Document Coversheet

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Phase Ib/II Study of Enzalutamide with Venetoclax (ABT-199) in Patients with Metastatic Castrate Resistant Prostate Cancer (mCRPC)

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1 OBJECTIVES

The purpose of this study is to evaluate the safety, efficacy, and pharmacokinetics of treatment with Enzalutamide and Venetoclax in patients with metastatic castrate resistant prostate cancer.

1.1 Phase 1b

1.1.1 Primary Objectives

- To determine the dose-limiting toxicity (DLT), maximum tolerated dose (MTD), and recommended Phase II dose (RP2D) of enzalutamide in combination with venetoclax in patients with metastatic castrate resistant prostate cancer (mCRPC).
- To characterize the safety and tolerability profile of enzalutamide in combination with venetoclax in patients with metastatic castrate resistant prostate cancer (mCRPC).

1.2 Phase II

1.2.1 Primary Objective

- To evaluate the efficacy of venetoclax in combination with enzalutamide in patients with metastatic castrate resistant prostate cancer (mCRPC) that are enzalutamide-naïve, as measured by 12-month progression free survival rate.

1.2.2 Secondary Objectives

- To assess PSA50 response as well as circulating tumor cell (CTC) response in patients who received venetoclax + enzalutamide.
- To estimate the proportion of patients who received venetoclax + enzalutamide and remain radiographic progression free at 6 months.
- To estimate the time to first skeletal-related event (SRE) and the time to first symptomatic skeletal event (SSE).
- To estimate the time to clinical progression.
- To estimate the time to initiation of new systemic treatment for prostate cancer
- Assess the effect of venetoclax + enzalutamide on overall survival.

1.3 Pharmacokinetic Objective

- The pharmacokinetic (PK) objective for this study is to characterize the PK profiles of enzalutamide and venetoclax when given in combination to patients with metastatic castrate resistant prostate cancer (mCRPC). As PK data are generated, and at the latest before starting the Phase II portion of this trial, exposure metrics for venetoclax and enzalutamide will be evaluated and compared with historical data.
- Comprehensive analyses of venetoclax levels to assure that they are in the therapeutic range.

1.4 Exploratory Biomarker Objectives

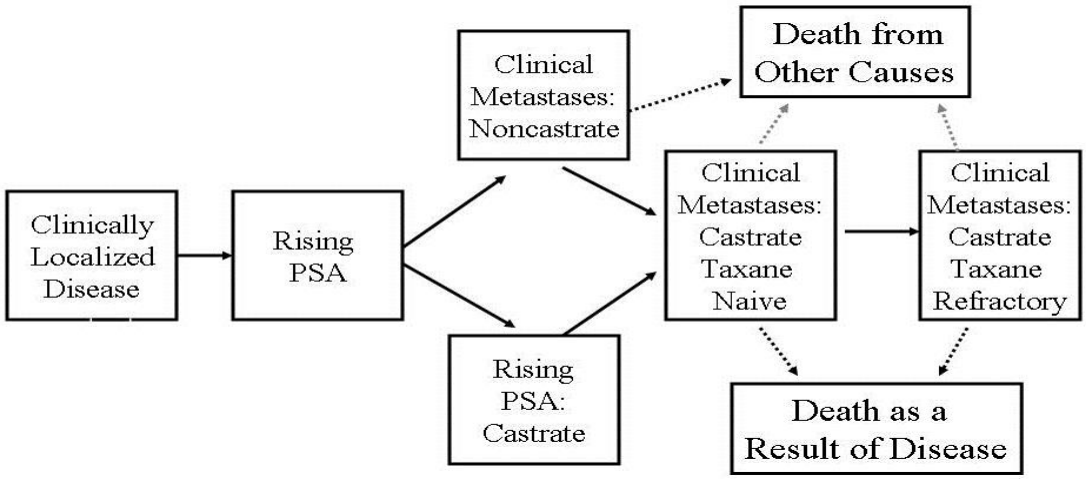
The exploratory biomarker objective for this study is to identify non-inherited biomarkers that are predictive of response to study treatment (i.e., predictive biomarkers), are associated with progression to a more severe disease state (i.e., prognostic biomarkers), are associated with acquired resistance to study treatment, are associated with susceptibility to developing adverse events, can provide evidence of study treatment activity, can increase the knowledge and understanding of prostate cancer biology or study treatment mechanism of action, or can contribute to improvement of diagnostic assays.

2 BACKGROUND

2.1 Disease Background

Prostate cancer is the second leading cause of cancer deaths in men in the US.[1] According to American Cancer Society estimates for 2018 as many as 164,690 American men will be diagnosed with prostate cancer, and nearly 29,430 will die of the disease.[1] The course of prostate cancer from diagnosis to death is best categorized as a series of clinical states (**Figure 1**).[2] These clinical states involve the complex interplay of a network of signaling molecules that collectively promote net cell proliferation relative to cell death.

Figure 1: Clinical States of Prostate Cancer



Androgen deprivation therapy with LHRH analogs is the standard of care first line treatment for metastatic prostate cancer. However, most patients despite initial response notice disease progression after median time of 18-24 months developing castration resistant prostate cancer (CRPC), defined as progression through suppression of gonadal androgens.[3] Treatment options for patients with castrate resistant prostate cancer (CRPC) are limited and until recently only docetaxel was shown to improve survival. In the last several years five new agents; abiraterone acetate, cabazitaxel, enzalutamide, radium-223 and sipuleucel-T were approved for use in CRPC.[4-8] However, the optimal sequencing of these agents is unknown. Moreover, all patients with CRPC are treated identically and there is no means of identifying which patient population

would benefit from a particular agent. Furthermore, irrespective of the sequencing of the above agents, metastatic CRPC is invariably fatal, with the median **survival being around 3 years**.

2.2 Treatment Background

Standard treatment approach after primary androgen ablation therapy

Over the past decade, second-generation androgen-targeted agents have demonstrated meaningful clinical benefit in men with CRPC. These agents include inhibitors of testosterone synthesis via CYP17A1, such as abiraterone and TAK-700, as well as competitive antagonists of testosterone at the androgen receptor (AR), including enzalutamide and apalutamide.

Enzalutamide inhibits androgen receptor (AR) signaling in three distinct ways: it inhibits 1) testosterone binding to ARs; 2) nuclear translocation of ARs; and 3) DNA binding and activation by ARs. Based on the AFFIRM trial, enzalutamide was approved by the FDA in 2012, and by the EMA in 2013, for the treatment of men with mCRPC who have progressed on or after first-line docetaxel chemotherapy.[9] Recently, Beer and colleagues (PREVAIL phase III trial) have shown that enzalutamide significantly reduced the risk for radiographic progression, delayed initiation of chemotherapy and improved overall survival (OS) in men with CRPC that have not received prior chemotherapy.[10] In September 2014, the U.S. Food and Drug Administration (FDA) expanded the use of enzalutamide to include men with CRPC that have not received prior chemotherapy. Thus, systemic enzalutamide therapy has become the standard of care for men with mCRPC, and since there is no need for systemic steroids, it is expected to be the predominant agent used in this pre-chemotherapy setting. In the PREVAIL study, enzalutamide had a beneficial effect on PSA response rate, time to PSA progression, time to initiation of chemotherapy, and overall survival. Although the magnitude of these treatment effects varied based on the characteristics of the enrolled populations, the time to PSA progression was approximately 11 to 12 months. However, resistance to enzalutamide and other second-generation androgen-targeted agents inevitably develops. Treatment with a subsequent androgen-targeted agent yields only a modest clinical benefit, with progression free survival in this setting being 3 to 4 months and overall survival being less than 12 months. [11-14]

2.3 Mechanisms of Resistance in Refractory CRPC and Response to Enzalutamide

Extensive work has been performed to elucidate the mechanisms of castrate resistance. Preclinical and clinical studies have demonstrated that alterations and heterogeneity in AR expression are central to both the emergence of CRPC and the subsequent development of refractory CRPC. In fact, AR protein expression is already heterogeneous with both AR+ and AR- cells co-existing in untreated primary tumors (**Figure 2**, top left) and the AR heterogeneity becomes accentuated in metastases and CRPCs.[15] By analyzing ~100 CRPC samples, our laboratory collaborators have noted that all CRPCs have 3 distinct patterns of AR protein expression, i.e., increased nuclear/cytoplasmic AR, low/negative (i.e., AR-), and mixed AR+/AR- (**Figure 2**).

Figure 2: AR heterogeneity in CRPC cells

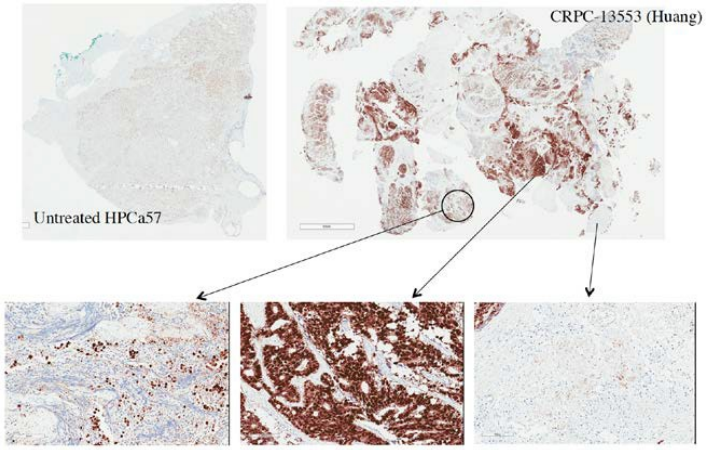


Figure 2: Shown on top are representative IHC images of AR staining from an untreated human PCa (HPCa57) sample (left) and a CRPC sample (CRPC-13553; right). AR staining was performed using an antibody against the N-terminus, which could pick up both the full-length AR as well as all C-terminus truncated AR splice variants. Shown below are three distinct patterns of AR staining in the same CRPC: negative (right panel; note the faint staining on the background is non-specific), high nuclear/cytoplasmic (middle panel), and mixed (left panel).

Preliminary data show that the AR+ PCa cells in xenograft CRPC models still respond to Enzalutamide and the AR- PCa cells do not. Clearly, loss of AR dependence is one mechanism of resistance that needs to be addressed with the generation of better, 2nd generation androgen targeted agents.

2.4 Background on Venetoclax

Venetoclax (synonymous with ABT-199 and GDC-0199) is a highly selective, orally available small−molecule BCL-2 family protein inhibitor that binds with high affinity (dissociation constant [Ki] < 0.10 nM) to BCL-2 and with lower affinity to other BCL-2 family proteins BCL-XL and BCL-W (> 480- and > 2000-fold lower affinity than to BCL-2, respectively). Overexpression of anti−apoptotic BCL-2 family proteins is associated with resistance to chemotherapy, and antagonism of the action of these proteins might overcome resistance and enhance response to therapy. Anti−apoptotic BCL-2 family members are associated with tumor initiation, disease progression, and drug resistance, making them compelling targets for anti-tumor therapy.[16]

A detailed discussion of the preclinical pharmacology, pharmacokinetics, and toxicology of venetoclax can be found in the Investigator's Brochure (v8.1).

2.4.1 Venetoclax mechanism of action and pharmacokinetics

Venetoclax is a selective and orally bioavailable small-molecule inhibitor of the anti-apoptotic protein BCL-2. Overexpression of BCL-2 has been demonstrated in CLL cells where it mediates tumor cell survival and has been associated with resistance to chemotherapeutics. Venetoclax helps restore the process of apoptosis by binding directly to the BCL-2 protein, displacing pro-apoptotic proteins like BIM, triggering mitochondrial outer membrane permeabilization and the

activation of caspases. In nonclinical studies, venetoclax has demonstrated cytotoxic activity in tumor cells that overexpress BCL-2.

Pharmacokinetic data for venetoclax are available from studies in subjects with cancer (CLL/SLL, AML, NHL, and MM), healthy subjects, and a single study in subjects with SLE. Following multiple-dose administration, the maximum plasma concentration of venetoclax was attained 5 to 8 hours after dosing. The harmonic mean terminal half-life (t1/2) ranged from 17 to 41 hours following a single oral dose of venetoclax. In subjects with CLL, venetoclax showed minimal accumulation, and steady-state AUC increased proportionally over the dose range of 150 to 800 mg. Venetoclax has been administered with food in all clinical studies, as food increased the bioavailability of venetoclax by approximately 3- to 5-fold. Venetoclax is highly bound to plasma proteins with unbound fraction (fu) < 0.01, and it is primarily eliminated as metabolites in feces with negligible renal elimination (< 0.1%).

Drug-drug interaction studies of venetoclax with ketoconazole, rifampin, warfarin, ritonavir, and digoxin were conducted to provide dosing recommendations for patients concomitantly taking CYP3A and/or P-gp inhibitors, inducers, and/or warfarin. Pharmacokinetic studies were conducted in healthy Chinese subjects and in Japanese subjects to provide dosing recommendations for those specific populations. Additionally, a dedicated study to evaluate the pharmacokinetics of venetoclax in subjects with hepatic impairment is ongoing. Based on the population pharmacokinetic analysis, age, sex, race, weight, mild and moderate renal or hepatic impairment do not have an effect on venetoclax clearance.

2.4.2 Preclinical safety pharmacology of venetoclax

Venetoclax was tested in a battery of safety pharmacology assays and produced no effects in central nervous system (CNS)/neurobehavioral, or respiratory studies in mice at oral doses up to 600 mg/kg. In dogs, mild reductions in cardiac contractility and cardiac output were observed at plasma concentrations of ≥ 16 µg/mL; concentrations greater than the concentration of venetoclax in humans (average maximum observed plasma concentration [Cmax] = 6.09 µg/mL at the 1200 mg dose). However, no effects on blood pressure, heart rate, or electrocardiogram (ECG) parameters were observed in dogs at a maximum drug concentration of 46 µg/mL.

2.5 Preclinical Studies with Venetoclax

In vitro, venetoclax demonstrated cell killing activity against patient-derived CLL, acute myeloid leukemia (AML) cells, and a variety of lymphoma and leukemia cell lines, including B-cell follicular lymphomas (FLs), mantle cell lymphomas (MCLs), diffuse large B-cell lymphomas (DLBCLs), AMLs, and multiple myeloma (MM) cell lines. Venetoclax was especially potent against non-Hodgkin lymphoma (NHL) cell lines expressing high levels of BCL-2. In vivo, venetoclax inhibits subcutaneous xenograft growth of human tumor cell lines derived from acute lymphoblastic leukemia (ALL), NHL, and AML, and is highly efficacious using various doses and when combined with other regimens. The drug is also active in a model of disseminated ALL and AML. Venetoclax enhanced the activity of a broad variety of chemotherapeutic agents in other human hematological models. Specifically, venetoclax enhances the efficacy of bendamustine plus rituximab (BR) in models of MCL and DLBCL. Furthermore, venetoclax demonstrated potential to enhance the efficacy of bortezomib in multiple models of MM.

The PK profile of venetoclax was evaluated in multiple animal species. In mice, rats, monkeys, and dogs, low plasma clearance and low volumes of distribution characterized the venetoclax PK profile. Half-lives ranged from 2.2 hours in monkeys to 12 hours in dogs. Food had a marked effect on the oral bioavailability in dogs.

Venetoclax demonstrated high protein binding to human, rat, dog, and monkey plasma proteins (> 99.9%). In rats, venetoclax was widely distributed into liver, kidneys, spleen, heart, lungs, small intestine, and white fat, but was poorly distributed in testes, brain, muscle, and bone. Liver metabolism was the major route of elimination with biliary excretion of the parent drug playing the secondary role in rats. Venetoclax showed moderate metabolic stability in in vitro hepatic systems across species tested, except for low to moderate stability in dog hepatocytes.

Venetoclax is metabolized by cytochrome (CYP) 3A4, is a moderate inhibitor of CYP2C8, and a potent inhibitor of CYP2C9. It is not a potent inhibitor of CYP3A4, CYP1A2, CYP2B6, CYP2C19, or CYP2D6 (IC50 > 30 μM) and does not induce CYP3A4 or CYP1A2 at concentrations up to 10 μM. Venetoclax has the potential to inhibit P-glycoprotein (P-gp).

2.6 Clinical Studies with Venetoclax

As of 28 November 2016, on the basis of data available in the AbbVie and Genentech/Roche clinical databases, a total of 2759 subjects have been exposed to at least 1 dose of venetoclax in the oncology and immunology development programs. A total of 2534 oncology subjects had data available in AbbVie and Genentech/Roche studies as of 28 November 2016. Of these 2534, 1435 subjects had CLL/small lymphocytic leukemia (SLL), 626 subjects had NHL, 178 subjects had MM, and 295 had AML. An additional 127 subjects were healthy volunteers. A total of 663 oncology subjects received the drug as monotherapy and 1871 received the drug in combination with other therapies. Additionally, 98 subjects were exposed to at least 1 dose of venetoclax in the AbbVie immunology study, Study M13-093, as of 28 November 2016.

2.6.1 Preliminary safety results of venetoclax in oncology subjects

Based on nonclinical and clinical data available with venetoclax administration, important identified risks are tumor lysis syndrome (TLS) and neutropenia. The risk of TLS is highest in CLL and mantle cell lymphoma. Infection is a potential risk. Other adverse events commonly observed with venetoclax include nausea, diarrhea, and other hematological effects (including, anemia, thrombocytopenia, and lymphopenia). Decreased spermatogenesis has been observed in nonclinical studies with dogs and could present a risk to male infertility. In addition, as venetoclax is being evaluated in subjects with R/R disease who had previously been treated with various cytotoxic agents, second primary malignancies are closely monitored.

2.6.2 Clinical results in hematologic and lymphoid malignancies

In the phase1b study of venetoclax with decitabine or azacitidine in elderly patients with previously untreated acute myeloid leukemia, primary endpoints included safety, pharmacokinetics, maximum tolerated dose and recommended phase 2 dose.[17] Patients were enrolled into one of three groups for the dose-escalation phase of this study: group A (venetoclax and intravenous decitabine 20 mg/m², group B (venetoclax and subcutaneous or intravenous azacitidine 75 mg/m², and group C (a venetoclax and decitabine substudy with the oral CYP3A inhibitor posaconazole, 300 mg twice on cycle 1, day 21, and 300 mg once daily from cycle 1,

days 22–28, to assess its effect on venetoclax pharmacokinetics). Dose escalation followed a standard 3 + 3 design with at least three evaluable patients enrolled per cohort; daily target doses of venetoclax for groups A and B were 400 mg (cohort 1), 800 mg (cohorts 2 and 3), and 1200 mg (cohort 4), and 400 mg for group C: fifty-seven patients were enrolled in the study. The recommended phase 2 dose was 400 mg once a day or 800 mg with an interrupted dosing schedule (safety expansion). The most common grade 3–4 treatment-emergent adverse events were thrombocytopenia (47%), febrile neutropenia (42%) and neutropenia (40%). The most common serious treatment-emergent adverse event in groups A and B was febrile neutropenia (30%) vs (32%), whereas in group C it was lung infection (33%). In total, four (7%) of 57 patients died within 30 days of the first venetoclax dose: sepsis (group B), bacteriemia (group A), lung infection (group C), and respiratory failure (group A). Overall, 35 (61%) of 57 patients achieved complete remission or complete remission with incomplete marrow recovery. In groups A and B, 27 (60%) of 45 patients had complete remission or complete remission with incomplete marrow recovery.[17]

In another phase1 study, patients with relapsed/ refractory MM received venetoclax monotherapy.[18]: following a 2-week lead-in with weekly dose escalation, daily venetoclax was given at 300, 600, 900, or 1200 mg in dose-escalation cohorts and 1200 mg in the safety expansion. Sixty-six patients were enrolled with a median of 5 prior therapies. Venetoclax was generally well tolerated. Most common adverse events included mild gastrointestinal symptoms (nausea [47%], diarrhea [36%], vomiting [21%]). Cytopenias were the most common grade 3/4 events, with thrombocytopenia (32%), neutropenia (27%), anemia (23%), and leukopenia (23%) reported. The overall response rate (ORR) was 21% (14/66), and 15% achieved very good partial response or better (≥VGPR). Most responses (12/14 [86%]) were reported in patients with t (11, 14). In this group, ORR was 40%, with 27% of patients achieving ≥VGPR. Biomarker analysis confirmed that response to venetoclax correlated with higher BCL2:BCL2L1 and BCL2:MCL1 mRNA expression ratios. Venetoclax monotherapy at a daily dose up to 1200 mg has an acceptable safety profile and evidence of single-agent antimyeloma activity in patients with relapsed/refractory MM, predominantly in patients with t (11, 14) abnormality and those with a favorable BCL2 family profile.[18]

2.6.3 Clinical results in metastatic breast cancer

In the first study to evaluate venetoclax in solid tumors, Lindeman et al (mBEP study) tested the safety and efficacy of venetoclax and tamoxifen combination in ER positive BCL2 positive metastatic breast cancer (BC).[19] Previous studies have shown that the anti-apoptotic protein BCL2 is overexpressed in ~85% of ER positive breast cancer. In the preclinical patient derived xenograft models venetoclax (ABT-199), a BCL2 inhibitor synergizes with tamoxifen by increasing apoptosis. A ‘3+3 design’ dose escalation phase 1b study enrolled women with ER positive (> 1%), BCL2 positive (> 10%, mod-high) and HER2 non-amplified metastatic BC. Patients received escalating doses of venetoclax 200, 400, 600 or 800 mg/day with tamoxifen 20 mg/day. The primary objective was to determine dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) over 4 weeks. There was no limit to the number of prior lines of therapy. Fifteen patients were enrolled (mean age 62 years, range 44-78; previous tamoxifen, 10 pts). Mean lines of prior therapy for metastatic BC was 2.5 (median 2, range 0-6) and included tamoxifen (6 pts). ESR1 mutations were present in ctDNA of 4 patients. Treatment was well tolerated, with no DLT observed. MTD was not reached; 6 patients received the maximal

planned dose (800 mg). The most common adverse event (AE) was lymphopenia (67% Grade 1-2; 13% Grade 3; No Grade 4), followed by nausea (46%, Grade 1-2), which was readily managed. Of 13 women with measurable disease (RECIST v1.1), 4 (31%) had a partial response and 5 (38%) had stable disease (clinical benefit rate, 69%). For patients with a partial response, tumor regression was rapid (evident at first restaging) and occurred in the 400-800 mg dose levels. Two patients with non-measurable bone-only disease had clinically stable disease (1 ongoing > 64 weeks). The median duration of response has not yet been reached (range, 12 to > 64 weeks). This study demonstrated the safety of tamoxifen and venetoclax in ER positive BCL2 positive metastatic BC, with preliminary evidence of clinically relevant activity. A dose expansion study including serial biopsy, ctDNA and PET scans is ongoing at the 800 mg/day recommended phase 2 dose.[19]

2.7 Background on Enzalutamide

Enzalutamide (MDV3100) is an AR signaling inhibitor that targets several steps in the AR signaling pathway. Enzalutamide competitively inhibits binding of androgens to ARs, inhibits nuclear translocation of receptors and inhibits the association of the AR with DNA, even in the setting of AR overexpression and in prostate cancer cells resistant to antiandrogens.

