

**TITLE: A Phase I/1b Study of Enzalutamide in Combination with Gemcitabine
and Cisplatin in Bladder Cancer**

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1. OBJECTIVES

1.1. Primary Objective

To determine the safety and tolerability of enzalutamide at doses of 80 mg and 160 mg each in combination with gemcitabine and cisplatin in advanced bladder cancer. Once the safety of the combination is established, the phase 1 cohort will be expanded to an additional cohort of 12 patients with stage IV bladder cancer (phase 1b), who express androgen receptor (AR) by immunohistochemistry (IHC), to determine the safety and tolerability of enzalutamide and gemcitabine and cisplatin in this expanded cohort of patients with AR+ bladder cancer.

1.2. Secondary Objectives

- To evaluate the response rate (RR) of enzalutamide in combination with gemcitabine and cisplatin in advanced bladder cancer. Evaluation of response would be based on Response Evaluation Criteria in Solid Tumors (RECIST) criteria for all solid tumors.
- To evaluate progression free survival.
- To evaluate overall survival.

1.3. Exploratory Objectives

- To determine if AR expression in bladder cancer correlates with outcome. For patients with positive AR expression on IHC, we will use Automated Quantitative Analysis (AQUA) for precise quantitative scoring and test for pAKT in addition to AR to better understand biological behavior of tumors, including responders and non-responders.
- To evaluate whether circulating tumor cell (CTC) count measurements at baseline and on day 1 of cycle 3 correlate with disease burden, and treatment response respectively. AR expression in CTCs will also be evaluated and correlated with response.

2. BACKGROUND

2.1 Bladder Cancer

Bladder cancer is the 4th most common malignancy in men and the 9th most common in women in the US, with an estimated 72,570 new case and 15,210 deaths in 2013. ¹ Approximately 25% of patients initially present with muscle invasive disease, which is life threatening. Despite radical cystectomy, approximately 50% of patients develop disease recurrence and require subsequent systemic chemotherapy within 5 years usually in the first 2-3 years. Transitional cell cancer is a chemotherapy-sensitive malignancy and systemic chemotherapy with platinum based regimens is an integral component of first-line therapy. However, durable responses are rarely seen and most patients with metastatic disease recur. Combination regimens with multiple conventional cytotoxics, have been developed. Methotrexate, vinblastine, doxorubicin, and cisplatin (M-VAC) combination chemotherapy demonstrated a median survival of

13 months, however the regimen was quite toxic. A follow-up phase 3 trial comparing M-VAC with the combination of gemcitabine and cisplatin (GC) was conducted, demonstrating similar efficacy (ORR of 49% versus 46%), and time to disease progression (7.4 months in both arms), and similar median survivals (14.8 months vs. 13.8 months), respectively. Patients in the GC arm also experienced less adverse events.² There are no standard 2nd line therapy options and taxane bases regimens are frequently employed with low response rates and very little options after progression on taxanes. Majority of patients with metastatic urothelial cancer will die of their disease in less than two years. Effective treatment of bladder cancer is an unmet medical need and novel treatment strategies are urgently required.

2.2 Androgen Receptor and Bladder cancer

Androgen receptor (AR) signaling has been reported to play a critical role in the development of prostate cancer and anti-androgen therapy is effective in stopping the growth and progression of prostate cancer.⁽¹⁰⁻¹³⁾

The higher incidence of urothelial cancer of urinary bladder in men could be related to AR being involved in the development of bladder cancer. The AR, a member of the nuclear receptor superfamily, is a ligand-dependent transcriptional factor that mediates the biologic effects of androgens. AR expression has been demonstrated in various bladder cancer cell lines, expression of the AR by immunohistochemistry (IHC) has been demonstrated in normal bladder epithelium, bladder smooth muscle and neuronal cells.^{3,4} Early studies using androgen-binding assays suggested the expression of AR in bladder tumors.⁵ Recent studies showed AR expression in more than half of bladder cancers using immunohistochemistry (IHC).⁶

It has been demonstrated by Miyamoto et al. that dihydrotestosterone (DHT) treatment increased the growth of TCC-SUP cells by 55 and UMUC3 cells by 45%. 95% CI = 18% to 73%) and the antiandrogen, hydroxyflutamide (HF) partially antagonized (up to 75% reduction) in both cell lines. Of note, no significant activity was seen with DHT and HF on cell growth in AR negative bladder cancer cell lines, thus indicating that androgen treatment increases proliferation of AR-positive bladder cancer cells.⁷ These findings were confirmed in xenograft mouse models as well, using androgen deprivation therapy via castration and/or antiandrogen like flutamide, treatment with an anti-AR compound ASC-J9, and treatment with AR-siRNA.⁷ TCC-SUP tumors in mice treated with castration and/or antiandrogen were statistically significantly smaller than those in the control mice at 16 weeks. When the tumors in the treatment groups were harvested, their weights were found to be reduced by 57% to 63%. Significant reduction in tumors was also seen in UMUC3 xenograft tumors, and only minimal effects of androgen deprivation therapy were seen in the AR-negative 5637 xenograft tumors. Androgen deprivation also decreased proliferation by up to 73%, induced apoptosis and reduced levels of angiogenic factors and metastasis-related factors in TCC-SUP tumors.

The effect of a novel AR antagonist, ASC-J9, a curcumin analog which directly targets the AR by dissociating AR coregulators from the AR, leading to selective degradation of the AR proteins were also studied in vitro and in vivo.^{8,9} ASC-J9 inhibited DHT-simulated growth of TCCSUP and UMUC3 cells and inhibited tumor growth by 58 %. Moreover, it decreased the proliferation index, increased the apoptotic index, and reduced levels of angiogenic factors and metastasis-related factors. ASC-J9 also reduced AR expression by 39% suggesting that directly targeting the AR can inhibit androgen-sensitive bladder cancer progression. It has been demonstrated that AR knockdown in AR-expressing bladder cancer cell lines by siRNA also decreased cell proliferation, even in androgen-depleted conditions, and castration and flutamide treatment had little effect on the development of AR-siRNA-expressing tumors, it is possible that AR signals (via androgen-mediated and non-androgen-mediated pathways) might contribute to the bladder cancer progression.⁶ These preclinical findings demonstrate that androgens increase the growth of AR-positive bladder cancer cells and androgen deprivation and or/ anti-androgen therapy, including novel AR antagonist suppressed cancer progression. This study supports the concept that androgens may be implicated in the growth of bladder cancer and effective blockade of androgen receptors offers a potentially new approach to treatment.

