A Pilot Study of the Combination of 5-azacitidine (5-AZA) and All-trans Retinoic Acid (ATRA) for Prostate Cancer (PCa) With PSA-only Recurrence After Definitive Local Treatment
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A pilot study of the combination of 5-azacitidine (5-AZA) and all-trans retinoic acid (ATRA) for prostate cancer (PCa) with PSA-only recurrence after definitive local treatment

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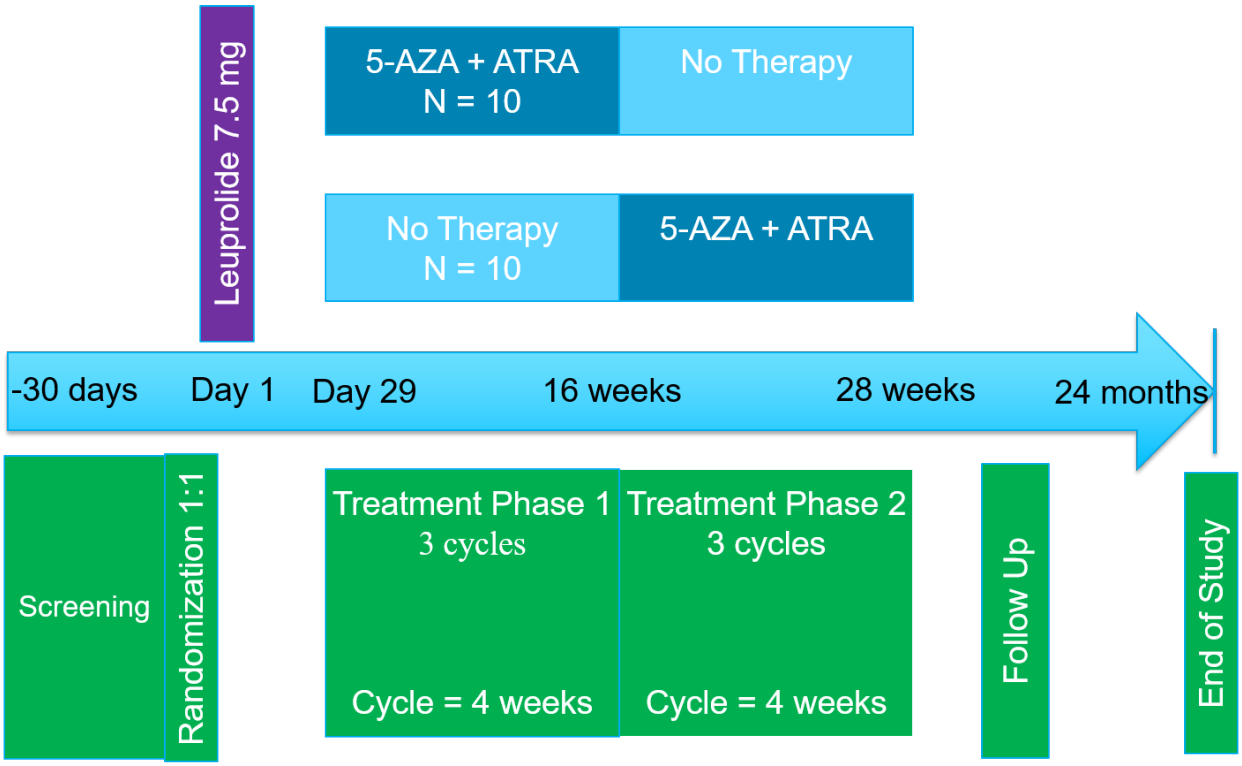
LIST OF ABBREVIATIONS

ADT	Androgen Deprivation Therapy
AE	Adverse Event
ASCO	American Society of Clinical Oncology
AST	Aspartate Aminotransferase
BM	Bone Marrow

CBC	Complete Blood Count
CMP	Comprehensive Metabolic Panel
CR	Complete Response
CT CA/P	Computed Tomography Scan of Chest Abdomen, and Pelvis
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
DSMB	Data and Safety Monitoring Board
DTCs	Disseminated Tumor Cells
EC	Ethics Committee
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator Brochure
IND	Investigational New Drug
IRB	Institutional Review Board
LDH	Lactate Dehydrogenase
mCRPC	Metastatic Castration Resistant Prostate Cancer
MRI	Magnetic Resonance Imaging
NIST	National Institute of Standard and Technology
PFS	Progression Free Survival
PHI	Protected Health Information
PI	Principle Investigator
PR	Partial Response
PSA	Prostate Specific Antigen
PSADT	Prostate Specific Antigen Doubling Time
PCWG2	Prostate Cancer Working Group 2
RECIST	Response Evaluation Criteria in Solid Tumors
rPFS	Radiographic Progression Free Survival
RSI	Reported Safety Information
SAE	Serious Adverse Event
SD	Stable Disease
SRM	Standard Reference Material
TTP	Time to Tumor Progression

STUDY SCHEMA



STUDY SUMMARY

Title	A pilot study of the combination of 5-azacitidine (5-AZA) and all-trans retinoic acid (ATRA) for prostate cancer with PSA-only recurrence
Short Title	Prostate Cancer Dormancy
Protocol Number	
Phase	Pilot, phase 2
Methodology	Randomized, open label
Study Center(s)	Single-center
Objectives	Primary 1) Evaluate disease progression-free rate at the end of Treatment Phase 1 between 5-AZA+ATRA and no-therapy. 2) Assess safety of the 5-AZA and ATRA combination therapy Secondary: 1) Evaluate time to disease progression from start of Treatment Phase 1 2) Correlate antitumor activity and disease progression with biomarkers of dormancy in BM aspirates and peripheral blood
Number of Subjects	20 evaluable patients; the target accrual number is set at 30 to account for screen failures.

Diagnosis and Eligibility Criteria	Diagnosis: Histologically confirmed adenocarcinoma of the prostate Main inclusion criteria: 1) Rising PSA 2) PSADT ≤ 10 months prior to initiation of ADT 2) 3) Received definitive local treatment 4) Indication for ADT 5) No evidence of regional or active distant metastases, except for regional metastases where salvage radiation therapy is not an option Main exclusion criteria: 1) Patients who have received ADT and/or other chemotherapy within 3 months prior to entering the study. 2) Patients who have had radiotherapy or surgery within 4 weeks prior to entering the study. Minimally-invasive procedures for the purpose of diagnosis or staging of the disease are permitted.
Study Product(s), Dose, Route, Regimen	5-azacitidine: 40 mg/m^2 SQ on days 1-5 All-trans retinoic acid: 45 mg/m^2 PO daily (divided into 2 doses) on days 3-7 One cycle of treatment: 28 days
Duration of administration	Drugs will be administered on a 28-day cycle
Reference therapy	No reference therapy
Statistical Methodology	We will compare the disease progression-free rate at end of treatment phase 1 and time to disease progression from start of Treatment Phase 1 between 5-AZA+ATRA and no-therapy. Descriptive summary will be provided for safety endpoints and dormancy marker panel. The effect of biomarker abundance on recurrence free survival will be determined using Kaplan-Meier curves stratified across biomarker abundance groups.
Estimated enrollment period	12
Estimated study duration	24 months

