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ARRx in Combination With Enzalutamide in Metastatic Castration Resistant Prostate Cancer

PROTOCOL UMCC 2017.055

ARRO-CITO: (UMCC 2017.055) Phase Ib/II Single-Arm Multi-Center Study of IONIS-AR-2.5$_{Rx}$, a Next Generation Androgen Receptor Antisense Oligonucleotide, in combination with Enzalutamide in Metastatic Castration Resistant Prostate Cancer

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Study Drug: IONIS-AR-2.5$_{Rx}$ or specifically ISIS 560131; formerly known as AZD5312)

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(COVID-19 Addendum) NOTE: The study was amended to address protocol-required items that the COVID-19 pandemic may temporarily affect. The potential changes are listed in Section 18.0 (COVID-19 Addendum).

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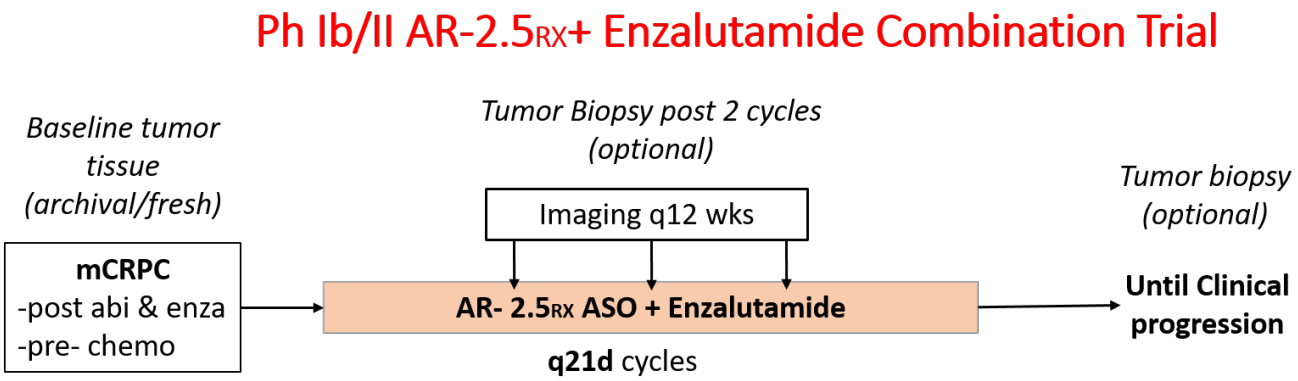
ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
ALC	Absolute Lymphocyte Count
AR	Androgen Receptor
ISIS 560131/IONIS-AR-2.5$_{Rx}$	Next Generation (Gen 2.5) Androgen Receptor Antisense Oligonucleotide
ASO	Antisense Oligonucleotide
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CMP	Comprehensive Metabolic Panel
CR	Complete Response
CRPC	Castrate Resistant Prostate Cancer
CT	Computed Tomography
CTC	Circulating Tumor Cells
CTCAE	Common Terminology Criteria for Adverse Events
CTSU	Clinical Trials Support Unit
DLT	Dose Limiting Toxicity
DSMC	Data and Safety Monitoring Committee
H&P	History & Physical Exam
HRPP	Human Research Protections Program
IND	Investigational New Drug
IRB	Institutional Review Board
IV (or iv)	Intravenously
mCRPC	Metastatic Castrate Resistant Prostate Cancer
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
ORR	Overall Response Rate
OS	Overall Survival
O-CTSU	Oncology-Clinical Trials Support Unit
PBMCs	Peripheral Blood Mononuclear Cells
PD	Progressive Disease
PDL1	Programmed Death Ligand 1
PFS	Progression Free Survival
PI	Principal Investigator
p.o.	per os/by mouth/orally
PR	Partial Response
PRC	Protocol Review Committee
RPFS	Radiographic Progression Free Survival

SAE	Serious Adverse Event
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SPGT	Serum Glutamic Pyruvic Transaminase
UaP	Unanticipated Problem
WBC	White Blood Cells

STUDY SCHEMA



STUDY SYNOPSIS

Title	**ARRO-CITO:** Phase Ib/II Single-Arm Multi-Center Study of IONIS-AR-2.5$_{Rx}$, an Androgen Receptor Antisense Oligonucleotide in combination with Enzalutamide in Metastatic Castration Resistant Prostate Cancer.
Phase	Phase Ib/II
Methodology	Open label, Single Arm, Phase Ib/II: IONIS-AR-2.5$_{Rx}$ (AR ASO) therapy + enzalutamide
Study Duration	• Subject accrual over 24 months • Anticipated median duration of protocol therapy of 12 months • Follow-up of 24months
Study Center(s)	Two-centers (University of Michigan, Karmanos Cancer Center)

Objectives/Endpoints	**Primary Objectives** 1. Phase Ib: To determine the maximum tolerated dose (MTD) of IONIS-AR-2.5$_{Rx}$ in combination with enzalutamide. 2. Phase II: To estimate the anti-tumor efficacy of IONIS-AR-2.5$_{Rx}$ in combination with enzalutamide as measured by PSA response rate. **Secondary Objectives** 1. To estimate secondary measures of efficacy of IONIS-AR-2.5$_{Rx}$ in combination with enzalutamide in CRPC subjects including radiographic progression-free survival (rPFS) by PCWG3-modified RECIST 1.1, PSA progression free survival. 30% PSA reduction proportion, Duration of Therapy (DOT), Duration of PSA Response (DOR), and OS. 2. To determine the safety and toxicity of IONIS-AR-2.5$_{Rx}$, in patients with metastatic castration resistant prostate cancer. **Exploratory/Correlative Objectives** 1. To determine the inhibition of androgen receptor driven signaling by IONIS-AR-2.5$_{Rx}$ in tumor tissues. 2. To identify molecular (including genomic, metabolic, and/or proteomic) biomarkers in blood and tissues that may be indicative of clinical response/resistance, safety, pharmacodynamic activity of IONIS-AR-2.5$_{Rx}$ in combination with enzalutamide. 3. To evaluate changes in health-related quality of life assessment from baseline using FACT-P and EuroQoL-5D. 4. To explore pharmacokinetic (PK) characteristics of IONIS-AR-2.5$_{Rx}$ in combination with enzalutamide.

	Primary Endpoint 1. Dose-limiting toxicity (DLT) and MTD during the first cycle of IONIS-AR-2.5Rx (in combination with enzalutamide) 2. PSA decline of 50% or greater from baseline using PCWG3 criteria at any time after the start of combination therapy **Secondary Endpoints** 1. Efficacy: Radiographic progression-free survival time (rPFS) by PCWG3-modifed RECIST 1.1, PSA progression-free survival time, PSA reductions by 30% and 50% from baseline, duration of therapy (DOT), duration of PSA Response (DOR), and overall survival time 2. Safety and toxicity: Adverse events as assessed by CTCAE criteria 5.0 and classified by body system, grade and attribution. Other toxicity endpoints include intrapatient dose delays and dose reductions. **Exploratory/Correlative Endpoints** 1. Androgen receptor driven signaling measures in tumor tissues 2. Measures of genomic metabolomics and proteomic biomarkers in blood and tissues 3. FACT-P and EuroQoL-5D scores with comparisons to baseline scores 4. Pharmacokinetic (PK) characteristics
Number of Subjects	35 evaluable subjects

Key Inclusion Criteria	1. Histologically confirmed prostate adenocarcinoma cancer, either pure or mixed. NO small cell/neuroendocrine differentiation is allowed in any quantity. 2. Castrate levels of serum testosterone (≤50 ng/dL). Patients must continue androgen deprivation therapy with an LHRH analogue or antagonist if they have not undergone bilateral orchiectomy. 3. Patients must have metastatic disease, either measurable OR non-measurable per RECIST 1.1. 4. Progressive disease despite ongoing treatment with Androgen Deprivation Therapy (ADT) based on **ANY ONE** of the following: • Progression on axial imaging per treating investigator. • PSA evidence for progressive prostate cancer consisting of a PSA level of at least 1 ng/ml which has risen on at least 2 successive occasions, at least one week apart. If the confirmatory PSA (#3) value is less (i.e., #3b) than the screening PSA (#2) value, then an additional test for rising PSA (#4) will be required to document progression for the purposes of eligibility. • Radionuclide bone scan: At least two new foci consistent with metastatic lesions. 5. Patients treated with first generation anti-androgen (e.g. bicalutamide, nilutamide, flutamide) as part of most recent systemic therapy must have at least 4 weeks elapsed from treatment discontinuation to start of protocol therapy with evidence of disease progression per criterion #4 following discontinuation of prior anti-androgen. 6. Minimum PSA at entry of 1 ng/mL is required.

Key Inclusion Criteria	7. ECOG Performance Status 0, 1 or 2. 8. Ability to understand and voluntarily agree to participate by providing written informed consent for the trial. 9. Be ≥18 years of age on the day of signing informed consent. 10. Demonstrate adequate organ function as defined below: Hematological: • ANC ≥1,500/uL • Platelets ≥100,000/uL • Hemoglobin ≥9 g/dL or ≥5.6 mmol/L with or without a red blood cell transfusion. Renal: • Serum creatinine ≤1.5X ULN OR calculated creatinine clearance ≥30 mL/min for subjects with creatinine levels >1.5X institutional ULN (GFR can also be used in place of creatinine or CrCl). Hepatic: • Serum total bilirubin ≤1.5X ULN OR Direct bilirubin ≤ULN for subjects with total bilirubin levels >1.5 ULN. • AST (SGOT) and ALT (SGPT) ≤2.5X ULN OR ≤5X ULN for subjects with active liver metastases. • Alkaline Phosphatase If >2.5X ULN, then liver fraction should be ≤2.5X ULN Coagulation: • INR PT or aPTT or PTT ≤1.5X ULN (can vary if subject is receiving anticoagulant therapy, as long as PT or PTT remains within therapeutic range of the anticoagulant's intended use). 11. Subjects must agree to use an adequate method of contraception as outlined in Section 8.1.1, starting with the time of informed consent through 120 days after the last dose of trial therapy.
Exclusion Criteria	1. Prior chemotherapy and/or enzalutamide for metastatic castration-resistant prostate cancer. Prior abiraterone is allowed but not required. Chemotherapy administered in the castration-sensitive setting is allowed. 2. Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigation device within 4 weeks of enrollment. 3. Has not recovered (i.e., AE ≤Grade 1 or at baseline) from AEs due to a previously administered agent. Subjects with ≤Grade 2 neuropathy or ≤Grade 2 alopecia are an exception to this criterion and are allowed if relevant toxicity is stabilized. 4. If subjects received major surgery they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting trial therapy.

Exclusion Criteria	5. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin and squamous cell carcinoma of the skin. 6. Has a known history of active tuberculosis (TB) (Bacillus tuberculosis). 7. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial. At the time of signing informed consent is a known regular user (including "recreational use") of any illicit drug(s) or had a recent history (within the last year) of drug or alcohol abuse. 8. Has a known history of human immunodeficiency virus (HIV) (HIV 1/2 antibodies). 9. Has known history of active hepatitis B (e.g. HBsAg reactive) or hepatitis C (e.g., HCV RNA [qualitative] is detected). 10. Has received a live virus vaccine within 30 days of planned start of trial therapy. 11. Has known active CNS metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they have stable brain metastases (stability is normally defined as a period of 1 to 3 months in which there is no evidence of new or enlarging CNS metastases). 12. Has symptomatic ascites or pleural effusion; a subject who is clinically stable following treatment for these conditions is eligible. 13. Has had a prior allogeneic stem cell or bone marrow transplant. 14. Has known contraindication to aspirin (81 mg).
Study Product(s), Dose, Route, Regimen	IONIS-AR-2.5$_{Rx}$ Androgen Receptor Antisense Oligonucleotide (AR ASO) at starting dose of 900 mg IV + enzalutamide 160 mg PO Qday in Phase Ib. IONIS-AR-2.5$_{Rx}$ (at MTD from Phase Ib) + enzalutamide in Phase II.
Duration of Administration	Until imaging/radiographic progression per PCWG3 criteria and/or until lack of clinical benefit as judged by the treating investigator. Clinical progression as judged by the treating investigator or unacceptable toxicity or withdrawal of informed consent or deterioration in general medical condition that is excessive per treating investigator would result in stopping therapy.
Reference Therapy	None

Statistical Methodology	This is a single dose-finding one-arm phase Ib/II trial to (i) estimate the MTD from among three dose levels of IONIS-AR-2.5$_{Rx}$ in combination with a fixed dose of enzalutamide, as described below, and (ii) obtain preliminary estimate of efficacy at this estimated MTD, as measured by PSA response rate. The MTD is defined as the highest dose of IONIS-AR-2.5$_{Rx}$ inducing a rate of DLT closest to but not larger than 35%, and we will assess the evidence that the PSA response rate at this dose level exceeds 5%. The planned enrollment is 35 evaluable patients. Success for the trial is defined as finding a dose level that is likely to be both tolerable and effective. **Study Design** ***Dose Assignment*** The first patient will be enrolled to dose level 3 of IONIS-AR-2.5$_{Rx}$, in combination with a fixed dose of enzalutamide. Subsequent dose assignments to dose levels 3, 2, or 1 will be based upon the statistical-model-based TITE-CRM design. This trial will seek to estimate the MTD, the true value of which is defined as the dose level with probability of DLT closest to but not exceeding 35%. ***Final Efficacy Analysis*** After all 35 evaluable patients are enrolled and the PSA response endpoint is recorded for all patients, we will fit the Bayesian isotonic regression model on all patients' data. If the probability that the PSA response rate at the final estimated dose exceeds 5% is at least 0.95, the trial will have successfully completed its primary objective. ***Interim Analysis/Criteria for Stopping Study*** Continuous monitoring of safety is built into the TITE-CRM model, and the trial will stop at any point in which all dose levels have a model-estimated probability of DLT exceeding 37.5%. Subject accrual is expected to be 15-18 patients per year per site for a total period of up to 24 months. We assume an anticipated median duration of protocol therapy of 12 months and a follow-up of 24 months.

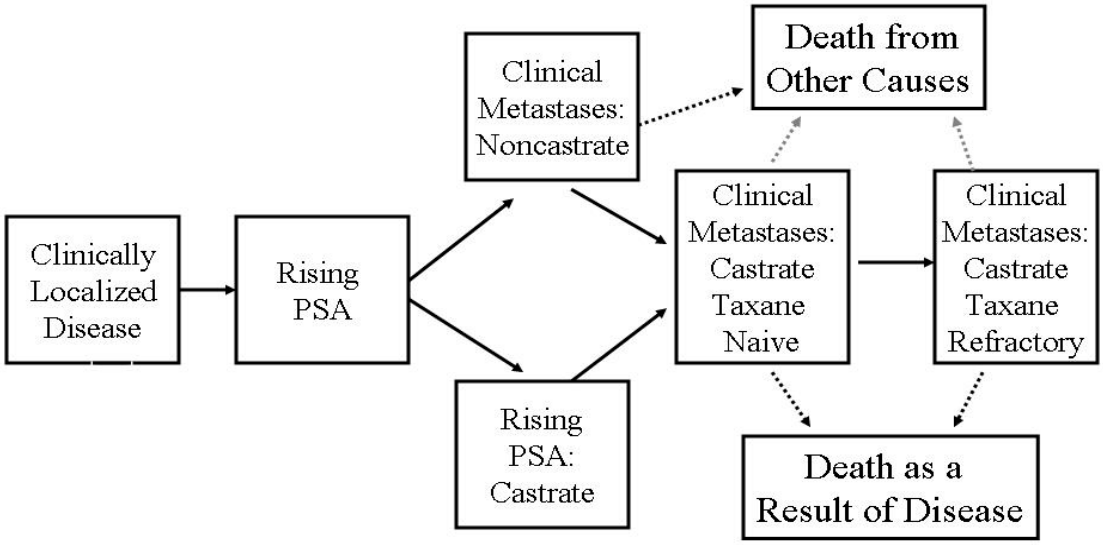
1.0 BACKGROUND AND RATIONALE

1.1 Disease Background

Prostate cancer is the second leading cause of cancer deaths in men. According to American Cancer Society[1] estimates in 2014, as many as 233,000 American men will be diagnosed with prostate cancer, and nearly 29,480 will die of the disease.

These clinical states involve the complex interplay of a network of signaling molecules that collectively promote net cell proliferation relative to cell death. The course of prostate cancer from diagnosis to death is best categorized as a series of clinical states.

Figure 1. Clinical states of prostate cancer

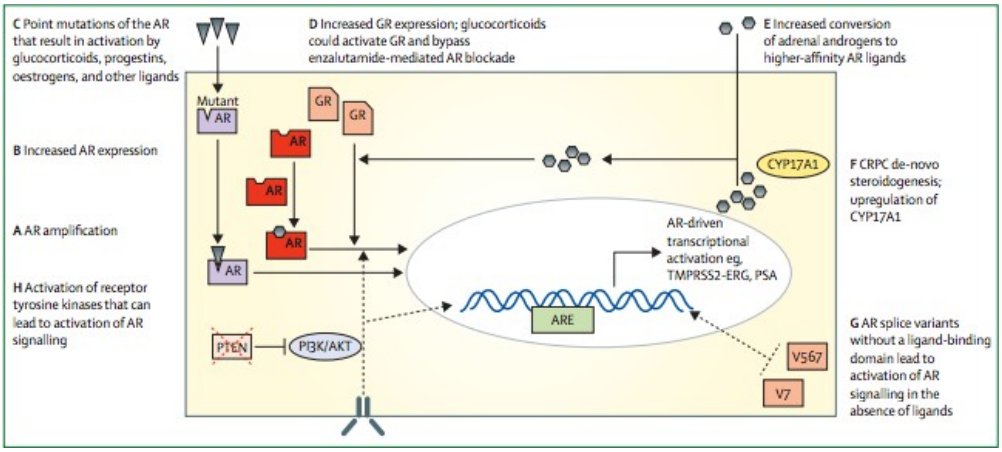


Treatment options for patients with castrate resistant prostate cancer (CRPC) are limited, and until recently only docetaxel was shown to improve survival. In the last several years, five new agents: abiraterone acetate, cabazitaxel, enzalutamide, radium-223 and sipuleucel-T, have been approved for use in this clinical state[2-8]. However, the optimal sequencing of these agents is unknown. Moreover, all patients with CRPC are treated with an identical paradigm, and there is currently no means of identifying which patient population would benefit from a particular agent.

