LLN - 1500/mm^3; Grade 2: ANC 1000 - <1500/mm^3; Grade 3: ANC 500 - <1000/mm^3; Grade 4: ANC <500/mm^3).
ANC=absolute neutrophil count; CTCAE=Common Terminology Criteria for Adverse Events; LLN=lower limit of normal.

[a] Table applies to all hematologic adverse reactions except lymphopenia (unless associated with clinical events, e.g., opportunistic infections).

Table 2. Palbociclib Dose Modification and Management – Non-Hematologic Toxicities

CTCAE Grade	Dose Modifications
Grade 1 or 2	No dose adjustment is required.
Grade ≥3 non-hematologic toxicity (if persisting despite medical treatment)	Withhold until symptoms resolve to: • Grade ≤1; • Grade ≤2 (if not considered a safety risk for the patient) Resume at the *next lower dose*.

Grading according to CTCAE 4.0.
CTCAE=Common Terminology Criteria for Adverse Events.

7.1.3. A dose reduction of 1 level will be pursued for all Grade 4 toxicities and also for any Grade 3 toxicity that in the opinion of the investigator will potentially impact the safety of the participant. Dose reductions based on the above criteria can occur at the start of a new cycle or at the time of retreatment.

- The dose of **palbociclib** will not be re-escalated once a dose reduction has occurred.
- Asymptomatic laboratory abnormalities would not be considered a serious adverse event unless the treating physician believes it may potentially impact the participant's safety.
- If **palbociclib** is delayed more than 4 weeks for any reason, or if a dose reduction below 75 mg daily is required, patients will be removed from protocol treatment.
- Doses should be held until toxicity resolution. Appropriate follow up assessments, as defined by the investigator, should be undertaken until adequate recovery occurs.
- Criteria required before treatment can resume are described in the Retreatment

criteria section above.

- Depending on when the adverse event resolved, a treatment interruption may lead to the patient missing all subsequent planned doses within that same cycle or even to delay in the initiation of the subsequent cycle.

- If the adverse event that led to the treatment interruption recovers within the same cycle, then re-dosing in that cycle is allowed. Doses omitted for toxicity are not replaced within the same cycle.

- The need for a dose reduction at the time of treatment resumption should be based on the criteria defined in the Dose Reductions Section unless an alternative plan is expressly agreed upon between the treating physician and the PI.

- If a dose reduction is applied in the same cycle, the patient will need to return to the clinic to receive new drug supply.

7.2 Bicalutamide Dose Hold

There are no dose reductions for Bicalutamide.

7.2.1. Diarrhea:
The major toxic effect of bicalutamide is moderate diarrhea which is rarely severe (exclusion of other causes of diarrhea should be considered in severe cases). The dose modifications for this will be as follows.

- Grade 2 diarrhea, treat symptomatically with anti-diarrhea drugs.
- Grade 2 diarrhea unresponsive to symptomatic treatment or Grade 3 or 4 diarrhea - discontinue bicalutamide until complete recovery.

Further tests are up to discretion of treating physician. Restart treatment as per protocol when diarrhea resolves to ≤ Grade 1.

7.2.2. Abnormal Liver Function Tests (SGOT/AST, SGPT/ALT, Bilirubin)
Grade 3 or greater toxicity - Stop bicalutamide. Hepatitis screening (A, B, C) should be done in all cases of abnormal LFTs which could be consistent with infectious hepatitis. To assess return to normal, abnormal liver function tests should be done weekly or more frequently, as per the discretion of the treating physician. Bicalutamide may be restarted after LFTs normalize (< or = Grade 1) if the event is deemed not related to Bicalutamide.

7.2.3. Patients may complain of flatulence, bloating and mild "gas pains" which should not result in changes in treatment. Symptomatic treatment should be employed with antacids, simethicone, etc.

7.2.4. If patient develops serious adverse event felt to be related to bicalutamide then bicalutamide can be discontinued while continuing LHRH agonist/
antagonist plus or minus **palbociclib** (based on arm).

7.2.5. For any other Grade 3-4 adverse events which the investigator believes to be related to bicalutamide and is clinically significant stop bicalutamide.

7.2.6 Asymptomatic Grade 3 or 4 laboratory findings, which are not considered clinically significant may not require dose hold. The decision to hold the dose should be based on the investigator's clinical judgment.

8. ADVERSE EVENTS

8.1 Adverse Events and Potential Risks Related to Palbociclib (see section 3.1).

- For the most recent safety update, please refer to the current Investigator's Brochure.
- For contraindications, interactions with other medications and known adverse reactions related to palbociclib please see section 3.1.

8.2 Adverse Event Reporting Requirements

8.2.1 Adverse event (AE) monitoring and reporting is a routine part of every clinical trial and is done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Data on adverse events will be collected from the time of the initial study treatment administration through 30 days after the last dose of study treatment. Any serious adverse event that occurs more than 30 days after the last study treatment and is considered related to the study treatment must also be reported. Serious Adverse Events (SAEs) will continue to be followed until:

- Resolution or the symptoms or signs that constitute the serious adverse event return to baseline;
- There is satisfactory explanation other than the study treatment for the changes observed; or
- Death.

8.2.2 The investigator is responsible for the detection, documentation, grading and assignment of attribution of events meeting the criteria and definition of an AE or SAE. The definitions of AEs and SAEs are given below. It is the responsibility of the Protocol principal investigator and site investigator to ensure that all staff involved in the trial are familiar with the content of this section.

8.2.3 Any medical condition or laboratory abnormality with an onset date before initial study treatment administration is considered to be pre-existing in nature. Any known pre-existing conditions that are ongoing at time of study entry should be considered medical history.

8.2.4 All events meeting the criteria and definition of an AE or SAE, as defined in Section 8.3, occurring from the initial study treatment administration through

30 days following the last dose of the study treatment must be recorded as an adverse event in the patient's source documents and on the CRF regardless of frequency, severity (grade) or assessed relationship to the study treatment.

8.2.5 In addition to new events, any increase in the frequency or severity (i.e., toxicity grade) of a pre-existing condition that occurs after the patient begins study treatment is also considered an adverse event.

8.3 Definitions

8.3.1 Adverse Event

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

Diagnostic and therapeutic non-invasive and invasive (i.e., surgical) procedures will not be reported as adverse events. However, the medical condition for which the procedure was performed must be reported if it meets the definition of an adverse event unless it is a pre-existing (prior to protocol treatment) condition.

- Symptoms of the original or targeted disease will be considered adverse events. The following symptoms are indicative of underlying disease (Metastatic Prostate Cancer) and will be reported as adverse events. Attribution to underlying disease versus therapy must be provided:

    - Bone Pain
    - Urinary Obstruction
    - Spinal Cord Compression
    - Fractures

- *Abnormal laboratory values or test results constitute adverse events if they induce clinical signs or symptoms or require therapy. They are to be captured under the signs, symptoms or diagnoses associated with them.*

- *Hy's Law Cases. Cases of potential drug-induced liver injury as assessed by laboratory test values ("Hy's Law Cases") are also reportable to Pfizer. If a Study subject develops abnormal values in aspartate transaminase (AST) or alanine transaminase or both, concurrent with abnormal elevations in total bilirubin and no other known cause of liver injury, that event would be classified as a Hy's Law Case. This reporting requirement is further explained in the training material provided by Pfizer-. As used in this Agreement, the term SAE will be understood to also include Hy's Law Cases."*

8.3.2 Serious Adverse Event

An adverse event is considered "serious" if, in the view of either the Site Investigator or the protocol PI it results in any of the following outcomes:

- Death
  If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.

- A life-threatening adverse event
  An adverse even is considered 'life-threatening' if, in the view of either the investigator [or sponsor], its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.

- Inpatient hospitalization or prolongation of existing hospitalization for > 24 hours.

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions

- A congenital anomaly/birth defect

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

- Previously planned (prior to signing the informed consent form) surgeries should not be reported as SAEs unless the underlying medical condition has worsened during the course of the study. Preplanned hospitalizations or procedures for preexisting conditions that are already recorded in the patient's medical history at the time of study enrollment should not be considered SAEs. Hospitalization or prolongation of hospitalization without a precipitating clinical AE (for example, for the administration of study therapy or other protocol-required procedure) should not be considered SAEs. However, if the preexisting condition worsened during the course of the study, it should be reported as an SAE.

- Exposure During Pregnancy:

Paternal exposure must be reported if a male has been exposed (e.g., due to treatment or environmental exposure) to the investigational product prior to or around the time of conception of his child and/or is exposed during the partner's pregnancy.