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1. BACKGROUND AND RATIONALE

1.1 Disease Background

Prostate cancer (PCa) causes significant morbidity and mortality in the US, disproportionally affecting African American men. The initial treatment for PCa consists of surgical resection and radiation therapy (RT). While these approaches reduce the primary tumor burden, the majority of patients have clinical recurrence and eventually die of metastases several years later. One explanation is the presence of disseminated tumor cells (DTCs) that are resistant to chemotherapy and ADT, but maintain the capacity to reconstitute incurable metastatic disease later in life. In prior studies, DTCs have been shown to survive in BM of PCa patients in a dormant or quiescent (G0/G1 arrest) state and evade therapies.[1] DTCs can be detected in the BM before overt disease is detected, suggesting that DTCs may help us understand how dormancy and reactivation to metastasis occurs. The mechanisms responsible for the onset of dormancy and the transition from DTC dormancy to reactivation have been mostly unknown.[2] Thus, a window of opportunity to target quiescent residual DTCs for elimination or maintenance of dormancy is missed. Our team has pioneered the study of cancer dormancy to understand the biology of DTCs. Based on prior literature and our pre-clinical models, we have developed a treatment strategy with available FDA-approved drugs (5-azacitidine and all-trans retinoic acid) to reprogram DTCs into a dormant state and ultimately, prolong time to clinical disease progression.

1.2 Rationale

Our long-term goal is to target the biology of dormant PCa DTCs to prevent relapse of clinical disease. In pre-clinical models, specific micro-environmental cues have been shown to activate a transcription factor (TF) network, which drives epigenetic programs of quiescence and survival in dormant tumor cells.[1, 3-5] Specifically, ATRA could induce a high p38/ERK activity ratio and dormancy in previously proliferative DTCs through TGFβ2 signaling in the BM and through upregulation of BMP7.[6] The p38 activation also induces TFs DEC2 and NR2F1 to induce a dormant state.[4] Overall, the dormancy TF network serves as a signature to predict longer metastasis-free periods and to identify dormant DTCs recovered from BM of prostate cancer patients. Interestingly, many dormancy genes are epigenetically silenced and can be re-expressed using a short-term pulse of low dose 5-AZA, a DNA methylating agent and DNMT1 inhibitor.[7, 8] When we introduced 5-AZA and ATRA in malignant prostate cancer cell lines for three days, many cells displayed upregulation of dormancy biomarkers and the response was maintained for at least 2 weeks.[1]

Our pre-clinical work led us to design a “reprogramming therapy” combining two available FDA-approved drugs (5-AZA, ATRA) to reprogram proliferative cells into stable induced dormant cancer cells (iDCCs).[1] Prior studies with the drugs of interest were performed in castration-sensitive PCa patients with prior exposure to ADT and with fast PSA doubling time (PSADT).[9-11] We will test if 5-AZA+ATRA extends the progression-free period and delays biochemical and clinical recurrence via induction of PCa cell dormancy. This study will allow us to temporally understand the impact of the reprogramming therapy on the delay in chemical and clinical progression, and to correlate clinical activity with biomarkers of dormancy in DTCs.

Currently, patients who have received definitive local treatment with either RT or surgery undergo serial measurements of PSA to detect early disease recurrence. Such monitoring leads to the identification of men with PSA-only (biochemical) recurrence without evidence of clinical symptoms, or radiographic evidence of recurrent or disseminated disease. Androgen deprivation therapy (ADT) is the standard of care for the initial systemic therapy, however the optimal timing for initiation of ADT remains controversial due to paucity of clinical trial data in this population.[12] Furthermore, ADT causes side effects such as hot flashes, loss of libido, decreased muscle mass, fatigue, and osteoporosis, which can significantly lower the quality of life in otherwise asymptomatic men.

Leuprolide is considered standard of care for prostate cancer with PSA-only recurrence. One dose of Leuprolide has a transient effect of decreasing PSA to negligible levels resulting in a similar baseline for

both groups. Based on historical data, in measuring primary endpoint at 12 weeks, we do not expect Leuprolideto impact disease progression.
To this end, we will conduct a randomized, pilot study in recurrent PCa patients with biochemical recurrence, as defined by rising PSA, of the combination of 5-azacitidine and all-trans retinoic acid to delay disease progression.

1.3 Correlative Studies

There is prior literature that shows that DTCs in head and neck squamous cell carcinoma[4] and PCa models[6] could persist in a dormant state in the BM. Mechanistically, we found that TGFβ2 mRNA was enriched in naïve BM and in PCa DTCs from patients with no evidence of disease for up to 18 years vs. DTCs from advanced metastatic patients.[3, 4] Upregulation of TGFβ2 signaling leads to activation of p38 and its associated quiescence program involving DEC2 and NR2F1.[1] Furthermore, we showed that NR2F1 induces RARb and patients with high levels of NR2F1 and RARb showed longer disease free periods than those with low levels of these transcription factors.[1, 6] We also found that inducible knock down of NR2F1 reactivated DTCs in different organs.[1]

DNA promoter methylation and/or changes in histone-H3 post-translational modifications (PTMs) contribute to NR2F1 silencing proliferative tumor cells and thus, drugs can be used to remodel the epigenome and restore dormancy.[1] ATRA was sufficient to induce NR2F1 and RARb but only transiently, suggesting that other epigenetic changes might be needed to sustain dormancy.[1] Whole genome changes in histone-H3 PTMs occur after low-dose 5-AZA and can be used to rewrite the epigenome for growth suppression.[7, 8] Many aggressive cancer cells including prostate cells treated with 5-AZA+ATRA displayed upregulation of dormancy biomarkers including NR2F1 and RARb mRNAs.[1] We hypothesize that the reprogramming of residual prostate cancer with 5-AZA and ATRA will be a new strategy to prevent or significantly delay time to clinical recurrence compared to current therapies.

2. STUDY OBJECTIVES

To our knowledge, no study has evaluated the combination of 5-AZA and ATRA in recurrent PCa. Our hypothesis is that this combination can reprogram DTCs to activate the dormancy pathway and thus delay disease progression, and can be safely tolerated.

2.1 Primary Objectives

The co-primary objectives of the study are:
- To compare disease progression-free rate at end of Treatment Phase 1 between 5-AZA + ATRA and no-therapy.
- To assess safety of the 5-AZA and ATRA combination therapy

2.2 Secondary Objectives

The secondary objectives are:
- To compare time to disease progression from start of Treatment Phase 1 during the 24-month study period between 5-AZA+ATRA and no-therapy.
- To correlate antitumor activity and progression with biomarkers of dormancy in BM aspirates and peripheral blood.

3. STUDY DESIGN

3.1 General Design

This is a prospective, open-label, randomized, pilot study of reprogramming therapy in patients with recurrent PCa based on rising PSA only. Reprogramming therapy will include 5-azacitidine and all-trans retinoic acid. Expected duration of subject participation is 24 months.