2.3 Enzalutamide

Enzalutamide (formerly MDV3100) is an androgen-receptor-signaling inhibitor chosen for clinical development on the basis of activity in prostate-cancer models with over expression of the androgen receptor. Enzalutamide is distinct from the currently available antiandrogen agents in that it inhibits nuclear translocation of the androgen receptor, DNA binding, and co activator recruitment. It also has a greater affinity for the receptor, induces tumor shrinkage in xenograft models (in which conventional agents only retard growth), and has no known agonistic effects.^{14,15} Enzalutamide, unlike conventional anti-androgens bicalutamide and flutamide, lacks agonist activity. In the landmark phase 3, double-blind, placebo-controlled trial (AFFIRM), 1199 men with castration-resistant prostate cancer after chemotherapy were randomized to receive oral enzalutamide at a dose of 160 mg per day (800 patients) or placebo (399 patients). The primary end point was overall survival. The study was stopped after a planned interim analysis at the time of 520 deaths. The median overall survival was 18.4 months in the enzalutamide group versus 13.6 months in the placebo group (hazard ratio for death in the enzalutamide group, 0.63; 95% CI, 0.53 to 0.75; $P<0.001$). The superiority of enzalutamide over placebo was shown with respect to all secondary end points: the proportion of patients with a reduction in the prostate-specific antigen (PSA) level by 50% or more (54% vs. 2%, $P<0.001$), the soft-tissue response rate (29% vs. 4%, $P<0.001$), the quality-of-life response rate (43% vs. 18%, $P<0.001$), the time to PSA progression (8.3 vs. 3.0 months; hazard ratio, 0.25; $P<0.001$), radiographic progression-free survival (8.3 vs. 2.9 months; hazard ratio, 0.40; $P<0.001$), and the time to the first skeletal-related event (16.7 vs. 13.3 months; hazard ratio, 0.69; $P<0.001$). Rates of fatigue, diarrhea, and hot flashes were higher in

the enzalutamide group and seizures were reported in five patients (0.6%) receiving enzalutamide¹⁶ Enzalutamide was approved by the FDA in 2012, and by the EMA in 2013, for patients with mCRPC progressing on docetaxel chemotherapy.

Enzalutamide has also been studied in men with chemotherapy-naïve asymptomatic or mildly symptomatic mCRPC in the PREVAIL study (randomized, double blind placebo controlled multinational phase 3 trial).¹⁷ A total of 1717 patients were randomized to enzalutamide 160mg orally daily or placebo. The interim analysis at 540 deaths showed a statistically significant benefit of enzalutamide over placebo with a 29% reduction in risk of death (OS: HR 0.71; 95% CI: 0.6-0.84, p<0.001 and an 81% reduction in risk of radiographic progression or death (rPFS: HR 0.19; 95% CI: 0.15-0.23; P< 0.001). Estimated median OS was 32.4 months with enzalutamide compared to 30.2 months with placebo. An updated survival analysis presented at ASCO 2014 with additional follow-up time and events demonstrated a median OS that was not reached with enzalutamide vs. 31 months for placebo (HR 0.73, p<0.001). Median rPFS was not reached in the enzalutamide arm vs 3.9 mo in the placebo arm. The Independent Data Monitoring Committee considered the benefit-risk ratio to favor enzalutamide and recommended stopping the study and crossing placebo patients to enzalutamide. Thus treatment with enzalutamide significantly improves OS and rPFS in men with chemotherapy-naïve mCRPC and is anticipated to receive FDA approval for this setting soon.

Based on the preclinical rationale for utilizing androgen blockade in bladder cancer, we will attempt to study whether it can be safely combined with cisplatin and gemcitabine in bladder cancer.

Enzalutamide has been safely combined with chemotherapy agents like docetaxel at doses of 160 mg and there was no incidence of increased toxicity.¹⁸

3. PATIENT SELECTION

3.1 Inclusion Criteria

3.1.1 Cytologically or histologically confirmed evidence of transitional cell carcinoma of bladder, renal pelvis, ureter or urethra.

3.1.2 Patients with Stage IV (locally advanced or metastatic) disease. The AJCC cancer staging manual, 7th edition will be used for staging (as below). RECIST 1.1 criteria will be used for measurable disease.

T4b	N0	M0
Any T	N1–3	M0
Any T	Any N	M1

3.1.3 Age ≥ 18 years.

3.1.4 Minimum of four weeks since any major surgery, completion of radiation.

3.1.5 Prior treatment with cytotoxic chemotherapy is not a requirement, but allowed only if used in neoadjuvant, adjuvant or for bladder preserving protocols, as long as was administered > 6 months prior to starting study.

3.1.6 ECOG performance status ≤ 2 .

3.1.7 Life expectancy 12 weeks or more.

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

- leukocytes $\geq 3,000/\mu\text{L}$
- absolute neutrophil count $\geq 1,500/\mu\text{L}$
- platelets $\geq 100,000/\mu\text{L}$
- hemoglobin $\geq 9 \text{ g/dL}$
- total bilirubin $\leq 2 \times$ institutional upper limit of normal
- AST(SGOT)/ALT(SGPT) $\leq 3 \times$ institutional upper limit of normal (≤ 5 if liver metastases present)
- creatinine $< 1.5 \text{ mg/dl}$
- INR < 1.3 (or < 3 if on warfarin or other anticoagulants)

Blood transfusion will be allowed for patients with hemoglobin less than 9 g/dl and G-CSF is allowed for neutropenic patients at time of enrollment.

Chemotherapy treatment can only be administered 48 hours post G-CSF therapy.

3.1.9 Women of childbearing potential must have a negative serum or urine pregnancy test within 14 days of the administration of the first study treatment. Women must not be lactating.

3.1.10 Sexually active women of childbearing age and men should be willing to use two acceptable methods of birth control (one of which must include a condom as a barrier method of contraception) starting at screening and continuing through 3 months after the final study drugs administration. Men must agree to avoid sperm donation while on study and 3 months after the final study drugs administration.

3.1.11 Signed informed consent to participate in the study, including participation in the expansion cohort after safety of enzalutamide and gemcitabine and cisplatin have been established, must be obtained from patients after they have been fully informed of the nature and potential risks by the investigator (or his/her designee) with the aid of written information.

3.1.12 Patients should be able to swallow enzalutamide and comply with study requirements.

3.2 Exclusion Criteria

3.2.1 Prior treatment with any cytotoxic chemotherapy in metastatic setting. Prior treatment with cytotoxic chemotherapy is allowed only if used in neoadjuvant, adjuvant or for bladder preserving protocols, as long as was administered > 6 months prior to starting study.

3.2.2 Patients who have undergone major surgery within 4 weeks prior to study enrollment

3.2.3 Chronic treatment with steroids or any other immunosuppressant drugs.

3.2.4 Patients should not receive immunization with attenuated live vaccines during study period or within 1 week of study entry.

3.2.5 Patients with a history of seizures, predisposing factors for seizures, including underlying brain injury with loss of consciousness within previous 12 months, transient ischemic attack within previous 12 months, cerebral vascular accident or brain arteriovenous malformation.

3.2.6 Untreated brain or leptomeningeal metastases, including patients who continue to require glucocorticoids for brain or leptomeningeal metastases.