8.3.3 Expected Adverse Events

An adverse event (AE) is considered "expected" if:

- For approved and marketed drugs or devices, those adverse events are described in the approved Package Insert (Label).
- For investigational new drugs, those adverse events are described in the FDA Investigator's Brochure.
- In clinical research studies, information on expected adverse events is also summarized in the protocol and in the consent document. See section 3.1 for the list of expected adverse events related to the drug under study.

8.3.4 Unexpected Adverse Event

An adverse event (AE) is considered "unexpected" if it is not described in the Package Insert, Investigator's Brochure, in published medical literature, in the protocol, or in the informed consent document.

8.4 Adverse Event Characteristics

8.4.1 CTCAE Term

(AE description) and grade: The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be down loaded from the CTEP web site. (http://ctep.cancer.gov)

8.4.2 Attribution of the AE

The investigator or co-investigator is responsible for assignment of attribution.

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

8.5 Serious Adverse Event Reporting Guidelines

8.5.1 The PI and the Coordinating Center must be notified within 24 hours of first awareness of the event (immediately if the event is fatal or life-threatening) meeting the criteria and definition of a serious adverse event, regardless of

attribution, occurring from after the first dose of Pfizer Product through 30 days after the last administration of the Pfizer Product. In addition, SAEs should be submitted any time after the administration of the last dose of the Pfizer Product if the Investigator suspects a causal relationship between the Pfizer Product and the SAE.

8.5.2 All serious adverse events must be reported to the Coordinating Center within 24 hours of first awareness of the event. Events should be reported using the Medwatch 3500A form. A copy of the Medwatch 3500A form should be sent to the University of Michigan Multi-site Coordinator via fax at 734 232 0744 or via email to bdwright@med.umich.edu, kemarsh@med.umich.edu or cto-multisite@med.umich.edu.

8.5.3 All serious adverse events will be reported to the IRB per current institutional standards.

8.5.4 The University of Michigan acting as the Coordinating Center will be responsible for reporting to Pfizer by facsimile any Serious Adverse Event that occur during the SAE reporting period. The coordinating center will report the SAE within 24 hours of first awareness of the event (immediately if the event is fatal or life-threatening).

8.5.5 Contact information for Principal Investigator for SAE Reporting:

Maha Hussain, M.D.
Division of Hematology Oncology
Northwestern Medicine
676 N. St. Clair, Suite 850
Chicago, Illinois 60611
Phone: 312-695-6180
Fax: 312-695-4530
e-mail: maha.hussain@northwestern.edu

8.5.6 The Coordinating Center will disseminate information regarding serious adverse events to the participating sites within 5 days of review of the information by the Principal Investigator (or her designee in the event of extended absence) only in the case that the event(s) is believed to be related (i.e., possibly, probably, or definitely) to the study medication.

The Coordinating Center, or designee will be responsible for reporting of events to the FDA and supporters, as appropriate (outlined in Section 8.6)

8.6 SERIOUS ADVERSE EVENT REPORTING TO THE FDA

It is the responsibility of the IND Sponsor to submit an IND for clinical trials conducted with investigational agents and to ensure that FDA and all participating investigators are promptly informed of significant new AEs or risks with respect to the investigational agent.

In this trial, serious unexpected adverse events believed to be definitely, probably or possibly related to the study treatment will be reported to the Food and Drug Administration via the MedWatch 3500A. The Coordinating Center, will coordinate with the IND Sponsor and/or designee for the reporting of any and all IND safety reports to the FDA as per the requirements outlined in 21 CFR 312.32.

9. TISSUE PROCESSING/CORRELATIVE/SPECIAL STUDIES

9.1 Tissue / Laboratory Correlative Studies

9.1.1. Collection and Handling of Specimens

From each patient, tissue specimens will be collected from:

a) A metastatic site of disease (via biopsy) or adequate metastatic tissue from prior biopsy or resection.

b) Archived prostatectomy or biopsy specimen from the primary prostate tumor (if available, via retrieval of previously archived specimens).

c) Blood/circulating tumor cells (pre-treatment, week 12, after 28 weeks of therapy, and at progression/or coming off protocol treatment).

The rationale, and a brief overview, for collection and handling of these specimens is as described below. Further technical details regarding biopsies of metastatic sites and handling of these biopsy specimens can be found in Appendices B and C, respectively.

9.1.1a Biopsy of metastatic site: Rationale and Procedure (Appendices B and C)

a. Preclinical and clinical data indicates that palbociclib primarily exerts its effect via inhibition of intact RB protein phosphorylation. Therefore, only patients with intact RB expression (as assessed by immunohistochemistry) will be randomized to palbociclib or control arms. Thus all patients enrolled in this trial will either undergo a biopsy of a metastatic site or have previously underwent a biopsy of metastatic disease with evaluable tissue to determine RB status prior to randomization.

b. Biopsies must be scheduled for Monday to Thursday to allow for overnight shipping to be received by Friday.

c. Patients will be scheduled for a CT-guided biopsy of a bone or soft tissue abnormality consistent with prostate cancer metastasis. The site of biopsy will be determined by the radiologist after review of available radiographs, CT, MRI, and/or bone scan, and will be selected based on whatever site is expected to result in the best access and best yield.

- An ultrasound (US) can be used for lymph node or soft tissue biopsies provided that the target lymph node or soft tissue mass is visible with the US and can be properly accessed.

- Verbal and written informed consent will be obtained after discussion of risks (bleeding, infection, soft tissue and bone injury, inconclusive biopsy results) of the procedure.

- Standard laboratory tests to assess coagulation and bleeding will also be completed prior to the procedure. **A standard operating procedure for bone biopsy is provided in Appendix B**. **A standard operating procedure for soft tissue biopsy is provided in Appendix C**.

- In order to ensure adequate tumor sample as long as it is deemed safe and based on accessibility, target lesion size, specimen integrity, specimen appearance, intra-procedural bleeding, tumor biopsy should attempt to obtain:

1. Bone biopsy: **2-8 bone biopsy samples**. In addition, at the time of biopsy, it will be determined if a bone marrow aspirate is feasible, and if performed, the bone marrow aspirate will be sent to University of Michigan for analysis.

2. Lymph node or soft tissue: If obtaining **a 1-cm-long 18-gauge core specimen, attempts should be made to obtain at least 6 cores. If obtaining a 2-cm-long 18-gauge core specimen, attempts should be made to obtain at least 4 cores.**

   If core biopsy is unsuccessful, a lymph node aspirate can be obtained, but is likely to be insufficient to allow Rb status determination and randomization.

d. The biopsy specimens and the aspirate will be collected and will be immediately frozen in dry-ice ethanol bath in Tissue-Tek O.C.T Compound (Sakura Finetek, Torrance, CA) to ensure the integrity of tissue and proteins for both immunoblotting and immunohistological analysis of samples. Upon enrollment of each patient on study, sample kits containing necessary reagents for tissue collection (Tissue-Tek standard-size cryomolds, Tissue-Tek OCT, blue pads, markers, and an insulated bucket) will be shipped to the enrolling site coordinator.

- It is expected that dry ice and isopentane will be provided by the enrolling site.

In addition, written SOPs, with specific details regarding tissue collection with this kit, along with a contact sheet with mailing addresses for sample shipment, will be mailed to each site coordinator.

e. Each Tissue-Tek cryomold will be labeled with the clinical protocol number, the patient's study registration number, the tissue type and the biopsy time and date. In addition, a separate information sheet accompanying each specimen will be filled out. This information sheet will include various details necessary for quality assurance of the specimen, including:

1) Date of collection
2) Specimen ID
3) Site from which biopsy obtained (bone versus soft tissue, and then location)
4) Type of biopsy device
5) Biopsy diameter: gauge, and length: cm
6) Time biopsy needle introduced
7) Time biopsy snap-frozen on dry ice
8) Time aspirate performed (if applicable)
9) Time aspirate snap-frozen on dry ice

A standard operating procedure for handling of the biopsy and aspirate specimens has been attached to this protocol as Appendix B.

Following biopsy, samples from metastatic sites need to be immediately frozen prior to shipment within 24 hours to the University of Michigan, per shipping directions described in 9.1.2.

9.1.1b Archived Prostate Cancer Samples: Rationale and Procurement Procedure

Occasionally, newly metastatic prostate cancer is identified by biopsy at the time of diagnosis or resection. This tissue may be utilized for RB status assessment, trial enrollment and randomization if it is of adequate quality for IHC analysis of RB status. Additionally, previous diagnostic tissue is being requested from all patients to assess whether RB status and other correlative markers are maintained between diagnostic and metastatic samples with or without previous hormonal therapy.

