Title: Systemic and tumor-directed therapy for oligometastatic prostate cancer

Technical Title: A Single-Arm, Open-Label, Phase II Study of Systemic and Tumor Directed Therapy for Newly Diagnosed Oligometastatic M1 Prostate Cancer

Sponsor:
VA West Los Angeles Medical Center

Principal Investigator:

Nicholas G. Nickols, M.D., Ph.D.
Radiation Oncology
VA West Los Angeles Medical Center

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STUDY TEAM ROSTER

Principal Investigator:
Nicholas Nickols, M.D., Ph.D.

VA West Los Angeles Medical Center
Radiation Oncology
11301 Wilshire Blvd
Los Angeles, CA 90073
Ph: 310-268-3390

UCLA Radiation Oncology
BOX 951714, B3-109 CHS
Los Angeles, CA 90095-1714
Ph: 310-825-8278
Fax: 310-206-1260

e-mail: nnickols@mednet.ucla.edu; Nicholas.nickols@va.gov

Co-Investigators:

Matthew Rettig, M.D.

VA West Los Angeles Medical Center
Hematology-Oncology
11301 Wilshire Blvd
Los Angeles, CA 90073

UCLA Institute of Urologic Oncology
Department of Urology and Division of Hematology and Oncology
300 Stein Plaza, 3rd Floor
Los Angeles, CA 90095
Ph: 310-794-3565
Fax: 310-794-3512

e-mail: mrettig@mednet.ucla.edu; matthew.rettig@va.gov

Isla P. Garraway, M.D, Ph.D.

VA West Los Angeles Medical Center
Urology
11301 Wilshire Blvd
Los Angeles, CA 90073

UCLA Urology
BOX 951738, 12-145 Factor
Los Angeles, CA 90095-1738
e-mail: IGarraway@mednet.ucla.edu; isla.garraway@va.gov

Steve P. Lee, M.D., Ph.D.

VA Long Beach Healthcare System
Radiation Oncology
5901 E 7th St
Long Beach, CA 90822

UCLA Radiation Oncology
BOX 956951, Ste B265
200 Medical Plaza
Los Angeles, CA 90095-6951
e-mail: splee@mednet.ucla.edu; steve.lee5@va.gov

Mark Jordan, M.D.

VA Long Beach Healthcare System
Urology
5901 E 7th St
Long Beach, CA 90822
Ph: 562-826-5562
Fax: 562-826-8117
e-mail: Mark.Jordan2@va.gov

Michael G. Chang, M.D.

Hunter Holmes McGuire VA Medical Center
Radiation Oncology
1201 Broad Rock Blvd
Richmond, VA 23249
e-mail: michael.chang@vcuhealth.org

Bhaumik B. Patel, M.D.

Hunter Holmes McGuire VA Medical Center
Department of Internal Medicine
Division of Hematology, Oncology & Palliative Care
1201 Broad Rock Blvd.
Richmond, VA 23249
e-mail: bhaumik.patel@vcuhealth.org

PARTICIPATING STUDY SITES

West LA VA, Los Angeles, CA, United States
(Nickols, Rettig, Garraway)

Long Beach VA, Long Beach, CA, United States
(Lee, Jordan)

Hunter Holmes McGuire VA Medical Center, Richmond, VA, United States
(Chang, Patel)

PRÉCIS/SUMMARY

Study Title

Systemic and tumor-directed therapy for oligometastatic prostate cancer

Objectives

Primary Objective:
The primary objective of this study is to assess the efficacy of combined modality therapy of systemic and tumor directed therapy for newly diagnosed (de novo) oligometastatic M1a,b prostate cancer. In this study, androgen deprivation therapy (ADT) is limited to a six-month duration. The primary endpoint of our study is the percent of patients achieving a serum PSA of <0.05 ng/mL six months after recovery of serum testosterone ≥150 ng/dL (i.e., undetectable disease burden off-therapy and after gonadal recovery) if primary tumor therapy is radical prostatectomy, and PSA of < nadir + 2 ng/mL six months after recovery of serum testosterone ≥150 ng/dL if primary tumor therapy is radiation therapy.

Secondary Objectives:
The secondary objectives of the study include a number of clinical endpoints enumerated below:

- Time to biochemical progression.
- Time to radiographic progression
- Time to additional antineoplastic therapy
- Prostate cancer specific survival
- Assessment of health related quality of life using the Functional Assessment of Cancer Therapy - Prostate (FACT-P) scale

Tissue available for optional correlative analyses:
Pre-treatment metastatic biopsies
Radical prostatectomy specimens (prior to systemic or radiotherapy)
Serial blood collections
Metastatic biopsies at radiographic progression (recommended)

Design

This is a single arm Phase II clinical trial in patients with newly diagnosed M1a,b prostate cancer and 1-5 radiographically visible metastases treated with radical prostatectomy (and post-operative fractionated radiotherapy for pT≥3a, pN1, or positive margins) or radiotherapy (including inclusion of the pelvic lymph nodes), metastasis directed SBRT, and complete ADT with LHRH analog leuprolide, abiraterone acetate with prednisone, and apalutamide (ARN-509) for a total of six months of systemic therapy. The primary endpoint of our study is the percent of patients achieving a serum PSA of <0.05 ng/mL six months after recovery of serum testosterone ≥150 ng/dL if primary tumor therapy is radical prostatectomy, and PSA of < nadir + 2 ng/mL six months after recovery of serum testosterone ≥150 ng/dL if primary tumor therapy is radiation therapy. [Based on prior studies, >90% of patients treated with ADT for six months will recover serum testosterone to ≥150 ng/dL within six months after cessation of ADT with a median time to recovery of three months (1,2)] This primary endpoint was selected to offer a rapid and sensitive assessment of treatment efficacy. PSA is a biomarker for disease burden in prostate adenocarcinoma and offers a non-invasive and sensitive assessment of disease control

after treatment in the vast majority of patients. Durable PSA control in the context of recovery of gonadal function (testosterone ≥150 ng/dL) after ADT is therefore a surrogate for disease control. See **Figure 1** for trial schema.

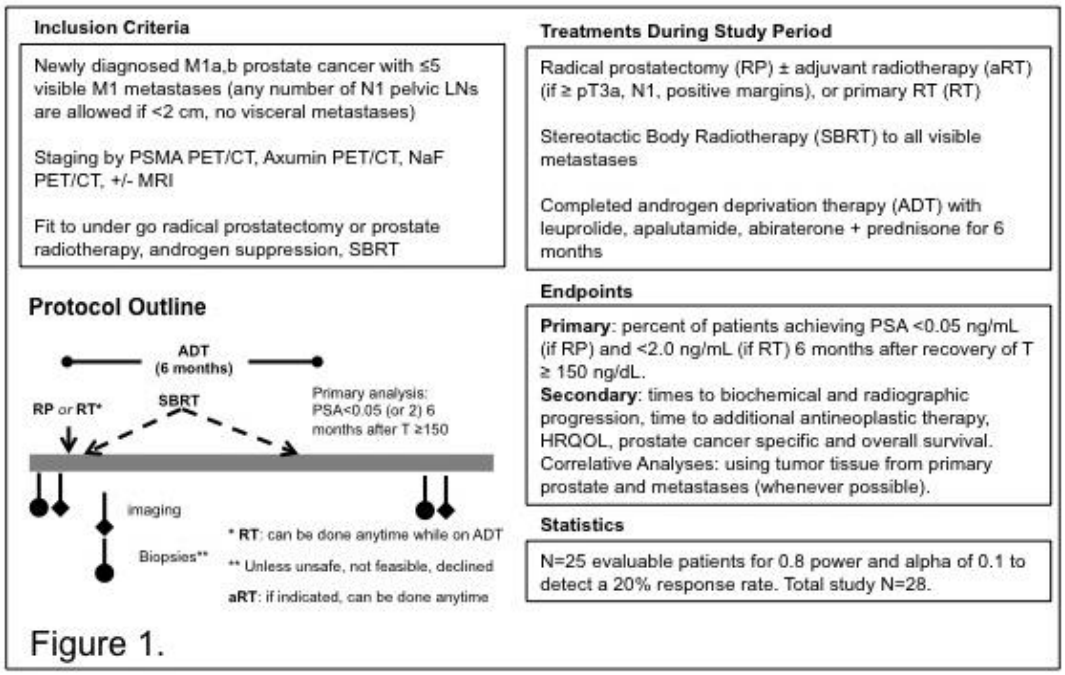

Figure 1.

Currently, metastatic prostate cancer patients are treated with ADT indefinitely, or intermittently. Based on data from prior trials of discontinuous ADT in prostate cancer patients with metastatic disease, essentially all patients will relapse with a rising PSA after discontinuation of ADT. The current study hypothesis is that 20% of patients will achieve a PSA <0.05 ng/mL six months after recovery of testosterone to >150 ng/dL, or PSA of < nadir + 2 ng/mL six months after recovery of serum testosterone ≥150 ng/dL if primary tumor therapy is radiation therapy. N=25 evaluable patients has 80% power to detect a 20% response rate at a significance (alpha) of 0.1. Assuming 10% of patients may not recover testosterone to ≥150 ng/dL or may drop out, we anticipate a total study N=28.

Apalutamide is a next-generation antiandrogen in ongoing trials in locally advanced, castrate resistant, and metastatic prostate cancer (3,4). Apalutamide is FDA approved for the treatment of non-metastatic castration resistant prostate cancer and metastatic castration sensitive prostate cancer. Abiraterone acetate is an androgen synthesis inhibitor approved for metastatic castrate resistant prostate cancer (mCRPC) (5) and is also FDA approved for high risk metastatic castration sensitive prostate cancer. SBRT directed to metastatic foci in prostate cancer achieves local of greater than 95% (9). Radiotherapy directed to the primary tumor is also now a standard of care for patients with oligometastatic castration sensitive prostate cancer (PMID:30355464).

This trial includes a radiographic directed biopsy of a metastatic lesion prior to initiation of therapy unless unsafe to perform, not feasible, or declined by patient. As such, evaluable tissues may include metastatic and primary tumor tissue acquired prior to initiation of hormone or radiotherapy. Additionally, blood is collected for correlative

analyses. A metastatic biopsy at the time of radiographic progression is recommended but not required.

Correlative Objectives, Overview

Background. This trial will yield evaluable tissue from previously untreated prostate cancer patients with metastatic disease. We will identify the primary tumor in each patient that gave rise to metastasis, and compare these potentially lethal primaries with the other tumors that likely did not yield metastases. We will also compare these potentially lethal primary tumors to their corresponding metastases whenever possible. In doing so, we may **(1)** identify characteristic features of potentially lethal primary prostate tumors that are destined for metastasis, and **(2)** identify which pathways become activated within these potentially lethal primary tumors during or after the metastatic event. Importantly, these investigations all use tissue that is acquired at the outset for each patient enrolled in our trial, and these investigations do not depend on the clinical outcome of the trial. See **Figure 2** for overview of the key correlative Aims.

Sources of Tissue. From VA West LA, fresh tissue from the prostatectomy or biopsy will be obtained in addition to FFPE tissue from the prostatectomy specimen or initial prostate biopsy. Fresh prostate tissue obtained at surgery at VA West LA will be shared with Dr Garraway's study VA #0001____. From the other sites, FFPE tissue from the prostatectomy specimen or initial prostate biopsy will be obtained, and fresh tissue will not be obtained due to issues of feasibility.

Correlative Aim 1. What genomic and transcriptomic features are present in lethal intraprostatic tumors that ultimately give rise to metastatic disease that are absent, or different, in those that do not? To answer this question, we aim to conduct whole exome deep sequencing (WES), RNA sequencing (RNA-seq), and comparative genomic hybridizations (CGH) of the metastatic and intraprostatic tumors of the 28 patients enrolled in our Phase II trial. This will enable identification of the "true" primary tumors from which the biopsied metastases arose. Then, we will combine the aforementioned genomic analyses with RNA-seq data to identify discriminating features of the "true" primary lesions that distinguish them from the intraprostatic tumors that did not yield these metastatic lesions.

1.1 To identify which intraprostatic tumor in each patient gave rise to metastasis.

1.2 To discover genomic and transcriptomic features whenever possible specific to the intraprostatic tumors that ultimately gave rise to metastasis versus those that did not.

Correlative Aim 2. What genomic and transcriptomic features are present in the metastases that are absent, or different, from the corresponding primary tumors from which they metastasized? This will involve comparison between the primary tumors and their metastases. We may also specifically interrogate selected markers of kinase activity (MEK/ERK) and differentiation (EMT).

2.1 To discover genomic and transcriptomic features specific to metastatic prostate tumors as compared to the primary tumors from which they arose.

2.2 To evaluate MAPK (ERK) activation and markers of epithelial to mesenchymal transition (EMT) in metastatic prostate cancer lesions as compared to their corresponding primary tumors.

Correlative Aim 3. What genomic and transcriptomic features are present in circulating tumor cells and DNA that predict for response? Are there changes in the immunophenotype of circulating lymphocytes over the course of treatment?

2.1 To evaluate biomarkers of response using circulating tumor cells (CTCs).

2.2 To evaluate biomarkers of response using circulating tumor DNA (ctDNA).

2.3 To evaluate immunophenotypes of circulating immune cells.

Interventions and Duration

Screening
Screening will occur between day -90 and day 0 (day 1 = date of surgery for patients undergoing radical prostatectomy as primary tumor therapy; day 1 = date of initiation of ADT/ +/-3 days apalutamide + abiraterone + prednisone for patients undergoing radiotherapy as primary tumor therapy). Screening evaluation will include a history and physical examination, performance status evaluation, laboratory studies, and biopsy of a metastatic lesion. Staging by NaF or PSMA PET-CT or Fluciclovine (Axumin) PSMA PET-CT (with diagnostic CT of the chest, abdomen/pelvis) is required. If biopsy is not diagnostic, or unsafe to perform, not feasible, or declined, then a secondary imaging modality (for example, MRI) must also be consistent with metastatic disease (unless PSMA PET-CT or Fluciclovine (Axumin) PSMA PET-CT was used for initial staging).

On Study Interventions
Patients who undergo radical prostatectomy as primary tumor therapy will undergo radical prostatectomy prior to initiation of systemic or radiotherapy. After prostatectomy, patients will begin six months of complete ADT with leuprolide, apalutamide, and abiraterone acetate. Patients who undergo radiotherapy as primary tumor therapy can undergo the radiotherapy anytime while on the six months of complete ADT with leuprolide, apalutamide, and abiraterone acetate, provided they will complete the radiotherapy while still on the ADT. All patients will undergo SBRT to all visible sites of metastases, unless the metastases would be included in pelvic RT volumes (i.e., common iliac M1a lymph nodes). The SBRT may be done anytime while the patient is still on ADT. Some patients who undergo prostatectomy will receive post-operative pelvic radiotherapy (for ≥pT3a, N1, positive margins). The total duration of systemic therapy is six months. Patients will be evaluated on treatment every 30 days, and subsequently after systemic therapy is complete every three months thereafter. A research biopsy of a metastatic lesion at the time of radiographic progression will be encouraged but not required.

Sample Size and Population

Study population. Patients to be enrolled will have untreated metastatic prostate cancer with 1-5 metastasis (exclusive of pelvic, N1, metastases) identified by NaF PET-CT or PSMA PET-CT or Fluciclovine (Axumin) PET-CT (with diagnostic CT of the chest,

abdomen, pelvis) that have undergone biopsy of a metastatic lesion.

Sample Size. Currently, metastatic prostate cancer patients are treated with ADT indefinitely or intermittently. Based on data from prior trials of discontinuous ADT in prostate cancer patients with metastatic disease, essentially all patients will relapse with a rising PSA after discontinuation of ADT (33,34). The current study hypothesis is that 20% of patients will achieve a PSA <0.05 ng/mL six months after recovery of testosterone to >150 ng/dL if primary tumor therapy is radical prostatectomy or PSA of < nadir + 2 ng/mL six months after recovery of serum testosterone ≥150 ng/dL if primary tumor therapy is radiation therapy.. A sample size of N=25 evaluable patients has an 80% power to test the null hypothesis response rate of 5.5% against a two sided alternative response rate of 20% at a significance level (alpha) of 0.1. Assuming 10% of patients may not recover testosterone to ≥150 ng/dL or drop out, we anticipate a total study N=28.

List of Abbreviations

AD = androgen dependent
ADT = androgen deprivation therapy
AE = adverse event
ALT = alanine aminotransferase
AR = androgen receptor
AST = aspartate aminotransferase
CBC= complete blood count
CFR= Code of Federal Regulations
CRF = case report form
CRPC = castration resistant prostate cancer (mCRPC, m denotes metastatic)
CT = computed-tomography
CTCAE = Common Terminology Criteria for Adverse Events
ctDNA = circulating tumor DNA
DEXA = dual energy x-ray absorptiometry
DiPSC = Differential Pathway Signature Analysis
DMC = Data Monitoring Committee
ECOG = Eastern Cooperative Oncology Group
EKG = electrocardiogram
EMT = epithelial to mesenchymal transition
ERK = extracellular signal related kinase (p-ERK = phosphorylated form of ERK)
FDA = Food and Drug Administration
FFPE = formalin fixed, paraffin-embedded
GnRH = gonadotropin-releasing hormone
IBC= Institutional biosafety committee
IHC = immunohistochemistry
IND = investigational new drug
INR= international normalized ratio
IRB = institutional review board
ISPRC = the Internal Scientific Peer Review Committee
LDH = lactate dehydrogenase
LFT= liver function test
LHRH = luteinizing hormone releasing hormone
LVEF = left ventricular ejection fraction

MAPK = mitogen activated protein kinase
MEK = mitogen-activated extracellular signal-related kinase
MRI = magnetic resonance imaging
NCI = National Cancer Institute
NIH= National Institute of Health
NYHA = New York Heart Association
OCT = optimal cutting temperature freezing compound
ORC = Office of Regulatory Compliance
ORSP = Office of Research Subject Protection
PARADOGM = Pathway Recognition using Data Integration on Genomic Models
PNBx = prostate needle biopsy
PSA = prostate specific antigen
PI= principal investigator
PI3K = phosphatidylinositol 3-kinase
PT= prothrombin time
PTEN = phosphatase and tensin homolog
RP = radical prostatectomy
RAS = proto-oncoprotein, guanosine-nucleotide-binding protein/GTPase
RECIST = Response Evaluation Criteria in Solid Tumors
SAE = serious adverse event.
SPORE = Specialized Program of Research Excellence (in prostate cancer)
SQ = subcutaneous
SRC = proto-oncoprotein (p-SRC = phosphorylated form of SRC)
SU2C = Stand Up To Cancer
TUNEL = assay of apoptosis
UCLA= University of California, Los Angeles
WCDT = West Coast Dream Team (SU2C WCDT)

1. STUDY OBJECTIVES

1.1 Primary Objective

1.1 The primary objective of this study is to assess the efficacy of combined systemic and tumor directed therapy for newly diagnosed (de novo) oligometastatic M1a,b prostate cancer patients with 1-5 metastases (exclusive of pelvic nodal N1 metastases). The primary endpoint of our study is the percent of patients achieving a serum PSA of <0.05 ng/mL six months after recovery of serum testosterone ≥150 ng/dL if primary tumor therapy is radical prostatectomy or PSA of < nadir + 2 ng/mL six months after recovery of serum testosterone ≥150 ng/dL if primary tumor therapy is radiation therapy. (i.e., undetectable disease burden off-therapy and after gonadal recovery).