3.2.7 Patients who have congestive heart failure (NYHA Class III or IV), unstable angina, sustained ventricular tachycardia, ventricular fibrillation, clinically significant bradycardia, advanced heart block or a history of acute myocardial infarction within the six months preceding enrollment.

3.2.8 Patients with known history of HIV.

3.2.9 Patients who have any severe and/or uncontrolled medical conditions or other conditions that could affect their participation in the study such as any active (acute or chronic) or uncontrolled infection/ disorders or nonmalignant medical illnesses that are uncontrolled or whose control may be jeopardized by the treatment with the study therapy

3.2.10 Women who are pregnant or breast feeding, or women/men able to conceive and unwilling to practice an effective method of birth control (one of which must include a condom as a barrier method of contraception) starting at

screening and continuing through 3 months after the final study drugs administration

3.2.11 Concurrent medications which strongly inhibit or induce CYP enzymes (gemfibrozil, Rifampin, carbamazepine, phenobarbital, phenytoin, rifabutin, rifampin, rifapentine, bosentan, efavirenz, etravirine, modafinil, nafcillin, St. John's Wort)

3.2.12 History of stage III or greater cancer, except basal or squamous cell skin cancers adequately treated or any other stage I or II cancer adequately treated and disease-free for ≥ 2 years. Incidental findings of stage I or II prostate cancer that is considered to be cured with radical cystoprostatectomy is allowed.

3.2.13 Prior use of enzalutamide.

3.2.14 Radiation therapy via external beam or brachytherapy within 28 days of registration.

3.2.15 Patients who are ineligible to receive cisplatin:
Creatinine clearance of less than 60 mL/minute, hearing loss of 25 dB at two contiguous frequencies, grade 2 or higher peripheral neuropathy, or New York Heart Association Class III or higher heart failure.

Hearing test will not be routinely done, it will only be done if patients report hearing loss at baseline or during treatment.

3.2.16 Allergy/sensitivity to any study drug (gemcitabine, cisplatin, enzalutamide), or drugs chemically related to study drug, or excipients .

3.2.17 Patients with brain metastases (including treated or stable brain metastases).

Although, positive AR expression is not a requirement for the dose escalation phase, it will be tested in archival tissue from formalin-fixed, paraffin-embedded, tumor specimen from either radical cystectomy or TURBT. If patients have received neoadjuvant chemotherapy, then pre-chemotherapy TURBT archival tissue specimen will be used and if there was no neoadjuvant chemotherapy administered, then either TURBT specimen or radical cystectomy specimen can be used. A fresh biopsy specimen would be obtained if archival tissue not available. Dose expansion Cohort: The inclusion and exclusion criteria for the expansion cohort are the same as above, except for the expansion cohort also required to express AR (score 1+ or more) by IHC in archival bladder cancer samples or fresh biopsy specimens (if archival tissue not available).

4. TREATMENT PLAN

4.1 Drug Administration

Treatment will be administered on an outpatient basis. Reported AEs and potential risks are described in Section 6. Appropriate dose modifications for enzalutamide, cisplatin, and gemcitabine are described in Section 5. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's cancer.

For the phase 1 dose escalation phase, the starting dose of enzalutamide will be 80 mg orally once a day (Level 1). The dosing regimen of cisplatin and gemcitabine will be at standard doses of Gemcitabine at 1000 mg/m² IV on days 1, 8 and cisplatin at 70 mg/m² IV on day 1, repeated every 21 days for total of 6 cycles.

Three patients will be treated dose level 1 (enzalutamide 80 mg daily). If 0 patients experience dose limiting toxicity (DLT), dose escalation will be done to level 2 of enzalutamide 160 mg daily. If 1 patient experiences DLT, 3 more patients will be treated at the same dose level; if 1 of 6 experiences DLT, escalate the dose to next level, and if 2 or more of 6 experiences DLT, the dose level 1 (80 mg enzalutamide) will be the recommended dose for dose expansion cohort.

Three patients have been treated with dose level 1 and one patients has completed dosel level 2 and completed the DLT period.

The cohort expansion will then be done by enrolling 12 patients with stage IV bladder cancer, who express AR staining of 1+ and above by IHC, to determine the safety and tolerability of cisplatin and gemcitabine with the recommended dose level of enzalutamide (80 mg or 160 mg, depending upon the safety results from dose escalation part) in this expanded cohort of patients with AR+ bladder cancer.

Enzalutamide would be continued after completion of 6 cycles of gemcitabine-cisplatin for patients exhibiting a response or stable disease, until they experience disease progression.

Dose Escalation Schedule			
Dose Level	Dose		
	Enzalutamide	Gemcitabine	Cisplatin
Level 1	80 mg PO daily	1000 mg/m ² IV day 1, and 8 every 21 days	70 mg/m ² every 21 days
Level 2	160 mg PO daily	1000 mg/m ² IV day 1, and 8 every 21 days	70 mg/m ² every 21 days

Regimen Description:

Days 1-21: Enzalutamide 80 mg or 160 mg PO daily

Day 1: Premeditations:

Aloxi 0.25mg IV Push, Fosaprepitant 150mg in 250mL 0.9% NaCl IVPB over 30 minutes
Dexamethasone 12mg PO

Treatment Drugs:

Gemcitabine: IV infusion in 250 ml 0.9% NaCl over 30 minutes
0.9% NaCl over 1 hour pre-Cisplatin

Mannitol 12.5 gm IVPB in 50ml 0.9% NaCl over 15 minutes pre-Cisplatin
Cisplatin: IV solution in 500mL NaCl 0.9% over 60-120 minutes
1000mL 0.9% NaCl + 8mEq MgSO4 + 20 mEq KCL over 2 hours post-Cisplatin

Day 8: Premedications:

Prochlorperazine 10mg po Pre-Chemo

Treatment Drug:

Gemcitabine: IV infusion in 250 ml 0.95 NaCl over 30-60 minutes

4.2 Definition of Dose-Limiting Toxicity

Dose-Limiting Toxicity (DLT) is defined as any of the following occurring in the first 21 days (cycle 1) of study participation that are considered at least **possibly related** to enzalutamide administration. Toxicities that are in the opinion of the investigator(s) attributable exclusively to gemcitabine or cisplatin will not be considered DLT.

- More than 7 consecutive missed doses (out of 21 doses) of enzalutamide in 21 days due to enzalutamide related toxicity.
- Missed day 8 dose of gemcitabine in cycle 1 will not be considered DLT.
- Delay of greater than 3 weeks from scheduled date in initiating cycle 2 due to enzalutamide, gemcitabine or cisplatin related toxicity.
Discontinuation of a patient due to enzalutamide related toxicity before completing cycle 1. **Hematologic toxicity**
- Hematologic toxicity is generally not expected from enzalutamide, the frequency of grade 3-4 neutropenia reported with enzalutamide is 1% and grade 3-4 thrombocytopenia is 0.5%. However, it is frequently seen with cytotoxic chemotherapeutic agents, like cisplatin and gemcitabine, mainly due to gemcitabine. For the purposes of this study, any grade 3/4 hematologic event resulting in neutropenic fever or neutropenia lasting more than 7 days in spite of optimal supportive medications or spontaneous bleeding will be likely from chemotherapy and not considered a DLT.