In recent studies of enzalutamide, a potent Androgen Receptor signaling inhibitor in combination with anti-PD1 blocking antibody immunotherapy[9] (NCT02312557 and Merck's ongoing and recently amended study Keynote-199, cohorts 4 and 5/UMCC 2016.055/NCT02787005), the hypothesis based on intriguing data (though early and limited dataset) is that sustained Androgen receptor signaling inhibition is needed to render prostate cancer tissue responsive to immunotherapy. This is in line with other recent preclinical data that inhibition of intracellular signaling pathways such as CDK4/6[10] and wnt[11] could render tumor cells more responsive to immunotherapy.

1.2 Treatment Background

Extensive work has been performed to increase the understanding of the major mechanism driving resistance to castration. Preclinical and clinical studies have demonstrated that alterations in the AR, specifically up-regulation, are central to the progression to CRPC[12]. Several novel agents targeting the androgen receptor have been in development.



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[7]. Currently, the NCCN guidelines recommend enzalutamide in this setting as well as for men with mCRPC who are not candidates for docetaxel. 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[2]. In September 2014, the U.S. Food and Drug Administration (FDA) expanded the use of enzalutamide to include men with CRPC who have not received prior chemotherapy. Thus, enzalutamide has become a standard of care as systemic therapy for men with mCRPC, and since there is no need for concurrent administration of systemic steroids, it is expected to be the predominant agent to be used in the pre-chemotherapy setting. However, enzalutamide as well as abiraterone acetate are associated eventual development of resistance and re-activation of the AR.

1.3 Study Agent(s) Background and Associated Known Toxicities

Therapeutic nucleic acid- based approaches, including antisense oligonucleotides (ASOs), offer the potential to develop drugs to targets that have proven to be intractable to alternative drug modalities[13]. With the ASO approach inhibitors can be rationally designed solely based on gene sequence information, thereby enabling the development of inhibitors to a wide range of target classes, including those regarded as undruggable. There have been several successes in the development of systemically administered ASOs in non-oncological settings[14,15] and the US Food and Drug Administration has now approved the first systemically administered ASO drug (Kynamro). Continued efforts to improve upon the stability and potency of ASOs has resulted in the discovery of a next generation class of ASOs that employ 2'-4' constrained ethyl (cEt) residues,[16,17]. These next generation cEt ASOs, Generation 2.5, exhibit enhanced in vitro and in vivo potency compared to earlier ASO chemistries, and recently a cEt ASO (AZD4785) targeting the oncogene KRAS has demonstrated robust activity in preclinical models of cancer[18] and another cEt ASO (AZD9150) targeting the previously undruggable target, the transcription factor STAT3, has shown single agent activity in highly treatment refractory cancer patients I phase I studies[19].

IONIS-AR-2.5$_{Rx}$ (specifically ISIS 560131, formerly known as AZD5312) is a novel Generation 2.5 (constrained ethyl bicyclic nucleic acid) phosphorothioate antisense oligonucleotide (ASO) designed to target full length, splice variant and mutated forms of the androgen receptor, specifically resulting in suppression of human AR expression.

IONIS-AR-2.5$_{Rx}$ has been shown in pre-clinical trials to cause dose-dependent down-regulation of full-length AR mRNA and protein, inhibits AR-dependent gene expression and induces apoptosis in prostate cancer cell lines[20]. IONIS-AR-2.5$_{Rx}$ was also shown to downregulate the splice variant AR-v7 and was also shown to inhibit proliferation of 22Rv1 cells, a human prostate carcinoma xenograft-derived epithelial cell line expressing PSA. Of note, this ASO was shown to have differential activity from enzalutamide in preclinical data, as enzalutamide does not inhibit 22Rv1 cell proliferation.

A first-time-in-patients (FTIP) phase I dose escalation, safety and pharmacokinetic study (Phase I Open Label Dose Escalation Study to Investigate the Safety & Pharmacokinetics of IONIS-AR-2.5$_{Rx}$ in Patients With Androgen Receptor Tumors NCT02144051/D5860C00001) was conducted in mCRPC patients who had previously failed standard of care treatment options including enzalutamide, abiraterone, and chemotherapy.[21] Patients were treated on a 28-day cycle in 4 cohorts (drug doses 150, 300, 600 and 900 mg). IONIS-AR-2.5$_{Rx}$ was given on days 1, 4, 8, 11, 15 and 22 of cycle 1, and on days 1, 8, 15 and 22 of each subsequent 28-day cycle.

Pharmacodynamic response was assessed at each cycle by PSA and circulating tumor cells (CTCs) and IHC and gene expression in a limited number of tissue biopsies. A maximum tolerated dose (MTD) of 900 mg once daily was established after treatment of 29 patients across the 4 cohorts. Dose-limiting toxicity was an increase in liver function tests at 900 mg (1 of 13 pts). Adverse events reported in preliminary data included fatigue, diarrhea, nausea, vomiting, dry skin, headache, insomnia, loss of appetite, mouth pain, muscle pain, pruritus, increased ALT/AST, elevated ALP and GGT, taste alteration, thrombocytopenia, gastrointestinal cramping, constipation, and weight loss.

Of note, after the cut-off date for review of emerging safety data, several AE incidents of venous thromboembolism (pulmonary embolism and deep venous thrombosis) were reported. At the time of reporting, 15 patients had been treated on the D5860C00001 study, and 4 episodes of grade 3 pulmonary embolism and 3 episodes of non-serious deep venous thrombosis were reported. We have noted this clinical finding and with recent unpublished work from the Palapattu lab at the University of Michigan showing platelet aggregation with other anti-sense oligonucleotides, built in a low dose aspirin of 81 mg daily into the protocol. We will continue to monitor this aspect closely in our proposed trial.

1.4 Other Agents

Enzalutamide (standard of care as monotherapy)

Enzalutamide is an androgen receptor antagonist which acts by competitively inhibiting (1) testosterone binding to androgen receptors, (2) nuclear translocation of androgen receptors, and (3) DNA binding and activation by androgen receptors. Enzalutamide was approved by the FDA in 2012 for the treatment of med with mCRPC who have progressed on or after first-line docetaxel chemotherapy. In September 2014, the FDA expanded the use of enzalutamide to include men with CRPC that have not received prior chemotherapy.

Clinical Trial Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

Three randomized clinical trials enrolled patients with metastatic prostate cancer that has progressed on androgen deprivation therapy (GnRH therapy or bilateral orchiectomy), a disease setting that is also defined as metastatic CRPC. Two trials were placebo-controlled (Studies 1 and 2), and one trial was bicalutamide-controlled (Study 3). In Studies 1 and 2, patients received enzalutamide 160 mg or placebo orally once daily. In Study 3, patients received

enzalutamide 160 mg or bicalutamide 50 mg orally once daily. All patients continued androgen deprivation therapy.

Patients were allowed, but not required, to take glucocorticoids.

The most common adverse reactions (≥ 10%) that occurred more commonly (≥ 2% over placebo) in the enzalutamide- treated patients from the two randomized placebo-controlled clinical trials were asthenia/fatigue, back pain, decreased appetite, constipation, arthralgia, diarrhea, hot flush, upper respiratory tract infection, peripheral edema, dyspnea, musculoskeletal pain, weight decreased, headache, hypertension, and dizziness/vertigo.

Study 1: Enzalutamide versus Placebo in Metastatic CRPC Following Chemotherapy
In this study of patients with metastatic CRPC who had previously received docetaxel, the median duration of treatment was 8.3 months with enzalutamide and 3.0 months with placebo. During the trial, 48% of patients on the enzalutamide arm and 46% of patients on the placebo arm received glucocorticoids.

Grade 3 and higher adverse reactions were reported among 47% of enzalutamide-treated patients and 53% of placebo- treated patients. Discontinuations due to adverse events were reported for 16% of enzalutamide-treated patients and 18% of placebo-treated patients. The most common adverse reaction leading to treatment discontinuation was seizure, which occurred in 0.9% of the enzalutamide-treated patients compared to none (0%) of the placebo-treated patients.

Table 1 shows adverse reactions reported in Study 1 that occurred at a ≥ 2% higher frequency in the enzalutamide arm compared to the placebo arm.

Table 1. Adverse Reactions in Study 1

	Enzalutamide **N = 800**		**Placebo N = 399**	
	Grade 1-4[a] (%)	**Grade 3-4 (%)**	**Grade 1-4 (%)**	**Grade 3-4 (%)**
General Disorders				
Asthenic Conditions[b]	50.6	9.0	44.4	9.3
Peripheral Edema	15.4	1.0	13.3	0.8
Musculoskeletal And Connective Tissue Disorders				
Back Pain	26.4	5.3	24.3	4.0
Arthralgia	20.5	2.5	17.3	1.8
Musculoskeletal Pain	15.0	1.3	11.5	0.3
Muscular Weakness	9.8	1.5	6.8	1.8
Musculoskeletal Stiffness	2.6	0.3	0.3	0.0
Gastrointestinal Disorders				
Diarrhea	21.8	1.1	17.5	0.3
Vascular Disorders				
Hot Flush	20.3	0.0	10.3	0.0
Hypertension	6.4	2.1	2.8	1.3
Nervous System Disorders				
Headache	12.1	0.9	5.5	0.0
Dizziness[c]	9.5	0.5	7.5	0.5
Spinal Cord Compression and Cauda Equina Syndrome	7.4	6.6	4.5	3.8
Paresthesia	6.6	0.0	4.5	0.0
Mental Impairment Disorders[d]	4.3	0.3	1.8	0.0
Hypoesthesia	4.0	0.3	1.8	0.0
Infections And Infestations				
Upper Respiratory Tract Infection[e]	10.9	0.0	6.5	0.3
Lower Respiratory Tract And Lung Infection[f]	8.5	2.4	4.8	1.3
Psychiatric Disorders				
Insomnia	8.8	0.0	6.0	0.5
Anxiety	6.5	0.3	4.0	0.0
Renal And Urinary Disorders				
Hematuria	6.9	1.8	4.5	1.0
Pollakiuria	4.8	0.0	2.5	0.0
Injury, Poisoning And Procedural Complications				
Fall	4.6	0.3	1.3	0.0
Non-pathologic Fractures	4.0	1.4	0.8	0.3
Skin And Subcutaneous Tissue Disorders				
Pruritus	3.8	0.0	1.3	0.0
Dry Skin	3.5	0.0	1.3	0.0

	Enzalutamide N = 800		Placebo N = 399	
	Grade 1-4[a] (%)	Grade 3-4 (%)	Grade 1-4 (%)	Grade 3-4 (%)
Respiratory Disorders				
Epistaxis	3.3	0.1	1.3	0.3

a CTCAE v5.0
b Includes asthenia and fatigue.
c Includes dizziness and vertigo.
d Includes amnesia, memory impairment, cognitive disorder, and disturbance in attention.
e Includes nasopharyngitis, upper respiratory tract infection, sinusitis, rhinitis, pharyngitis, and laryngitis.
f Includes pneumonia, lower respiratory tract infection, bronchitis, and lung infection.

Study 2: Enzalutamide versus Placebo in Chemotherapy-naïve Metastatic CRPC
Study 2 enrolled 1717 patients with metastatic CRPC who had not received prior cytotoxic chemotherapy, of whom 1715 received at least one dose of study drug. The median duration of treatment was 17.5 months with enzalutamide and 4.6 months with placebo. Grade 3-4 adverse reactions were reported in 44% of enzalutamide-treated patients and 37% of placebo-treated patients. Discontinuations due to adverse events were reported for 6% of enzalutamide-treated patients and 6% of placebo-treated patients. The most common adverse reaction leading to treatment discontinuation was fatigue/asthenia, which occurred in 1% of patients on each treatment arm. Table 2 includes adverse reactions reported in Study 2 that occurred at a ≥ 2% higher frequency in the enzalutamide arm compared to the placebo arm.

Table 2. Adverse Reactions in Study 2

	Enzalutamide N = 871		Placebo N = 844	
	Grade 1-4[a] (%)	Grade 3-4 (%)	Grade 1-4 (%)	Grade 3-4 (%)
General Disorders				
Asthenic Conditions[b]	46.9	3.4	33.0	2.8
Peripheral Edema	11.5	0.2	8.2	0.4
Musculoskeletal And Connective Tissue Disorders				
Back Pain	28.6	2.5	22.4	3.0
Arthralgia	21.4	1.6	16.1	1.1
Gastrointestinal Disorders				
Constipation	23.2	0.7	17.3	0.4
Diarrhea	16.8	0.3	14.3	0.4
Vascular Disorders				
Hot Flush	18.0	0.1	7.8	0.0
Hypertension	14.2	7.2	4.1	2.3
Nervous System Disorders				
Dizziness[c]	11.3	0.3	7.1	0.0
Headache	11.0	0.2	7.0	0.4
Dysgeusia	7.6	0.1	3.7	0.0
Mental Impairment Disorders[d]	5.7	0.0	1.3	0.1
Restless Legs Syndrome	2.1	0.1	0.4	0.0
Respiratory Disorders				

Dyspnea[e]	11.0	0.6	8.5	0.6
Infections And Infestations				
Upper Respiratory Tract Infection[f]	16.4	0.0	10.5	0.0
Lower Respiratory Tract And Lung Infection[g]	7.9	1.5	4.7	1.1
Psychiatric Disorders				
Insomnia	8.2	0.1	5.7	0.0
Renal And Urinary Disorders				
Hematuria	8.8	1.3	5.8	1.3
Injury, Poisoning And Procedural Complications				
Fall	12.7	1.6	5.3	0.7
Non-Pathological Fracture	8.8	2.1	3.0	1.1
Metabolism and Nutrition Disorders				
Decreased Appetite	18.9	0.3	16.4	0.7
Investigations				
Weight Decreased	12.4	0.8	8.5	0.2
Reproductive System and Breast disorders				
Gynecomastia	3.4	0.0	1.4	0.0

a CTCAE v5.0
b Includes asthenia and fatigue.
c Includes dizziness and vertigo.
d Includes amnesia, memory impairment, cognitive disorder, and disturbance in attention.
e Includes dyspnea, exertional dyspnea, and dyspnea at rest.
f Includes nasopharyngitis, upper respiratory tract infection, sinusitis, rhinitis, pharyngitis, and laryngitis.
g Includes pneumonia, lower respiratory tract infection, bronchitis, and lung infection.

Study 3: enzalutamide versus Bicalutamide in Chemotherapy-naïve Metastatic CRPC
Study 3 enrolled 375 patients with metastatic CRPC who had not received prior cytotoxic chemotherapy, of whom 372 received at least one dose of study drug. The median duration of treatment was 11.6 months with enzalutamide and 5.8 months with bicalutamide. Discontinuations with an adverse event as the primary reason were reported for 7.6% of enzalutamide-treated patients and 6.3% of bicalutamide-treated patients. The most common adverse reactions leading to treatment discontinuation were back pain and pathological fracture, which occurred in 3.8% of enzalutamide-treated patients for each event and in 2.1% and 1.6% of bicalutamide-treated patients, respectively. Table 3 shows overall and common adverse reactions (≥ 10%) in enzalutamide-treated patients.

Table 3. Adverse Reactions in Study 3

	Enzalutamide (N=183)		Bicalutamide (N=189)	
	Grade 1-4 [a] (%)	**Grade 3-4 (%)**	**Grade 1-4 [a] (%)**	**Grade 3-4 (%)**
Overall	94.0	38.8	94.2	37.6
General Disorders				
Asthenic Conditions[b]	31.7	1.6	22.8	1.1
Musculoskeletal And Connective Tissue Disorders				
Back Pain	19.1	2.7	18.0	1.6
Musculoskeletal Pain[c]	16.4	1.1	14.3	0.5
Vascular Disorders				
Hot Flush	14.8	0	11.1	0
Hypertension	14.2	7.1	7.4	4.2
Gastrointestinal Disorders				
Nausea	14.2	0	17.5	0
Constipation	12.6	1.1	13.2	0.5
Diarrhea	11.5	0	9.0	1.1
Infections And Infestations				
Upper Respiratory Tract Infection[d]	12.0	0	6.3	0.5
Investigational				
Weight Loss	10.9	0.5	7.9	0.5

a CTCAE v 5.0
b Including asthenia and fatigue.
c Including musculoskeletal pain and pain in extremity
d Including nasopharyngitis, upper respiratory tract infection, sinusitis, rhinitis, pharyngitis, and laryngitis

Laboratory Abnormalities
In the two randomized placebo-controlled clinical trials, Grade 1-4 neutropenia occurred in 15% of patients treated with enzalutamide (1% Grade 3-4) and in 6% of patients treated with placebo (0.5% Grade 3-4). The incidence of Grade 1-4 thrombocytopenia was 6% of patients treated with enzalutamide (0.3% Grade 3-4) and 5% of patients treated with placebo (0.5% Grade 3-4). Grade 1-4 elevations in ALT occurred in 10% of patients treated with enzalutamide (0.2% Grade 3-4) and 16% of patients treated with placebo (0.2% Grade 3-4). Grade 1-4 elevations in bilirubin occurred in 3% of patients treated with enzalutamide (0.1% Grade 3-4) and 2% of patients treated with placebo (no Grade 3-4).