2.7.1 Enzalutamide mechanism of action and pharmacokinetics

Enzalutamide is an androgen receptor inhibitor that acts on different steps in the androgen receptor signaling pathway.[20] Enzalutamide has been shown to competitively inhibit androgen binding to androgen receptors and inhibit androgen receptor nuclear translocation and interaction with DNA. A major metabolite, N-desmethyl enzalutamide, exhibited similar in vitro activity to enzalutamide. Enzalutamide decreased proliferation and induced cell death of prostate cancer cells in vitro and decreased tumor volume in a mouse prostate cancer xenograft model.

The pharmacokinetics of enzalutamide and its major active metabolite (N-desmethyl enzalutamide) were evaluated in patients with metastatic castration-resistant prostate cancer and healthy male volunteers. The plasma enzalutamide pharmacokinetics is adequately described by a linear two-compartment model with first-order absorption.

Absorption: Following oral administration of enzalutamide (XTANDI®) 160 mg daily, in patients with metastatic castration-resistant prostate cancer, the median time to reach maximum plasma enzalutamide concentrations (Cmax) is 1 hour (range 0.5 to 3 hours). At steady state, the plasma mean Cmax values for enzalutamide and N-desmethyl enzalutamide are 16.6 μg/mL (23% CV) and 12.7 μg/mL (30% CV), respectively, and the plasma mean predose trough values are 11.4 μg/mL (26% CV) and 13.0 μg/mL (30% CV), respectively. With the daily dosing regimen, enzalutamide steady state is achieved by Day 28, and enzalutamide accumulates approximately 8.3-fold relative to a single dose. Daily fluctuations in enzalutamide plasma concentrations are low (mean peak-to-trough ratio of 1.25). At steady state, enzalutamide showed approximately dose proportional pharmacokinetics over the daily dose range of 30 to 360 mg. A single 160 mg oral dose of XTANDI was administered to healthy volunteers with a high-fat meal or in the fasted condition. A high-fat meal did not alter the AUC to enzalutamide or N-desmethyl enzalutamide.

Metabolism: Following single oral administration of 14C-enzalutamide 160 mg, plasma samples were analyzed for enzalutamide and its metabolites up to 77 days post dose. Enzalutamide, N-

desmethyl enzalutamide, and a major inactive carboxylic acid metabolite accounted for 88% of the 14C-radioactivity in plasma, representing 30%, 49%, and 10%, respectively, of the total 14C-AUC0-inf. In vitro, human CYP2C8 and CYP3A4 are responsible for the metabolism of enzalutamide. Based on in vivo and in vitro data, CYP2C8 is primarily responsible for the formation of the active metabolite (N-desmethyl enzalutamide).

Elimination: Enzalutamide is primarily eliminated by hepatic metabolism. Following single oral administration of 14C-enzalutamide 160 mg, 85% of the radioactivity is recovered by 77 days post dose: 71% is recovered in urine (including only trace amounts of enzalutamide and N-desmethyl enzalutamide), and 14% is recovered in feces (0.4% of dose as unchanged enzalutamide and 1% as N-desmethyl enzalutamide). The mean apparent clearance (CL/F) of enzalutamide in patients after a single oral dose is 0.56 L/h (range 0.33 to 1.02 L/h). The mean terminal half-life (t1/2) for enzalutamide in patients after a single oral dose is 5.8 days (range 2.8 to 10.2 days). Following a single 160 mg oral dose of enzalutamide in healthy volunteers, the mean terminal t1/2 for N-desmethyl enzalutamide is approximately 7.8 to 8.6 days.

2.8 Clinical Studies with Enzalutamide

The efficacy of enzalutamide in patients with metastatic prostate cancer who progressed on androgen deprivation therapy has been demonstrated in two randomized controlled phase III studies including PREVAIL and AFFIRM studies.[9, 10] The AFFIRM study randomized metastatic CRPC patients who had received prior docetaxel chemotherapy. Median survival was improved with enzalutamide from 13.6 to 18.4 months (HR 0.63, P <0.001). Survival was improved in all subgroups analyzed. Secondary endpoints also were improved significantly, which included the proportion of men with >50% PSA decline (54% vs 2%), radiographic response (29% vs 4%), radiographic progression-free survival (8.3 vs 2.9 months), and time to first SRE (16.7 vs 13.3 months). Adverse events were mild, and included fatigue (34% vs 29%), diarrhea (21% vs 18%), hot flushes (20% vs 10%), headache (12% vs 6%), and seizures (0.6% vs 0%).[9]

In the PREVAIL study enzalutamide was randomly assigned to chemotherapy naïve metastatic prostate cancer.[10] The study was stopped early due to benefits shown in the treatment arm. Compared to the placebo group, the enzalutamide group showed improved median progression-free survival (20.0 vs 5.4 months) and median overall survival (35.3 vs 31.3 months). Time to radiographic progression at 12 months was 65% compared to 14%. The benefit of enzalutamide was shown with respect to all secondary end points, including the time until the initiation of cytotoxic chemotherapy (hazard ratio, 0.35), the time until the first skeletal-related event (hazard ratio, 0.72), a complete or partial soft-tissue response (59% vs. 5%), the time until prostate-specific antigen (PSA) progression (hazard ratio, 0.17), and a rate of decline of at least 50% in PSA (78% vs. 3%). Fatigue and hypertension were the most common clinically relevant adverse events associated with enzalutamide treatment.

Two randomized clinical trials have reported that enzalutamide may be superior to bicalutamide for cancer control in metastatic CRPC. The TERRAIN study randomized 375 men with treatment naïve, metastatic CRPC to 160 mg/d enzalutamide or 50 mg/d bicalutamide.[21] The enzalutamide group had significantly better progression-free survival (defined as PSA progression, soft tissue progression or development of additional bony metastases) compared to bicalutamide group (15.7 vs 5.8 months). The STRIVE trial randomized patients with M0 or M1

treatment naïve CRPC to enzalutamide or bicalutamide.[22] The primary endpoint in this study was progression-free survival; defined as either PSA progression, radiographic progression disease or, death from any cause. Enzalutamide reduced risk of progression or death by 75% compared to bicalutamide. These studies demonstrated that enzalutamide extended progression-free survival better than bicalutamide in men choosing an anti-androgen for secondary hormonal therapy treatment of CRPC.

The FDA approved dose of enzalutamide, 160 mg daily, will be used in the study. The benefit of enzalutamide for men with prostate cancer is consistently observed across studies of differing patient populations and across the majority of efficacy endpoints assessed.

2.9 Study Rationale and Benefit-Risk Assessment

2.9.1 Rationale for combining enzalutamide and BCL2 inhibitor venetoclax (ABT-199) to treat CRPC

AR expression is heterogeneous at the outset in CRPC

Dr. Tang and his colleagues have been focused on understanding prostate cancer (PCa) cell heterogeneity for over 12 years. They have provided convincing evidence that human PCa harbors a population of prostate cancer stem cells (PCSCs) that are generally AR- in untreated tumors [15, 23-33] and, have also shown that ADT, which blocks androgen production and/or AR nuclear functions, may both enrich AR- PCa cells and cause genomic amplification of AR in AR+ PCa cells, leading to two castration-resistant PCa cell populations. Both AR- and AR+ cell populations may function as CRPC propagating cells.[15] Most current anti-PCa therapeutic efforts have been directed towards targeting AR+ PCa cells, while largely ignoring the AR- cell population. Since AR- cells can also propagate CRPC, simultaneously targeting the AR- population at the outset of ADT resistance will be beneficial, and may prevent resistance to second generation agents.

CRPC xenograft models demonstrate unique molecular changes with respect to AR and BCL-2

Dr. Tang and his colleagues established 4 pairs of androgen-dependent (AD) and androgen-independent (AI) PCa xenograft models, i.e., LNCaP, VCaP, LAPC9, and LAPC4 (**Figure 3**).

Figure 3: Androgen-dependent (AD) and Androgen-independent (AI) PCa Xenograft Models

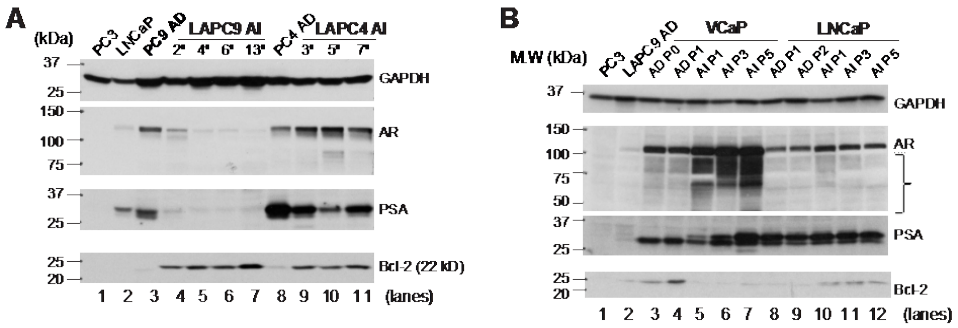


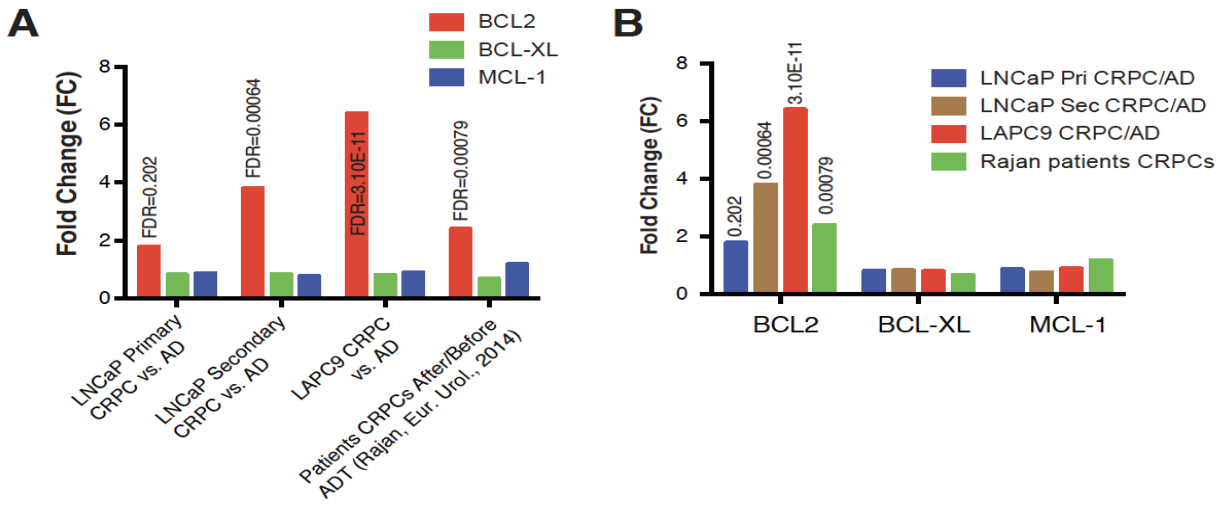
Figure 3: Four CRPC xenograft models show different molecular changes regarding AR and BCL-2: Shown are the 4 xenograft models serially passaged in castrated male NOD/SCID-γ (NSG; for LNCaP) or NOD/SCID (the rest) mice. The AI (passages indicated) and their corresponding AD tumors were harvested for WB for the molecules indicated. PC3 cells were used as negative control for AR. 60 μg WCL was loaded each lane and WB of GADPH was run as loading controls (top and bottom). (**A**) The LAPC9 (PC9) and LAPC4 (PC4) models. Cultured LNCaP cells were used as control for AR and PSA. (**B**) The VCaP and LNCaP models. LAPC9 AD tumor was run for comparison.

Interestingly, although 3 AI models including LNCaP, LAPC4, and VCaP express significant amount of AR, the castration-resistant LAPC9 AI tumors largely lost the expression of AR and its target PSA (**Figure 3**). Strikingly, ***BCL-2***, a major anti-apoptotic protein critical for stem cell survival and functions, was overexpressed in 3 of the 4 models, i.e., LNCaP, LAPC4, and LAPC9.

<u>BCL2, but NOT BCL-XL or MCL-1, is a critical driver of Enzalutamide resistant CRPC</u>

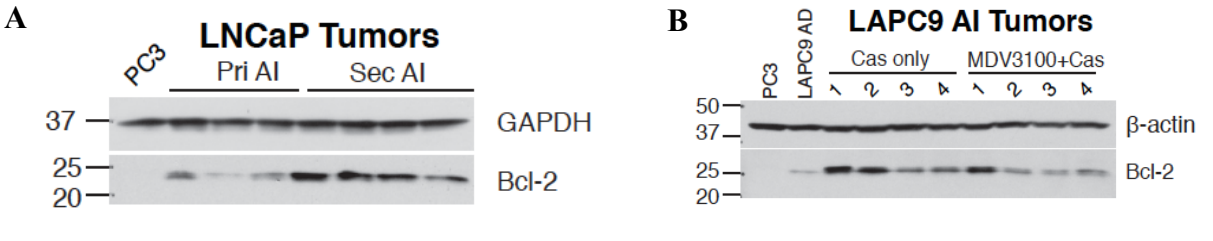
As shown in **Figure 4** below (A and B represent two different ways to present the data), BCL2 mRNA is selectively upregulated in CRPC xenograft models as well as in patients with CRPC. Numerous literature reports have linked BCL-2 to prostate cancer development, progression, and castration resistance. In contrast, although there are some in vitro studies showing that experimental BCL-XL overexpression can confer apoptosis resistance in prostate cancer cells, there is NO in vivo evidence linking castration resistance to BCL-XL induction. Nor is there any report that selective inhibition of BCL-XL can inhibit/prevent CRPC. We interrogated the mRNA levels for the 3 MAJOR anti-apoptotic molecules BCL-2, BCL-XL, and MCL-1 in 4 deep RNA-Seq data sets, including one patient derived CRPC dataset[34], and 3 of our in-house CRPC datasets, i.e., LNCaP primary CRPC (induced by tumor propagation in surgically castrated male NOD/SCID mice) over AD (androgen-dependent) tumors, LNCaP secondary (tumors resistant to both castration AND Enzalutamide) CRPCs vs. AD tumors, and LAPC9 CRPCs (induced by castration of hosts) vs. AD tumors. The results clearly indicate that ONLY BCL-2 mRNA levels are induced in response to castration (note: an FDR value of <0.25 is considered statistically significant in analyzing RNA-Seq data).

Figure 4: Selective Upregulation of BCL2 mRNA in CRPC Xenograft Models and in Patients with CRPC



As demonstrated previously, and in **Figure 5**, castration in LNCaP and LAPC9 xenograft models results in upregulation of BCL-2 protein. Enzalutamide (MDV3100) further upregulates BCL-2 in the LNCaP model (**Figure 5A**), but has limited impact on LAPC9 tumors (**Figure 5B**). Using primary cultures of patient derived PCa cells, we further demonstrate enzalutamide treatment leads to upregulation of BCL-2 (**Figure 5C**). Overall, these results suggest that BCL-2 plays a key role in regulating resistance to enzalutamide treatment.

Figure 5: BCL-2 Protein Upregulation Resulting from Castration in LNCaP and LAPC9 Xenograft Models



C

Patient	Age	Gleason	Tumor involvement
HPCa229	71	7 (3+4)	30%
HPCa230	59	7 (3+4)	100%
HPCa231	72	8 (4+3)	100%
HPCa232	72	7 (4+3)	100%

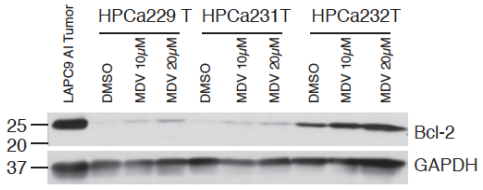


Figure 5: A-B: LNCaP primary (Pri) and secondary (Sec) CRPCs (or AI, androgen-independent tumors) (A) or LAPC9 AI tumors (B) were probed for BCL2 protein in Western blotting (WB). C: Enzalutamide induces BCL2 protein expression in patient tumor-derived cells. Shown are the results from 3 patient tumors (right).

Enzalutamide and ABT-199 combination prevents emergence of CRPC

In the LNCaP model, surgical castration led to increased AR and BCL-2 expression (**Figure 3B**). We asked a clinically relevant question: Is the upregulated AR in LNCaP primary AI tumors (i.e., the AI induced by surgical castration) functional and can it be targeted by Enzalutamide (MDV 3100)? We performed a therapeutic experiment by treating LNCaP AI-tumor bearing animals with i.p. injections of Enzalutamide (**Figure 6A**). The results revealed that Enzalutamide suppressed the growth of AI LNCaP for the first 6.5 weeks (**Figure 6B**), suggesting that the upregulated nuc-AR is causally mediating the primary CRPC in the LNCaP model.

Figure 6: Enzalutamide with ABT199 Significantly Prevent LNCaP CRPC Emergence

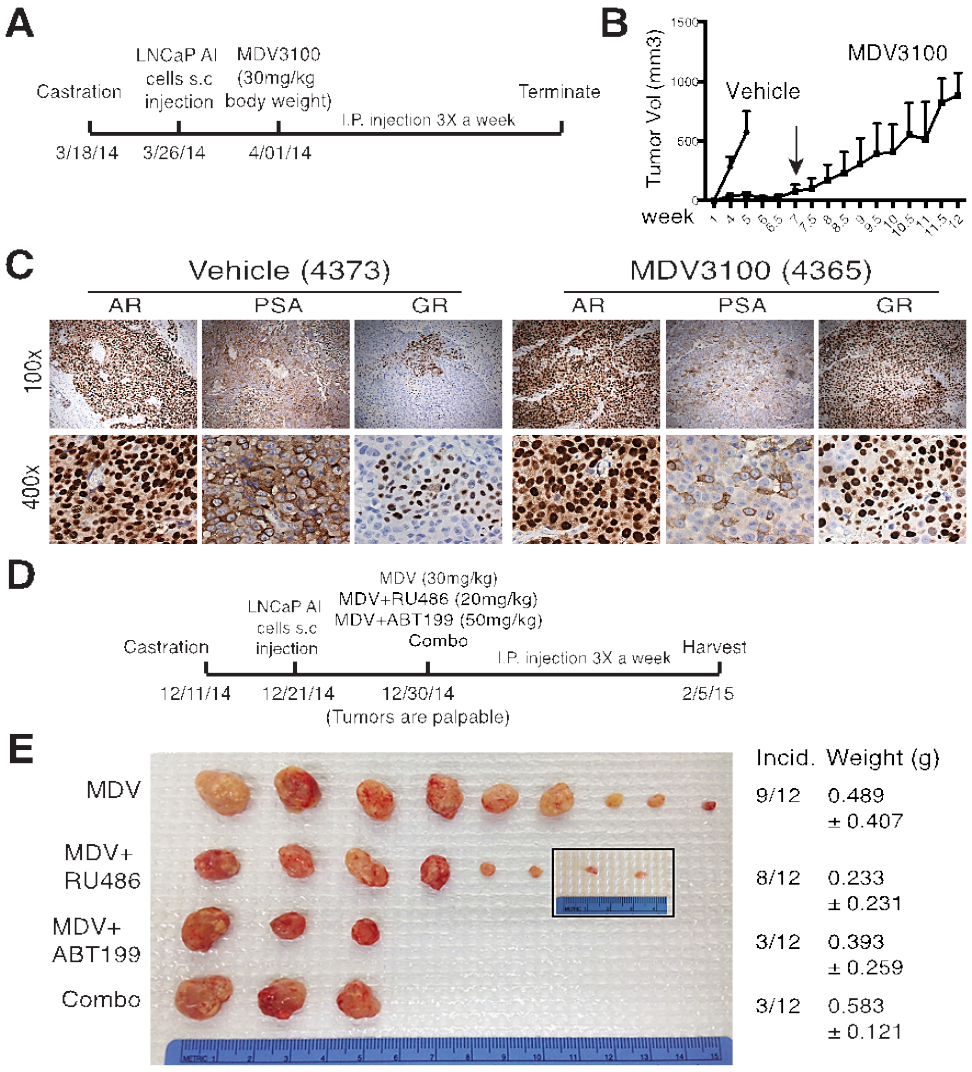


Figure 6: Enzalutamide with ABT199 significantly prevent LNCaP CRPC emergence. (**A**) Experimental timeline. (**B**) Treatment response of the AI LNCaP tumors to MDV3100. The arrow indicates the time when MDV3100-resistant secondary CRPC began to emerge. (**C**) Representative IHC images of AR, PSA, and GR in one each vehicle- and MDV3100-treated endpoint tumors. (**D**) Timeline for a pilot secondary CRPC treatment. (**E**). Endpoint tumor images with tumor incidence and tumor weights indicated on the right. The two small tumors in the rectangle box were harvested two weeks later than the rest.

Unfortunately, LNCaP AI tumor response to MDV3100 was not permanent (Figure 5B; arrow). The MDV3100-resistant tumors, termed **Secondary AI tumors** (or secondary CRPC, which were resistant to both surgical castration and MDV3100) demonstrated further increases in nuc-AR and GR (**Figure 6C**). Strikingly, *BCL-2* showed the most prominent upregulation in response to MDV3100 (data not shown). Therefore, we performed a "targeted" therapy experiment by treating LNCaP *primary AI tumors* with MDV3100 PLUS the BCL-2 antagonist *ABT-199* (**Figure 6D**).[35, 36]

For comparisons, we also treated cohorts of tumor-bearing animals with MDV3100 alone, MDV3100 + GR antagonist RU486, and MDV3100+ABT199+ RU486 (Combo). The results were *striking*; MDV3100 + ABT199 as well as the Combo greatly suppressed the emergence of LNCaP *secondary CRPC* ($P<0.0001$; **Figure 6E**). In contrast, MDV3100+ RU486 only inhibited tumor growth but did not reduce incidence (**Figure 6E**).