Non-Hematologic toxicity

- Grade ≥ 3 nausea, vomiting or diarrhea lasting more than 72 hours in the setting of optimal supportive care and medications.
- Any other Grade 3 non-hematological toxicity (except for electrolytes abnormalities that are reversible or hair loss which is not dose-limiting) that results in greater than 7 days interruption of therapy.
- Intra-patient dose de-escalation will be allowed, i.e. if patients have Grade 3-4 AEs on 160 mg enzalutamide, they will be allowed to try 80 mg enzalutamide. If they cannot tolerate 80 mg enzalutamide, enzalutamide will be stopped and they can receive only gemcitabine-cisplatin off protocol.
- Occurrence of seizure, which can rarely occur with enzalutamide, will be considered a DLT.
- Any other grade 3 or 4 non-hematologic toxicity that is definitely attributable to enzalutamide and not gemcitabine or cisplatin.

- Hepatic toxicity: Hepatic enzyme and bilirubin increase which meet Hy's law criteria (ALT/AST > 3 X upper limits of normal, total bilirubin > 2 X upper limits of normal and alkaline phosphatase < 2 X upper limits of normal).

The DLT evaluation set is defined as all patients who receive at least one dose of the enzalutamide, gemcitabine and cisplatin and complete Cycle 1 or discontinue due to toxicity related to enzalutamide. DLT will be derived from the toxicities observed during the first cycle (21 days) for each dose level.

Any patient who does not complete the evaluation period for DLTs in cycle 1 for reasons other than study drug-related toxicity will be replaced.

Adverse events will be graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Version 4.0.

At dose level 1, if 0 patients experience dose limiting toxicity (DLT), dose escalation will be done to level 2 of 160 mg daily.

For dose level 1 (80 mg enzalutamide), if 1 patient experiences DLT, 3 more patients will be treated at the same dose level; if 1 of 6 patients experiences DLT, escalate to dose to next level (160 mg enzalutamide), and if 2 or more of 6 experiences DLT on 160 mg enzalutamide, the recommended dose of enzalutamide will be 80 mg. If at dose level 1 (80 mg), 2 or more patients experience DLT, the study would be stopped.

The cohort expansion will then be done by enrolling 12 patients with stage IV bladder cancer, who express AR staining of 1+ and above by IHC, to determine the safety and tolerability of the recommended dose of enzalutamide with gemcitabine and cisplatin in this expanded cohort of patients with AR+ bladder cancer.

4.3 Supportive Care Guidelines

Anti-emetics are permitted for prevention and treatment of nausea and vomiting. Patients will be given the standard anti-emetics used for gemcitabine and cisplatin as outlined above. For patients who are allergic, have unacceptable side effects or have limited benefit from the above anti-emetics, other agents including ondansetron can be used for both prophylaxis and treatment. Prophylactic dexamethasone at doses of 4 mg Po BID can be used day after cisplatin chemotherapy. Anti-diarrheal agents can be used for the treatment of diarrhea.

G-CSF is allowed for treatment and prevention of neutropenia. The dose and schedule are at the discretion of the investigators. Chemotherapy treatment can only be administered 48 hours post G-CSF therapy.

Packed red cell transfusion and platelet transfusion will be allowed as clinically indicated.

4.4 **Duration of Therapy**

In the absence of treatment delays due to AEs, treatment may continue until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable AEs
- Patient decides to withdraw from the study
- Any changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

For cycle 2 and beyond, if there is a treatment cycle delay of more than 3 weeks, patients will be removed from the study.

4.5 **Duration of Follow Up**

Patients will be followed for 30 days for AEs after removal from study or until death, whichever occurs first. Patients with significant or unacceptable AEs at the time of removal from the study therapy will be followed until the toxicities resolve or are deemed irreversible. Patients will be followed for survival every 2-3 months by telephone call for up to 1 year.

4.6 **Patient Replacement**

Any patient who does not complete the evaluation period for DLTs in cycle 1 for reasons other than study drug (enzalutamide)-related toxicity will be replaced.

4.7 **Discontinuation Criteria**

Patients would be discontinued from the study for any of the following:

- Any AE that is intolerable to the subject and that cannot be ameliorated by the use of adequate medical intervention, or that in the opinion of the investigator would lead to undue risk to the subject if dosing continued
- Any treatment emergent seizure or any condition that significantly predisposes the patient to have seizures, like brain metastases or clinically evident stroke will be a criteria for discontinuation from the study.
- Symptomatic or radiographic disease progression
- Any current illness that prevents further administration of treatment

- Subject who is, in the opinion of the investigator, grossly non-compliant with the protocol's requirements

5. DOSE DELAYS/DOSE MODIFICATIONS

The treatment can be interrupted if the treatment criteria are not met as mentioned in section 5.1 and 5.2. If a patient requires treatment cycle interruption of more than 3 weeks, the patient will be taken off study. Patient can continue to receive gemcitabine and cisplatin off protocol at the discretion of treating physician.

Dose modifications for certain events may be limited to just one of the drugs based on what the event is and which drug may be most likely causing it. For example, nausea/vomiting, ototoxicity or neuropathy with cisplatin, diarrhea with gemcitabine, hot flashes with enzalutamide etc. However, if improvement does not occur following reduction or cessation of dosing of the suspected investigational agent, other drug(s) should be considered as a contributing factor. Once the dose has been reduced, patients cannot be dose escalated to previous level. The treatment can be held at investigator discretion, if in the opinion of the investigator it is not safe to proceed with the treatment. For cycle 2 and beyond, dose delay for day 8 Gemcitabine is allowed for 1 week and if patients are unable to get gemcitabine on day 15 as well, they will not receive it as part of that cycle.

5.1 Dose Modification Guidelines for Hematologic Toxicity

The dose modification guidelines for gemcitabine and cisplatin for hematologic toxicity on day 1 of cycle 2 and subsequent cycles is mentioned below.

ANC		Platelet count	Gemcitabine	Cisplatin
$\geq 1,500/\text{mm}^3$	and	$\geq 100,000/\text{mm}^3$	Give full dose	
$< 1500/\text{mm}^3$	or/and	$< 100,000/\text{mm}^3$	Delay treatment by 1 week.	

The dose modification guidelines for gemcitabine for hematologic toxicity on day 8 of each cycle of the treatment are mentioned below:

ANC		Platelet count	Gemcitabine
$\geq 1,000/\text{mm}^3$	and	$\geq 75,000/\text{mm}^3$	Give full dose
$\geq 1,000/\text{mm}^3$	and	50,000-74,999	Give 75% dose
$< 1,000/\text{mm}^3$	or/and	$< 50,000/\text{mm}^3$	Skip treatment. Reduce dose by 25% for next treatment.