Infections
In Study 1, 1% of patients treated with enzalutamide compared to 0.3% of patients treated with placebo died from infections or sepsis. In Study 2, 1 patient in each treatment group (0.1%) had an infection resulting in death.

Falls and Fall-related Injuries
In the two randomized placebo-controlled clinical trials, falls including fall-related injuries, occurred in 9% of patients treated with enzalutamide compared to 4% of patients treated with placebo. Falls were not associated with loss of consciousness or seizure. Fall-related injuries were more severe in patients treated with enzalutamide and included non- pathologic fractures,

joint injuries, and hematomas.

Hypertension
In the two randomized placebo-controlled trials, hypertension was reported in 11% of patients receiving enzalutamide and 4% of patients receiving placebo. No patients experienced hypertensive crisis. Medical history of hypertension was balanced between arms. Hypertension led to study discontinuation in < 1% of patients in each arm.

1.5 Rationale

Metastatic castration resistant prostate cancer (CRPC) is an area of urgent unmet clinical need, especially in the clinical state characterized by resistance to or progression on monotherapy with androgen signaling inhibitors (ASI) such as abiraterone acetate and/or enzalutamide. In the CRPC setting, androgen receptor (AR) signaling continues to remain active and drives cancer progression. More potent inhibitors of AR-driven signaling, especially those that circumvent mechanisms of castration resistance involving the AR (e.g. AR splice variants or AR mutations), could help prolong the duration of benefit from ASI such as enzalutamide by avoiding or delaying development of resistance. We therefore propose a Phase Ib/II Single-Arm Multi-Center Study of IONIS-AR-2.5$_{Rx}$, the Androgen Receptor Antisense Oligonucleotide in combination with enzalutamide in Metastatic Castration Resistant Prostate Cancer.

1.6 Correlative Studies

IONIS-AR-2.5$_{Rx}$ acts by targeting full length and all variants of AR mRNA by targeting them for degradation. AR mRNA levels and those of the AR mutant and variants are expected to be reduced upon treatment with IONIS-AR-2.5$_{Rx}$. We seek to determine the inhibition of androgen receptor driven signaling by IONIS-AR-2.5$_{Rx}$ in tumor tissues. Secondly, we seek to identify other molecular (including genomic, metabolic, and/or proteomic) biomarkers in blood and tumor tissue potentially indicative of clinical response/resistance, safety, pharmacodynamic activity and/or the mechanism of action of IONIS-AR-2.5$_{Rx}$ in combination with enzalutamide. We will explore pharmacokinetic (PK) characteristics of IONIS-AR-2.5$_{Rx.}$ Finally, we will explore the quality of life impact of IONIS-AR-2.5$_{Rx}$ in combination with enzalutamide.

2.0 STUDY OBJECTIVES/ENDPOINTS

2.1 Primary Objective

Phase Ib: To determine the maximum tolerated dose (MTD) of IONIS-AR-2.5$_{Rx}$ in combination with enzalutamide.

Phase II: To estimate the anti-tumor efficacy of IONIS-AR-2.5$_{Rx}$ in combination with enzalutamide as measured by PSA response rate in patients with metastatic castration resistant prostate cancer.

2.2 Secondary Objectives

2.2.1 To estimate secondary measures efficacy of IONIS-AR-2.5$_{Rx}$ in combination with enzalutamide in CRPC subjects including radiographic progression-free survival (rPFS) by PCWG3-modified RECIST 1.1, 30% PSA reduction proportion, PSA progression-free survival, Duration of Therapy (DOT), Duration of Response (DOR), PFS and OS.

2.2.2 To determine the safety and toxicity of IONIS-AR-2.5$_{Rx}$, the Androgen Receptor AntiSense Oligonucleotide (AR ASO) in patients with metastatic castration resistant prostate cancer.

2.3 Exploratory/Correlative Objectives

2.3.1 To determine the inhibition of androgen receptor driven signaling by IONIS-AR-2.5$_{Rx}$ in tumor tissues.

2.3.2 To identify molecular (including genomic, metabolic, and/or proteomic) biomarkers in blood and tissues that may be indicative of clinical response/resistance, safety, pharmacodynamic activity and/or the mechanism of action of IONIS-AR-2.5$_{Rx}$ in combination with enzalutamide.

2.3.3 To evaluate changes in health-related quality of life assessment from baseline using FACT-P and EuroQoL-5D.

2.3.4 To explore pharmacokinetic (PK) characteristics of IONIS-AR-2.5$_{Rx}$ in combination with enzalutamide.

2.4 Primary Endpoint

2.4.1 Dose-limiting toxicity (DLT) during the first cycle of IONIS-AR-2.5$_{Rx}$ (in combination with enzalutamide)

2.4.2 PSA decline of 50% or greater from baseline using PCWG3 criteria at any time after the start of combination therapy

2.5 Secondary Endpoints

2.5.1 Efficacy: Radiographic progression-free survival time (rPFS) by PCWG3-modifed RECIST 1.1, PSA reduction from baseline, PSA progression-free survival time, duration of therapy (DOT), duration of PSA Response (DOR), progression-free survival time and overall survival time

2.5.2 Safety and toxicity: Adverse events as assessed by CTCAE criteria 5.0 and classified by body system, grade and attribution. Other toxicity endpoints include intrapatient dose delays and dose reductions.

2.6 Exploratory/Correlative Endpoints

2.6.1 Androgen receptor driven signaling measures in tumor tissues

2.6.2 Measures of genomic metabolomics and proteomic biomarkers in blood and tissues

2.6.3 FACT-P and EuroQoL-5D scores with comparisons to baseline scores

2.6.4 Pharmacokinetic (PK) characteristics

3.0 PATIENT ELIGIBILITY

Subjects must meet all of the inclusion and exclusion criteria to be enrolled to the study. Study treatment may not begin until a subject is enrolled. No eligibility waivers will be granted for this clinical trial.

3.1 Inclusion Criteria

3.1.1 Ability to understand and voluntarily agree to participate by providing written informed consent for the trial.

3.1.2 Histologically confirmed prostate adenocarcinoma cancer, either pure or mixed. Small cell/neuroendocrine differentiation is not allowed.

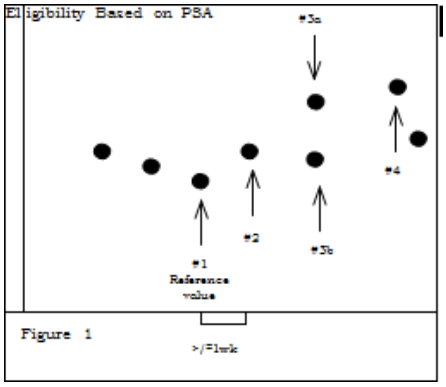
3.1.3 Castrate levels of serum testosterone (≤ 50 ng/dL). Patients must continue androgen deprivation therapy with an LHRH analogue or antagonist if they have not undergone bilateral orchiectomy.

3.1.4 Patients must have metastatic disease; either non-measurable disease OR measurable disease per RECIST 1.1.

3.1.5 Progressive disease despite ongoing treatment with Androgen Deprivation Therapy (ADT) based **on ANY one of the following**:

1) Progression on axial imaging per treating investigator

2) PSA evidence for progressive prostate cancer consists of a PSA level of at least 1 ng/ml which has risen on at least 2 successive occasions, at least one week apart. If the confirmatory PSA (#3) value is less (i.e., #3b) than the screening PSA (#2) value, then an additional test for rising PSA (#4) will be required to document progression for the purposes of eligibility.



3) Radionuclide bone scan: At least two new foci consistent with metastatic lesions

3.1.6 Patients treated with first generation anti-androgen as most recent systemic therapy (e.g. bicalutamide, nilutamide) must have at least 4 weeks elapsed from treatment discontinuation to start of protocol therapy with evidence of disease progression per criterion 3.1.5 f*ollowing* discontinuation of prior anti-androgen.

3.1.7 Minimum PSA at entry of 1 ng/mL is required.

3.1.8 ECOG Performance Status 0,1 or 2

3.1.9 Be ≥18 years of age on the day of signing informed consent.

3.1.10 Demonstrate adequate organ function as defined below:

System	Laboratory Value
Hematological	
ANC	≥1,500/uL
Platelets	≥100,000/uL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L with or without a red blood cell transfusion within 2 weeks ofenrollment
Renal	
Serum creatinine **OR** calculated creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5X ULN **OR** ≥30 mL/min for subjects with creatinine levels >1.5X institutional ULN
Hepatic	
Serum total bilirubin	≤1.5X ULN **OR** Direct bilirubin ≤ULN for subjects with total bilirubin levels >1.5 ULN
AST (SGOT) and ALT (SGPT)	≤2.5X ULN **OR** **≤**5X ULN for subjects with active liver metastases
Alkaline Phosphatase	If >2.5X ULN, then liver fraction should be ≤2.5X ULN
Coagulation	
INR PT aPTT or PTT	≤1.5X ULN This can vary if subject is receiving anticoagulant therapy, as long as PT or PTT remains within therapeutic range of the anticoagulant's intended use

3.1.11 Subjects must agree to use an adequate method of contraception as outlined in Section 8.1.1. starting with the time of informed consent through 120 days after the last dose trial therapy.

3.2 Exclusion Criteria

3.2.1 Prior chemotherapy and/or enzalutamide for metastatic castration-resistant prostate cancer. Chemotherapy administered in the castration-sensitive setting is allowed provided last dose of chemotherapy was greater than 6 months prior to study entry.

3.2.2 Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigation device within 4 weeks of enrollment.

3.2.3 Has not recovered (i.e., AE ≤Grade 1 or at baseline) from AEs due to a previously administered agent. Subjects with ≤Grade 2 neuropathy or ≤Grade 2 alopecia are an exception to this criterion and are allowed if relevant toxicity is stabilized.

3.2.4 If subjects received major surgery they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting trial therapy.

3.2.5 Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin.

3.2.6 Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial. At the time of signing informed consent is a known regular user (including "recreational use") of any illicit drug(s) or had a recent history (within the last year) of drug or alcohol abuse.

3.2.7 Has a known history of human immunodeficiency virus (HIV) (HIV 1/2 antibodies).

3.2.8 Has known active hepatitis B (e.g., HBsAg reactive) or hepatitis C (e.g., HCV RNA [qualitative] is detected).

3.2.9 Has received a live virus vaccine within 30 days of planned start of trial therapy.

3.2.10 Has known active CNS metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they have stable brain metastases (stability is normally defined as a period of 1 to 3 months in which there is no evidence of new or enlarging CNS metastases).

3.2.11 Has symptomatic ascites or pleural effusion; a subject who is clinically stable following treatment for these conditions is eligible.

3.2.12 Has had a prior allogeneic stem cell or bone marrow transplant.

3.2.13 Has known contraindication to aspirin (81 mg).

4.0 SUBJECT SCREENING AND REGISTRATION PROCEDURES

Patient registration for this trial will be centrally managed by the Coordinating Center of the University of Michigan Rogel Cancer Center as described below:

A potential study subject who has been screened for the trial and who has signed the Informed Consent document will be initially documented by the participating site on the Screening and Enrollment Log provided by the Coordinating Center.

It is the responsibility of the local site investigator to determine patient eligibility prior to submitting patient registration request to the Coordinating Center. After patient eligibility has been determined, a copy of the completed Eligibility Worksheet together with all the pertinent de-identified source documents will be submitted by the requesting site to the Coordinating Center, by email to CTSU-Oncology-Multisite@med.umich.edu.

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

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

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

5.0 TREATMENT PLAN

5.1 Treatment Dosage and Administration

Protocol treatment must start within 14 business days of enrollment to the study.

5.1.1 Overall Treatment Plan:

Phase Ib/II: All registered subjects will be treated with IONIS-AR-2.5$_{Rx}$ (ASO) in combination with enzalutamide. IONIS-AR-2.5$_{Rx}$ will be given intravenously in 60-minute infusions on Days 1, 4, 8, 11, 15 on cycle 1, then on days 1,8,15 in subsequent 21-day cycles. Enzalutamide will be taken daily in 21 day cycles starting Day 1 of cycle 1. Treatment will continue until clinical or radiologic progression or unacceptable toxicity. The infusion duration may be extended to 120 minutes for individuals experiencing infusion reactions.

- Baseline archival CRPC tumor tissue will be collected (mandatory) as well as tumor biopsies (optional) after 2 cycles of IONIS-AR-2.5$_{Rx}$ and, tumor biopsies (optional) at progression.

Phase	Agent	Premedications; Precautions	Starting Dose	Route	Schedule
Phase Ib and II	IONIS-AR-2.5$_{Rx}$	Per institutional standards for low emetogenic risk	Assigned Dose Level (See table in 5.1.2)	IV infusion over 60 minutes (+/-15 minutes)	*Days 1,4, 8, 11 and 15 of cycle #1. *Days 1, 8, 15 of each subsequent 21-day cycle
Phase Ib and II	Enzalutamide	No premedications mandated but may be used at investigator discretion/institutional standards	160 mg Qday	Oral	Day 1-21 of each 21-day cycle

Starting on Cycle 1 Day 1, all subjects should take ASA 81 mg PO Qday unless contraindicated.

As noted in section 1.2, the primary study drug IONIS-AR-2.5$_{Rx}$ has been associated with an increased risk of venous thromboembolism (VTE) in early clinical data. Prostate cancer is not associated with a high baseline incidence of VTE, and therefore routine VTE prophylaxis is not required in this patient population based on current NCCN guidelines for cancer-associated VTE. Low-dose aspirin for VTE prophylaxis has been recommend in multiple myeloma patients based on these NCCN guidelines (https://www.nccn.org/professionals/physician_gls/f_guidelines.asp). The overall likelihood of increased risk of VTE events with use of IONIS-AR-2.5$_{Rx}$ in prostate cancer patients is unknown, but given the relatively increased rate of VTE events in the initial study population, VTE prophylaxis appears to be warranted in further clinical study populations.

Patients in the current phase II trial will receive low dose aspirin (81 mg) daily while receiving study drug to mitigate the apparent increased risk of VTE.

Clotting and bleeding functions including platelet counts will be closely monitored on this study including weekly CBCPD, PT/PTT/INR and reviewed continuously as well at the interim analysis time points. If so needed and indicated by the data, including at interim safety analysis and continually by the Data and Safety Monitoring Board, we would modify the above strategy towards either escalating the prophylactic anti-coagulation to enoxaparin 40 mg daily, for example, or removing the 81 mg daily dose of aspirin.

5.1.2 Upon enrollment, each patient will be assigned a dose level from the statistician to initiate treatment. (See Section 11 for further details.)
Assigned Dose level (TITE-CRM):

Assigned Dose Level	Enzalutamide Dose	IONIS-AR-2.5$_{Rx}$ Dose
3	160 mg	900 mg
2	160 mg	750 mg
1	160 mg	600 mg

5.1.3 Definition of Dose Limiting Toxicity (DLT):

Excessive toxicity/dose limiting toxicity (DLT) will be defined as any Grade 3 or higher toxicity (as defined by CTCAE v5.0) that occurs during the DLT evaluation period (2 cycles plus labs prior to start of cycle 3). Toxicity that is clearly and directly related to the primary disease or to another etiology is excluded from this definition. The following will be DLTs:

- Liver transaminase elevation Grade 2 lasting 14 calendar days or more OR Liver transaminase elevation Grade 3 or 4
- Grade 3 or Grade 4 febrile neutropenia will be a DLT regardless of duration or reversibility
- Grade 3 thrombocytopenia that IS associated with clinically significant bleeding that requires medical intervention

The DLT Definition excludes the following conditions:

- Grade 3 fatigue lasting ≤ 7 days
- Grade 3 endocrine disorder (thyroid, pituitary, and/or adrenal insufficiency) that is managed with or without systemic corticosteroid therapy and/or hormone replacement therapy and the subject is asymptomatic
- Grade 3 infusion-related reaction (first occurrence and in the absence of steroid prophylaxis) that resolves within 6 hours with appropriate clinical management
- Grade 3 or 4 lymphopenia
- Grade 3 or 4 neutropenia that is not associated with fever or systemic infection that improves within 7 days
- Grade 3 thrombocytopenia that is not associated with clinically significant bleeding that requires medical intervention, and improves by at least 1 grade within 3 days
- Isolated Grade 3 electrolyte abnormalities that are not associated with clinical signs or symptoms OR are reversed with appropriate maximal medical intervention within 3 days
- Grade 3 laboratory abnormalities that are responsive to oral supplementation OR deemed by the investigator to be clinically non-significant

A withdrawal or death within the first 2 cycles (42 days) not related to treatment will not be considered a DLT.

DLTs will be counted based on the number of subjects with DLT at a given dose level, not the absolute number of DLTs. No single subject can trigger more than one DLT event.