Overall, our data provides direct evidence that BCL-2 and NOT BCL-XL represents a therapeutic target for nuc-AR mediated primary CRPC and that combined 'therapy' with MDV3100 (enzalutamide) and ABT-199 inhibits development of enzalutamide-resistant secondary CRPC. We have also observed that ABT-199, as a single agent, inhibited the growth of AR-/lo LAPC9 CRPCs (data not shown), suggesting that ABT199 should be effective in targeting both AR+/hi and AR-/lo CRPC cells.

Finally, it should be noted that our results in PCa are consistent with emerging evidence that ABT199 is effective in eradicating undifferentiated and therapy-resistant leukemia/lymphoma and breast cancer stem cells. Our proposed prostate cancer trial is similar to the current ABV-10437 trial, initiated in 2015, to combine venetoclax (ABT199) with tamoxifen to treat luminal breast cancer patients and thereby delay/prevent Tamoxifen resistance.[36] Similarly, our proposed trial is driven by the hypothesis that the addition of ABT199 to enzalutamide in CRPC at treatment initiation, will delay the emergence of enzalutamide resistance.

2.9.2 Rationale for study design

As discussed in Section 2 (Background), despite significant therapeutic progress with the use of 2nd generation AR directed therapy, metastatic CRPC essentially remains a fatal disease, highlighting the urgent need for novel therapeutic approaches for these patients.

The impetus for the current trial comes from directly targeting one of the principle mechanisms of AR resistance, i.e., the presence of an $AR^{-/lo}$ and $BCl\text{-}2^{+/hi}$ population of progenitor cells. Concomitant therapy with enzalutamide and venetoclax should prevent the emergence of this resistant progenitor population, given that enzalutamide is a potent AR antagonist, and the venetoclax is a potent BCL-2 inhibitor.

2.9.3 Rationale for patient population

Treatment options for men with metastatic CRPC, progressing through AR directed therapy are minimal and the disease is invariably fatal.

2.9.4 Rationale for dose and schedule

We propose to use a fixed dose of Enzalutamide. After a 4 week "lead in" phase with Enzalutamide, to establish stable levels of the drug, Venetoclax will be added and increased based on a standard 3 + 3 design.

As described above, Enzalutamide is a potent CYP3A4 inhibitor, which with time also induces 3A4 [37]. Thus, it is anticipated that if combined immediately this would lead to higher than expected venetoclax levels, a situation we are hoping to avoid with the lead-in. With the lead-in and long term use, the predominance of induction of CYP3A4 by enzalutamide may result in lower and even sub-therapeutic venetoclax levels. It is for this exact reason that we propose the graduated dose escalation and the intensive PK sampling to evaluate drug – drug interactions. As PK data are generated, and at the latest before starting the phase II portion of this trial, exposure metrics for venetoclax and enzalutamide will be evaluated and compared with historical data.

The Phase 2 study is a *single-arm*, *single-stage trial during which venetoclax and enzalutamide will be administered at their recommended Phase 2 doses (RP2D)*, as determined from the Phase Ib study. This study is intentionally small and, if venetoclax significantly delays enzalutamide resistance, it will form the basis for a larger confirmatory trial.

2.9.5 Rationale for primary and secondary endpoints

Primary endpoint is safety, determining a RP2D for the combination, and a thorough evaluation of potential drug-drug interactions.

The efficacy parameters proposed, should give a sufficient signal to help plan a definitive study which may lead to approval of a novel life-extending combination in men with metastatic CRPC.

2.9.6 Correlative Studies: Rationale for biomarker assessments

Rationale for studying Treatment Related Biomarkers

Enzalutamide and venetoclax target androgen receptor (AR) and BCL-2 dependent cells, respectively. Assessment of these target molecules is critical for complete understanding of therapeutic outcome. These two molecules are intimately linked in PCa, as it has been demonstrated by us that enzalutamide treatment leads to the upregulation of the anti-apoptotic BCL-2 in AR dependent tumors. Furthermore, BCL-2 is found to be highly up-regulated in AR-/lo (androgen independent) tumors. Patient tumors are likely to be heterogeneous for expression of AR and BCL-2 at start of treatment. Patient's responding to therapy would be expected to initially express full length AR and modest expression of BCL-2. Once treatment starts, AR-dependent cells are expected to undergo apoptosis and die. Upregulation of BCL-2 as a survival strategy by AR-dependent, or independent cells will initially be unsuccessful due to venetoclax treatment. It will be critical to understand how expression of these markers, as well as others listed in Table X, change in Patients progressing on combination therapy. Tumors may be expected to upregulate ARv7, shed AR in association with upregulation of neuroendocrine CRPC markers (SYP and CHGA), or upregulate other BCL-2 family members. Lastly, since venetoclax is a known substrate for drug resistance pumps, ABCB1 and ABCG2 will be evaluated in patients with progressive disease. These biomarker analyses will give us some ideas about how patients are progressing, and whether progression is related to known mediators of enzalutamide resistance, or possibly mediators of venetoclax resistance.

Rationale for studying "Stemness" gene expression related biomarkers

We, and others, have demonstrated extensively that PCa progression and resistance to therapy is highly associated with an increase in PCa stem-like cells (PCSCs). PCSCs have an increased capacity for tumor propagation and are inherently more resistant to therapeutic interventions. CRPCs expressing higher levels of "stemness" associated genes may be expected to be more resistant to therapeutic intervention. It will be interesting to determine the relationship of "stemness" as a prognostic marker for treatment outcome, as well as a marker for treatment resistance in this patient cohort

Rationale for studying BCL-2 in PBMCs from whole blood

Given the complex drug interactions between enzalutamide and venetoclax, it will be nice to establish a reliable pharmacodynamic readout for venetoclax activity. Venetoclax has been heavily studied in hematologic malignancies, but very little data is available on the effect of venetoclax in normal BCL-2 expressing PBMCs. BCL-2 regulates apoptosis in lymphoid tissues, functions in elimination of autoreactive lymphocytes, and plays a role in immune response resolution. Furthermore, experimental studies in rodents demonstrate a clear role for BCL-2 in immune cell homeostasis, with BCL-2 deficient mice exhibiting loss of both B- and T-cells.[38] Given the important role of BCL-2 in regulating lymphoid cells (a major component of PBMCs) as well as other PBMC cell types, it's rational to look for deregulation of BCL-2 in this population. Decreases of lymphocytes in peripheral blood and in lymphoid tissues are a prominent finding in all nonclinical species tested (mice, rats, and dogs) and are considered to be an on-target effect of venetoclax.[39]

Likewise, a decrease in lymphocytes in humans has also been observed, in both diseased and healthy volunteers. Therefore, we will use changes to BCL-2 expression, as well as changes in PBMC population dynamics (% B-Cells) as a surrogate marker for venetoclax activity.

Rationale for studying CTC enumeration and biomarker expression

Multiple lines of evidence have shown that CTC enumeration can be directly tied to therapeutic outcome in patients. Here we will count CTCs in pre-treated and treated blood samples from patients and assess CTCs as a prognostic biomarker for treatment outcome. Additionally, we will be assessing the expression of therapy related biomarkers in enriched CTCs using the flow cytometry based ImageStream. As with tumor biopsies, expression of AR and BCL-2 are expected to correlate with patient responsiveness and may be able to predict when patients are no longer responding.

2.10 Benefit-Risk Assessment

Metastatic prostate cancer growth, survival, and progression are intrinsically linked to the activity of the AR. Thus, first line therapies target AR either through suppression of androgen production or direct inhibition of the AR, resulting in the inability of the receptor to bind to chromatin and initiate AR-directed gene expression. Recurrent AR activity is the critical driver of CRPC; therefore, novel AR-targeted therapeutics were developed that show transient clinical benefit. Despite these advances, the vast majority of patients with CRPC succumb to disease within 2-3 years, indicating the need for metrics of precision medicine, and development of additional therapeutics.

Our preclinical studies indicate, tumors with high BCL2 expression proved to be exquisitely responsive to BCL2 inhibitors, revealing cytostatic and cytotoxic effects specific to the tumor. Our study showed that: i) BCL-2 and not BCL-XL represents a therapeutic target for nuc-AR mediated primary CRPC ii) BCL2 protein is induced by castration in both xenograft models as well as patient primary tumor cells treated with enzalutamide iii) ABT-199, as a single agent, inhibited the growth of AR-/lo LAPC9 CRPCs; and iv) Most importantly, MDV3100 (enzalutamide) + ABT199 (venetoclax) combination greatly suppressed the emergence of LNCaP secondary CRPC. These preclinical findings suggest that the addition of ABT199 (venetoclax) to enzalutamide in CRPC patients at treatment initiation, will delay the emergence of enzalutamide resistance, thus providing the rationale for combination of venetoclax and enzalutamide in patients with mCRPC.

Since the venetoclax and enzalutamide have not been combined together in patients, the first phase will be a dose escalating study to find a safe dose of the combination. The standard doses of enzalutamide will be used and there will be three dose levels of venetoclax. A traditional 3x3 dose escalation schema will be used to establish the safe dose of venetoclax to combine with enzalutamide. The safe dose of the combination will be used in the phase II portion of the study. The primary endpoint of the phase II portion of the study will be a 12 month progression-free survival. Secondary endpoints will be rPFS, OS and safety. If there is clinical activity noted with the combination further randomized testing will be required to establish clinical benefit of the venetoclax combination.

3 INCLUSION AND EXCLUSION CRITERIA

Men of all races and ethnic groups with metastatic castrate resistant prostate cancer who meet the following criteria are eligible for this study.

3.1 Inclusion Criteria

To be included in this study, participants must meet the following criteria:

1. Male with age ≥ 18 years of age.
2. Histological or cytological documentation of diagnosis of prostate cancer.
3. Documented progressive metastatic castrate resistant prostate cancer (mCRPC) based on <u>at least one</u> of the following criteria:
    a. *PSA progression* defined as 25% increase over baseline value with an increase in the absolute value of at least 2 ng/mL that is confirmed by another PSA level with a minimum of a 1week interval and a minimum PSA of 2 ng/mL.
    b. *Soft-tissue progression* defined as an increase ≥ 20% in the sum of the longest diameter (LD) of all target lesions based on the smallest sum LD since treatment started or the appearance of one or more new lesions.
    c. Progression of bone disease (evaluable disease) or, new bone lesion(s), by bone scan.
    d. If on an anti-androgen, must have documented progression 6 weeks after stopping anti-androgen therapy
4. Willing to undergo a biopsy, if readily available biopsy site present (i.e., nodal or visceral metastasis).

5. Have an ECOG Performance Status of 0 - 1. Refer to **Appendix A**.
6. Have testosterone level of < 50 ng/dL. ***Note****:* Patients must continue primary androgen deprivation with an LHRH analogue (agonist or antagonist) if they have not undergone orchiectomy.
7. Patient has adequate bone marrow and organ function as defined by the following laboratory values, obtained *within 14 days* prior to treatment start:
    - White blood cells ≥ 1.5 x 10^9/L
    - Platelets (UNVPLT) ≥ 100 × 109/L
    - Hemoglobin (HGB) ≥ 9 g/dL
    - Potassium (K), total calcium (CA) (corrected for serum albumin), magnesium, sodium (NA) and phosphorus within normal limits for the institution or corrected to within normal limits with supplements before first dose of study medication
    - INR ≤ 1.5
    - Adequate renal function as demonstrated by a creatinine clearance ≥ 30 mL/min; calculated by the Cockcroft Gault formula (refer to **Appendix K**)
    - Adequate hepatic function defined as:
        - Aspartate transaminase (AST) and alanine transaminase (ALT) ≤ 3 X ULN. If the patient has liver metastases, ALT and AST must still be ≤ 3 x ULN. Patients with liver metastases and AST/ALT above this limit will not be enrolled.
        - Total serum bilirubin ≤1.5 x ULN (unless bilirubin rise is due to Gilbert's syndrome or of non-hepatic origin); or total bilirubin (TBILI) ≤ 3.0 x ULN with direct bilirubin within normal range in patients with well documented Gilbert's Syndrome.
8. Ability to swallow and retain oral medication (without crushing, dissolving or chewing tablets).
9. Phase Ib only: Prior enzalutamide treatment and/or other approved treatments for CRPC are acceptable
10. Phase II only: Participants MUST be treatment naïve in the CRPC setting: i.e., no prior exposure to abiraterone acetate other specific CYP-17 inhibitors; no prior exposure to enzalutamide or investigational androgen receptor (AR) targeted agents; and no prior exposure to chemotherapy and or RAD-223.
11. Sexually active males must agree to use a condom during intercourse while taking the study drug and for at least 3 months after stopping study treatment. Sexually active males should not father a child during this period. A condom is required to be used by vasectomized men in order to prevent delivery of the drug via seminal fluid. Should a woman become pregnant or suspect she is pregnant while her partner is participating in this study, she should inform her treating physician immediately.
12. Participant or legal representative must understand the investigational nature of this study and voluntarily sign and date an Independent Ethics Committee/Institutional Review

Board approved written informed consent form prior to receiving any study related procedure.

Refer to **Appendix B** for the ***ELIGIBILITY VERIFICATION FORM: INCLUSION CRITERIA*** checklist.

3.2 Exclusion Criteria

Participants will be excluded from this study for the following (all criteria apply to Phase Ib and Phase II, unless otherwise noted):

1. **Phase II only**: Prior exposure to abiraterone acetate.
2. **Phase II only**: Prior exposure to BCL-2 inhibitors agents like venetoclax.
3. **Phase II only**: Prior chemotherapy for castration resistant disease. Chemotherapy given in the castration-sensitive setting is permissible if stopped at least 4 weeks prior to treatment start.
4. **Phase II only**: Prior isotope therapy with strontium-89, samarium or radium-223 within 12 weeks of treatment start.
5. Subject has acute promyelocytic leukemia.
6. Subject has known active CNS involvement with AML.
7. Participants with known symptomatic brain metastases.
8. Participant has a concurrent malignancy or malignancy within 3 years of treatment start, with the exception of adequately treated, basal or squamous cell carcinoma, non-melanomatous skin cancer or curatively resected cervical cancer.
9. Participant has a known history of HIV infection (testing not mandatory).
10. Chronic hepatitis B virus (HBV) or hepatitis C (HCV) requiring treatment. **Note:** subjects with serologic evidence of prior vaccination to HBV (i.e. hepatitis B surface (HBs) antigen negative-, anti-HBs antibody positive and anti-hepatitis B core (HBc) antibody negative) or positive anti-HBc antibody from intravenous immunoglobulins (IVIG) may participate.
11. Uncontrolled and/or active systemic infection (viral, bacterial or fungal).
12. Participant has clinically significant, uncontrolled heart disease and/or recent events including any of the following:

    - History of acute coronary syndromes (including myocardial infarction, unstable angina, uncontrolled hypertension, uncontrolled arrhythmia, stroke, coronary artery bypass grafting, coronary angioplasty, or stenting) or symptomatic pericarditis within 12 months prior to treatment start
    - Cardiac history of CHF requiring treatment or Ejection fraction ≤ 50% or chronic stable angina
    - A cardiovascular disability status of New York Heart Association Class > 2 (refer to **Appendix L)**:
        - Class 2 is:

  - Defined as cardiac disease in which patients are comfortable at rest but ordinary physical activity
  - Results in fatigue, palpitations, dyspnea, or angina pain
- On screening 12 lead ECG, any of the following cardiac parameters: bradycardia (heart rate < 50 at rest), tachycardia (heart rate > 90 at rest), PR interval > 220 msec, QRS interval > 109 msec or, QTcF > 450 msec. Congenital long QT syndrome or family history of long QT syndrome.

13. DLCO ≤ 65% or FEV1 ≤ 65%.
14. Treatment with any of the following **within 7 days** prior to the first dose of study drug:
    - Steroid therapy for anti-neoplastic intent
    - Moderate or strong cytochrome P450 3A (CYP3A) inhibitors (refer to **Appendix G**)
    - Moderate or strong CYP3A inducers (refer to **Appendix G**)
    - Medications that have a known risk to prolong the QT interval or induce Torsades de Pointes
    - Herbal preparations/medications
15. Participants receiving any medications or substances that are inhibitors or inducers of CYP2C8 enzymes are ineligible (refer to Appendix H).
16. Administration or consumption of any of the following **within 3 days** prior to the first dose of study drug:
    - grapefruit or grapefruit products
    - Seville oranges (including marmalade containing Seville oranges)
    - star fruit
17. Patient who has received radiotherapy ≤ 4 weeks prior to start of treatment or limited field radiation for palliation ≤ 2 weeks prior to treatment start and, who has not recovered to Grade 1 or better from related side effects of such therapy (exceptions include alopecia) and/or in whom ≥ 30% of the bone marrow was irradiated.
18. Patients with central nervous system (CNS) involvement.
19. Patients with seizure disorder.
20. Patient has not recovered from all toxicities related to prior anticancer therapies to NCI CTCAE (version 5.0) Grade ≤1 (*Exception to this criterion:* patients with any grade of alopecia are allowed to enter the study).
21. Participant has any other concurrent severe and/or uncontrolled medical condition that would cause, in the investigator's judgment, an unacceptable safety risk.
22. Unwilling or unable to follow protocol requirements.
23. Any condition which in the Investigator's opinion deems the participant an unsuitable candidate to receive study drug.

Refer to **Appendix C** for the ***ELIGIBILITY VERIFICATION FORM: EXCLUSION CRITERIA*** checklist.

4 LOCAL AND STUDY-WIDE NUMBER OF SUBJECTS

Phase Ib: Approximately 15 to 18 patients will be accrued in the dose escalation phase (Phase Ib) at Roswell Park will be enrolled. The number of participants required for the Phase Ib portion is a function of the unknown dose-toxicity relationship. A Phase Ib expansion (n=3-6) is allowed at the MTD for patients with soft tissue disease to better assess the safety profile.

Phase II: 21 patients will be accrued in the dose expansion phase (Phase II).

5 LOCAL AND STUDY-WIDE RECRUITMENT METHODS

Participants will be identified/recruited/screened from patients at the GU clinic at Roswell Park and from multi-disciplinary conference discussion.

6 MULTI-SITE RESEARCH

This is a multi-center study

7 STUDY TIMELINES

A maximum of 39 participants will be enrolled at Roswell Park and other centers. Accrual is expected to take approximately 3 years, with follow-up for 1 year from the time of treatment discontinuation or death, whichever occurs first. Upon completion of the 1 year follow-up period, patient survival status will be monitored every 3 months.

8 STUDY ENDPOINTS

8.1 Phase 1b

8.1.1 Primary Endpoints

- The MTD will be determined based on the rate of dose-limiting toxicities (DLTs). Adverse events will be categorized and graded according to NCI CTCAE version 5.0 (see **Section 9.1** for additional information).
- The RP2D will be selected based on the overall tolerability of the regimen, but will not exceed the MTD.

8.2 Phase 2

8.2.1 Primary Endpoint

- 12-month progression free survival rate of patients with enzalutamide-naïve metastatic castrate resistant prostate cancer (mCRPC) treated with venetoclax plus enzalutamide.
    - PFS defined as time from start of treatment combination therapy to disease progression
        - PCWG3 will be used to evaluate PSA response and progression as well as progression on bone scans.

- RECIST 1.1 will be used to assess response and progression for nodal and visceral metastasis.

8.2.2 Secondary Endpoints

- PSA50 is defined as the proportion of patients with a ≥ 50% reduction in PSA from baseline.
- CTC conversion: In patients who enter the trial with unfavorable CTCs (five or more cells per 7.5 mL of blood), conversion to favorable status is defined as four or fewer cells per 7. mL of blood
- rPFS is defined per PCWG3 as time from day (D) 1 of treatment to the date when the first site of disease is found to progress (using a manifestation-specific definition off progression), or death, whichever occurs first.
    - The proportion of patients who remain radiographic progression free at 6 months is defined per PCWG3.
    - For patients with measurable soft tissue disease, ORR is defined as the proportion of patients with a CR or PR per PCWG3. DOR is measured from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that radiographic progression is documented per PCWG3.
- Time to first SRE is defined as the time from D1 of treatment to the date of first SRE.
- Time to clinical progression is defined as the time from D1 of treatment to the date of clinical progression.
- Time to initiation of new systemic treatment for prostate cancer is defined as the time from D1 of treatment to the date any new systemic treatment for prostate cancer is initiated.
- Overall survival is determined from the time of initiation of the combination therapy until death from any cause.

8.3 Pharmacokinetic Endpoints

- Observed serum concentration of enzalutamide at specified time points
- Observed serum concentration of venetoclax at specified time points

Note: *Following Phase Ib there will be a comprehensive analysis of venetoclax levels to assure that they are in the therapeutic range before proceeding with Phase II enrollment (refer to Section 19.6.1).*

A detailed PK profile of venetoclax will be determined when administered in combination with enzalutamide. PK interactions between venetoclax and enzalutamide will also be evaluated (see Section 11.5).

PK parameters will include: area under the concentration versus time curves (AUC), maximum concentration (C_{max}), time to maximum concentration (Tmax), minimum (trough) concentration (C_{trough}), elimination half-life (T1/2), volume of distribution (Vd)/ bioavailability or fraction absorbed (F), clearance (CL)/F, peak-to-trough ratio, and accumulation ratio.

Note: Adjustments will be made if PK characteristics for a given individual are outside the 95% predicted range for venetoclax or enzalutamide

8.4 Exploratory Endpoints

Non-inherited biomarkers that may be predictive of response to the study treatment will be identified from the following patient samples:

- Tumor tissue samples from pre-treatment core needle, excisional, or incisional tumor biopsy is required for confirmation of PCa diagnosis, and exploratory research on candidate biomarkers. Cytological or fine-needle aspiration samples are not acceptable:
    - Biopsies will be either (1) snap frozen for molecular characterization by RNA-sequencing, (2) processed by formalin fixation and paraffin embedding (FFPE) for biomarker analysis (see Table 1), or (3) enzymatically digested to generate live organoid cultures. Each form of processing will be used to assess pre- and post-treatment changes associated with combination enzalutamide/venetoclax.
- Tumor tissue samples obtained at the time of progression (unless no adequate tumor site is accessible) for exploratory research on candidate biomarkers (see **Table 1**). Samples will be processed similar to pre-treatment biopsies.
- Whole blood samples for isolation of peripheral blood mononuclear cells and circulating tumor cells (CTCs) for exploratory research on candidate biomarkers (see **Table 1**).