It is recommended that G-CSF is administered if patients develop neutropenia. Primary prophylaxis with G-CSF should be considered with subsequent cycles if patients develop neutropenic complications in the immediate previous cycle. If febrile neutropenia requiring antibiotic therapy or grade 4 thrombocytopenia occurs, the gemcitabine dose will be reduced to 75% for subsequent treatment cycles. The dose of cisplatin may be reduced to 75% for subsequent treatment cycles at the discretion of treating physician. The dose of enzalutamide will not be modified for hematologic toxicity as the incidence of cytopenias with enzalutamide is less than or equal to 1%.

5.2 Dose Modification Guidelines for Non-Hematologic Toxicity

The dose modification guidelines for cisplatin and gemcitabine for non-hematologic toxicities on day 1 and day 8 of each cycle (as applicable) is mentioned below:

Non-Hematologic Toxicity*	Gemcitabine and Cisplatin
Grade 1 or 2	Give full dose.
Grade 3 or 4	Give 50% dose or hold dose (per treating physician discretion). Delay treatment until resolves to \leq Grade 2. Reduce dose by 25% for next treatment.

Non Hematologic Toxicity*	Enzalutamide
Grade 1 or 2	Treat as scheduled
Grade 3 or 4	<p>Delay treatment until resolves to \leq Grade 2.</p> <p><u>For dose escalation portion:</u> At dose level 1 (80 mg enzalutamide), if 1 patient experiences DLT, 3 more patients will be treated at the same dose level, if 2 or more patients experience DLT, the enzalutamide would be stopped.</p> <p>If 1/6 patients experiences DLT, the enzalutamide dose will be escalated to 160 mg. If 2 or more of 6 patients experience DLT on 160 mg enzalutamide, the recommended dose of enzalutamide will be 80 mg for dose expansion portion.</p> <p><u>For dose expansion portion:</u> Intra-patient dose de-escalation will be allowed, i.e. if patients has Grade 3-4 AEs on 160 mg enzalutamide, they will be allowed to try 80 mg enzalutamide. If they cannot tolerate 80 mg enzalutamide, enzalutamide will be stopped and they can receive only gemcitabine-cisplatin off protocol. If the recommended dose expansion phase dose is 80 mg, and patients cannot tolerate it, they will be allowed to hold it for 7-10 days, and if symptoms do not improve, and they cannot resume this dose, enzalutamide will be stopped and they can receive only gemcitabine-cisplatin off protocol.</p>

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*Except for alopecia, clinically insignificant laboratory abnormalities, and inadequately treated nausea, vomiting and diarrhea.

Serum creatinine will be evaluated on the first treatment day of each cycle. The treatment will be delayed by 1 week if the serum creatinine was >1.5 mg/dl; cisplatin will be reduced by 25% or held for subsequent cycles if the repeat serum creatinine level a week later is 1.6–2 or >2 mg/dl, respectively. The dose of cisplatin will also be decreased by 25% in the event of severe neurotoxicity

6. PHARMACEUTICAL INFORMATION

6.1 Enzalutamide

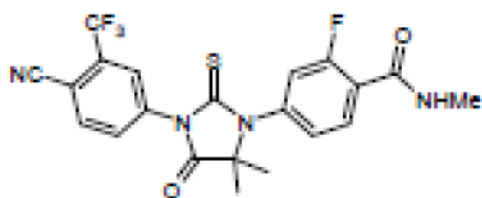
Enzalutamide (MDV3100) is an AR signaling inhibitor that targets several steps in the AR signaling pathway. It is approved for the treatment of castrate resistant prostate cancer. Refer to the package insert for full information about enzalutamide.

Chemical Name

4-{3-[4-Cyano-3-(trifluoromethyl)phenyl]-5,5-dimethyl-4-oxo-2-sulfanylideneimidazolidin-1-yl}-2-fluoro-*N*-methylbenzamide

Structural Formula

The molecular formula is C₂₁H₁₆F₄N₄O₂S. The structure of enzalutamide is represented in the figure below.



Packaging and Storage

Enzalutamide is supplied as soft gelatin capsules. Each capsule 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. The drug is stored at room temperature

($\leq 25^{\circ}\text{C}$).

Reported Adverse Events and Potential Risks

The safety profile of enzalutamide is derived from multiple clinical studies. More than 1400 patients have been treated with enzalutamide, predominantly patients with prostate and breast cancer. Overall enzalutamide has been well tolerated by patients with prostate cancer with less than 10% of patients discontinuing the treatment due to treatment-emergent AEs. The following treatment related AEs are believed to be associated with enzalutamide treatment and are considered adverse drug reactions: seizure (<1%), fatigue (34%), diarrhea (21%), hot flash (20%), headache (20%), hypertension, anxiety, hallucinations, cognitive/memory impairment, falls, non-pathologic fractures, pruritis and dry skin. The most clinically important treatment-emergent AE, seizure, occurred in less than 1% (7/800) of enzalutamide-treated patients in a phase 3 study involving castrate resistant prostate cancer. In the phase 3 AFFIRM trial, \geq grade 3 AEs were reported in 45% of the patients as compared to 53% in the placebo group.^[16] Most common \geq grade 3 AEs in the enzalutamide group included fatigue (6%), diarrhea (1%), musculoskeletal pain (1%), headache (<1%), cardiac disorder (1%), seizure (<1%), and liver function abnormalities (<1%).

Availability

Enzalutamide is provided by Medivation/Astellas

Agent Accountability

The Investigator, pharmacist or qualified designee is responsible for making an inventory of study drug(s) upon their receipt. All used and unused study drug supplies should be retained until final reconciliation or as indicated by the investigator. The study drug is to be prescribed by the principal investigator or appropriately qualified site personnel named on the delegation of authority log. Under no circumstances will the investigator allow the study drug to be used other than as directed by this protocol. Although appropriate personnel may be designated to administer/dispense drug and maintain drug accountability records, the Principal Investigator is ultimately responsible for all drug accountability.

The Investigator or designee must maintain accurate records of the receipt and disposition of study drug supplies.

After study drug is reconciled, drug may be destroyed as per Institutional Policy, or returned to Astellas. The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of all agents.

6.2 Gemcitabine

Gemcitabine is a nucleoside inhibitor that is indicated for the treatment of various cancers, including bladder cancer. Gemcitabine is commercially available and will be supplied by the site's pharmacy. Refer to the package insert for full information about gemcitabine.

6.3 Cisplatin

Cisplatin is a platinum agent indicated for treatment of various cancers, including bladder cancer. Cisplatin is administered intravenously in normal saline for treatment of solid malignancies. Cisplatin is commercially available and will be supplied by the site's pharmacy. It is infused intravenously. Refer to the package insert for full information about cisplatin.