5.2 Toxicities and Dosing Delays/Dose Modifications

Any patient who receives treatment on this protocol will be evaluable for toxicity. Each patient will be assessed for the development of toxicity according to the Time and Events Table (Section 6.4). Toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 5.0. Dose adjustments should be made according to the system showing the greatest degree of toxicity.

5.2.1 IONIS-AR-2.5$_{Rx}$

IONIS-AR-2.5 $_{Rx}$ Dose Modification Levels

Dose reduction levels	Assigned Dose Level = 1	Assigned Dose Level = 2	Assigned Dose Level = 3
Starting Dose	600 mg	750 mg	900 mg
-1	450 mg	600 mg	750 mg
-2	300 mg	450 mg	600 mg
-3	150 mg	300 mg	450 mg

Hematological Toxicities

Hematological Toxicity Dose Reductions for IONIS-AR-2.5$_{Rx}$ (AR ASO)		
ANC[1]	**Platelets**	**Action**
≥ 1,500/μL or	≥100,000/μL	None.
1000-1499/μL or	75,000-99,000/μL	-*1st Occurrence:* Hold current dose until ANC ≥ 1,500/μL and platelets ≥ 100,000/μL. Do not replace missed doses. Restart IONIS-AR-2.5$_{Rx}$ at the next lower dose level as available. -*2nd Occurrence*: Hold current dose until ANC ≥ 1,500/μL and platelets ≥ 100,000/μL. Do not replace missed doses. Restart IONIS-AR-2.5$_{Rx}$ at the next lower dose level as available. -*3rd Occurrence:* Hold current dose until ANC ≥ 1,500/μL and platelets ≥ 100,000/μL. Do not replace missed doses. Restart IONIS-AR-2.5$_{Rx}$ at the next lower dose level as available. -4th Occurrence: Discontinue protocol therapy.
500-999/μL or	50,000-74,000/μL	-*1st Occurrence*: Hold current dose until ANC ≥ 1,500/μL and platelets ≥ 100,000/μL. Do not replace missed doses. Restart IONIS-AR-2.5$_{Rx}$ at the next lower dose level as available. -*2nd Occurrence*: Hold current dose until ANC ≥ 1,500/μL and platelets ≥ 100,000/μL. Do not replace missed doses. Restart IONIS-AR-2.5$_{Rx}$ at the next lower dose level as available. -*3rd Occurrence*: Hold current dose until ANC ≥ 1,500/μL and platelets ≥ 100,000/μL. Do not replace missed doses. Restart IONIS-AR-2.5$_{Rx}$ at the next lower dose level as available. -4th Occurrence: Discontinue protocol therapy.

<500/μL or	<50,000/μL	-*1st Occurrence*: Hold current dose until ANC ≥ 1,500/μL and platelets ≥ 100,000/μL. Restart IONIS-AR-2.5$_{Rx}$ at the next lower dose level as available. -*2nd Occurrence*: Hold current dose until ANC ≥ 1,500/μL and platelets ≥ 100,000/μL. Do not replace missed doses. Restart IONIS-AR-2.5$_{Rx}$ at the next lower dose level as available. -3rd Occurrence: Discontinue protocol therapy.
[1]Note: G-CSF (Filgrastim) may be added for low ANC on day of treatment *BEFORE* a dose reduction is instituted at treating physician's discretions. Neulasta® is NOT allowed.		

Dose Modifications for Hepatic Toxicity

Patients who develop abnormal liver function tests as defined below, for any reason while on study, will have treatment held and/or reduced according to the following schedules:

HEPATOTOXICITY (BILIRUBIN, SGPT/ALT, SGOT/AST)	
TOTAL BILIRUBIN without ALT/AST increase above baseline value	
Grade 1 (> ULN – 1.5 x ULN)	Maintain IONIS-AR-2.5$_{Rx}$ level with LFTs monitored bi-weekly.
Grade 2 (> 1.5 – 3.0 x ULN)	Interrupt dosing of IONIS-AR-2.5$_{Rx}$. If resolved to ≤ grade 1 in ≤ 28 days, then reduce by 1 dose level and resume IONIS-AR-2.5$_{Rx}$ therapy. If toxicity recurs after three dose reductions OR does NOT resolve to ≤ grade 1 in ≤ 28 days, discontinue IONIS-AR-2.5$_{Rx}$ therapy.
Grade 3 (> 3.0 – 10.0 x ULN)	Interrupt dosing of IONIS-AR-2.5$_{Rx}$. If resolved to ≤ grade 1 in ≤ 28 days, lower by 1 dose level of IONIS-AR-2.5$_{Rx}$. If toxicity does NOT resolve to ≤ grade 1 in ≤ 28 days or toxicity recurs, discontinue IONIS-AR-2.5$_{Rx}$
Grade 4 (> 10.0 x ULN)	Discontinue IONIS-AR-2.5$_{Rx}$.
Confounding factors and/or alternative causes for increase of total bilirubin should be excluded before dose interruption/reduction. They include but are not limited to: evidence of obstruction, such as elevated ALP and GGT typical of gall bladder or bile duct disease, hyperbilirubinemia due to the indirect component only (i.e. direct bilirubin component ≤ 1 x ULN) due to hemolysis or Gilbert Syndrome, pharmacologic treatment, viral hepatitis, alcoholic or autoimmune hepatitis, other hepatotoxic drugs. For patients with Gilbert Syndrome, these dose modifications apply to changes in direct bilirubin only. Bilirubin will be fractionated if elevated.	

AST or ALT	
AST or ALT without bilirubin elevation > 2 x ULN	
Same grade as baseline or increase from baseline grade 0 to grade 1	No dose adjustment required with LFTs monitored weekly if same grade as baseline or bi-weekly in case of increase from baseline grade 0 to 1.

Increase from baseline to grade 2 (> 3.0– 5.0 x ULN) OR from baseline grade 2 to grade 3 (> 5.0 – 20.0 x ULN)	Interrupt dosing of IONIS-AR-2.5$_{Rx}$. If resolved to ≤ grade 1 in ≤ 28 days, then reduce by one dose level and resume IONIS-AR-2.5$_{Rx}$ therapy. If toxicity recurs, then reduce by 1 dose level and resume IONIS-AR-2.5$_{Rx}$. If toxicity recurs after three dose reductions OR does NOT resolve to ≤ grade 1 in ≤ 28 days, discontinue IONIS-AR-2.5$_{Rx}$ therapy.
Increase from baseline grade 0 or 1 to grade 3 (> 5.0– 20.0 x ULN)	Interrupt dosing of IONIS-AR-2.5$_{Rx}$ until resolved to ≤ baseline value, then lower 1 dose level of IONIS-AR-2.5$_{Rx}$. If toxicity recurs, discontinue IONIS-AR-2.5$_{Rx}$. If toxicity does NOT resolve to ≤ baseline value in ≤ 28 days, discontinue IONIS-AR-2.5$_{Rx}$ therapy.
Grade 4 (> 20.0 x ULN)	Discontinue IONIS-AR-2.5$_{Rx}$.

AST or ALT and concurrent Bilirubin elevation	
AST or ALT ≥ grade 2 (> 3 x ULN) in patients with normal values at baseline and total bilirubin > 2 x ULN OR AST or ALT ≥ grade 3 (> 5 x ULN) in patients with grade 1 or 2 at baseline, and total bilirubin > 2 x ULN	Discontinue IONIS-AR-2.5$_{Rx}$.
Confounding factors and/or alternative causes for increased transaminases should be excluded before dose interruption/reduction. They include but are not limited to: concomitant medications, herbal preparations or dietary supplements, infection, hepato-biliary disorder or obstruction, new or progressive liver metastasis, and alcohol intake.	

Additional follow-up for hepatic toxicities

Hepatic toxicity monitoring includes the following LFTs: albumin, ALT, AST, total bilirubin (fractionated if total bilirubin > 2 x ULN), alkaline phosphatase, and GGT. For patients with Gilbert Syndrome: total and direct bilirubin must be monitored, intensified monitoring applies to changes in direct bilirubin only.

Close observation is **recommended** in case of AST, ALT, and/or bilirubin increase requiring dose interruption, which involves:

- Repeating liver enzyme and serum bilirubin tests **two or three times weekly**. Frequency of re- testing can decrease to once a week or less if abnormalities stabilize or return to normal values.
- Obtaining a more detailed history of current symptoms.
- Obtaining a more detailed history of prior and/or concurrent diseases.
- Obtaining a history of concomitant drug use (including non-prescription medications, herbal and dietary supplements), alcohol use, recreational drug use, and special diets.
- Ruling out acute viral hepatitis types A, B, C, D, and E; hepatotropic virus infections (CMV, EBV or HSV); autoimmune or alcoholic hepatitis; NASH; hypoxic/ischemic hepatopathy; and biliary tract disease.

- Obtaining a history of exposure to environmental chemical agents.
- Obtaining additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin).
- Considering gastroenterology or hepatology consultations.
- Assessing cardiovascular dysfunction or impaired liver oxygenation, including hypotension or right heart failure as possible etiologies for liver dysfunction.

Hypersensitivity infusion reaction.
Patients will be monitored for signs and symptoms of infusion-related reactions including rigors, chills. wheezing, pruritus, flushing, rash, hypotension, hypoxemia, and fever. For severe (Grade 3) or life-threatening (Grade 4) infusion-related reactions, stop infusion of IONIS-AR-2.5$_{Rx}$. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

Dose Modifications for other Non-Hematologic Toxicity

- For treatment regimen-related toxicities $\geq$ grade 3 (excluding the hematologic, liver toxicities outlined above, as well as grade 3 nausea/vomiting/diarrhea/fatigue lasting less than 48 hours with optimal medical management, any grade alopecia, or non-clinically significant laboratory abnormalities that resolve within 48 hours with optimal medical management), IONIS-AR-2.5$_{Rx}$ should be withheld until resolution to $\leq$ grade 1 (or to baseline if baseline was greater than grade 1). Treatment may be restarted at next lower dose level if the toxicity improves to grade 1 or lower within 14 days of onset of adverse event. If toxicity recurs at grade 3 or higher, patients will be required to discontinue IONIS-AR-2.5$_{Rx}$ therapy.

- IONIS-AR-2.5$_{Rx}$ treatment days should be skipped for grade 2 related toxicities and restarted at the original dose level once recovered to grade ≤ 1. If the same toxicity recurs at grade 2, interrupt IONIS-AR-2.5$_{Rx}$ until recovery to grade ≤ 1, then restart IONIS-AR-2.5$_{Rx}$ at the next lower dose level. Discontinue IONIS-AR-2.5$_{Rx}$ therapy IF toxicity recurs after three dose reductions or does not improve to grade 1 or lower within 28 days of onset of adverse event.

- IONIS-AR-2.5$_{Rx}$ should be discontinued for Grade 4 non-hematologic toxicities that are possibly related to the study treatment, unless otherwise specified in the protocol (excluding: grade 4 nausea/vomiting/diarrhea/fatigue lasting less than 48 hours with optimal medical management, any grade alopecia, or non-clinically significant laboratory abnormalities that resolve within 48 hours with optimal medical management).

5.2.2 Enzalutamide

Enzalutamide Dose Modification Levels

Dose reduction levels	enzalutamide
(starting dose)	160 mg/day
-1	120 mg/day
-2	80 mg/day

Hepatic Toxicity

For ≥ Grade 2 ALT or AST or Bilirubin (see Investigations in CTCAE v. 5.0), treatment days should be skipped for grade 2 related toxicities and restarted at the original dose level once recovered to grade ≤ 1. If the same toxicity recurs at ≥ grade 2, interrupt enzalutamide. Restart enzalutamide at one dose level reduction when ALT, AST, and bilirubin recover to Grade 1. Discontinue enzalutamide therapy IF toxicity recurs after two dose reductions or does not improve to grade 1 or lower within 28 days of onset of adverse event.

For hepatic failure (see Hepatobiliary Disorders in CTCAE v. 5.0), discontinue enzalutamide.

Neurologic toxicity
Discontinue enzalutamide for any seizure.

Reversible posterior leukoencephalopathy syndrome (RPLS)
For signs and symptoms suggestive of reversible posterior leukoencephalopathy syndrome (RPLS) such as confusion, headache, seizures, and cortical blindness, hold enzalutamide for up to 4 weeks. Suspected RPLS should be investigated with MRI. If diagnosis of RPLS is confirmed, enzalutamide should be permanently discontinued. If RPLS is ruled out via MRI and signs and symptoms attributed to another cause, enzalutamide should resume.

Hypersensitivity
Discontinue enzalutamide.

Ischemic Heart Disease
Optimize management of cardiovascular risk factors. Discontinue enzalutamide for Grade 3-4 events.

Falls and Fractures
Falls and fractures occurred in 10% and 8% of patients receiving enzalutamide, respectively. Evaluate patients for fracture and fall risk, and treat patients with bone-targeting agents according to established guidelines.

Other Non-Hematologic Grade 3 or 4 Toxicity
For patients who experience a Grade 3 or greater toxicity attributed to enzalutamide, enzalutamide should be held for one week or until symptoms improve to ≤ Grade 2. Patients may be re-started on enzalutamide at the same or one dose level reduction. If treatment is held for more than 4 weeks, discontinue protocol therapy.

5.3 Concomitant Medications/Treatments

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for any medication or vaccination specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Study PI. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician. However, the decision to continue the subject on trial therapy or vaccination schedule requires the mutual agreement of the investigator, the Sponsor and the subject.

Use of bisphosphonates/RANKL inhibitors are standard in CRPC and are allowed on the study.

Subjects are prohibited from receiving the following therapies during the Screening and Treatment of this trial:

- Antineoplastic systemic chemotherapy or biological therapy except for ongoing androgen deprivation therapy (e.g. with LHRH agonist/antagonist)
- Immunotherapy
- Chemotherapy not specified in this protocol
- Investigational agents
- 5 α-reductase inhibitors (e.g., finasteride, dutasteride).
- Any herbal product known to decrease PSA levels (eg. Saw Palmetto and PC-SPES)
- Neulasta®
- Radiation therapy
    - Local intervention is discouraged unless medically unavoidable. Radiation therapy to a symptomatic solitary lesion/area may be considered on a

case-by-case basis after consultation with the Principal Investigator (except during screening). Subjects receiving local intervention (e.g., palliative radiation) are allowed to continue to receive study treatment at the investigator's discretion. Subjects who receive local intervention may be considered not evaluable (and may be assigned a conservative censoring or progression date).

- Live vaccines within 30 days prior to the first dose of treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, intranasal influenza, rabies, BCG (tuberculosis vaccine), and typhoid vaccine.
- Systemic glucocorticoids or other immunosuppressive drugs for any purpose other than to modulate symptoms from a drug-related AE of immunologic etiology (refer to Section 5.2 – Dose Modification). The use of physiologic doses of corticosteroids may be approved after consultation with the Study PI.
    - Use of prophylactic corticosteroids to avoid allergic and other adverse reactions (e.g., to IV contrast dye or transfusions) is permitted.
    - Use of intermittent inhaled steroids or local injection of corticosteroids is permitted upon consultation with the Principal Investigator.
    - Physiologic doses of prednisone ≤10 mg (or equivalent) per day.
- Avoid strong CYP2C8 inhibitors CYP3A4 inducers as they can increase the plasma exposure to enzalutamide. If co-administration is necessary, reduce the dose of enzalutamide.
- Avoid CYP3A4, CYP2C9, and CYP2C19 substrates with a narrow therapeutic index as enzalutamide may decrease the plasma exposure of these drugs.
- The exclusion criteria describe other medications prohibited in this trial.

5.4 Other Modalities or Procedures

No other modalities or procedures will be used in this protocol

5.5 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue until any one or more of the following criteria apply:

- Disease progression
- Inter-current illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient voluntarily withdraws from treatment
- General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the treating investigator **OR**
- Investigator determination that the patient is no longer benefitting from protocol therapy.

If treatment is stopped due to one or more of the criteria above, both enzalutamide and IONIS-AR-2.5$_{Rx}$ ASO will be discontinued.

5.6 Off Treatment Criteria

Patients will be removed from protocol specified therapy when any of the respective criteria listed in Section 5.5 apply.

Document in the source the reason for ending protocol therapy and the date the patient was removed from treatment.

All patients who discontinue treatment should comply with protocol specific follow-up procedures. The only exception to this requirement is when a subject withdraws consent for all study procedures or loses the ability to consent freely.

5.7 Duration of Follow-Up

Patients will be followed for up to 24 months after removal from treatment or until death, whichever occurs first. Patients removed from treatment for unacceptable adverse events will be followed until resolution or stabilization of the adverse events.

5.8 Off Study Criteria

Patients can be taken off study at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation from study will be documented and may include:

5.8.1 Patient withdraws consent (termination of treatment and follow-up);

5.8.2 Loss of ability to freely provide consent through imprisonment or involuntary incarceration for treatment;

5.8.3 Patient is unable to comply with protocol requirements;

5.8.4 Treating physician determines continuation on the study would not be in the patient's best interest;

5.8.5 Development of second malignancy (except for basal cell carcinoma or squamous cell carcinoma of the skin) that requires treatment, which would interfere with this study;

5.8.6 Lost to Follow-up. If a research subject cannot be located to document survival after a period of 2 years, the subject may be considered "lost to follow-up." All attempts to contact the subject during the two years must be documented;

5.8.7 Termination of the study by the University of Michigan;

5.8.8 Patient completes protocol treatment and follow-up criteria.

5.9 Patient Replacement

Patients who do not receive any dose of the study drug IONIS-AR-2.5$_{Rx}$ will be replaced.