Exploratory biomarker research may include, but will not be limited to, the biomarkers listed in **Table 1**.

Table 1: Proposed Non-Inherited Biomarkers

Sample Type	Timing	Proposed Non-Inherited Biomarkers
Fresh pre-treatment, and post-treatment (progressed disease) tumor tissue	Prior to study (baseline, pre-treatment); at disease progression (post-treatment)	• Treatment related biomarkers: AR, ARv7, SYN, CHGA, BCL-2, BCL-XL, MCL-1, ABCB1, and ABCG2 • "Stemness" related gene expression (mRNA) or protein expression (IHC) changes associated with response or potential resistance.
Whole Blood Samples	Baseline and subsequent time points during and after treatment	• PBMC Analysis: BCL-2 expression by flow cytometry and WB. • CTC enumeration and biomarker analysis: Immunophenotyping with EPCAM, PSMA, PanCKs, CD45, AR, and BCL-2.

AR = androgen receptor; ARv7 = AR variant 7; SYN = synaptophysin; CHGA = chromogranin A; BCL-2 = B cell lymphoma 2; BCL-XL = B cell lymphoma – extra-large; MCL-1 = Myeloid cell leukemia 1; ABCB1 = ATP binding cassette subfamily B, member 1; ABCG2 = ABC subfamily G, member 2; IHC = immunohistochemistry; PBMC = peripheral blood mononuclear cell; WB = western blotting; CTC = circulating tumor cell; EPCAM = epithelial cell adhesion molecule; PSMA = prostate specific membrane antigen; PanCKs = pan-cytokeratins; CD45 = leukocyte common antigen. Note: Exploratory biomarker research may include, but will not be limited to, the biomarkers listed in this table.

9 DESIGN

This is a Phase Ib/II, open-label, multi-center, non-randomized study that will evaluate the safety, efficacy, and pharmacokinetics of venetoclax when administered with Enzalutamide in patients with metastatic castrate resistant prostate cancer (mCRPC). A maximum of 39 participants will be enrolled. The study schema is depicted in **Figure 7**.

Figure 7 Study Schema*

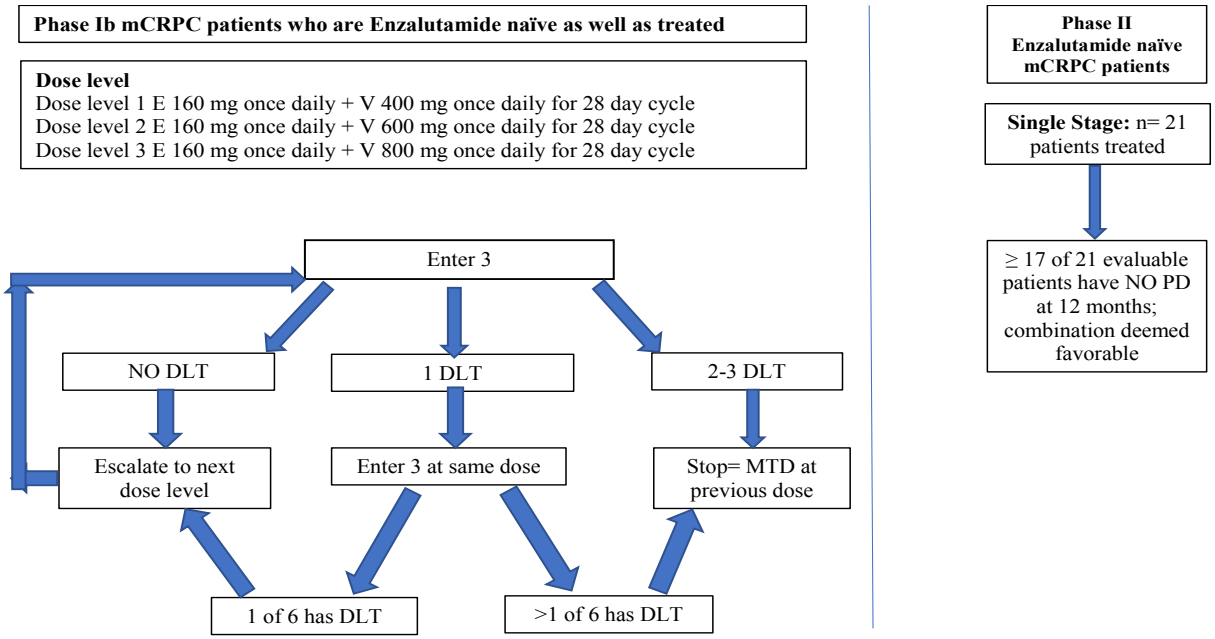


Figure 7: Schema of study design for Phase Ib/II. Radiographic assessments will be performed after every 12 weeks: Radiographic progression-free survival (rPFS) is a composite endpoint defined as the time from treatment start to disease progression in bone or soft-tissue, or death, whichever occurs first. All assessments of disease should be collected at the same time interval (e.g., bone scan, CT scan, and PSA at 12-week intervals). In addition to PSA, confirm posttreatment changes in measurable target lesions, radionuclide bone scans, and symptoms. Adverse events will be assesses using NCI-CTCAE v5.0.

E=Enzalutamide; V=Venetoclax; DLT=Dose Limiting Toxicity; MTD=Maximum Tolerated Dose; PD=Progressive Disease; mCRPC=Metastatic Castrate Resistant Prostate Cancer

***Note: There will be a 4 week "lead-in" with Enzalutamide alone in both the Phase Ib and Phase II components of the trial, prior to the addition of Venetoclax.**

The study will include an initial Phase IB dose-escalation trial followed by a Phase II single-stage trial during which venetoclax and enzalutamide will be administered at their recommended phase 2 doses (RP2D).

A maximum of 18 patients will be accrued in the Phase IB dose-escalation trial and another 21 patients will be accrued in the Phase II single-stage trial, for a maximum of 39 patients.

All patients will be closely monitored for adverse events throughout the study until the time of disease progression. Adverse events will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), Version 5.0. The data safety monitoring committee (DSMC) of the Roswell Park Early Phase Clinical Trials Committee will monitor patient safety throughout the study.

To characterize the PK properties of enzalutamide and venetoclax, blood samples will be obtained at various time points before and during study treatment administration (see Section 11.5).

Response and progression will be evaluated in this study using a combination of the international criteria proposed by the Response Evaluation Criteria in Solid Tumors version 1.1(RECIST 1.1)[40] and the guidelines for prostate cancer endpoints developed by the Prostate Cancer Clinical Trials Working Group (PCWG3).[41]. Refer to Section **11.2.4** for details on tumor assessments.

9.1 Design Rationale

9.1.1 Dose-Escalation Phase

The purpose of the Phase Ib dose-escalation phase is to identify the RP2D for venetoclax when combined with a fixed dose of Enzalutamide. The RP2D will be based on the maximum tolerated doses (MTDs) and the totality of data for venetoclax and enzalutamide.

Patients will be closely monitored for adverse events during a DLT assessment window, which will be defined as the first treatment cycle (from Day 1 of Cycle 1 to Day 1 of Cycle 2). Adverse events that meet the criteria for DLT, as defined in **Section 10.3**, will be reported to the Sponsor within 24 hours (see **Section 17.4**).

Patients who discontinue from the study prior to completion of the DLT assessment window for reasons other than a DLT will be considered non-evaluable for dose escalation decisions and MTD assessments and will be replaced by an additional patient at that same dose level. Patients who miss five consecutive daily doses of venetoclax during the DLT assessment window for reasons other than a DLT will also be replaced and considered non-evaluable for dose-escalation decisions.

The **Phase Ib portion** of this trial will follow a standard 3+3 design, starting with dose level 1, and escalating to the next higher dose cohort if 3 patients are treated and no DLTs are observed in the first cycle of therapy. DLT is defined in **Section 10.3**. If one DLT is recorded then three additional patients will be enrolled into the present cohort. If no further DLTs occur (1 out of 6 DLTs) then the next patient will be enrolled in the next higher cohort. If 2 out 6 patients have an observed DLT in any given cohort, 3 additional patients will be treated at one dose level lower, and if no additional DLTs are observed, this will be the maximum tolerated dose. The maximum

tolerable dose (MTD) is defined as the highest dose at which no more than 1 out of 6 patients experiences DLT in cycle 1, with a cycle duration of 28 days. If multiple toxicities are seen, the presence of DLT will be based on the most severe toxicity experienced.

Patients will also be monitored for tumor lysis (known to occur with venetoclax in hematologic malignancies). However in the context of solid tumors (including prostate cancer) tumor lysis is considered to be very unlikely.

If no DLTs are observed in all three cohorts then the RP2D will be dose level 3 for the Phase II study. At the completion of the Phase I portion of the study, the investigators will review the toxicity profile and determine the RP2D. PK levels of venetoclax will be evaluated following the completion of accrual in each cohort.

A small expansion cohort (n=3-6) of soft tissue disease will be enrolled at the MTD/RP2D to further evaluate the safety profile.

9.1.2 Phase II

Phase II is designed to further assess the safety and efficacy of venetoclax when combined with enzalutamide over a longer time period in patients with mCRPC.

All patients enrolling in this phase will be enzalutamide naïve. After a 4 week "lead in" phase with enzalutamide, oral venetoclax will be added at the RP2D. Cycle 1 will be the first 4 weeks of combination therapy.

Response and progression will be evaluated in this study using a combination of the international criteria proposed by the Response Evaluation Criteria in Solid Tumors version 1.1(RECIST 1.1)[40] and the guidelines for prostate cancer endpoints developed by the Prostate Cancer Clinical Trials Working Group (PCWG3) (refer to Section 11.2.4 for additional details on tumor assessments).

10 TREATMENT

One cycle of therapy = 28 days

Enzalutamide and venetoclax are both metabolized via CYP3A4. Furthermore, enzalutamide is a potent CYP3A4 inhibitor that can initially inhibit the metabolism of CYP3A4 substrates and subsequently induce their metabolism (including its own). Hence it is anticipated, that initially levels of both enzalutamide and venetoclax will fluctuate. In order to establish steady state levels of enzalutamide, patients will only be administered venetoclax after at least 4 weeks (1 cycle) of prior exposure to enzalutamide [37], as outlined in the study schema **Figure 7**.

10.1 Dosing and Administration

10.1.1 Enzalutamide

Enzalutamide will be administered at a starting dose of 160 mg (40 mg x 4 pills) orally once daily. Enzalutamide can be taken with or without food. Capsules must be swallowed whole, and should not be chewed, dissolved, or opened.

10.1.2 Venetoclax

After a minimum of 4 weeks prior exposure to enzalutamide, venetoclax will be administered at a starting dose of 400 mg and, the dose will be escalated from 400 mg, 600 mg, and 800 mg as shown below in **Table 2**.

Venetoclax will be taken orally once daily for 28 consecutive days. During cycle 1 and cycle 2 Venetoclax should be taken in the morning with breakfast, to accommodate for the PK schedule. Administration of Venetoclax can be taken at the time of day the patient prefers, as long as Venetoclax is taken with a meal for subsequent cycles.

Each dose of venetoclax will be taken orally, once daily, with approximately 240 mL of water approximately 30 minutes after the completion of a meal. A meal containing approximately 30% of the total caloric content from fat is recommended to ensure adequate absorption of venetoclax. The Standard American Heart Association Healthy (Low-Fat) Breakfast includes 1 box cereal (30-40 g), skim milk (240 mL), 1 boiled egg, 1 slice of toast, and margarine (10 g) for approximately 520 Kcal with approximately 17 grams of fat.

Patients must avoid consumption of grapefruit, Seville oranges or products containing the juice of each during the entire study and preferably 7 days before the first dose of study medications, due to potential CYP3A4 interaction with the study medications. Orange juice is allowed.

If vomiting occurs within 15 minutes after taking venetoclax and all expelled tablets are still intact, another dose may be provided. If tablets are not identified, or if any are not intact, no replacement dose is to be given. In cases where a dose of venetoclax is missed or forgotten, the patient should take the dose as soon as possible and ensure that the dose is taken with food within 8 hours after the scheduled time of the missed dose. Otherwise, the missed dose should not be taken. Patient compliance with the assigned daily dose of venetoclax will be assessed by standard pill counts. Bottles that contain venetoclax tablets will be given to patients at regular scheduled visits. Previously distributed bottles will be returned to the clinic, and tablets will be counted. Any discrepancy will be resolved with the patient at each clinic visit and documented in the patient record.

<u>On days with PK, ECG sampling, chemistry panel and/or lipid panel sampling, the following additional guidelines should be followed (Cycle 1 Day 1 and Cycle 2 Day 1 only):</u>

- On days that pre and post-dose PK sampling is required, the patient's first meal of the day (e.g., breakfast) will be consumed in the morning at the clinic, and venetoclax dosing (and immediately thereafter, enzalutamide) will occur in the clinic (under the supervision of the Investigator or designee) after completion of the meal to facilitate PK sampling. On all other days patients may take the study treatment at home.
- On a day of chemistry panel and/or lipid panel sampling, patients must be fasting from all food and drink for at least 8 hours overnight. Water is allowed during all fasting periods; however, coffee, tea and juice are not permitted during the fasting period. Patients must also take the study treatment in the clinic under the supervision of the Investigator or designee. On all other days patients may take the study treatment at home.
- Pre-dose samples should be drawn prior to dosing. The sampling time of the PK samples and the dosing time must be precisely recorded in the CRF. Furthermore, the dosing date

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and time the study medication was taken on the day before the PK assessment must be precisely recorded in the CRF (see

- **Appendix** J).
- Post-dose PK samples should be collected after study treatment dosing.

Guidelines for venetoclax dosage modification and treatment interruption or discontinuation are provided in **Section 10.4**. Reported adverse events (AEs) and potential risks are described in **Section 13**. Treatment is intended for an outpatient setting. However, at the investigator's/physician's discretion, the participant may receive treatment as an inpatient, if deemed necessary.

Table 2 Phase Ib Dose Escalation Scheme

Dose	Enzalutamide	Venetoclax
Level (-)1*	120 mg po QD	400 mg po QD
Level 1 (starting dose)	160 mg po QD	400 mg po QD
Level2	160 mg po QD	600 mg po QD
Level 3	160 mg po QD	800 mg po QD

Specific to the dose level 1 cohort: Dose-reduce enzalutamide to 120 mg or 80 mg if 2 or more DLTs are observed (and are determined to be attributable to enzalutamide) within the dose level 1 cohort (venetoclax will remain al Level 1 dose). If DL-1 is tolerable, we will consider escalating Venetoclax after discussion with AbbVie.

10.2 Cohort Management

Management of treatment cohorts/dose levels will be conducted by the Roswell Park's Early Phase Clinical Trials (EPCT) program.

10.2.1 Phase Ib: Dose Escalation

During the dose-escalation phase, the dose of venetoclax for each patient will depend on dose assignment (400 mg, 600 mg, or 800 mg). Enzalutamide will be dosed-reduced according to recommended guidelines.

10.2.2 Phase II: Expansion Phase

During the expansion phase, the dose of venetoclax for each patient will depend on the RP2D established during the Phase Ib dose-escalation phase. Enzalutamide will be administered according to recommended guidelines.

10.3 Definition of Dose-Limiting Toxicity

In this study, a DLT is defined as any one of the following events that occurs during the first cycle of the treatment combination and is assessed by the investigator as related to study treatment that is not attributed to disease progression or another clearly identified cause:

i) Any adverse event of any grade that leads to a delay of more than 14 days in the start of the next treatment cycle

ii) Any Grade 3 or 4 non-hematologic adverse event, **with the following exceptions:**

   a. Grade 3 diarrhea that responds to therapy within 72 hours

b. Grade 3 nausea or vomiting that occurs in the absence of premedication and responds to adequate therapy within 72 hours

c. Grade 3 fatigue that resolves to Grade < 2 within 7 days

d. Grade 3 laboratory abnormality that is asymptomatic and deemed by the investigator not to be clinically significant

iii) Hematologic adverse event that meets any of the following criteria:

a. Grade 3 or 4 neutropenia in the presence of sustained fever of > 38°C (lasting > 5 days) or a documented infection

b. Grade 4 neutropenia lasting > 7 days

c. Grade 3 or 4 thrombocytopenia that results in significant bleeding per investigator judgment

d. Grade 4 thrombocytopenia lasting > 7 days

Other toxicities occurring during the first cycle that are considered clinically relevant and related to study treatment, as determined by the investigator, may also be considered DLTs.

Management and dose modifications associated with the above AEs are outlined in **Section 10.4**.

10.4 Dose Modifications and Treatment Delays

Dose delays and modifications will be made as indicated below. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for dose delays and dose modifications.

10.4.1 Venetoclax dose adjustments for drug-related hematological adverse events (AEs)

Venetoclax has been associated with hematologic adverse events, including neutropenia. Therefore, the combination of enzalutamide and venetoclax is anticipated to may have overlapping hematologic toxicity and will be closely monitored. In addition to the standard hematologic monitoring, patients enrolled in this study will be closely monitored for evidence of infections.

There is the identified risk of TLS *occurring after initiation of treatment* with venetoclax, *with most cases occurring in hematological malignancies. TLS is rare in solid tumors and particularly rare in prostate cancer.* Even so, overlapping toxicity in regard to TLS cannot be excluded.

TLS in Non-CLL Oncology Indications

The risk of TLS is being closely monitored in non-CLL indications. The available data suggest that in non-CLL (with the exception of mantle cell lymphoma) subjects the risk of TLS is low but present. In general, before initiating venetoclax, subjects risk for developing TLS should be assessed. Prophylaxis with hydration and uric-acid reducing agents is recommended. Clinical chemistries should be corrected. Dose initiation and ramp up to reach the final dose is implemented based on the risk assessment in each of the non-CLL indications. Monitor clinical chemistries and manage electrolyte abnormalities promptly, as clinically indicated.

- Blood chemistry changes or symptoms suggestive of TLS (Any Occurrence):

- Withhold the next day's dose. If resolved within 24 to 48 hours of last dose, resume at the same dose.
- For any blood chemistry changes requiring more than 48 hours to resolve, resume at a reduced dose.
- For any events of clinical TLS* resume at a reduced dose following resolution.

* Clinical TLS defined as laboratory TLS with clinical consequences such as acute renal failure, cardiac arrhythmias, or sudden death and/or seizures.

Study-specific Monitoring Guidelines for TLS

All patients receiving venetoclax will be monitored closely for tumor lysis syndrome (TLS). The available data suggest that in non-CLL (with the exception of mantle cell lymphoma) subjects the risk of TLS is low but present. In general:

- Subjects at risk for developing TLS will be assessed
- Prophylaxis with hydration and uric-acid reducing agents will be assessed

Signs and symptoms of TLS (e.g., hyperuricemia, hyperkalemia, hypocalcemia, hyperphosphatemia, acute renal failure, and elevated LDH) will be closely monitored weekly through cycle 1 and on Day 1 of all subsequent cycles and when clinically indicated.

Neutropenia

Neutropenia is an important identified risk for venetoclax. Clinical data from the oncology studies suggest that the neutropenia adverse events are observed among subjects who receive venetoclax as a single agent or in combination with other therapeutic agents, with slightly higher frequency observed in some combination studies. Serious adverse events of neutropenia or neutropenia events that lead to discontinuations are few across the entire venetoclax oncology program. Granulocyte colony stimulating factors can be used for supportive measures; however the guidance for their use in non-venetoclax CLL indications, especially in AML, is per routine local oncology practice, as well as protocol-specific.

NOTE: Colony stimulating factors will only be permissible from Cycle 2 onwards, in the event of G4 febrile neutropenia of > 5 days in Cycle 1.

Monitor complete blood counts throughout the treatment period. Interrupt dosing or reduce dose for severe neutropenia. Consider supportive measures including antimicrobials for signs of infection and use of growth factors.

For Grade 3 or 4 neutropenia with infection or fever; or Grade 4 hematologic toxicities (except lymphopenia)

- *1st occurrence*: Interrupt venetoclax. To reduce the infection risks associated with neutropenia, granulocyte-colony stimulating factor (G-CSF) may be administered with venetoclax if clinically indicated. Once the toxicity has resolved to Grade 1 or baseline level, venetoclax therapy may be resumed at the same dose.
- *2nd and subsequent occurrences*: Interrupt venetoclax. Consider using G-CSF as clinically indicated. Follow dose reduction guidelines found in the venetoclax

package insert (Table 4) when resuming treatment with venetoclax after resolution. A larger dose reduction may occur at the discretion of the physician.

Infections

Infections have been reported in the oncology clinical studies; however, these events are confounded by the underlying disease, comorbidities, and other immunosuppressive medications. To date, no clear relationship has been noted between serious infectious events and neutropenia. The types of infectious events observed generally have been consistent with those anticipated in the elderly population of heavily pretreated subjects with hematologic malignancies and are similar across all indications. Infections are closely monitored in venetoclax program across all indications. The NCCN Clinical Practice Guidelines: Prevention and Treatment of Cancer-Related Infections will be followed, if clinically indicated, for anti-infective prophylaxis.[42]

10.4.2 Venetoclax dose adjustments for non-hematologic drug-related AEs

The guidelines outlined in **Table 3** are for dose interruptions and reductions for non-hematologic AEs considered related to venetoclax.