If resection specimens are available (preferred): The block with the highest volume of representative tumor histology from the index focus OR 16 unstained, unbaked sections (4-5 microns in thickness) on glass slides (charged or uncharged) should be shipped at room temperature to the University of Michigan.

If biopsy specimens are available: Three blocks with the highest volume of representative tumor histology OR 15 unstained, unbaked sections (4-5 microns in thickness) on glass slides (charged or uncharged) from up to three blocks should be shipped at room temperature to the University of Michigan. If unstained sections are sent, they should be labeled with the block number/letter. If less than three blocks have

cancer, then all blocks with cancer OR as many unstained sections as can be cut from blocks with cancer without exhaustion should be sent.

9.1.1c: Circulating Tumor Cells: Rationale and Sample Collection Procedure

Blood will be collected pre-treatment, week 12, after 28 weeks of therapy, and at progression/or coming off protocol treatment. Blood samples will be drawn into 10-mL evacuated blood draw tube (Cell-Free DNA; Streck) (one at each time point). These blood samples can be maintained at room temperature, **but must be shipped to Epic Sciences within 48 hours after collection**, per shipping directions described in 9.1.2.

9.1.1d: Circulating Tumor DNA: Rationale and Sample Collection Procedure

Blood will be collected pre-treatment, week 12, after 28 weeks of therapy, and at progression/or coming off protocol treatment. Blood samples will be drawn into 10-mL evacuated blood draw tubes (Cell-Free DNA; Streck) (one at each time point). These blood samples can be maintained at room temperature, **but must be shipped to University of Michigan within 48 hours after collection**, per shipping directions described in 9.1.2.

9.1.2 Shipping of Specimens after Collection

All samples, except the blood samples for CTC analysis, must be shipped to the University of Michigan, at the following address:

Javed Siddiqui, MS, MT(ASCP, CLsp (MB))
Technical Director
Michigan Center for Translational Pathology
Room 1138
2900 Huron Parkway Suite 100
Ann Arbor, MI 48105
Phone: (734) 232-0829
Fax: (734) 232-0805
siddiqui@med.umich.edu

Shipping must occur only Monday-Thursday, and an e-mail or phone call to the contact listed on the laboratory contact sheet, alerting them to the shipment, is required.

9.1.2a Biopsies and aspirates from metastatic sites: If biopsies are performed on metastatic sites on Monday through Thursday, the biopsy and aspirate samples should be shipped overnight, on dry ice, to the University of Michigan.

9.1.2b Archived Primary Prostate Cancer Tissue: The tumor samples can be shipped via standard mail, at room temperature, to the University of Michigan. It is requested that efforts be made to ship the primary tumor samples within a month of patient enrollment onto the trial.

9.1.2c Blood samples NOT for CTC analysis: Blood samples should be obtained on Monday through Thursdays, and can be shipped overnight, at room temperature, to the University of Michigan.

9.1.2d Blood samples for CTC analysis: The blood samples should be shipped overnight, at room temperature, to Epic Sciences, at the following address:

Epic Sciences
9381 Judicial Drive, STE 200
San Diego, CA 92121
858-356-6610

Shipping must occur only Monday-Thursday, and an e-mail should be sent to partners@epicsciences.com, alerting to the shipment, the inclusion of the sample ID, number of samples, tracking number, and time of blood draw, is required. Please note that the Epic Sciences shipping address is to be used ONLY FOR THE CTC SAMPLES, and that the remainder of the samples (metastases, archived primary tumor, other blood samples) should be sent to the University of Michigan address listed above section 9.1.2.

For further details regarding shipping, please refer to Appendix D, E & F which are the standard operating procedures for the shipment of samples.

9.1.3 Sites Performing Correlative Studies

Aside from the circulating tumor cell studies, all correlative studies will be performed at the University of Michigan, within the Michigan Center for Translational Pathology (MCTP). Dr. Felix Feng, translational co-investigator, and Dr. Scott Tomlins, study pathologist, will oversee these correlative studies, with additional consultation provided Dr. Karen Knudsen, translational co-investigator. The circulating tumor cell studies will be performed by Epic Sciences.

9.1.4 Processing of Specimens after Receipt at the University of Michigan

a. With the receipt of each biopsy specimen, Javed Siddiqui at the University of Michigan (or a pre-specified designee) will place the samples in the -80˚C tissue repository described below and will record two additional items for further quality assurance:

1) The date/time of receipt of the biopsy specimen at the University of Michigan
2) The date/time that the biopsy specimen is placed at -80°C

b. Biopsies from metastatic sites: The frozen tissue samples from either biopsy or from preexisting metastatic tissue, obtained for research from patients taking part in this protocol will be held in the University of Michigan Prostate Oncology Program Tissue Bank. The Tissue Bank is located in Room 1143, 2900 Huron Parkway, Suite 100, University of Michigan, Ann Arbor, Michigan 48105. This laboratory has multiple -80˚C freezers, one of which will be devoted to the biopsies from this trial. The freezers

have an alarm system and self-dialing emergency system. It is anticipated that at least two samples will be obtained from each metastatic biopsy.

From bone biopsies, these metastases may consist of two biopsies or a biopsy and an aspirate. One sample from each metastatic biopsy (the biopsy sample in cases where there is a biopsy sample and an aspirate) will be fixed in formalin shortly after receipt at the University of Michigan. Eight 5 um section slides will be cut from this formalin fixed sample -- one for H&E staining to confirm the presence of tumor, two for determination of RB status and the others for correlative studies. The remaining formalin-fixed metastatic biopsy sample will be utilized for targeted transcriptome/genome sequencing. Where feasible, transcriptome sequencing and whole exome capture will be performed on remaining frozen metastatic biopsy materials.

c. Primary tumor samples: Retrieved primary tumor samples will be stored at room temperature in the University of Michigan Prostate Oncology Program Tissue Bank, and will be assessed for RB pathway biomarkers, as well as for genetic alterations by focused targeted transcriptome/genome sequencing.

d. Blood samples for circulating tumor DNA studies: Blood samples received will have genomic DNA extracted per standard protocols. Samples will then be stored in -80C freezer. Further analyses of these blood samples will proceed once the genomic studies have been performed on the biopsy samples.[24-28]

e. Blood samples for circulating tumor cell studies: The Epic Sciences platform will be utilized for circulating tumor cell analyses.[24-28] Peripheral blood samples will be drawn at accruing sites following informed consent and processed following standard operating procedures. Using a standardized and ISO-certified shipping process, samples are transferred to the Epic Sciences central laboratory. Samples are then processed after 48 hours of blood draw. The red blood cells are lysed and the remaining nucleated cells are plated on custom glass slides and frozen for storage. As needed, blood sample slides are thawed and processed to bind the appropriate cell markers with fluorescently labeled antibodies. Depending on peripheral blood counts, up to 16 slides are generated from each subject. Two to four processed slides, representing one patient test, the identification of CTCs consists of three markers (DAPI-nucleus, cytokeratin – epithelial marker, and CD-45 – white blood cell marker) and are imaged on custom-built automated slide imagers. The resulting images are then automatically processed to produce data for technical analysis which consists of removing imaging artifacts and selecting potential CTC candidates which are then presented in a web-based report to a pathologist for final review. Remaining slides are stored for subsequent analysis. These subsequent analyses will include immunofluorescence for the androgen receptor (AR), including AR C-terminal and AR N-terminal expression.

9.2 Description of RB Assessment and Correlative Studies

9.2.1 RB Status Determination

As part of the patient selection for this trial, patients will undergo immunohistochemistry (IHC) for total RB status in a CLIA certified laboratory, per a previously published RB IHC protocol.[32] IHC will be performed for RB using a mouse-

anti-RB monoclonal antibody (BD Biosciences, San Jose, CA, G3-245) using automated IHC staining on the Ventana Medical Systems Benchmark Ultra autostainer. Slides are pre-treated with CC1 for 40 minutes, incubated with primary antibody for 16 minutes at 37 C (1:1600 dilution in Ventana diluent 90040). Tumor RB status will be determined by assessing staining in at least 100 tumor cells (or all cells if less than 100 cells are present). Nuclear RB staining intensity in tumor cells will be assessed as negative (0), weak (1+), moderate (2+) or strong (3+). Inflammatory, endothelial and/or stromal cells will be used as internal positive controls. Based on validation studies using samples with known RB retention or deletion, tumor samples with >5% of cells showing at least moderate RB expression or 20% of tumor cells showing at least weak RB expression will be considered positive and eligible for the trial. For correlative studies, RB staining will be quantified in positive patients by multiplying the percentage of positive tumor cells by the average intensity of positive tumor cells (1=weak, 2=moderate, 3=strong).

