COVER SHEET

A phase II randomized trial of RAdium-223 and SABR Versus SABR for oligomEtastatic prostate caNcerS (RAVENS)

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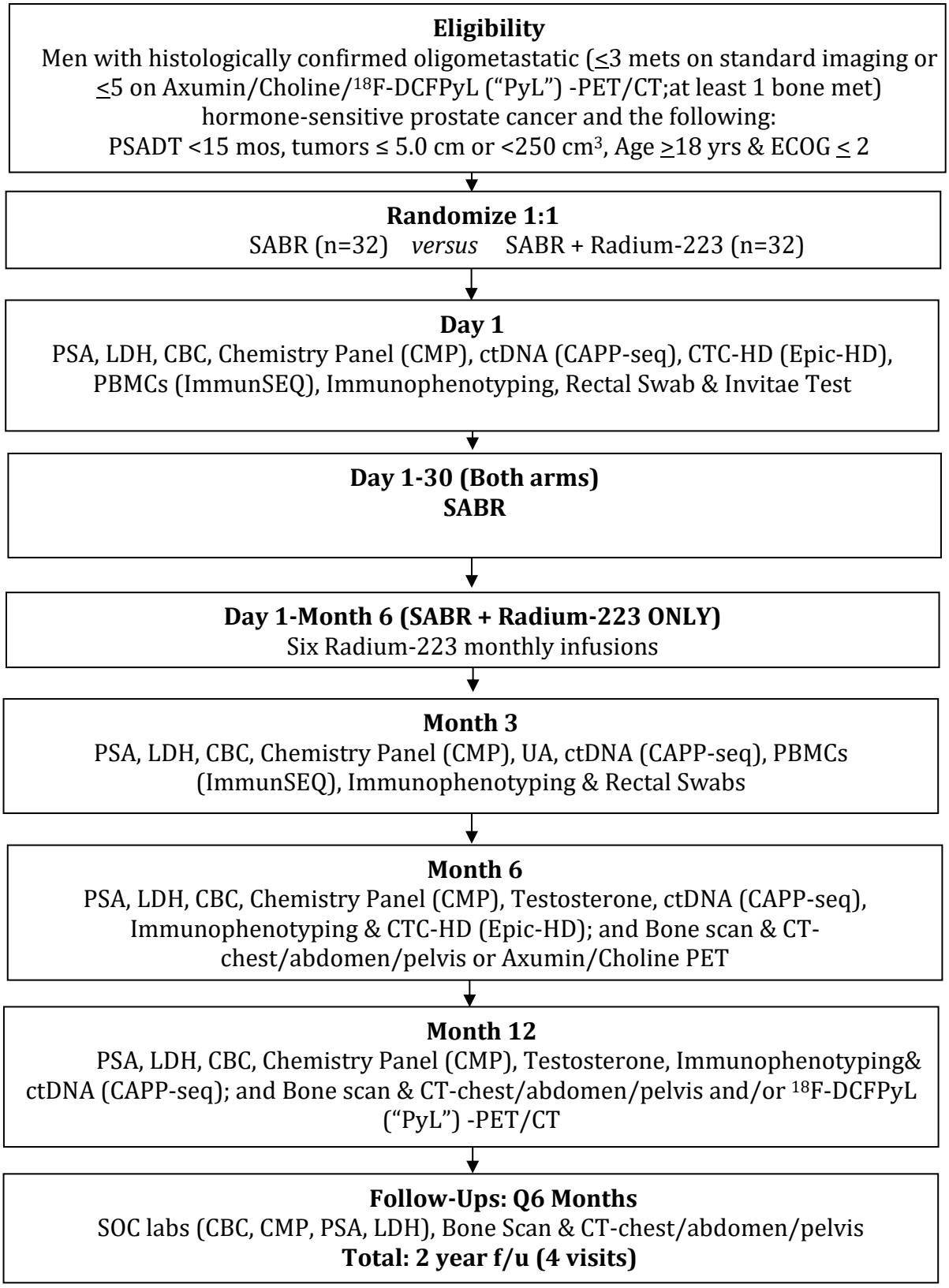

1. PROTOCOL SYNOPSIS

TITLE	A phase II randomized trial of RAdium-223 and SABR Versus SABR for oligomEtastatic prostate caNcerS (RAVENS)
STUDY PHASE	Phase II
INDICATION	Men with oligometastatic disease (≤3 mets) and at least one bone metastasis
INVESTIGATIONAL PRODUCT	Radium-223 (Xofigo®) is an alpha-emitting radioisotope developed by Algeta ASA. Radium-223 is indicated for treatment of men with castrate-resistant prostate cancer (CRPC) with symptomatic bone metastases and no known visceral metastatic disease. The approved dose and schedule of radium-223 is 50 kBq (or 1.35 microcurie) (55 kBq/kg (or 1.49 microcurie)) after implementation of the NIST update) per kg body weight intravenous (IV) infused at 4 week interval for 6 injections
PRIMARY OBJECTVES	• To assess progression-free survival of men who have oligometastatic prostate cancer after randomization to stereotactic ablative radiation therapy (SABR) *versus* SABR and Radium-223
SECONDARY OBJECTIVES	• To assess the toxicity of SABR + Radium-223 in patients with oligometastatic disease • To determine local control at 12-months after SABR *versus* SABR + Radium-223 in patients with oligometastatic disease • To assess time to locoregional progression, time to distant progression, time to new metastasis and duration of response • To assess ADT-free survival after randomization to SABR *versus* SABR + Radium-223 • To assess quality of life following SABR *versus* SABR + Radium-223

CORRELATIVE SCIENCE	• To enumerate circulating tumor cells (CTC) using EPIC HD-CTC platforms at baseline, months 3, 6 and 12 from randomization. • To enumerate circulating tumor DNA (ctDNA) using Cancer Personalized Profiling by deep sequencing (CAPP-Seq) at baseline, months 3, 6 and 12 from randomization for SABR *versus* SABR + Radium-223. • To quantitatively sequence T-cell receptor (TCR) repertoires using peripheral blood monocytes and the ImmunoSEQ platform at baseline, months 3, 6 and 12 from randomization. • To evaluate immunophenotypes by running PBMCs through a Cytek Aurora machine, which allows for simultaneous detection of up to 25 markers; to evaluate metabolic fitness of the total PBMCs using the mitochondrial stress test on the Seahorse analyzer; and to determine the relative capacity of peripheral T cells to metabolically respond to T cell receptor stimulation through activation of CD3 and CD28 by running functional assays on PBMCs. • To determine frequency of germline DNA repair mutations in the oligometastatic state • Descriptive statistics will be performed to correlate the metrics above with clinical outcomes.
HYPOTHESES	SABR + Radium-223 will increase median progression-free survival from 10 months in the SABR arm to 20 months in the SABR + Radium-223 arm
STUDY DESIGN	Men with oligometastatic prostate cancer lesions will be randomized (1:1) to SABR *versus* SABR + Radium-223. The study will NOT be blinded. Within three weeks of the initial treatment planning, SABR(1-

	5 fractions) will be administered for all men. For the SABR + Radium-223 the 1st radium-223 infusion will be within four weeks from randomization.
SAMPLE SIZE BY TREATMENT GROUP	64 patients total
SUMMARY OF SUBJECT ELIGIBILITY CRITERIA	1.Metastatic bone or nodal sites ≤ 3 on standard imaging (or ≤ 5 on Axumin, Choline with at least one bone metastasis. 2.PSADT <15 months 3.Tumor ≤ 5.0 cm or <250 cm3. 4.Age ≥ 18 years. 5.ECOG perfomance status ≤ 2. 6.Histologic confirmation of malignancy (primary or metastatic tumor).
CONTROL GROUP	N/A
PROCEDURES	1. Physical exam 2. CT/MRI and Bone scan and/or ^{18}F-DCFPyL (“PyL”) PET/CT of Involved Site 3. Randomization 4. Blood draws, Rectal Swab and Invitae Test 5. SABR *versus* SABR + Radium-223 6. CT/MRI Scan and Bone Scan or Axumin/Choline-PET of Involved Site at 6 months 8. ^{18}F-DCFPyL (“PyL”) PET/CT at 360 Days from first “PyL”

2. ABBREVIATIONS AND DEFINITIONS OF TERMS

ADL	Activities of daily living
AE	Adverse event
BID	Twice daily
BSA	Body surface area
CBC	Complete blood count
CI	Confidence interval
CMAX	Maximum concentration of drug
CNS	Central nervous system
CRF	Case report/Record form
CR	Complete response
CRPC	Castration-Resistant Prostate Cancer
CTC	Circulating Tumor Cell

CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
GI	Gastrointestinal
Hgb	Hemoglobin
HIV	Human Immunodeficiency Virus
HPF	High-power field
HTN	Hypertensions
IRB	Institutional Review Board
IV	Intravenous
LLN	Lower limit of normal
MTD	Maximum tolerated dose
OS	Overall survival
PLT	Platelet
PD	Progressive diseased
PFS	Progression free survival
PR	Partial response
PSADT	PSA Doubling Time
PSMA	Prostate Specific Membrane Antigen
QD	Once daily
RECIST	Response evaluation criteria in solid tumors
RR	Response rate
SABR	Stereotactic ablative radiation therapy
SAE	Serious adverse event
SBRT	Stereotactic body radiation therapy
SD	Stable disease
TTP	Time to progression
ULN	Upper limit of normal
UNK	Unknown
WBC	White blood cell

3. STUDY DESIGN AND OBJECTIVES

3.1 Study Design

Phase II non-blinded randomized, multi-center, study evaluating men with oligometastatic prostate cancer lesions randomized (1:1) to stereotactic ablative radiation therapy (SABR) *versus* SBAR + Radium-223.