7. CORRELATIVE/SPECIAL STUDIES

7.1 AR Expression

The AR expression in bladder cancer is not validated and this study would aim to evaluate AR expression and outcomes in bladder cancer. All the patients would be included for AR analysis. While AR + by IHC is not an inclusion criteria for the dose finding phase and is an inclusion criteria only for the expansion cohort, for evaluation of outcomes, even AR- patients from the dose finding study will be included.

Archival tissue from formalin-fixed, paraffin-embedded, tumor specimen from either radical cystectomy or TURBT used to establish the diagnosis of urothelial carcinoma would be used for AR evaluation by IHC.

If patients have received neoadjuvant chemotherapy, then pre-chemotherapy TURBT archival tissue specimen will be used and if there was no neoadjuvant chemotherapy administered, then either TURBT specimen or radical cystectomy specimen can be used. If these archival tissues are unavailable or insufficient, a fresh core needle or incisional biopsy from a metastatic lesion would be used for AR evaluation. AR expression will be detected in tissue sections of formalin-fixed urothelial cancer specimens from bladder biopsy or cystectomy using standard IHC procedure.

We have validated AR (SP107) rabbit monoclonal antibody (Cell Marque) in Moffitt's CLIA certified pathology lab and will use this for the purposes of this study. The slides would be reviewed by a genitourinary pathologist for absence or presence of AR expression, based upon number of tumor cells exhibiting AR and the intensity of staining. Positive AR staining would be in 1% or more tumor cells.

For patients with positive AR expression, we will use Automated Quantitative Analysis (AQUA) for precise quantitative scoring. Automated image acquisition will be performed using the APERIO ScanScope FL and digital images will be analyzed using AQUAnalysis® software version 2.3.4.1. Briefly, high-resolution monochromatic 8-bit digital images will be obtained for each WTS slide using

separate filters to define the nuclear (DAPI), tumor (Cy3), and AR biomarker target (Cy5) compartments. An analysis algorithm will be constructed to identify tumor cell nuclei and calculate the nuclear AQUA score, defined as the average concentration of Cy5 (AR) pixel intensity within the tumor nuclear area for each WTS.

AQUA can be better calibrated using internal controls to overcome preanalytical variations that could lead to false results.

7.2 **PTEN loss**

The PI3K-AKT-mTOR signaling pathway is one of the most frequently dysregulated pathways in cancer and is thought to play a major role in many cancers, including bladder cancer.¹⁹⁻²¹ PTEN is a potent inhibitor of the PI3K/Akt/mTOR pathway and PTEN alterations have been described in several cancers, including bladder cancer, leading to tumorigenesis and poor prognosis. We will evaluate for loss of PTEN (which would imply the activation of the PI3K-AKT-mTOR pathway) in the bladder cancer tissues to better understand biological behavior of tumors, including responders and non-responders. The PTEN analysis will be done with a rabbit monoclonal antibody, clone Y184 using AQUA, as described in section 7.1. Loss of PTEN will be described as <10% of PTEN expression. We will also study the correlation between AR and loss of PTEN in bladder cancer.

Tumor biopsy for clinically responding and stable subjects at treatment discontinuation is highly encouraged to evaluate for any changes in AR signaling and apoptosis. Tumor biopsies that are inaccessible or contraindicated due to subject safety concerns are exempt from this requirement.

7.3 **Circulating Tumor Cells**

Several studies in patients with circulating tumor cells (CTCs) in metastatic prostate, breast and colon cancer have established their prognostic and treatment-predictive value.²²⁻²⁶ The molecular features of CTCs in advanced cancers have the potential to study pharmacodynamic effects, treatment selection, and possible mechanisms of resistance to treatment. Currently, there are no biologic markers for bladder cancer that may serve as a prognostic or predictive marker. Some studies have looked at the utility of CTCs in both high grade non-invasive and metastatic bladder cancer.²⁷⁻²⁹ Gallagher et al. evaluated CTCs in 33 patients with metastatic bladder cancer, 44% of them had a positive assay with significantly higher number of CTCs correlated with disease burden; moreover one-third of patients had 5 or more CTCs making it a potential marker to monitor response to chemotherapy.²⁸

We propose to measure CTCs at baseline, and on day 1 of cycle 3 in all patients, if budget allows. Baseline counts and changes in counts at the time of day 1 of cycle 3 will be evaluated for association with disease burden, RECIST response and/or changes in bone scan if applicable. CTC counts will be measured with CellSearch (Veridex, LLC, Huntingdon Valley, PA, USA), an analytically valid assay approved by the US FDA and report as number of CTCs per 7.5 mL of blood, as previously

described.²⁶ We will also test the CTCs for AR expression, to look at presence or absence of AR and in cases of positive AR in CTCs at baseline, we will monitor the AR expression while on treatment. Correlation will be done between presence or absence of AR in CTCs and response. We will use the AR (D6F11) XP rabbit mAB for detection of AR in CTCs. From available data from 2 patients enrolled in the dose escalation portion of the study, 1 patient expressed 3 CTCs of which 1 CTC expressed AR.

8. STUDY CALENDAR/PROCEDURES

8.1 Study Calendar

		Cycle1		Cycles 2-6		Cycle 7 and beyond			
	Screening within 14 days of C1d1	Day1	Day 8	Day1	Day 8	Day1	End of treatment visit \pm 14 days	End of study visit 30 ± 7 days	Followup for 1 year
Gemcitabine		x	x	x	x				
Cisplatin		x		x					
Enzalutamide ¹		x-----x							
Pre-Screening Consent	x								
Consent	x								
Demographics	x								
Medical history	x	x		x		x	x	x	
Concurrent meds	x	x-----x							
Physical exam	x	x		x		x	x	x	
Vital signs	x	x	x	x	x	x	x	x	
Weight	x	x	x	x	x	x	x	x	
ECOG PS	x	x		x		x	x	x	
CBC w/diff	x	x	x	x	x	x	x	x	
CMP, Magnesium	x	x	x	x	x	x	x	x	
Urine β -HCG ²	x								
Adverse event evaluation		x-----x							
Radiologic evaluation ³	x			x					
Archival tissue	x								

for AR ⁴							
Tumor Biopsy ⁵					X		
CTCs	X		X Cycle 3, day 1				
Telephone call for survival							X

All scheduled procedures/visits can be performed +/- 3 days unless specifically mentioned.

¹Enzalutamide must be administered at the time of completing gemcitabine and cisplatin infusion on cycle 1 day 1 of treatment.

²Serum pregnancy test will be performed within 14 days of starting treatment only in women of childbearing potential.

³CT scan of thorax, abdomen, pelvis should be performed within 4 weeks of starting treatment and every 2 cycles (anytime after day 15 dosing of the preceding cycle and prior to starting next cycle).

⁴ While AR expression in tumor tissue will be tested for all patients in dose escalation and dose expansion phases, positive AR expression is only a requirement for enrollment in the dose expansion phase. Pre-screening consent for AR testing from archival tissue will be obtained for all patients in the dose expansion phase.