9.2.2 Identification of Biomarkers Predictive of Responsiveness to Palbociclib

The RB pathway can be disrupted in human cancers via multiple mechanisms,[5] and because alterations in the RB pathway could modulate the response to palbociclib, correlative studies will assess for potential biomarkers along the RB pathway via multiplexed IHC. Because the RB protein can be inactivated by posttranslational modifications that are the subject of oncogenic (e.g., cyclin D1 overexpression/amplification) or tumor suppressive (e.g., p16ink4a silencing) alterations, IHC for cyclin D1, cyclin D1B (a variant associated with aggressive disease), and p16 will be performed.[33] Additionally, as RB also plays a critical role in the response to many antiproliferative stresses that are engaged by therapeutic agents,[5] immunohistochemistry will also be performed for Ki-67, a common marker of cell proliferation. As previous studies from our group have defined a signature of RB loss,[7] immunohistochemistry will be performed for key RB target genes (such as Cyclin A and MCM7). Finally, we will also assay the status of the RB interactor, E2F1, via IHC. In both the primary tumor samples and metastatic biopsies, IHC biomarkers will be quantified by multiplying the percentage of positive tumor cells by the average intensity of positive tumor cells (0=negative to 3=strong) by experienced pathologists. For Ki-67, proliferative index will be determined by quantifying the percentage of tumor cells with nuclear staining. Statistical analyses plans for these correlative studies are described in Section 13.

9.2.3 Targeted DNA sequencing and expression profiling. While the ultimate goal would be to perform comprehensive sequencing (see below) on all the biopsy studies from this clinical trial, the tissue limitations regarding tumor quantity may preclude comprehensive sequencing on a subset of samples. Specifically, in patients in which only one biopsy core can be obtained, this single core will be converted to a formalin-fixed paraffin embedded (FFPE) specimen, to allow assessment of the primary biomarker (RB) using a validated assay (IHC) in a CLIA-certified laboratory. While comprehensive sequencing cannot be reliably performed on FFPE biopsy specimens, targeted sequencing and expression profiling can be performed on these limited FFPE tissues. Specifically, we will assess a novel panel of recurrent alterations identified

across prostate cancer profiling studies.

DNA and RNA will be co-isolated using a commercially available kit (Qiagen All Prep DNA/RNA FFPE) according to the manufacturer's instructions. For DNA based alterations, a Haloplex custom capture panel (Agilent Technologies) will be utilized with Ion Torrent-based sequencing on the PGM/Proton sequencing systems according to the manufacturer's instructions. Our team (S Tomlins) has previously developed a Haloplex Custom Design (Agilent) for capture-based targeted sequencing of complete coding sequences of 80 genes previously reported as significantly mutated in prostate cancer, 46 therapeutic targets, and 50 causal cancer genes; this approach has been optimized for 200 ng of genomic DNA. We will adapt this platform to include potential biomarkers of response as identified in Section 9.2.1. Identified variants will be assessed for potential as drivers of resistance to CDK4/6 inhibition or ADT based on known roles in oncogenesis and prostate cancer, comparison to Oncomine and COSMIC mutational data, and comparison to CRPC tissue expression profiles (as previously published by our group and others[16,34]), and integration with AR chip-seq profiles.[35]

qPCR for targeted gene expression profiling will be performed using a previously validated panel of 48 prostate cancer related genes, supplemented with potential biomarkers of response as identified in Section 9.2.1. Reverse transcription will be performed on 300ng of isolated RNA from each tissue sample using gene specific priming with subsequent qPCR using FFPE optimized TaqMan assays. Genes have been selected to assess AR signaling status (including both AR induced/repressed and neuroendocrine genes), known prostate cancer fusions (through 5' and 3' assays for ETS genes, FGFR2, RAF/RAS family members, etc), potential therapeutic targets in other malignancies (such as 5' and 3' assays for ALK and RET over-expression), as well as housekeeping genes. qPCR will be performed on the ABI Quantstudio 12K flex system using TaqMan Universal PCR Mastermix and 384 well TLDA cards.

9.2.4 Comprehensive next-generation sequencing. For specimens in which enough tumor tissue was present (i.e., additional frozen biopsy specimens), exome sequencing and RNA-sequencing using Illumina based technology will be performed as described using robust protocols and pipelines previously optimized by our team.[16,35-39] Standard pipelines will be employed as described to quantify gene expression, and identify gene fusions, point mutations, indels and copy number variants.

9.2.5 Circulating tumor DNAs as predictive biomarkers of response to androgen deprivation therapy alone or in combination with Palbociclib.

Detection of DNA in the serum of patients with metastatic prostate cancer, may allow determination of tractable biomarkers of disease status and responsiveness to therapies. Here we will collected and analyze serum for circulating tumor DNA to determine if we can identify markers of disease state and responsiveness.[40] In particular, potential biomarkers identified via targeted sequencing and expression profiling of the metastatic biopsy samples (see 9.2.3) will be assessed within the blood samples as well, to determine if these blood samples can serve as a less invasive approach of querying relevant biomarkers.

9.2.6 Circulating Tumor Cell (CTC) Count and Heterogeneity as predictive biomarkers of response to androgen deprivation therapy alone or in combination with Palbociclib. CTCs will be enumerated using the Epic Sciences System utilizing automated fluorescence- based microscopy systems that permit computer-generated reconstruction of cellular images. In addition, immunofluoresence-based approaches assessing for CK, CD45, DAPI, and AR expression will be used to determine the extent of CTC heterogeneity (distribution of CTC phenotypes, such as nucleoli CTCs, CTC clusters, CK- CTCs, CK speckled CTCs, small CTCs, AR nuclear CTCs, and AR cytoplasmic CTCs). Associations between outcome and CTC count/heterogeneity will be performed as per Section 13.4.

10. STUDY CALENDAR

		Cycle 1					Cycle 2-7					Subsequent Cycles		Off Treatment [g]
Required Treatment or Studies	Pre-Study	Day 1	Day 8	Day 15	Day 21	Day 28	Day 1	Day 8	Day 15	Day 21	Day 28	ARM 1 ADT Alone	ARM 2 palbociclib + ADT[h]	
Bicalutamide		X----------				----------X						Daily	Daily	
LHRH Therapy		X----------				----------X						Continuous	Continuous	
PD-0332991 (Arm 2) 3 weeks on /1 week off[h]		X----------				X	X----------				X	NA	Days 1-21	
Informed consent	X													
History & Physical	X	X					X					Every 12 weeks	Every 12 weeks	X
Weight & PS	X	X					X					Every 12 weeks	Every 12 weeks	X
Height	X													
Concurrent meds	X	X					X					Every 12 weeks	Every 12 weeks	
Metastatic disease biopsy	X													
Serum PSA[a]	X	X					X					Every 4 weeks	Every 4 weeks	X
CBC w/diff, plts[b]	X	X	X	X			X		X[b]			Every 12 weeks	Every 4 weeks	X
Serum chemistry[c]	X	X	X	X			X					Every 12 weeks	Every 4 weeks	X
PT/PTT/INR	X													
Correlative Blood Samples for circulating tumor DNA and CTC analysis[d]		X					X[d]					X[d]	X[d]	X[d]
EKG [e]	X						X[e]							
Archived Tissues	X													
Adverse event evaluation		X----------				----------X						X	X	X
Tumor measurement	X	Measured after 28 weeks +/- 1 week										Measure every 24 weeks(+/- 1 week) for 2 years then annually unless clinically indicated		X
Radiologic evaluation [f]	X													X

a: For patients who are initiated on ADT prior to registration, pre androgen deprivation PSA should be recorded.
b: Arm 2 may consider adding additional interim CBCPD during additional cycles if clinically indicated. Arm 1 patients do not need repeat CBC during any cycle unless medically indicated.
c: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium, magnesium. Arm 1 patients do not need repeat Chemistry during cycle 1 unless medically indicated.
d: Pretreatment, week 12, after 28 weeks of therapy, and at progression/or coming off protocol treatment.
e: EKG should be done on day 1 of cycle 2 for arm 2 patients. If there is no abnormality, then no additional EKG's are needed. If abnormal, then monthly EKG for 7 cycles can be performed. Arm 1 patients do not need repeat EKG unless clinically indicated
f. Include bone scan, CT/MRI abdomen and pelvis, CT/MRI chest or CXR as appropriate. All disease sites must be reassessed.
g: Follow-up for patients taken off treatment for reasons other than progression will be done every 12 weeks. These visits will include H&P and labs as outlined in subsequent cycle arm 1 column. Imaging will be done q 24 weeks. Clinical, Lab and imaging will be done for 2 years or until progression whichever comes first.

h: For patients coming every 12 weeks appropriate supply of palbociclib can be provided per institutional guidelines and careful instructions regarding the dosing.