3.2 Primary Objective

To determine the progression-free survival of men who have oligometastatic prostate cancer with at least one bone metastasis with stereotactic ablative radiation therapy (SABR) *versus* SABR + Radium-223.

3.3 Secondary Objectives

To assess the toxicity of SABR + Radium-223 in patients with oligometastatic disease.

To determine local control at 12-months after SABR + Radium-223 in patients with oligometastatic disease.

To assess time to locoregional progression, time to distant progression, time to new metastatsis and duration of response after randomization to SABR *versus* SABR + Radium-223.

To assess ADT-free survival after randomization to SABR *versus* SABR + Radium-223.

To assess quality of life following completion of SABR + Radium-223.

3.4 Correlative Objectives

To enumerate circulating tumor cells (CTC) using EPIC HD-CTC platforms at baseline and 6 months from randomization.

To enumerate circulating tumor DNA (ctDNA) using Cancer Personalized Profiling by deep sequencing (CAPP-Seq) at baseline, months 3, 6 and 12 from randomization for SABR *versus* SABR + Radium-223 arms.

To quantitatively sequence T-cell receptor (TCR) repertoires using peripheral blood monocytes and the ImmunoSEQ platform at baseline and 3 months from randomization.

To evaluate immunophenotypes by running PBMCs through a Cytek Aurora machine, which allows for simultaneous detection of up to 25 markers; to evaluate metabolic fitness of the total PBMCs using the mitochondrial stress test on the Seahorse analyzer; and to determine the relative capacity of peripheral T cells to metabolically respond to T cell receptor stimulation through activation of CD3 and CD28 by running functional assays on PBMCs.

To determine frequency of germline DNA repair mutations in the oligometastatic state.

Descriptive statistics will be performed to correlate the metrics above with clinical outcomes.

4. BACKGROUND

4.1 Oligometastatic Disease

Cancer is the second leading cause of death in the United States, chiefly from an inability to control metastatic disease. Systemic therapy alone is not curative for

patients with most metastatic solid tumors (1). The metastatic capacity of cancers behaves along a spectrum of disease progression, such that some tumors have spread widely before clinical detectability and others never metastasize. Contained within this spectrum, is an oligometastatic state where metastases are limited in number and location. The presence of an oligometastatic state was originally proposed by Hellman who suggested that these oligometastatic patients would benefit from effective local therapy in addition to systemic therapy (1). In agreement with this hypothesis, surgery and chemotherapy for isolated pulmonary metastases can result in long term disease-free periods (2). Additionally, some 25% of patients following resection and chemotherapy for colorectal cancer and isolated liver metastases can similarly have long-term disease free survival (3)(4)(5).

4.2 Stereotactic Body Radiation Therapy (SBRT) or Stereotactic Ablative Radiation Therapy (SABR)

Conventional moderate dose radiation for metastatic disease is given primarily for palliation. Recent advancements in radiation delivery now make it possible to image and treat precisely within any anatomical region of the body (6, 7). As a result, the capacity to deliver tumor killing radiation doses in a single or few (1-5) outpatient radiation treatments is now possible (8-12). In addition, by minimizing the irradiation of surrounding healthy tissue, it should also be possible to decrease the rate of complications. Intracranial stereotactic body radiation therapy (SBRT) [also known has stereotactic ablative radiation therapy (SABR)] has been shown to be a highly effective treatment for brain metastases (13). Data suggests that selective small extracranial tumors (either primary or metastatic tumors) may be effectively controlled by similar focal high-dose SBRT/SABR. There is an increasing experience with extracranial SBRT as effective local therapy for metastatic lesions. Local control in excess of 75% has been reported for metastatic tumors of the spine, lung and liver, which is significantly higher than standard conventional moderate dose radiation (9, 11, 12, 14)(15-27). Toxicity has been minimal in multiple U.S., European and Japanese trials of extracranial stereotactic radiotherapy to the lung, liver, spine, pelvis and abdomen despite the use of very high biological equivalent doses for patients with both organ confined and metastatic cancer.

4.3 Rationale for Use of SBRT/SABR in Oligometastatic Disease

Historically, aggressive local therapy of metastases has not been fruitful secondary to further progression from microscopic metastatic deposits. Chemotherapy and even molecular-targeted agents rarely eradicate macroscopic metastases permanently. However, as systemic treatments for microscopic metastatic disease have improved the importance of local therapy in metastatic disease has been re-examined. It is now recognized that some patients with “oligo,” or few sites of metastases, may have isolated sites of metastases that can be potentially eradicated with aggressive local therapy. The term “oligometastases” was coined to refer to this stage of distant metastases. Typically, the entire burden of disease

can be recognized as a finite number of discrete lesions. Although there is no strict definition of oligometastases, it is commonly interpreted to be no more than 5-10 metastatic sites.

Although there have been a number of retrospective patient series showing a putative benefit of MDT to oligometastatic patients (5, 9, 15, 28-32), there are recent encouraging randomized prospective data for total consolidation of macroscopic metastases in aggressive histologies such as non-small cell lung cancer (NSCLC). Two recent randomized phase II trials in non-small cell lung cancer (NSCLC) patients with up to 3-5 metastases recently showed approximate tripling of progression-free survival (PFS) with the addition of metastasis-directed therapy (MDT) to maintenance treatment as compared to maintenance treatment alone, possibly due to ablation of systemic therapy resistant subpopulations that may have otherwise led to subsequent dissemination of treatment-refractory disease. In-line with this hypothesis, Gomez et. al. demonstrated that local consolidation of macroscopic metastases delayed time to development of new metastases.

4.4 Rationale for Radium-223 for Bone Metastases in Prostate Cancer

Radium-223 is an alpha-emitting radioisotope which is a bone-seeking calcium mimetic. It selectively targets the area with increased bone turnover, especially within the microenvironment of osteoblastic or sclerotic metastases. It has been approved by FDA for the treatment of metastatic castrate-resistant prostate cancer (mCRPC) with symptomatic bone metastases and without known visceral disease based on its ability to extend overall survival as shown its pivotal phase III trial. Unlike beta particle emitting radiopharmaceuticals, radium-223 emits high-energy alpha particles of short range (<100 μm) thus saving the surrounding healthy tissues and significantly reducing unwanted myelotoxicity.

Radium-223 was approved by the US FDA on May 15, 2013 based on the interim results from a Phase III randomized trial, the ALSYMPCA study (Alpharadin in the treatment of patients with symptomatic bone metastases in castration-resistant prostate cancer). In this trial, 921 patients who had received, were not eligible to receive, or declined docetaxel were randomly assigned in a 2:1 ratio to receive six injections of radium-223 (at a dose of 50 kBq per kilogram of body weight intravenously (55 kBq/kg after implementation of NIST standard update) every 4 weeks or matching placebo. At the interim analysis, which involved 809 patients, radium-223, as compared with placebo, significantly improved overall survival (median, 14.0 months vs. 11.2 months; HR=0.70; 95% CI, 0.55 to 0.88; two-sided P=0.002). The updated analysis involving 921 patients confirmed the radium-223 survival benefit (median, 14.9 months vs. 11.3 months; HR=0.70; 95% CI, 0.58 to 0.83; P<0.001). The rate of AEs was consistently lower in the radium-223 group than in the placebo group for all AEs (93% vs. 96%), grade 3 or 4 AEs (56% vs. 62%), and serious AEs (47% vs. 60%). There were no clinically meaningful differences in the frequency

of grade 3 and 4 hematologic AEs. Only one patient developed grade 3 febrile neutropenia in the radium-223 group. For serious AEs that occurred in at least 5% of patients in the radium-223 group or the placebo group, the respective frequencies were as follows: disease progression (11% and 12%), bone pain (10% and 16%), anemia (8% and 9%), and spinal cord compression (4% and 5%). Also a significantly higher percentage of patients who received radium-223 had a meaningful improvement in the quality of life according to the Functional Assessment of Cancer Therapy-Prostate (FACT-P) total score during the period of study-drug administration (25% vs. 16%, P=0.02).