6.0 STUDY PROCEDURES

Time and Events Table

All assessments have a window of ± 3 business days except for imaging/tumor measurements where a window of ± 7 business days will apply or unless otherwise specified.

Cycle length = 21 days

Trial period	Screening	Treatment								Safety Follow-up/EOT	Follow-up Visits
Treatment Cycle/Title	Screening Visit	Cycle 1					Cycle 2+ (every 21 days) (+/-3 business days)			30 days from last dose of IONIS-AR-2.5Rx (+/- 7 business days)	Every 12 weeks (+/-7 business days) For up to 24 months
Scheduling Window (Days)	**-28 to -1**	**D 1**	**D 4**	**D 8**	**D 11**	**D 15**	**D 1**	**D 8**	**D 15**		
Administrative Procedures:											
Informed Consent	X										
Review Eligibility Criteria	X										
Demographics and Medical History	X	X	X	X	X	X	X	X	X		
Prior and Concomitant Medications	X	X	X	X	X	X	X	X	X		
Survival Status											X[6]
Clinical Assessments:											
Review Adverse Events	X	X	X	X	X	X	X	X	X	X	
Physical Examination	X	X		X		X	X				
Vital Signs		X	X	X	X	X	X	X	X	X	
Height[5], Weight	X	X					X			X	
ECOG Performance Status	X	X					X			X	
Laboratory Assessments:											
PT/INR and PTT/aPTT	X	X		X		X	X	X	X		
CBC with differential, platelets	X	X	X	X	X	X	X	X	X	X	
Comprehensive Metabolic Panel[1], D-dimer	X	X	X	X	X	X	X	X	X	X	
PSA	X	X					X			X	X
Testosterone	X	X								X	
TSH, free T3, free T4 (If Clinically Indicated: FSH, LH, ACTH)	X									X	
Treatment:											
IONIS-AR-2.5$_{Rx}$[4]		X	X	X	X	X	X	X	X		
Enzalutamide		PO daily in 21-day cycles									

Trial period	Screening	Treatment								Safety Follow-up/EOT	Follow-up Visits
Treatment Cycle/Title	Screening Visit	Cycle 1					Cycle 2+ (every 21 days) (+/-3 business days)			30 days from last dose of IONIS-AR-2.5Rx (+/- 7 business days)	Every 12 weeks (+/-7 business days) For up to 24 months
Scheduling Window (Days)	**-28 to -1**	**D 1**	**D 4**	**D 8**	**D 11**	**D 15**	**D 1**	**D 8**	**D 15**		
Imaging Tumor Assessments[2]											
CT chest/CXR	X									X	X
MRI Abdomen/Pelvis or CT Abdomen/Pelvis	X									X	X
Bone Scan	X									X	X
QoL questionnaires[3]		X					X			X	
Correlatives											
Whole blood for banking		X pre-dose									
Tumor Tissue Archival CRPC	X										
Tumor Tissue Biopsy[7]	X								X	X	
PK Plasma IONIS-AR-2.5_{Rx} concentration (pre-dose)[8,10]		X	X	X	X	X	X	X	X		
PK Plasma IONIS-AR-2.5_{Rx} concentration (end of infusion)[8,9,10]		X	X	X	X	X	X	X	X		

1. Comprehensive metabolic panel (CMP) to include: albumin, alkaline phosphatase, ALT/SGPT, AST/SGOT, BUN, creatinine, electrolytes (sodium, potassium, calcium, chloride, bicarbonate), glucose, and total bilirubin; LDH, D-dimer.

2. Imaging Assessments: Every 12 weeks or as clinically indicated.

3. QoL questionnaires: FACT-P and EuroQoL-5D.

4. Starting Cycle 1 Day 1, all subjects should take ASA 81 mg PO q day unless contraindicated.

5. Height only at baseline.

6. Survival status every 6 months.

7. Tumor Tissue Biopsy at Screening is required only if Archival Tissue is not available. Tissue biopsy collection at post cycle 2 and Safety Follow-up/EOT is optional if subject consents; +/- 7working days.

8. Plasma PK samples may also be used for profiling of drug binding proteins, bioanalytical method development and/or validation purposes, stability assessments, metabolite assessments, immunogenicity assessments or to assess other actions of IONIS-AR-2.5_{Rx} with plasma constituents.

9. Collected within 2 minutes before infusion is stopped

10. Collected Cycle 1 Days 1, 4, 8, 11 and 15 and Cycle 2 Day 1, 8 and 15 only.

7.0 MEASUREMENT OF EFFECT

7.1 Antitumor Effect- Solid Tumors

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee [*JNCI* 92(3):205-216, 2000]. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST v1.1 criteria.

7.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with IONIS-AR-2.5Rx.

Evaluable for PSA response. Only those patients who have received at least 1 dose of IONIS-AR-2.5Rx, and have at least 2 PSA measures at least 1 week apart after initiating treatment will be considered evaluable for PSA response. (Note: Patients who exhibit objective disease progression prior to the end of cycle 2 will also be considered evaluable and will be classified as PSA non-response).

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least 1 dose of IONIS-AR-2.5Rx, and have had their disease re-evaluated will be considered evaluable for objective 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 4 will also be considered evaluable).

7.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

- 10mm by CT scan (irrespective of scanner type) for studies with a slice thickness of ≤5mm or twice the slice thickness or MRI
- 10mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable)
- 20mm by chest X-ray (if clearly defined and surrounded by aerated lung)

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥15mm 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.

Note: Tumor lesions that are situated in a previously irradiated area are not considered measurable.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <20 mm with conventional techniques or <10 mm using CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total 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 organ, but in addition should be those that lend themselves to reproducible repeated measurements.

Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumor. Pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of ≥15mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumor. Nodal size is normally reported as two dimensions in the plane in which the image is obtained (for CT scan this is almost always the axial plane; for MRI the plane of acquisition may be axial, sagittal or coronal). The smaller of these measures is the short axis. For example, an abdominal node which is reported as being 20mm x 30mm has a short axis of 20mm and qualifies as a malignant, measurable node. In this example, 20mm should be recorded as the node measurement. All other pathological nodes (those with short axis ≥10mm but <15 mm) should be considered non-target lesions. Nodes that have a short axis <10mm are considered nonpathological and should not be recorded or followed.
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 as noted above, 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 pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as ‘present’, ‘absent’, or in rare cases ‘unequivocal progression’ (more details to follow). In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the case record form (e.g. ‘multiple enlarged pelvic lymph nodes’ or ‘multiple liver metastases’).

7.1.3 Guidelines for Evaluation of Measurable Disease

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start 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 should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and >10mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5mm or less. When 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).

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

Tumor markers: Tumor markers alone cannot be used to assess objective tumor response. If markers are initially above the upper normal limit, however, they must normalize for a patient to be considered in complete response. Because tumor markers are disease specific, instructions for their measurement should be incorporated into protocols on a disease specific basis. Specific guidelines for PSA response (in recurrent prostate cancer), have been published

Cytology, Histology: These techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain). When effusions are known to be a potential adverse effect of treatment (e.g. with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment can be considered if the measurable tumor has met criteria for response or stable disease in order to differentiate between response (or stable disease) and progressive disease.

7.2 Response Criteria for RECIST 1.1 subjects with measurable disease

7.2.1.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions, determined by two separate observations conducted not less than 4 weeks apart. There can be no appearance of new lesions.
Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD. There can be no appearance of new lesions.

Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started, or the appearance of one or more new lesions.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.

7.2.1.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level.

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

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.

7.2.1.3 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.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Response for this Category Also Requires:
CR	CR	No	CR	≥4 wks. confirmation
CR	Non-CR/SD	No	PR	≥4 wks. confirmation
PR	Non-PD	No	PR	
SD	Non-PD	No	SD	documented at least once ≥4 wks. from baseline
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD*	Yes or No	PD	
Any	Any	Yes	PD	

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

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

Note: If subjects respond to treatment and are able to have their disease resected, the patient's response will be assessed prior to the surgery.

7.2.2 PSA Response

Per PCWG3 criteria, PSA response rate will be defined as the proportion of subjects who have a PSA response defined by a 50% decline or more in PSA (ng/ml) from baseline measured twice at least 3 weeks apart. Based on the PCWG3 criteria, a favorable effect on PSA may be delayed for ≥ 12 weeks. PSA will be monitored every cycle, but treatment will be planned to continue through early rises for 12 weeks unless other evidence of progression, such as radiographic progression. Therefore, early rises in PSA before 12 weeks will not be considered progression when determining PSA response.

7.2.3 PSA Progression by PCWG3 criteria

PSA evidence for progressive prostate cancer consists of a PSA level of at least 1 ng/ml which has risen

on at least 2 successive occasions, at least one week apart. If the third PSA (#3) value is less (i.e., #3b) than the screening PSA (#2) value, then an additional test for rising PSA (#4) will be required to document progression by PSA. All PSA progression as above will require confirmation of equal or higher PSA value at least 4 weeks later (from the #3a or #4 value as applicable). **Based on the PCWG3 criteria, a favorable effect on PSA may be delayed for ≥ 12 weeks. PSA will be monitored every cycle, but treatment will be planned to continue through early rises for 12 weeks unless other evidence of progression, such as radiographic progression. Therefore, early rises in PSA before 12 weeks will not be considered progression when determining PSA progression by PCWG3 criteria without evidence of other progression.**

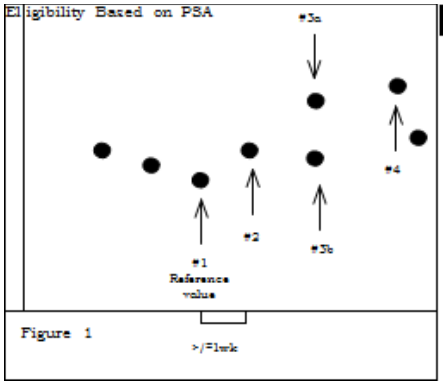


Figure 1

7.2.4 Bone Scan

On bone scan, radiographic progression will be defined by PCWG3 criteria as ≥ 2 new bone lesions (Appendix B). However, for the first reassessment scan only, patients should remain on study and have a confirmatory scan performed 12 weeks later. If this confirmatory scan shows 2 or more additional new lesions, this defines progression. The date of progression is the date of the first reassessment bone scan. If the confirmatory scan does not show any additional new lesions, patient remains on study. If progression is observed on subsequent bone scans, a confirmatory scan is not required; the date of this bone scan is the date of progression (Appendix B).

7.2.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for response (whether PSA response and/or RECIST 1.1 response when applicable) 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 recurrent disease is objectively documented.

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

7.2.6 Progression-Free Survival

Progression-free survival (PFS) is defined as the duration of time from start of treatment to time of progression whether PSA progression by PCWG3 criteria (see section 7.2.3 above) and/or RECIST 1.1 criteria as applicable.

7.3 Safety/Tolerability

Analyses will be performed for all patients having received at least one dose of study drug. The study will use the CTCAE version 5.0 for reporting of non-hematologic adverse events.

8.0 ADVERSE EVENTS

8.1 Experimental Therapy

For the most recent safety update, please refer to the current Investigator's Brochure or Study Agent Prescribing Information.

8.1.1 Special Warnings and Precautions for Use

Contraception:
Subjects receiving IONIS-AR-2.5$_{Rx}$ are advised to use two acceptable (to treating investigator) methods of birth control (one of which must include a condom as a barrier method of contraception) starting at the time of informed consent and continuing throughout the course of treatment and for at least 120 days after last dose of trial therapy.

8.2 Adverse Event Reporting Requirements

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

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

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

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

All events meeting the criteria and definition of an AE or SAE, as defined in Section 8.3, occurring from the initial study treatment administration or intervention through 30 days following the last dose of the study treatment or study intervention must be recorded as an adverse event in the patient's source documents and on the CRF regardless of frequency, severity (grade) or assessed relationship to the study treatment or intervention.

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

8.3 Definitions

8.3.1 Adverse Event

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

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

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

8.3.2 Serious Adverse Event

An adverse event is considered “serious” if, in the view of the sponsor investigator, it results in any of the following outcomes:

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

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

- Inpatient hospitalization or prolongation of existing hospitalization for $\geq$ 24 hours.

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

- A congenital anomaly/birth defect

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

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

- Procedure in case of Pregnancy
  If during the conduct of the clinical trial, a male subject impregnates his partner, the subject should report the pregnancy to the investigator. The investigator should report the pregnancy to the Coordinating Center as an SAE within 24 hours of awareness of the event. The expected date of delivery or expected date of the end of the pregnancy, last menstruation, estimated fertility date, pregnancy result and neonatal data etc., should be included in this information.

  The investigator should report the outcome of the pregnancy (independent of outcome, e.g., full term delivery, pre-term delivery, spontaneous abortion, induced abortion, stillbirth, death of newborn, congenital anomaly [including anomaly in a miscarried fetus, etc.) in accordance with the same reporting procedure as for SAEs. The date of outcome of the pregnancy, gestational age, date of birth and neonatal data etc., should be included in this information.

8.3.3 Expected Adverse Events

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

- For approved and marketed drugs or devices, those adverse events are described in the approved Package Insert (Label).
- For investigational new drugs or devices, those adverse events are described in the FDA Investigator's Brochure.
- In clinical research studies, information on expected adverse events is also summarized in the protocol and in the consent document. See section 9.1 for the list of expected adverse events related to the drug under study.

8.3.4 Unexpected Adverse Event

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

8.4 Adverse Event Characteristics

8.4.1 CTCAE Term

(AE description) and grade: The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be down loaded from the CTEP web site. (http://ctep.cancer.gov)

8.4.2 Attribution of the AE

The investigator or co-investigator is responsible for assignment of attribution.
Definite – The AE *is clearly related* to the study [treatment/intervention].
Probable – The AE *is likely related* to the study [treatment/intervention].
Possible – The AE *may be related* to the study [treatment/intervention].
Unlikely – The AE *is doubtfully related* to the study [treatment/intervention].
Unrelated – The AE *is clearly NOT related* to the study [treatment/intervention].

8.5 Serious Adverse Event Reporting Guidelines

All serious adverse events (SAEs) and unanticipated problems (UPs), regardless of causality to study drug, will be reported to the Principal Investigator and also to the Coordinating Center. All SAEs and UPs must be reported to the Coordinating Center within 24 hours of first awareness of the event. Events should be reported using the Coordinating Center SAE form as available in the study database. A copy of the Coordinating Center SAE form should be sent to the Coordinating Center via fax at 734-232-0744 or via email to CTSU-Oncology-Multisite@med.umich.edu within 24 hours of the site's knowledge of the event.

Principal Investigator Contact Information for SAE reporting:

Ajjai Alva, MD
Internal Medicine - Hematology/Oncology
University of Michigan
7316 Cancer Center
1500 E. Medical Center Drive
Ann Arbor, MI 48109
Phone: (734) 936-0091
Fax: (734) 615-2719
Email: ajjai@umich.edu

Follow-up information must also be reported within 24 hours of receipt of the information by the investigator.

All SAEs and UPs will be reported to the local IRBs per current local institutional standards.

The Coordinating Center will disseminate information regarding SAEs and UPs to the participating sites within 5 days of review of the information by the Coordinating Center's Principal Investigator (or designee in the event of extended absence) only in the case that the event(s) is believed to be related (i.e., possibly, probably, or definitely) to the study drug. The Coordinating Center will be responsible for reporting of events to the FDA and supporters, as appropriate (outlined below).

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

8.5.1 SERIOUS ADVERSE EVENT REPORTING TO THE FDA

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

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

8.6 Routine Reporting

All other adverse events such as those that are expected, or are unlikely or definitely not related to the study participation are to be reported annually as part of regular data submission.

8.7 Reporting of Unanticipated Problems

There are types of incidents, experiences and outcomes that occur during the conduct of human subjects research that represent unanticipated problems but are not considered adverse events. For example, some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased risk of harm, but no harm occurs.

Upon becoming aware of any incident, experience, or outcome (not related to an adverse event) that may represent an unanticipated problem, the investigator should assess whether the incident, experience, or outcome represents an unanticipated problem. The incident, experience or outcomes is considered unanticipated if it meets all of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency);

2. Related or possibly related to participation in the research; and
3. Suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized.

If the investigator determines that the incident, experience, or outcome represents an unanticipated problem, the investigator must report it to the local IRB as per local guidelines.

8.8 Unblinding Procedures

Not applicable

8.9 Stopping Rules

See section 11.1 **Safety Monitoring and Stopping Rule.** The DSMC will have oversight on the safety and data integrity of the study on an ongoing basis.

9.0 DRUG INFORMATION

9.1 IONIS-AR-2.5$_{Rx}$ (or specifically ISIS 560131; formerly known as AZD5312)

IONIS-AR-2.5$_{Rx}$ is an ASO targeting a region of the AR that results in selective and specific inhibition of full length, mutated, and splice-variant AR proteins.

IONIS-AR-2.5$_{Rx}$ was designed with the purpose of specifically suppressing human AR expression, thereby providing potential therapeutic benefit to the patients who became unresponsive to traditional anti-hormonal therapies. Preclinical studies indicate that IONIS-AR-2.5$_{Rx}$ suppresses several other AR-regulated genes, suggesting a potential for this agent to be used as a cancer therapy where aberrant AR-signaling drives the disease progression.