A cycle is 28 days. For both arms scans to assess disease will be performed after 28 weeks calculated from "Day 1" of protocol. Return visits after initiation of therapy can be done within ± 4 days from actual due date. Lab draws for Arm 2 (CBC w/diff, plts and Serum chemistry) on Cycle 1 (days 8 and 15), and Cycles 2-7 (D15) can be done ± one day.

11. MEASUREMENT OF EFFECT

- For the purposes of this study, patients will have a PSA evaluation at the start of each new cycle with primary endpoint assessment after 28 weeks of therapy. Radiologic response will be determined after 28 weeks plus or minus 1 week (or as prompted by symptoms) and every 24 weeks plus or minus 1 week thereafter while on study for 2 years, then annually and otherwise if clinically indicated.

- Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1).[41] Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with ADT with or without palbociclib.

Evaluable for primary endpoint. All patients randomized to either Arm 1 or Arm 2 and receive at least one cycle of therapy or are removed from treatment due to toxicity are evaluable. Patients who are randomized but do not complete 1 cycle of treatment due to reasons other than toxicity will be replaced.

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

Evaluable non-target disease response. Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

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

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. Decision should be made in consultation with primary investigator.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, **a lymph node must be $\geq$15 mm in short axis when assessed by CT scan** (CT

scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

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

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

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

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

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

11.2 Response and Progression Criteria

11.2.1 Outcomes based on post-therapy PSA changes

- Primary Endpoint will be stable or declining PSA ≤ 4ng/mL after 28 weeks of therapy

- Secondary Endpoint will be rate of undetectable PSA after 28 weeks of therapy (≤0.2ng/mL)

- These definitions are intended to characterize the PSA changes on study for the purpose of reporting of results.

  Complete Response (CR): Undetectable PSA (≤0.2 ng/ml) that is confirmed by another PSA level at no less than 4 weeks interval.

  Partial Response (PR): PSA level between 0.2ng/ml and 4ng/ml..

  Stabilization (SD): Patients who do not meet the criteria for PR or PD for at least 90 days on study (2 cycles of treatment) will be considered stable

  Progression (PD): 25% increase over baseline or nadir whichever is lower and an increase in the absolute value of PSA level by 2 ng/ml that is confirmed by another PSA level at no less than 4 weeks interval.

11.2.2 Outcomes based on evaluation of target lesions

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

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

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

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

11.2.3 Evaluation of Non-Target Lesions

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

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

Progressive Disease (PD): Appearance of one or more new lesions and/or

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

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

11.2.4 Outcomes based on Radionuclide bone scans

The subjectivity in interpreting serial changes in a radionuclide bone scan is well recognized. Thus, the primary outcome will be whether the scan is stable or improved, vs. worse or progression. Changes in intensity will not be used as an outcome measure.

Stable or Improved: A stable or improved classification requires that no new lesions appear or that new pain has not developed in an area that was previously visualized.

Progression (Non-Response): Appearance of **two or more** new skeletal lesions. *An increase in the size or intensity of known skeletal lesions will not be considered progression*.

11.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 6 weeks before registration.

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

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

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

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT

scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

Bone disease: Bone disease will be evaluated using Radionuclide bone scan.

11.4 Progression-Free Survival:

Progression-free survival (PFS) is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

11.5 Evaluation of Best Overall Response

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

For Patients with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	**Non-Target Lesions**	**New Lesions**	**Overall Response**	**Best Overall Response when Confirmation is Required***
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	

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

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

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

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

For Patients with Non -Measurable Disease (*i.e.*, Non Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

11.6 Duration of Response

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

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

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

12. DATA MANAGEMENT AND MONITORING

12.1 Data and Safety Monitoring Procedures

The Data and Safety Monitoring Board (DSMB) of The University of Michigan Comprehensive Cancer Center (UMCCC) is the DSMB for this study. This committee is responsible for monitoring the safety and data integrity of the trial.

Each participating site is required to have its own Data and Safety Monitoring Committee (DSMC) for the study. This committee will be composed of the local site principal investigator, site co-investigator(s), site data manager or study coordinator and other members of the study staff involved in the conduct of the trial. During the committee's quarterly meeting, the site principal investigator will discuss matters related to:

- Enrollment rate relative to expectations, characteristics of participants
- Safety of study participants (Serious Adverse Event & Adverse Event reporting)
- Adherence to protocol (protocol deviations)
- Completeness, validity and integrity of study data
- Retention of study participants

These meetings are to be documented by the site data manager or study coordinator using the Protocol Specific Data and Safety Monitoring Report (DSMR), signed by the site principal investigator or co-investigator. Each site is required to submit the completed DSMR to the Multi-Site Coordinator at the University of Michigan Clinical Trials Office on a quarterly basis together with other pertinent documents.

Similarly, protocol deviations are to be documented using the Notice of Protocol Deviation Form and requires the signatures of both the sites data manager or study coordinator and the site principal investigator or co-investigator. These reports are to be sent to the University of Michigan Clinical Trials Office within 7 calendar days of awareness of the event and on a quarterly basis with the Protocol Specific Data and Safety Monitoring Report.

The Clinical Trials Office is responsible for collating all the Data and Safety Monitoring Reports from all the participating sites, and providing the information to the Data Safety Monitoring Board.

12.2 Clinical Monitoring Procedures

Clinical studies coordinated by The University of Michigan Comprehensive Cancer Center (UMCCC) must be conducted in accordance with the ethical principles that are consistent with Good Clinical Practices (GCP) and in compliance with other applicable regulatory requirements. Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 8.0 (Adverse Events: List and Reporting Requirements).

This study will be monitored by a representative of the Clinical Trials Office (CTO) of the UMCCC. Monitoring visits will be made during the conduct of the study and at study close-out.

Prior to subject recruitment, a participating site will undergo site initiation meeting to be conducted by the Clinical Trials Office. This will be done as an actual site visit; teleconference, videoconference, or web-based meeting after the site has been given access to the study database and assembled a study reference binder. The site's principal investigator and his study staff should make every effort in attending the site initiation meeting. Study–related questions or issues identified during the site initiation meeting will be followed-up by the appropriate UMCCC personnel until they have been answered and resolved.

Monitoring of this study will include both 'Centralized Monitoring', the review of source documents at the CTO and 'On-site Monitoring', an actual site visit. The first 'Centralized' visit should occur after the first subject enrolled completes the first treatment cycle**.** The study site will send the de-identified source documents to the CTO for monitoring. 'Centralized' monitoring may be requested by the CTO if an amendment requires changes to the protocol procedures. The site will send in pertinent de-identified source documents, as defined by the CTO for monitoring.

The first annual 'On-site' monitoring visit should occur after the first five study participants are enrolled or twelve months after a study opens, whichever occurs first. The annual visit may be conducted as a 'Centralized' visit if less than three subjects have enrolled at the study site. The type of visit is at the discretion of the CTO. At a minimum, a routine monitoring visit will be done at least once a year, or once during the course of the study if the study duration is less than 12 months. The purpose of these visits is to verify:

- Adherence to the protocol
- Completeness and accuracy of study data and samples collected
- Proper storage, dispensing and inventory of study medication
- Compliance with regulations

During a monitoring visit to a site, access to relevant hospital and clinical records must be given by the site investigator to the CTO representative conducting the monitoring visit to verify consistency of data collected on the CRFs with the original source data. While most patient cases will be selected from patients accrued since the previous monitoring visit, any patient case has the potential for review. At least one or more unannounced cases will be reviewed, if the total accruals warrant selection of unannounced cases.

The Clinical Trials Office expects the relevant investigational staff to be available to facilitate the conduct of the visit, that source documents are available at the time of the visit, and that a suitable environment will be provided for review of study-related documents. Any issues identified during these visits will be communicated to the site and are expected to be resolved by the site in a timely manner. For review of study-related documents at the CTO, the site will be required to ship or fax documents to be reviewed.

Participating site will also undergo a site close-out upon completion, termination or cancellation of a study to ensure fulfillment of study obligations during the conduct of the study, and that the site Investigator is aware of his/her ongoing responsibilities. In general, a site close-out is

conducted during a site visit; however, site close-out can occur without a site visit if all of the following apply:

- No patient has signed the Informed Consent Form and has enrolled into the study
- Investigational agent has not been dispensed
- All investigational agent and materials have been returned as defined for the study or destroyed and accounted for properly.