1.2 Secondary Objectives

1.3.1 Time to biochemical progression.
1.3.2 Time to additional antineoplastic therapy
1.3.3 Prostate cancer specific survival

1.3.4 Safety and tolerability
1.3.5 Assessment of health related quality of life using the Functional Assessment of Cancer Therapy - Prostate (FACT-P) scale.

1.3 Correlative Objectives

Correlative Aim 1

1.1 Identification of metastasis-generating intraprostatic tumors.
1.2 Determination of differences between metastasis-generating intraprostatic tumors and other intraprostatic tumors.

Correlative Aim 2

2.1 Determination of differences between metastatic and metastasis-generating primary tumors.
2.2 Determination of ERK activation and markers of EMT between metastatic and primary tumors

Correlative Aim 3.

2.1 To evaluate biomarkers of response using circulating tumor cells (CTCs).
2.2 To evaluate biomarkers of response using circulating tumor DNA (ctDNA).
2.3 To evaluate immunophenotypes of circulating immune cells.

2. BACKGROUND AND RATIONALE

2.1 Background on Condition, Disease, or Other Primary Study Focus

Metastatic prostate cancer is incurable and treatment of metastatic prostate cancer is palliative. The current standard of care is ADT (androgen deprivation therapy) (10); however, relapse on ADT is inevitable. Modern cohorts of patients treated with ADT alone have a median time to failure and overall survival of 11 and 42 months, respectively (11). Patients are maintained on ADT continuously or intermittently, until death.

2.2 Study Rationale

New therapeutics that suppress androgen signaling include abiraterone acetate and enzalutamide, which interfere with androgen synthesis and androgen receptor-androgen binding, respectively, are approved for castrate resistant prostate cancer (5,12,13) and these and apalutamide are currently used also for castration sensitive metastatic prostate cancer and under evaluation for high-risk localized prostate cancer in combination with radiotherapy (RT) (4,14). Stereotactic body RT (SBRT) enables safe and accurate delivery of ablative doses of ionizing radiation to metastatic prostate cancer lesions with local control exceeding 95% (9). The addition of definitive local therapy to systemic therapy in high-risk localized prostate cancer improves survival (15,16). Clinical data also support the notion that the addition of local therapy in node positive (17–19) and M1 patients also favorably impact survival (20). The STAMPEDE trial evaluated the role of prostate radiotherapy and lifelong conventional ADT in M1 patients and found improved survival of prostate radiotherapy for oligometastatic M1 patients (21). Osseous metastases account for 90% of all prostate cancer metastases and bones are the first site

of metastatic spread in more than 80% of patients (22–24). The identification of metastatic sites in prostate cancer has greatly improved through use of NaF PET-CT and PSMA PET-CT scans (25,26), with reported sensitivity and specificity >95% (27), superior to that of conventional technetium bone scans and CT scans (27,28).

With this confluence of treatment and diagnostic advances, the question arises if a multimodal approach with aggressive, early treatment of newly diagnosed M1 prostate cancer should be attempted with curative intent.

A striking difference in prostate cancer specific mortality is noted in newly diagnosed M1b patients with 5 or fewer visible metastases (exclusive of pelvic lymph nodes) versus those with more than 5 (**Figure 3**) (unpublished). This retrospective data supports the hypothesis that metastatic prostate cancer patients comprise a heterogeneous group with a divergent clinical course. To at least a first approximation, clinical course appears to be associated with the number of metastases. This analysis led our team to select patients with newly diagnosed metastatic prostate cancer with 5 or fewer visible metastases for inclusion in this prospective Phase II clinical trial.

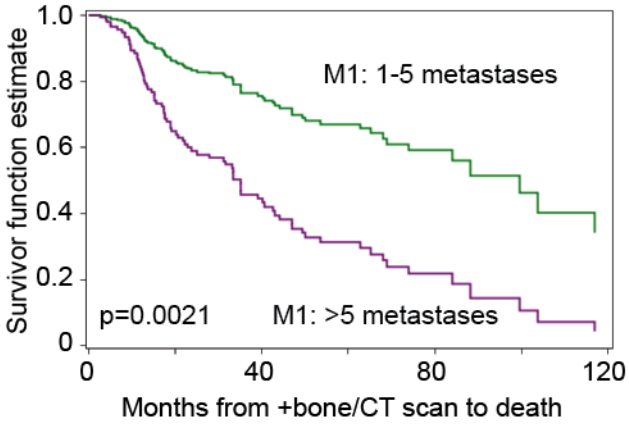

Figure 3. Prostate cancer specific mortality in patients at one of our institutions diagnosed with M1 prostate cancer and with 1-5 sites of metastases (n=23) and >5 (n=79). Patients with 1-5 have significantly better prostate cancer survival after diagnosis.

Both localized and metastatic castrate resistant prostate cancers (mCRPCs) are characterized by frequent copy number alterations, gene fusions, and rearrangements (29,30). Some characteristic differences between primary and mCRPCs have been identified, which include a greater frequency of androgen receptor gene amplification or pathway alteration (50% vs 90-100%), *pten* loss or PI3K/AKT activation (50% vs 90-100%), *tp53* mutations (3-20% vs 50%), and MAP Kinase (ERK) pathway activation (30% vs 90-100%), for primary and mCRPC, respectively (31). Elucidation of the critical pathways associated with emergence of metastases from the primary prostate cancer could identify actionable targets for systemic therapy to be used both early in the disease course and in patients with treatment refractory mCRPC.

Understandably, prior studies assessing metastatic prostate cancer analyzed castrate resistant tumors taken from heavily pre-treated patients on long term ADT (30,32). As such, in these studies the *initial* genomic events driving metastasis may be obscured through subsequent genomic and transcriptomic adaptations occurring within tumors during systemic therapy. Therefore, our study, which incorporates genomic analysis of both primary and metastatic sites pre-treatment, is poised to address the specific question of which gene expression pathways, mutations, and copy number alterations are associated with early metastases, *de novo*, independent of therapeutic pressure.

- **Hypothesis.** The combination of aggressive local, metastases directed, and systemic therapy is an effective treatment for metastatic prostate cancer patients with limited metastatic disease burden. The correlative analyses will identify key

features unique to intraprostatic tumors that metastasize, the proximal drivers of the metastatic event, and biomarkers associated with response to this multimodal treatment strategy.

3. STUDY DESIGN

This is a single arm Phase II clinical trial in patients with newly diagnosed M1a,b prostate cancer and 1-5 radiographically visible metastases treated with primary radical prostatectomy (and post-operative fractionated radiotherapy for pT≥3a, pN1, or positive margins) or primary radiotherapy (and pelvic lymph node radiotherapy), metastasis directed SBRT, and complete ADT with GnRH analog leuprolide, androgen synthesis inhibitor abiraterone acetate, and antiandrogen apalutamide for a total of six months of systemic therapy. The primary endpoint of our study is the percent of patients achieving a serum PSA of <0.05 ng/mL six months after recovery of serum testosterone ≥150 ng/dL for patients treated with primary radical prostatectomy and PSA of < nadir + 2 ng/mL six months after recovery of serum testosterone ≥150 ng/dL for patients treated with primary radiotherapy.

- Type of trial: Phase II, open-label, single-arm.
- Outcomes:
    - **Primary outcome** is a sustained undetectable disease burden after gonadal recovery:
        - The primary endpoint of our study is the percent of patients achieving a serum PSA of <0.05 ng/mL six months after recovery of serum testosterone ≥150 ng/dL for patients treated with primary radical prostatectomy and PSA of < nadir + 2 ng/mL six months after recovery of serum testosterone ≥150 ng/dL for patients treated with primary radiotherapy. [Based on prior studies, >90% of patients treated with ADT for six months will recover serum testosterone to ≥150 ng/dL within six months after cessation of ADT with a median time to recovery of three month (1,2).] This primary endpoint was selected to offer a rapid and sensitive assessment of treatment efficacy. PSA is a biomarker for disease burden in prostate adenocarcinoma and offers a non-invasive and sensitive assessment of disease control after treatment in the vast majority of patients. Durable PSA control in the context of recovery of gonadal function (testosterone ≥150 ng/dL) after ADT is therefore a surrogate for disease control. It is expected that determination of the primary endpoint for each patient will occur between 9 and 12 months after completion of systemic therapy.
    - **Secondary outcomes** are:
        - Time to biochemical progression
        - Time to radiographic progression
        - Time to initiation of alternative antineoplastic therapy
        - Prostate cancer specific survival
        - Assessment of health related quality of life using the Functional Assessment of Cancer Therapy - Prostate (FACT-P) scale
        - Safety and tolerability
    - **Correlative objectives**:
        - Correlative Aim 1

1.1 Identification of the metastasis-generating intraprotatic tumor.
1.2 Determination of differences between metastasis-generating intraprotatic tumors and other intraprostatic tumors

- Correlative Aim 2
  - 2.1 Determination of differences between metastatic and metastasis-generating primary tumors.
  - 2.2 Determination of ERK activation and markers of EMT between metastatic and primary tumors
- Correlative Aim 3
  - 3.1 Analysis of ctDNA for predictors of response
  - 3.2 Analysis of CTC's for predictors of response.
  - 3.3 Analysis of changes in circulating immunophenotypes

- Study population: Patients with metastatic prostate cancer with 1-5 sites of metastases (exclusive of pelvic nodal N1 metastases) that have not undergone prior treatment. Additionally, the patient must have at least 1 metastatic site that has been biopsied (unless deemed unsafe to perform, not feasible, or declined by patient) prior to enrollment on the study. If biopsy is not diagnostic, or unsafe to perform, then a secondary imaging modality (for example, MRI) must also be consistent with metastatic disease (unless PSMA PET-CT or Fluciclovine (Axumin) PET-CT) was used as initial staging).

- Number of patients: Currently, metastatic prostate cancer patients are treated with ADT indefinitely. Based on data from prior trials of discontinuous ADT in prostate cancer patients with metastatic disease, essentially all patients will relapse with a rising PSA after discontinuation of ADT (33,34). The current study hypothesis is that 20% of patients will achieve a PSA <0.05 ng/mL six months after recovery of testosterone to >150 ng/dL. N=25 evaluable patients has 80% power to detect a 20% response rate at a significance (alpha) of 0.1. Assuming 10% of patients may not recover testosterone to ≥150 ng/dL or drop out, we anticipate a total study N=28.

- Study Sites:
  - West LA VA Medical Center (Los Angeles, California, United States of America)
  - Long Beach VA Medical Center (Long Beach, California, United States of America)
  - Hunter Holmes McGuire VA Medical Center (Richmond, Virginia, United States of America)
  - Other VA sites may be added

- Duration of study:
  - Entire study: ~48 months.
  - Individual patient enrollment: ~15-18 months (screening period, active treatment, follow-up to primary endpoint).

- Interventions:
  - Pre-treatment biopsy of a metastatic lesion during the screening period unless not feasible, unsafe, or declined by patient. Biopsy site will be determined by safety. This biopsy, if done, is used both for diagnostic

confirmation of metastatic disease as well as correlative analyses.

- Biopsy of a metastatic lesion at progression. This biopsy is not required. However, it is encouraged. This biopsy is a research biopsy that will provide tissue for correlative analyses.
- Study treatments summary (detailed in **Section 5.1**)

  - **Tumor directed Therapy.**

    - **Primary Tumor therapy**. Patients may undergo either surgery or radiotherapy as primary tumor therapy. The decision is via shared decision making between the investigators and the patient. Either are acceptable.

      - **Surgery**. Patients who undergo surgery as primary tumor treatment will undergo radical prostatectomy (open or robotic assisted) with pelvic node dissection by an experienced urologic surgeon.
        - **Post-operative pelvic RT**. Patients found after surgery to be stage pT3a or higher, have positive margins, or pathologically involved pelvic nodes, will receive post-operative fractionated IMRT delivered to the pelvic nodes and prostate bed. This pelvic RT may extend after completion of ADT.

      - **Primary tumor radiotherapy**. Patients who undergo radiotherapy as primary tumor treatment will undergo radiotherapy to the prostate, seminal vesicles, and pelvic lymph nodes. Allowed dose fractionations include:
        - **Prostate**: 40 Gy in 5 fractions or 60 Gy in 20 fractions or 79.2 Gy in 44 fractions
        - **SVs:** 25-40 Gy in 5 fractions, or 42-43 Gy in 20 fractions, or 45 Gy in 25 fractions.
        - **Pelvic LNs:** 25 Gy in 5 fractions or 42-43 Gy in 20 fractions or 45 Gy in 25 fractions.

        The selection of dose and fractionation will be made at the discretion of the investigator, and typically depends on anatomical considerations and baseline urinary and bowel function. Gross disease may be prescribed a higher dose at the investigator's discretion.

      - **Metastasis Directed Therapy.**

        SBRT. Patients will undergo SBRT to all radiographically visible sites of M1 metastases within two months of initiation of ADT. SBRT offers ablative

dose-escalation to tumor targets with simultaneous dose-restraint to normal tissues, which is not possible using conventionally radiotherapy. SBRT utilizes high-precision radiotherapy delivery machines, integrated image guidance systems, advanced planning software, and a high level of professional and technical expertise.

- **Systemic Therapy**. Complete ADT with GnRH analog leuprolide (6 month depot, 45 mg, once), antiandrogen apalutamide (240 mg PO daily), and abiraterone acetate co-administered with prednisone (1,000 mg and 10 mg PO daily), will begin immediately after surgery for patients who undergo radical prostatectomy for primary tumor therapy and at day 1(+/-3 days) for patients who undergo radiotherapy as primary tumor therapy, and continue for a total of 6 months.

- Pre-treatment assessment of eligibility:
  - **Staging**. To ensure sensitive and accurate staging, patients must undergo NaF PET-CT or PSMA PET-CT or Fluciclovine (Axumin) PET-CT (with fused diagnostic CT of the chest abdomen and pelvis), and have 1-5 radiographically visible metastases (pelvic nodal metastases are not included). At least one site with radiographic suspicion for metastases will be biopsied for both diagnosis and for correlative genomic analyses, unless deemed to be unsafe to perform, or not feasible, or declined by patient, then a secondary imaging modality (for example, MRI) must also be consistent with metastatic disease (unless PET-CT was or Fluciclovine (Axumin) PET-CT used as initial staging). Patients must not have visceral metastases, but may have one or more pelvic lymph nodes involved, up to a maximum diameter of 2 cm. Metastatic sites (exclusive of N1 pelvic nodes) must be amenable to treatment via SBRT.

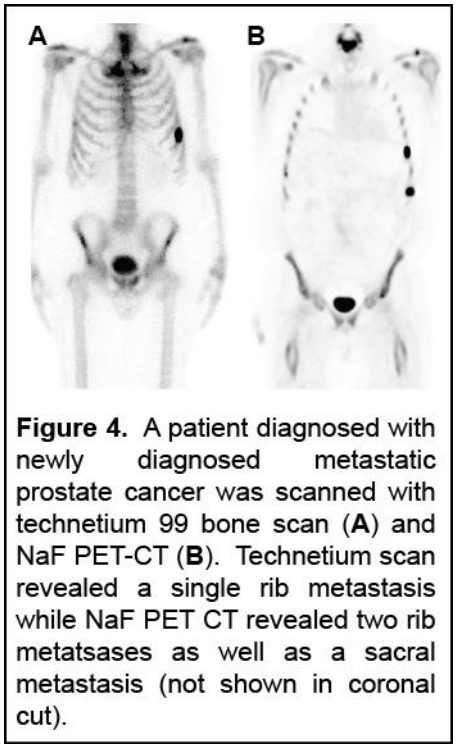

Figure 4. A patient diagnosed with newly diagnosed metastatic prostate cancer was scanned with technetium 99 bone scan (**A**) and NaF PET-CT (**B**). Technetium scan revealed a single rib metastasis while NaF PET CT revealed two rib metatsases as well as a sacral metastasis (not shown in coronal cut).

The reported sensitivity and specificity of NaF PET-CT for detection of osseous metastases in prostate cancer patients both exceed >95% when interpreted by an experienced physician. (The specificity

of this technique derives from thoughtful correlation of PET tracer intensity with underlying sclerotic foci on the fused high resolution CT scan). In contrast, the sensitivity and specificity of technetium 99 bone scans in this population are 70%, 57%, respectively. The value of using NaF PET-CT for staging in this trial is illustrated in **Figure 4**, where a patient with newly diagnosed metastatic prostate cancer underwent both technetium 99 and NaF PET-CT scans prior to treatment. The technetium 99 scan revealed a single metastasis to the left seventh rib, while the NaF PET CT revealed an additional ninth rib metastasis as well as a sacral metastasis. Although not widely available at this time in the United States, we anticipate that PSMA-PET CT will become available over the next few years. Therefore, we also allow for staging by PSMA PET-CT in this trial. In addition, fluciclovine (Axumin) PET/CT was recently FDA approved for prostate cancer. As such, we also allow staging by fluciclovine (Axumin) PET/CT.