Nomenclature
Laboratory Code: IONIS-AR-2.5$_{Rx}$ pentadecasodium

General properties
IONIS-AR-2.5$_{Rx}$ is an amorphous powder and a pentadecasodium salt. IONIS-AR-2.5$_{Rx}$ pentadecasodium is freely soluble in water and has solubility >250 mg/mL at 25°C.

Presentation
The drug product is presented as a sterile solution for injection/infusion filled into 2 mL clear glass vials. Each vial contains an extractable volume of at least 1.2 mL of a 167 mg/mL solution of IONIS-AR-2.5$_{Rx}$ (as the free acid) giving a maximum dose per vial of 200 mg. The product can be administered by direct injection, or by dilution into 0.9% sodium chloride for infusion. The formulation should be prepared and administered according to the handling instructions provided. The vials are intended for single use only.

Composition
The composition of IONIS-AR-2.5$_{Rx}$ solution for injection/infusion is IONIS-AR-2.5$_{Rx}$, water for injection (Ph Eur or USP), pH adjusted as needed with either sodium hydroxide or hydrochloric acid to pH 8.

Storage conditions
The product should be stored in the pack provided and used as detailed in the handling instructions. Store refrigerated at 2-8°C protected from light.

Intravenous Dose Preparation
1. Obtain required materials:
    - 250 mL Normal Saline I.V. Bag
    - Study Drug Vials

• Filter Needles 19G or 18G, 5 micron (Becton Dickinson Part # 305200 or 305201 or equivalent)

• Regular 18-22 Gauge Needles

2. Volume of drug to yield the intended dose of 900 mg for AR

3. Warm vial(s) to room temperature for approximately 15 minutes and record the patient number on each vial before dose preparation.

4. Use a filter needle 19G or 18G, 5 micron (Becton Dickinson Part # 305200 or 305201 or equivalent) to withdraw study drug from vials.

5. Remove the used filter needle, replacing it with a regular 18-22-gauge needle.

6. Transfer the study drug to the 250 mL Normal Saline I.V. bag. Vials are for single-use only.

7. Label the I.V. bag per your institution's procedures. Include a label with the statement "Caution: New Drug – Limited by Federal (or United States) law to investigational use". Record on the label the total volume contained in the I.V. bag. Be sure to include in the total volume, the overfill (assume 25 mL
unless otherwise known) and the volume of study drug that was added.

8. Start infusion within 2 hours of preparation if kept at room temperature or within 24 hours if refrigerated at 2-8°C.

Availability

- Provided by Ionis Pharmaceutical
- Under no circumstance will the study medication IONIS-AR-2.5$_{Rx}$ be used other than as directed by the protocol.

Drug Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the investigational drug IONIS-AR-2.5$_{Rx}$. The drug accountability records will capture drug receipt, drug dispensing, drug return and final disposition.

Clinical Experience with IONIS-AR-2.5$_{Rx}$:

The first-time-in-patients (FTIP) study, D5860C00001: A Phase I, open-label, multicenter dose-escalation study to investigate the safety and pharmacokinetics of IONIS-AR-2.5$_{Rx}$ in patients with advanced solid tumors where the androgen receptor pathway is a potential factor was conducted. Patients are administered IONIS-AR-2.5$_{Rx}$ IV on Days 1, 4, 8, 11, 15, and 22 of Cycle 1 and Days 1, 8, 15, and 22 of each subsequent 28-day cycle in an accelerated 3+3 dose escalation. Preliminary PK and safety data are presented in the following sections.

Pharmacokinetics and drug metabolism in humans

Study D5860C00001: The pharmacokinetics of the dose levels of 150, 300, 600, and 900 mg were assessed in 4 cohorts. Intravenous infusion doses of IONIS-AR-2.5$_{Rx}$ were administered over 1 hour in Cycle 1 on Days 1, 4, 8, and 11 (±2 days). Blood samples were obtained over a 24-hour period on Day 1 and prior to dosing on Days 4, 8, and 11. Patients also received weekly treatments on Days 15 and 22 to complete Cycle 1. During subsequent cycles, patients received once weekly treatments on Days 1, 8, 15, and 22 (±2 days). In Cycle 2, blood samples were obtained over a 24-hour period on Day 22 to characterize the PK profile on that day.

Additional samples were taken prior to the first dose during Cycles 3 and 4, during which doses were administered on Days 1, 8, 15, and 22 (±2 days).

Cohort 1 (150 mg)
During Cohort 1, PK data was collected for 3 patients on Cycle 1 Day 1 and for 2 patients on Cycle 2 Day 22. The Cycle 1 Day 1 Cmax values averaged 32.4 µg/mL, Tmax occurred at the end of the infusion sampling time (1 hour).

The results of the Cycle 2 Day 22 PK parameter analysis were very similar to Cycle 1 Day 1 results; Cmax values averaged 26.1 µg/mL, Tmax occurred at the end-of-infusion sampling time (1 hour).

Cohort 2 (300 mg)
During Cohort 2, one of the 3 patients had blood samples taken after the end of infusion rather than at the beginning, both on Cycle 1 Day 1 and on Cycle 2 Day 22, so the peak concentrations were not determined for that patient, but rather for the 2 other patients in the cohort. The Cycle 1 Day 1 Cmax values averaged 49.0 µg/mL, Tmax occurred at the end-of-infusion sampling time (1 hour).

For Cycle 2 Day 22, Cmax values averaged 42.5 µg/mL, Tmax occurred at the end-of-infusion sampling time (1 hour).

Cohort 3 (600 mg)
During Cohort 3, one of the 3 patients had blood samples taken after the end of infusion rather than at the beginning, both on Cycle 1 Day 1 and on Cycle 2 Day 22, so the peak concentrations were not determined for that patient, but rather for the 2 other patients in the cohort. The Cycle 1 Day 1 Cmax values averaged 61.3 µg/mL, Tmax occurred at the end-of-infusion sampling time (1 hour).

For Cycle 2 Day 22, Cmax values averaged 58.3 µg/mL, Tmax occurred at the end-of-infusion sampling time (1 hour).

Cohort 4 (900 mg)
During Cohort 4, PK data was collected for 5 patients on Cycle 1 Day 1 and for 2 patients on Cycle 2 Day 22. The Cycle 1, Day 1 Cmax values averaged 102 µg/mL, Tmax occurred at the end-of-infusion sampling time (1 hour).

For Cycle 2 Day 22, Cmax values averaged 72.5 µg/mL, Tmax occurred at the end-of-infusion sampling time (1 hour).

Urinary excretion
Twenty-four-hour urinary collections were made in 2 intervals (0 to 8 and 8 to 24 hours) on Cycle 1 Day 1 and Cycle 2 Day 22. The results of the analysis showed:
-Cohort 1 (150 mg) - the amount of unchanged drug excreted in the urine ranged from 1.04 to 9.96 mg, accounting for 0.694% to 6.94% of the administered dose.
-Cohort 2 (300 mg) - the amount of unchanged drug excreted in the urine ranged from 14.4 to 30.2 mg, accounting for 4.79% to 10.1% of the administered dose.
-Cohort 3 (600 mg), the amount of unchanged drug excreted in the urine ranged from 17.3 to 84.6 mg, accounting for 2.87% to 14.1% of the administered dose.
-Cohort 4 (900 mg), the amount of unchanged drug excreted in the urine ranged from 121 to 245 mg, accounting for 13.5% to 27.2% of the administered dose.

Overall, 0.694% to 27.2% of IONIS-AR-2.5$_{Rx}$ was excreted unchanged in the urine. There was a trend toward increasing percentage excreted with increase in dose.

Safety and efficacy

Hepatic Function test abnormalities: Review of laboratory data has identified a trend for increases in ALT/AST and ALP in the 600 mg and 900 mg dose cohorts, however, which was most apparent at 900 mg. Elevations tend to develop slowly and have been mostly Grade 1. One

patient was noted to have Grade 2 ALT and Grade 1 AST elevations at Cycle 2 Day 8 leading to a hold of study treatment. The patient returned 1 week later and ALT and AST had returned to normal range, and the patient restarted study medication with no new issues identified at this time. Adverse events reported in preliminary, unaudited safety information include fatigue, diarrhea, nausea, vomiting, dry skin, headache, insomnia, loss of appetite, mouth pain, muscle pain, pruritus, increased ALT/ AST, elevated ALP and GGT, taste alteration, thrombocytopenia, gastrointestinal cramping, constipation, and weight loss. Most AEs were Grade 1 and 2. One patient in Cohort 2 (300 mg) experienced an immediate Grade 3 infusion reaction at the Cycle 5 Day 15 dose (hypotension and flushing), which resolved on treatment with steroids. The patient received prophylactic pre-medication following standard local procedures prior to the Cycle 5 Day 22 dose, but still experienced another infusion reaction (arthralgia, chills). The patient has been discontinued due to disease progression. No serious AEs that were considered to be related to the study drug by an Investigator have been reported up to the cut-off date, and no fatal events have been reported.

Venous thromboembolism: After the data cut-off date for inclusion in the above review of emerging safety data, a number of AE reports of venous thromboembolism (pulmonary embolism, and deep vein thrombosis) were received for patients in study D5860C00001. By 18 August 2015 there were a total of 4 events of Grade 3 pulmonary embolism in 4 patients reported to AstraZeneca for patients in study D5860C00001. At that date, 29 patients had been treated with IONIS-AR-2.5$_{Rx}$ in that study. Of the 4 patients, 3 were symptomatic (and were incidental findings on routine CT restaging), and 1 patient presented with shortness of breath. All patients continued on study after diagnosis and treatment. Initially 2 of these events were assessed as unrelated to IONIS-AR-2.5$_{Rx}$ by the site investigators, but considering the cluster of pulmonary embolism events, AstraZeneca reassessed these events as possibly related to study therapy. In addition, 3 other events of non-serious deep venous thrombosis were reported, and constitute similar thromboembolic phenomena.

Marketing experiences:
IONIS-AR-2.5$_{Rx}$ 2 is an investigational drug and is not available as a marketed product in any region.

Emerging safety profile:
This section is the Reference Safety Information for IONIS-AR-2.5$_{Rx}$ and presents those events that are to be regarded as expected for safety reporting purposes:
Venous thromboembolism (pulmonary embolism and deep vein thrombosis) – Occurs at a Council for International Organizations of Medical Sciences (CIOMS) III frequency of very common (>10%). The majority of pulmonary embolism reports were incidental.

SUMMARY OF DATA AND GUIDANCE FOR THE INVESTIGATORS:
Contraindications
IONIS-AR-2.5$_{Rx}$ is an investigational drug and should only be used in accordance with the relevant clinical protocol. Patients must fulfil the Inclusion Criteria and not violate any of the Exclusion Criteria specified within the relevant protocol.

Warnings and precautions
Pregnancy
Reproductive and developmental toxicity studies and juvenile toxicity studies have not been conducted with IONIS-AR-2.5$_{Rx}$ to date, and it is unknown whether the drug is excreted in human milk. Therefore, men taking IONIS-AR-2.5$_{Rx}$ and their female partners of childbearing potential should agree to use adequate contraception for the duration of study participation, and for 120 days following last study treatment.

Male subjects should be fully informed of the lack of reproductive toxicity testing. The clinical protocol gives details on the requirements to report pregnancy and its outcome.

Interaction potential
No interaction studies have been conducted with IONIS-AR-2.5$_{Rx}$ to date.

Phosphorothioate oligonucleotides are not substrates for the classic CYP metabolic pathways and are thus unlikely to interact with other drugs that modulate or are modulated by CYP, and the same would again be expected for IONIS-AR-2.5$_{Rx}$, though not yet evaluated.

Overdose
IONIS-AR-2.5$_{Rx}$ must only be used in accordance with the relevant clinical protocol. An overdose is defined is a dose of IONIS-AR-2.5$_{Rx}$ 2 administered to a patient that is greater than the protocol defined dose for the patient. Symptomatic and non-symptomatic overdose must be documented in accordance with the protocol.

There is currently no specific treatment in the event of an overdose with IONIS-AR-2.5$_{Rx}$ and possible symptoms of overdose have not been established. In the event of overdose, the patient should be observed and appropriate symptomatic treatment given.

IONIS-AR-2.5$_{Rx}$ toxicity observed in preclinical studies
The anticipated AE profile and potential risks seen with IONIS-AR-2.5$_{Rx}$[22]in mice and monkeys, include accumulation of compound as basophilic granules in multiple tissues, complement activation and prolongation of activated partial thromboplastin time (aPTT) in monkeys and pro-inflammatory effects in mice are consistent with expected effects of ASOs (Burel et al 2013).[22]

Evidence of effects on AR signaling, including tissue weight and histopathological changes, are in line with expected pharmacodynamics.

Safety considerations for phosphorothioate oligonucleotides.
More than twenty 2′-MOE-modified phosphorothioate ASOs, a chemical class similar to IONIS-AR-2.5$_{Rx}$, have been administered to human subjects, including ASOs that are under evaluation for the treatment of cancer. Clinical safety results of such 2′-MOE-modified ASOs can provide guidance to the potential attributes of IONIS-AR-2.5$_{Rx}$. Some profile differences exist between class members and extrapolations from one ASO to another should be made cautiously[23,24].

Potential class-related effects are informed by prior clinical experience with IV-administered 2nd generation ASOs and include transient constitutional symptoms associated with early doses and transient prolongation of aPTT[24]. Also, due to prior preclinical findings for one or more members of the drug class, the following additional areas are monitored in the clinical trials of ASOs: renal effects, liver effects, complement activation, and proinflammatory effects[23,24].

<u>Constitutional symptoms</u>
Transient fever, chills, body aches, nausea, and other constitutional symptoms have been experienced following the first few administrations of some ASOs[24]. In general, these symptoms appear within hours of ASO administration, are mild in intensity, and resolve spontaneously overnight. The occurrence and intensity of these symptoms generally diminishes over the course of repeated administrations. The proportion of patients that experience infusion-associated constitutional symptoms and the symptom spectrum is dose dependent.

At doses in the range of 200 to 400 mg, mild body aches, and general discomfort are the most frequent complaints. However, at higher doses, fever and chills become more prominent complaints. The relationship between dose and constitutional symptoms is variable between ASOs.

Constitutional symptoms associated with dose administration should be monitored through adverse event recording, and should be managed as required.

Prolongation of aPTT
Prolongation of aPTT associated with ASO administration has been shown to be the result of low-affinity oligonucleotide binding to the tenase complex (Factor IXa-Factor VIIIa phospholipid) and the effect is proportional to concentration of oligonucleotide in plasma (Cmax)[24,25]. In monkeys, there were acute, transient increases in aPTT at 12 mg/kg IONIS-AR-2.5_{Rx} and above. There was no evidence of associated hemorrhage or bleeding. Changes in activity of the intrinsic clotting pathway reverse when ASO is cleared from plasma. Coagulation effects should be monitored by adverse event recording.

Renal effects
The kidney is the organ of greatest ASO distribution. The dose-dependent presence of basophilic granules in proximal tubular epithelial cells is a common finding for animals treated with ASOs[23]. They arise from the accumulation of ASOs in endosomal vesicles and as such are an indication of ASO uptake and compartmentalization.

No toxicological effects have been associated with the presence of basophilic granules. Renal effects should be monitored through adverse event recording, clinical chemistry evaluations, and urinalysis.

Liver effects
The liver is the organ having the second highest distribution of ASOs. In humans and monkeys, elevations of transaminases or other signs of hepatotoxicity are not a class-effect of phosphorothioate oligonucleotides. Two to three-fold increases in transaminases are routinely observed in mice and other rodents; however, the elevations are secondary to the proinflammatory effects of ASOs in this most sensitive species (manifested by inflammatory cell infiltrates in multiple organs including the liver). In some cases, liver enzyme elevations have occurred after administration of oligonucleotides in humans.

Liver effects should be monitored through adverse event recording and clinical chemistry parameters.

Complement activation
Complement pathway activation has been observed in monkeys treated with high doses of phosphorothioate oligonucleotides. The effect arises from the interaction of oligonucleotides with Factor H, an inhibitor of complement activation, and occurs when oligonucleotide levels in plasma exceed threshold concentrations (approximately >100 µg/mL for most ASOs after IV administration)[23,26]. Monkeys are known to be more sensitive to complement activation than humans[27].

Proinflammatory effects
Pro-inflammation is a well described non-specific class effect of ASOs[28,29]. In preclinical toxicology studies, particularly in studies conducted in rodents, proinflammatory effects are often observed for the phosphorothioate oligonucleotide chemical class. In general, rodents appear to be more sensitive to ASO-mediated pro-inflammatory effects than primates[23].

Pro-inflammatory effects should be monitored by adverse event recording.

9.2 Enzalutamide

Other names for the drug: XTANDI®

Description:
Enzalutamide is a white crystalline non-hygroscopic solid. Enzalutamide is provided as a liquid-filled soft gelatin capsule for oral administration. Each capsule contains 40 mg of enzalutamide as a solution in caprylocaproyl polyoxylglycerides.

Classification: type of agent: androgen receptor inhibitor

Mode of action:
Enzalutamide is an androgen receptor inhibitor that acts on different steps in the androgen receptor signaling pathway. Enzalutamide has been shown to competitively inhibit androgen binding to androgen receptors and inhibit androgen receptor nuclear translocation and interaction with DNA.

Pharmacokinetics:

Absorption: rapid with time to peak concentration in 1 hour (range 0.5-3 hours). A high-fat meal did not alter the AUC to enzalutamide or N-desmethyl enzalutamide.