⁵ Tumor biopsy for clinically responding and stable subjects at treatment discontinuation is highly encouraged to evaluate for any changes in AR signaling and apoptosis.

9. MEASUREMENT OF EFFECT

Patients will be evaluated for response at the end of every 2 cycles. Each cycle lasts for 3 weeks.

9.1 Antitumor Effect

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1). 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.

All the patients would be included for analysis. While AR + by IHC is not an inclusion criteria for the dose finding phase and is an inclusion criteria only for the expansion cohort, for evaluation of outcomes, even AR- patients from the dose finding study will be included.

9.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their

first treatment with enzalutamide.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

9.1.2 Disease Parameters

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

Note: Tumor lesions that are situated in a previously irradiated area might be considered measurable if the lesion has increased in size since the radiation.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

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

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

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and

should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

9.1.3 Methods for Evaluation of Measurable Disease

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

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

Conventional CT Scans This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

9.1.4 Response Criteria

9.1.4.1 Evaluation of Target Lesions

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

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

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

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

9.1.4.2 Evaluation of Non-Target Lesions

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

short axis).

Non-CR/Non-PD: Persistence of one or more non-target lesion(s).

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

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

9.1.4.3 Evaluation of Best Overall Response

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

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-CR/Non-PD/not evaluated	No	PR
SD	Non-CR/Non-PD/not evaluated	No	SD
PD	Any	Yes or No	PD
Any	PD*	Yes or No	PD
Any	Any	Yes	PD
* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.			

9.1.5 Duration of Response

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

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

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

9.1.6 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

10. REGULATORY CONSIDERATIONS

10.1 Adverse Event

An AE is any untoward medical occurrence or worsening of a pre-existing medical condition in a subject. AE will be graded using the National Cancer Institute CTCAE version 4.0. A laboratory test abnormality considered clinically relevant, e.g., causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an AE.

Serious Adverse Event

AEs are classified as serious or non-serious. A *serious adverse event* (SAE) is any AE that is:

- 1) fatal
- 2) life-threatening
- 3) requires or prolongs hospital stay
- 4) results in persistent or significant disability or incapacity
- 5) a congenital anomaly or birth defect
- 6) an important medical event. Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

10.2 Reporting Adverse Events

All SAEs should be reported to H. Lee Moffitt Cancer Center & Research Institute through Oncore for review by our Protocol Review & Monitoring Committee. *All SAEs related to enzalutamide should be reported to Astellas.* SAEs are reported to the reviewing IRB as well per the institution policy. All SAEs regardless of relationship to investigational product will be collected from the time a subject signs the informed consent to participate until the end of the follow-up period (30 days after last dose).

All AEs will be recorded on the appropriate CRF and in the subject's medical records. The Investigator will also identify the date of onset, date of resolution, seriousness, outcome, and the relationship to study drug (enzalutamide). Every effort should be made to determine the cause of each AE and whether or not it is related to enzalutamide. The relationship of the AE to enzalutamide must be rated and recorded following the guidelines outlined in the CTCAE v4.0. The 5 categories for AE grading are:

- 1- **Not related**
- 2- **Not Likely**
- 3- **Possible**
- 4- **Probable**
- 5- **Definite**

Safety events of interest that may require expedited reporting and/or safety evaluation include, but are not limited to overdose of enzalutamide, suspected abuse/misuse of enzalutamide, medication error with or without subject/patient exposure to enzalutamide, e.g. name confusion)

10.3 Investigator Responsibilities

Investigator responsibilities are set out in the ICH guideline for Good Clinical Practice and in the US Code of Federal Regulations. Investigators, or designee, must enter study data onto a data collection system. The Investigator will permit study-related monitoring visits and audits IRB/EC review, and regulatory inspection(s) (e.g., FDA, EMEA, and TPP), providing direct access to the facilities where the study took place, to source documents, to the data collection system, and to all other study documents. The Investigator, or a designated member of the Investigator's staff, must be available at some time during monitoring visits to review data and resolve any queries and to allow direct access to the subject's records (e.g., medical records, office charts, hospital charts, and study related charts) for source data verification. The data collection must be completed prior to any visits.

10.4 Institutional Review Board Approval

The protocol for this study has been designed in accordance with the general ethical principles outlined in the Declaration of Helsinki. The review of this protocol by the IRB and the performance of all aspects of the study, including the methods used for obtaining informed consent, must also be in accordance with principles enunciated in the declaration, as well as ICH Guidelines, Title 21 of the Code of Federal Regulations (CFR), Part 50 Protection of Human Subjects and Part 56 Institutional Review Boards. The Investigator will be responsible for preparing documents for submission to the relevant IRB and obtaining written approval for this study. The approval will be obtained prior to the initiation of the study. The Investigator is also responsible for notifying the IRB of any serious deviations from the protocol, or anything else that may involve added risk to subjects. Any advertisements used to recruit subjects for the study must be reviewed and approved by the IRB prior to use.

10.5 Informed consent

The Investigator must obtain informed consent of a subject or his/her designee prior to any study related procedures as per Good Clinical Practices (GCP) as set forth in the CFR and ICH guidelines. Documentation that informed consent occurred prior to the subject's entry into the study and the informed consent process should be recorded in the subject's source documents. The original consent form signed and dated by the subject and by the person consenting the subject prior to the subject's entry into the study, must be maintained in the Investigator's study files.

10.6 Study Records Requirements

The Investigator must ensure that the records and documents pertaining to the conduct of the study and the distribution of the study drug (enzalutamide), that is copies of data collection and source documents (original documents, data, and records [e.g., hospital records; clinical and office charts; laboratory notes; memoranda; subject's diaries or evaluation checklists; pharmacy dispensing records; recorded data from automated instruments; copies or transcriptions certified after verification as being accurate copies; subject files; and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical study; documents regarding subject treatment and study drug accountability; original signed informed consents, etc.]) be retained by the investigator for as long as needed to comply with national and international regulations (generally 2 years after discontinuing clinical development or after the last marketing approval). At any time the Investigator may be subject to a field audit by regulatory authorities (e.g., FDA, TPP, EMEA) in order to validate the participation of subjects in the study. Therefore, careful attention should be paid to seeing that all study documents/records are complete, accurate, and filed and retained by the Investigator.

10.7 Patient Registration

All subjects must be registered with the MCRN Coordinating Center to be able to participate in a trial. The participating site must fax or email the completed study specific eligibility checklist and registration forms, supporting documents and signed informed consent to the Coordinating Center. Unsigned or incomplete forms will be returned to the site. Once documents are received, the MCRN Research Coordinator will review them to confirm eligibility and to complete the registration process. If eligibility cannot be confirmed, the research coordinator will query the site for clarification or additional documents as needed. Subjects failing to meet all study eligibility requirements will not be registered and will be unable to participate in the trial.