- Blood tests to assess for adequate organ function - liver, kidney, bone marrow, coagulation

- Assessment of the PSA and testosterone, total testosterone must be >200 ng/dL prior to ADT. (The optimal time to measure total testosterone is between 8 and 9 am)

- Adequate performance status and labs

- Randomization: This is a non-randomized study.

- Other: All molecular and pathologic analyses germane to the study objectives will be performed under direction of the investigators. This process leverages the established infrastructure enabled by existing collaborative programs.

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

Patient Recruitment

Patients with metastatic prostate cancer that:
i. have not received prior therapy for prostate cancer,
ii. have at least 1 metastatic lesion that has been biopsied (unless deemed to be unsafe, not feasible, or declined by patient) will be considered for this trial.

Patients will be recruited from the urology, medical oncology, and radiation oncology clinics at the participating centers. This clinical trial is listed on the clinicaltrials.gov website.

4.1 Inclusion Criteria

Patients must meet all of the following inclusion criteria to participate in this study.

1. Biopsy confirmed diagnosis of prostate adenocarcinoma (primary small cell carcinoma of the prostate is not allowed, however adenocarcinoma with neuroendocrine differentiation is allowed)

2. Age ≥18

3. Presence of 1-5 visible metastases (by NaF PET-CT or PSMA PET-CT or Fluciclovine [Axumin] PET-CT including diagnostic CT of the chest, abdomen, and pelvis)

    a. At least one metastasis must be M1a-b

    b. Visceral metastases are not allowed

    c. Patients may have any number of pelvic nodal metastases (but largest must be <2 cm in shortest diameter)

    d. Metastases must be amenable to treatment with SBRT

    e. Biopsy of one metastasis must be attempted, unless unsafe or not feasible to perform, or declined by patient. If biopsy is not diagnostic, or unsafe to perform, or declined by patient, then a secondary imaging modality (for example, MRI) must also be consistent with metastatic disease (unless PSMA PET-CT or Fluciclovine [Axumin] PET-CT was used as initial staging).

4. Patient must be fit to undergo radical prostatectomy or prostate radiotherapy, SBRT to all visible sites of metastases, ADT,

5. Total testosterone >200 ng/dL prior to ADT (optimal time to measure total testosterone is between 8 and 9 am)

6. Adequate performance status (ECOG 0-1)

7. Clinical laboratory values at screening:

    a. Hemoglobin ≥9.0 g/dL, independent of transfusion and/or growth factors within 3 months prior to randomization

    b. Platelet count ≥100,000 x 10^9/μL independent of transfusion and/or growth factors within 3 months prior to randomization

    c. Serum albumin ≥3.0 g/dL

    d. GFR ≥45 mL/min

    e. Serum potassium ≥3.5 mmol/L

    f. Serum total bilirubin ≤1.5 × ULN (Note: In subjects with Gilbert's syndrome, if total bilirubin is >1.5 × ULN, measure direct and indirect bilirubin and if direct bilirubin is ≤1.5 × ULN, subject may be eligible)

    g. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) <2.5 × ULN

8. Medications known to lower the seizure threshold (see list under prohibited medications) must be discontinued or substituted at least 4 weeks prior to study entry.

4.2 Exclusion Criteria

All candidates meeting any of the following exclusion criteria will be excluded from participation in the study.

1. Any evidence of spinal cord compression (radiological or clinical)
2. Prior pelvic malignancy
3. Prior pelvic radiation
4. Concurrent malignancy aside from superficial skin cancers or superficial bladder tumors
5. Inability to undergo prostatectomy, radiotherapy, or ADT
6. Primary small cell carcinoma of the prostate (prostate adenocarcinoma with neuroendocrine differentiation is allowed)
7. Inflammatory bowel disease or active collagen vascular disease
8. History of any of the following:
    a. Seizure or known condition that may pre-dispose to seizure (e.g. prior stroke within 1year to randomization, brain arteriovenous malformation, Schwannoma, meningioma, or other benign CNS or meningeal disease which may require treatment with surgery or radiation therapy)
    b. Severe or unstable angina, myocardial infarction, symptomatic congestive heart failure, arterial or venous thromboembolic events (eg, pulmonary embolism, cerebrovascular accident including transient ischemic attacks), or clinically significant ventricular arrhythmias within 6 months prior to randomization
9. Current evidence of any of the following:
    a. Uncontrolled hypertension
    b. Gastrointestinal disorder affecting absorption
    c. Active infection (eg, human immunodeficiency virus [HIV] or viral hepatitis)
    d. Any chronic medical condition requiring a higher dose of corticosteroid than 10 mg prednisone/prednisolone once daily
    e. Any condition that in the opinion of the investigator would preclude participation in this study
    f. Concomitant strong CYP3A4 inducers. (If a strong CYP3A4 inducer must be co-administered, abiraterone acetate dose frequency will be adjusted).

g. Treatment with CYP2D6 substrates that have a narrow therapeutic index. If an alternative treatment cannot be used, a dose reduction of the CYP2D6 substrate may be considered.

h. Baseline severe hepatic impairment (ChildPugh Class B & C)

i. Concomitant CYP2C8 inhibitors with narrow therapeutic index. (If a concomitant CYP2C8 inhibitor with narrow therapeutic index must be co-administered patients should be monitored closely for signs of toxicity related to the CYP2C8 inhibitor with a narrow therapeutic index if used concomitantly with abiraterone acetate)

10. Presence of visceral metastases (i.e., stage M1c)

4.3 Study Enrollment Procedures

The PI and co-PIs will ensure that this study is conducted in full compliance with the FDA standards for human research as specified in 21 CFR 312. The study will not be initiated at a clinical site until the informed consent form has been approved by the clinical site's Institutional Review Board (IRB). All potential revisions of the protocol must be reflected in the informed consent form and reviewed by the IRB & Janssen. Separate informed consent will be obtained for all surgical procedures and biopsies that may be performed as part of the patient's standard medical management.

Patients will be provided complete informed consent that will be sought in the participating clinics by the Study PI's, one of the designated sub-investigators or by appropriately designated investigators at other participating sites. Subjects will have the option of having family and/or anyone else they deem appropriate present during the informed consent process. The PI and/or co-investigators will discuss the study with patients verbally. The patients will be given the informed consent forms and encouraged to take the forms home for careful reading and review at each patient's leisure. After reading the informed consent form, the patients will be encouraged to return to the clinic to meet with the study investigators to ask any and all questions that the patients may have. The investigators will in turn pose questions that verify that the patients understand the nature and risks of the study.

Subjects will be informed that they may refuse participation in or withdraw from this study at any time without prejudice or any negative effect on subsequent care. In addition, subjects will be informed that their health care provider may be one of the investigators on this study and a conflict of interest may therefore exist in that the investigator may be interested in both the patient's welfare and in the conduct of the study.

The patient should agree to use a condom (even men with vasectomies) and another effective method of birth control if he is having sex with a woman of childbearing potential or agrees to use a condom if he is having sex with a woman who is pregnant while on study drug and for 3 months following the last dose of study drugs. Must also agree not to donate sperm during the study and for 3 months after receiving the last dose of study drug.

5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

5.1.1. Study Drugs.

Study groups, medications, and dosing:

This is a single-arm study. All enrolled patients will initiate leuprolide, apalutamide and abiraterone acetate beginning immediately (within 0-3 days) after radical prostatectomy. Patients will receive a single dose of leuprolide, SQ, 45 mg, a six month depot, and start at the recommended dose of apalutamide (ARN-509, 240 mg PO daily), and abiraterone acetate co-administered with prednisone (1,000 mg PO and 10 mg PO daily, respectively), by mouth daily, for a duration of six months. Based on the experience from clinical trials with apalutamide and abiraterone acetate in prostate cancer these are thought the optimal doses and are well tolerated when given on a continuous daily basis. These drugs will be continued for six months. Based on the experience from clinical trials in metastatic prostate cancer this is thought the optimal doses and is tolerated with acceptable toxicity.

- **Leuprolide.** Leuprolide (or another GnRH analog) is the standard of care for patients with M1 prostate cancer, and expected side effects for a six-month course include loss of libido, fatigue, and hot flashes. Leuprolide has been used in multiple previous and ongoing Phase III trials in prostate cancer patients.

- **Apalutamide.** Apalutamide is FDA approved for the treatment of non-metastatic castration resistant prostate cancer and also metastatic castration sensitive prostate cancer. It is thus part of standard of care for these patients. It has been evaluated in several previous and ongoing trials for local and metastatic prostate cancer and is tolerated in combination with ADT, radiotherapy, and surgery. It can be associated with fatigue and rare convulsions.

- **Abiraterone acetate** is also part of standard of care for these patients and is also approved for mCRPC in combination with prednisone, and is well tolerated in combination with ADT, radiotherapy, and surgery. It can be associated with mineralocorticoid excess, hepatotoxicity, osteoporosis, and has some drug-drug interactions through inhibition of CYP2D6. After discontinuation of abiraterone, prednisone will be continued, but tapered. A recommended prednisone taper schedule is to decrease the dose by 1 mg/day every two weeks (see **Appendix 14.4**).

A Material Safety Data Sheet (MSDS) describing the occupational hazards and recommended handling precautions for the study drugs (available from Janssen for apalutamide and abiraterone acetate) *will be provided to site staff if required by local laws or will otherwise be available upon request.*

Toxicity will be attributed to specific study drugs based on judgment of the treating

physician, or study PI or co-investigators. Toxicity will be judged as likely related, possibly related, or unlikely related to the study drug. If likely or possibly related, then dose modifications to the study drug in question will be made. In the situation where an unexpected toxicity is observed but not clearly attributable to a study drug or procedure, then attempts will be made to identify the source of toxicity along with treatment and/or palliation of the toxicity. In this situation, dose modifications may be made at the treating physician, study PI or co-investigators' discretion.

Apalutamide

- Acquisition: Supplied by Janssen. Will be sent to the sponsor site (West LA clinical research pharmacy) and West LA VA will distribute the drug to additional sites. The contents of the label will be in accordance with all applicable regulatory requirements.

- No special preparation of apalutamide is required. Under normal conditions of handling and administration, apalutamide is not expected to pose significant safety risks to site staff.

- Dosing: 240 mg by mouth daily. Apalutamide will be supplied as 60 mg tablets to be administered with or without food. If a subject misses a dose, subject may take the dose if the next scheduled dose is at least 12 hours later. If the next scheduled dose is due in less than 12 hours, subject should skip the dose and resume dosing the next day at the regular time.

- Patients will be supplied with a drug diary. They will be asked to annotate daily when they take the medication and to regard all other medications, supplements, they are taking – dose and frequency.

- Thyroid stimulating hormone (TSH) will be evaluated throughout the study (with T3 and T4 done only if TSH is abnormal) as follows: screening, day 1, and monthly during treatment.

- Dose modifications for apalutamide are described below.

- **Safety Monitoring** Thyroid stimulating hormone (TSH) is evaluated throughout the study (with T3 and T4 done only if TSH is abnormal) as follows: Screening, and every 30 days for the duration of treatment.

Apalutamide Investigational Product Details

	Investigational Product
Product name:	Apalutamide (formerly ARN-509)
Dosage form:	Tablet
Unit dose strength(s):	60 mg
Dosage level(s):	240 mg
Route/Administration:	Oral, continuous once daily dosing
Dosing instructions:	Apalutamide may be taken with or without food.

Manufacturer/source of procurement:	Janssen

Risks of Apalutamide include:

Very common (≥ 10% of people experience these)

- Fatigue
- Skin rash
- Joint pain and muscle spasms (Arthralgia)
- Weight loss
- Fall
- Fracture
- Increased blood pressure (hypertension)
- Hot flush
- Diarrhea
- Decreased appetite**

Common (≥1% -<10% of people experience these):

- Itching
- Changes in thyroid function (hypothyroidism)
- Increases in cholesterol and blood triglycerides
- Change in experience of taste (dysgeusia)
- Reduced or blocked blood flow to the heart, including heart attack (ischemic heart disease, including myocardial infarction).
- Reduced or blocked blood flow to the brain, including stroke (ischemic cerebrovascular disorders)
- Alopecia (hair loss)

Uncommon (≥ 0.1% - <1% of people experience these):

- Seizures
- Inflammation with the lungs that may lead to permanent damage (Interstitial lung disease)*

Rare (≥0.01% - <0.1%)

***Life-threatening rash with blisters and peeling over much of the body (Stevens-Johnson syndrome/Toxic epidermal necrolysis)

***Skin rash with fever, and blood cell abnormalities including increase in white blood cells (lymphocytes and eosinophils), a decrease in platelets and potential life threatening inflammation of internal organs (Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS)

*This is information provided voluntarily by doctors using apalutamide in routine clinical practice where frequency of this event can be estimated from clinical trials and is assessed as uncommon (0.1%)

** This information provided voluntarily by doctors using apalutamide in routine clinical practice where frequency cannot be estimated; however, frequency of this event can be estimated form clinical trials and is assessed as very common.

***this information provided voluntarily by doctors using apalutamide in routine clinical practice where frequency cannot be estimated; however, frequency of this event can be estimated from clinical trials and is assessed as rare.

Other information on apalutamide:

History of seizures: Seizures have been observed very rarely in subjects taking part in apalutamide studies. Subjects will be confirmed that they have no history of seizures and will be checked throughout the study that they are not taking other medications that can increase their risk of seizures.

Development of rash: More than 1 in 10 patients have developed a rash. Some rashes may need medical attention. The rash may be confined to one area of the body or may spread across the body. Rashes can be painful, blister on or near the lips, eyes or genital and peel on areas of skin surface. Subject's may be given medicines to apply to the skin or take by mouth to help the signs and symptoms of rash. Also the study medication may be temporarily held. In most cases, rashes can be treated with topical or oral medications so that subjects can continue apalutamide treatment.

Difficulty breathing: Scarrring of the inner lining of the lung (Interstitial lung disease) has been observed in patients taking apalutamide. Subject's will be checked for history of lung problems and monitored throughout study for symptoms of shortness of breath, breathing difficulty, cough or fever.

SAEs to apalutamide are reported to Janssen and Janssen determines respec attribution to SAEs.

Dose reductions or discontinuation of apalutamide will be made due to toxicities that are attributed to apalutamide, or not clearly attributable to another drug in this study (e.g., abiraterone acetate, leuprolide).

Dose modifications for toxicity attributed to apalutamide given concurrently with abiraterone acetate will be guided by the Investigator Brochure provided by the drug manufacturer, as detailed below:

Toxicity*	Dose of abiraterone acetate	Dose of apalutamide	Dose of prednisone
Grade 1 or 2	No change	No change	No change

≥Grade 3 or higher	No change	Hold until Grade 1 or baseline, resume at 180 mg (3 tablets). **	No change
First Recurrence ≥Grade 3	No change	Hold until Grade 1 or baseline, resume at 120 mg (2 tablets). **	No change
Second Recurrence ≥Grade 3	No change	Permanently Discontinue	No change
First occurrence of seizure of any grade or any Grade 4 toxicity of any duration*** neurotoxicity	No change	Permanently Discontinue	No change

* Toxicity that is attributed to apalutamide or not clearly attributed to another drug on this study.

** Patients who require a treatment interruption > 2 weeks should discontinue apalutamide if it is believed to be responsible for the treatment interruption.

*** Individual exclusions to this criteria may be allowable for grade 4 emesis or clinically insignificant laboratory abnormalities that resolve within two days of optimum treatment at the investigators' discretion.

Rash

Dose modifications for rash are allowed only for apalutamide and are summarized in below table.

If the skin rash has any component of desquamation, mucosal involvement, or pustules, stop dosing with apalutamide, refer to dermatologist for evaluation, and a skin biopsy is recommended (in addition to the interventions listed in below Table) If the skin rash is Grade 3 or higher, asking the subject to consent to documentation by a photograph and further evaluation by a dermatologist should also be considered.

5.1.1.1 **Severity**	5.1.1.2 **Intervention**

5.1.1.3 Grade 1	• Continue apalutamide at current dose
	• Initiate dermatological treatment[a]
	  o Topical steroid cream AND
	  o Oral Antihistamines
	• Monitor for change in severity[a]

5.1.1.4 Grade 2 (or symptomatic Grade 1)[b]	• Hold apalutamide for up to 28 days
	• Initiate dermatological treatment[a]
	  o Topical steroid cream AND
	  o Oral Antihistamines
	• Monitor for change in severity[a]
	  o If rash or related symptoms improve, reinitiate apalutamide when rash is Grade≤1. Consider dose reduction at a 1 dose level reduction[c].

5.1.1.5 Grade ≥3[d]	• Hold apalutamide for up to 28 days
	• Initiate dermatological treatment[a]
	  o Topical steroid cream AND
	  o Oral Antihistamines AND
	  o Consider short course of oral steroids
	• Reassess after 2 weeks (by site staff), and if the rash is the same or has worsened, initiate oral steroids (if not already done) and refer the subject to a dermatologist
	  o Reinitiate apalutamide at a 1 dose level reduction[e] when rash is Grade≤1.
	  o If the dose reduction will lead to a dose less than 120mg, the study drug must be stopped (discontinued)
	• If after 28 days, rash has not resolved to Grade≤1, contact PI to discuss further management and possible discontinuation of study drug.

Note: Rash may be graded differently according to the type of rash and associated symptoms. For example, maculo-papular rash is graded by body surface area covered and not severity of the rash. Please consult NCI-CTCAE Version 4.03 for specific grading criteria for other types of rash.

a Obtain bacterial/viral cultures if infection is suspected
b Subject presents with other rash related symptoms such as pruritus, stinging, or burning
c 1 dose level reduction = 60mg (1 apalutamide tablet)
d If there is blistering or mucosal involvement, stop apalutamide dosing immediately and contact PI
e If a subject previously started oral corticosteroids, continue for at least 1 week after resumption of reduced dose of apalutamide. If the proposed total oral steroid use will exceed 28 days, contact Janssen.