All study will be assigned in a 1:1 randomization using computer random number generator to either the '5-AZA+ATRA' group or the 'no therapy' group. All enrollees will receive Leuprolide 7.5 mg x 1 on Day 1. On Day 29, they will initiate treatment phase 1. During this phase, patients in the '5-AZA + ATRA' group will receive treatment on a 28-day cycle, in the absence of prohibitive toxicities, for 3 cycles. During treatment phase 2, the 'no therapy' group will receive 5-AZA + ATRA on a 28-day cycle, in the absence of prohibitive toxicities, for 3 cycles. The treatment effect will be determined via comparing the intra-individual disease progression outcome at the end of the treatment phase 1, with period effect being separated through randomization.[17] After treatment phase 1 and phase 2, all patients will be followed for a total of 24 months from the start of the study or until the events leading to discontinuation as outlined in Section 7.1 are observed. 5-AZA will be given subcutaneously on days 1-5 at a dose of 40 mg/m^2. ATRA, dosed at 45 mg/m^2 daily (divided into 2 doses), will be taken orally on days 3-7 of each cycle.

3.2 Primary study endpoints

The primary clinical endpoints of this study are the disease progression-free rate at the end of treatment phase 1 and safety of the combination of 5-AZA and ATRA. Disease progression is defined as a composite of PSA or radiographic progression.

3.2.1 PSA Progression

PSA Progression is as defined by PCWG2 criteria.[13] Baseline PSA will be defined as the serum PSA level measured prior to start of treatment.

In patients who have a decline in PSA value from baseline, progression is defined by:
- An increase in PSA by 25% above the nadir, AND
- An increase in PSA by a minimum of 2 ng/ml, or an increase in PSA to the pre-treatment PSA value, AND
- Confirmation by a second PSA at least 3 weeks apart, AND
- There is no objective evidence of disease response.

In patients whose PSA value has not declined from baseline, progression is defined by:
- An increase in PSA by 25% above either the pre-treatment level, or the nadir PSA level (whichever is lowest), AND
- An increase in PSA by a minimum of 2 ng/ml, AND
- Confirmation by a second PSA at least 3 weeks apart, AND
- There is no objective evidence of disease response.

3.2.2 Radiographic Progression

Radiographic progression will be evaluated by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 criteria, with definitions of CR, PR, SD, and PD for target and non-target lesions as defined below. Baseline imaging defined as the CT and bone scan obtained prior to starting treatment. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 6 weeks before the beginning of the treatment.

Lesions are categorized as either measurable or non-measurable based on the criteria below. All measurements should be taken and recorded in metric notation using a ruler or calipers. Tumor lesions

that are situated in a previously irradiated area will not be considered measurable. 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.

Target Lesions

All measurable lesions up to a maximum of 5 lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameters (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize objective tumor response.

Non-Target Lesions

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ or total of all involved organs as well as non-measurable lesions. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

3.2.2.1 Response Criteria for Target Lesions

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

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

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. The sum must also demonstrate an absolute increase of at least 5 mm. The appearance of one or more new lesions is also considered progression.

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.

3.2.2.2 Response Criteria for Non-Target Lesions

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

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 two or more new lesions and/or unequivocal progression of existing non-target lesions.

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

3.2.2.3 Outcomes Based on Radionuclide Bone Scans

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

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

3.2.3 Safety

Safety and tolerability of the combination of 5-AZA and ATRA will be assessed by the recording of adverse events, monitoring of vital signs and physical examinations, safety laboratory evaluations, and 12-lead ECG. Adverse events will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.[14] This will be assessed at baseline examination and every 4 weeks.

3.3 Secondary Study Endpoints

Secondary end points include time to tumor progression from start of treatment phase 1 and correlation of antitumor activity and tumor progression with levels of dormancy biomarkers.

3.3.1 Time to disease progression

Time to disease progression is defined as a composite of either PSA or radiographic progression, whichever occurs first. Please refer to 3.2 for further details.

3.3.2 Dormancy biomarkers

We will measure the dormancy biomarkers TGFβ2, BMP7, BMP4, GAS6, retinoic acid, NR2F1, in BM and/or blood supernatants of our study patients. We will correlate the levels of these biomarkers with clinical response to treatment and disease progression.

4. ELIGIBILITY CRITERIA

4.1 Patient Eligibility

Eligibility waivers are not permitted. Subjects must meet all of the inclusion and must not meet any of the exclusion criteria to be registered to the study. Study treatment may not begin until a subject is registered.

4.1.1 Inclusion Criteria

- Histologically confirmed adenocarcinoma of the prostate
- Rising PSA
- PSADT ≤ 10 months prior to initiation of ADT
- No evidence of regional or active distant metastases, except for regional metastasis where salvage radiation therapy is not an option
- Indication for ADT after receiving definitive local therapy
- Males ≥ 18 years.
- ECOG performance status of ≤ 2
- Men must agree to use a condom and not father a child or donate sperm for the duration of the study and for 90 days after completion of therapy
- Ability to understand and the willingness to sign a written informed consent
- Ability to adhere to the study visit schedule and requirements of the protocol

4.1.2 Exclusion Criteria

- Patients who have received ADT and/or other chemotherapy within 3 months prior to entering the study.
- Patients who have had radiotherapy or surgery within 4 weeks prior to entering the study. Minimally-invasive procedures for the purpose of diagnosis or staging of the disease are permitted.
- Patients may not be receiving any other investigational agents.

- Patients with known brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.
- History of allergic reactions attributed to compounds of similar chemical or biologic composition to 5-AZA and ATRA.
- Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
- Significant active cardiac disease within the previous 6 months
- Inadequate organ and marrow function as defined below:

- leukocytes	≤ 3,000/mcL
- absolute neutrophil count	≤ 1,500/mcL
- platelets	≤ 100,000/mcl
- total bilirubin	above normal institutional limits
- AST(SGOT)/ALT(SPGT)	≥ 2.5 X institutional upper limit of normal
- creatinine	above normal institutional limits

4.2 Pretreatment Evaluation

All patients must sign a written informed consent form before study specific screening procedures are performed. Informed consents may be obtained up to 30 days prior to day 1 of study. Screening procedures to evaluate patient eligibility for the study will be conducted within 30 days prior to day 1. The pretreatment evaluation includes a complete medical history and physical examination, laboratory studies including complete blood count (CBC) with differential, comprehensive metabolic panel (CMP), lactate dehydrogenase (LDH), PSA, testosterone panel, and baseline EKG. A bone scan, and CT chest abdomen/pelvis will also be performed up to 60 days prior to Day 1 of treatment. 18F-Fluciclovine (Axumin) PET/CT may be substituted for pre-treatment CT chest/abdomen/pelvis and bone scan and can be obtained up to 60 days prior to Day 1 of treatment. If the patient meets eligibility, they will return on Day 1 for Leuprolide administration.

5. DRUG INFORMATION

5.1 5-azacitidine

Please see product package insert for complete details.

5.1.1 Availability

5-azacitidine is commercially available

5.1.2 Formulation

5-azacitidine is available as a reconstituted suspension in vials containing 100 mg of 5-azacitidine. Commercial supplies of 5-azacitidine will be used for the study

5.1.3 Storage, preparation, and administration

Azacitidine is associated with a moderate emetic potential; antiemetics are recommended to prevent nausea and vomiting.