12.3 Quality Assurance and Audits

The Data Safety Monitoring Board can request a 'for cause' audit of the trial if the board identifies a need for a more rigorous evaluation of study-related issues. A "for cause" audit would be conducted by the Quality Assurance Review Committee (QARC) of the University of Michigan Comprehensive Cancer Center.

A regulatory authority (e.g., FDA) may also wish to conduct an inspection of the study, during its conduct or even after its completion. If an inspection has been requested by a regulatory authority, the site investigator must immediately inform the Clinical Trials Office that such a request has been made.

13. STATISTICAL CONSIDERATIONS

13.1 Statistical Design

The primary analysis will be assessment of the proportion of patients who achieve a PSA $\leq$ 4ng/mL after 28 weeks of treatment in each arm. For purposes of the primary endpoint, day 1 will be the randomization date for patients with pre-registration ADT treatment and the treatment start date for patients' naïve to ADT at registration. The difference in proportions will be reported with the mid p-value test of the 2X2 table with a one-sided type I error of 10%. The binomial proportions with the corresponding Wilson score 90% binomial confidence intervals will also be reported by arm.

The primary endpoint of the study is to compare the proportion of patients who have a PSA $\leq$ 4ng/mL after 28 weeks of protocol treatment between patients stratified by pre-registration ADT treatment and extent of disease and randomized to combined androgen deprivation (ADT: LHRH agonist + bicalutamide) and those randomized to combined ADT + palbociclib. ADT alone (Arm 1) is expected to result in 70% of patients achieving PSA $\leq$ 4ng/mL after seven months of protocol treatment. It is hypothesized that the ADT + palbociclib arm (Arm 2) will have a 20% absolute increase in proportion to 90% (H_A: $p_2 - p_1 > 0$). The null hypothesis is no difference in proportions (H_0: $p_2 - p_1 \leq 0$). Patients will be randomized using a 1:2 (Arm 1: Arm 2) schema stratified by extent of disease. Up to 95 patients may need to be registered (enrolled) in order to obtain the 60 evaluable patients. All patients randomized will be evaluable if they receive 1 cycle of treatment or are removed during treatment due to toxicity. Patients who are randomized but do not complete 1 cycle of treatment due to reasons other than toxicity will be replaced. With 20 evaluable patients on ADT alone and 40 evaluable patients on combination ADT + palbociclib, there is 64.2% power to

detect a 20% difference in proportions with a one-sided type I error of 0.10 using the mid p-value method of the Fisher's exact test.

With regard to power of the study, the focus of this trial is feasibility and to detect a signal, as described by our aims for this small targeted early phase II trial. The compromise of the power was done because of the overwhelming benefit to include a control population since there is no data on the rate of PSA declines after 28 weeks of ADT in the targeted RB positive population. The plan is to use the mid p-value test as it is a very close approximation to the one margin condition and is more powerful compared to the Fisher's exact test, which is very conservative due to conditioning on both margins.[42]

13.2 Analysis Plan

The primary analysis will be assessment of the proportion of patients who achieve a PSA $\leq$ 4ng/mL after 28 weeks of treatment in each arm. The difference in proportions will be reported with the mid p-value method of the Fisher's exact test of the 2X2 table. The binomial proportions with the corresponding Wilson score 90% binomial confidence intervals will also be reported by arm.

Secondary endpoints include safety and tolerability. Frequency of adverse event types will be reported for each arm by attribution and grade. Duration of therapy, dose modifications and treatment delays will be reported to describe tolerability within each arm.

The proportion of patients who achieve undetectable PSA (<0.2ng/mL), PSA response, and radiographic response will be reported by arm using methods described in the primary analysis. Biochemical progression-free survival will begin from treatment start until the event of biochemical (PSA) progression (section 11.2) or death, whichever occurs first. Patients who do not have either event will be censored at their last known PSA date. Clinical progression-free survival will begin from treatment start until the event of clinical progression or death, whichever occurs first. Patients who do not have either event will be censored at the date of their last known clinical measure. Biochemical PFS and clinical PFS will be described by arm using Kaplan-Meier methods and log-rank p-values will be reported.

13.3 Correlative Endpoints Analyses

13.3.1 To determine whether cyclin D1, Cyclin D1B, Cyclin D3, p16, CDK4, Cyclin A or MCM7 levels in prebiopsy tissue as measured by IHC predict a subset of tumors responsive to palbociclib or overall response rates, logistic models will be used. Logistic models including one marker in each model and response as the outcome will be performed. The models for response rates will explore the one marker, treatment group, and the interaction of marker and treatment group to determine if the marker is associated with response by palbociclib. Models of response rates with each marker without treatment group will be explored if treatment associations are not found. Exploration using multiple markers may be investigated using logistic modeling if there is indication that more than one marker

is associated with response. This is an exploratory aim for discovery so multiple testing control will not be included.

13.3.2 Concordance in RB status will be described between primary tumor, and metastases within patients using a generalized kappa. Pairwise concordance will be described with proportions, McNemar's test and kappa statistic.

13.3.3 To evaluate targeted sequencing and gene expression biomarkers that predict response to palbociclib, patients will be classified as responders or non-responders and individual biomarkers will be assessed for associations with response by Fisher's exact tests or logistic regression. A comparison of the results for the subset of markers tested in 13.3.1 will be reported. Similar approaches will be used with transcriptome/exome sequencing data.

13.3.4 The number of and heterogeneity in circulating tumor cells at baseline, during treatment, and after treatment will be described by treatment arm. Analyses will be exploratory. Models may be used to explore baseline CTCs enumeration and molecular characterization to predict response.

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APPENDIX A

Performance Status Criteria

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

APPENDIX B

Standard Operating Procedures for CT-guided Biopsy of Metastatic Prostate Cancer Lesion in Bone

Patients will be scheduled for a CT-guided biopsy of a bone or soft tissue abnormality consistent with prostate metastasis. The site of biopsy will be determined after review of available radiographs, CT, MRI, and/or bone scan (where applicable). The decision regarding which metastatic lesion to biopsy should depend on three main factors:

1) Size of lesion (with preference towards the largest lesion)
2) Safety (with obvious preference towards least risk to the patient)
3) For bone lesions, the intensity of lesion activity on bone scan (with preference towards the most intense activity)

Verbal and written informed consent will be obtained after discussion of risks (bleeding, infection, soft tissue and bone injury, inconclusive biopsy results) of the procedure. Standard laboratory tests to assess coagulation and bleeding will also be completed prior to the procedure. Conscious sedation will be used as necessary using intravenous Fentanyl and Midazolam with appropriate nursing support. In order to ensure adequate tumor sample as long as it is deemed safe and based on accessibility, target lesion size, specimen integrity, specimen appearance, intra-procedural bleeding, tumor biopsy should attempt to obtain 2-8 bone biopsy samples in addition to possible bone marrow aspirate, to be determined at the time of biopsy.

The patient will be initially scanned with a clinically-available multislice CT scanner for the purpose of biopsy planning. Biopsies will be performed using sterile technique and lidocaine local anesthetic. For bone lesions, a clinically-available 11-to-14 gauge bone cutting needle, with or without a co-axial trocar, will be used to obtain **two to eight** biopsy samples of the selected lesion. Options for obtaining multiple samples of the biopsy can include:

a) Sequential advancement of needle passes through a fixed trocar, such that each needle pass progresses more deeply into the bone lesion
b) Re-insertion and re-direction of the needle and/or trocar through another region of the bone lesion
c) Aspiration of the lesion will be attempted in conjunction with biopsy, by attaching a 20 cc sterile syringe to the cannula of the biopsy needle and aspirating into this syringe, with repeat aspirations as needed.

At the completion of the procedure, CT imaging will be repeated to assess for immediate complications. The patient will be observed for 2 – 4 hours before discharge.

APPENDIX C

Standard Operating Procedures for CT-guided Biopsy of Metastatic Prostate Cancer Lesion in Soft Tissue

The patient's imaging will be reviewed and all soft tissue or nodal lesions deemed suspicious for metastasis will be considered. A target for sampling will be selected based on: 1) perceived percutaneous access with respect to safety and technical feasibility, and 2) suspected likelihood of obtaining malignant tissue. In the cases where both bone and soft tissue lesions are safely accessible and have high likelihoods of containing malignant tissue, it would be preferable to biopsy the soft tissue lesion.