Abiraterone Acetate

- Acquisition: Supplied by Janssen. Will be sent to the sponsor site (West LA VA clinical research pharmacy) and West LA VA will distribute the drug to additional sites. The contents of the label will be in accordance with all applicable regulatory requirements.

- Based on its mechanism of action, abiraterone acetate may harm a developing fetus. Therefore, women who are pregnant or women who may be pregnant should not handle abiraterone acetate without protection e.g., gloves.

  Dosing: 1000 mg by mouth daily. Abiraterone acetate will be supplied as 250 mg tablets to be taken without food. Abiraterone acetate must be taken on an empty stomach. No food should be consumed for at least two hours before the dose is taken and for at least one hour after the dose of abiraterone acetate is taken. Do not crush or chew the tablets. If a subject misses a dose, subject may take the dose if the next scheduled dose is at least 12 hours later. If the next scheduled dose is due in less than 12 hours, subject should skip the dose and resume dosing the next day at the regular time. Abiraterone acetate is administered with prednisone, 5 mg, PO to be taken twice daily.

  Patients will be supplied with a drug diary. They will be asked to annotate daily when they take the medication and to regard all other medications, supplements, they are taking – dose and frequency.

  LFTs will be assessed every 2 weeks for the first three months, and then at each subsequent blood draw (monthly) thereafter until abiraterone acetate and prednisone are discontinued.

  LFTs are also assessed monthly after discontinuation of ADT (leuprolide, abiraterone acetate, and apalutamide) for the first six months, and then every three months thereafter.

- Dose modifications for abiraterone acetate in combination with apalutamide are described below.

Abiraterone acetate Investigational Product Details

	Investigational Product
Product name:	Abiraterone acetate
Dosage form:	Tablet
Unit dose strength(s):	250 mg
Dosage level(s):	1000 mg
Route/Administration:	Oral, continuous once daily dosing
Dosing instructions:	Abiraterone acetate should be taken without food.
Manufacturer/source of procurement:	Janssen

Risks of abiraterone acetate include:

Frequent (≥ 20%) [May occur in 20 or more patients in 100]

- hypokalaemia (low blood potassium, a mineral that helps regulate heart rate/function, fluid balance in the body and is needed for adequate body function)
- hypertension (high blood pressure)

Very Common (10% to 19%) [May occur between 10 and 19 patients in 100]

- edema peripheral (swelling of the legs as a result of the body keeping too much fluid)

Common (5% to 9%) [May occur between 5 to 9 patients in 100]

- dyspepsia (uncomfortable feeling in upper belly, indigestion)
- hematuria (presence of blood in the urine)
- fractures (a break in the bone)
- alanine aminotransferase increased and/or aspartate aminotransferase increased (enzymes in the blood that measure the function of the liver). It is common to see increases in liver enzymes but this does not mean the liver is not functioning normally. Monthly blood tests are done to monitor acceptable levles.
- urinary tract infection

Less Common (< 5% [May occur in fewer than 5 patients in 100]

- hypertriglyceridemia (high levels of fats (triglycerides) in the blood)
- angina pectoris (chest pain)
- atrial fibrillation (a fast and irregular heartbeat)
- tachycardia (rapid heartbeats)

Uncommon (< 1%) [May occur between 1 and 9 patients in 1000]

- adrenal insufficiency (decreased function of adrenal glands that normally help maintain blood pressure, balance minerals and fluid in your body)
- Cardiac failure (heart failure, the heart is unable to supply enough blood flow to meet the body's needs)

- Arrhythmia (changes in the rhythm of the heart)
- Abnormal ECG with QT prolongation (an abnormal finding on the ECG)
- Bone density decreased (loss of strength of bones)
- Myopathy (muscle weakness and /or muscle pain)

Unknown (frequency isn't determined since data was derived from post-marketing experience and there was no report from clinical studies)

- failure of the liver to function (called acute liver failure)
- allergic alveolitis (swelling and irritation of the lung)
- Rhabdomyolysis (breakdown of muscle tissue)
- Torsades de Pointes (rapid or irregular heart rate which can cause sudden cardiac death)

There is a small chance of severe allergic reaction to the drug which may be life-threatening.

Other information on abiraterone acetate:

- Abiraterone acetate may cause harm to the liver. About 13% of patients taking abiraterone acetate have had abnormal blood levels of liver enzymes. Rarely, liver failure can occur. Subject's liver function will be monitored closely by blood tests every two weeks for the first 3 months of the study and monthly thereafter. If elevations in the liver function tests show abnormalities, the dose of the study medication will be adjusted or discontinued.

- Abiraterone acetate should be used with caution in patients with a history of heart disease**.** Before treatment with abiraterone acetate, high blood pressure must be controlled and low potassium must be monitored and corrected, when low.

Use With Glucose-lowering Agents Isolated cases of hypoglycemia have been reported when abiraterone acetate and prednisone was administered to patients with pre-existing diabetes receiving pioglitazone and repaglinide. Blood sugar should be monitored in patients with diabetes.

SAEs to abiraterone acetate are reported to Janssen and Janssen determines respective attribution to SAEs.

Dose modifications for LFT abnormalities attributed to abiraterone acetate given concurrently with apalutamide will be guided by the prescribing information provided by the drug manufacturer, as detailed below:

Toxicity	Dose of abiraterone acetate	Dose of apalutamide	Dose of prednisone

Grade 1 or 2	No change	No change	No change
Grade 3	Hold until return to baseline or to AST or ALT ≤2.5 x ULN and total bilirubin ≤1.5 x ULN, resume at 750 mg (3 tablets) only after discussion and agreement with medical monitor	Hold until return to baseline	No change
Recurrence Grade 3	Hold until return to baseline or to AST or ALT ≤2.5 x ULN and total bilirubin ≤1.5 x ULN, resume at 500 mg (2 tablets) only after discussion and agreement with medical monitor	Hold until return to baseline	No change
Grade 4	Discontinue AA treatment	Hold until return to baseline	No change or consider tapering if AA discontinued
Concurrent elevation of AST/ALT > 3x ULN with bilirubin >2x ULN (unless the concurrent elevation is related to biliary obstruction or other causes unrelated to study treatment)	Discontinue AA treatment		No change or consider tapering if AA discontinued

Dose modifications for hypokalemia attributed to abiraterone acetate given concurrently with apalutamide will be guided by the prescribing information provided by the drug manufacturer, as detailed below:

Toxicity	Dose of abiraterone acetate	Dose of apalutamide	Dose of prednisone
Grade 1 or 2	Initiate oral potassium supplementation, titrate to ≥3.5 to ≤5.0 mmol/L, maintenance at ≥4.0 mmol/L recommended	No change	No change
≥Grade 3	Hold and initiate IV potassium and cardiac monitoring, resume only after discussion and approval by the medical monitor	No change	No change or consider tapering if AA is discontinued

Dose modifications for hypertension and edema/fluid retention attributed to abiraterone acetate given concurrently with apalutamide will be guided by the

prescribing information provided by the drug manufacturer, as detailed below:

Toxicity	Dose of abiraterone acetate	Dose of apalutamide	Dose of prednisone
Grade 1 or 2	No change	No change	No change
≥Grade 3	Hold until Grade 1 or baseline, resume at full dose	No change	No change
First Recurrence ≥Grade 3	Hold until Grade 1 or baseline, resume at 750 mg (3 tablets)	No change	No change
Second Recurrence ≥Grade 3	Hold until Grade 1 or baseline, resume at 500 mg (2 tablets)	No change	No change
Third Recurrence ≥Grade 3	Discontinue	No change	No change or consider tapering if AA is discontinued

5.1.2. Surgery.

Radical Prostatectomy
Radical prostatectomy with pelvic lymph node dissection will be performed at the VA hospital by a urologic oncologist. The prostatectomy may be performed as an open or robot-assisted procedure at the discretion of the treating urologist. Surgery will be completed after radiographic directed metastatic biopsy but prior to systemic or radiotherapy.

5.1.3. Radiotherapy

Primary prostate radiotherapy. Patients who undergo radiotherapy as primary tumor therapy will undergo radiotherapy directed to the prostate, seminal vesicles, and pelvic lymph nodes.

- Allowed dose fractionations include:
  - **Prostate**: 40 Gy in 5 fractions or 60 Gy in 20 fractions or 79.2 Gy in 44 fractions
  - **SVs:** 25-40 Gy in 5 fractions, or 42-60 Gy in 20 fractions, or 45-29.2 Gy in 44 fractions.
  - **Pelvic LNs:** 25 Gy in 5 fractions or 42-43 Gy in 20 fractions or 45 Gy in 25 fractions.

The selection of dose and fractionation will be made at the discretion of the investigator, and typically depends on anatomical considerations and baseline urinary and bowel function. Gross disease may be prescribed a higher dose at the investigator's discretion. If a pelvic node is >1.5 cm on the simulation CT scan (RT planning scan) or was enlarged or PSMA positive on the staging scan, then the enlarged node may be boosted to a higher dose while respecting normal tissue dose constraints. This radiation may be done anytime while the patient is on ADT. Standard RTOG contouring guidelines are recommended but not required.

SBRT. SBRT offers ablative dose-escalation to tumor targets with simultaneous dose-restraint to normal tissues, which is not possible using conventional radiotherapy. SBRT utilizes high-precision radiotherapy delivery machines, integrated image guidance systems, advanced planning software, and a high level of professional and technical expertise. Unlike conventional RT, which is delivered in multiple fractions of 1.8-2 Gy (Gy is the unit of measurement for dose delivered), SBRT is delivered in one to five fractions of typically 8 Gy or greater. Patients will undergo SBRT to all visible sites of metastases within two months of initiation of ADT. Prior to SBRT, patients may be re-imaged with a NaF or PSMA PET-CT or Fluciclovine (Axumin) PET-CT in the treatment position for RT planning. (Patients suspected of having >5 sites of metastases at this time will be censored from primary analysis but will be treated per protocol at patients and investigators discretion and the tissue used for the correlative analyses). Simulation CT scan will be fused to the NaF or PSMA PET-CT or Fluciclovine (Axumin) PET-CT. SBRT will be delivered in 1 to 5 fractions. SBRT 5 fraction regimens should deliver a total dose of 25 to 50 Gy. Acceptable 3 fraction regimens should deliver a total dose of 27-36 Gy. Single fraction regimens should deliver a dose of at least 18 Gy. Spinal metastases may be treated with a single fraction of 15 to 22 Gy, or 3 fractions to a total dose of 24-36 Gy, or 5 fractions to a total dose of 30-35 Gy. Dose selection will depend on adequate sparing of normal tissue with maximization of dose delivered to metastatic sites. A biologically equivalent dose (BED) to tumor of >100 Gy (for an alpha-beta ratio of 3) is a goal but is not required (11). Dose constraints per NRG (RTOG) B001 are recommended (35).

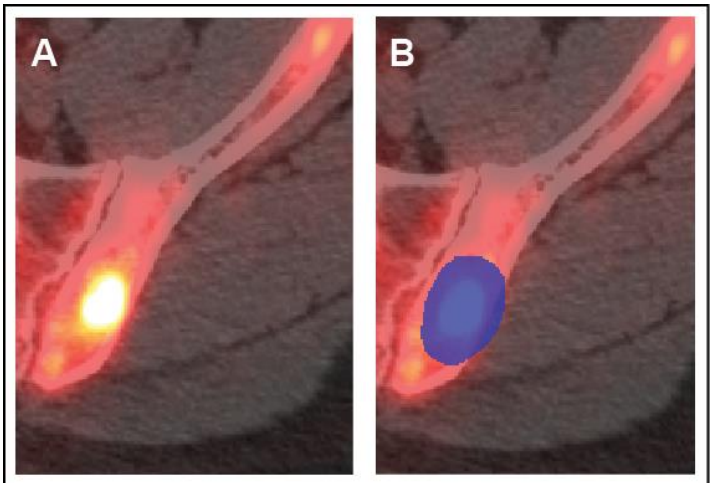

Figure 5. SBRT as metastasis directed treatment for oligometastatic prostate cancer. **A.** NaF PET-CT fused to diagnostic CT scan reveals a hyper matabolic focus in the left ilium corresponding to an underlying sclerotic lesion. **B.** Isodose distribution of ablative, single fraction SBRT to a dose of 20 Gy. Note the coverage of the PET positive focus and areas of bony sclerosis with simultaneous sparing of adjacent normal structures.

Figure 5 shows a recent SBRT treatment plan for a patient with biopsy proven de-novo oligometastatic prostate cancer. Image fusion between the simulation CT scan and the NaF PET was used to delineate the target. This patient received a single dose of 20 Gy (BED = 153 Gy) to this site of osseous metastasis without toxicity. Note the sharp dose fall-off and conformity of the dose delivered to the metastatic lesion, made possible by SBRT technique. (Image is taken from the RT planning MIM software package.)

Post-operative pelvic RT. Patients found to have pT3a or higher, positive margins, or pathologically involved nodes after radical prostatectomy, will receive post-operative fractionated IMRT delivered to the pelvic nodes and prostate bed.

The pelvic nodes will receive 45-50.4 Gy in 25-28 fractions and the prostate bed will receive 66-72 Gy in 34-40 fractions with daily image guidance. If a pelvic node is >1.5 cm on the simulation CT scan (RT planning scan) or was enlarged or PSMA positive on the staging scan, then the enlarged node may be boosted to a higher dose while respecting normal tissue dose constraints. This pelvic RT can extend beyond or be completed after ADT. Standard RTOG contouring guidelines are recommended but not required.

Tissue handling Recommendations:

Research Biopsies:

- A pre-treatment radiographic directed metastatic biopsy for both diagnostic and correlative analysis is required for enrollment unless not safe to perform, not feasible, or declined. If this biopsy is not safe, not feasible, or not diagnostic of metastatic prostate cancer, then secondary imaging modality [for example, MRI] must also be consistent with metastatic disease [unless PSMA PET-CT or Fluciclovine (Axumin) PET-CT was used as initial staging]. A second biopsy obtained at time of progression will be encouraged but is optional. The biopsy site will be determined primarily by safety. Biopsies will be performed by Interventional Radiology.
- Lesions will be chosen based upon the strength of the evidence suggesting the presence of metastasis and with the goal of minimizing patient risk. The biopsies will be performed in an interventional radiology suite with radiological guidance (typically CT or MRI) in accordance with institutional standards. CT or MRI will confirm designated lesions immediately prior to biopsy. Once the target lesion is identified, up to six biopsies will be performed. Preferably, a 16 gauge BonoptyTM needle or biopsy needle with an equivalent 16g bore will be used to biopsy the metastatic lesion. If the lesion is a bone metastasis, the Bonopty needle will be passed through the cortical bone and into the target lesion. Optimal results are obtained when the biopsies are performed on medulary bone directly adjacent to blastic lesion. Soft tissue biopsies should be taken so that a core of approximately 10 to 20 mm in length is obtained. Core biopsies will be extracted: at least 3 will be placed in neutral-buffered formalin and 3 will be immediately frozen on a pre-frozen bed of OCT (Optimal Cutting Temperature compound used for frozen sections), covered with additional OCT, and kept on dry ice or at -80° C. Recommended procedures for biopsies are included in **Appendix 14.4**.

Research Blood Samples:

- Blood samples will be collected during screening, at 1 month, 2 months, at the end of systemic treatments, and at each follow-up visit (q30 days for the first 6 months, then q3 months thereafter) whenever possible.
- Blood will ideally be collected in 2 x 5 ml Streck Cyto-Chex® BCT tubes and 1 x 10 mL Cell-Free DNA BCT@ tubes, and 1 x 10 mL Cell-Free RNA BCT@ tubes. The blood is used for immunophenotyping, CTC, ctDNA, and ctRNA analyses. Blood in these tubes are stable at room temperature for shipping. Upon receipt, blood is processed for immunophenotyping, DNA, and RNA isolation.

Recommendation for processing of prostatectomy specimens:

- After resection, the prostate is sectioned into multiple slices (total number depends on the size of the prostate, but usually 5-8). Alternating slices are frozen, while

other slices are fixed in formalin and embedded in paraffin.

- A GU pathologist will identify distinct loci of prostate adenocarcinoma by histopathology and the corresponding (adjacent) frozen and formalin slices will be processed for genomic/transcriptomic analyses and sectioning for immunohistochemistry, respectively.
- Each focus of prostate cancer will be laser capture microdissected from frozen tumor sections prior to "omics" analyses if necessary.
- When a single lesion exhibits areas of differing Gleason grade, an area representative of each component will be separately microdissected and subjected to analysis.
- For sites aside from VA West LA, FFPE blocks of the primary tumor and initial biopsy may be sent.
- For VA West LA, the fresh (tissue collected after enrollment which has not been exposed to fixative; ie: non-FFPE) prostate tissue may also be shared with Dr Garraway's Study VA #0001.

Shipment of tissue:

- All specimens (blood, frozen biopsies, frozen prostatectomy specimen slices) are delivered in person to the laboratory of Dr. Nicholas Nickols at UCLA, if acquired at West LA VA. Frozen tissues are packed on dry-ice. Blood is shipped room temperature.
- Formalin fixed biopsies tissue and prostatectomy specimen slices are shipped after diagnostic evaluation by the institution. These are also shipped to the laboratory of Dr Nicholas Nickols.
- Samples shipped can be accompanied by a Sample Shipment Log (supplied by Sponsor, West LA VA).
- Emails will be sent to Nicholas.nickols@va.gov / nnickols@mednet.ucla.edu (PI) within three working days prior to shipment whenever possible.
- Samples should be shipped Monday through Thursday and Saturday Delivery is not allowed, unless special arrangements are made in advance.
- **Shipping Address**:
  Nicholas Nickols MD PhD
  UCLA Radiation Oncology
  BOX 951714, B3-109 CHS
  Los Angeles, CA 90095-1714

Tissue will be stored in the laboratory of Nicholas Nickols in appropriately secure laboratory research areas.