Table 3 Dose Adjustments for Non-Hematologic Drug-Related AEs

Grade	Occurrence	Dose modification
1 or 2	Any time	No dose reduction.
3 (or persistent grade 2: consider similar dose adjustments if persistent and not responding to optimal management in the opinion of PI and treating physician)	1st	• Hold venetoclax • Resume venetoclax at prior dose if recovery to ≤ Grade 1 or baseline occurs within 14 days. • If toxicity persists for 15-28 days, hold therapy and resume at ONE dose level below current dose (see Table 6-1) ONLY after recovery of toxicity to ≤ Grade 2. • Dose re-escalation to prior dose of venetoclax is permitted if tolerated and no recurrent Grade 3 or 4 toxicities occur over 4 weeks while on the lower dose level.
	2nd	• Hold venetoclax • Follow until toxicity ≤ Grade 2. • Resume at TWO dose levels below current dose for venetoclax ONLY after recovery of toxicity to ≤ Grade 2. • Dose re-escalation by one dose level every 4 weeks of venetoclax is permitted if tolerated and no recurrent Grade 3 or 4 toxicities occur over the 4 weeks while on the lower dose level.
	3rd	• Stop venetoclax and discontinue patient from trial. For patients with clinical benefit/response we will consider continuation if toxicities resolve to ≤ grade 1 and with proper dose adjustments after consultation with the PI)
4	Any time	• Stop venetoclax and discontinue patient from trial. For patients with clinical benefit/response we will consider continuation if toxicities resolve to ≤ grade 1 and with proper dose adjustments after consultation with the PI)

10.4.3 Dose modifications for enzalutamide

All dose adjustments should be made as per the local approved prescribing information and investigator's best judgment. An example from the prescribing information is provided below:

- If a patient experiences a ≥ Grade 3 toxicity or an intolerable side effect, withhold dosing for one week or until symptoms improve to ≤ Grade 2, then resume at the same or a reduced dose (120 mg or 80 mg), if warranted.

10.4.4 Concomitant Therapy, Prohibited Food, and Additional Restrictions

10.4.5 Permitted Therapy

Medications required to treat AEs, manage cancer symptoms, concurrent diseases and supportive care agents, such as pain medications, anti-emetics and antidiarrheal are allowed.

The patient must be told to notify the investigational site about any new medications he takes after the start of the study treatment. All medications (other than study drugs) and significant non-drug therapies (including vitamins, herbal medications, physical therapy and blood transfusions) administered within 30 days of study entry and during the study must be entered into the patient's eCRF.

Bisphosphonates and denosumab
Bisphosphonates and denosumab are permitted for the treatment of osteoporosis and prevention of skeletal related events for patients with bone metastases. Chronic concomitant bisphosphonate/denosumab therapy for the prevention of bone metastasis *is not* permitted.

Hematopoietic growth factors
Hematopoietic growth factors may be used according to ASCO guidelines.[43]

Palliative radiotherapy
Palliative radiation is permitted if done solely for bone pain relief. It should not be delivered to a target lesion and it should not encompass more than 25% of irradiated bone marrow.

Prophylactic treatment with antibiotics should be administered as per standard practice. Necessary supportive measures for optimal medical care will be given throughout the study according to institutional standards.

10.4.6 Prohibited and Cautionary Therapy for Concomitant Medications

Excluded Food (From3 Days Prior to Study Administration until Last Day of Treatment)

- Grapefruit and grapefruit products
- Seville Oranges (including marmalade containing Seville oranges)
- Starfruit

Excluded and Cautionary Medications

Please refer to **Appendix F** and **Appendix G** for examples of excluded and cautionary medications and suggested treatment guidelines**.**

Live attenuated vaccines should not be administered prior to, during, or after treatment with venetoclax until B-cell recovery occurs. The safety and efficacy of immunization with live attenuated vaccines during or following venetoclax therapy have not been studied. Patients should be advised that vaccinations may be less effective.

10.5 Duration of Treatment

Participants may remain on study and continue to receive treatment in the absence of disease progression, unacceptable toxicity and withdrawal from study, intercurrent illness that prevents further administration of treatment, participant demonstrates an inability/refusal to comply with oral medication regime or, participant withdraws from study.

10.5.1 Post-Trial Access to Venetoclax

Currently, AbbVie does not have any plans to provide venetoclax, or any other study treatments or interventions to patients who have completed the study. AbbVie will evaluate whether to continue to provide venetoclax in accordance to their Policy.

10.6 Compliance

Patients will be provided with a diary (**Appendix I**) in which to record their intake of venetoclax and will be asked to bring their diary (as well as venetoclax bottle) to each clinic visit. The actual number of tablets taken by the patient must be calculated from the number of tablets dispensed and returned. Patients must be instructed to return unused study drugs to the site at discontinuation or completion of treatment.

11 PROCEDURES INVOLVED

The study-specific assessments are detailed in this section and outlined in **Appendix D** [Table 1 (Phase Ib) and Table 2 (Phase II)]**:** Schedule of Procedures and Observations. Results of standard-of-care tests or examinations performed prior to obtaining informed consent, and within the defined window, may be used as screening and baseline assessments (see **Appendix D**); such tests do not need to be repeated for screening purposes (e.g., screening tumor assessment).

Study treatment should be initiated within 28 days after the Informed Consent Form has been signed.

Unless otherwise defined in the written protocol text, all procedures/assessments will be conducted in accordance with Roswell Park Clinical Research Services Standard Operating Procedures.

11.1 Participant Registration

Eligibility of each participant will be established prior to enrollment.

Informed consent ***MUST*** be completed prior to receiving any study related procedures.

- Informed Consent Forms for enrolled patients and for patients who are not subsequently enrolled will be maintained at the study site.
- All screening evaluations must be completed and reviewed to confirm that patients meet all eligibility criteria before enrollment. The investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable.

11.2 Study Assessments

11.2.1 Medical History and Demographic Data

Medical history includes clinically significant diseases, surgeries, reproductive status, smoking history, and alcohol and drug abuse. In addition, all medications (e.g., prescription drugs, over-the-counter drugs, herbal or homeopathic remedies, nutritional supplements) used by the patient within 7 days prior to the screening period will be recorded.

Demographic data will include age, sex, and self-reported race/ethnicity.

The following clinical parameters relative to disease history, diagnosis, and prognostic indices will be recorded at screening:

- Date of initial diagnosis
- ECOG Performance Status

- Previous lines of treatments and response to prior therapy, date of disease progression in relation to start date of prior therapy, and date of last dose of prior therapy

11.2.2 Physical Examinations

A complete physical examination will be performed at screening and will include an evaluation of the head, eyes, ears, nose, and throat, and the cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, genitourinary, and neurological systems. Any abnormality identified at baseline should be recorded on the General Medical History and Baseline Conditions eCRF.

At subsequent visits (or as clinically indicated), targeted (limited, symptom-directed) physical examinations should be performed. Targeted physical examinations should be limited to systems of primary relevance (e.g., cardiovascular and respiratory systems), systems associated with symptoms (newly emergent or monitored from baseline), and areas associated with tumor assessment.

Changes from baseline abnormalities should be recorded in patient notes. New or worsened clinically significant abnormalities should be recorded as adverse events on the Adverse Event eCRF.

11.2.3 Vital Signs

Vital signs will include measurements of respiratory rate, pulse rate, body temperature, and systolic and diastolic blood pressures while the patient is in a seated position. Height will be recorded at baseline only.

Vital sign measurements will be performed as outlined in the Schedule of Procedures and Observations (see **Appendix D**), but the associated data, other than the data collected at screening, does not need to be recorded on the eCRF (except in the case of an adverse event).

11.2.4 Tumor and Response Evaluations

11.2.4.1 Radiographic Assessments

A bone scan (99mTc-methylene diphosphonate radionuclide bone scintigraphy) should be done during Screening, Day 1 of every 3rd cycle ± 7 days (starting with Cycle 3-Day 1), and at the End of Treatment Visit.

Patients who discontinue study treatment for reasons other than radiographic progression will continue to have their disease evaluated by bone scan every 12 weeks until another anti-cancer treatment is started, radiographic progression of disease, death, loss to follow up or withdrawal of consent, whichever comes first.

If an appropriate bone scan was performed before the patient's screening for the study began, but was obtained within 6 weeks before C1D1, then that scan may be used as the baseline scan. Imaging data (imaging studies and derived assessments) will be stored according to usual practice and will be available upon request for review by the Sponsor or an independent radiology reviewer.

Imaging of the chest, abdomen, and pelvis using a CT scan with contrast with ≤ 5 mm axial slices is advised for all patients. For those intolerant of contrast, a cross-sectional MRI scan of the abdomen and pelvis, with a noncontrast CT scan of the chest, may be considered.

- If appropriate CT/MRI scans were performed before the patient's screening for the study began, but were obtained within 6 weeks before C1D1, then those scans may be used as the baseline scans.
- These will be done during Screening, Day 1 of every 3rd cycle ± 7 days (starting with Cycle 3-Day 1) and at the End of Treatment Visit.

Patients who discontinue study treatment for reasons other than radiographic progression will continue to have their disease evaluated by CT/MRI every 12 weeks until another anti-cancer treatment starts, radiographic progression of disease, death, loss to follow up or withdrawal of consent (whichever occurs first).

11.2.4.2 Hematology and Chemistry Assessments

- Hematology (CBC with automated differentials): WBC, RBC, HGB, HCT, platelet, MCV, MCH, MCHC, % neutrophils, absolute neutrophils, % monocytes, absolute monocytes, % eosinophils, absolute eosinophils, % basophils, absolute basophils, % lymphocyte, absolute lymphocyte, platelet confirmation (as clinically indicated), and differential confirmation (as clinically indicated).
- Chemistry (CMP): chloride, CO_2, potassium, sodium, BUN, glucose, calcium, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT, A/G ratio, BUN/creatinine ratio, osmol (Calc), anion gap).
    - LDH
    - Serum PSA
- Coagulation: prothrombin time (PT) or International normalized ratio (INR)
- Lipid panel: cholesterol (CHOL), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides (TRIG)
- Urinalysis
- Serum testosterone
- Phosphorus
- Uric Acid

11.2.5 Electrocardiograms

Single, resting, 12-lead ECG recordings will be obtained at screening, as outlined in the Schedule of Procedures and Observations (see **Appendix D**), and may be obtained at unscheduled time points as clinically indicated.

ECGs for each patient should be obtained using the same machine wherever possible.

Interpretation of the ECG should be performed by the investigator.

11.2.6 Multigated Acquisition Scan/Echocardiogram

MUGA scans or Echocardiogram may be used.

11.2.7 Pulmonary Function Test (PFT) Screen Spirometry or PFT without Bronchodilator

PFT Screen Spirometry and PFT without Bronchodilator may be used.

11.3 Post-Treatment Follow-Up Evaluations

Follow-up safety evaluations will occur 30 days (± 3 days) after last dose of study drug or until resolution of any drug-related toxicity (telephone contact is acceptable).

Patients who discontinue treatment for reasons other than disease progression will undergo assessments every 3 months during the post treatment follow-up period, which will continue until disease progression, the start of new CRPC treatment, or the end of the study (as defined in **Section 11.4**), whichever occurs first.

Patients who experience disease progression will be evaluated for survival status and initiation of new treatment every 3 months until the end of the study.

Participants who are unavailable for follow-up evaluations should be classified as lost to follow-up for 1 of the following reasons:

- Lost to follow-up: For a participant to be considered lost to follow-up, the investigator must make two separate attempts to re-establish contact with the participant. The attempts to re-establish participant contact must be documented (e.g., certified letter).
- Death: Date and cause of death will be recorded for those participants who die within 30 days after last dose of study drug (telephone verification is acceptable).

11.4 End of Study

The end of this study is defined as the time when the following criteria are met:

- All enrolled mCRPC patients will be followed till death or at least 1 year after they have discontinued study treatment, whichever occurs first.

11.5 Pharmacokinetic Blood Sample Collection and Processing

Blood samples for Pharmacokinetic analysis of venetoclax and enzalutamide (and its metabolite, metabolite N-desmethyl-enzalutamide) levels will be collected via venipuncture) using 1, 10 mL, purple-top tube (K_2EDTA) per time point.

Note: Beginning with Cycle 1-Day 8 there is a window of (± 2 days) for assessments.

Phase I

Pharmacokinetic sample collection will be obtained on:

- Cycle 1-Day 1:
  - Pre-dose: Prior to daily enzalutamide and initial dose of venetoclax
  - 1 hr (± 15 min), 2 hr (± 15 min), 4 hr (± 15 min), 6 hr (± 15 min), 8 hr (± 15 min), following enzalutamide and venetoclax combination
- Cycle 1-Day 2: Prior to daily dose of enzalutamide and venetoclax. ***Note***: Blood draw must be **within 22-26 hr (± 1 hr)** from the time of drug dosing on Cycle 1-Day 1.

- Cycle 1-Day 8: Pre-daily dose of enzalutamide and venetoclax
- Cycle 1-Day 15: Pre-daily dose of enzalutamide and venetoclax
- Cycle 1-Day 21: Pre-daily dose of enzalutamide and venetoclax
- Cycle 2-Day 1: Pre-dose (prior to daily enzalutamide and initial dose of venetoclax taken at the same time), 1 hr (± 15 min), 2 hr (± 15 min), 4 hr (± 15 min), 6 hr (± 15 min) and, 8 hr (± 15 min) following enzalutamide and venetoclax combination.
- Cycle 3-Day 1: Pre-daily dose of enzalutamide and venetoclax.

Phase II

Pharmacokinetic sample collection will be obtained on:

- Cycle 1-Day 1:
    - Pre-dose: Prior to daily enzalutamide and initial dose of venetoclax
    - A sample within the window of 2-4 hr, following enzalutamide and venetoclax combination
- Cycle 1-Day 2: Prior to daily dose of enzalutamide and venetoclax. Note: Blood draw must be within 22-26 hr (± 1 hr) from the time of drug dosing on Cycle 1-Day 1.
- Cycle 1-Day 8: Pre-daily dose of enzalutamide and venetoclax
- Cycle 1-Day 15: Pre-daily dose of enzalutamide and venetoclax
- Cycle 1-Day 21: Pre-daily dose of enzalutamide and venetoclax
- Cycle 2-Day 1: Pre-dose (prior to daily enzalutamide and venetoclax taken at the same time), and a sample within the window of 2-4 hr, following enzalutamide and venetoclax combination.
- Cycle 3-Day 1: Pre-daily dose of enzalutamide and venetoclax

NOTE: ***On days when indicated that blood draw is pre-dose, patients will be instructed to delay their daily dose of enzalutamide and venetoclax until blood has been drawn. In addition, the PK questionnaire*** **(**

Appendix J***) is to be completed before every pre-dose blood draw***.

Samples will be processed in the Hematological procurement facility. Plasma will be separated from whole blood and aliquoted into 2, 1.8 mL cryovials per time-point. The screw cap polypropylene cryogenic tube will be labeled with the participant's MR number, participant's initials, participant's study number, clinical study number, protocol time point, dose, and protocol day.

The processed samples will immediately be frozen at -80ºC or below and sent on dry ice to Dr. Dean Tang's laboratory for storage (see Section 11.6.1 for laboratory location and contacts). Frozen samples will then be batch shipped from Dr. Tang's laboratory (upon completion of each cohort) to Dr. Beumer (Director of the UPMC Hillman Cancer Center) for analysis. Samples taken at UPMC will be kept for storage and processing at UPMC in Dr. Beumer's lab.

Jan Hendrick Beumer, Pharm.D., Ph.D., D.A.B.T.
Director Cancer Pharmacokinetics and Pharmacodynamics Facility
UPMC Hillman Cancer Center
Research Pavillon, Suite G.27e
5117 Centre Avenue
Pittsburgh, PA 15213-1863
Tel. office: (412) 623-3216
Tel. lab: (412) 623-3248
Fax: (412) 623-1212
Email: beumerjh@upmc.edu

For additional information regarding the handling of pharmacokinetic samples please contact Darren Gilmartin at (412) 623-1213.

Note: All investigator or analyzing research laboratories housing research samples need to maintain current **Temperature Logs** and study-specific **Sample Tracking and Shipping Logs**. The Principal Investigator/Laboratory Manager **must** ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

*Please see **Appendix E** for additional information on the pharmacokinetic sampling and time point rationale.

11.6 Correlative Studies

11.6.1 Correlative Blood Sampling

Blood samples will be collected via venipuncture for isolation of peripheral blood mononuclear cells and plasma for exploratory research on candidate biomarkers. Samples will be collected using 4, 10 mL purple top K_2EDTA collection tubes.

Samples for the correlative analyses will be obtained on:

- Pre-treatment (prior to initiation of 28 day enzalutamide-only treatment)
- Cycle 1-Day 1:Pre-dose (prior to daily enzalutamide and initial dose of venetoclax)

- Cycle 3-Day 1: Pre-dose (prior to daily enzalutamide and venetoclax)
- Cycle 6-Day 1: Pre-dose (prior to daily enzalutamide and venetoclax)
- Cycle 9-Day 1: Pre-dose (prior to daily enzalutamide and venetoclax)
- Cycle 12-Day 1: Pre-dose (prior to daily enzalutamide and venetoclax)
- Day 1 of every 3rd cycle following Cycle 12 (i.e., Cycle 15-Day 1, …) and at End of treatment or disease progression (whichever occurs first)

The collection tubes will be labeled with the participant's MR number, participant's initials, participant's study number, clinical study number, protocol time point, dose, and protocol day. Dr. Tang's lab will be notified immediately upon receipt of sample by Heme Procurement, and samples will be picked up immediately for processing by lab personnel:

Roswell Park Comprehensive Cancer Center
Tang Laboratory
CGP 4th floor, Rm. L4-126
Attn: Study Number – I 63428
Elm & Carlton Streets
Buffalo, NY 14263
Office: 716-845-1254
Lab: 716-845-3058
Fax: 716-845-8857
Dean.Tang@roswellpark.org
Jason.Kirk@roswellpark.org

For any questions regarding specimen handling, please contact Jason Kirk at (716) 845-3058 or email at Jason.Kirk@RoswellPark.org

NETWORK SITES: Follow directions above for sample collection. One of the four collection tubes should be processed for PBMCs. PBMCs can be pelleted and snap frozen as a pellet. PBMC samples can be frozen and batched shipped to the above address upon request. The remaining 3 collection tubes should be collected and immediately shipped to Roswell Park Overnight at ambient temperature for processing. The sample tubes will be labeled with the Subject ID # (unique to Network patients), initials, the participant's study number, clinical study number, protocol time point, dose, and protocol day.

Note: PBMC samples cannot be frozen in glass tubes – cryogenic vials must be used.

Frozen samples will be shipped via Fed Express Overnight on dry ice with delivery on Mon-Fri. NO SATURDAY DELIVERY. Do not ship on a Friday or the day before a holiday.

Address shipments and any questions regarding specimen processing to:

Jason S. Kirk
Roswell Park Comprehensive Cancer Center
CGP, L4-126
Elm and Carlton Streets
Buffalo, NY 14263
Office: 716-845-3058

Cell: 716-481-3327

Jason.kirk@roswellpark.org

Note: All investigator or analyzing research laboratories housing research samples need to maintain current **Temperature Logs** and study-specific **Sample Tracking and Shipping Logs**. The Principal Investigator/Laboratory Manager **must** ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

11.7 Pathology

11.7.1 Pre-treatment tissue

In the presence of soft tissue or visceral metastatic disease, a tumor biopsy may be performed at screening, as would be dictated by standard of care.

The following tissue samples to be used for the correlative analyses will be requested if available, but are not mandatory for enrollment:

- One (1) core of fresh tissue to be transported on ice to Dr. Tang's laboratory for processing and analysis. Media for transport will be provided by Dr. Tang's laboratory prior to study initiation.
- One (1) core of flash-frozen tissue to be transported to Dr. Tang's laboratory on dry ice.
- One (1) core FFPE tissue.

For **Network Sites,** de-identified tissue samples using study-specific subject ID number and tissue accession# (GCP requires at least 2 identifiers) are to be sent to RPCI Correlative Science Pathology Office (Attn: Protocol Lab Team). The shipping label should read as follows:

De-identified samples using study-specific subject ID number and date collected are to be sent to Roswell Park Correlative Science Pathology Office (Attn: Protocol Lab Team).

Roswell Park Comprehensive Cancer Center
Elm & Carlton Streets
Correlative Science Pathology Office
Attention: Protocol Lab Team
Gratwick Basic Science Building, S-636
Attn: Protocol Lab Team, I 63418 Samples
Buffalo, NY 14263
(716) 845-8917

Email: CRSLabPathTeam@RoswellPark.org

Samples should then be forwarded to Dr. Tang's laboratory for additional processing and analysis. . Fresh tissue samples should be sent to Dr. Tang's lab as soon as possible from the time of biopsy.

11.7.2 Tissue biopsy at time of disease progression

A second *optional* tumor biopsy will be requested for subjects at the time of disease progression:

- One (1) core fresh tissue to be transported on ice to Dr. Tang's laboratory for processing and analysis. Media for transport will be provided by Dr. Tang's laboratory prior to study initiation.
- One (1) core flash-frozen tissue to be transported to Dr. Tang's laboratory on dry ice.
- One (1) core FFPE tissue.

De-identified samples using study-specific subject ID number and tissue accession# are to be forwarded to Dr. Tang's laboratory for additional processing and analysis.

Roswell Park Comprehensive Cancer Center
Tang Laboratory
CGP 4th floor, Rm. L4-126
Attn: Study Number – I 63428
Elm & Carlton Streets
Buffalo, NY 14263
Office: 716-845-1254
Lab: 716-845-3058
Fax: 716-845-8857
Dean.Tang@roswellpark.org
Jason.Kirk@roswellpark.org

For any questions regarding specimen handling, please contact Jason Kirk at (716) 845-3058 or email at Jason.Kirk@RoswellPark.org

12 WITHDRAWAL OF SUBJECTS

12.1 Treatment Discontinuation

Upon treatment discontinuation all end of treatment evaluations and tests will be conducted. All participants who discontinue due to an AE must be followed until the event resolves or stabilizes. Appropriate medical care should be provided until signs and symptoms have abated, stabilized, or until abnormal laboratory findings have returned to acceptable or pre-study limits. The final status of the AE will be reported in the participant's medical records and the appropriate eCRF.

Patients have the right to voluntarily withdraw from the study at any time for any reason. In addition, the investigator has the right to withdraw a patient from the study at any time.

Reasons for withdrawal from the study may include, but are not limited to, the following:

- Death
- Progressive disease
- Toxicity; treatment related or unrelated

- Investigator judgment
    - The Investigator may discontinue a participant if, in his/her judgment, it is in the best interest of the participant to do so.
- Noncompliance
- Participant voluntary withdrawal
    - A participant may withdraw from the study at any time, for any reason. If a participant discontinues treatment, an attempt should be made to obtain information regarding the reason for withdrawal.
- Sponsor decision.

Every effort should be made to obtain information on patients who withdraw from the study. The primary reason for withdrawal from the study should be documented on the appropriate eCRF. However, patients will not be followed for any reason after consent has been withdrawn. If a patient withdraws consent, this request must be documented in the source documents and signed by the investigator. Study personnel may use a public information source (e.g., county records) to obtain information about survival status.