- For lymph nodes or soft tissue lesions a CT scan will be utilized. An ultrasound may be utilized, provided that the target lymph node or soft tissue mass is visible and accessible.

- In order to ensure adequate tumor sample as long as it is deemed safe and based on accessibility, target lesion size, specimen integrity, specimen appearance, intra-procedural bleeding, tumor biopsy should attempt to obtain:
    - at least 6 cores if obtaining a 1-cm-long 18-gauge core specimen.
      or
    - at least 4 cores if obtaining a 2-cm-long 18-gauge core specimen. The needle should be directed into the center of the lesion.

Options for obtaining multiple samples include similar principles as described for bone lesions, including sequential advancement through the lesion, re-insertion/re-direction, or aspiration where appropriate.

Mitigating factors may include but are not limited to: 1) safety of obtaining multiple core specimens, 2) friability of the core specimens on visual inspection, 3) target size, 4) perceived likelihood of obtaining additional diagnostic tissue by continued sampling. After biopsy is performed, the biopsy would be snap frozen, as described in Appendix E. No aspirate will be performed for soft tissue biopsies.

Appendix D: Standard Operating Procedure (SOP) for Handling Samples

1.0 PURPOSE

To standardize the method for handling frozen needle tumor biopsies, to optimize specimen use for molecular analyses.

2.0 MATERIALS AND EQUIPMENT REQUIRED

2.1 Sample kit containing:

Dual sample kit will be sent to each enrolling site immediately following patient registration

A) Cell Free DNA 2 tubes

B) Sheet for recording biopsy details

This sample kit will be sent to each enrolling site immediately following patient registration.

2.2 Other materials which will be sent to each enrolling site at the initiation of the clinical trial (to be kept for use over serial cases):

a) One box Tissue Tek™ cryomolds
b) Large bottle of Tissue Tek™ OCT
c) Blue Pads, Markers
d) Sterile tweezers
e) Shipping labels
f) A supply of additional Cell-Free DNA tubes
g) Boxes for shipment of Cell-Free DNA samples (see Appendix F)

2.3 Materials that will be provided by the enrolling site:

a) Dry ice
b) -80°C freezer (particularly in the case of Friday biopsies)

3.0 OPERATING PROCEDURES

3.1 At least 24 hours prior to the biopsy, the research coordinator is to notify the institutional research team involved in this protocol, of the scheduled sample collections. A laboratory technician should pick up the sample kit (see 2.1 above), and prepare cryomolds prior to the biopsy, by labeling them, using the provided alcohol-proof marker, with the following information:

Clinical protocol number
Specimen ID
Biopsy time and Date

The research coordinator and the laboratory technician should also confirm, on the day prior to biopsy, the availability of materials needed for handling the biopsies, as specified in Section 2 above.

3.2 The laboratory technician should arrive at the biopsy collection site at least 15 min ahead of the scheduled biopsy to allow sufficient time to set up laboratory supplies and ensure rapid

transport of specimens to the laboratory after collection. He or she should also re-confirm, at that time, the availability of all specimens from Section 2. He should also, immediately before the biopsy, fill the insulated bucket with dry ice and isopentane.

3.3 Immediately after the biopsy is performed, the freshly collected specimen should be placed in the cryomold. A single drop of Tissue Tek™ OCT should be placed on the specimen, and the sterile tweezers should be used to gently hold one end of the freshly collected needle biopsy and to push the biopsy to the bottom of the cryomold cassette with forceps. Make sure biopsy is as flat as possible. The cryomold should then be filled with OCT, and the cryomold should be immediately placed in direct contact with the dry ice/isopentane cocktail until the bottom of the OCT freezes and turns white. Only the bottom of the cryomold should contact the dry ice/isopentane--none of the dry ice/isopentane should spill inside the cryomold itself and contact the specimen.

This process can be repeated using separate cryomold cassettes for separate biopsy samples.

3.4 Once frozen, place cryomolds on dry ice for transport. The used isopentane should be poured back into its bottle using funnel.

3.5 For bone lesions, an aspirate of the site of bone biopsy may be performed, as described in Section C, using a 20 cc sterile syringe. The contents of this syringe (clotted aspirate) should be transferred into a 1.7 mL eppendorf tube. Several drops of Tissue Tek™ OCT should be added to the tube, such that the clotted aspirate is covered by OCT. The eppendorf tube should then be closed, and placed on dry ice for snap freezing.

3.6 The cryopreserved biopsy and aspirate specimen(s) should be stored at -80°C until shipment. Ideally, shipment should occur on the same day as the biopsy, unless the biopsy occurs on a Friday, in which case the specimen should be preserved at -80°C until shipment on the following Monday.

4. Quality Assurance Process

After completion of each biopsy, the following information should be recorded on the information sheet shipped with each sample kit:

<u>Biopsy collection</u>

Date:
Specimen ID:
Time guide needle placement confirmed:
Needle Type:
Needle diameter: gauge; and length: cm
Time biopsy needle introduced:
Time biopsy snap-frozen on dry ice:
Number of specimens:
Time aspirate performed (for bone lesions):
Time aspirate snap-frozen on dry ice:
Date/time of biopsy specimen(s) (and aspirate specimens, if applicable) placed at -80°C:
Date/time of biopsy specimen(s) (and aspirate specimens, if applicable) shipped:
Notes, including any deviations from the standard operating procedure:

Appendix E: Standard Operating Procedure for Shipment of non-CTC Clinical Samples

I. Preparing for Shipment

A. Send an e-mail to Siddiqui@med.umich.edu prior to shipping to advise recipient of scheduled shipping time.

B. Generate a shipping list recording the number of samples being shipped, type of sample (frozen biopsy, blood, or archived formalin-fixed biopsy), and clinical protocol number of the patient

C. Make sure that the following packing materials are available:

- Cardboard shipping box with Styrofoam insert.
  --Shipper Boxes 13.38L x 9.25W x 6 in.D; Outside Dimensions: 15.5L x 11.5W x 8 in.H; Wall Thickness: 1 in. Fischer Scientific Catalog # 03-528-27
- Dry ice (amount varies depending on size of Styrofoam insert; usually about 15 kg's)
- 6 in x 6 in cardboard specimen box, with fiberboard box dividers- holds 81, 16mm vials
  --Revco Fiberboard Storage Boxes, 12/PAK
  --Revco Fiberboard Box Dividers, Holds 81 16mm vials, 12/PAK
  Fischer Scientific Catalog # 11-678-24A, 13-989-218 (resp.)
- Plastic bag (to go over the cardboard box –in case of leakage)
- Absorbent strip (to go inside the plastic bag that the boxes are in; can soak up to 250 cc of liquid)
  --Absorbent Strips 250/Cs
  --Fischer Scientific Catalog # NC9193000
- UN 3373 label (2 in x 2 in)
  --Labelmaster UN3373 Labels > 2W x 2.75 In. L
  --Labels; Biological Substance; 2W x 2.75 In. L; Paper; 500/RL
  Fischer Scientific Catalog # NC9493045
- Dry Ice Label with designated UN 1845 diamond (can also be ordered from Fischer Scientific)
  --5 1/2 x 5 1/2 in.; complies with DOT (49 CFR 173.217) and IATA (Packing Instructions 904)
  --Fischer Scientific Catalog #22-130-065
- Packing tape

II. Packaging the specimens

1. Pull sample tubes from temporary storage freezers and place in dry ice, make sure the labels are securely placed on the tube and tubes properly labeled and easy to read.
2. Ensure that caps are tightly secured and place in 6 in x 6 in cardboard specimen box (which should be kept on dry ice during this transfer).
3. Be sure to **check off** EACH specimen being shipped and verify the contents of the package match the shipping list
4. Label the top of the cardboard specimen box with number of samples; clinical protocol/specimen number, and contact information of sender, as well as contact of receiver.