5.2 Handling of Study Interventions

Acquisition of study drugs:

- Apalutamide and abiraterone acetate will be provided by Janssen and sent directly to West LA VA (West LA VA Clinical Research Pharmacy). Drug will be stored per manufacturers' recommendations in the Clinical Research Pharmacy. West LA VA will distribute to other participating centers.
- Leuprolide will be purchased from commercial suppliers.

Drug accountability: The Clinical Research Pharmacy will maintain an inventory of the study drugs, including the lot number, amount shipped, date of shipment, dates/amounts

dispensed, and remaining amounts of drug. A minimum amount of drug will be kept on hand at all times (enough drug to treat 5 patients for four weeks). Unused or expired study medication will be destroyed following the institution's or drug vendor policies and procedures.

5.3. Concomitant Interventions

5.3.2 Allowed interventions:
All except 5.3.3.

5.3.3 Required Interventions:
NaF-PET CT or PSMA PET-CT or Fluciclovine (Axumin) PET-CT, (If biopsy is not diagnostic, or unsafe to perform, or not feasible, or declined, then a secondary imaging modality [for example, MRI] must also be consistent with metastatic disease [unless PSMA PET-CT or Fluciclovine (Axumin) PET-CT was used as initial staging]) as per protocol and for determining eligibility.
Laboratory assessment as per protocol and for determining eligibility.
Treatments as described in this protocol.

5.3.4 Prohibited Interventions

- Herbal products that may have hormonal anti-prostate cancer activity and/or are known to decrease PSA levels (e.g., saw palmetto),

- Any medication outside the trial agents known to affect serum androgen levels or PSA.

- **Prohibited Concomitant Medications During Trial.** As a class effect, AR antagonists have been associated with seizures due to an off-target mechanism of action (gamma amino butyric acid chloride channel [$GABA_A$] inhibition). Drugs known to lower the seizure threshold or cause seizures are prohibited and a representative list is included below:

    - Atypical antipsychotics (e.g. clozapine, olanzapine, risperidone, ziprasidone)
    - Bupropion
    - Lithium
    - Meperidine and pethidine
    - Phenothiazine antipsychotics (eg, chlorpromazine, mesoridazine, thioridazine)
    - Tricyclic antidepressants (eg, amitriptyline, desipramine, doxepin, imipramine, maprotiline, mirtazapine
    - Concomitant strong CYP3A4 inducers (e.g., phenytoin, carbamazepine, rifampin, rifabutin, rifapentine, phenobarbital) will be avoided during abiraterone acetate treatment. Although there are no clinical data with this dose adjustment in patients receiving strong CYP3A4 inducers, because of the potential for an interaction, if a strong CYP3A4 inducer must be co-administered, increase the abiraterone

acetate dosing frequency to twice a day only during the co-administration period (e.g., from 1,000 mg once daily to 1,000 mg twice a day). Reduce the dose back to the previous dose and frequency, if the concomitant strong CYP3A4 inducer is discontinued.

- **Restricted Concomitant Medications.**

  - **Apalutamide** is metabolized primarily by human CYP3A4, thus co-administration with strong inhibitors or inducers of CYP3A4 should be avoided as much as possible. Apalutamide may also induce CYP3A4; therefore, caution should be taken when administered in conjunction with CYP3A4 substrates that have a narrow therapeutic index. Examples of the strong CYP3A4 inhibitors and inducers include the following:

    - Strong CYP3A4 inhibitors: itraconazole, clarithromycin, erythromycin, diltiazem, verapamil, delavirdine, atazanavir, indinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, voriconazole, grapefruit juice (or grapefruits); co-administration with any of these agents may increase apalutamide plasma concentrations. Strong CYP inducers: phenytoin, carbamazepine, rifampin, rifabutin, rifapentin, phenobarbital, efavirenz, tipranivir, St. John's wort; co-administration with any of these agents may decrease apalutamide plasma concentrations. The potential for drug-drug interaction between apalutamide and warfarin (eg, Coumadin) is unknown at present. If a subject is taking warfarin, re-assess PT (prothrombin time)/international normalized ratio (INR) as clinically indicated and adjust the dose of warfarin accordingly.

  - **Abiraterone acetate** is a substrate of CYP3A4. In a dedicated drug interaction trial, co-administration of rifampin, a strong CYP3A4 inducer, decreased exposure of abiraterone by 55%. Avoid concomitant strong CYP3A4 inducers during abiraterone acetate treatment. If a strong CYP3A4 inducer must be co-administered, increase the abiraterone acetate dosing frequency. In a dedicated drug interaction trial, co-administration of ketoconazole, a strong inhibitor of CYP3A4, had no clinically meaningful effect on the pharmacokinetics of abiraterone acetate. Abiraterone acetate is an inhibitor of the hepatic drug-metabolizing enzymes CYP2D6 and CYP2C8. In a CYP2D6 drug-drug interaction trial, the Cmax and AUC of dextromethorphan (CYP2D6 substrate) were increased 2.8- and 2.9-fold, respectively, when dextromethorphan was given with abiraterone acetate 1,000 mg daily and prednisone 5 mg twice daily. Avoid co-administration of abiraterone acetate with substrates of CYP2D6 with a narrow therapeutic index (e.g., thioridazine). If alternative treatments cannot be used, exercise caution and consider a dose reduction of the concomitant CYP2D6 substrate drug. In a CYP2C8 drug-drug interaction trial in healthy subjects, the AUC of pioglitazone (CYP2C8 substrate) was increased by 46% when pioglitazone was given together

with a single dose of 1,000 mg abiraterone acetate. Therefore, patients should be monitored closely for signs of toxicity related to a CYP2C8 substrate with a narrow therapeutic index if used.

5.4 Adherence Assessment

Adherence to study drugs (apalutamide and abiraterone acetate) will be defined as at least 80% of treatment intervention pills taken as determined by pill count during follow-up visits and assessment of drug diaries at scheduled visits. Patients must undergo radical prostatectomy and/or all required radiation treatments.

6. STUDY PROCEDURES

6.1 Schedule of Evaluations	Treatment Visits (q30d)					Follow up (visits are q30 d for first 6 M; q3 M thereafter)
Visit	-90 to 0	Day 1	Day 30 (1 Mo)	31-60 days	2, 3, 4, 5, 6 Month	Follow up
Window (days)		1	+/-7		+/-7	+/-7
Informed consent	x					
Inclusion/exclusion criteria	x					
Demographics	x					
Scan used to find out if cancer has spread to the bone (with CT of the chest, abdomen and pelvis)	x					
Biopsy or MRI if this biopsy is unsafe to perform, not feasible or declined	x					
Quality of Life Questionnaire	x		x		x	x
Medical assessment of quality of patient life	x		x		x	x
Medical history	x		x	x	x	x
Physical Examination	x		x	x	x	
Vital signs, including weight	x		x	x	x	x
Current Medications/Drug Diary Review	x		x	x	x	x
Blood Draws to check for thyroid and liver function	x		x		x	x
PSA, total testosterone	x		x		x	x
Research Blood [2]	x		x		x	x
Urine collection	x		x		x	x
Surgery (Radical Prostatectomy) if this is chosen		x				
SBRT to all sites of metastases [6]				x		
Pelvic RT (if indicated) [3]					X (may extend beyond first 6 months)	
Adverse Events		x	x	x	x	x

Leuprolide Injection [54]		x				
Start apalutamide, abiraterone acetate , prednisone daily (6 months)		x				
Radiotherapy (prostate, seminal vesicles, pelvic lymph nodes)		Done any time during 6 months systemic therapy after ADT start				

1. *LFTs are drawn with each blood chemistry. In addition, for the first 3 months LFTs are also drawn q2 weeks. (After the first 3 months, they are drawn with the scheduled blood chemistries).*
2. *Research blood draws: 2 x 5 ml Streck Cyto-Chex BCT@ tubes, 1 x 10 mL Cell-Free DNA BCT@ tube, and 1 x 10 mL Cell-Free RNA BCT@ tube.*
3. *Pelvic (post operative) RT may be done anytime, and may be completed after systemic therapy. It is done only for patients with pT3+, positive margins, or pN1.*
4. *Leuprolide, apalutamide, and abiraterone acetate will be initiated on Day 1(+/- 3 days), if primary tumor therapy is radiotherapy or after radical prostatectomy, within 0-3 days, if primary tumor therapy is surgery*
5. *Apalutamide, abiraterone acetate, and prednisone are stopped 6 months (+/- 7 days) after leuprolide injection. Prednisone will be tapered down after discontinuation of study drugs.*
6. *SBRT can be done anytime within the first 2 months of ADT, but preferably during the second month.*

6.2 Description of Evaluations

6.3.1 Screening Evaluation

Patients with metastatic prostate carcinoma that have not undergone prior treatment for prostate cancer and have undergone a metastatic biopsy will be evaluated for this study unless deemed unsafe or not feasible to perform, or declined by patient. Patients must be staged with NaF or PSMA PET-CT or Fluciclovine (Axumin) PET-CT. If the biopsy is not diagnostic, declined, or not feasible, MRI must be consistent with metastatic prostate cancer.

Consenting Procedure

Before any screening procedure is performed, informed consent will be obtained. One consenting process and a single informed consent form will be used to describe both the screening and study procedures. One of the study investigators will approach patients who may be eligible for this study from the Urology Clinic, Radiation Oncology clinic, or medical oncology clinic within the West LA VA or Long Beach VA Health System. At participating sites, consent will be obtained by one of the sub-investigators at that site. The nature and purpose of the study will be described in detail, the patients will be given the IRB-approved consent form, and patients will be encouraged to ask questions and read the consent form at their leisure (including taking it home to read it and ask questions at a subsequent time). An enrollment note in the electronic medical record will document the informed consent process has been appropriately completed.

Screening Assessments

Screening studies must be completed within 28 days of enrollment as defined by initiation of study drug treatment.

- History and physical.
- Confirmation of carcinoma of the prostate pathology.
- Laboratories- blood tests, urine analysis
- PSA and testosterone assessment
- Radiographic directed biopsy of metastatic lesion unless deemed unsafe or

not feasible to perform, or declined by patient

- Imaging studies (NaF or PSMA PET-CT, diagnostic CT scan of the chest, abdomen/pelvis). If biopsy is not diagnostic, or unsafe to perform, or not feasible, or declined by patient, then a secondary imaging modality (for example, MRI) must also be consistent with metastatic disease (unless PSMA PET-CT was used as initial staging).
- Blood samples to determine circulating tumor genetic information and immunophenotyping
- Assessment of performance status (e.g., ability to perform daily activities)

6.3.2 Enrollment, Baseline, and/or Randomization

Enrollment (Day 1)

Enrollment will occur when patients have met all eligibility criteria and initiate treatment, which begins with radical prostatectomy, if primary tumor therapy is surgery. Enrollment must occur within 90 days of the initiation of screening, which starts at the time that informed consent is signed.

6.3.3 Treatment and Follow-up Visits

Scheduled treatment visits will be on days 31 (1 month), and then every 30 days (monthly), until patient completes the treatment study period (all systemic and radiotherapy completed), and then every 30 days for six months, and then every 3 months thereafter (+/- 7 days). See 6.1 Schedule of Evaluations. At these visits, the following will occur:

- Review of medical history and physical examination and vital signs.
- Laboratories- blood tests, urine analysis.
- Pill count of study drugs, review of drug diary with concomitant medications
- Adverse events assessment.
- Laboratory assessments

6.3.4 Completion/Final Evaluation

Each patient will remain on study until unacceptable toxicity, disease progression, or withdrawal of consent, or two years, whichever occurs first. The patient will have a follow up visit 2 to 4 weeks after going off study or as clinically indicated. At this visit, the following will occur:

- Review of medical history and physical, and vital signs
- Blood and urine tests
- Review of concomitant medications
- Review of Adverse Events (AE), or any side effects from the study drugs or other interventions

7. SAFETY ASSESSMENTS

7.1 Specification of Safety Parameters

Toxicities of the study drugs will be monitored with frequent laboratory assessments and exams. Safety during radiotherapy will be assessed by standard on treatment visits and standard operating procedures for quality assurance of radiotherapy. An individual Case Safety Report (ICSR) will be used to document safety events to be reported.

Individual Case Safety Report (ICSR)

A valid ICSR must contain the four minimum criteria required to meet regulatory reporting requirements.

- an identifiable subject (but not disclosing personal information such as the subject's name, initials or address)
- an identifiable reporter (investigational site)
- a Janssen medicinal product
- an adverse event, outcome, or certain special situations

The minimum information required is:

- suspected Janssen medicinal product (doses, indication)
- date of therapy (start and end date, if available)
- batch or lot number, if available
- subject details (subject ID and country)
- gender
- age at AE onset
- reporter ID
- adverse event detail (AE verbatim in English), onset date, relatedness, causality, action taken, outcome, (if available)
- Janssen protocol ID

Product Quality Complaint (PQC)

A product quality compliant is defined as any suspicion of a product defect related to a potential quality issue during manufacturing, packaging, release testing, stability monitoring, dose preparation, storage or distribution of the product, or delivery system. Not all PQCs involve a subject. Lot and batch numbers are of high significance and need to be collected whenever available.

Examples of PQC include but not limited to:

- Functional Problem: e.g., altered delivery rate in a controlled release product
- Physical Defect: e.g. abnormal odor, broken or crushed tablets/capsules
- Potential Dosing Device Malfunction: e.g., autoinjector button not working, needle detaching from syringe
- Suspected Contamination
- Suspected Counterfeit

Hospitalization

For reports of hospitalization, it is the sign, symptom or diagnosis which led to the hospitalization that is the serious event for which details must be provided.

Any event requiring hospitalization or prolongation of hospitalization that occurs during the study must be reported as a serious adverse event, except hospitalizations for the following:

- Hospitalizations not intended to treat an acute illness or adverse event (e.g., social reasons such as pending placement in long-term care facility)
- Surgery or procedure planned before entry into the study. [Note: Hospitalizations that were planned before the start of data collection and where the underlying condition for which the hospitalization was planned has not worsened will not be considered serious adverse events. Any adverse event that results in a prolongation of the originally planned hospitalization is to be reported as a new serious adverse event.]

Life-Threatening Conditions

The cause of death of a subject in a study within 30-days of the last dose of Apalutamide or Abiraterone acetate, whether or not the event is expected or associated with the study drug, is considered a serious adverse event.

Disease progression should not be recorded as an adverse event or serious adverse event term; instead, signs and symptoms of clinical sequelae resulting from disease progression/lack of efficacy will be reported if they fulfill the serious adverse event definition.

Unlisted (Unexpected) Adverse Event/Reference Safety Information

An adverse event is considered unlisted if the nature or severity is not consistent with the applicable product reference safety information. For a medicinal product(s) with a marketing authorization, the expectedness of an adverse event will be determined by whether or not it is listed in the applicable product information.

For abiraterone acetate, the link to the package insert is:

http://www.zytiga.com/sites/default/files/pdf/full_product_information.pdf

For apalutamide, the link to the package insert is:
http://www.janssenlabels.com/package-insert/product-monograph/prescribing-information/ERLEADA-pi.pdf

Special Reporting Situations

Safety events of interest for a Janssen medicinal product that require expediting reporting and/or safety evaluation include, but are not limited to:

- Drug exposure during pregnancy (maternal and paternal)

- Overdose of a Janssen medicinal product
- Exposure to a Janssen medicinal product from breastfeeding
- Suspected abuse/misuse of a Janssen medicinal product
- Inadvertent or accidental exposure to a Janssen medicinal product
- For Abiraterone acetate only, any failure of expected pharmacological action (i.e., lack of effect) of a Janssen medicinal product
- Medication error involving a Janssen medicinal product (with or without patient exposure to the Janssen medicinal product, e.g., name confusion)
- Suspected transmission of any infectious agent via administration of a medicinal product
- For abiraterone acetate only, unexpected therapeutic or clinical benefit from use of a Janssen medicinal product

These safety events may not meet the definition of an adverse event; however, from a Janssen Scientific Affairs perspective, they are treated in the same manner as adverse events. Special situations should be recorded on the Adverse Event page of the CRF.

Any special situation that meets the criteria of a serious adverse event should be recorded on a Serious Adverse Event Report Form and be reported to Janssen Scientific Affairs **within 24 hours of becoming aware of the event.**

7.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

Safety of the study interventions, including study drug treatments, and SBRT, and research biopsies will be assessed by frequent monitoring by:

- History and physical
- Laboratories
- Pill counts, drug diary review

7.3 Adverse Events and Serious Adverse Events

Adverse Event (AE)

An adverse event is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An adverse event does not necessarily have a causal relationship with the treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non- investigational) product, whether or not related to that medicinal (investigational or non-investigational) product. (Definition per International Conference on Harmonisation [ICH]).

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

Adverse Events of Special Interest. There are no adverse events of special interest identified for apalutamide or abiraterone acetate.

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. An adverse event is any adverse change (developing or worsening) from the patient's baseline (pre-treatment) condition, including intercurrent illness, which occurs during the course of a clinical study after treatment has started, whether considered related to treatment or not. Adverse event data will be collected at each patient visit.

Adverse reaction: An adverse reaction is defined as any adverse event caused by the use of a drug or other intervention. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug or intervention caused the event.

Suspected adverse reaction: A suspected adverse reaction is defined as any adverse event for which there is a reasonable possibility that the drug or intervention caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" indicates that there is evidence to suggest a causal relationship between the drug or intervention and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

Unexpected adverse reaction: An adverse event or suspected adverse reaction is considered unexpected if it is not consistent with the risk information described in the general investigational plan or elsewhere in the protocol.

A **SERIOUS** adverse event (SAE) is defined as follows:

Death: Report of any patient's death for any reason should be reported as a serious adverse event.

Suspected Transmission Of Any Infectious Agent Via A Medicinal Product: Report any transmission of any infectious agent if suspected to be transmitted by a medicinal product.

Medically Important Event*: any medically important event should be reported as a serious adverse event.

* Medical and scientific judgment should be exercised in deciding whether expedited reporting is also appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should usually be considered serious.