Distribution: 110 L; protein binding of parent drug is 97-98% to primarily albumin; protein binding of active metabolite is 95% to plasma proteins

Metabolism: Hepatic via CYP2C8 (formation of active metabolite N-desmethyl enzalutamide) and CYP3A4

Excretion: 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: Urine (71%), feces (14%) primarily as inactive metabolite

T1/2: Enzalutamide: 5.8 days (range 2.8-10.2 days); N-desmethyl enzalutamide: 7.8-8.6 days.

Side effects:
The most common adverse reactions (≥ 10%) are asthenia/fatigue, back pain, decreased appetite, constipation, arthralgia, diarrhea, hot flush, upper respiratory tract infection, peripheral edema, dyspnea, musculoskeletal pain, weight decreased, headache, hypertension, and dizziness/vertigo.

Drug Interactions: Please refer to the package insert for enzalutamide.

Storage and stability: Store enzalutamide at 20º to 25ºC (68º to 77ºF) in a dry place and keep the container tightly closed. Excursions permitted to 15º to 30ºC (59º to 86º F).

Administration:
Enzalutamide is administered orally once daily with or without food for 21 day cycles. Patients should be instructed to swallow capsules whole, and not to open or crush the capsules. Subjects will be asked to keep a pill diary noting the date they take enzalutamide. They will be asked to bring their pill diary on day one of each cycle and all used and unused medication bottles and remaining pills in the bottle(s) will be counted to document compliance. This information should be recorded on the patient's pill diary and/or in the patient data file.

Availability: Commercially available

Drug Accountability:
The investigator, or a responsible party designated by the investigator, must maintain a record of the disposition of enzalutamide. The drug accountability records should capture drug dispensing, drug return and final disposition.

10.0 CORRELATIVES/SPECIAL STUDIES

The goal of the planned laboratory correlative studies is to ascertain if ISIS 560131 effectively inhibits AR signaling in the tissues, confirm its mechanism of action as postulated, profile the tumor cell intracellular pathways and understand mechanisms of resistance.

- Submission of tissue samples for correlative studies is mandatory at baseline (archival/fresh biopsy).

- Submission of tissue samples for correlative studies is optional at 2 cycles post therapy and at progression.

- Whole blood samples collected at C1D1 pre-dose will be banked for possible future analysis including to identify potential molecular biomarkers that may be indicative of clinical response/resistance. Refer to Lab Manual.

- Blood samples will be collected pre and post dose of IONIS-AR-2.5$_{Rx}$ for pharmacokinetic analysis for the first two cycles only. Plasma PK samples may also be used for profiling of drug binding proteins, bioanalytical method development and/or validation purposes, stability assessments, metabolite assessments, immunogenicity assessments or to assess other actions of IONIS-AR-2.5$_{Rx}$ with plasma constituents. Refer to Lab Manual.

10.1 Sample Collection Guidelines (Refer to Lab Manual)

Tumor Tissue

Tumor tissue should be processed and shipped to the Michigan Center for Translational Pathology (below). From each patient, tissue specimens will be collected at time points indicated in the Study Calendar. Generally, tissue specimens will be collected from:

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

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

Archived Prostate Cancer Samples:

Occasionally, castration resistant prostate cancer tissue may have been obtained previously for the subject and archived. This tissue may be utilized for baseline tissue purposes.

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

- If biopsy specimens are available: Three blocks with the highest volume of representative tumor histology OR 15 unstained, unbaked sections (4-5 microns in thickness) on glass slides (charged or uncharged) from up to three blocks should be shipped at room temperature to the University of Michigan. If unstained sections are sent, they should be labeled with the block number/letter. If less than three blocks have cancer, then all blocks with cancer OR as many unstained sections as can be cut from blocks with cancer without exhaustion should be sent.

Whole blood and PK Plasma IONIS-AR-2.5$_{Rx}$ Samples:

Whole blood and PK plasma samples should be processed and shipped according to the Lab Manual.

11.0 STATISTICAL CONSIDERATIONS

This is a single dose-finding one-arm, phase Ib/II trial to (i) estimate the MTD from among three dose levels of IONIS-AR-2.5$_{Rx}$ in combination with a fixed dose of enzalutamide, as described below, and (ii) obtain preliminary estimate of efficacy at this estimated MTD, as measured by PSA response rate. The MTD is defined as the highest dose of IONIS-AR-2.5$_{Rx}$ inducing a rate of DLT closest to but not larger than 35%, and we will assess the evidence that the PSA response rate at

this dose level exceeds 5%. The planned enrollment is 35 evaluable patients. Success for the trial is defined as finding a dose level that is likely to be both tolerable and effective.

There are two novel elements relative to standard Phase Ib/II designs. First, statistical-model-based between-patient dose modifications of IONIS-AR-2.5$_{Rx}$ accounting for cumulative toxicity outcomes will be implemented during the entire trial. Second, instead of using only patients at the MTD to determine the PSA response rate, a monotonic model of PSA response using all response evaluable patients will be used to estimate the PSA response rate at the MTD. The only formal statistical mechanism for stopping the trial early is due to exceeding toxicity (safety) thresholds.

11.1 Study Design

Dose Assignment in Phase 1b*:* The first patient will be enrolled to dose level 3 of IONIS-AR-2.5$_{Rx}$, in combination with a fixed dose of enzalutamide. Subsequent dose assignments to dose levels 1, 2 or 3 will be based upon the statistical-model-based TITE-CRM design. This trial will seek to estimate the MTD, the true value of which is defined as the dose level with probability of DLT closest to but not exceeding 35%.

Final Efficacy Analysis After all 35 evaluable patients are enrolled and the PSA response endpoint is recorded for all patients, we will fit the Bayesian isotonic regression model on all patients' data. If the probability that the PSA response rate at the final estimated dose exceeds 5% is at least 0.95, the trial will have successfully completed its primary objective.

Interim Analysis/Criteria for Stopping Study Continuous monitoring of safety is built into the TITE-CRM model, and the trial will stop at any point in which all dose levels have a model-estimated probability of DLT exceeding 37.5%.

11.2 Endpoints

11.2.1 Primary Endpoints

The primary endpoints are (i) dose-limiting toxicity (DLT) during the first cycle of IONIS-AR-2.5$_{Rx}$ (in combination with enzalutamide) and (ii) PSA response (PSA decline of 50% or greater from baseline) at any time after the start of combination therapy.

11.2.2 Definition of Secondary Endpoints

The secondary endpoints of efficacy are radiographic progression-free survival time (rPFS) by PCWG3-modified RECIST 1.1, 30% PSA reduction from baseline, PSA progression-free survival time, duration of therapy (DOT), duration of PSA Response (DOR), progression-free survival time and overall survival time.

The secondary endpoints for safety and toxicity are adverse events as assessed by CTCAE criteria 5.0 and classified by body system, grade, and attribution. Other toxicity endpoints include intrapatient dose delays and dose reductions.

11.2.3 Exploratory/Correlative Endpoints

Androgen receptor driven signaling measures in tumor tissues, measures of genomic, metabolomics, and proteomic biomarkers in blood and tissues, FACT-P and EuroQoL-5D scores with comparisons to baseline scores, and pharmacokinetic (PK) characteristics will be the exploratory and correlative endpoints.

11.3 Sample Size and Accrual

We will enroll a total of up to 35 toxicity evaluable patients, depending on if the trial stops early for safety (toxicity). Justification for this design and sample size is based upon 250 simulated trials for each of 28 true data-generating scenarios (seven sets of dose-DLT probabilities crossed with four sets of dose-efficacy probabilities).

The results of the simulation study are given in Tables 1A-1C. The true probabilities of DLT for the three dose levels of IONIS-AR-2.5$_{Rx}$ are given in the rows. The true probabilities of PSA response for the three dose levels of IONIS-AR-2.5$_{Rx}$ are given in the columns.

Table 1A gives the probability of making the correct MTD determination. The true MTD is always defined as the dose level closest to but not exceeding 35% by more than 2.5% in the simulations. From Table 1A, we can see that this probability is high (>0.85) when the true MTD is dose level 3 or >67% when all dose levels are unsafe and lower when the true DLT probabilities are closer to each other or when dose level 1 is close to being too toxic.

Table 1B give the simulation-based probabilities of declaring success, i.e. finding a dose level that statistically appears to be both safe and sufficiently efficacious. These probabilities approximately represent the 'type I error' and 'power' of our design. These probabilities are low (<0.45) in the left-hand columns (non-efficacious scenarios) and/or bottom rows (unsafe scenarios). Conversely, they are high (>0.70) in the top-right cells (efficacious, safe scenarios).

Finally, Table 1C gives the 25th and 75th percentiles for total enrollment. Because the trial will only stop for safety (and not futility), expected enrollment decreases only in the unsafe scenarios. The trial will generally stop after 9 - 35 patients when no dose levels are safe.

Table 1A: Simulation-based probability of correctly identifying the true MTD (highlighted in **bold** in the left column**)**, regardless of efficacy. The correct determination in the last row is to stop for toxicity, because no dose levels have true rate of DLT less than 35%.

	True PSA Response Probabilities (when DLT is observed)			
True DLT probabilities	{3%,8%,10%}	{5%,13%,20%}	{5%,10%,25%}	{20%,28%,35%}
{8%,13%,**25%**}	0.97	0.97	0.97	0.97
{10%,20%,**30%**}	0.86	0.87	0.82	0.84
{14%,21%,**35%**}	0.72	0.72	0.73	0.72
{20%,**27%**,40%}	0.46	0.45	0.43	0.45
{25%,**33%**,45%}	0.48	0.46	0.46	0.46
{**30%**,45%,55%}	0.46	0.45	0.42	0.40
{40%,50%,70%}	0.67	0.66	0.66	0.71

Table 1B: Simulation-based probability of 'declaring success', i.e. finding any dose level that is estimated to be safe AND efficacious.

	True PSA Response Probabilities (when DLT is observed)			
True DLT probabilities	{3%,8%,10%}	{5%,13%,20%}	{5%,10%,25%}	{20%,28%,35%}
{8%,13%,25%}	0.47	0.92	0.94	0.99
{10%,20%,30%}	0.35	0.84	0.84	0.97
{14%,21%,35%}	0.37	0.74	0.73	0.92
{20%,27%,40%}	0.17	0.54	0.48	0.86
{25%,33%,45%}	0.14	0.37	0.32	0.69
{30%,45%,55%}	0.04	0.07	0.05	0.46
{40%,50%,70%}	0.008	0.04	0.01	0.15

Table 1C: Simulation-based interquartile range of number of patients enrolled over all simulated trials.

	True PSA Response Probabilities (when DLT is observed)			
True DLT probabilities	{10%,12%,15%}	{12%,16%,23%}	{16%,26%,30%}	{10%,13%,33%}
{8%,13%,25%}	(35, 35)	(35, 35)	(35, 35)	(35, 35)
{10%,17%,30%}	(35, 35)	(35, 35)	(35, 35)	(35, 35)
{14%,21%,35%}	(35, 35)	(35, 35)	(35, 35)	(35, 35)
{20%,27%,42%}	(35, 35)	(35, 35)	(35, 35)	(35, 35)
{25%,38%,46%}	(35, 35)	(35, 35)	(35, 35)	(35, 35)
{33%,46%,60%}	(22, 35)	(23, 35)	(28, 35)	(25, 35)
{38%,50%,70%}	(10, 35)	(9, 35)	(9, 35)	(10, 35)

11.4 Analysis Datasets

All subjects who enroll and receive at least one dose of each treatment in the combination therapy will be used for updating the TITE-CRM model. Patients who withdraw prior to completing their DLT window and without the occurrence of DLT will have their likelihood contribution downweighted as described in the first paragraph of Section 11.6.1 and will be replaced.

All subjects who have completed one or more cycles of treatment or who are removed from protocol due to progression or toxicity will be evaluable in the efficacy analyses.

Population	Definition
Enrolled	This will comprise all subjects who meet the eligibility criteria and are registered onto the study.
Evaluable	This will comprise all subjects who receive at least one cycle of treatment or who are removed from protocol due to progression or toxicity.
Intention-to-treat (ITT)	This will comprise all subjects who meet the eligibility criteria and are registered onto the study irrespective of their compliance to the planned course of treatment. (See ITT principle below*).
Per Protocol Set (Valid Cases, Efficacy Sample, Evaluable Subjects Sample)	This will comprise all subjects who completed at least one cycle of protocol therapy and have a post-baseline PSA drawn.
Safety	This will comprise all subjects who receive any protocol treatment.
Treated	This will comprise all subjects who have been exposed to the planned course of treatment to any extent.

11.5 Assessment of Safety

All subjects who enroll and receive at least one dose of the combination therapy (one dose each of IONIS-AR-2.5$_{Rx}$ and Enzalutamide) will be eligible for DLT assessment. Formal assessment of safety is built into the dose-escalation design. No dose level having a model-estimated probability of DLT exceeding 35% plus a 2.5% margin will ever be assigned, and patient enrollment will stop entirely if dose level 1 ever has a model-estimated probability of DLT exceeding 37.5% after the first 6 patients.

11.6 Data Analysis Plans

11.6.1 Primary Objective Analyses

Dose Assignment with toxicity assessment The first patient will be assigned to dose level 3. For each patient after the first, we will estimate the one-parameter power model, Pr (Tox|dose j) = $(d_j)^{\exp\{b\}}$, where $\{d_1,d_2,d_3\}=\{0.10, 0.20, 0.30\}$ is the pre-specified skeleton (the anticipated probabilities of DLT at each dose level) and the parameter *b* is to be estimated, using its posterior mean from a Bayesian analysis. We will place a Normal $(0, 0.6^2)$ prior on *b*. Each patient will be assigned to dose level j, j=1,2,3, such that the model estimated probability of DLT at that dose level, $(d_j)^{\exp\{b\}}$, is closest to 0.35 but not exceeding 0.375. Any patients who are free of DLT but have only finished *t* days of their first cycle, $t < 42$, will have their likelihood contribution down-weighted by a factor $(t^2/42^2)$. At any point after the first 6 patients, if the model-estimated rate of DLT at dose level 1 exceeds 0.375, the trial will stop for toxicity.

Efficacy Analysis The efficacy analysis will occur in the PSA response evaluable dataset after the patients have their PSA Response endpoint recorded. Let $\{m_1, m_2, m_3\}$ denote the true, unknown probability that a patient exhibits PSA response at the three dose levels of IONIS-AR-2.5$_{Rx}$ (with fixed dose of enzalutamide), respectively. We will use weakly informative, independent Beta prior distributions: m_1~Beta (0.1, 1.9), m_2~Beta (0.3,1.7), and m_3~Beta (0.5,1.5), or the information equivalent of 2 historical patients' worth of data at each dose level, with historical response rates of 0.05, 0.15, and 0.25, respectively. The posterior distributions will also be Beta: add the number of PSA responders at each dose level to the first shape parameter (0.1, 0.3, or 0.5), and add the number of PSA non-responders at each dose level to the second shape parameter (1.9, 1.7, or 1.5). So, for example, if 4 patients received dose level 1, and one of these patients exhibited a PSA response, then posterior Beta distribution for m_1 would be Beta (0.1+1, 1.9+3) = Beta (1.1,4.9). We will then independently sample a large number, e.g., 200,000, of draws from each of these posterior Beta distributions. In order to preserve the isotonic assumption that the potential for response is not decreasing with dose level, we will only keep posterior samples satisfying $m_1 <= m_2 <= m_3$. To estimate the Bayesian probability that the response rate at the MTD exceeds 5%, we will calculate the proportion of isotonic draws at the m_i exceeding 0.05, where dose level i is the estimated MTD. We will calculate the Bayesian probability that the probability of PSA response at the final estimated MTD exceeds 5%. If the proportion of posterior draws at the estimated MTD exceeding 0.05, subject to the isotonic constraint, is at least 0.95, the trial will have successfully completed its primary objective.

11.6.2 Secondary Objective Analyses

The time-to-event secondary endpoints of efficacy including rPFS, time to PSA progression, PSA progression-free survival time, DOT, DOR, PFS and OS will be analyzed using Kaplan-meier methods. Percentiles of each will be reported with associated 95% confidence intervals and a Kaplan-meier plot. The proportion of patients who are able to reach a 30% or greater PSA reduction from baseline will be reported with the associated 95% binomial confidence interval. Each of the above will be reported overall and by dose level during cycle 1.

Safety and toxicity will be described by dose level. Adverse events as assessed by CTCAE criteria 5.0 and classified by body system, grade, and attribution will be reported

using counts and proportions. Intrapatient dose delays and reductions will be described for each starting dose level.

11.6.3 Exploratory/Correlative Analyses

Inhibition of androgen receptor driven signaling in tumor tissues by IONIS-AR-2.5Rx will be reported using descriptive statistics.

To identify molecular (including genomic, metabolic, and/or proteomic) biomarkers in blood and tissues that may be indicative of clinical response/resistance, safety, pharmacodynamic activity and/or the mechanism of action of IONIS-AR-2.5Rx, each biomarker will be described in the overall treated population and compared between PSA response/non-response outcome. If a biomarker is found to be interesting, Kaplan-Meier methods may be used to explore the relationship with secondary endpoints of rPFS and PSA-PFS.

Changes in health-related quality of life assessment from baseline using FACT-P and EuroQoL-5D will be described using descriptive statistics.

Pharmacokinetic (PK) characteristics of IONIS-AR-2.5Rx will be described using summary descriptive statistics and figures.