Upon completion of registration, the MCRN Research Coordinator will provide the participating site with the study sequence number and randomization information, if indicated. Within 24-48 hours after registration, it is the site's responsibility to:

- Enter the demographic and on-study patient information into the Oncore database

- Order investigational agent(s) if indicated per protocol

It is the responsibility of the participating Investigator or designee to inform the subject of the research treatment plan and to conduct the study in compliance with the protocol as agreed upon with Moffitt Cancer Center and approved by the site's IRB.

To register a patient send the completed signed eligibility checklist along with supporting documentation to [REDACTED]

10.8 Data Management

Data Collection

The Clinical Research Coordinators and Investigators of each site will be responsible for the recording of the site's data into the electronic data capture system, ONCORE.

Protocol Monitoring Committee

The Protocol monitoring committee (PMC) will be composed of medical and statistical independent reviewers and will meet to review the efficacy and safety data and determine a risk/benefit analysis in this subject population. The purpose of the PMC is to advise on serious safety considerations, lack of efficacy and any other considerations within the charge to the Committee. The PMC may request additional meetings or safety reports as deemed necessary upon discussion with the principal investigator. The PMC may stop the study following review of results from each interim analysis.

10.9 Study Monitoring and Auditing

Investigator Responsibilities

Investigator responsibilities are set out in the ICH guideline for Good Clinical Practice (GCP) and in the US Code of Federal Regulations. The principal investigator is responsible for every aspect of the design, conduct and actions of all members of the research team as well as final analysis of the protocol.

Investigators, or a designated member, must enter study data onto CRFs or other data collection system. The Investigator will permit study-related monitoring visits and audits by Karyopharm Therapeutics or its representatives, IRB/EC review, and regulatory inspection(s) (e.g., FDA, EMEA, TPP), providing direct access to the facilities where the study took place, to source documents, to CRFs, and to all other study documents.

The Investigator, or a designated member of the Investigator's staff, must be available at some time during monitoring visits to review data and resolve any queries and to allow direct access to the subject's records (e.g., medical records, office charts, hospital charts, and study related charts) for source data verification. The data collection must be completed prior to each visit and be made available to the Astellas representative so that the accuracy and completeness may be checked.

Site Responsibilities

Before the study can be initiated at any site, the site will be required to provide regulatory documentation to the Moffitt Clinical Research Network (MCRN) at Moffitt Cancer Center.

Sites must provide a copy of their informed consent to the MCRN coordinating center for review and approval prior to submission of any documents to the site's IRB. Any changes requested by the site's IRB must be provided to the MCRN staff for review and approval prior to resubmission to the IRB.

The MCRN Coordinating Center must receive the following trial specific documents either by hardcopy, fax, or email before a site can be activated for any trial:

1. IRB Approval Letter that includes the protocol version and date
2. FDA Related Forms 1572/1571/310 as appropriate
3. Signed Protocol Title Page
4. IRB Approved Consent Form
5. Site Delegation of Authority Log
6. Signed Financial Interest Disclosure Forms (principal and sub investigators)
7. Updated Investigator/Personnel documents (CVs, licenses, Conflict of Interest statements, etc.) as needed
8. Updated Laboratory Documents (certifications, normal ranges, etc.) as needed
9. Signed protocol specific Task Order

A study initiation teleconference will be held prior to the start of any study related activity at the site. Attendance is required for:

- The site PI and appropriate research staff
- Moffitt PI and MCRN research coordinator

The requirements of the protocol and all associated procedures and processes will be reviewed and agreed upon prior to the activation of the study. The MCRN utilizes the EDC system, Oncore. Oncore training will be scheduled, if indicated, with the appropriate staff from the site.

A conference call/study meeting will be held weekly for the phase I and monthly for the phase II to review patient enrollment and accrual, safety and toxicity data, and treatment results, as available.

10.9.3 Monitoring

Data will be captured in Oncore, Moffitt's Clinical Trials Database. Regulatory documents and case report forms will be monitored internally according to Moffitt Cancer Center Monitoring Policies. Monitoring will be performed regularly to verify data is accurate, complete, and verifiable from source documents; and the conduct of the trial is in compliance with currently approved protocol / amendments, good clinical practice (GCP) and applicable regulatory requirements. Monitoring at external sites will be per Moffitt policy.

11. STATISTICAL CONSIDERATIONS

11.1 Study Design/Endpoints

The primary goal of this study is to determine the safety and tolerability of enzalutamide used in combination with standard doses of cisplatin and gemcitabine. Secondary goals are to assess objective tumor response, time to progression, overall survival.

Exploratory objectives include 1) correlation of AR expression with outcomes (quantitative and qualitative AR expression using AQUA), and co-expression of pAKT; 2) Correlation of CTCs at baseline and on day 1 of cycle 3 with disease burden, and treatment response. AR expression in CTCs will also be evaluated and correlated with response.

Standard “3+3” design will be used for the dose defining portion of the study. The maximum number of patients in the dose finding phase 1 portion will be 12, and in addition, there will be cohort expansion to 12 more patients with Stage IV bladder cancer once safety of the combination is established. The assessment of objective response, time to progression, and overall survival will be done; objective response will be summarized as frequency counts and percentages; and time to progression and overall survival will be summarized using the method of Kaplan and Meier.

11.2 Sample Size

The maximum number of patients in phase 1 portion of the study will be 12. Cohort expansion will be done to 12 more patients with stage IV bladder cancer who are planned to be treated and eligible to receive gemcitabine and cisplatin; to assess response rate, safety profile, progression free survival and overall survival in patients with bladder cancer who expresses AR.

The estimated total number of patients in the study (Phase 1 and 1b included) will be 24.

11.3 Analysis of Secondary Endpoints

The secondary objective is to evaluate the efficacy of the combination regimen consisting of enzalutamide, gemcitabine and cisplatin. The secondary endpoints for efficacy evaluation will be response rate, progression free survival, and overall survival. The intention to treat population will include all patients who have received at least one dose of the drug. Efficacy outcomes will be reported for all patients, and subset analysis for patients in the dose expansion cohort (all of whom have AR positive tumor). The Kaplan-Meier method and the Cox proportional will be used for time-to-event variable. The response rate and best overall response along with 95% confidence interval will be reported. The logistic regression model will be used to explore the association of response with other potential predictors. A two-side p-value of <0.05 will be considered statistically significant.

For toxicity assessment, incidence of AEs will be described along with drug attribution. If the AE is at least possibly related to the study drug, the AE will be attributed to the drug.

11.4 Analysis of Exploratory Endpoints

Quantitative assessments and analysis of AR expression will be done using AQUA, and correlated with treatment response, and evaluation of correlation between CTCs with disease burden, and treatment response. AR expression in CTCs will also be evaluated and correlated with response.

12.0 Publications

The study of these patients and results of all laboratory studies are considered private and confidential. The progress and results of this study will not be presented without approval by the principal investigator.

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