4.5 Rationale for SBRT/SABR for Bone Oligometastases in Prostate Cancer

With advances in detection and primary treatment, both with radical prostatectomy and definitive radiotherapy, the 92% of men diagnosed with prostate cancer in the local or regional stage can expect to live for decades with appropriate intervention. The long disease-specific and overall survival observed with treated hormone sensitive prostate cancer, particularly in the localized setting, are due not solely to primary treatment modalities, but also the ever-evolving armamentarium of treatment approaches for men who recur, either locally or distantly, after definitive treatment. The management of locoregional recurrent prostate cancer comprises local salvage approaches including external beam radiotherapy (EBRT), brachytherapy, prostatectomy, or cryotherapy, along with re-staging to assess for nodal or distant metastases.

Metachronous or oligorecurrent (oligoprogressive) prostate cancer comprises a large number of men, possibly the majority of men following failed primary therapy. Assuming that these patients are possibly in a curable state before castration-resistance develops, we need additional treatment strategies for this potentially large number of men.

For patients with metastatic prostate cancer disease recurrence, the established approach is to offer androgen deprivation therapy (ADT) which improves survival but inevitably leads to castration resistance, and can be associated with significant adverse effects. Many men can remain on ADT treatment for years before progression or failure of ADT. However, similar to chemotherapy for other metastatic malignancies, ADT and even newer androgen receptor signaling inhibitory agents rarely eradicate metastatic disease permanently. In addition, ADT has been shown conclusively to adversely affect patient quality of life. Thus, even the ability to defer ADT initiation in men with oligometastatic prostate cancer represents a considerable clinical advance.

Major advancements in radiation treatment planning and delivery have resulted in resurgence in the use of radiation therapy (RT) as a treatment for bone metastases. SBRT/SABR is defined as highly focused, stereotactic localized, high-dose RT delivered in a hypofractionated course. In selected patients, very high local control rates have been observed, with minimal toxicity. Bone

metastases represent the major metastatic site (>90%) in men with rising PSA following primary treatment for their prostate cancer.

The prospective randomized phase II STOMP trial reported the ability of metastasis-directed therapies (MDT), primarily SABR, to forestall initiation of ADT in men with hormone-sensitive metastatic prostate cancer with three or fewer detectable metastases. STOMP showed ADT-free survival was lengthened in men randomized to MDT versus observation. The authors reported median ADT-free survival of 21 vs 13 months for the MDT and surveillance arms, respectively. All patients for whom ADT was initiated due purely to local (n = 6) or symptomatic (n = 3) progression were from the surveillance arm, underlining the high efficacy of MDT for local control of individual metastatic lesions. Nearly equal numbers of men in the MDT (n = 19) and surveillance (n = 16) arms received ADT due to polymetastatic progression, suggesting there may have been distinct subpopulations within this oligorecurrent STOMP cohort, i.e. those who had oligometastatic disease and where MDT impacted future macroscopic metastatic colonization versus those who had so-called “OligoVisible” polymetastatic disease already beyond the practical reach of MDT alone.

Our own prospective randomized phase II ORIOLE trial is similar to STOMP and nearly completed. Although preliminary, our data show a significant ability of SABR to delay progression in men with oligorecurrent prostate cancer. In addition, these combined preliminary data suggest that progression at microscopic metastatic sites is the predominant mode of progression in men with oligorecurrent prostate cancer treted with SABR.

Forestalling systemic therapy is attractive in order to shield patients from adverse effects, but the combination of MDT with systemic therapy also warrants additional investigation. In patients not receiving MDT, immediate initiation of ADT can improve survival over delayed therapy and patients with low-volume disease appear to have improved overall survival on ADT and gain more benefit from ADT than those with greater disease burden. An overall survival benefit from single-modality chemotherapy as management for oligometastatic prostate cancer has not been appreciated in large studies to date, but such a benefit may exist in patients prone to castrate-resistance. The combination of hormonal agents, chemotherapy or radiopharmaceutical agents such as Radium-223 with MDT is clinically relevant for the treatment of oligorecurrent prostate cancer patients. On the basis of this clinical evidence and because SABR and radium-223 separately are known to be safe, we propose a phase II study of SABR + Radium-223 in patients with oligometastatic prostate cancer.

In general metastatic disease carries an extremely high mortality rate. Current therapies provide only partial palliation of symptoms and mild to moderate prolongation of survival. Patients are rarely cured of this disease; consequently, better treatment is clearly needed. The proposed treatment represents a logical

extension of the current state-of-the-art radiation therapy. It has the potential to translate into more effective palliation and longer patient survival free of intiation of ADT systemic treatment.

4.6 Rationale for Prostate specific membrane antigen (PSMA) Functional Imaging to Help Refine Selection of Bone Lesions to Target with SBRT/SABR in Oligometastatic Prostate Cancer

Conventional imaging modalities, *i.e.,* bone scintigraphy, CT and MR imaging, are currently used to detect metastatic prostate cancer for staging (4). Positron emission tomography (PET) imaging, particularly [^{18}F]fluorodeoxyglucose positron emission tomography ([^{18}F]-FDG PET), has gained an important role in the clinical management of cancer patients, more notably for staging and assessment of response to therapy (32, 33). However, studies employing [18F]-FDG PET have demonstrated low uptake in prostate cancer except for advanced metastatic disease (34-37). A number of novel PET radiotracer are being investigated for use in prostate cancer but have yielded mixed results and have yet to gain widespread clinical use (38, 39). [11C]Choline is the most widely studied PET radiotracer for prostate cancer detection and has demonstrated the ability to detect lymph node and bone metastases (40-42). [11C]Acetate is another emerging radiotracer, which has been evaluated in a limited number of studies and appears to demonstrates comparable uptake in comparison to [11C]Choline for detection of prostate tumor and metastases (43). One limitation of these radiotracers include nonspecific uptake at sites of inflammation. 18F-Fluoride-PET (NaF-PET) for identifying bone metastases has proven very sensitive but is unable to differentiate between viable metastatic prostate tumor from chronic reactive bone changes (44, 45). Other promising radiopharmaceuticals for prostate cancer include anti-1-amino-3-18F-fluorocyclobutane-1-carboxylic acid (18F-FACBC) and 18F-fluorodihydrotestosterone (18F-FDHT), which are also actively undergoing clinical evaluation in a variety of settings (46-48). Further work needs to be done to compare the merits of [11C]Choline and other emerging PET radiotracers in the detection and management of prostate cancer (49).

Prostate specific membrane antigen (PSMA) is a promising well-characterized biomarker specific for prostate cancer which has also been associated with prostate tumor aggressiveness. Histologic studies have associated high PSMA expression with metastatic spread (50-52), androgen independence (53), and expression levels have be found to be predictive of prostate cancer progression (54, 55). However, previous attempts to image PSMA by single-photon-emission computed tomography (SPECT) (Prostacint^{TM}) demonstrated poor performance due to inherent limited antibody and imaging characteristics (poor tumor penetration, slow blood pool clearance, low SPECT resolution) (56, 57).

[^{18}F]DCFBC (N-[N-[(S)-1,3-Dicarboxypropyl]Carbamoyl]-4-[18F]Fluorobenzyl-L-Cysteine) (DCFBC) is a promising clinically practical small molecule PET imaging agent specific for prostate cancer with superior pharmacodynamic and pharmacokinetic characteristics than existing prostate cancer imaging agents. This is a small molecule urea-based analog inhibitor of PSMA radiolabeled with a PET radiotracer fluorine-18 which was rationally designed based on knowledge of the crystal structure of PSMA (58, 59). DCFBC is a high affinity inhibitory ligand for the prostate specific membrane antigen (PSMA). The relative affinity of DCFBC for PSMA was determined by evaluating ability of DCFBC to inhibit the N-acetylaspartylglutamate (NAAG) peptidase activity of PSMA using a previously developed NAAG peptidase assay. The IC50 value for the inhibitory capacity of DCFBC for PSMA was 13.9 nM, as determined by a NAAG peptidase inhibition assay, in keeping with other compounds of this class.