12.2 Study Treatment Discontinuation

Study treatment should be permanently discontinued in patients who experience any of the following:

- Anaphylaxis, acute respiratory distress
- Any adverse event that meets criteria for permanent discontinuation per guidelines provided in Section 10.4
- Disease progression

The primary reason for study treatment discontinuation should be documented on the appropriate eCRF.

Patients who discontinue study treatment will not be replaced, except as outlined below:

- During the dose-escalation phase, patients who discontinue study treatment prior to completion of the DLT assessment window for reasons other than a DLT will be replaced by an additional patient at that same dose level.
- Patients who discontinue before having received at least one dose of each component of the combination will be replaced.

12.3 Study and Site Discontinuation

The provider of venetoclax (AbbVie) has the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- The incidence or severity of adverse events in this or other studies indicates a potential health hazard to patients.
- Patient enrollment is unsatisfactory.

AbbVie will notify the investigators if they decide to discontinue the study. AbbVie has the right to terminate the study at any time. Reasons for terminating a study may include, but are not limited to, the following:

- Excessively slow recruitment
- Poor protocol adherence
- Inaccurate or incomplete data recording
- Non-compliance with the International Conference on Harmonization (ICH) guideline for GCP
- No study activity (i.e., all patients have completed the study and all obligations have been fulfilled)

13 RISKS TO SUBJECTS

13.1 Enzalutamide

The most common adverse reactions (≥ 5%) that have been reported for enzalutamide are: asthenia/fatigue, back pain, diarrhea, arthralgia, hot flush, peripheral edema, musculoskeletal pain, headache, upper respiratory infection, muscular weakness, dizziness, insomnia, lower respiratory infection, spinal cord compression and cauda equina syndrome, hematuria, paresthesia, anxiety, and hypertension.

13.2 Venetoclax

The most common adverse reactions (≥20%) that have been reported for venetoclax (in CLL patients) are: neutropenia, diarrhea, nausea, anemia, upper respiratory tract infection, thrombocytopenia, and fatigue.

14 POTENTIAL BENEFITS TO SUBJECTS

The addition of venetoclax to enzalutamide has the potential to reverse enzalutamide resistance (in Phase 1B) and delay Enzalutamide resistance (in Phase 2). In men with metastatic CRPC, the development of enzalutamide resistance is inevitable, with limited subsequent treatment options. Thus, all interventions that prolong response to enzalutamide may potentially have a survival advantage, over and above current standard of care therapies.

15 DATA AND SPECIMEN BANKING

Samples taken for pharmacokinetic analysis will be stored in Dr. Tang's laboratory at Roswell Park until being shipped to Jan Hendrick Beumer, Pharm.D., Ph.D., D.A.B.T (Director Cancer Pharmacokinetics and Pharmacodynamics Facility) at the UPMC Hillman Cancer Center. Upon completion of sample assays the analyzing laboratory will provide results to Roswell Park Biostatistics and to Roswell Park Data Management in a SAS compatible EXCEL file.

Any clinical data that is associated with the samples will be stored on a secured server, will be accessible only by the PI, co-investigators and PI-designated data manager and, will be password protected. All computer entry and networking programs will be done using PIDs only.

Note: All investigator or analyzing research laboratories housing research samples need to maintain current Temperature Logs and study-specific Sample Tracking and Shipping Logs. The Principal Investigator/Laboratory Manager must ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

16 MEASUREMENT OF EFFECT

16.1 Solid Tumors

Response and progression will be evaluated in this study using a combination of the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [40], and the guidelines for prostate cancer endpoints developed by the Prostate Cancer Clinical Trials Working Group 3 (PCWG3) [41].

Tumor response will be assessed after the 3rd cycle and every 12 weeks thereafter using PCWG3 criteria. Conventional RECIST 1.1 will also be documented during the trial however; RECIST 1.1 will not be the primary criteria used to determine disease progression.

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.

For the purposes of this study, patients should be re-evaluated for response every 12 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 12 weeks following initial documentation of objective response.

- PCWG3 will be used to evaluate PSA response and progression as well as progression on bone scans.
- RECIST 1.1 will be used to assess response and progression for nodal and visceral metastasis

16.2 Other Response Parameters

- PSA Progression-free survival: Defined as PSA progression of $\geq$ 25% increase and $\geq$ 2ng/mL increase from baseline beyond 12 weeks
- OPTIONAL:
    - Radiographic progression-free survival (rPFS) is a composite endpoint defined as the time from treatment start to disease progression in bone or soft-tissue, or death, whichever occurs first. All assessments of disease should be collected at the same time interval (e.g., bone scan, CT scan, and PSA at 12-week intervals). In addition to PSA, confirm posttreatment changes in measurable target lesions, radionuclide bone scans, and symptoms.

17 SAFETY EVALUATION

17.1 Adverse Events

An adverse event or adverse experience (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Therefore, an AE can be ANY unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product (attribution of 'unrelated', 'unlikely', 'possible', 'probable', or 'definite').

An AE is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan in other study-related documents.

17.1.1 Diagnosis Versus Signs and Symptoms

If known, a diagnosis should be recorded on the CRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be clinically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE or SAE on the CRF. If a diagnosis is subsequently established, it should be reported as follow-up information.

17.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the CRF. However, clinically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the CRF. For example, if a severe gastrointestinal hemorrhage leads to renal failure, both events should be recorded separately on the CRF.

17.1.3 Abnormal Laboratory Values

Only clinically significant laboratory abnormalities that require active management will be recorded as AEs or SAEs on the CRF (e.g., abnormalities that require study drug dose modification, discontinuation of study treatment, more frequent follow-up assessments, further diagnostic investigation, etc.).

If the clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 x the upper limit of normal associated with cholecystitis), only the diagnosis (e.g., cholecystitis) needs to be recorded on the Adverse Event CRF.

If the clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded as an AE or SAE on the CRF. If the laboratory abnormality can be characterized by a precise clinical term, the clinical term should be recorded as the AE or

SAE. For example, an elevated serum potassium level of 7 mEq/L should be recorded as "hyperkalemia".

Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded as AEs or SAEs on the CRF, unless their severity, seriousness, or etiology changes.

17.1.4 Preexisting Medical Conditions (Baseline Conditions)

A preexisting medical condition should be recorded as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on an Adverse Event CRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

17.2 Grading and Reporting Adverse Events

17.2.1 Grading and Relationship to Drug

The descriptions and grading scales found in the CTEP Version 4 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. CTEP Version 5.0 of the CTCAE is identified and located at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

AEs not covered by specific terminology listed should be reported with common medical terminology, and documented according to the grading scales provided in the CTCAE Version 5.0. The relationship of event to study drug will be documented by the Investigator as follows:

- **Unrelated:** The event is clearly related to other factors such as the participant's clinical state, other therapeutic interventions or concomitant drugs administered to the participant.
- **Unlikely:** The event is doubtfully related to investigational agent(s). The event was most likely related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Possible:** The event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the participant's clinical state, other therapeutic interventions or concomitant drugs.
- **Probable:** The event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The event cannot be reasonably explained by other factors such as the participant's clinical state, therapeutic interventions or concomitant drugs.
- **Definite:** The event follows a reasonable temporal sequence from the time of drug administration, follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the participant's condition, therapeutic interventions or concomitant drugs; AND occurs immediately following study drug administration, improves upon stopping the drug, or reappears on re-exposure.

17.2.2 Reporting Adverse Events

Routine AEs occurring between the start date of intervention until 30 days after the last intervention, or until the event has resolved, the study participant is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible, will be reported. New information will be reported after it is received.

Guidelines for Routine Adverse Event Reporting for Phase 1 Studies (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated	X	X	X	X
Unlikely	X	X	X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

Guidelines for Routine Adverse Event Reporting for Pilot, Phase 2, and Phase 3 Studies (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated			X	X
Unlikely			X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

17.3 Serious Adverse Events

A serious adverse event (SAE) is any adverse event (experience) that in the opinion of either the investigator or sponsor results in **ANY** of the following:

- Death.
- A life-threatening adverse event (experience). Any AE that places a participant or participants, in the view of the Investigator or sponsor, at immediate risk of death from the reaction as it occurred. It does NOT include an AE 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 or birth defect.

- Important Medical Event (IME) that, based upon medical judgment, may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

17.3.1 Reporting Serious Adverse Events

All new SAEs occurring from the date the participant signs the study consent until 30 days after the last intervention or a new treatment is started, whichever comes first, will be reported. The ROSWELL PARK SAE Source Form is to be completed with all available information, including a brief narrative describing the SAE and any other relevant information.

SAEs occurring after the 30 day follow-up period that the investigator determines to be possibly, probably or definitely related to the study intervention should be reported.

SAEs identified as an Unanticipated Problem by the Investigator must be reported. Please refer to Section 17.6 for details on reporting Unanticipated Problems.

17.4 Investigator Reporting: Notifying AbbVie

In addition to compliance with all FDA reporting requirements pursuant to 21 CFR 312, the Principal Investigator shall:

- Report to AbbVie all serious adverse events experienced by a study subject receiving an AbbVie product within 24 hours of learning of the event regardless of the relationship of the event to the AbbVie product. The Principal Investigator shall make available to AbbVie promptly such records as may be necessary and pertinent to investigate any such event, if specifically requested by AbbVie; and in addition, report all non-serious adverse events of tumor lysis syndrome for studies involving venetoclax.

And,

- Notify AbbVie upon any subjects receiving an AbbVie Product whose pregnancy has resulted in a negative outcome or untoward event during the course of pregnancy or upon delivery.

AbbVie's contact for reporting serious adverse drug experiences, pregnancy experiences, and non-serious adverse events of tumor lysis syndrome shall be:
PPDINDPharmacovigilance@abbvie.com

Product Complaints

In addition to compliance with all FDA requirements pursuant to 21 CFR 211 and 21 CFR 820, the Principal Investigator will report to AbbVie within 24 hours any suspected quality defect in an AbbVie Product or its AbbVie-provided packaging, labeling, or medical device component (collectively, "Product Complaint"). The Principal Investigator will report Product Complaints that involve an AbbVie Product, whether AbbVie has supplied the AbbVie Product used in the Study or not. AbbVie's contact for reporting Product Complaints shall be
RD_PQC_QS@abbvie.com

17.5 Follow-Up for Serious Adverse Events

All related SAEs should be followed to their resolution, until the study participant is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible. New information will be reported when it is received.

17.6 Unanticipated Problems

An Unanticipated Problem (UP) is any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given:
  a) The research procedures that are described in the study-related documents, including study deviations, as well as issues related to compromise of participant privacy or confidentiality of data.
  b) The characteristics of the participant population being studied.
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research).
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized and if in relation to an AE is also deemed Serious per Section 17.3.

17.6.1 Reporting Unanticipated Problems:

The Reportable New Information (RNI) Form will be submitted to the CRS Compliance Office within 1 business day of becoming aware of the Unanticipated Problem. After review, CRS Compliance will submit the RNI to the IRB.

When becoming aware of new information about an Unanticipated Problem, submit the updated information to CRS Compliance with an updated Reportable New Information Form. The site Investigator or designated research personnel will report all unanticipated problems to the IRB in accordance with their local institutional guidelines.

18 DATA MANAGEMENT AND CONFIDENTIALITY

18.1 Data Collection

Full build studies are managed by Roswell Park CRS Data Management for analysis by Roswell Park Biostatisticians. All electronic case report form (eCRF) data are captured for these studies.

Data management activities are performed using a CTMS system that enables the collection, cleaning and viewing of clinical trial data. CRS data management designs the study-specific database and facilitates development by the Information Technology team. Once the database design is approved by the Investigator, Statistician, and Clinical Research Coordinator, the database is put into production and data entry can begin. Data can be entered and changed only by those with the rights to do so into the eCRFs.

18.2 Maintenance of Study Documents

Essential documents will be retained per Roswell Park's policy for 6 years from the study termination date. These documents could be retained for a longer period, however, if required by the applicable local regulatory requirements or by an agreement with Roswell Park.

18.3 Revisions to the Protocol

ROSWELL PARK may make such changes to the protocol as it deems necessary for safety reasons or as may be required by the U.S. FDA or other regulatory agencies. Revisions will be submitted to the IRB/ERC for written approval before implementation.

18.4 Termination of the Study

It is agreed that, for reasonable cause, either the Roswell Park Investigators or AbbVie, may terminate this study, provided a written notice is submitted within the time period provided for in the Clinical Trial Agreement. In addition, Roswell Park may terminate the study at any time upon immediate notice if it believes termination is necessary for the safety of participants enrolled in the study.

18.5 Confidentiality

Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a limited access environment. All computer entry and networking programs will be done using PIDs only. Information will not be released without written authorization of the participant.

19 STATISTICAL PLAN

The study will include an initial Phase IB dose-escalation trial followed by a Phase II single-stage trial during which venetoclax and enzalutamide will be administered at their recommended phase 2 doses (RP2D).

Proposed Statistical Analysis:

The **Phase 1 portion** of this trial will follow a standard 3+3 design starting at dose level 1 (refer to **Table 2**: Section 10.1.2). The given dose level cohort will start with three patients, with three patients added to any cohort if we observe 1 out of 3 dose limiting toxicities (DLTs). If 2 out of 3 to 6 subjects have a DLT we will not proceed to the next cohort.

We anticipate enrolling 15 to 18 patients in the Phase Ib portion. Prior exposure to enzalutamide and/or other treatments for CRPC will be permitted.

The dosing regimen for the Phase II portion of the trial will be informed from the Phase Ib portion of the trial. The primary endpoint ***will be PFS rates at 12 months.*** Only enzalutamide-naïve patients will be accrued.

The design for the Phase II component of this trial will consist of using a single arm one-stage design for enzalutamide naïve subjects. The primary endpoint will be 12 month progression free survival fractions. A true response of greater than greater than 0.65 (one-sided) is considered

efficacious and evidence of such will deem the treatment worthy of further study in that population. The primary test will be carried out using a one-sided exact binomial test.

19.1 Sample Size Determination

A maximum of 39 participants (Phase Ib and Phase II) will be enrolled in this study.

In the Phase 1B portion, a total of 15 to 18 patients will be enrolled, depending on the dose-limiting toxicity relationship. We anticipate accruing 3 patients per month.

In the Phase 2 portion, the sample size calculation is based on testing the hypotheses concerning the proportion of the population with a response to the treatment. The nominal significance level of this design is alpha=0.10. This one-stage design requires 21 subjects for the enzalutamide-naïve cohort in order to achieve approximately 1-beta=0.80 power to detect differences of 0.20 percentage points greater than the null hypothesized value (rate of 0.85 v 0.65). Thus if there are greater than or equal to 17 responses out of 21 (0.81), the trial will be deemed to be positive.

This effect size was determined based on Beer et al. (2014), where the difference in the 12 month PFS rate between enzalutamide and placebo was 65%-14%=51%. We designed the current trial to detect an effect size less than half of the original study at 85%-65%=20% relative to a target for moving this investigation forward into the Phase III setting.

We anticipate accruing 2-3 patients per month in this phase.

Accrual is expected to take approximately 24 to 30 months.

The endpoints for both the Phase 1b and Phase II study are summarized in **Section 8**.

19.2 Demographics and Baseline Characteristics

Descriptive statistics (as appropriate: n, percent, mean, median, min and max) will be used to summarize demographic and baseline characteristics.

19.3 Efficacy Analysis

The efficacy of the combination will be evaluated on the PCWG 3 criteria for response to treatment. Objective tumor response will be tabulated overall (and by dose level if appropriate). Only participants who have completed at least 2 cycles will be evaluable for response.

Secondary Analysis

The Kaplan-Meier product-limit estimator will be used to estimate PFS and OS distributions. Secondary examinations the association of PK and biomarker data with PFS and OS time will be carried out using a Cox regression model. PK and biomarker data will be summarized using standard descriptive statistics.

19.4 Safety Analysis

DLTs are the primary endpoint of this study and are used in the estimation of the MTD and the accompanying of the dose escalation decisions. However, no formal analyses of DLTs are planned. Participants who do not have a DLT and who do not complete a full cycle of treatment will be considered non-evaluable for DLT.

19.4.1 Adverse Event

The frequency of toxicities will be tabulated by grade across all dose levels and cycles. The frequency of toxicities will also be tabulated for the dose estimated to be the MTD. All participants who receive any study treatment will be considered evaluable for toxicity.

19.5 Interim Analysis and Criteria for Early Termination of the Study

The Phase 1B portion of the study will be monitored and discussed by Roswell Park's Early Phase Clinical Trials Committee, which meets on a regular basis per the Roswell Park Data Safety Monitoring Plan. Drug safety will be monitored and evaluated continuously throughout the study including 30 day safety follow-up period by obtaining, reviewing and analyzing data on AEs, changes in laboratory values, vital signs, electrocardiograms (ECGs), and physical examination findings. Potential early termination decisions are an inherent part of the Phase 1 study monitoring.

19.6 Correlative Data Analysis

Exploratory analyses of biomarkers related to tumor biology and study treatment mechanisms of action will be conducted. Analyses will assess the prognostic and/or predictive value of candidate biomarker.

19.6.1 Pharmacokinetic Analysis and Modeling

To characterize the pharmacokinetic (PK) properties of enzalutamide and venetoclax, blood samples will be obtained at various time points before and during study treatment admin (see Section 11.5). PK levels of venetoclax will be evaluated at completion of accrual of each cohort. Summary statistics of concentration data will be computed for each scheduled sampling time for each analyte after appropriate grouping.

PK model parameters for enzalutamide and venetoclax will be determined: specifically clearances, and volumes of distribution for both compounds. Inter-individual variability will be determined and individual specific concentration exposure metrics calculated (e.g., area under the curve, average concentration). Compartmental, non-compartmental, and/or population approaches will be considered as appropriate. We will also develop a PK/PD model exploring the relationship and specific concentration exposure as captured by the population pharmacokinetic model for enzalutamide and venetoclax and adverse events in phase I.

20 PROVISIONS TO MONITOR THE DATA TO ENSURE THE SAFETY OF SUBJECTS

All Roswell Park Phase I studies will be reviewed at the scheduled Early Phase Clinical Trial Committee meetings and the minutes are forwarded to the IRB for review.

In addition, the Roswell Park Data Safety Monitoring Committee will assess the progress of the study, the safety data, and critical efficacy endpoints (Phase I studies will be reviewed quarterly Phase II, III and pilot investigator-initiated studies will be reviewed semi-annually). The DSMC will review the study and will make recommendations that include but not limited to; (a) continuation of the study, (b) modifications to the design (c) or termination of the study.

21 VULNERABLE POPULATIONS

Not Applicable.

22 COMMUNITY-BASED PARTICIPATORY RESEARCH

Not Applicable.

23 SHARING OF RESULTS WITH SUBJECTS

Individual response data is shared with the participant as a part of their clinical care.

24 SETTING

All treatment will be conducted on an outpatient basis at Roswell Park's Genitourinary Clinic within Roswell Park. Potential study participants will be identified and recruited from current Genitourinary Clinic patients and from community referral.

25 PROVISIONS TO PROTECT THE PRIVACY INTERESTS OF SUBJECTS

Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a limited access environment. All computer entry and networking programs will be done using PIDs only. Information will not be released without written authorization of the participant.

26 RESOURCES AVAILABLE

Not Applicable.

27 PRIOR APPROVALS

Not Applicable.

28 COMPENSATION FOR RESEARCH-RELATED INJURY

If the subject believes they have been injured as a direct result of their participation in this research study, they will be advised to notify the Roswell Park Patient Advocate at (716) 845-1365 or the Study Doctor at (716) 845-8675.

Medical diagnosis and treatment for the injury will be offered, and a determination will be made regarding appropriate billing for the diagnosis and treatment of the injury. A financial counselor (716-845-3161) will be able to provide an explanation of coverage and to answer questions the subject may have regarding study related billing.

The subject is not prevented from seeking to collect compensation for injury related to malpractice, fault, or blame on the part of those involved in the research.

29 ECONOMIC BURDEN TO SUBJECTS

The participants will not be subject to any economic burden.

30 CONSENT PROCESS

The Roswell Park SOP: Informed Consent Process for Research (HRP-090) will be followed:

This study will not be initiated until the protocol and informed consent document(s) have been reviewed and approved by a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). Each participant (or legal guardian) shall read, understand, and sign an instrument of informed consent prior to performance of any study-specific procedure. It is the responsibility of the investigator to ensure that the participant is made aware of the investigational nature of the treatment and that informed consent is given.

The Investigator is responsible for the retention of the participant log and participant records; although personal information may be reviewed by authorized persons, that information will be treated as strictly confidential and will not be made publicly available. The investigator is also responsible for obtaining participant authorization to access medical records and other applicable study specific information according to Health Insurance Portability and Accountability Act regulations (where applicable).

This study will be conducted in compliance with all applicable laws and regulations of the state and/or country and institution where the participant is treated. The clinical trial should be conducted in accordance with the ethical principles embodied in the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, consistent with good clinical practice and the applicable regulatory requirements and according to the guidelines in this protocol, including attached appendices.

31 PROCESS TO DOCUMENT CONSENT IN WRITING

The Roswell Park “SOP: Written Documentation of Consent (HRP-091)” will be followed:

The Investigator (or IRB specified designee) is responsible for obtaining written consent from each participant or the participant's legally authorized representative in accordance with GCP guidelines using the approved informed consent form, before any study specific procedures (including screening procedures) are performed. The informed consent form acknowledges all information that must be given to the participant according to applicable GCP guidelines, including the purpose and nature of the study, the expected efficacy and possible side effects of the treatment(s), and specifying that refusal to participate will not influence further options for therapy. Any additional information that is applicable to the study must also be included. Additional national or institutionally mandated requirements for informed consent must also be adhered to. The participant should also be made aware that by signing the consent form, processing of sensitive clinical trial data and transfer to other countries for further processing is allowed.

The Investigator shall provide a copy of the signed and dated consent form to the participant and the signed and dated original shall be maintained in the Investigator File. A copy of the signed and dated consent form must be filed in the participant file. At any stage, the participant may withdraw from the study and such a decision will not affect any further treatment options.