SubQ: The manufacturer recommends equally dividing volumes >4 mL into 2 syringes and injecting into 2 separate sites; however, policies for maximum SubQ administration volume may vary by institution; interpatient variations may also apply. Rotate sites for each injection (thigh, abdomen, or upper arm). Administer subsequent injections at least 1 inch from previous injection sites; do not inject into tender, bruised, red, or hard areas. Allow refrigerated suspensions to come to room temperature (up to 30 minutes) prior to administration. Resuspend by inverting the syringe 2 to 3 times and then rolling the syringe between the palms for 30 seconds.

5.1.4 Mechanism of Action

Promotes hypomethylation of DNA to restore normal gene differentiation and proliferation.

5.1.5 Drug dosing rationale

The proposed dosing of 5-AZA is based on the FDA-approved doses for MDS. 5-AZA has been tested in phase II trials of PCa patients. Sonpavde et al[10] conducted a phase II trial in 26 patients with CRPC and PSADT < 3 months. A dose of 40 mg/m^2 SQ on days 1-5 was administered every 28 days. The primary endpoint was prolongation of PSADT by greater than 3 months and was noted in 19 patients (55%). PSA declines were noted in 14 of 36 evaluable patients. Median PSADT was 2.8 months after treatment, compared with 1.5 months prior to starting ($p<0.01$).

5-azacitidine is generally well tolerated, as demonstrated by a phase II trial in chemo-naive castration-resistant metastatic prostate cancer.[10] The toxicities were generally mild and manageable. In those patients with toxicities, the most common were fatigue (41.2%), constipation (41.2%), nausea (35.3%), injection site reaction (32.4%), vomiting (26.5%), anorexia (20.6%), anemia (20.6%), and neutropenia (17.6%). The most common grade 3 toxicities were fatigue (11.8%) and neutropenia (5.9%). There were no grade 4 toxicities. Only 4 out of 36 patients discontinued therapy due to toxicities.

5.1.6 Adverse Events

Incidence rates of adverse events associated with 5-azacitidine are provided in the product package insert. The most common adverse events treatment are listed below.

Cardiovascular: Peripheral edema (7% to 19%), chest pain (16%)

Central nervous system: Fatigue (13% to 36%), rigors (26%), headache (22%), dizziness (19%), anxiety (5% to 13%), depression (12%), malaise (11%), pain (11%), insomnia (9% to 11%)

Dermatologic: Erythema (7% to 17%), pallor (16%), skin lesion (15%), skin rash (10% to 14%), pruritus (12%), diaphoresis (11%)

Endocrine & metabolic: Weight loss (≤16%), pitting edema (15%), hypokalemia (6% to 13%)

Gastrointestinal: Nausea (48% to 71%), vomiting (27% to 54%), constipation (34% to 50%), diarrhea (36%), anorexia (13% to 21%), abdominal pain (11% to 16%), abdominal tenderness (12%)

Hematologic & oncologic: Thrombocytopenia (66% to 70%; grades 3/4: 58%), anemia (51% to 70%; grades 3/4: 14%), neutropenia (32% to 66%; grades 3/4: 61%), leukopenia (18% to 48%; grades 3/4: 15%), bruise (19% to 31%), petechia (11% to 24%), febrile neutropenia (14% to 16%; grades 3/4: 13%), bone marrow depression (nadir: days 10 to 17; recovery: days 28 to 31)

Local: Injection site reactions (14% to 29%): Erythema (35% to 43%; more common with IV administration), pain (19% to 23%; more common with IV administration), bruising (5% to 14%)

Neuromuscular & skeletal: Weakness (29%), arthralgia (22%), limb pain (20%), back pain (19%), myalgia (16%)

Respiratory: Cough (11% to 30%), dyspnea (5% to 29%), pharyngitis (20%), epistaxis (16%), nasopharyngitis (15%), upper respiratory infection (9% to 13%), pneumonia (11%), rales (9% to 11%)

Miscellaneous: Fever (30% to 52%)

5.2 All-trans retinoic acid

Please see product package insert for complete details.

5.2.1 Availability

ATRA is commercially available

5.2.2 Formulation

ATRA is available as an oral capsule of 10 mg.

5.2.3 Storage, preparation, and administration

Dosage calculations will be rounded to the nearest 10 mg increment and given in two divided doses per day.

ATRA will be taken orally with meals.

5.2.4 Mechanism of Action

Promotes hypomethylation of DNA to restore normal gene differentiation and proliferation.

5.2.5 Drug dosing rationale

The proposed dosing of ATRA is based on the FDA-approved doses for acute promyelocytic leukemia. ATRA has been studied in several phase II trials. Trump et al[11] gave ATRA 50 mg/m^3 daily x 14 days every 3 weeks to 17 CRPC patients and saw no clinical responses. However, Kelly et al[9] showed in a trial of ATRA 45 mg/m^2 daily combined with cis-retinoic acid + IFN that 4 patients with hormone-sensitive PCa had stabilization in PSA kinetics.

ATRA has been studied in multiple phase II trials of PCa patients.[9, 11] In both studies, the toxicities were mild and manageable. In the study by Trump et al., only one patient required a 50% dose reduction due to intolerable headaches occurring on day 1 of therapy. Most patients reported xeroderma, cheilosis, and mild conjunctivitis. Two men developed painful balanitis controlled with emollients. Six patients had headaches; two had mild and four had moderate headaches. No other important toxicities were noted. In the study by Kelly et al., only one patient required dose reduction, which was for fatigue. Xeroderma and chelitis were common adverse events, which were successfully treated with skin emollients. Other mild toxicities (grade 1 or 2) included elevated transaminases (79%), headaches (57%), constipation (50%), and nausea (29%). The most common hematologic toxicities were mild anemia and thrombocytopenia except for two patients, who had grade 3 anemia.

5.2.6 Adverse Events

Incidence rates of adverse events associated with ATRA are provided in the product package insert. The most common adverse events are listed below.

Cardiovascular: Peripheral edema (52%), chest discomfort (32%), edema (29%), cardiac arrhythmia (23%), flushing (23%), hypotension (14%), hypertension (11%), localized phlebitis (11%)

Central nervous system: Headache (86%), malaise (66%), shivering (63%), pain (37%), dizziness (20%), anxiety (17%), paresthesia (17%), depression (14%), insomnia (14%), confusion (11%)

Dermatologic: Xeroderma (≤77%), skin rash (54%), diaphoresis (20%), pruritus (20%), alopecia (14%), skin changes (14%)

Endocrine & metabolic: Hypercholesterolemia (≤60%), hypertriglyceridemia (≤60%), weight gain (23%), weight loss (17%)

Gastrointestinal: Dry mucous membranes (≤77%), nausea (≤57%), vomiting (≤57%), gastrointestinal hemorrhage (34%), abdominal pain (31%), mucositis (26%), diarrhea (23%), anorexia (17%), constipation (17%), dyspepsia (14%), abdominal distention (11%)

Hematologic & oncologic: Hemorrhage (60%), leukocytosis (40%), disseminated intravascular coagulation (26%)

Hepatic: Increased liver enzymes (50% to 60%)

Infection: Infection (58%)

Neuromuscular & skeletal: Ostealgia (77%), APL differentiation syndrome (≤25%), myalgia (14%)

Ophthalmic: Eye disease (17%), visual disturbance (17%)

Otic: Otalgia (23%; ear fullness)

Renal: Renal insufficiency (11%)

Respiratory: Upper respiratory complaint (63%), dyspnea (60%), respiratory insufficiency (26%), pleural effusion (20%), pneumonia (14%), rales (14%), wheezing (expiratory: 14%)

Miscellaneous: Fever (83%)

5.2.7 Drug-drug interactions

ATRA is a major CYPC28 substrate:

CYP2C8 Inducers (Strong): May increase the metabolism of CYP2C8 Substrates (High risk with Inducers).
Management: Consider an alternative for one of the interacting drugs. Some combinations may be specifically contraindicated. Consult appropriate manufacturer labeling.