2-(3-{1-carboxy-5-[(6-[^{18}F]fluoro-pyridine-3-carbonyl)-amino]-pentyl}-ureido)-pentanedioic acid, DCFPyL, is a second generation compound that includes the pyridine moiety intended to improve pharmacokinetics through altered lipophilicity and potential pH dependent intra-tumoral sequestration. Extensive study of both DCFBC and DCFPyL in pre-clinical models has been performed and published, attesting to their suitability for clinical translation – including with respect to dosimetry. While the first generation compound (DCFBC) provided isogenic PSMA+ human PC3 PIP tumor to PSMA- PC3 flu tumor ratios of 20:1 at 3 h post-injection, DCFPyL demonstrated ratios on the order of 400:1. Neither compound demonstrates appreciable de-fluorination *in vivo* and PSMA+ PIP to bone ratios are sufficient to enable imaging of bone metastases, as demonstrated below for DCFBC. After synthesis of ~100 compounds, including those with several new scaffolds designed to target PSMA, DCFPyL emerged as the best *in vivo* (63). In our recently published ORIOLE trial, we have also shown that men with oligometastatic hormone-sensitive prostate cancer that have all their DCFPyL PET-CT lesions ablated with SABR have a significant improvement in median PFS (11.8 mos *versus* not reached, HR = 0.26, p =0.006). On the basis of this promising clinical evidence with DCFPyL PET-CT, we propose incorporating this a better means to identify metastatic lesions for SBRT treatment in oligometastatic prostate cancer.

4.7 Rationale for Correlative Science

Novel Models of Metastasis and Circulating Tumor Cell (CTCs). Older models of metastasis portray the unidirectional flow of circulating tumor cells (CTCs) leaving the primary tumor and seeding a metastasis at a distant site (64). However, recent preclinical data using diverse experimental models of breast cancer, colon cancer and melanoma suggest metastasis is a multidirectional process where CTCs seed both distant sites as well as the original primary tumor – a process termed “self-seeding”(65, 66). Proponents of self-seeding have posited that CTC self-seeding of established macroscopic

tumor sites likely requires less or no adaptation of CTCs to the recipient microenvironment in comparison to the colonization of CTCs to a distant and foreign site. In addition, self-seeding CTCs have already undergone selection for movement into and out of the circulation as well as resistance to anoiksis. Pre-clinical data have shown that self-seeding CTCs home back and extravasate into the primary in reaction to signals from the recipient primary tumors cells and tumor stroma. These self-seeding CTCs appear to be the most aggressive fraction of the CTC population (65). This feed forward loop of increasingly more aggressive cancer cells interacting with the tumor stroma results in the release of signals that foster tumor growth, angiogenesis, immune evasion, and ultimately macroscopic metastases.

Interestingly, genomic lineage tracing data of metastases from a rapid autopsy series of men who died of metastatic prostate cancer suggest macroscopic metastases represent *communal sanctuaries* that are composed of prostate cancer cells from many other metastatic sites throughout the body of patients (67). These *communal sanctuaries* are favorable niches that allow prostate cancer cells the ability to gain competence for the development of future macroscopic metastases. These human data are consistent with the preclinical concept of “self-seeding” or a multidirectional flow of CTCs. If these provocative data hold true, then SBRT/SABR to all macroscopic metastases in oligometastatic patients may eliminate these *sanctuaries* and alter the natural history of metastatic patients. Following change in CTC numbers and biology following SABR may allow us to interrogate this hypothesis. Interventions that effectively target these macroscopic metastatic sanctuaries, such as SBRT/SABR, in combination with immune mediated therapies appear poised to arrest self-seeding and subsequent maturation of macroscopic metastases.

EPIC High-Definition analysis of single CTCs. The High Definition-CTC (HD-CTC) method can be used for the longitudinal CTC enumeration and to assess for AR expression (68). The assay employs as little as 1 mL of blood and is an unbiased protocol to distinguish CTCs among the surrounding leukocytes based on their cytokeratin positive (CK+) phenotype by using a high resolution immunofluorescence imaging. All cells are captured, and AR- cells can be evaluated. In addition, the HD-CTC technology preserves the cell morphology in such a way that enables the morphometric and the indirect quantification of AR and CK protein expression levels for all the CTCs identified in the blood sample (Fig. 1). Recently, the HD-CTC method has been used to examine expression of immune checkpoint molecules relevant to our proposal such as programmed cell death-1 (PD-1) on CTCs (data not shown). The HD-CTC assay was technically validated with cell line spiking experiments to reach an R2=0.9997 on linearity testing as previously reported. These experiments were performed using SK-BR-3 cell lines and 0 to 3x102 cells per mL of normal donor control blood. The coefficient of variation is 16% and inter-processor correlation is R2 = 0.979. Sample preparation process adhered to standard operating procedures for patient samples through a bar coded system for all consumables and instrumentation. All off-the-shelf instrumentation was calibrated according to analytical validation protocols established during commissioning.

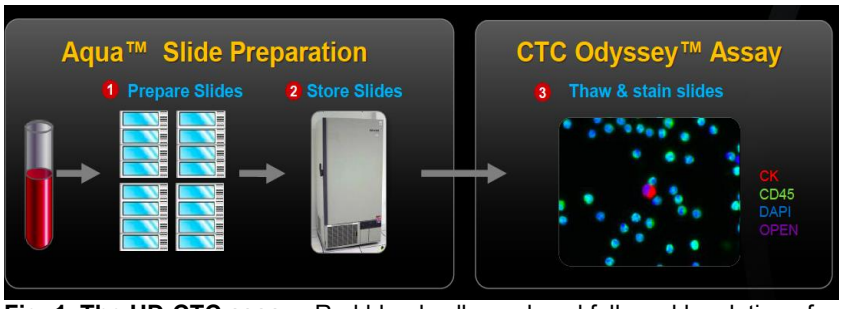

Fig. 1. The HD-CTC assay. Red blood cells are lysed followed by plating of nucleated cells on custom made cell-adhesion glass slides. Slides are stained for cytokeratin (CK), CD45 and DAPI, then CTCs are identified among leukocytes using computerized high resolution immunofluorescence imaging.

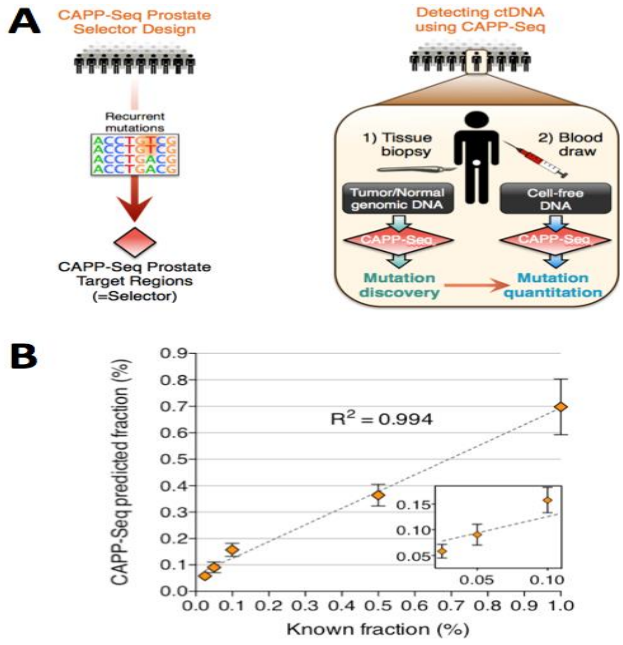

Fig. 2. CAPP-Seq ctDNA detection. (**A**) Analysis of exome sequencing from many tumors is used to select mutated genomic regions for a custom library of biotinylated oligonucleotides that are used for hybrid capture selection of tumor and germline genomic DNA and ctDNA. (**B**) Dilution series analysis of expected versus observed frequencies of mutant alleles, assessed by spiking fragmented HCC78 DNA into control cell free DNA.

Cancer personalized profiling by deep sequencing (CAPP-Seq) to detect circulating tumor DNA. Circulating tumor DNA (ctDNA) is a promising biomarker for non-invasive assessment of cancer burden, but existing ctDNA

detection methods have either insufficient sensitivity and/or lack broad clinical applicability. Dr. Max Diehn's laboratory recently published Cancer Personalized Profiling by deep sequencing (CAPP-Seq) (69), an economical and ultrasensitive method for quantifying ctDNA. CAPP-Seq was initially implemented for lung cancer with a design covering multiple classes of somatic alterations that identified mutations in >95% of tumors. We detected ctDNA in 100% of patients with stage II–IV lung cancer and in 50% of patients with stage I, with 96% specificity for mutant allele fractions down to ~0.02%. Levels of ctDNA were highly correlated with tumor volume and distinguished between residual disease and treatment-related imaging changes, and measurement of ctDNA levels allowed for earlier response assessment than radiographic approaches from 1.5 mL of blood (**Fig. 2**). We have designed a prostate cancer-specific CAPP-Seq selector that identifies at least 1 mutation in ≥95% of prostate tumors with a median of ~4 mutations per tumor. We will apply CAPP-Seq to detect and monitor prostate cancer response to SBRT/SABR.