32 DRUGS OR DEVICES

32.1 Enzalutamide

32.1.1 Active Substance and Source

Each capsule of enzalutamide contains 40 mg of enzalutamide as a solution in caprylocaproyl polyoxylglycerides. The inactive ingredients are caprylocaproyl polyoxylglycerides, butylated hydroxyanisole, butylated hydroxytoluene, gelatin, sorbitol sorbitan solution, glycerin, purified water, titanium dioxide, and black iron oxide.

32.1.2 Drug Shipment

Drug will not be supplied by the study. Enzalutamide will be paid for by the patient's insurance carrier as part of the standard of care treatment.

32.1.3 Storage and Stability

Store enzalutamide capsules at 20°C to 25°C (68°F to 77°F) in a dry place and keep the container tightly closed. Excursions permitted from 15°C to 30°C (59°F to 86°F).

32.2 Venetoclax

32.2.1 Active Substance and Source

Venetoclax will be supplied by AbbVie as oral film-coated tablets of 100 mg strength in high-density polyethylene plastic bottles or blister cards. For information on the formulation and handling of venetoclax, see the Package Insert and the Venetoclax Investigator's Brochure (v8.1).

32.2.2 Drug Shipment

Venetoclax will be provided by AbbVie and will be shipped to Roswell Park IDS.

32.2.3 Storage and Stability

The Investigator or designate will be responsible for ensuring that the investigational product is securely maintained in a locked, limited-access facility, as specified and, in accordance with the applicable regulatory requirements.

Store at 15-25°C. Storage conditions are described in the label attached to the clinical drug supplies.

Drug storage temperature will be maintained and recorded, as applicable.

32.2.4 Handling and Disposal

All products dispensed will be recorded on a product accountability record. Records of product lot numbers and dates received will be entered on a product accountability form. This record will be reviewed on a regular basis. It is the Investigator's responsibility to ensure that an accurate record of investigational drug issued and returned is maintained.

Excess drug will be destroyed according to standard practices after properly accounting for the dispensing. Excess drug will not be used for other participants.

Under no circumstances will the Investigator supply investigational drug to a third party or allow the investigational drug to be used in a manner other than as directed by this protocol.

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34 APPENDICES/ SUPPLEMENTS

Appendix A ECOG Performance Status Scores

Description	Status
Fully active, able to carry on all pre-disease performance without restriction.	0
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.	1
Ambulatory and capable of all self-care but unable to carry out any work activities.	2
Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	3
Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	4
Dead	5

Appendix B ELIGIBILITY VERIFICATION FORM: INCLUSION CRITERIA

Participant Name: _____

Medical Record No.:_____

Title: Phase Ib/II Study of Enzalutamide with Venetoclax (ABT-199) in Patients with Metastatic Castrate Resistant Prostate Cancer (mCRPC)

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "Yes" or "N/A" for participant enrollment.**	**Date**
☐	☐	☐	1. . Male with age ≥ 18 years of age	
☐	☐	☐	2. Histological or cytological documentation of diagnosis of prostate cancer.	
☐	☐	☐	3. Documented progressive metastatic castrate resistant prostate cancer (mCRPC) based on <u>at least one</u> of the following criteria: a. *PSA progression* defined as 25% increase over baseline value with an increase in the absolute value of at least 2 ng/mL that is confirmed by another PSA level with a minimum of a 1week interval and a minimum PSA of 2 ng/mL. b. *Soft-tissue progression* defined as an increase ≥ 20% in the sum of the longest diameter (LD) of all target lesions based on the smallest sum LD since treatment started or the appearance of one or more new lesions. c. Progression of bone disease (evaluable disease) or, new bone lesion(s), by bone scan. d. If on an anti-androgen, must have documented progression 6 weeks after stopping anti-androgen therapy	
☐	☐	☐	4. Willing to undergo a biopsy, if readily available biopsy site present (i.e., nodal or visceral metastasis).	
☐	☐	☐	5. Have an ECOG Performance Status of 0 - 1. Refer to Appendix A.	
☐	☐	☐	6. Have testosterone level of < 50 ng/dL. ***<u>Note</u>**:* Patients must continue primary androgen deprivation with an LHRH analogue (agonist or antagonist) if they have not undergone orchiectomy.	
☐	☐	☐	7. Patient has adequate bone marrow and organ function as defined by the following laboratory values, obtained *within 14 days* prior to treatment start: • White blood cells ≥ 1.5 x 10^9/L • Platelets (UNVPLT) ≥ 100 × 109/L • Hemoglobin (HGB) ≥ 9 g/dL • Potassium (K), total calcium (CA) (corrected for serum albumin), magnesium, sodium (NA) and phosphorus within normal limits for the institution or corrected to within normal limits with supplements before first dose of study medication	

PROTOCOL TITLE: Phase Ib/II Enzalutamide and Venetoclax (ABT-199) in Patients with mCRPC

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "Yes" or "N/A" for participant enrollment.**	**Date**
			• INR ≤ 1.5 • Adequate renal function as demonstrated by a creatinine clearance ≥ 30 mL/min; calculated by the Cockcroft Gault formula (Appendix K) • Adequate hepatic function defined as: o Aspartate transaminase (AST) and alanine transaminase (ALT) ≤ 3 X ULN. If the patient has liver metastases, ALT and AST must still be ≤ 3 x ULN. Patients with liver metastases and AST/ALT above this limit will not be enrolled. • Total serum bilirubin ≤1.5 x ULN (unless bilirubin rise is due to Gilbert's syndrome or of non-hepatic origin); or total bilirubin (TBILI) ≤ 3.0 x ULN with direct bilirubin within normal range in patients with well documented Gilbert's Syndrome.	
☐	☐	☐	8. Ability to swallow and retain oral medication (without crushing, dissolving or chewing tablets).	
☐	☐	☐	9. **Phase Ib only**: Prior enzalutamide treatment and/or other approved treatments for CRPC are acceptable.	
☐	☐	☐	10. .**Phase II only**: Participants MUST be treatment naïve in the CRPC setting: i.e., no prior exposure to abiraterone acetate other specific CYP-17 inhibitors; no prior exposure to enzalutamide or investigational androgen receptor (AR) targeted agents; and no prior exposure to chemotherapy and or RAD-223	
☐	☐	☐	11. Sexually active males must agree to use a condom during intercourse while taking the study drug and for at least 3 months after stopping study treatment. Sexually active males should not father a child during this period. A condom is required to be used by vasectomized men in order to prevent delivery of the drug via seminal fluid. Should a woman become pregnant or suspect she is pregnant while her partner is participating in this study, she should inform her treating physician immediately.	
☐	☐	☐	12. Participant or legal representative must understand the investigational nature of this study and voluntarily sign and date an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.	

Investigator Signature: _____ **Date:** _____

Printed Name of Investigator: _____

PROTOCOL TITLE: Phase Ib/II Enzalutamide and Venetoclax (ABT-199) in Patients with mCRPC

Appendix C ELIGIBILITY VERIFICATION FORM: EXCLUSION CRITERIA

Participant Name: _____

Medical Record No.: _____

Title: Phase Ib/II Study of Enzalutamide with Venetoclax (ABT-199) in Patients with Metastatic Castrate Resistant Prostate Cancer (mCRPC)

EXCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "No" or "N/A" for participant enrollment.**	**Date**
☐	☐	☐	1. **Phase II only**: Prior exposure to abiraterone acetate.	
☐	☐	☐	2. **Phase II only**: Prior exposure to BCL-2 inhibitors agents like venetoclax.	
☐	☐	☐	3. **Phase II only**: Prior chemotherapy for castration resistant disease. Chemotherapy given in the castration-sensitive setting is permissible if stopped at least 4 weeks prior to treatment start.	
☐	☐	☐	4. **Phase II only**: Prior isotope therapy with strontium-89, samarium or radium-223 within 12 weeks of treatment start.	
☐	☐	☐	**5.** Subject has acute promyelocytic leukemia.	
☐	☐	☐	6. Subject has known active CNS involvement with AML.	
☐	☐	☐	7. Participants with known symptomatic brain metastases.	
☐	☐	☐	8. Participant has a concurrent malignancy or malignancy within 3 years of treatment start, with the exception of adequately treated, basal or squamous cell carcinoma, non-melanomatous skin cancer or curatively resected cervical cancer.	
☐	☐	☐	9. Participant has a known history of HIV infection (testing not mandatory).	
☐	☐	☐	10. Chronic hepatitis B virus (HBV) or hepatitis C (HCV) requiring treatment. **Note**: subjects with serologic evidence of prior vaccination to HBV (i.e. hepatitis B surface (HBs) antigen negative-, anti-HBs antibody positive and anti-hepatitis B core (HBc) antibody negative) or positive anti-HBc antibody from intravenous immunoglobulins (IVIG) may participate	
☐	☐	☐	11. Uncontrolled and/or active systemic infection (viral, bacterial or fungal).	
☐	☐	☐	12. Participant has clinically significant, uncontrolled heart disease and/or recent events including any of the following: • History of acute coronary syndromes (including myocardial infarction, unstable angina, uncontrolled hypertension, uncontrolled arrhythmia, stroke, coronary artery bypass grafting, coronary angioplasty, or stenting) or symptomatic pericarditis within 12 months prior to treatment start • Cardiac history of CHF requiring treatment or Ejection Fraction ≤ 50% or chronic stable angina • A cardiovascular disability status of New York Heart Association Class > 2 (refer to **Appendix L**): • Class 2 is:	

EXCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "No" or "N/A" for participant enrollment.**	**Date**
			○ defined as cardiac disease in which patients are comfortable at rest but ordinary physical activity ○ results in fatigue, palpitations, dyspnea, or angina pain • On screening 12 lead ECG, any of the following cardiac parameters: bradycardia (heart rate < 50 at rest), tachycardia (heart rate > 90 at rest), PR interval > 220 msec, QRS interval > 109 msec or, QTcF > 450 msec. Congenital long QT syndrome or family history of long QT syndrome.	
☐	☐	☐	13. DLCO ≤ 65% or FEV1 ≤ 65%	
☐	☐	☐	14. Treatment with any of the following **within 7 days** prior to the first dose of study drug: • Steroid therapy for anti-neoplastic intent • Moderate or strong cytochrome P450 3A (CYP3A) inhibitors (refer to Appendix G) • Moderate or strong CYP3A inducers (refer to **Appendix G**) • Medications that have a known risk to prolong the QT interval or induce Torsades de Pointes • Herbal preparations/medications	
☐	☐	☐	15. Participants receiving any medications or substances that are inhibitors or inducers of CYP2C8 enzymes are ineligible (refer to Appendix H).	
☐	☐	☐	16. Administration or consumption of any of the following within 3 days prior to the first dose of study drug: • grapefruit or grapefruit products • Seville oranges (including marmalade containing Seville oranges) • star fruit	
☐	☐	☐	17. Patient who has received radiotherapy ≤ 4 weeks prior to start of treatment or limited field radiation for palliation ≤ 2 weeks prior to treatment start and, who has not recovered to Grade 1 or better from related side effects of such therapy (exceptions include alopecia) and/or in whom ≥ 30% of the bone marrow was irradiated.	
☐	☐	☐	18. Patients with central nervous system (CNS) involvement.	
☐	☐	☐	19. Patients with seizure disorder.	
☐	☐	☐	20. Patient has not recovered from all toxicities related to prior anticancer therapies to NCI CTCAE (version 5.0) Grade ≤1 (*Exception to this criterion:* patients with any grade of alopecia are allowed to enter the study).	
☐	☐	☐	21. Participant has any other concurrent severe and/or uncontrolled medical condition that would cause, in the investigator's judgment, an unacceptable safety risk.	
☐	☐	☐	22. Unwilling or unable to follow protocol requirements.	

PROTOCOL TITLE: Phase Ib/II Enzalutamide and Venetoclax (ABT-199) in Patients with mCRPC

EXCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "No" or "N/A" for participant enrollment.**	**Date**
☐	☐	☐	23. Any condition which in the Investigator's opinion deems the participant an unsuitable candidate to receive study drug.	

Participant meets all entry criteria: ☐ **Yes** ☐ **No**
If "NO", do not enroll participant in study.

Investigator Signature: _____ **Date:** _____

Printed Name of Investigator: _____

APPROVED RPCI IRB
7/30/2020

Appendix D Schedule of Procedures and Observations

Table 1: Schedule of Study Procedures and Assessments-Phase Ib

Table 2: Schedule of Study Procedures and Assessments-Phase II

Appendix D Table 1

Schedule of Study Procedures and Assessments: Phase Ib

Assessment/Procedure Phase Ib	Screening[15] (prior to start of enzalutamide only)		Enzalutamide only[13]	Cycle 1 Combination enzalutamide + venetoclax					Cycle 2 (±2 days)	Cycle 3 (± 2 days)	Cycle 4 (± 2 days)	Cycle 5 (± 2 days) and beyond[12]	End of Treatment or Treatment Discontinuation	Post Treatment follow up[1]
Study Day/Visit Day	Baseline			Day 1	Day 2	Day 8±2 d	Day 15±2 d	Day 21±2 d	Day 1	Day 1	Day 1			
	-28 to 0	-14 to 0												
Informed consent	X													
Inclusion/exclusion criteria	X													
Medical History and Demographics	X													
Pre-Existing Conditions	X													
Physical Examination, vital signs	X			X		X	X	X	X	X	X	X	X	
ECOG Performance Status	X			X		X	X	X	X	X	X	X	X	
Concomitant Meds	X			X		X	X	X	X	X	X	X	X	
AE Assessment			X[14]	X	X	X	X	X	X	X	X	X	X	
Survival Status														X
TLS Monitoring[10]				X		X	X	X	X	X	X		X	
Laboratory Procedures														
Hematology: CBC w/ auto differential		X		X		X	X	X	X	X	X	X	X	
Chemistry (CMP)		X		X		X	X	X	X	X	X	X	X	
Magnesium		X												
Urinalysis/BUN		X							X	X	X		X	
Serum PSA		X		X		X	X	X	X	X	X	X	X	

Assessment/Procedure Phase Ib	Screening[15] (prior to start of enzalutamide only)		Enzalutamide only[13]	Cycle 1 Combination enzalutamide + venetoclax					Cycle 2 (±2 days)	Cycle 3 (± 2 days)	Cycle 4 (± 2 days)	Cycle 5 (± 2 days) and beyond[12]	End of Treatment or Treatment Discontinuation	Post Treatment follow up[1]
Study Day/Visit Day	Baseline			Day 1	Day 2	Day 8±2 d	Day 15±2 d	Day 21±2 d	Day 1	Day 1	Day 1			
	-28 to 0	-14 to 0												
LDH		X		X		X	X	X	X	X	X		X	
Uric acid		X		X		X	X	X	X	X	X		X	
PT/ INR		X												
Lipid Panel[2]		X									X			
Phosphorus		X		X					X	X	X		X	
Pharmacokinetics[3]		X		X[3a]	X[3b]	X[3c]	X[3d]	X[3e]	X[3f]	X[3g]				
12-lead Electrocardiogram[9]		X												
ECHO/MUGA		X												
Serum Testosterone		X												
PFT screen spirometry or PFT w/o bronchodilator		X												
Imaging Procedures														
CT/MRI of abdomen & pelvis, CT or X-ray of chest)[4]	X									X			X	
Bone Scan[4]	X									X			X	
Tumor Measurement[4]	X									X			X	
Study Drugs														
Venetoclax[5]				Daily during each cycle										
Enzalutamide			X[6]	Daily during each cycle										
Correlative Procedures														

Assessment/Procedure **Phase Ib**	**Screening[15] (prior to start of enzalutamide only)**		**Enzalutamide only[13]**	**Cycle 1 Combination enzalutamide + venetoclax**					**Cycle 2 (±2 days)**	**Cycle 3 (± 2 days)**	**Cycle 4 (± 2 days)**	**Cycle 5 (± 2 days) and beyond[12]**	**End of Treatment or Treatment Discontinuation**	**Post Treatment follow up[1]**
Study Day/Visit Day	**Baseline**			**Day 1**	**Day 2**	**Day 8±2 d**	**Day 15±2 d**	**Day 21±2 d**	**Day 1**	**Day 1**	**Day 1**			
	-28 to 0	**-14 to 0**												
Tumor biopsy	X[7]												X[8]	
Blood draw for correlative studies[11]	X			X						X[11a]			X	

Phase Ib footnotes:

1. Follow-up safety evaluations will occur 30 days (± 3 days) after last dose of study drug or until resolution of any drug-related toxicity (telephone contact is acceptable). Patients who discontinue treatment for reasons other than disease progression will undergo assessments every 3 months during the post treatment follow-up period for 1 year or until disease progression, the start of new CRPC treatment, or the end of the study, whichever occurs first.
   Patients who experience disease progression will be evaluated for survival status and initiation of new treatment every 3 months until the end of the study
2. Every 4th cycle
3. PK assessment schedule (Refer to Section 11.5):
   3a: C1D1Pre-dose (prior to daily enzalutamide and venetoclax taken at the same time), 1 hr (± 15 min), 2 hr (± 15 min), 4 hr (± 15 min), 6 hr (± 15 min) and, 8 hr (± 15 min) following enzalutamide and venetoclax combination;
   3b:C1D2: Within 22-26 hr (± 1 hr) from the time of drug dosing on Cycle 1-Day 1;
   3c: CID8: Pre-daily dose of enzalutamide and venetoclax
   3d: C1D15: Pre-daily dose of enzalutamide and venetoclax
   3e:C1D21: Pre-daily dose of enzalutamide and venetoclax
   3f: C2D1: Pre-dose (prior to daily enzalutamide and venetoclax taken at the same time), 1 hr (± 15 min), 2 hr (± 15 min), 4 hr (± 15 min), 6 hr (± 15 min) and, 8 hr (± 15 min) following enzalutamide and venetoclax combination
   3g: C3D1: Pre-daily dose of enzalutamide and venetoclax.
4. Baseline imaging assessments can be done within 6 weeks of treatment start. Imaging will be repeated every 12 weeks (± 7 days) on treatment.
5. Subjects will take venetoclax daily throughout each cycle.
6. Enzalutamide will be administered as per recommended guidelines.
7. In the presence of soft tissue or visceral metastatic disease, a tumor biopsy may be performed if it is part of standard of care (refer to Section 11.7.1).
8. A second optional tumor biopsy will be requested for subjects at disease progression (refer to Section 11.7.2).
9. Single, resting, 12-lead ECG recordings will be obtained at screening, and may be obtained at unscheduled time points as clinically indicated.
10. Signs and symptoms of TLS (e.g., hyperuricemia, hyperkalemia, hypocalcemia, hyperphosphatemia, acute renal failure, and elevated LDH) will be closely monitored weekly through cycle 1 and on Day 1 of all subsequent cycles and, when clinically indicated.

11. Correlative blood sampling: Refer to Section 11.6.1.
    11a: Following Cycle 3, correlative blood sampling will be done on Day 1 of every 3rd cycle thereafter (e.g., Day 1 of Cycles 6, 9, 12, etc…)
12. Patient may remain on study and continue to receive treatment until disease progression, the start of a new therapy or death; whichever occurs first). CBC, CMP, and PSA to be performed on day 1 (± 2 days) of each cycle from cycle 5 and forward.
13. A minimum of 4 weeks prior to combination therapy.
14. Participants will be monitored for toxicity as per standard of care.
15. For patients already on enzalutamide, screening assessments will occur prior to Cycle 1 Day 1 of combination therapy with Venetoclax.

Appendix D Table 2

Table 2 Schedule of Study Procedures and Assessments: Phase II

Assessment/Procedure Phase II	Screening(prior to start of enzalutamide only)		Enzalutamide only	Cycle 1 Combination enzalutamide + venetoclax					Cycle 2 (± 2 days)	Cycle 3 (± 2 days)	Cycle 4 (± 2 days)	Cycle 5 (± 2 days) and beyond)[12]	End of Treatment or Treatment Discontinuation	Post Treatment follow up[1]
Study Day/Visit Day	Baseline			Day 1	Day 2	Day 8 ±2 d	Day 15±2 d	Day 21±2 d	Day 1	Day 1	Day 1			
	-28 to 0	-14 to 0												
Informed consent	X													
Inclusion/exclusion criteria	X													
Medical History and Demographics	X													
Pre-Existing Conditions	X													
Physical Examination, vital signs	X			X					X	X	X	X	X	
ECOG Performance Status	X			X					X	X	X	X	X	
Concomitant Meds	X			X					X	X	X	X	X	
AE Assessment			X[13]	X					X	X	X	X	X	
Survival Status														X
TLS Monitoring [9]				X					X	X	X		X	
Laboratory Procedures														
Hematology: CBC w/ auto differential		X		X					X	X	X	X	X	

Assessment/Procedure Phase II	Screening(prior to start of enzalutamide only)		Enzalutamide only	Cycle 1 Combination enzalutamide + venetoclax					Cycle 2 (± 2 days)	Cycle 3 (± 2 days)	Cycle 4 (± 2 days)	Cycle 5 (± 2 days) and beyond)[12]	End of Treatment or Treatment Discontinuation	Post Treatment follow up[1]
Study Day/Visit Day	Baseline -28 to 0	Baseline -14 to 0		Day 1	Day 2	Day 8 ±2 d	Day 15±2 d	Day 21±2 d	Day 1	Day 1	Day 1			
Chemistry (CMP)		X		X					X	X	X	X	X	
Magnesium		X												
Urinalysis/BUN		X							X	X	X		X	
Serum PSA		X		X					X	X	X	X	X	
LDH		X		X					X	X	X		X	
Uric acid		X		X					X	X	X		X	
PT/ INR		X												
Lipid Panel[2]		X									X			
Phosphorus		X		X					X	X	X		X	
Pharmacokinetics[10]				X[10a]	X[10b]	X[10c]	X[10d]	X[10e]	X[10f]	X[10g]				
12-lead Electrocardiogram[8]		X												
ECHO/MUGA		X												
Serum Testosterone		X												
PFT screen spirometry or PFT w/o bronchodilator		X												
Imaging Procedures														
CT/MRI of abdomen & pelvis, CT or X-ray of chest)[3]	X									X			X	

Assessment/Procedure Phase II	Screening(prior to start of enzalutamide only)		Enzalutamide only	Cycle 1 Combination enzalutamide + venetoclax					Cycle 2 (± 2 days)	Cycle 3 (± 2 days)	Cycle 4 (± 2 days)	Cycle 5 (± 2 days) and beyond)[12]	End of Treatment or Treatment Discontinuation	Post Treatment follow up[1]
Study Day/Visit Day	**Baseline**			**Day 1**	**Day 2**	**Day 8 ±2 d**	**Day 15±2 d**	**Day 21±2 d**	**Day 1**	**Day 1**	**Day 1**			
	-28 to 0	**-14 to 0**												
Bone Scan[3]	X									X			X	
Tumor Measurement[3]	X									X			X	
Study Drugs														
Venetoclax[4]				Daily during each cycle										
Enzalutamide			X[5]	Daily during each cycle										
Correlative Procedures														
Tumor biopsy	X[6]												X[7]	
Blood draw for correlative studies[11]	X			X						X[11a]			X	

Phase II footnotes:

1 Follow-up safety evaluations will occur 30 days (± 3 days) after last dose of study drug or until resolution of any drug-related toxicity (telephone contact is acceptable). Patients who discontinue treatment for reasons other than disease progression will undergo assessments every 3 months during the post treatment follow-up period for 1 year or until disease progression, the start of new CRPC treatment, or the end of the study, whichever occurs first.
Patients who experience disease progression will be evaluated for survival status and initiation of new treatment every 3 months until the end of the study.