CYP2C8 Inhibitors (Moderate): May decrease the metabolism of CYP2C8 Substrates (High risk with Inhibitors).
Management: Monitor during therapy.

CYP2C8 Inhibitors (Strong): May decrease the metabolism of CYP2C8 Substrates (High risk with Inhibitors).

Management: Consider an alternative for one of the interacting drugs. Some combinations may be specifically contraindicated. Consult appropriate manufacturer labeling.

Table 1. Therapeutic agents: Dosing and administration schedule

Agent	Dose	Route	Schedule	Cycle Length
5-AZA	40 mg/m^2	SQ	Days 1-5 x 12 weeks	4 weeks (28 days)
ATRA	45 mg/m^2 daily (divided into 2 doses)	PO	Days 3-7 x 12 weeks	

Note: Height and weight will be obtained at Day 1 of each treatment cycle to determine treatment dosing.

The daily dosing for ATRA will be rounded to the nearest 10 mg increment and given in two divided doses per day.

Table 2. Administration schedule per cycle

	Cycle							
Drug	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8-28**
5-AZA	X	X	X	X	X			
ATRA			X	X	X	X	X	

The treatment schedule maybe adjusted for up to 7 days if any scheduling conflicts related to patient availability and/or holidays.

6. ADVERSE EVENTS

An adverse event or experience is defined as any symptom, sign, illness, or untoward experience (including a clinically significant laboratory finding classified as grade ≥ 3 by the National Cancer Institute's Common Terminology Criteria for Adverse Events [CTCAE][14] that develops or worsens during the study, whether the event is considered related to study drug, and should be recorded only after the first dose of study drug is taken. Serious adverse events are recorded from the time the informed consent form is signed.

6.1 Definitions

Serious adverse event (SAE): any untoward medical occurrence that at any dose:

- Results in death,
- Is life threatening, (Note: the term "life-threatening" refers to an event/reaction in which the patient was at risk of death at the time of the event/reaction; it does not refer to an event/ reaction which hypothetically might have caused death if it were more severe),
- Requires inpatient hospitalization or results in prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity,
- Is a congenital anomaly/birth defect, or
- Is a medically important event or reaction. Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious, such as important medical events that might not be immediately life-threatening or result in death or hospitalization, but might

jeopardize the patient or might require intervention to prevent one of the other outcomes listed in the definition above.

Related Adverse Event, i.e. Adverse Drug Reaction (ADR): There is a reasonable possibility per the IST/ISS sponsor that the product may have caused the event.

Unexpected Adverse Drug Reaction: An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product). An expected ADR with a fatal outcome should be considered unexpected unless the local/regional product labeling specifically states that the ADR might be associated with a fatal outcome.

6.2 Management of Study Drug Events and Drug Dosing Modifications

Any patient who receives treatment on this protocol will be evaluated for toxicity. Each patient will be assessed for the development of toxicity. Toxicity will be assessed per the NCI Common Toxicity Criteria for Adverse Events (CTCAE), version 4.03.[14] Dose adjustments should be made according to the system showing the greatest degree of toxicity. Concurrent participation in another clinical trial or treatment with any other anti-cancer therapy is not permitted. The Investigator may prescribe any other concomitant medications as deemed necessary.

The proposed dosing of 5-AZA is based on the FDA-approved doses for MDS. 5-azacitidine is generally well tolerated, as demonstrated by a phase II trial in chemo-naive castration-resistant metastatic prostate cancer.[10] The toxicities were generally mild and manageable and only 4 out of 36 patients discontinued therapy due to toxicities. ATRA has been studied in multiple phase II trials of PCa patients.[9, 11] In both studies, the toxicities were mild and manageable. Only one patient required dose reduction in each trial.

Sequential boundaries will be used to monitor the toxicity rate.[14] In this trial, we conservatively plan that the accrual will be halted if there is sufficient evidence that the toxicity rate exceeds the acceptable rate of 10% by more than 15% (unacceptable toxicity rate of 25%). The discrete toxicity boundary values are reported in following Table 4. The cumulative number of patients experiencing the toxicities described in Table 6 and 7 will be compared with Pocock's stopping boundary values and if greater than the associated boundary value, then the trial will stop early. This toxicity monitoring plan controls the overall type I error rate of 10%, i.e., probability of stopping trial early under acceptable toxicity rate of 10%, and 80% power, i.e., probability of stopping trial early under unacceptable toxicity rate of 25%.

Table 4: Progressive toxicity boundary rules for cumulative number of patients experiencing toxicity.

Looks	5	10	15	20
Boundary	1	2	3	4

If among the 20 patients, 2 or more of the first 5, 3 or more of the first 10, 4 or more of the first 15, or 5 or more of the first 20 patients experience recurrent grade 3 or 4 hematological or non-hematological toxicities defined in Table 6 and 7 or any toxicity deemed unacceptable by the patient or investigator during the 12-week treatment window and up to 4 weeks after treatment window, we will stop the trial early.

The statistical operating characteristics of the above toxicity monitoring rule are as follows:

Table 5: Probability of stopping early based on the true toxicity rate.

Toxicity probability	10%	13%	16%	19%	22%	25%

Early stopping probability	14%	24%	36%	48%	60%	71%

6.2.1 Hematologic Toxicities

Table 6. Hematologic toxicity dose reductions for 5-AZA

Grade	ANC[a]	Platelets	Hemoglobin	Action
1	<LLN to 1,500/μL	<LLN to 75,000/μL	< LLN to 10 g/dL	None
2	1000-1499/μL	50,000 to 74,999/μL	8 – 10 g/dL	None
3	500-999/μL	25,000 to 49,999/μL	< 8 g/dL	-*1st Occurrence:* Hold current dose until ANC ≥ 1,000/μL, platelets ≥ 50,000/μL, and hemoglobin ≥ 8 g/dL. Do not replace missed doses. Restart next treatment at **30 mg/m²** dose. -*2nd Occurrence*: Discontinue protocol therapy. -If therapy held > 3 weeks: Discontinue protocol therapy.
4	<500/μL	<25,000/μL	Life-threatening consequences; urgent intervention indicated	-*1st Occurrence:* Hold current dose until ANC ≥ 1,000/μL, platelets ≥ 50,000/μL, and hemoglobin ≥ 8 g/dL. Do not replace missed doses. Restart next treatment at **30 mg/m²** dose**.** -*2nd Occurrence*: Discontinue protocol therapy. -If therapy held > 3 weeks: Discontinue protocol therapy.
	[a]Note: G-CSF (Filgrastim) 5 mcg/kg/day may be added for low ANC on day of treatment *BEFORE* a dose reduction is instituted at treating physician's discretion. This will be continued until anticipated nadir has passed and ANC ≥ 1000. Neulasta® is NOT allowed.			