5. Cover the specimen box, and either tape the sides down or place a rubber band around the box, to ensure that the cover will not come off.
6. Place inside the plastic Ziploc bag with the absorbent strip and seal. Keep on dry ice until ready to transfer to the larger shipping box. (can fit two of these specimen boxes in a shipper box that is the designated size above)
7. Fill the shipper box about half way with dry ice (about 1-2 inches in height) and place the specimen box inside with shipper.
8. Add more dry ice to cover the specimen boxes. (about 1 or 2 inches in height again and 1-2 inches on the sides the boxes as well). Note: there should be sufficient dry ice to maintain the samples at -20°C for at least 72 h.
9. DO NOT place dry ice inside the specimen boxes or inside the plastic bag.
10. Close the Styrofoam box, and tape the packing list to the top. DO NOT seal the Styrofoam box with tape.
11. Ship the specimens with a copy of the shipping list and the completed quality assurance record (see Appendix D) for all specimens. Retain copies of the completed shipping list and quality assurance record in your records.
12. Seal the shipping box by taping the flaps of the insulated box along the top edges.
13. At this point, you should check that your final packaging consists of three components (triple packaging):
    (a) a primary receptacles (the specimen tubes)
    (b) a secondary packaging (6 in x 6 in cardboard specimen box)
    (c) a rigid outer packaging (larger cardboard shipping box)
14. Should you decide to use alternative packaging materials instead of those listed above, please note the following requirements:
    The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packagings must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging. The primary container (i.e. test tube) must be leak proof, as should the secondary container. The outer packaging (tertiary container) does not have to be leak proof. Packaging must comply with the IATA packing instructions 650 which are summarized below; for further questions, please refer to the IATA website:
    http://www.iata.org/SiteCollectionDocuments/DGR51_PI650_EN.pdf

III. <u>Labelling and sending the shipping box</u>

A. Fill out the black and white dry ice label to reflect the weight of the dry ice in the box, note 2lbs= 1kg. Place completed black and white dry ice label on the shipping box

B. All shipping boxes must be affixed with two labels:
    "DIAGNOSTIC SPECIMENS PACKED IN COMPLIANCE WITH IATA PACKING INSTRUCTION 650"
    and
    "UN 3373 Biological Substance Category B"

C. Complete the air bill online and attach packing labels to the outside of the shipper. Schedule a pick up time and ship overnight to:

Javed Siddiqui, MS, MT(ASCP, CLsp (MB))
Michigan Center for Translational Pathology
Room 1138
2900 Huron Parkway Suite 100
Ann Arbor, MI 48105
Phone: (734) 232-0829
Fax: (734) 232-0805
siddiqui@med.umich.edu

D. All shipping boxes are required to ship out Priority Overnight (or another comparable shipping service that results in delivery by 10:30 AM the following day).

E. Biopsies on Monday through Thursday should be shipped on the same day as the biopsy. Biopsies on Friday should be stored at -80 degrees Celsius until the following Monday, and then should be shipped via Priority Overnight (or a comparable service).

F. The sender would need to complete an "air waybill" form

G. All diagnostic or investigational specimens shipped on this trial should be classified as “Diagnostic Specimens” not “Infectious Substances” when shipping via a standard carrier. (i.e. UPS, FED-EX, DHL/AIRBORNE). Diagnostic Specimens DO NOT require a Shipper’s Declaration of Dangerous Goods form.

IV. Questions

For any questions regarding shipping, please contact Javed Siddiqui at siddiqui@med.umich.edu or phone: (734) 232-0829.

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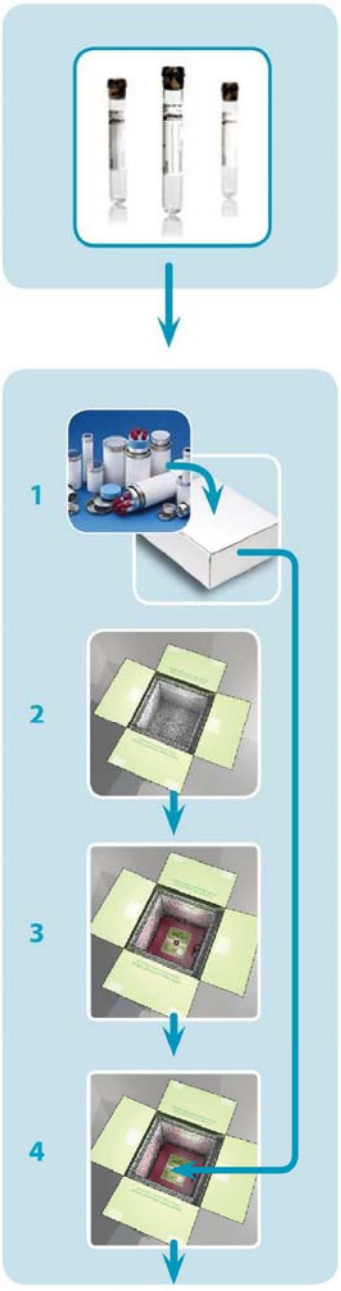
BLOOD SPECIMEN COLLECTION AND SHIPPING INSTRUCTIONS



Blood Specimen Collection

- Collect blood in one Cell-Free DNA blood collection tube and invert twice after the blood draw to mix the anti-coagulatant
- Be sure each tube is at least half way filled or cell preservative will not work optimally
- Keep blood tubes at room temperature with no agitation until shipment

Blood Specimen Shipment

1 Place blood tube(s) into foam insert and place in aluminum canister. Place canister into supplied cardboard box

2 Open pre-assembled reusable specimen shipping container, and remove insulation panel

3 Place two liquid (red) E23 panels that have been stored at room temperature in the bottom of the shipping container rotated 90 degrees from each other

4 Place box containing canister with blood specimen(s) directly on top of previously placed panel

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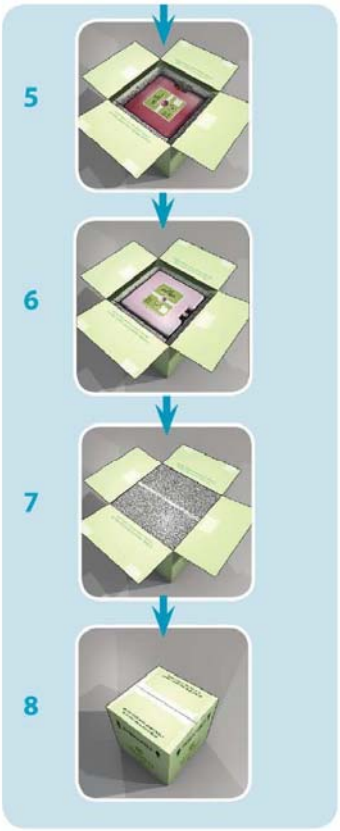

5 Place one liquid (red) E23 panel that has been stored at room temperature on top of the box rotated at 90 degrees from the panel below it

6 Place one solid (red) E23 panel that has been stored at 4°C on top of box

7 Place locking lid on top of the 4th panel with the seam facing up to ensure insulation

8 Close shipping box and tape appropriately

9 **Ship to:**

Epic Sciences
c/o Dena Marrinucci
9381 Judicial Drive
Suite 200
San Diego, CA 92121
tel: 858.356.6610

Samples may be mailed to Epic Sciences for Monday–Friday delivery

10 **Send Email to:**

partners@epicsciences.com

In the Email Include:

1.
2. Tracking number
3. The number of samples being shipped
4. The date and time of each blood draw
5. Case report form including white blood cell count of patient(s)

Appendix G
PATIENT'S MEDICATION DIARY

Today's date _____ **Agent: Palbociclib**

Patient Name_____(*initials acceptable)* **Patient Study ID** _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each cycle of treatment.
2. You will take **Palbociclib**
   Dose:
   ___75 mg capsules
   ___ 100 mg capsules
   ___ 125 mg capsules

   Take Palbociclib daily, days 1-21. Take with food. Do not crush, break, or chew it.
3. Record the date, the number of tablets of each size of tablet that you took, and when you took them.
4. If you have any comments or notice any side effects, please record them in the Comments column.
5. Please bring this form, bottles and **extra tablets** when you return for each appointment.
6. Please record missed or skipped doses. Do not share your study drug supply, and wash your hands after touching the pills

Day	Date	Time of Dose	# of capsules taken	Comments
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				

Patient's signature_____

*****FOR CLINIC STAFF USE ONLY*****

STUDY STAFF SIGNATURE:		# of capsules dispensed to patient:	
DATE:		# of capsules returned:	

Appendix H
PATIENT'S MEDICATION DIARY

Today's date _____ **Agent: Bicalutamide**

Patient Name_____(*initials acceptable)* **Patient Study ID** _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each cycle of treatment.
2. You will take **Bicalutamide**
   Dose: 50mg
   Take Bicalutamide daily every day of the month. Do not crush, break, or chew it.
3. Record the date and when you took it.
4. If you have any comments or notice any side effects, please record them in the Comments column.
5. Please bring this form, bottles and **extra tablets** when you return for each appointment.
6. Please record missed or skipped doses. Do not share your study drug supply, and wash your hands after touching the pills

Day	Date	Time of dose	# of tablets taken	Comments
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28				

Patient's signature_____

STUDY STAFF SIGNATURE:	
DATE:	