Life-Threatening: Report if the patient was at substantial risk of dying at the time of the adverse event or it is suspected that the use or continued use of the product would result in the patient's death.

Examples**:** Pacemaker failure; gastrointestinal hemorrhage; bone marrow suppression; infusion pump failure which permits uncontrolled free flow resulting in excessive drug dosing.

Hospitalization (initial or prolonged): Report if admission to the hospital or prolongation of a hospital stay results because of the adverse event.

Examples: Anaphylaxis; pseudo-membranous colitis; or bleeding causing or prolonging hospitalization.

Disability: Report if the adverse event resulted in a significant, persistent, or permanent change, impairment, damage or disruption in the patient's body function/structure, physical activities or quality of life.

Examples: Stroke due to drug-induced hypercoagulability; peripheral neuropathy.

Congenital Anomaly: Report if there are suspicions that exposure to a medical product prior to conception or during pregnancy resulted in an adverse outcome in the child.

Examples: Vaginal cancer in female offspring from diethylstilbestrol during pregnancy; malformation in the offspring caused by thalidomide.

Medically Significant or Requires Intervention to Prevent Permanent Impairment or Damage: Report if you suspect that the use of a medical product may result in a condition, which required medical or surgical intervention to preclude permanent impairment or damage to a patient.

Examples: Acetaminophen overdose-induced hepatic toxicity requiring treatment with acetylcysteine to prevent permanent damage; burns from radiation equipment requiring drug therapy; breakage of a screw requiring replacement of hardware to prevent poor union of a fractured long bone.

7.4 Reporting Procedures

All adverse events encountered during the clinical study will be reported on the case report form (CRF).

The intensity of clinical adverse events will be graded according to the Common Terminology Criteria for Adverse Events v 4.0 (CTCAE) grading system in the toxicity categories. The investigator must evaluate and document the adverse event for severity, grade it according to the CTCAE v 4.0, judge causal relationship to the study drug under study, take appropriate action to care for the patient and to document the outcome.

All information recorded in the case report form must be verifiably documented in the source (i.e., medical record or physician office chart).

When specific adverse events are not listed in the CTCAE, they will be graded by the Investigator as *none*, *mild*, *moderate* or *severe* according to the following grades and definitions:

Grade 0	No AE (or within normal limits)
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate; minimal, local, or noninvasive intervention (e.g., packing, cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL)
Grade 3:	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
Grade 4:	Life-threatening consequences; urgent intervention indicated
Grade 5:	Death related to AE

When an adverse event occurs, it is the responsibility of the investigator to evaluate and record into the source documents, the nature of the symptom, prescribe the appropriate remedy and to report the event. The adverse event must be reported to the IRB, pharmaceutical companies sponsoring the study drugs, FDA and any other appropriate agencies (e.g., IBC and NIH for gene medicine trials.)

Any clinical adverse event or abnormal event or abnormal laboratory test value that is serious (including death or congenital anomaly) occurring during the course of the study must be reported to the Institutional Review Board and FDA in writing as soon as is practical and in accordance with international and local laws and regulations, with a copy forwarded to pharmaceutical companies sponsoring the study drugs.

If the event is Serious and Unexpected, the event should be reported immediately (in writing within 10 days, 48 hours for death) by the Principal Investigator to the IRB, Clinical Sciences Research and Development (CSR&D) centralized Data Monitoring Committee (DMC), pharmaceutical companies sponsoring the study drugs‘ NIH and the VA IBC for gene medicine trial and to any other appropriate agency;

Any clinical adverse event is to be recorded on the case report form, managed medically as appropriate, and the event is followed until resolution. Follow-up report to the initial serious adverse event report is required to document final resolution or outcome of the event.

Abnormal Laboratory Test Values
In the event of unexplained abnormal laboratory test values, the tests should be repeated immediately and followed up until they have returned to the normal range and/or an adequate explanation of the abnormality is found.

7.5 Follow-up for Adverse Events

All SAEs occurring during the course of the study will be reported to the Principle Investigator and IRB within 24 hours of the knowledge of the occurrence. Where the initial report is made verbally or by telephone, a written confirmation within a further 24 hours must follow.

Within 24 hours of first awareness of the event (immediately if the event is fatal or life-threatening), Principal Investigator will report to Janssen Scientific Affairs, LLC, by facsimile any Serious Adverse Event (“SAE,” as defined below) that occurs during the SAE reporting period (as defined below) in a Study subject. PI will report such SAEs using

an FDA MEDWATCH form and a Serious Adverse Event Fax Cover Sheet. SAEs should be reported as soon as they are determined to meet the definition, even if complete information is not yet available.

The SAEs that are subject to this reporting provision are those that occur from after systemic treatment initiation through 30 days after last treatment.

The details regarding SAE reporting to Janssen Scientific Affairs, LLC are as follows:

SAE reporting to Janssen Scientific Affairs, LLC

Within 24 hours of awareness of a serious adverse event, whether or not related to a study drug, the Investigator will complete and submit a Medwatch 3500A Form to FDA, containing all required information (reference 21 CFR 312.32). The Investigator will submit a copy of this MedWatch 3500A form to Janssen Scientific Affairs, LLC by either e-mail or fax, within the same timeframe. If submission of this SAE to FDA or Janssen Scientific Affairs, LLC or is not possible within 24 hours, the Investigator's local drug safety contact (IRB, etc.) should be informed by phone.

The SAE documentation, including the MedWatch 3500A Form and available source records should be emailed or faxed to Janssen Scientific Affairs, LLC.

The following minimum information is required:

- Study number/IIT regulatory identifier
- Subject number, sex and age
- The date of report
- A description of the SAE (event, seriousness of the event)
- Causal relationship to the study drug or intervention

Follow-up information for the event should be sent within promptly (within 7 days) as necessary.

Non-Serious AEs. All non-serious adverse events should be reported to Janssen Scientific Affairs according to the timeframe outlined in the Research Funding Agreement section entitled Reporting of Data

Pregnancy. Because the Janssen medicinal product may have an effect on sperm, pregnancies in partners of male subjects exposed to a Janssen medicinal product will be reported by the principal investigator within 24 hours of their knowledge of the event using the Serious Adverse Event Form. Depending on local legislation this may require prior consent of the partner. Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required

Product Quality Complaint (PQC) Reporting. A PQC may have an impact on the safety and efficacy of the product. Timely, accurate, and complete reporting and analysis of PQC information from studies are crucial for the protection of patients, investigators, and Janssen Scientific Affairs, and are mandated by regulatory agencies worldwide. Janssen Scientific Affairs has established procedures in conformity with regulatory requirements

worldwide to ensure appropriate reporting of PQC information. Lot and/or Batch #s shall be collected or any reports failure of expected pharmacological action (i.e., lack of effect). The product should be quarantined immediately and if possible, take a picture. All initial PQCs involving a Janssen medicinal product under study must be reported to Janssen Scientific Affairs by the principal investigator within 24 hours after being made aware of the event. The Janssen contact will provide additional information/form to be completed. If the defect for a Janssen medicinal product under study is combined with either a serious adverse event or non-serious adverse event, the principal investigator must report the PQC to the Janssen Scientific Affairs according to the serious adverse event reporting timelines. A sample of the suspected product should be maintained for further investigation if requested by Janssen Scientific Affairs.

SAE Follow-up

For all SAEs, the investigator must submit follow-up reports to the IRB. Institutional Review Board is to be notified regarding the patient's subsequent course until the SAE has subsided, or until the condition stabilizes, the patient dies, or receives alternative therapy.

Local reporting for data and safety monitoring for the protocol will require SAE's to be reported to the Office of Research Subject Protection (ORSP using the Adverse Event Reporting form and the FDA MEDWATCH SAE reporting form.

On the anniversary date of the IRB approved protocol, the principal investigator will be required to report to the IRB, the number of patients entered on the trial, the number of patients treated, a summary of all adverse events reported to date using CTCAE version 4.0 grading, a specific list of serious adverse events requiring immediate reporting, and significant literature reporting developments that may affect the safety of participants or the ethics of the study.

The IRB will review annual data and safety monitoring reports and make recommendations on whether the study should continue unchanged, require modification/amendment, or be closed based on unacceptable risk to participants.

7.6 Safety Monitoring

Data and Safety Monitoring Plan

Overview. The VA Clinical Sciences Research and Development (CSR&D) centralized Data Monitoring Committee (DMC) will monitor this study.

The CSR&D DMC provides an ongoing independent evaluation of the progress of studies, including participant accrual and retention, adverse events monitoring, and analysis plan. This is a service that is provided by CSR&D to ensure independent oversight of the safety and integrity of this project. No other DMC or Data and Safety Monitoring Board (DSMB) review is needed.

The DMC office is located within the Cooperative Studies Program Coordinating Center (CSPCC) at the Edward Hines, Jr. VA Hospital in Hines, IL.

The DMC Office at Hines then communicates directly with the PI to provide DMC related information.

After the DMC has recommended approval for this trial to begin and the first participant has been randomized, a progress report will be due in approximately four months and then every four months thereafter (April 15, August 15 and December 15). The PI typically will not need to call in for the DMC review after the initial review.

The DMC makes recommendations to the director of CSR&D for endorsement. The recommendations range from approval (unconditionally or with conditions to be addressed) to probation to termination.

Following each DMC meeting, the PI will receive meeting minutes containing any action items for which a response will be requested typically within 30 days. Then the process starts all over again for the next reporting period.

In addition to the quarterly progress reports, all Serious Adverse Events are to be reported to the DMC.

Level of Risk of a Study

Level of Risk of a Study

The intensity level of study oversight is determined by the risk category. Some of the factors that are considered when assigning the Level of Risk category include:

- A biostatistical design and appropriate procedures for proper data management so that the information collected can be properly validated.
- Appropriate Serious Adverse Event reporting procedures must be in place.
- The study duration must be appropriate and must be based on a realistic rate of enrollment.
- Data collection and data management must be adequate to verify and ensure subject eligibility.

Assignment of risk

Assigning risk ensures that the data and safety monitoring is based on the level of risk (low, medium, or high) to ensure that the data and safety monitoring activities are appropriate. Below are some of the criteria used to make a decision regarding the assignment of risk:

- Expected duration of the study based upon the estimated rate of enrollment.
- Type of study population (e.g., children, geriatric)
- The procedures used in the trial are commensurate with the degree of risk.
- Adequate data management systems in place and appropriate case report forms
- Proper serious adverse event reporting procedures in place
- Proper biostatistical design and data analysis procedures in place.

Level of Risk

Level 2

- Compliance Officer meets with PI/Staff prior to study initiation; review regulatory requirements and operating system. Compliance Officer provides real time monitoring to determine eligibility prior to enrollment onto the protocol.
- Real time QA monitoring of the subjects and data collection occurs for all subjects entered onto the trial.
- Comprehensive QA auditing within first year or first 10 subjects enrolled, whichever comes first. Subsequent audit frequency will be annually.
- Frequency of DSMB Summary Report is typically on a biannual basis or approximately every six months.

8. INTERVENTION DISCONTINUATION

Intervention can be stopped at any time per patient or treating physician choice.

Study follow up will be discontinued for the following reasons:

- Unacceptable toxicity
- Patient no longer under the care of enrolling physician.
- Withdrawal of informed consent (Subject's decision to withdraw for any reason)

In the event of an adverse event, further study interventions may be discontinued as judged by the physician based on the severity and nature of the event.

Patients must maintain adherence to study drugs and other treatments (≥ 80% of treatment intervention pills taken as determined by pill count during follow-up visits at Days 30, and during scheduled visits until last day of study drug treatment; patients must complete all SBRT treatments and undergo prostatectomy. Patients who do not meet these criteria will be censored, replaced, and accordingly, additional patients will be recruited and enrolled for this purpose.

9. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

This is a single arm Phase II clinical trial in patients with newly diagnosed M1a,b prostate cancer and 1-5 radiographically visible metastases treated with radical prostatectomy (and post-operative fractionated radiotherapy for pT≥3a, pN1, or positive margins) or radiotherapy (including inclusion of the pelvic lymph nodes), metastasis directed SBRT, and complete ADT with LHRH analog leuprolide, abiraterone acetate, and apalutamide (ARN-509) for a total of six months of systemic therapy. The primary endpoint of our study is the percent of patients achieving a serum PSA of <0.05 ng/mL six months after recovery of serum testosterone ≥150 ng/dL for patients treated with primary radical prostatectomy and PSA of < nadir + 2 ng/mL six months after recovery of serum testosterone ≥150 ng/dL for patients treated with primary radiotherapy. [Based on prior studies, >90% of patients treated with ADT for six months will recover serum testosterone to ≥150 ng/dL within six months after cessation of ADT with a median time to recovery of three month (1,2).] This primary endpoint was selected to offer a rapid and sensitive assessment of treatment

efficacy. PSA is a biomarker for disease burden in prostate adenocarcinoma and offers a non-invasive and sensitive assessment of disease control after treatment in the vast majority of patients. Durable PSA control in the context of recovery of gonadal function (testosterone ≥150 ng/dL) after ADT is therefore a surrogate for disease control.

9.2 Sample Size

Currently, metastatic prostate cancer patients are treated with ADT indefinitely. Based on data from prior trials of discontinuous ADT in prostate cancer patients with metastatic disease, essentially all patients will relapse with a rising PSA after discontinuation of ADT (33,34).

The current study hypothesis is that 20% of patients will achieve a PSA <0.05 ng/mL six months after recovery of testosterone to >150 ng/dL. A sample size of N=25 evaluable patients has an 80% power to test the null hypothesis response rate of 5.5% against a two sided alternative response rate of 20% at a significance level (alpha) of 0.1. Assuming 10% of patients may not recover testosterone to ≥150 ng/dL, we anticipate a total study N=28.

9.3 Outcomes

9.3.1 Primary outcomes

The primary endpoint of our study is the percent of patients achieving a serum PSA of <0.05 ng/mL six months after recovery of serum testosterone ≥150 ng/dLfor patients treated with primary radical prostatectomy and PSA of < nadir + 2 ng/mL six months after recovery of serum testosterone ≥150 ng/dL for patients treated with primary radiotherapy. PSA and testosterone are checked every 30 days after completion of systemic therapy for the first six months and then every three months thereafter.

9.3.2 Secondary outcomes

- Time to biochemical progression
- Time to radiographic progression
- Time to initiation of alternative antineoplastic therapy
- Prostate cancer specific survival
- Assessment of health related quality of life using the Functional Assessment of Cancer Therapy - Prostate (FACT-P) scale
- Safety and tolerability
- Correlative analyses (described in appendix)

9.4 Data Analyses

Primary outcome.
The primary endpoint of our study is the percent of patients achieving a serum PSA of <0.05 ng/mL six months after recovery of serum testosterone ≥150 ng/dL for patients treated with primary radical prostatectomy and PSA of < nadir + 2 ng/mL six months after recovery of serum testosterone ≥150 ng/dL for patients treated with primary radiotherapy. [Based on prior studies, >90% of patients treated with ADT for six months will recover serum testosterone to ≥150 ng/dL within six months after cessation of ADT with a median time to recovery of three month (29,30).] This primary endpoint was selected to offer a rapid and sensitive assessment of treatment efficacy. PSA is a biomarker for disease

burden in prostate adenocarcinoma and offers a non-invasive and sensitive assessment of disease control after treatment in the vast majority of patients. Durable PSA control in the context of recovery of gonadal function (testosterone ≥150 ng/dL) after ADT is therefore a surrogate for disease control. It is expected that determination of the primary endpoint for each patient will occur between 9 and 12 months after completion of systemic therapy. PSA and testosterone are checked at least every 90 days after completion of systemic therapy.

Correlative Analyses.
Described in Section 15.

10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

Data will be collected for each patient, including age and general medical history. To assess for eligibility and primary/secondary outcomes, the following will be performed and collected: routine blood tests, urine analysis, PSA and testosterone, NaF PET with diagnostic CT scans of the chest/abdomen/pelvis. Additionally, tissue from the primary tumor and metastatic biopsy prior to therapy, blood, and (recommended) biopsy at progression are used to perform correlative analysis.

IHC studies will be performed in the Pathology Core of the Prostate Cancer SPORE and data maintained on an Excel Spreadsheet. The sequencing studies will be performed and only coded data transmitted for analysis. Codes will be maintained on a password-protected computer in the PI's office.

10.2 Data Management

Access to individual identifiable patient information will be available to the PI, the co-PIs, and study staff. Case report forms will be generated to track clinical, laboratory, and radiographic data. The study site will maintain records for its patients; data will be compiled by the PI and co-PIs for overall analysis.

10.3 Quality Assurance

10.3.1 Training

All staff has and will continue to undergo appropriate training related to human subjects.

10.3.2 Protocol Deviations

Protocol deviations will be recorded at the site at which they occur and documented with a note to chart. Deviations will be reported to the coordinating site and sponsor (West LA VA).

11. PARTICIPANT RIGHTS AND CONFIDENTIALITY

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki as stated in 21 CFR §312.120(c)(4); consistent with GCP and all applicable regulatory requirements. The Investigator must comply with the applicable regulations in Title 21 of the Code of Federal Regulations (21 CFR §50, §54,

and §312), GCP/ICH guidelines, and all applicable regulatory requirements. The IRB must comply with the regulations in 21 CFR §56 and applicable regulatory requirements.

11.1 Institutional Review Board (IRB) Review

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB responsible for oversight of the study. Subject recruitment materials and any other written information to be provided to subjects are also reviewed and approved by the IRB before any protocol related procedures are performed on any subjects. The study will also be approved by the local IRB's at the other sites prior to initiation.

11.2 Informed Consent Forms

A signed consent form will be obtained from each participant. For participants who cannot consent for themselves, such as those with a legal guardian (e.g. person with power of attorney), this individual must sign the consent form. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy will be given to each participant or legal guardian and this fact will be documented in the participant's record.

11.3 Participant Confidentiality

All patient reports and clinical samples will be identified by an assigned coded number/letter to maintain patient confidentiality (e.g. sample 1B). The PI will maintain a log of patients' codes, names, and contact information, which will be kept in a locked cabinet in his office, which in turn will remain locked in his absence. Every effort will be made to keep all documents with patient identifiers under the strictest confidentiality. Information that is collected during the study will be stored at the research site: paper copies will be kept in the safe, and computer files will be protected by passwords.