ImmunoSEQ allows Unprecedented Profiling of the Anti-Tumor Immune Response. There are now an established body of pre-clinical and emerging clinical literature demonstrating that SABR can profoundly modify anti-tumor immune responses. Radiation induced activation of antigen presenting cells has been demonstrated in animal models to enhance tumor antigen cross-presentation in the draining lymph node and result in activation and proliferation of tumor specific cytotoxic T-cells (78). The cellular adaptive immune system generates a remarkable breadth of diversity in antigen-specific TCRs by combinatorial recombination of gene segments in lymphocytes. The TCR is composed of two peptide chains, encoded by the TCRA and TCRB or TCRG and TCRD genes, respectively. There are thus two types of T-cell receptors, αβ and γδ, that differ by the TCR heterodimer type and immune function. The antigenic specificity of T-lymphocytes is in large part determined by the amino acid sequence in the hypervariable complementarity-determining region 3 (CDR3) regions of the T-cell receptors. Because of the potential diversity of receptors (a healthy adult has approximately 10 million different TCRB chains contained within their 10^{12} circulating T-cells (70)) it is highly improbable to randomly converge on the same TCRB nucleotide CDR3 sequence, effectively making each CDR3 sequence a unique tag for a T-cell clone. Adaptive Biotechnologies' ImmunoSEQ assay, a multiplex PCR-based method that amplifies rearranged TCR CDR3 sequences and exploits the capacity of high throughout sequencing technology characterizes tens of thousands of TCRB CDR3 chains simultaneously. Thus, the assay captures the full TCR repertoire including specific individual clones. The ImmunoSEQ assay provides a novel method to identify and track the presence and frequency of common and rare clones, in the context of the total adaptive immune system. Recently, ImmunoSEQ showed profound evolution and diversification of the TCR repertoire of men with mCRPC treated with the immune stimulatory agent ipilimumab (71). Improved clinical outcomes were associated with less T-cell clonotype loss, consistent with the maintenance of high-frequency TCR

clonotypes during treatment reported by ImmunoSEQ. These clones may have represented the presence of preexisting high-avidity T cells that may be relevant in the antitumor response.

Rational for Invitae. The evolution of human genome sequencing has enabled the assessment of genetic anomalies in routine clinical practice. According to a recent multicenter study in the *New England Journal of Medicine*, it was reported that inherited mutations in DNA-repair genes were much higher than expected in men with lethal metastatic castrate resistant prostate cancer (79). The study isolated germline DNA of 692 men with metastatic prostate cancer (unselected for family history of cancer or age at diagnosis), and identified 84 deleterious germline DNA-repair gene mutations in 82 of those men (79). This was substantially higher than in men with non-metastatic hormone sensitive localized disease. The prevalence of germline DNA-repair mutations in the oligometastatic hormone sensitive or castrate-resistant state is unknown.

In the Invitae's Multi-Cancer Panel genetic test, Invitae uses a sequencing platform to analyze the risk of developing hereditary cancer due to inheritance of a pathogenic mutation in 84 cancer predisposition genes (81). The Invitae panel, covers 12 of 16 genes (BRCA1, BRCA2, MSH2, MSH6, PMS2, CHECK2, ATM, NBN, BRIP1, RAD51C, and RAD51D) that account for 92% of the germline mutations reported in Pritchard et al NEJM. Thus, inherited prostate cancer genomic data provided by Invitae can further allow investigators to personalize screening and target specified effective therapies for each patient disease (80).

5. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

All patients will be eligible to receive systemic therapy alone at the time of clinical or radiographic disease progression.

5.1 Inclusion Criteria

5.1.1 Patient must have at least one and up to three asypmtomatic metastatic tumor(s) of the bone or soft tissue (with at least one bone metastasis) within the past 3-months that are ≤ 5.0 cm or <250 cm^3 as seen on either CT/MRI scan and/or bone scan. Up to five lesions are allowed on advanced funcitonal imaging such as fluciclovine (Axumin), choline or ^{18}F-DCFPyL ("PyL") PET-CT scan within the past 6-months. (PET-CT scan is reasonable for study entry imaging as an alternative to CT/MRI scan and/or bone scan)

5.1.2 Patient must have had their primary tumor treated with surgery and/or radiation.

5.1.3 Histologic confirmation of malignancy (primary or metastatic tumor).

5.1.4 PSADT <15 months. PSA doubling time (PSADT) will be calculated using as many PSA values that are available from time of relapse (PSA > 0.2). To calculate PSADT, the Memorial Sloan Kettering Cancer Center Prostate Cancer Prediction Tool will be used. It can be found at the following web site: https://www.mskcc.org/nomograms/prostate/psa-doubling-time.

5.1.5 Patient may have had prior systemic therapy and/or ADT associated with treatment of their primary prostate cancer. Patient may have had ADT

associated with salvage radiation therapy (to the primary prostate cancer or pelvis is allowed).

5.1.6 PSA ≥ 0.5 but ≤50.

5.1.7 Any testosterone lab within the past 6 months > 50ng/dL.

5.1.8 Patient must be ≥ 18 years of age.

5.1.9 Patient must have a life expectancy ≥ 12 months.

5.1.10 Patient must have an ECOG performance status ≤ 2.

5.1.11 Patient must have normal organ and marrow function as defined as:

Before the first administration of Xofigo, the absolute neutrophil count (ANC) should be ≥ 1.5 x 109/L, the platelet count ≥ 100 x 109/L and hemoglobin ≥ 10 g/dL.

5.1.12 Patient must have the ability to understand and the willingness to sign a written informed consent document.

5.1.13 LDH lab performed within 6 months of enrollment.

5.2 Exclusion Criteria

5.2.1 No more than 3 years of ADT is allowed, with the most recent ADT treatment having occurred greater than 6 months prior to enrollment.

5.2.2 Castration-resistant prostate cancer (CRPC).

5.2.3 Spinal cord compression or impending spinal cord compression.

5.2.4 Suspected pulmonary and/or liver metastases (greater ≥10 mm in largest axis).

5.2.5 Patient receiving any other investigational agents.

5.2.6 Patient receiving abiraterone and prednisone.

5.2.7 Patient is participating in a concurrent treatment protocol.

5.2.8 Serum creatinine > 3 times the upper limit of normal.

5.2.9 Total bilirubin > 3 times the upper limit of normal.

5.2.10 Liver Transaminases > 5-times the upper limit of normal.

5.2.11 Unable to lie flat during or tolerate PET/MRI, PET/CT or SBRT.

5.2.12 Prior salvage treatment to the primary prostate cancer or pelvis is allowed.

5.2.13 Refusal to sign informed consent.

6. PATIENT REGISTRATION AND TREATMENT PLAN

6.1 Multicenter/Participating Site Registration

Central registration for this study will take place at *SKCCC, Johns Hopkins University.*

Patient registration at each study site/institution will be conducted according to the institution's established policies. Before registration, patients will be asked to sign and date an Institutional Review Board (IRB)-approved consent form and a research authorization/HIPAA form. Patients must be registered with their local site/institution and also with the sponsor or Lead Site before beginning any treatment.

6.1.1 General Guidelines

Eligible patients will be entered on study centrally at the Sidney Kimmel Comprehensive Cancer Center at the Johns Hopkins University by the Lead Study Coordinator, Noura Radwan. All sites should call/email the coordinating

center at nradwan1@jh.edu for registration. The Eligibility Worksheet will be supplied to each participating site.

Subjects who sign a consent form, but do not initiate protocol treatment for any reason (e.g., subjects who are screen failures), will be replaced and will not count towards our accrual goal. The Coordinating Center should be notified as soon as possible.