12.0 DATA MANAGEMENT

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

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

The data submission schedule is as follows:

- At the time of registration
    - Subject entry into EDC
        - Subject Status
        - Demographics
- During study participation

All data should be entered online within 10 business days of data acquisition. *[Information on dose limiting toxicity events must be entered within one business day.]* Information on Serious Adverse Events must be entered within the reporting timeframe specified in Section 8 of the protocol.

13.0 DATA AND SAFETY MONITORING

This study will be monitored in accordance with the NCI approved University of Michigan Rogel Cancer Center Data and Safety Monitoring Plan, with oversight by the Rogel Cancer Center Data and Safety Monitoring Committee (DSMC).\

The Sponsor-Investigator (S-I)/Study Principal Investigator will provide ongoing monitoring of data and patient safety in this trial and conduct regular data review with participating sites. :

The Sponsor-Investigator (S-I)/Study Principal Investigator and/or the Project Manager/Delegate will review data and patient safety issues with participating sites per a defined monthly meeting cadence. Depending on the protocol activity, the meeting cadence may be more frequent. This data review meeting may be achieved via a teleconference or another similar mechanism to discuss matters related to:

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

Participating sites are required to ensure all pertinent data for the review period are available in the database at the time of the discussion.

Participating sites unable to participate in the data review meeting are required to provide written confirmation that their site has reviewed the relevant data and patient safety issues for the review period and their site's data are in alignment with the data reported in the database. Written confirmation is to be provided to the Project Manager/Delegate within the timeline requested to retain compliance with monitoring timelines.

Documentation of the teleconference or alternate mechanism utilized to review items above is to be retained in the Trial Master File.

The Project Manager/Delegate is responsible for collating the data from all participating sites and completing the Protocol Specific Data and Safety Monitoring Report (DSMR) form to document the data review meeting discussion.

The DSMR will be signed by the Sponsor-Investigator (S-I)/Study Principal Investigator or designated Co-Investigator and submitted to the DSMC on a monthly basis for independent review.

14.0 QUALITY ASSURANCE AND AUDITS

The Data and Safety Monitoring Committee can request a 'for cause' quality assurance audit of the trial if the committee identifies a need for a more rigorous evaluation of study-related issues.

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

15.0 CLINICAL MONITORING PROCEDURES

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

This study will be monitored by a representative of the Coordinating Center of the University of Michigan Rogel Cancer Center. Monitoring visits will be made during the conduct of the study and at study close-out.

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

Monitoring of this study will include both 'Centralized Monitoring', the review of source documents at the Coordinating Center, and 'On-site Monitoring', an actual site visit. The first 'Centralized' visit should occur after the first subject enrolled completes the first treatment cycle. The study site will send the de-identified source documents to the Coordinating Center for monitoring. 'Centralized' monitoring may be requested

by the Coordinating Center if an amendment requires changes to the protocol procedures. The site will send in pertinent de-identified source documents, as defined by the Coordinating Center for monitoring.

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

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

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

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

Participating site will also undergo a site close-out upon completion, termination or cancellation of a study to ensure fulfillment of study obligations during the conduct of the study, and that the site Investigator is aware of his/her ongoing responsibilities. In general, a site close-out is conducted during a site visit; however, site close-out can occur without a site visit.

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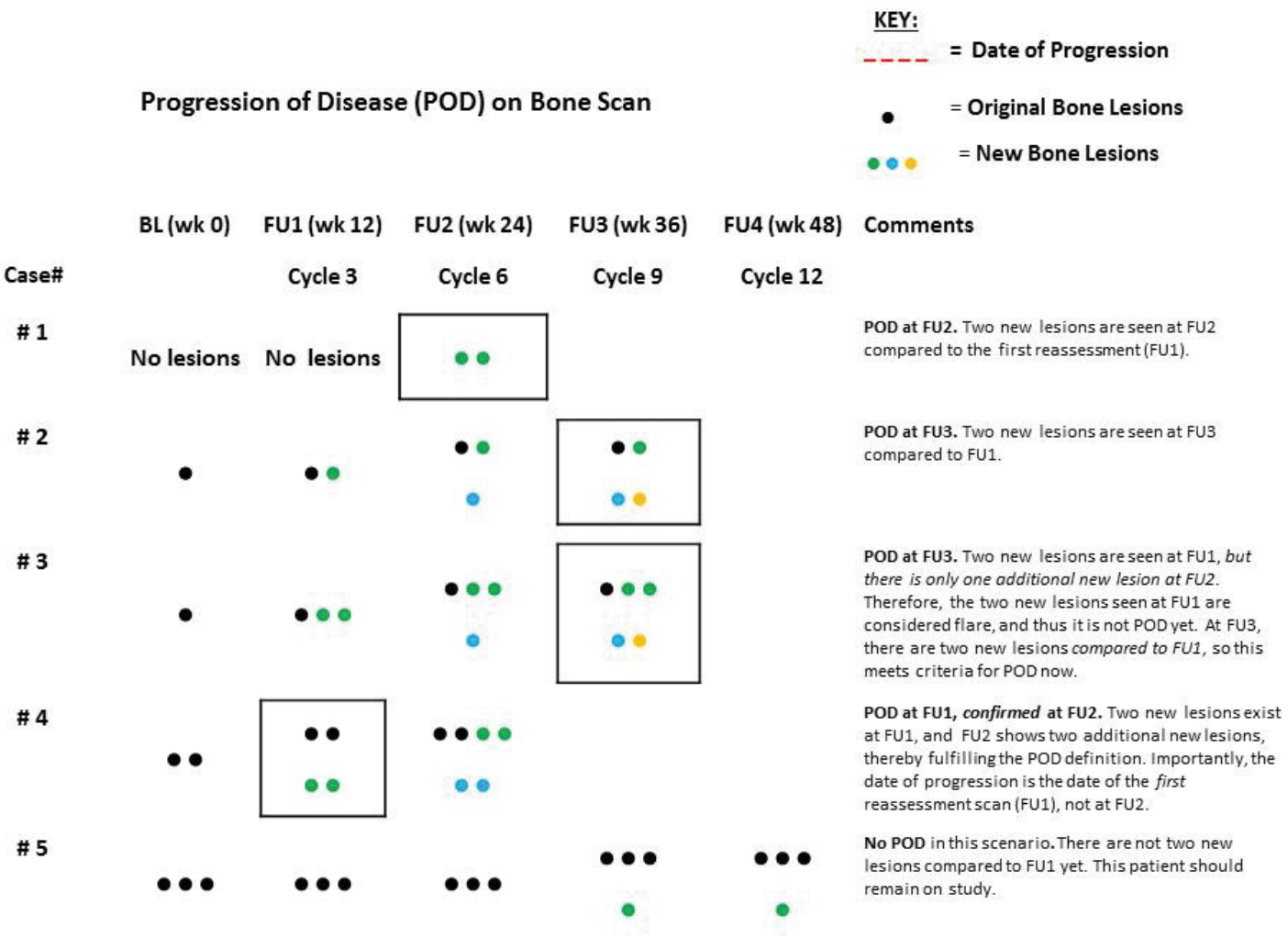
17.0 APPENDICES

APPENDIX A

ECOG Performance Status Scale	
Grade	**Description**
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

Appendix B

Assessment of Progression on Bone Scan



APPENDIX C

Health Questionnaire

English version for the USA

Check the ONE box that best describes your health TODAY.

MOBILITY

I have no problems walking ❑
I have slight problems walking ❑
I have moderate problems walking ❑
I have severe problems walking ❑
I am unable to walk ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

We would like to know how good or bad your health is TODAY.

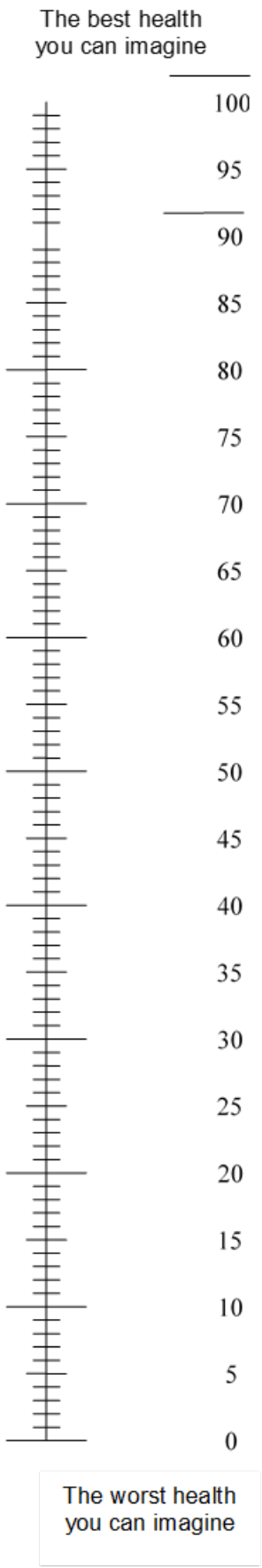
This scale is numbered from 0 to 100.

100 means the best health you can imagine.
0 means the worst health you can imagine.

Mark an X on the scale to indicate how your health is TODAY.

Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



APPENDIX D FACT-P

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

PHYSICAL WELL-BEING		Not at all	A little bit	Some what	Quite a bit	Very much
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

SOCIAL/FAMILY WELL-BEING		Not at all	A little bit	Some what	Quite a bit	Very much
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

EMOTIONAL WELL-BEING		Not at all	A little bit	Some what	Quite a bit	Very much
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

FUNCTIONAL WELL-BEING		Not at all	A little bit	Some what	Quite a bit	Very much
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days.</u>

	ADDITIONAL CONCERNS	Not at all	A little bit	Some what	Quite a bit	Very much
C2	I am losing weight	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4
P1	I have aches and pains that bother me.	0	1	2	3	4
P2	I have certain parts of my body where I experience pain.	0	1	2	3	4
P3	My pain keeps me from doing things I want to do	0	1	2	3	4
P4	I am satisfied with my present comfort level	0	1	2	3	4
P5	I am able to feel like a man	0	1	2	3	4
P6	I have trouble moving my bowels	0	1	2	3	4
P7	I have difficulty urinating	0	1	2	3	4
BL2	I urinate more frequently than usual	0	1	2	3	4
P8	My problems with urinating limit my activities	0	1	2	3	4
BL5	I am able to have and maintain an erection	0	1	2	3	4

APPENDIX E Statistical Appendix to track Amendment changes to the Design and Prior Simulation Results

After 2 patients were treated at Dose 3 and found to have toxicity that the study team felt should require dose modification, an amendment to the trial to modified the DLT definition to include Grade 2 liver transaminase toxicity and expanded the DLT window of toxicity from 1 cycle to include 2 cycles plus labs prior to initiation of cycle 3. These changes required re-estimation of the trial characteristics. Below are the trial characteristics with a toxicity window of 1 cycle (21 days). The current statistical section in 11.7 includes trial characteristics based upon sumulations with a toxicity window of 2 cycles plus labs prior to initiation of cycle 3 (42 days).

Sample Size and Accrual

We will enroll a total of up to 35 toxicity evaluable patients, depending on if the trial stops early for safety (toxicity). Justification for this design and sample size is based upon 250 simulated trials for each of 28 true data-generating scenarios (seven sets of dose-DLT probabilities crossed with four sets of dose-efficacy probabilities).

The results of the simulation study are given in Tables 1A-1C. The true probabilities of DLT for the three dose levels of IONIS-AR-2.5$_{Rx}$ are given in the rows. The true probabilities of PSA response for the three dose levels of IONIS-AR-2.5$_{Rx}$ are given in the columns.

Table 1A gives the probability of making the correct MTD determination. The true MTD is always defined as the dose level closest to but not exceeding 35% by more than 2.5% in the simulations. From Table 1A, we can see that this probability is high (>0.85) when the true MTD is dose level 3 or >67% when all dose levels are unsafe and lower when the true DLT probabilities are closer to each other or when dose level 1 is close to being too toxic.

Table 1B give the simulation-based probabilities of declaring success, i.e. finding a dose level that statistically appears to be both safe and sufficiently efficacious. These probabilities approximately represent the 'type I error' and 'power' of our design. These probabilities are low (<0.45) in the left-hand columns (non-efficacious scenarios) and/or bottom rows (unsafe scenarios). Conversely, they are high (>0.70) in the top-right cells (efficacious, safe scenarios).

Finally, Table 1C gives the 25th and 75th percentiles for total enrollment. Because the trial will only stop for safety (and not futility), expected enrollment decreases only in the unsafe scenarios. The trial will generally stop after 9 - 35 patients when no dose levels are safe.

Table 1A: Simulation-based probability of correctly identifying the true MTD (highlighted in **bold** in the left column**)**, regardless of efficacy. The correct determination in the last row is to stop for toxicity, because no dose levels have true rate of DLT less than 35%.

	True PSA Response Probabilities (when DLT is observed)			
True DLT probabilities	{3%,8%,10%}	{5%,13%,20%}	{5%,10%,25%}	{20%,28%,35%}
{8%,13%,**25%**}	0.98	0.98	0.99	0.96
{10%,20%,**30%**}	0.88	0.85	0.87	0.85
{14%,21%,**35%**}	0.70	0.70	0.70	0.70
{20%,**27%**,40%}	0.42	0.45	0.41	0.46
{25%,**33%**,45%}	0.46	0.46	0.44	0.44
{**30%**,45%,55%}	0.45	0.47	0.42	0.40
{40%,50%,70%}	0.67	0.70	0.66	0.68

Table 1B: Simulation-based probability of 'declaring success', i.e. finding any dose level that is estimated to be safe AND efficacious.

	True PSA Response Probabilities (when DLT is observed)			
True DLT probabilities	{3%,8%,10%}	{5%,13%,20%}	{5%,10%,25%}	{20%,28%,35%}
{8%,13%,25%}	0.42	0.90	0.99	0.996
{10%,20%,30%}	0.38	0.85	0.87	0.98
{14%,21%,35%}	0.31	0.72	0.71	0.96

{20%,27%,40%}	0.24	0.59	0.51	0.88
{25%,33%,45%}	0.16	0.34	0.31	0.71
{30%,45%,55%}	0.03	0.10	0.08	0.42
{40%,50%,70%}	0.004	0.02	0.01	0.16

Table 1C: Simulation-based interquartile range of number of patients enrolled over all simulated trials.

	True PSA Response Probabilities (when DLT is observed)			
True DLT probabilities	{10%,12%,15%}	{12%,16%,23%}	{16%,26%,30%}	{10%,13%,33%}
{8%,13%,25%}	(35, 35)	(35, 35)	(35, 35)	(35, 35)
{10%,17%,30%}	(35, 35)	(35, 35)	(35, 35)	(35, 35)
{14%,21%,35%}	(35, 35)	(35, 35)	(35, 35)	(35, 35)
{20%,27%,42%}	(35, 35)	(35, 35)	(35, 35)	(35, 35)
{25%,38%,46%}	(35, 35)	(35, 35)	(35, 35)	(35, 35)
{33%,46%,60%}	(33, 35)	(35, 35)	(34, 35)	(18, 35)
{38%,50%,70%}	(9, 35)	(10, 35)	(10, 35)	(9, 35)

18.0 COVID-19 Addendum

18.1 Purpose

The purpose of this addendum is to address protocol-required items that the COVID-19 pandemic may temporarily affect.

18.2 Study Visit Schedules/Schedule of Events

During the COVID-19 pandemic, and in accordance with regulatory requirements, temporary visit or follow-up visit adjustments may be made at the site level specific to the scenario (e.g., In the event of virtual visits per clinician/subject discretion). For instances where protocol-specified assessments cannot be made and/or data are unable to be collected, the study team is expected to document the specific reason(s) (e.g., identifying the specific limitation imposed by COVID-19 leading to the inability to perform the protocol-specified assessment), and report as appropriate. For reporting to Coordinating Center, this should be documented using the Protocol Deviation form.

Safety Follow-up/EOT visits

Adjustments to the imaging and bone scan window (30 days from last dose of IONIS-AR-2.5RX (+/- 7 business days) are permitted by the Sponsor-Investigator if on schedule visit not possible due to COVID-19.

Follow-Up Visits

Adjustments to the imaging window (every 12 weeks (+/- 7 business days) are permitted by the Sponsor-Investigator if on schedule visit not possible due to COVID-19.

Virtual Clinic Visits

In the event of virtual clinic visits", Vitals will likely not be collected; If subject has items such as blood pressure cuff, thermometer, weight scale, etc. at home, clinical staff may request the subject to use such apparatuses and record values in source documents – noting that home equipment was used for the reading.

18.3 Fresh Tumor Biopsy

During the COVID-19 pandemic, the Post-Cycle 2 and EOT biopsy may be temporarily unable to be processed at the receiving lab due to COVID-19 outages. The recommendation is to not collect if the lab is still unavailable at the time of the next scheduled time point (please consult with the Coordinating Center). If not collecting, this should be documented using the Protocol Deviation form, specifically addressing impact from COVID-19. If the sample is obtained (patient consented to biopsy), please store specimen at -80°C until notification to deliver to the receiving lab is received.

18.4 Blood draws

During the COVID-19 pandemic, the Cycle 2+ PK Draws for D1, D8 and D15 may be temporarily unable to be processed at the receiving lab due to COVID-19 outages. The recommendation is to not collect if the lab is still unavailable at the time of the next scheduled time point (please consult with the Coordinating Center). If not collecting, this should be documented using the Protocol Deviation form, specifically addressing impact from COVID-19.