However, by signing the consent form, patients are authorizing the use and disclosure of identifiable health information relevant to this study. This information comes from medical records and research study-specific information that is obtained for the purposes of this study.

Patient records, the research information, and the informed consent forms may be inspected by a representative of the California Department of Health, the FDA, the National Institutes of Health, the IRB, and Janssen Scientific Affairs, LLC. Thus, because of the possibility that information may be released to one of these regulatory institutions, absolute confidentiality cannot be guaranteed. Results of this study may be published, but the names or identities of subjects will not be revealed, and medical records will remain confidential unless the disclosure is required by law.

Research study files and specimens will be kept for up to 10 years after completion of the study. Remaining samples and research files will have the sample number removed and files will be destroyed as described in Health Authority (i.e. the FDA or EMA) guidelines. Specimens (tissue and blood) may be used outside of what is outlined in this protocol, if these investigations may lead to improved understanding of cancer biology and treatments.

11.4 Study Discontinuation

The study may be discontinued at any time by the IRB, the NIH, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research participants are protected.

12. FUNDING AND INSURANCE

The study sponsor (VA Greater Los Angeles Healthcare System, with drug support from Janssen Scientific Affairs, LLC) is funding the non-standard of care interventions.

13. PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by the policies and procedures of the IRB's of the participating sites. Patient confidentiality will be maintained in any presentation, abstract, or manuscript.

14. SUPPLEMENTS/APPENDICES

14.1 *A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep/cancer.gov).*

14.2 Investigators' Brochures (see attached).

14.3 ECOG Performance Status.

ECOG PERFORMANCE STATUS*

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	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	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

*As published in Am. J. Clin. Oncol.:*Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J ClinOncol 5:649-655, 1982.*

14.4 Recommended Procedures for Radiographic-Directed Biopsy.

Patients are positioned by radiology and conscious sedation may be administered to improve patient comfort. This is based on the judgment of the interventional radiologist and may be restricted in some centers due to the availability of supportive services.

During the image-guided biopsy procedure we recommend the following general rules when choosing a metastatic site and a specific location within the metastatic tumor for biopsy:

1. If the patient has bone only disease, the pelvis is the preferred site for biopsy.
2. Any biopsy kit capable of obtaining core biopsies from bone is acceptable. The Bonopty Coaxial biopsy system with eccentric drill (http://www.vasocare.co.kr/product04-5.html).http://www.vasocare.co.kr/product04-5.html) is a recommended system. Internal diameter is 1.3mm. This is the smallest for collection of usable material.
3. In the bone, extremely blastic lesions seldom yield usable material. Yields are greater if biopsies are performed in marrow of abnormal signal intensity directly adjacent to blastic lesions. Often, we pass the needle tangential to the blastic lesion in the marrow space for our most successful collections.
4. In soft tissue, do not biopsy from regions of metastatic lesions that appear necrotic on CT or MRI due to extremely poor yield.
5. It is important that excessive compressive force is not required or used to expel the biopsy from the biopsy needle. This obscures cellular morphology. If it is difficult to expel the biopsy from the core needle, alternative biopsy kits should be used.

The Principal Investigator, Dr. Nickols, should be contacted directly with any questions regarding the most appropriate approach to collect biopsy material from specific patients enrolled in the trial.

Please follow the directions below when processing the biopsy material:

Required Materials
• Disposable cryo-molds 15x15x5mm
• Sakura Tissue-Tek OCT compound
• Metal plate or tray
• Sarstedt black permanent marker
• Tissue-Tek Mega-Cassettes
• Styrofoam cooler (part of shipment box, a new box will be returned once the biopsies arrive at UCSF)
• Container of neutral buffered formalin (2 provided, keep extra, an additional vial will be included with the new box)
• Dry Ice (pellet form, smaller pellet size works best)
• Syringe needle (core manipulation; ≤19g)
• Forceps (fine toothed)

Recommended Protocol for Biopsies
1. Prepare a cold working surface by filling the supplied styrofoam cooler container roughly half full of dry ice. Shake the container to form a fairly even layer.
2. At the collection site, lay out supplies needed for sample handling.
3. Place several cryomolds on the dry ice allowing them to cool down prior to the biopsy.
4. Label 5 tissue cassettes, then place on dry ice to pre-chill before use during the procedure.
5. Take the cooler setup to the location of the biopsy and be sure to have easy access to it during the procedure. Be sure to take the formalin vial in the zip-lock bag as well.
6. Once the radiologist says a core is about ready, fill one of the cryomold trays

roughly half-full of OCT so that the compound is partially frozen by the time the core is added. The OCT compound will appear opaque as it solidifies.

7. Transfer a core to the partially solidified OCT layer in the cryomold. The best method of tissue transfer from the biopsy needle to the pallet will depend on the type of needle used. The core biopsy can be directly placed into the cryomold of OCT, but care must be taken to ensure the core is lying as close to flat as possible as it freezes. Bonopty and other hollow core needles will yield a cylindrical core that is ejected via a plunger needle. The pressure created by the plunger directly onto the core can unpredictably eject the core from the needle. If the core cannot be placed directly on the OCT, it can be ejected onto a gauze pad and transferred immediately onto the OCT so as to minimize time for RNA degradation and protein de-phosphorylation. This helps to maintain needle sterility and allows for more careful manipulation of the core away from the sterile procedure cart. Manipulation of the core from gauze pad to cryomold is done with a syringe needle.
8. Positioning of the core is very important. Be sure to place the core flat down on the center of the OCT layer. The tissue can freeze on contact if the OCT layer has solidified, thus preventing manipulation once the tissue is on the OCT surface. Adding the core to OCT prior to solidification is ideal since the tissue and OCT will then freeze at the same time, resulting in better sectioning later. Layers frozen in different stages tend to cause separation during sectioning.
9. Immediately cover the core with OCT, working in a slow circular motion around the core such that OCT fills in the sides, then surrounds the top. Contact of the liquid OCT with the tissue core is important to preserve the tissue and facilitate in cryo sectioning later.
10. Once the OCT becomes opaque in each cryomold, the tissue block is ready to be transferred to the labeled cold cassette.
11. In addition to collecting fresh-frozen biopsy cores, up to 3 core must be collected for paraffin embedding. This core should be placed in the container of neutral buffered formalin. Close the jar tightly, and return to the zip-lock bag.
12. Package the biopsies into the provided insulated box. The cold cryo cassettes should be placed in dry ice in the insulated section of the box, and the formalin vial contained within the zip-lock bag should be placed outside of the insulated region of the box. The appropriate spot for the formalin vial will be labeled.
13. Include with the shipment, the sample shipment log (provided by sponsor, West LA VA) that should include comments regarding core quality (long cylindrical core, bone shards, mostly blood clot, etc), the quantity of cores and any irregularities in the freezing process. The technician performing the collection should be sure to note their unique patient ID, the biopsy date, and the time of the procedure as well.
14. Prior to any shipments, the study coordinator and designated laboratory staff at West LA VA must be notified. Notification should come in the form of email at least 2 – 3 days or, if possible, a week prior to an anticipated shipment. The email should consist of the date of scheduled biopsy, unique patient ID, participating site name and site contact; this should be sent to Nicholas.nickols@va.gov / nnickols@mednet.ucla.edu (study PI).
15. Mail by Express Overnight delivery. Unless special arrangements are made, do not ship on Fridays, Saturdays, or Sundays.

14.5 Recommended Prednisone Taper after discontinuation of abiraterone acetate.

Weeks after discontinuing abiraterone acetate	*Prednisone dose (mg/day)*
0	*9*
2	*8*
4	*7*
6	*6*
8	*5*
10	*4*
12	*2*
14	*1*
16	*0.5*
18	*0*

15. ***CORRELATIVE ANALYSES***

Our prospective Phase II trial of prostatectomy, metastasis directed stereotactic body radiation therapy (SBRT), and systemic therapy in newly diagnosed M1a,b oligometastatic prostate will yield evaluable tissue (metastatic lesions through biopsy and the primary malignant prostate by prostatectomy) from previously untreated prostate cancer patients with metastatic disease. We will identify the primary tumor in each patient that gave rise to the biopsied metastasis, and compare these potentially lethal primaries with the other intraprostatic tumors that likely did not yield metastases. We will also compare these potentially lethal primary tumors to their corresponding metastases. In doing so, we will **(1)** identify characteristic features of potentially lethal primary prostate tumors that are destined for metastasis, and **(2)** identify which pathways become activated within these potentially lethal primary tumors during or after the metastatic event. Importantly, these investigations all use tissue that is acquired at the outset for each patient enrolled in our trial, and these investigations do not depend on the clinical outcome of the trial.

I. SPECIFIC AIMS FOR CORRELATIVE ANALYSES

Aim 1. What genomic and transcriptomic features are present in lethal intraprostatic tumors that ultimately give rise to metastatic disease that are absent, or different, in those intraprostatic tumors that do not? To answer this question, we will conduct whole exome deep sequencing (WES), RNA sequencing (RNA-seq), and comparative genomic hybridizations (CGH) of the metastatic and intraprostatic tumors of the 28 patients enrolled in our Phase II trial. This will enable identification of the “true” primary tumors from which the biopsied metastases arose. Then, we will combine the aforementioned genomic analyses with RNA-seq data to identify discriminating features of the “true” primary lesions that distinguish them from the intraprostatic tumors that did not yield these metastatic lesions.

1.1 To identify the intraprostatic/ primary tumor in each patient gave rise to the biopsied metastatic lesion.

1.2 To discover genomic and transcriptomic features specific to the intraprostatic /primary tumors that ultimately gave rise to metastases versus those that did not.

Aim 2. What genomic and transcriptomic features are present in the metastases that are absent, or different, from the corresponding primary tumors from which they metastasized? This will involve comparison between the “true” primary tumors identified

in **Aim 1.1** and their metastases. We will also specifically interrogate selected markers of kinase activity (MEK/ERK) and differentiation (EMT).

2.1 To discover genomic and transcriptomic features specific to metastatic prostate tumors as compared to the primary tumors from which they arose.

2.2 To evaluate MAPK (ERK) activation and markers of epithelial to mesenchymal transition (EMT) in metastatic prostate cancer lesions as compared to their corresponding primary tumors.

Aim 3. What genomic and transcriptomic features are present in circulating tumor cells and DNA that predict for response? Are there changes in the immunophenotype of circulating lymphocytes over the course of treatment?

3.1 To evaluate biomarkers of response using circulating tumor cells (CTCs).
3.2 To evaluate biomarkers of response using circulating tumor DNA (ctDNA).
3.3 To evaluate immunophenotypes of circulating immune cells.

II. **Unique opportunities for translational investigation**. Our trial, which offers the opportunity to assess both the untreated malignant prostates as well as the matching metastatic tumors in the same homogenous and untreated cohort, thereby lends itself to the following provocative correlative studies:

1. **Identification of the intraprostatic lesion from which the metastatic tumors arose.** It is likely that 80% or more of clinically significant malignant prostates are multifocal (36,37). Multiple prior studies lend credence to the notion that the multiple tumors within a malignant prostate may arise independently (38–40). A recent study specifically assessing the genomic heterogeneity in multifocal prostate tumors found minimal overlap in single nucleotide variants and no overlap in copy number alterations (CNAs) between the different tumor foci within malignant prostates (36), supporting a multi-clonal model for separate tumor foci. Another recent study using tumor tissue derived from warm autopsies on 63 patients with mCRPC revealed limited genomic intra-individual heterogeneity between metastases (41), supporting the hypotheses that a single metastatic biopsy may give a reasonable portrait of the genomic landscape of metastatic disease burden within an individual patient, and that metastases within most patients likely arise from a single tumor within the primary. Although notable exceptions have been reported (42), most published studies support the notion that rare subclones from within a primary tumor initially seed metastasis, which then go on to seed additional sites, often metastasis-to-metastasis (43). In **Aim 1.1**, we identify the primary lesion that gave rise to the biopsied metastasis in each patient in our trial.

2. **Discovery of genomic features of potentially lethal primary prostate tumors that give rise to metastatic lesions versus those that do not.** It has been hypothesized that the index lesion (largest lesion with the highest grade) within a malignant prostate may be primarily responsible for disease progression (44,45). Based on this hypothesis, a number of clinical trials aimed to investigate focal therapies (such as HIFU) directed against the index lesion, some with a high failure rate (46). A critical question remains: what genomic features characterize the potentially lethal primary prostate tumors from the more indolent intraprostatic tumors co-existing within the same malignant prostate? Prior studies have traced the evolutionary history of prostate tumors over time using genome sequencing. In

one report, surprisingly, the lethal metastatic clone arose from a region of low-grade tumor within the prostate in the presence of other, larger lesions of higher grade (47). In **Aim 1.2,** we compare the prostate tumors that gave rise to the biopsied metastases to those that did not, in order to better define the distinct biology of potentially lethal primary prostate cancers.

3. **Discovery of pathways directly associated with the emergence of metastases.** Both localized and metastatic castrate resistant prostate cancers (mCRPCs) are characterized by frequent CNAs, gene fusions, and rearrangements (29,30). Some characteristic differences between primary and mCRPCs have been identified, which include a greater frequency of androgen receptor gene amplification or pathway alteration (50% vs 90-100%), *pten* loss or PI3K/AKT activation (50% vs 90-100%), *tp53* mutations (3-20% vs 50%), and MAP Kinase (ERK) pathway activation (30% vs 90-100%), for primary and mCRPC, respectively (31). Understandably, prior studies assessing metastatic prostate cancer analyzed castrate resistant tumors taken from heavily pre-treated patients on long term ADT (30,32), and principally compared them to localized prostate tumors in The Cancer Genome Atlas (TCGA) (32). As such, in these studies the *initial* genomic events driving metastasis may be obscured through subsequent genomic and transcriptomic adaptations occurring within tumors during systemic therapy. Furthermore, the prostate tumors represented in the TCGA include some potentially lethal primary tumors but also a large number of primary tumors that would likely never have progressed (48). In our study, we will be able to compare, directly, the pathways activated in the metastatic lesions versus the specific primary tumor from which these metastases arose. We conduct this analysis in **Aim 2**.

4. **Discovery of genomic biomarkers and pathways in patients with oligometastatic prostate cancer who have a favorable response to treatment.** The total duration of active therapy in our trial is a relatively brief 6 months from prostatectomy to completion of systemic therapy. Our trial uses a rapid efficacy endpoint: PSA <0.05 ng/mL six months after recovery of testosterone to 150 ng/dL. We will use this marker of treatment efficacy (responders vs non-responders) to perform pattern recognition/discovery of factors that may be characteristic of the tumors from patients with a favorable response. This may allow for identification of markers characteristic of a favorable response to therapy in oligometastatic prostate cancer patients. Although this analysis is not a part of this proposal (because not all patients will have a primary endpoint determination within two years), we will conduct this analysis in due course.

5. **"Liquid biopsy" by detection of circulating tumor cells.** We will incorporate evaluation of circulating tumor cells (CTCs) detected in enrolled patients pre-treatment using VortexChip, a technology enabling of detection of CTCs and downstream evaluation by next generation sequencing on as few as three CTCs per sample.

III. PRELIMINARY DATA

A. Epithelial to Mesenchymal Transition (EMT) is associated with acquisition of the metastatic phenotype. Our group has a strong interest in understanding the

mechanisms that underlie the development of metastasis. EMT is a key developmental process in which epithelial cells acquire a migratory and invasive phenotype characterized by the coordinated loss and gain of various epithelial (e.g. E-cadherin) and mesenchymal (e.g. N-cadherin and vimentin) markers, respectively. In our model systems, we have shown that EMT is a common response of both normal and malignant prostates to castration, which in the case of prostate cancer is sufficient to cause metastasis (49–51). The expression of an EMT has been validated in gene expression datasets of metastatic prostate cancer (49–52). Preclinical studies by our groups demonstrate that therapeutic blockade of EMT or EMT-associated genes can inhibit metastasis and can synergize with androgen ablation to prevent castration resistance (49–51). Numerous studies by our group and others have implicated SRC in the development of castration resistance (53,54). We have shown that SRC activation can induce castration resistance and also EMT (43,52,54). SRC activation was associated with activation of the MAPK pathway, a finding that suggests that cross-talk between SRC and the MAPK pathway may be operative in the development of castration resistance, EMT, and metastasis. We therefore postulated that development of EMT, driven by SRC and MAPK, may underly development of metastasis.

B. MAPK: a potential driver of metastases. Our team, as part of the Stand up to Cancer / West Coast Dream Team (SU2C/WCDT), investigated transcriptomic differences between mCRPC and localized prostate cancer through RNA-sequencing of laser capture microdissected tumor tissue obtained from metastatic biopsies of patients with mCRPC (n=53 in this analysis) as compared to localized prostate cancer (TCGA, n>600). To identify evidence of activation of protein kinase pathways from RNA-seq data sets, we employed a bioinformatics tool known as Virtual Inference of Protein-activity by Enriched Regulon analysis (VIPER analysis), which infers kinase activity based on gene expression changes attributable to the kinase itself as well as downstream transcription factors. ERK was identified as the most hyperactivated kinase in the mCRPC cohort compared to the primary prostate cancer cohort (**Figure 6A**) and its activation was more than 140-fold higher in mCRPC metastases (**Figure 6B**), although there is some overlap between the cohorts at the upper and lower extremes of the TCGA and SU2C cohorts, respectively. Additionally, phosphorylated ERK, a marker of MAPK activation, was detected in 80% of bone metastases in a separate series of 30 patients with mCRPC. These results suggest MAPK activation is a characteristic of mCRPC and has led our team to embark on a PCF funded Phase II clinical trial of the MEK inhibitor trametinib in patients with mCRPC. (ERK is the phosphorylation target of MEK).