2 Every 4th cycle

3 Baseline imaging assessments can be done within 6 weeks of treatment start. Imaging will be repeated every 12 weeks (± 7 days) on treatment.

4 Subjects will start taking venetoclax daily from cycle 1 onwards throughout each cycle.

5 Enzalutamide will be administered as per recommended guidelines. Subjects will have been taking enzalutamide for at least 4 weeks prior to first administration of venetoclax.

6 In the presence of soft tissue or visceral metastatic disease, a tumor biopsy may be performed if it is part of standard of care (refer to Section 11.7.1).

7 A second optional tumor biopsy will be requested for subjects at POD (refer to Section 11.7.2).

8 Single, resting, 12-lead ECG recordings will be obtained at screening, and may be obtained at unscheduled time points as clinically indicated.

9 Signs and symptoms of TLS (e.g., hyperuricemia, hyperkalemia, hypocalcemia, hyperphosphatemia, acute renal failure, and elevated LDH) will be closely monitored weekly through cycle 1 and on Day 1 of all subsequent cycles and, when clinically indicated.

10 PK assessment schedule (Refer to Section 11.5):
10a: C1D1Pre-dose: Prior to daily enzalutamide and initial dose of venetoclax combination, 2-4 hr following enzalutamide + venetoclax combination;
10b:C1D2: Prior to daily dose of enzalutamide and venetoclax. ***Note***: Blood draw must be **within 22-26 hr (± 1 hr)** from the time of drug dosing on Cycle 1-Day 1;
10c: CID8: Pre-daily dose of enzalutamide and venetoclax
10d: C1D15: Pre-daily dose of enzalutamide and venetoclax
10e:C1D21: Pre-daily dose of enzalutamide and venetoclax
10f: C2D1: Pre-dose (prior to daily enzalutamide and venetoclax taken at the same time) and, 2-4 hr following enzalutamide + venetoclax combination
10g: C3D1: Pre-daily dose of enzalutamide and venetoclax.

11 Correlative blood sampling: Refer to Section 11.6.1.
11a: Following Cycle 3, correlative blood sampling will be done on Day 1 of every 3rd cycle thereafter (e.g., Day 1 of Cycles 6, 9, 12, etc…)

12 Patient may remain on study and continue to receive treatment until disease progression, the start of a new therapy or death; whichever occurs first). CBC, CMP, and PSA to be performed on day 1 (± 2 days) of each cycle from cycle 5 and forward

13 Participants will be monitored for toxicity as per standard of care.

Appendix E Characterization of Pharmacokinetic Properties of Enzalutamide and Venetoclax in patients with mCRPC

To characterize the pharmacokinetic (PK) properties of enzalutamide and venetoclax, blood samples will be obtained at various time points before and during study treatment admin (refer to **Section 11.5**). PK levels of venetoclax will be evaluated at completion of accrual of each cohort (without holding up accrual). As PK data are generated, and at the latest before starting the phase II portion of this trial, exposure metrics for venetoclax and enzalutamide will be evaluated and compared with historical data.

PK model parameters for enzalutamide and venetoclax will be determined: specifically clearances, and volumes of distribution for both compounds. Inter-individual variability will be determined and individual specific concentration exposure metrics calculated (e.g., area under the curve, average concentration). We will also develop a PK/PD model exploring the relationship and specific concentration exposure as captured by the population pharmacokinetic model for enzalutamide and venetoclax and adverse events in phase I.

Pre-first venetoclax dose (day 1). This will serve as a "trough" concentration for the enzalutamide as patients will have been taking this drug for at least one month prior to venetoclax initiation. The sample will be evaluated for venetoclax and serve as a "blank" to ensure there are not interfering compounds present and that the assay is working correctly. In addition to this pre-first venetoclax sample, samples will be taken after the administration of venetoclax at, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours and upon return of the patient the next day between 22 and 26 hours after the dose (but prior to the second dose). This will ensure that a full first dose profile is available and provides support for estimation of individual specific exposure utilizing the pharmacokinetic model for venetoclax published by Jones et al [44] and the enzalutamide model published by Gibbons J et al [37].

After one week, two weeks and three weeks of venetoclax administration, patients will return with the dose held on that day until they arrive in clinic. A blood sample will be taken pre-dose at each of those weeks to serve as a "trough" sample. Exact timing of the sample as well as the nominal reported time of dosing by the patient will be essential for this. At week four, a blood sample will be drawn pre-dose on day 7 (+/- 1 day) with an observed dose and samples taken again at 1 hour, 2 hours, 4 hours, 6 hours and 8 hours. The pre-dose sample will be combined with the samples after the dose at 7 days to provide a full dosage interval for the population PK modeling. Finally, a pre-dose sample will be taken at week 8 to evaluate whether the pharmacokinetic properties of venetoclax in conjunction with Enzalutamide are stable.

Individual exposures and parameter estimates, mainly focused on the apparent clearance of the drug and the area under the concentration time curve will be calculated for each cohort and compared with historically reported values. If the exposures are excessively high or low, appropriate adjustment will be made with respect to dosage.

Enzalutamide concentrations can be determined in fewer samples than those needed for venetoclax. Essentially, the pre-dose concentrations may be sufficient to determine overall exposures for enzalutamide. Specific population PK parameters values to be utilized in POP-PK modeling efforts to determine individual exposures based on measured values will be obtained from Jones et al [44] and Gibbons J et al [37].

Dr. Beumer, Director of the UPMC Hillman Cancer Center, Cancer Pharmacokinetics and Pharmacodynamics Facility, will analyze the samples for venetoclax, enzalutamide and the equipotent metabolite N-desmethyl-enzalutamide. The assay for enzalutamide and its metabolite was validated to FDA guidance and is currently employed to support multiple clinical trials, including a phase III trial run through the ALLIANCE.[45] The assay for venetoclax will be developed and validated to FDA guidance by Dr. Beumer. He has extensive experience in this respect with numerous new chemical entities and chemotherapeutic agents.

Appendix F Excluded and Cautionary Medications

Excluded
• **Anticancer therapies including chemotherapy, radiotherapy, or other investigational therapy, including targeted small molecule agents:** Excluded 5 half-lives prior to first dose and throughout venetoclax administration • **Biologic agents (e.g., monoclonal antibodies) for anti-neoplastic intent:** Excluded 30 days prior to first dose and throughout venetoclax administration
Excluded during ramp-up phase and Cautionary at the Cohort Designated Dose:
• **Strong and Moderate CYP3A inhibitors:** Exclude during ramp-up phase and consider alternative medications. If subject requires use of these medications at the cohort designated dose, use with caution and reduce the venetoclax dose by 50% for moderate inhibitors and at least 75% for strong inhibitors during co-administration. After discontinuation of CYP3A inhibitor, wait for 2 to 3 days before venetoclax dose is increased back to the initial maintenance/target dose. • **Strong and Moderate CYP3A inducers:** Exclude during ramp-up phase and consider alternative medications. If subject requires use of these medications at the cohort designated dose, use with caution and contact AbbVie medical monitor for guidance.
Cautionary
• Warfarin: Note: Closely monitor International Normalized Ratio (INR). • P-gp substrates • BCRP substrates • OATP1B1/1B3 substrates • P-gp inhibitors • BCRP inhibitors

Appendix G Sample List of Excluded and Cautionary Medications

Excluded during ramp-up phase and Cautionary at the Cohort Designated Dose
• **Strong CYP3A inducers**: avasimibe, carbamazepine, enzalutamide, mitotane, phenytoin, rifampin, St. John's wort • **Moderate CYP3A inducers**: bosentan, efavirenz, etravirine, modafinil, nafcillin • **Strong CYP3A inhibitors:** boceprevir, clarithromycin, cobicistat, conivaptan, danoprevir/ritonavir, elvitegravir/ritonavir, idelalisib, indinavir, itraconazole, ketoconazole, mibefradil, lopinavir/ritonavir, nefazodone, nelfinavir, ritonavir, paritaprevir/ritonavir combinations, posaconazole, saquinavir, telaprevir, telithromycin, tipranavir/ritonavir, voriconazole • **Moderate CYP3A inhibitors:** amprenavir, aprepitant, atazanavir, cimetidine, ciprofloxacin, clotrimazole, crizotinib, cyclosporine, darunavir/ritonavir, diltiazem, erythromycin, fluconazole, fosamprenavir, imatinib, isavuconazole, tofisopam, verapamil
Cautionary
• Warfarin • P-gp substrates • Aliskiren, ambrisentan, colchicine, dabigatran etexilate, digoxin, everolimus, fexofenadine, lapatinib, loperamide, maraviroc, nilotinib, ranolazine, saxagliptin, sirolimus, sitagliptin, talinolol, tolvaptan, topotecan • BCRP substrates • Methotrexate, mitoxantrone, irinotecan, lapatinib, rosuvastatin, sulfasalazine, topotecan • OATP1B1/1B3 substrates • Atrasentan, atorvastatin, ezetimibe, fluvastatin, glyburide, rosuvastatin, simvastatin acid, pitavastatin, pravastatin, repaglinide, telmisartan, valsartan, olmesartan • P-gp inhibitors • Amiodarone, azithromycin, captopril, carvedilol, dronedarone, felodipine, quercetin, quinidine, ranolazine, ticagrelor • BCRP inhibitors • Gefitinib

Appendix H CYP2C8 inducers/inhibitors

CYP2C8 Inducer	CYP2C8 Inhibitor
Primidone	Gemfibrozil
Rifampicin	Clopidogrel
Secobarbital	Lopinavir
Phenobarbital	Fluvoxamine
	Felodipine

Appendix I Study Medication Calendar for Venetoclax

Study No.: _____ Patient Name: _____

Drug Name: _____Medical Record No.: _____

Cycle Number: _____

Study Medication Calendar for Venetoclax

You should swallow your medication, unchewed, with a large glass of water (at least 8 ounces) at approximately the same time every day, once a day and approximately 30 minutes after a meal **You should take you medication after breakfast (or your first meal of the day) for the first two months of being on the study medication.** If you miss a dose and it is within 8 hours of the missed dose-take the missed dose as soon as possible with food. If it is longer than 8 hours since the missed dose-do not take any of the drug that day, but wait until the next day to take your regularly scheduled dose (see attached sheet for more detailed instructions).

Please complete this calendar on a daily basis immediately after you take your tablets. Fill in the date for each day, write the drug dose that you take each day, and write the total number of tablets you take each day.

Start Date: _____

Take _____ tablets once a day and approximately 30 minutes after a meal.

Cycle Day	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Date							
# of Pills							
Time tablets taken							

Cycle Day	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Date							
# of Pills							
Time tablets taken							

Cycle Day	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
Date							
# of Pills							
Time tablets taken							

Cycle Day	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
Date							
# of Pills							
Time tablets taken							

Please remember to bring this calendar and your study medication bottle (including any unused tablets) with you to your next clinic appointment.

Coordinator's Use Only

$$\%\ Compliance = \left(\frac{Number\ of\ Pills\ Dispensed - Number\ of\ Pills\ Returned}{Number\ of\ Pills\ Scheduled}\right) \times 100$$

$$___\%\ Compliance = \left(\frac{\quad - \quad}{\quad\quad\quad\quad}\right) \times 100$$

Subject's Signature: _____ Date: _____

PROTOCOL TITLE: Phase Ib/II Enzalutamide and Venetoclax (ABT-199) in Patients with mCRPC

CRC signature: _____ Date: _____

Patient instructions for taking Venetoclax

Venetoclax will be taken orally, once daily, for 28 consecutive days.

Each dose of venetoclax will be taken orally, once daily, with approximately 8 ounces (1 cup) of water and approximately 30 minutes after finishing a meal:

- A meal containing approximately 30% of the total caloric content from fat is recommended to ensure adequate absorption of venetoclax. The Standard American Heart Association Healthy (Low-Fat) Breakfast includes:
    - box cereal (approximately 1 cup),
    - skim milk (approximately 1 cup),
    - 1 boiled egg,
    - 1 slice of toast, and margarine (approximately 2 teaspoons),

for approximately 520 Kcal with approximately 17 grams of fat.

Avoid the consumption of grapefruit, Seville oranges or products containing the juice of each during the entire study and preferably 7 days before the first dose of study medications, due to potential interactions with the study medications. Orange juice is allowed.

If vomiting occurs within 15 minutes after taking venetoclax and all expelled tablets are still intact, another dose may be taken.

- If tablets are not identified, or if any are not intact, no replacement dose should be taken
- In cases where a dose of venetoclax is missed or forgotten, you should take the dose as soon as possible and ensure that the dose is taken with food within 8 hours after the scheduled time of the missed dose. Otherwise, the missed dose should not be taken.

You will receive bottles that contain venetoclax tablets at your regularly scheduled visits. You should bring your venetoclax bottle and any tablets that may be remaining to your scheduled clinic visits.

On days with PK, ECG sampling, chemistry panel and/or lipid panel sampling, the following additional guidelines should be followed (Cycle 1 Day 1 and Cycle 2 Day 1 Only):

- On days that pre and post-dose PK sampling is required, ***your first meal of the day (e.g., breakfast) will be consumed in the morning at the clinic***, and ***Venetoclax dosing (and immediately thereafter, enzalutamide) will occur in the clinic*** (under the supervision of the Investigator or designee) after completion of the meal to facilitate PK sampling. On all other days you will take the study treatment at home.
- On a day of chemistry panel and/or lipid panel sampling, you must be ***fasting*** from all food and drink ***for at least 8 hours overnight***. Water is allowed during all fasting periods; however, coffee, tea and juice are not permitted during the fasting period. ***You must also take the study treatment in the clinic*** under the supervision of the Investigator or designee. On all other days you may take the study treatment at home.

Appendix J PK Questionnaire

Note: This form is to be completed for all PK blood-draw time points.

On Cycle 1-Day 1, Cycle 1-Day 2, Cycle 1-Day 8, Cycle 1-Day 15, Cycle 1-Day 21, Cycle 2-Day 1, and Cycle 3-Day 1, this questionnaire is to be administered by a nurse/CRC, completed by the patient, and entered into the CRF by site staff.

Note: Study medication should be taken *after* providing the "Pre" blood samples.

Patient Initials: _____

Patient ID #: _____

Study #: _____

Cycle #: _____ Day #: _____

Options:

[Cycle 1 D1] / [Cycle 1 D2], [Cycle 1 D8], [Cycle 1 D15], [Cycle 1 D21], [Cycle 2 D1], [Cycle 3 D1]

Date: _____

The following question refers to yesterday (1 day ago):

What time did you take your study medication (*enzalutamide* + *venetoclax*)?
__ __:__ __ AM / PM

All days:

Pre dose blood sample:

Date: _____ PK sample time __ __:__ __AM /PM

Only for Cycle 1 Day 1 and Cycle 2 Day 1:

After having your Pre Dose blood sample drawn, what time did you take your medication?
Enzalutamide + venetoclax dose __ __:__ __ AM / PM

Time of PK samples after taking your enzalutamide + venetoclax dose:

1 h PK sample __ __:__ __ AM / PM

2 h PK sample __ __:__ __ AM / PM

4 h PK sample __ __:__ __ AM / PM

6 h PK sample __ __:__ __ AM / PM

8 h PK sample __ __:__ __ AM / PM

THE FOLLOWING QUESTION ONLY APPLIES IF YOU HAVE TAKEN YOUR STUDY MEDICATION BEFORE BEING SEEN IN THE CLINIC TODAY:
The following question refers to today:

What time did you take your study medication (*enzalutamide* + *venetoclax*)?
__ __:__ __ AM / PM?

Appendix K Calculation for Creatinine Clearance

Cockroft-Gault Equation*

Men: CrCl = [(140-YR) x IBW] / (SCr x 72)

Women: CrCl = 0.85 x [(140-YR) x IBW] / (SCr x 72)

Where:

CrCl is creatine clearance (mL/min)

IBW is ideal body weight (kg)

SCr is serum creatinine (mg/dL)

YR is age (years)

*Cockcroft D, W, Gault M, H, Prediction of Creatinine Clearance from Serum Creatinine. Nephron. 1976; 16 (1):31-41)

Appendix L NYHA Functional Classification System

New York Heart Association (NYHA) Functional Classification System[a]	
Class	**Functional Capacity**
Class I	Patients with cardiac disease but without resulting limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.
Class II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.
Class III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.
Class IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased

[a] The Criteria Committee of the New York Heart Association. (1994). *Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels.* (9th ed.). Boston: Little, Brown & Co. pp. 253–256.

Appendix M Instructions for Multi-Site Studies

1. CONTACT INFORMATION

All questions related to the protocol or study implementation should be directed to:
Roswell Park Cancer Institute
Beth Cahill, Quality Assurance Coordinator
CRS Multi-Site Quality Assurance (QA) Office
1930 GBSB
Buffalo, New York 14263
Telephone: 716-845-7117
Monday - Friday; 8:30 AM to 5:00 PM EST
After hours, weekends, and holidays request the Roswell Park Investigator
716-845-2300
Fax: 716-845-8743

2. INFORMED CONSENT

- Informed consent must be obtained by the **site Investigator/designee** from any participants wishing to participate, **prior to any procedures or treatment**.
- An informed consent template is provided by Roswell Park and can be amended to reflect institutional requirements.
- All consent changes **must** be reviewed by Roswell Park CRS Multi-Site QA Office prior to submission to the site IRB.
- The informed consent must be IRB approved.
- Always check that the most up to date version of the IRB approved consent is being used.
- Within 5 business days, notify the Roswell Park CRS Multi-Site QA Office of all participant withdrawals or consent to limited study participation and appropriately document the discontinuation and the reason(s) why.

3. PARTICIPANT REGISTRATION

Roswell Park does not grant exceptions to eligibility criteria.

Phase 2 Protocol Registration Instructions

The Subject Screening and Enrollment Log must be faxed or emailed to the Roswell Park CRS Multi-Site QA Office within 1 business day of the date the participant is consented. Once the Investigator has determined that eligibility has been met, complete the eligibility check list and fax or email it to the Roswell Park Multi-Site QA Coordinator at 716-845-8743.

4. STUDY DEVIATIONS

- If a deviation has occurred to eliminate hazard, this must be reported to the Roswell Park Multi-Site QA Department, site IRB and any other regulatory authority involved in the study.
- ALL study deviations will be recorded on the **Study Deviation Log**.

- Participants inadvertently enrolled with significant deviation(s) from the study-specified criteria will be removed from the study, at the discretion of the Principle Investigator.

5. STUDY DOCUMENTATION

- Study documents must be filled out completely and correctly. Ditto marks are not allowed.
- If an entry has been documented in error put a single line through the entry and initial and date the change. The Roswell Park Multi-Site QA Coordinator must be able to read what has been deleted.
- Do **NOT** use white-out, magic marker, scratch-outs.
- Do **NOT** erase entries.
- Use only black ink for documentation on the accountability form and any other study forms.
- It is the responsibility of Roswell Park to inform the Investigator/ institution as to when these documents no longer need to be retained. If, for any reason, the Investigator desires to no longer maintain the study records, they may be transferred to another institution, another investigator, or to Roswell Park upon written agreement between the Investigator and Roswell Park.

6. DRUG ACCOUNTABILITY

Drug accountability must be strictly maintained.

- Responsibility rests solely with the Investigator but can be delegated as appropriate (e.g., to pharmacy personnel).
- A drug accountability record form (DARF) will record quantities of study drug received, dispensed to participants and wasted, lot number, date dispensed, participant ID number and initials, quantity returned, balance remaining, manufacturer, expiration date, and the initials of the person dispensing the medication.
- Study drug supply will only be used in accordance with the IRB approved study.
- Drug accountability forms are protocol and agent specific, they are study source documents and will be used to verify compliance with the study.
- An inventory count must be performed with each transaction. Any discrepancies shall be documented and explained.
- Drug accountability forms must be stored with study related documents.
- Each medication provided for this study and each dosage form and strength must have its own DARF.
- Dispensing the wrong study supply is considered a **medication error**.
- **NEVER** replace investigational agents with commercial product.
- Do **NOT** “transfer”, “borrow” or “replace” supplies between studies.

7. SERIOUS ADVERSE EVENT REPORTING

The site Investigator or designated research personnel will report all SAEs, whether related or unrelated to the investigational agent(s) to the **IRB in accordance with their local institutional**

guidelines. The site will notify the Roswell Park Multi-Site QA Coordinator within 1 business day of being made aware of the SAE. A preliminary written report must follow within 1 business day of the first notification using the following forms:

- Roswell Park SAE Source form

Add instructions for notifying pharmaceutical company, if appropriate.

A complete follow-up report must be sent to the Roswell Park Multi-Site QA Coordinator when new information becomes available.

8. UNANTICIPATED PROBLEM REPORTING

An unanticipated problem (UP) is any incident, experience, or outcome that meets all of the criteria in **Section 17.10**.

For all adverse events occurring that are unanticipated and related or possibly related to the research drug, biologic or intervention, the participating physician or delegated research staff from each site will notify their local **IRB in accordance with their local institutional guidelines**. The site must also notify the Roswell Park Multi-Site QA Coordinator within 1 business day of being made aware of the Unanticipated Problem by completing the **Roswell Park Unanticipated Problem Report Form** and faxing or emailing it to the Roswell Park Multi-Site QA Coordinator.