6.2.2 Non-hematologic toxicities

5-AZA will be continued at the original dose if the adverse event is grade 0-2. For grade 3-4 toxicities related to study drug, 5-AZA will be held until improvement of symptoms to grade 2 or below. After the first episode, the dose will be reduced by 25%. After the second episode, the protocol therapy will be discontinued. The investigator will determine whether the toxicity is related to study drug. The investigator may prescribe any concomitant medications as necessary, except for other anti-cancer therapy, for the management of adverse effects.

ATRA will be continued at the original dose if the adverse event is grade 0-2. For grade 3-4 toxicities related to study drug, ATRA will be held until improvement of symptoms to grade 2 or below. After the first episode,

the dose will be reduced by 25%. After the second episode, the protocol therapy will be discontinued. The investigator will determine whether the toxicity is related to study drug. The investigator may prescribe any concomitant medications as necessary, except for other anti-cancer therapy, for the management of adverse effects.

Table 7. Non-hematologic toxicity dose reductions for 5-AZA and ATRA

NCI CTC Grade	5-AZA	ATRA
0-2	No change from original starting dose	No change from original starting dose
3 -4	Hold until resolved to ≤ Grade 2, then reduce to **30 mg/m²**	Hold until resolved to ≤ Grade 2, then reduce to **34 mg/m²**
Second episode of grade 3 or 4 toxicity	Remove subject from trial	Remove subject from trial

The administration of 5-AZA will be reported in a log book by the clinical trial staff**.** All patients will receive a study medication diary to log medication administration and adverse events for both 5-AZA and ATRA (See Appendix A).

7. FOLLOW UP, LABORATORY TESTING, AND DIAGNOSTIC IMAGING

Assessments performed exclusively to determine eligibility for this study will be done only after obtaining informed consent. All screening procedures must be performed within 30 days prior to registration unless otherwise stated in section 4.2.

Baseline:
Assessments performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values even if the studies were done before informed consent was obtained as outlined in section 4.2.

Treatment Phase(s):
Patients will be evaluated on day 1 of each cycle for assessment of adverse events and laboratory values. Peripheral blood will also be collected at day 1 of study prior to Leuprolide administration and day 1 of each cycle to perform dormancy signature assays. Patients will have an option to undergo BM biopsy to obtain aspirate at the end of the study period for dormancy signature assays. A restaging bone scan and CT A/P will be performed after every 3 cycles.

Follow Up Period:
Patients will be assessed with physical examination, AE monitoring, and laboratory values. CT abdomen/pelvis and bone scan assessments will be performed as deemed appropriate by the clinical investigator. Patients will continue with follow up for total of 24 months from day 1 of study, unless there is evidence of radiographic progression and/or initiation of other cancer-directed therapies.

Table 8. Assessment schedule

Procedures	Treatment Phase(s)	Follow Up

Physical Examination	Q4 weeks ± 7 days	Q12 weeks ± 7 days
Vital Signs/Weight	Q4 weeks ± 7 days	Q12 weeks ± 7 days
CBC with differential	Q4 weeks ± 7 days[++]	Q12 weeks ± 7 days
CMP	Q4 weeks ± 7 days	Q12 weeks ± 7 days
PSA	Q4 weeks ± 7 days	Q12 weeks ± 7 days
Testosterone	Q4 weeks ± 7 days	Q12 weeks ± 7 days
LDH	Q4 weeks ± 7 days	Q12 weeks ± 7 days
AE Monitoring	Q4 weeks ± 7 days	Q12 weeks ± 7 days
CT A/P	Q12 weeks ± 7 days	as per clinical investigator
Bone Scan	Q12 weeks ± 7 days	as per clinical investigator
Blood samples for dormancy biomarkers*^	Q4 weeks ± 7 days	Q12 weeks ± 7 days

[++]In order to evaluate for cytopenias while on 5-AZA, patients must obtain CBC with differential on Day 5 and Day 12 (+/- 3 days) while on treatment arm for all three cycles. Patients have the option to obtain the CBC at their nearest local laboratory facility and then send results to clinical trial investigators.

* 10 CC of whole blood should be placed in one green top (heparinized) tube following the timeline in the protocol. Blood should be collected in addition to bone marrow (optional). Label the tube with the patient's ID#, the date of collection, diagnosis, relapse (Y/N), and the source of the material (i.e. blood). The tubes should be kept on ice and handed over immediately to Aguirre-Ghiso Lab for processing.

^ Optional - 10 CC of bone marrow aspirate will be collected by Bobby Liaw/co-investigators at the end of 24-month study period. It should be placed in one green top (heparinized) tube. Same procedures as above.

7.1 Duration of Therapy

Treatment may continue for the 3-cycle treatment phase or until:

- Inter-current illness that prevents further administration of treatment
- Unacceptable adverse event(s) of the study regimen despite appropriate dose reduction and best supportive management
- Patient decides to withdraw from the study
- Noncompliance with study regimen or follow up appointments
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

The principal investigator will be notified and a case report form will be completed including the reason for study removal and the date the patient was removed. The patient should be followed up per protocol.

8. STATISTICAL CONSIDERATIONS

8.1 Study Design/Study Endpoints

The primary endpoints will be the disease progression-free rate at the end of the treatment phase 1 and safety of the combination of 5-AZA and ATRA. Disease progression is defined by composite of PSA or radiographic progression relative to period baseline (start of treatment phase 1), whichever occurs first. PSA levels will be measured on day 1 of each cycle. For assessment of PSA progression, see section 3.2.1 above. CT A/P and bone scan will be performed every 3 cycles. For assessment of radiographic progression, see section 3.2.2 above. Safety will be assessed by the recording of adverse events, monitoring of vital signs and physical examinations, safety laboratory evaluations, and 12-lead ECG. Adverse events will be graded per the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.[14] Therapy will be continued at the original dose if the adverse event is grade 0-2. For grade 3-4 toxicities, therapy will be held until improvement of symptoms to grade 2 or below. The dose will then be reduced by 25% of original for each episode. A maximum of 2 dosage reductions will be allowed. The third occurrence will result in removal of the patient from the study.

The secondary endpoints will be time to tumor progression from start of treatment phase 1 and the measurement of dormancy biomarker levels in relation to disease states: tumor progression and disease progression. To determine the relation between dormancy biomarkers and disease states, every 3 cycles, we will measure the dormancy biomarkers, TGFβ2, BMP7, BMP4, GAS6, retinoic acid, NR2F1, in BM aspirates and peripheral blood.