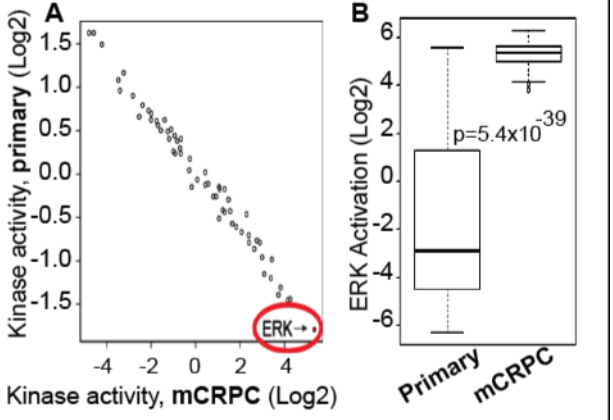

Figure 6. ERK is hyperactivaated in metastatic mCRPC as compared to primary prostate cancer. **A.** Scatter plot shows ERK is the most highly activated kinase (of 61 kinases evaluated) in metastases from mCRPC patients (SU2C WCDT cohort) as compared to primary prostate tumors (TCGA cohort). **B.** Box plot illustrating magnitude of ERK activity in primary versus mCRPC.

However, a slightly different interpretation of the aforementioned data implicating MEK/ERK activity in mCRPC, which is consistent with our prior work implicating MAPK in the acquisition of EMT, is that the ERK hyperactivation in mCRPC may, in fact, constitute an early event required for metastasis, and that the small but noticeable overlap in ERK activation between the mCRPC and TCGA cohorts, represents those potentially lethal primary tumors (or their subclones) within TCGA that ultimately metastasized. We are currently supplementing this analysis by correlating p-ERK staining of primary prostate cancer TMAs with clinical outcomes in our database that includes clinical outcomes over the last 25 years (since 1991).

In fact, there is pre-clinical evidence that MAPK activation may be an *early* event driving prostate cancer metastasis. In an autochthonous murine model of localized prostate cancer, activation of MAPK induced widespread metastases associated with epithelial to mesenchymal transitions in the absence of androgen suppression (51). In this model, pharmacologic inhibition of MEK suppressed metastatic growth with minimal effect on the primary tumor (51). We therefore hypothesize that MAPK activation is an *early* event in prostate cancer metastases, which may occur prior to, and independent of, androgen suppression. (This hypothesis does not preclude additional crosstalk between the MAPK pathway and other pathways associated with later emergence of castration resistance).

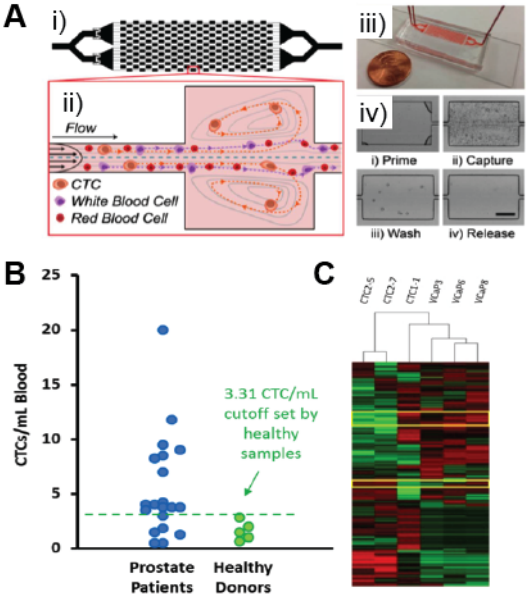

Figure 7. Vortex Chip. **A.** Overview. i) Parallelized with 16 parallel channels and 12 serial reservoirs in each channel. ii) Laminar microvortices trap larger cancer cells while smaller blood cells pass through. iii) The device consists of molded PDMS bonded to glass and contains two inlets, one for sample and one for wash, and one outlet. iv) Sample processing involves priming, infusing, washing and releasing. **B.** More than three CTCs are detected in each mL of blood in 75% of patients with metastatic prostate cancer (N=20). **C.** RNA-sequencing using three CTCs from 2 patients. The two CTCs from the same patient cluster together. RNA-sequencing of three VCaP cell lines also cluster together.

C. Circulating tumor cells (CTCs) represent a promising method to obtain genetic information about tumors as they evolve in response to treatment, as a "liquid biopsy." Many studies have shown the promise and potential of analysis of CTCs, though many currently available methods are protracted and require lengthy preprocessing and processing steps, limiting the viability of collected cells. To circumvent these limitations, the 'Vortex Chip' technology for isolating CTCs using microfluidic chips (55,56) (**Figure 7**). This technology is inexpensive, and utilizes vorticeal fluid flows to capture cells by size alone based on differential trapping and without the need for surface labeling. Accordingly, cells that have an epithelial phenotype as well as those that have undergone EMT are captured with our Vortex Chip. The processing of an 8 mL sample is rapidly completed within ten minutes (55,56), and typically achieves CTC purity of >80%. Furthermore, captured CTCs are

viable and can be cultured or sent for downstream analyses such as immunohistochemistry or DNA and RNA sequencing.

We have successfully performed CTC isolation and downstream analyses on dozens of patients with advanced prostate cancer, including DNA and RNA sequencing on individual and pooled CTCs. **Figure 7C** demonstrates representative results from three CTCs isolated from two distinct patients. RNA sequencing was performed on each of these cells and compared to the results of single cell RNA sequencing of three individual VCaP cells. Single cell RNA-seq data was mapped on hg19 human genome normalized to exonic reads, by length of the gene and by reads per kilobase per million mapped exonic reads (RPKM). Clustering was then performed using Pearson's correlation with average linkage. The results show clustering between CTCs from patient #2 (CTC2-5 and CTC2-7) and CTC from patient #1 clusters separately. VCaP cells cluster tightly together.

IV. HYPOTHESES

- The primary tumors that give rise to metastases may have genomic features that are distinguishable from those lesions that do not.
- Genomic analyses of these *de novo* metastatic prostate cancers compared concurrently to the specific primary tumors from which they arose will identify pathways and genomic alterations directly associated with the emergence of metastasis. These analyses will not be obscured by adaptive responses to prior therapies, or to genomic features of indolent prostate tumors without metastatic potential. (These are some limitations of prior studies that have evaluated the genomics of metastatic prostate cancer as compared to localized prostate cancer).
- EMT and MAPK activation are associated with the emergence of metastasis in prostate cancer.
- The tumors in patients with low volume metastatic prostate cancers that have a durable response to local, metastases directed, and systemic therapy may have biology distinct from the tumors in patients that do not have a durable response to this therapy.

V. APPROACH.

First, we will identify the primary tumor that gave rise to the biopsied metastatic tumor for each patient. Then, we will examine differences between these primary tumors that gave rise to metastases versus those that did not (**Aim 1**). Finally, we will examine differences between the metastatic tumors versus their “true” primary prostate tumors (**Aim 2**).

A. Aim 1.

We will determine the genomic relationship between the metastatic tumors to those of the tumors within the malignant prostate to identify the “true” primary tumor that gave rise to the metastatic lesion in each patient. This will involve comparative genomic hybridizations, whole exome deep sequencing, and RNA-sequencing of the laser captured microdissected metastatic and primary tumors.

1. **Rationale.** Primary prostate tumors are frequently multifocal, with malignant prostates exhibiting two or more spatially distinct foci of tumor. It has been hypothesized that the “index lesion”, defined by size and/or grade, may be responsible for disease progression. However, recent investigations call into question this hypothesis. Identification of the intraprostatic tumors in our patient cohort that gave rise to the biopsied metastases will enable specific characterizations of these lethal primary tumors.

2. **Recommended Tissue procurement and handling (for both Aims 1 and 2).**

   a. **Primary tumor tissue.** After resection, the prostate is sectioned into multiple slices. Alternating slices are kept fresh on ice, while other slices are fixed in formalin and embedded in paraffin. A GU pathologist will identify distinct loci of prostate adenocarcinoma by histopathology and the corresponding (adjacent) frozen and formalin slices will be processed for genomic/transcriptomic analyses and sectioning for immunohistochemistry. Each focus of prostate cancer will be laser capture microdissected from frozen tumor sections prior to "omics" analyses. When a single lesion exhibits areas of differing Gleason grade, an area representative of each component will be separately microdissected and subjected to analysis.

   b. **Metastatic tumor tissue**. As part of the SU2C/WCDT, our group has experience in conducting radiographically directed biopsies with 68% of attempted bone biopsies yielding tissue sufficient for sequencing analysis (with an overall success of 78% taking all metastatic sites into account). Cores (n=6-8) will be freshly frozen (n=4-6) and formalin-fixed for paraffin embedding (n=2). Laser capture microdissection of frozen specimens from biopsies will also be performed to enrich samples for tumor tissue for sequencing analyses, as is standard practice for metastatic biopsies in our SU2C/WCDT cohort.

3. **Experimental.**

   a. **For Aim 1.1**, we will perform unsupervised clustering based on CNAs (determined by CGH) of the metastatic and primary tumors for each patient. As well, we will perform unsupervised clustering based on somatic mutational status in exons, and transcript levels by RNA-sequencing. These analyses are expected to identify the intraprostatic primary tumor foci most closely related to the metastatic tumor for each patient (i.e. the metastasis generating intraprostatic tumor, or "true" primary).

   b. **For Aim 1.2**, genomic analyses will be performed for pattern recognition/discovery of factors that may be characteristic of the "true" primary tumor versus other non-metastasizing intraprostatic tumors. We will apply various bioinformatic tools for comparisons amongst the primary tumors: Pathway Recognition using Data Integration on Genomic Models (PARADIGM) and Differential Pathway Signature Analysis (DiPSC) (57–59). PARADIGM analysis integrates omics level datasets (i.e. mRNA expression, CNAs, and mutation status) to determine pathway activity and key signaling nodes. DiPSC utilizes a statistical bootstrap and Pearsons Correlation to determine the relationship of phenotype (in this case, the potentially lethal "true" primaries versus indolent primaries) to these patterns of pathway activation, for determination of discriminative networks characteristic of each phenotype. These analyses are similar to those used in the context of the SU2C/WCDT project and have been developed and applied by Ted Goldstein, PhD (UCSC).

4. **Considerations for genomic analyses (for both Aims 1 and 2).**

   Comparative genomic hybridizations will be performed on the microdissected metastatic and primary tumors for each patient and normalized against germline DNA (PBMCs) from each individual. The circular binary segmentation algorithm of

the DNAcopy Bioconductor package will be used to segment DNA copy number data and identify regions with abnormal copy number. **Data Pre-Processing**: Our pre-processing pipeline for exome and RNA-sequencing involves the following steps: *Quality control*, *Mapping reads*, *Counting reads per feature*, and *Normalization, filtering and correction for batch effects*. **Quality control**. Quality control will be performed on the raw sequence data files from exome capture and RNA sequencing experiments, which contain multiple short-read sequences with Phred quality score information. Useful tools for QC and pre-processing are FastQC and FASTX-Toolkit. FastQC aims to provide a QC report that can spot problems which originate either in the sequencer or in the starting library material. The QC metrics we will be looking at include: a) Per-base sequence quality; b) Per-sequence GC content for possible library contamination; c) Over-representing sequence potential adapter contamination; d) Duplicating sequences for enrichment bias. Once we have identified the quality issues, a collection of command-line tools called FASTX-Toolkit will be used to perform a series of pre-processing on the FASTA/FASTQ files. Our experience with similar data confirms that performing quality control and necessary pre-processing with the raw sequence data files will produce better mapping results. **Mapping reads.** Once the raw sequence data files are cleaned and quality control is performed, we will map short reads of each sample to the reference genome hg19 by a short read aligner TopHat2. TopHat2 aligns exome and RNA-sequencing reads to mammalian-sized genomes using the ultra-fast high-throughput short read aligner Bowtie2 as its core read-alignment engine. After mapping, a SAM/BAM file will be generated for each sample containing the aligned sequence data. We will use the flagstat command in SAMTools to perform quality check on the alignment files. **SNV calling.** For exome reads, VarScan will be utilized to annotate mutations and to generate single nucleotide variant (SNV) and indel calls. **Counting reads per feature**. The final step of data preprocessing is to summarize and count the aligned reads by genomic features (e.g., genes, transcripts) for each sample. A useful tool for this task is HTSeq-count. HTSeq-count counts the reads that align to each exon and sum them up for each gene. For dealing with reads that overlap more than one feature, we will use the 'union' mode setting. **Normalization, filtering and correction for batch effects**. For both exome and RNA sequencing, we will use a Bioconductor package 'edgeR' to perform normalization, filtering and batch effects correction. We will implement Trimmed Mean of M Values (TMM) normalization, which estimates scale factors between samples that can be incorporated into currently used statistical methods. We will also filter out tags with very low counts to reduce the number of features being tested and thus an increase in statistical power. For batch-effect correction, our strategy would be to include potential batch effects as additive terms when performing statistical modeling and testing. We will compute the false discovery rate (FDR) of Benjamini and Hochberg to correct for multiple testing.

B. Aim 2. We will determine genomic and transcriptomic features specific to the metastatic prostate cancers as compared to the "true" primary tumors from which they arose (identified in **Aim 1.1**), and also investigate evidence of EMT and MAPK activation in these metastatic versus primary tumors.

1. **Rationale.** Prior studies evaluating genomic and transcriptomic features characteristic of metastatic prostate cancer have relied upon tumor tissue derived from heavily pre-treated patients, potentially obscuring the drivers of the initial

metastatic event, and compared these metastatic cancers to localized prostate cancers, many of which would never have yielded metastases. Therefore, in order to specifically interrogate the immediate drivers of a metastatic event, one would ideally compare metastatic tumors to the specific intraprostatic tumor from which these metastases arose. Our own data suggest an EMT may accompany or drive metastasis, and that MAPK activation may be a characteristic feature of metastatic prostate cancers and potentially primary prostate cancers with high metastatic potential.

2. **Experimental.**

   a. **For Aim 2.1,** we will determine genomic and transcriptomic features specific to the metastatic prostate cancers versus the "true" primary tumors that gave rise to these metastases. The genomic analyses will be performed for pattern recognition/discovery of factors that may be characteristic of metastatic versus the primary tumors using the bioinformatic tools described in **Aim 1** (PARADIGM and DiPSC).

   b. **For Aim 2.2**, we will assess MAPK activity (p-ERK), and EMT markers (N-cadherin and vimentin) in each metastasis and its corresponding "true" primary tumor. IntMax scores (scale of 0-300) = (% of positive tumor cells [scale of 0-100]) X (intensity of staining based on 0-3 scale [scale of 0-3]) will be measured by quantitative image analysis for p-ERK as well as EMT markers (N-cad, and vimentin) by immunohistochemistry. Comparisons will be made between tissues from metastatic sites versus the primary tumors. Average IntMax will be compared using a paired t-test or Wilcoxon signed-rank test (if the IntMax scores do not follow a normal distribution). Fifteen evaluable tumors per group (metastasis vs primary) provides 90.5% power to detect a mean difference of 1.5 standard deviations (SDs) between groups, and 78.4% power to detect a mean difference of 1.3 SDs. The effect sizes used for this power analysis are based on our preliminary data in EMT markers expressed in local versus metastatic prostate cancer specimens and from animal model systems. We, in fact, expect to have at least 19 evaluable metastatic tumors (total N=28 x 68% successful biopsies yielding tissue for analysis = 19 evaluable specimens). Therefore, our study is likely sufficiently powered to detect differences in these histological markers.

C. **Aim 3.** Evaluation of CTCs, ctDNA, and immunophenotypes in blood. We will incorporate evaluation of circulating tumor cells (CTCs) detected in enrolled patients pre-treatment. VortexChip isolates highly purified CTCs by size alone and without need for antibody capture. Not relying on detection of epithelial markers, it allows capture of circulating tumor cells that have undergone EMT. It also enables downstream analyses by next generation sequencing.

1. We have detected CTCs in 80% of patients with metastatic prostate cancer (N=20, **Figure 3B**). In a pilot experiment, we successfully conducted RNA-sequencing in three cells from two patients with mCRPC (**Figure 3C**), and we have shown that VortexChip can capture prostate cancer CTCs that have undergone EMT and express vimentin and N-cadherin (not shown).

2. We will obtain blood from the patients at their baseline visit and capture the resulting CTCs using Vortex Chip. Samples will be included in the study if at least

5 CTCs are present for analysis. We will perform RNA sequencing and whole exome sequencing analysis on up to 20 CTCs isolated from each specimen, with a minimum of 5 cells. We will sort CTCs by expression of vimentin and N-cadherin to detect CTCs that may have undergone EMT. We will use clustering analysis of SNVs in the CTCs, and matched primary and metastatic tumors in each patient in an attempt to determine the site of origin of the CTCs.

3. Circulating nucleic acids (RNA, DNA) will be isolated using the Qiagen Circulating Nucleic Acids Kit, and quantified by the Agilent Bioanalyzer High Sensitivity DNA Kit. DNA samples will then be whole genome amplified with an equal amount of normal reference DNA.

4. PBMCs will be isolated over Ficoll-gradients and stored frozen until analysis. Lymphoid and myeloid subset phenotyping will be assessed by antibody staining and multi-color flow cytometry (LSR-Fortessa, BD). Circulating cytokines, PGE2, prostate-tumor specific antibodies, HMGB-1 protein and the tryptophan/kynurenine ratio in plasma will be quantified by ELISA. Tumor-specific CD4 and CD8 T cell activities to known HLA-matched, immunodominant, tumor-associated antigens will be assessed ex vivo by IFNγ-ELISPOT and dextramer staining. The tumor antigens of choice will include PSA and PSMA.

5. We anticipate that most patients in the trial will have detectable CTCs based on our prior experience in the detection of CTCs in metastatic prostate cancer patients, although a smaller frequency of CTC positivity is possible due to a lower expected burden of disease in this oligometastatic patient cohort. We also anticipate that the CTCs likely originate from the “true” primary tumors or metastatic tumors. However, it is possible that we may trace them to the other intraprostatic tumors in one or more patients. We anticipate that changes in lymphoid and myeloid subsets may be occur over time, as well as tumor specific T cell responses.

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