6.1.2 Registration Process

To register a patient, the following documents should be completed (and redacted from any PMI if being completed by a site outside of SKCCC) by the Research Nurse or Study Coordinator and emailed to nradwan1@jhmi.edu, the lead RAVENS Study Coordinator:

- Signed Patient Informed Consent Form
- Elgibility Screening Checklist
- Copies of the following documents:
    - Diagnostic pathology and surgical reports
    - Baseline CT, Bone, MRI, and/or PET/CT scan report (scans that are applicable for enrollment)
    - Laboratory Reports:
        - Complete blood count (CBC) with differential (including absolute lymphocyte count) and direct platelet count
        - Chemistry (CMP): Albumin, SGOT (AST), SGPT (ALT), Bilirubin (total) (Direct only if clinically indicated), Calcium, Creatinine, Glucose, Total protein, Urea nitrogen, (Uric Acid only if clinically indicated), Electrolytes (including sodium, potassium, chloride and bicarbonate).
        - PSA
        - LDH
        - TESTOSTERONE
    - Treating physician's medical note mentioning/indicating all inclusion/exclusion criteria, Full Physical Examination, ECOG (performance status), Past Medical/Surgical History
    - Other documents, if requested.

Study treatment cannot begin until the patient is registered.

The Research Nurse or Study Coordinator at the participating site will then e-mail the lead RAVENS Study Coordinator at nradwan1@jhmi.edu at the Coordinating Center to verify eligibility. To complete the registration process, the Coordinating Center will:

- Assign a patient study number
- Register the patient on the treatment portion/randomization of the study with the Sidney Kimmel Comprehensive Cancer Center's Clinical Research Office

- Email the patient study number to the participating site

6.2 Randomization

Eligibility work-up will include a complete blood count, serum chemistries, PSA, and radiographic studies (of involved sites) and bone scan (unless PET indicated). Once a signed informed consent has been obtained and after confirming patient eligibility, the lead data coordinator will assign a unique Study ID Number.

Treatment must not commence until the patient has received his identification number.

Stratification:
Subjects who meet eligibility criteria and qualify for enrollment will be stratified according to the following:

1) Instiution
2) Initial treatment with surgery vs. radiation therapy
2) Prior hormonal therapy vs. no prior hormonal therapy
3) PSADT <6 months vs. 6-14.9 months

Process for Randomization:
The research team will utilize an interactive web response system (IWRS) to obtain the patient's randomization assignment. The lead coordinator will enter demographic and baseline characteristics in IWRS. The lead coordinator will obtain the patient's randomization assignment from the IWRS and appropriately notify the study site. Randomization will be 1:1 for SABR: SABR + Radium-223 arms and be stratified as above. Minimization approach will be applied to ensure balanced assignment to each treatment arm. The **ON STUDY date for protocol entry** will be the day that the study subject is randomized. Within 30 days from the date of randomization, is considered DAY 1 of SABR and/or SABR + Radium-223 Arm.

All institutions should allow 48 hours for patients to be randomized from the time they provide all elgibility documentation.

6.3 Diagnostic Procedures

A bone scan and CT (and/or MRI of questionable sites) must be available or will be obtained within 3-months of randomization for confirmation of oligometastatses. And/or DCFPyL, Axumin- or Choline-PET/CT scan must be made available and obtained within 3-months of randomization A CT- and/or MRI-simulation scan will be performed for tumor localization and radiation planning using rigid immobilization appropriate for stereotactic treatment.

6.4 Therapeutic Procedures

Upon confirmation of eligibility and enrollment in the study, the following will be confirmed or completed:

1) Demographics review, medical history and clinical exam
2) Review of concurrent medications
3) Vital signs, height and weight
4) PSA, Testosterone, CBC, Chemistry Panel, LDH, CAPP-seq, EPIC HD-CTC and ImmunoSEQ. Immunophenotyping will only occur at Coordinating Center. Research Labs are voluntary and based on site participation.
5) CT and/or MRI scan of the involved site(s) (if not previously conducted within 3 month of enrollment). CT/MRI/Bone Scan can be supplemented if PET-CT is completed.
6) Bone scan (if not previously conducted within 3 month of enrollment).
7) Invitae Test
8) ^{18}F-DCFPyL ("PyL") PET/CT Diagnostic Imaging Protocol**. Only for participating sites.**

The subjects accrued at the outside insitutions may be scanned on a different scanner than that used at Johns Hopkins. This therapeutic procedure is only applicable for participating sites.

- There are no dietary or food restrictions for this research study.
- Fasting is not required prior to ^{18}F-DCFPyL (PyL) dosing and PET/CT imaging. Increased fluid intake should occur before and after image acquisition to maintain proper hydration throughout the study period, decrease radiation exposure to the urinary bladder and improve image quality.
- Subjects should be encouraged to void as frequent as possible. At a minimum void post study drug injection, immediately before imaging, and after imaging.
- A single administration of ^{18}F-DCFPyL Injection will be administered prior to PET/CT imaging
- ^{18}F-DCFPyL Study Drug Administration:
    - Place an IV catheter in an antecubital vein or an equivalent venous access.
    - Ensure patency of the line with a saline flush.
    - Inject a bolus of the prescribed dose of 18F-DCFPyL (9 mCi or 333 MBq) of into the IV line or equivalent venous access by slow push from the appropriately shielded syringe according to normal local practices.
    - Administer an intravenous flush (e.g., 5-10 ml sterile Sodium Chloride Injection, 0.9%), to ensure full delivery of the dose.

- ^{18}F-DCFPyL PET/CT imaging will be performed using local PET/CT scanners with low dose CT for attenuation correction and anatomic localization.
- Subjects will be asked to void prior to imaging. After voiding, a whole-body CT and PET scan will be acquired starting from the mid-thigh through the skull. Whole body PET/CT scans must be obtained within 60-120 minutes following the ^{18}F-DCFPyL dosing.

The study PI, co-I's or study coordinator will contact the patient around 7 days (3 - 10 days) after the PET/CT study to inquire about delayed side effects. If there are suspected problems the patient will be asked to return for a clinic visit for a follow-up clinic visit.

9) SBRT/SABR treatment planning

CT- and/or MRI-simulation will then be performed with fabrication of a radiation therapy immobilization device (such as the Alpha Cradle) which will be custom made for each patient. The treating radiation oncologist will identify the location of the tumor. Gross tumor volume (GTV) delineation will be performed with a diagnostic radiologist on sequential axial computed tomography images. A radiosurgical treatment plan will be developed based on tumor geometry and location. The clinical tumor volume (CTV) will equal the GTV. The dose will be prescribed to the minimal isodose line that completely covers the planning target volume (PTV) PTV (=CTV plus a 3-5 mm margin). Adjacent normal structures including but not limited to the heart, esophagus, aorta, spinal cord, kidneys, rectum, bowel, liver, and stomach within 5 cm of the CTV will be identified for the purpose of limiting incidental radiation to these structures.

In addition, prior to treatment delivery, a four-dimensional cone beam CT study will be performed on individual patients to assess respiration in these patients and to determine tumor targeting accuracy for those tumors that may be subject to respiratory motion such as those in the bones of the thorax. If tumor motion is greater than 5 mm, PTV will be expanded to account for respiration.

SBRT/SABR will be delivered in 1 to 5 fractions, and the dose and fractionation schedule will depend on the size and location of the lesion and the surrounding normal tissue constraints in accordance with AAPM Task Group 101 recommendations. Typical doses include 16 – 24 Gy in 1 fraction, 48 – 50 Gy in 4 fractions, and 50 – 60 Gy in 5 fractions. For example, isolated osseous lesions will be treated in a single fraction, lesions close to the lung and liver lesions will be treated in 3 to 5 fractions depending on their size (5 fractions for ≥ 3 cm or central tumors in close proximity to the mediastinum), and bone lesions will be treated in 5 fractions if small-bowel constrains fewer doses.

Within three weeks of the initial treatment planning imaging study, SBRT/SABR will be administered using image-guidance. An Alpha Cradle (or equivalent

immobilization device) will be used to minimize movement of the chest, spine, and abdomen during treatment. During treatment, real time cone beam CT images of the patient's body site of interest will be obtained. Cone beam CT scan will be obtained immediately prior to treatment and will be repeated until the treatment shift, required to align the CT planning scan and the cone beam CT scan performed on the day of treatment cone beam CT, is within tolerance for the body site.

Patients will be evaluated for adverse events/toxicities during their treatment.

The dose limits for surrounding critical structures are as follows:

Spinal Cord: maximal allowable dose should be = 800 cGy in 1 fraction

Lung: 2/3 of the lung volume should be kept under 500 cGy.

Heart: 50 % of the heart volume should be kept under 1000 cGy.

Esophagus: 50 % of the esophagus volume should be kept under 1000 cGy and no single point dose in the esophagus should exceed 2000 cGy.

Brachial Plexus: maximal allowable point dose = 1000 cGy

Liver: One third of the uninvolved liver or approximately 700 cc <15 Gy.

Kidneys: 75% of volume of each kidney <5 Gy.

Small Bowel: <5% of bowel limited to <20 Gy.