8.2 Sample Size and Accrual

The primary endpoint is disease progression free rate at the end of treatment phase 1. Disease progression is defined as either PSA progression or radiographic progression relative to baseline. The baseline value is defined as the PSA level at the beginning of treatment phase 1 (Day 29 of study). The previous Phase II studies on 5-AZA and ATRA individually demonstrated their efficacy in delaying PSA rise among prostate cancer patients. Preclinical in-vivo study demonstrated that in adjuvant setting following primary tumor surgery, animals treated with the drug combination for 4 weeks have 73% reduction on the median cell count of disseminated tumor cells in lung comparing to control animals (Personal communications between Dr. Aguirre-Ghiso and Dr. Jia).

Thus, we assume the difference in progression free rates at end of the treatment periods between drug combination and no-therapy ranges between 30% to 60% and listed various scenarios of progression-free rates in Table 1. We use McNemar test statistic to calculate the range of sample size required to differentiate the treatment from no-therapy under each scenario.[15, 18] The maximum sample size is required when the PSA rise is independent in the two treatment periods for the same patient. The minimum sample size is required when the PSA progression status in the two treatment periods is highly concordant. With the planned 20 patients in total, we would have >80% power to detect the difference of PSA progression rate of 20% vs 60%, with two-sided type I error of 10%. All calculation was done using PASS software.

Table 1: Sample size using McNemer test statistic, with 80% power and 10% two-sided type I error rate.

Sample size	P1=0.6	P2=0.7	P2=0.8
P1=0.2	14-19	8-10	10-13
P1=0.25	16-26	12-16	9-11
P1=0.3	19-35	14-20	10-13

With accrual rate of 2 patients per month, we expect to finish enrollment in 10 months. Patients who did not finish 8 weeks of drug combination treatment will be replaced but will included for toxicity stopping rule.

8.3 Randomization Schema

Since prior PSA doubling time is a known prognostic factor to disease progression, the randomization will be stratified on PSA doubling time: PSADT<=3 months vs PSADT>3 months. A stratified block randomization of block size 1 will be used[19, 20] and separate randomization lists are generated in different strata (See appendix). The enrollment will continue until total number of 20 evaluable patients is reached. The target accrual number is set at 30 to account for screen failures.The maximum imbalance in each stratum is 1 and maximum total imbalance across strata is 2.

8.4 Data analyses

Analysis on efficacy and safety:

Patients will receive at least 8 weeks of treatment in order to be eligible for efficacy analysis. Patients who discontinued due to toxicity after 8 weeks of treatment will be included in efficacy analysis. Descriptive statistics such as mean/median/range and frequency will be provided for continuous and categorical variables separately, and the distributions of period baseline variables are contrasted between 5-AZA+ATRA and placebo using Wilcoxon statistics and Fisher's exact test statistics for continuous and categorical variables separately. Waterfall plot will be provided for PSA change relative to period baseline for all patients for each treatment arm and period. Analysis will follow the two-step procedure prescribed in[17] for randomized crossover study[16] for binary endpoint. To test for carryover effect, Wilcoxon rank-sum test stratified by prior PSADT will be performed to assess whether the sum of outcome variables from both periods is different between the randomization arms. If the test yields insignificant results, a similar test will be performed to compare the within-patient outcome difference between period 1 and 2 between the randomization arms to assess the treatment effect. If the carryover effect is significant, the primary analysis will be based on the progression free rates at the end of first treatment period using Chi-square test. In addition, general linear mixed model will be applied to assess the treatment effect, carryover effect, and period effect, adjusting for the stratification factor prior PSADT. The time to disease progression is defined as time to either PSA progression or radiographic progression whichever occurs first since discontinuation of ADT. Time to event endpoints will be summarized using Kaplan-Meier method. The time to event endpoints will be estimated using rank preserving failure time model (RPFTM) to obtain the counter-factual event times assuming cross-over did not occur (R package "rpfstm").[21 20]

Descriptive summary will be provided for the safety endpoints. All patients who received at least one dose of study drug (treated set) will be included in the safety analysis.

Correlative analysis and biomarkers:

The micro-environmental biomarkers and dormancy biomarkers collected in this study will be described with summary statistics (mean, median, standard deviation, range) at each time point, and compared between the 5-AZA+ATRA and placebo using the Wilcoxon rank sum test statistics. Distribution of biomarkers will be examined and linear mixed model with random effects will be applied to analyze the biomarker trajectories over time. The biomarkers will be correlated with disease progression outcomes. Biomarker analysis will be performed on all patients with at least one biomarker measure at one time point (biomarker set).

9. DATA HANDING AND RECORD KEEPING

9.1 Electronic Data Capture (EDC) System

All eCRFs will be entered directly into a web-based electronic research application portal known as eRAP. eRAP's role-based access control security and audit capability ensures that the research data is protected from unauthorized access, modification, and exposure. Key features of this web-based database system include allowing access and data entry from multiple sites, with each site having a separate pool of data as necessary. All data stored in the eRAP system is backed up daily. Detailed audit services include any data field level changes, who made the changes, when the changes were made, the old value and the new value. Data from eRAP can easily be extracted to excel or flat text file formats for easy import into SAS or other statistical software packages.

9.2 Verification of EDC System

Once the database has been set up, the PI will test the system. The testing will include confirmation of the proper functioning of valid value checks, subject ID generation, derived variable computations, data extracts, and system reports.

9.3 Entering data

Since electronic CRFs will be used, data entry will be conducted onsite by clinicians who have been granted appropriate access to do so.

9.4 Data Validation Process

As part of the data validation process, edit check programs will be embedded in the database thru valid-value, valid-range and missing value alerts specified for each field as necessary. Valid-value edit checks include:

- Requiring the user to enter a coded value taken from a list that is presented on the CRF. If a value not on the list is entered the user will be prompted to check the value and enter a value on the list.
- Requiring the user to enter a value within a particular range of possible values for a continuous measure. If a value outside the range is entered the user will be prompted to check the value and re-enter.
    - In addition to the univariate field alerts specified above, there will be multivariate alerts built into the database design:
- Confirming that only valid options are selected in a 'choose all that apply' multiple choice field, where the range of options deemed valid depends on some other parameter.
- Confirming that diastolic blood pressure reading is less than the associated systolic blood pressure reading.
- Confirming that "other, specify" is completed when "other" is selected.
- Confirming that eligibility criteria are met.
- As well as cross-module alerts such as:
    - Comparing the dates and times of all assessment time points to confirm that they occur in an appropriate sequence.

9.5 Data Cleaning and Discrepancy Management

Any discrepancies identified through this process will be highlighted and corrected in the system.

9.6 Data Security

The EDC database is approved by Mount Sinai IT Security and HIPAA and operates using high-end servers located in the Secured Mount Sinai Data Center. All data stored in the eRAP system is backed up daily. Access to the database is controlled by policies requiring the PI to authorize user and user roles. Quick view audit service allows authorized user to see who accessed records for view or edit and detailed audit services include seeing any data field level changes, who made the changes, when the changes were made, as well as the value before and after the change.

9.7 Quality Control Procedures

Comprehensive edit checks will be used to clean data. Patient data will be entered continuously. All changes to the data and the database structure will be recorded in an automatic audit trail. Random checks will be done by the PI to ensure data accuracy and completeness. A final database will be declared when all data has been entered, the data entry verified, the data validated and the database defined as clean. After declaration of a final database the data will be exported from eRAP to excel for import to SAS and both the database and the SAS datasets will be locked and protected from changes. All statistical analyses for the final analysis will be performed on the locked SAS datasets.

9.8 Medical and Adverse Event Coding

Coding of Adverse Events occurring during the study will be performed by the Clinical Research Manager according to the revised CTCAE v4.0 while coding of concomitant medications, prior anti-cancer therapy and further therapy will be performed according to the World Health Organization-Drug Dictionary Enhanced (WHO-DDE). These dictionaries contain the respective classifications of adverse events and drugs in proper classes. When dictionary entries and verbatim terms (either for adverse events for medications) do not directly match, the PI will review the entry as well as other related information such as comment fields to identify the most appropriate match. In the event there are compound verbatim events (either AEs or medications) listed in the CRF, such events must be split into more than one record for purposes of medical coding. The PI will be responsible for splitting the compound events into separate entries.

9.9 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For

subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.10 Records Retention

The Investigator must retain drug disposition records (if applicable), source documents, and case histories designed to record all observations and other data pertinent to the investigation (e.g. case report form) for the maximum period required by applicable regulations and guidelines, or Institution procedures.
If a change in the PI occurs, the records shall be transferred to a mutually agreed upon designee (e.g., another Investigator, IRB).

9.11 Subject Privacy

Patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. Following this discussion, they will be asked to sign the HIPAA form and informed consent documents. The original signed document will become part of the patient's medical records, and each patient will receive a copy of the signed document. The use and disclosure of protected health information will be limited to the individuals described in the informed consent document.

It is the responsibility of the research staff to ensure that protocol subjects received, understands, and signs the informed consent document before enrolling the patient onto this trial. Personnel must provide a HIPAA form and obtain acknowledgment before the subject participates in this study.
All subject data will be identified by a subject identification number and subject initials only, to protect the subject's privacy. The data will be blinded accordingly in all data analysis. However, in compliance with federal guidelines, the investigator will permit a representative from Mount Sinai Health System audit committee to review that portion of the subject's medical record that is directly related to the study. This will include all relevant study documentation including medical histories to verify eligibility, laboratory test results to verify transcription accuracy, X-ray reports, admission/discharge summaries for hospital/outpatient admissions while the subject is on-study and autopsy reports for deaths occurring during the study. As part of the required content of informed consent, the subject will be informed that his medical record may be reviewed. Should access to the medical record require a separate waiver or authorization, it is the PI's responsibility to obtain such permission from the patient in writing before the subject is entered into the study.

10. ETHICAL CONSIDERATIONS

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See

Attachment D for a copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

11. FINANCIAL CONSIDERATIONS

See attached clinical trial and correlative study budget spreadsheets (appendix B). Funding was obtained from the Jimmy V Foundation – Translational Research Award Grant. Drug funding for 5-AZA and ATRA will be obtained from the Tisch Cancer Institute Grant.

12. REFERENCES

1. Sosa MS, Parikh F, Maia AG, et al. NR2F1 controls tumour cell dormancy via SOX9- and RARbeta-driven quiescence programmes. Nat Commun. 2015;6: 6170.
2. Klein CA. Framework models of tumor dormancy from patient-derived observations. Curr Opin Genet Dev. 2011;21: 42-49.
3. Chery L, Lam HM, Coleman I, et al. Characterization of single disseminated prostate cancer cells reveals tumor cell heterogeneity and identifies dormancy associated pathways. Oncotarget. 2014;5: 9939-9951.
4. Bragado P, Estrada Y, Parikh F, et al. TGF-beta2 dictates disseminated tumour cell fate in target organs through TGF-beta-RIII and p38alpha/beta signalling. Nat Cell Biol. 2013;15: 1351-1361.
5. Aguirre-Ghiso JA. Models, mechanisms and clinical evidence for cancer dormancy. Nat Rev Cancer. 2007;7: 834-846.
6. Kobayashi A, Okuda H, Xing F, et al. Bone morphogenetic protein 7 in dormancy and metastasis of prostate cancer stem-like cells in bone. J Exp Med. 2011;208: 2641-2655.
7. Tsai HC, Li H, Van Neste L, et al. Transient low doses of DNA-demethylating agents exert durable antitumor effects on hematological and epithelial tumor cells. Cancer Cell. 2012;21: 430-446.
8. Komashko VM, Farnham PJ. 5-azacytidine treatment reorganizes genomic histone modification patterns. Epigenetics. 2010;5: 229-240.
9. Kelly WK, Osman I, Reuter VE, et al. The development of biologic end points in patients treated with differentiation agents: an experience of retinoids in prostate cancer. Clin Cancer Res. 2000;6: 838-846.
10. Sonpavde G, Aparicio AM, Zhan F, et al. Azacitidine favorably modulates PSA kinetics correlating with plasma DNA LINE-1 hypomethylation in men with chemonaive castration-resistant prostate cancer. Urol Oncol. 2011;29: 682-689.
11. Trump DL, Smith DC, Stiff D, et al. A phase II trial of all-trans-retinoic acid in hormone-refractory prostate cancer: a clinical trial with detailed pharmacokinetic analysis. Cancer Chemother Pharmacol. 1997;39: 349-356.
12. van den Bergh RC, van Casteren NJ, van den Broeck T, et al. Role of Hormonal Treatment in Prostate Cancer Patients with Nonmetastatic Disease Recurrence After Local Curative Treatment: A Systematic Review. Eur Urol. 2016;69: 802-820.
13. Scher HI, Halabi S, Tannock I, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. J Clin Oncol. 2008;26: 1148-1159.
14. Modified from Common Terminology Criteria for Adverse Events (CTCAE)*. Clinical Gynecologic Oncology: Elsevier, 2012:682.
15. Machin D, Campbell MJ, Tan SB, Tan SH. Sample Size Tables for Clinical Studies. Wiley-Blackwell, 2008.
16. Schröder FH, Roobol MJ, Boevé ER, et al. Randomized, Double-Blind, Placebo-Controlled Crossover Study in Men with Prostate Cancer and Rising PSA: Effectiveness of a Dietary Supplement. European Urology. 2005;48: 922-931.

17. Wellek S, Blettner M. On the proper use of the crossover design in clinical trials: part 18 of a series on evaluation of scientific publications. Dtsch Arztebl Int. 2012;109: 276-281.
18. Schork PDMA, Williams PDGW. Number of observations required for the comparison of two correlated proportions. Communications in Statistics - Simulation and Computation. 1980;9: 349-357.
19. randomization.com. Available from URL: www.randomization.com.
20. Simon LJ, Chinchilli VM. A matched crossover design for clinical trials. Contemporary Clinical Trials. 2007;28: 638-646.
21. Wu Y, Zhao L, Hou Y, Li K, Zhou X. Correcting for non-compliance in randomized non-inferiority trials with active and placebo control using structural models. Stat Med. 2015;34: 950-965.