9) Radium-223

Dosage Forms and Strength:
Radium-223 is available in single-use vials containing 6 mL of solution at a concentration of 1,000 kBq/mL (27 microcurie/mL) (1,100 kBq/mL (29.7 microcurie/mL)after implementation of the NIST update) at the reference date with a total radioactivity of 6,000kBq/vial (162 microcurie/vial) (6,600 kBq/vial (178 microcurie/vial) after implementation of the NIST update) at the reference date.

Preparation:
Radium-223 is a ready-to-use solution and should not be diluted or mixed with any solutions. Each vial is for single use only. Parenteral drug products should be inspected visually forparticulate matter and discoloration prior to administration, whenever solution and container permit.

Before the first administration of Xofigo, the absolute neutrophil count (ANC) should be ≥ 1.5 x 109/L, the platelet count ≥ 100 x 109/L and hemoglobin ≥ 10 g/dL. Before subsequent administrations of Xofigo, the ANC should be ≥ 1 x 109/L and the platelet count ≥ 50 x 109/L. If ANC is not within normal limit prior to protocol window, then CBC can be drawn, per treating physician advisement. All doses 6 doses should still be administered regardless of

treatment delay due to ANC. Unless, there is no recovery to these values within 6 to 8 weeks after the last administration of Xofigo, despite receiving supportive care, then further treatment with Xofigo should be discontinued. Patients with evidence of compromised bone marrow reserve should be monitored closely and provided with supportive care measures when clinically indicated. Discontinue Xofigo in patients who experience life-threatening complications despite supportive care for bone marrow failure.

Dosage:

The dose regimen of radium-223 is 50 kBq (1.35 microcurie) (55 kBq/kg (1.49 microcurie/kg) after implementation of the NIST update) per kg body weight, given at 4 week intervals for 6 injections.

The volume to be administered to a given patient should be calculated using the:

– Patient's body weight (kg)
– Dosage level 50 kBq/kg body weight (55 kBq/kg after implementation of the NIST update) or 1.35 microcurie/kg body weight (1.49 microcurie after implementation of the NIST update)
– Radioactivity concentration of the product (1,000 kBq/mL; 27 microcurie/mL (1,100 kBq/mL after implementation of the NIST update;29.7 microcurie/mL) at the reference date
– Decay correction factor to correct for physical decay of radium-223.

The total amount (volume to be drawn into the syringe) to be administered to a patient should be calculated as follows:

Volume to be administered (mL) =
Body weight (kg) x dose (50 kBq/kg b.w)[a]/
DK factor x 1,000 kBq (0.027 mCi)/mL[b]

[a] 55 kBq/kg after implantation of the NIST update
[b] 1,100 kBq (29.7 microcuriei)/mL after implantation of the NIST update

Decay Correction Factor Table

Days from Reference Date	Decay Factor	Days from Reference Date	Decay Factor
-14	2.296	0	0.982
-13	2.161	1	0.925
-12	2.034	2	0.870
-11	1.914	3	0.819
-10	1.802	4	0.771
-9	1.696	5	0.725
-8	1.596	6	0.683
-7	1.502	7	0.643
-6	1.414	8	0.605
-5	1.330	9	0.569
-4	1.252	10	0.536
-3	1.178	11	0.504
-2	1.109	12	0.475
-1	1.044	13	0.447
		14	0.420

The Decay Correction Factor Table is corrected to 12 noon Central Standard Time (CST). To determine the decay correction factor, count the number of days before or after the reference date. The Decay Correction Factor Table includes a correction to account for the 7 hour time difference between 12 noon Central European Time (CET) at the site of manufacture and 12 noon US CST, which is 7 hours earlier than CET.

Immediately before and after administration, the net patient dose of administered radium-223 should be determined by measurement in an appropriate radioisotope dose calibrator that has been calibrated with a National Institute of Standards and Technology (NIST) traceable radium-223 standard (a new reference vial will be sent to each center corresponding to the updated NIST reference material) and corrected for decay using the date and time of calibration. The dose calibrator must be calibrated with nationally recognized standards, carried out at the time of commissioning, after any maintenance procedure that could affect the dosimetry and at intervals not to exceed one year.

Each center must perform the Radium Ra 223 dichloride dial setting on their relevant dose calibrator(s) (upon notification by Bayer each center is required to update the dial setting to correspond to the new NIST standard). The current dial settings are to remain in effect until Bayer obtains FULL approval from the FDA for implementation. In preparation for implementation of the NEW dial setting, the clinical study center will receive a sealed vial labeled NIST standard containing a Radium Ra 223 dichloride solution for calibration only. The vial is identical to the vials used for study treatment. The amount of Radium Ra 223 dichloride in the vial will be stated on the label. Instructions for the dial setting, including the calibration log form, will be enclosed with the dispatch of the calibration sample.

NIST Standardization Update:
The qualification of radium-223 radioactive in Xofigo (radium-223 dichloride; BAY 88-8223) is based on the primary standardization performed by the US NIST. National Institute of Standards and Technology prepares the standard reference material (SRM) using an official dial setting (primary standardization) as published [20]. The NIST SRM is used to calibrate the instruments in production and quality control for both the drug substance and drug product. Additionally, the NIST SRM is used to prepare the NIST traceable Ra-223 reference materials which are then sent to the end-users (e.g., nuclear medicine laboratory physicians or technicians) for dial-setting of their dose calibrators, to allow verification of the patient dose.

In 2014, NIST performed a re-assessment of the primary standardization based on preliminary information suggesting a potential discrepancy of approximately 8-10% between the published NIST primary standardization [20] and results obtained by other national metrology institutes (United Kingdom, Germany, Japan). After completion of the re-assessment, NIST reported their findings [21] and had issued a revised NIST SRM in 2015. The discrepancy in the NIST standardization was determined to be -9.5% between activity values obtained using the old reference standard relative to the new primary standardization. Consequently, the current numerical values need to be corrected by approximately +10.5%.

The current NIST standard for radium-223 dichloride will remain in effect until the FDA has fully approved the regulatory variation submitted for Xofigo and is anticipated in the 2nd quarter of 2016. All sites are expected to begin preparation for the updated NIST standardization and obtain all necessary IRB approvals. Bayer will continue to notify sites about the status of the regulatory approval and the date that the updated NIST standardization is to be implemented. Upon notification, and prior to the implementation, all sites are expected to add a new dial setting to their dose calibrators for the new NIST standardization for radium-223 dichloride, which should be documented on the appropriate study forms.

The change in the numerical description of the patient's dose, product strength and labeled vial activity does not impact the safety or efficacy of Xofigo. The change in the NIST radium-223 standard has no impact on subjects; dose subjects are receiving, and will continue to receive. Subjects will receive the same actual dose and volume that was studied in Study 15245 (BCI-06 dosimetry study) and is associated with the proven safety and efficacy of radium-223 dichloride, though the stated nominal radiation dose received is being updated to reflect the new standard. The formula for the calculation of the volume to be administered has to be changed respectively. (see dose section) Subjects who are on-treatment at the time the new NIST reference standard goes into effect should be notified of this change and

should be required to sign a Patient Information Sheet to acknowledge that they have received information on the updated NIST standard calibration. All patients randomized after the new reference standard is in effect should sign a revised Informed Consent Form that contains the updated NIST standardization.

Administration:
Radium-223 should be administered by slow IV injection over 1 minute. The IV access line or cannula should be flushed with isotonic saline before and after injection of radium-223.

Handling and Patient Protection:
Radium-223 is primarily an alpha emitter, with a 95.3% fraction of energy emitted as alphaparticles. The fraction emitted as beta-particles is 3.6%, and the fraction emitted as gammaradiation is 1.1%. The external radiation exposure associated with handling of patient doses is expected to be low, because the typical treatment activity will be below 8,000 kBq (216 microcurie). (8,800 kBq (238 microcurie) after implementation of the NIST update) In keeping with the **A**s **L**ow **A**s **R**easonably **A**chievable (ALARA) principle for minimization of radiation exposure, it is recommended to minimize the time spent in radiation areas, to maximize the distance to radiation sources, and to use adequate shielding. Any unused product or materials used in connection with the preparation or administration are to be treated as radioactive waste and should be disposed of in accordance with local regulations. The gamma radiation associated with the decay of radium-223 and its daughters allows for the radioactivity measurement of radium-223 and the detection of contamination with standard instruments.

General warning:
Radium-223 should be received, used and administered only by authorized persons in designated clinical settings. The receipt, storage, use, transfer and disposal of radium-223 are subject to the regulations and/or appropriate licenses of the competent official organization.

Radium-223 should be handled by the user in a manner which satisfies both radiation safety and pharmaceutical quality requirements. Appropriate aseptic precautions should be taken.

Radiation protection:
The administration of radium-223 is associated with potential risks to other persons (e.g., medical staff, caregivers and patient’s household members) from radiation or contamination from spills of bodily fluids such as urine, feces, or vomit. Therefore, radiation protection precautions must be taken in accordance with national and local regulations.

For drug handling:

Follow the normal working procedures for the handling of radiopharmaceuticals and use universal precautions for handling and administration such as gloves and barrier gowns when handling blood and bodily fluids to avoid contamination. In case of contact with skin or eyes, the affected area should be flushed immediately with water. In the event of spillage of radium-223, the local radiation safety officer should be contacted immediately to initiate the necessary measurements and required procedures to decontaminate the area. A complexing agent such as 0.01 M ethylene-diaminetetraacetic acid (EDTA) solution is recommended to remove contamination.

For patient care:
Whenever possible, patients should use a toilet and the toilet should be flushed several times after each use. When handling bodily fluids, simply wearing gloves and hand washing will protect caregivers. Clothing soiled with radium-223 or patient fecal matter or urine should be washed promptly and separately from other clothing.

6.5 Treatment Compliance

Study drugs will be administered or dispensed only to eligible patients under the supervision of the investigator or identified sub-investigator(s). The appropriate study personnel will maintain records of study drug receipt and dispensing.

6.6 Drug Accountability

Study drugs should be kept in a secure place and must be administered only to patients in the trial. A log of study drug (received, administered to patients and destroyed) must be maintained and signed by the person responsible for drug handling at each center. Study site/institution personnel will record all study drug administered during this trial on the drug-dispensing log. Description of monitoring of the overall drug accountability will be detailed in study and site specific procedures.

The nuclear medicine specialist or radiation oncologist at the center is responsible for drug accountability for radium-223. When the drug accountability has been monitored, the vials can be destroyed in accordance with hospital procedure for the handling of radioactive material, normally after storage for 4 months from date of receipt (>10 half-lives) before disposal; they may then be disposed as non-radioactive waste.

6.7 Follow-Up Procedures

6.7.1 Blood Draws

Subsequent to randomization, patients will be followed clinically and radiographically. A detailed medical and physical examination with blood draws for PSA, LDH, CBC w/Diff, Serum Chemistry panel (CMP), ctDNA (CAPP-seq), immune cell profiling (ImmunoSEQ), CTCs (EPIC HD-CTC), immunophenotyping, and testosterone will be performed within the first month, months 3, 6 and 12 (from date of randomization) for all patients. Correlatives at outside sites are optional and not mandatory. In-clinic visits are not required otherwise, unless indicated by laboratory tests. For men randomized to the SABR + Radium-223, they will also receive monthly CBCs as per standard of care for radium-223 infusions (within 7-10 days of radium-223 injection). Following 12-months patients will be followed by any physician as per standard of care which may include but not be limited to every 3-6 month clinic visits, laboratory evaluations for PSA and imaging, if appropriate. Further labs and imaging is at the discretion of the treating physician. This information of two year followup will be collected by the clincal study team for all participating sites.

Correlative science blood draws will be performed as above (details for these blood draws are contained within the APPENDIX III-V).

Briefly:

CAPP–Seq: 30-ml whole blood in purple top (EDTA) tubes (see APPENDIX III for details). The blood is stored in a freezer, then shipped in batches as per APPENDIX III. Baseline, months 3, 6 and 12 from randomization for both arms.

EPIC HD-CTC: 4-10 ml Streck Cell-Free DNA BCT tubes (see APPENDIX IV for details). Whole blood is shipped out the same day as it is drawn as per APPENDIX IV. Baseline and day 180 from all men.

ImmunoSEQ: 10-ml whole blood in purple top (EDTA) tubes (see APPENDIX V for details). The patient samples are stored in a 80 Degree Celsius freezer and shipped in batches as per APPENDIX V. Baseline and day 90 from all men.

Immunophenotyping: 2-10-mL whole blood in green top (heparin) tubes (see APPENDIX VIII for details).

Only participating sites that have the resources to do so, will participate in bloow draw collection and shipment.

6.7.2 Imaging

Bone scan and CT/MRI of metastatic site(s) will be at 6- and 12-months for all patients, unless indicated more frequently by clinical or laboratory findings.

If applicable, bone scan and ^{18}F-DCFPyL (“PyL”) -PET/CT of metastatic site(s) will be at 6 months for all patients, unless indicated more frequently by clinical or laboratory findings. The results of the ^{18}F-DCFPyL (“PyL”) -PET/CT will be made available to the treating physicians prior to enrollment to determine treatment.

The CT portion of the ^{18}F-DCFPyL ("PyL") -PET/CT scans at 6 months will be used to determine radiographic response based on RECIST 1.1 criteria for soft tissue lesions. Similarly, the CT or MRI scans at 6- and 12-months will be used to determine radiographic response based on RECIST 1.1 criteria for soft tissue lesions.

6.7.3 Rectal Swab

The collected rectal swab (see APPENDIX VI for details) will provide researchers with a tool to analyze the gut microbiome of patients in an effort to investigate potential correlations between the gut microbiome and response to cancer therapies. Consented patients will be asked to self-collect two rectal swabs during an office visit by inserting a cotton tipped application approximately 3 cm (about 1 inch) into the anal canal and rotating it. The swab can then be returned to the provided tube. Rectal swabs (two per specified time-point) will be collected at enrollment within 1-mos of randomization but prior to SABR or radium-223 infusion and at the 3-month time points.

Only participating sites that have the resources to do so, will participate in rectal swab collection.

6.7.4 Rectal Swab Processing

The collected swabs will be immediately stored at 4° C to prevent bacteria growth before being moved to -20/-80° C. Collected swabs will be processed for DNA extraction and eventual next generation sequencing to provide a genomic profile of the bacteria present in the gut microbiome of patients.

6.7.5 Invitae Genomic Testing

Consented patients will be asked to provide a blood or saliva sample during an office visit or at home, via an Invitae collection kit (see APPENDIX VII for details). **This collection will be performed on all enrolled patients, at any time point during study or after completion of study (F/U), regardless of their randomization arm.** The Invitae collection kit will then be activated online and dropped in the mail. Invitae will then analyze the genes from the patient's blood or saliva sample. Once the patient's results are ready, an email will be sent by Invitae prompting the PI to log into his established Invitae account and view the results. The patient will be allowed to make an appointment with one of the Invitae's genetic counselors at no cost. Further, Invitae will also keep the patient updated if any information related to their results changes.

6.8 Duration of Therapy

Within three weeks of the initial treatment planning imaging study, SABR will be administered in a 1-5 fractions to each treated site (within 60 days from randomization) of the study and coincides with delivery of fraction 1.

Within four weeks of randomization to the SABR + Radium-223 arm, men will receive their 1st of 6 monhtly infusions of radium-223.

6.9 Duration of Study Accrual

A trial with a similar patient population took 2 years to screen 60-70 patients. Thus we anticipate this study will last 3 years.

6.10 Duration of Follow-Up

Patients will be followed for 2-years, after Day one. Importantly, this follow-up period will include the collection of all Skeletal Related events.

6.11 Criteria for Patient Removal

Diagnosis of >3 bone and/or soft tissue metastases on entry imaging studies before SBRT/SABR. PSA >50 ng/ml before SABR. Progression as defined in section 9.5.5.

Radium-223 administration may be delayed by no more than four weeks for recovery of adverse events. In case of a treatment delay greater than four weeks, treatment should be discontinued. It is important to note that in general (unless otherwise agreed), in cases where study drug has been ordered, the time window is reduced to -3 days to + 3 days, due to decay. If administration has to be postponed more than 3 days after drug has been ordered, replacement of the drug order is required. Dose reduction for radium-223 will not be permitted and subjects with any toxicity requiring radium-223 dose reduction must discontinue radium-223 treatment.

Any toxicity requiring radium-223 discontinuation will be recorded as an AE in the subject's medical record and on the CRF.

6.12 Alternatives

The study has been designed to minimize potential risks to participants. First, this population of asypmtomatic oligometastatic men is heavily pre-selected based on institutional data, STOMP and on-going ORIOLE phase II randomized data indicating that similar men will progress in 6-12-months (72-74). Secondly, the level of clinical-radiographic interrogation will allow both arms to be safely transitioned to standard of care treatments that can be initiated within a typical interval between clinic visits (72-74). Lastly, the SABR dose has been shown to be safe in previous trials. Risks to confidentiality will be minimized by having access to study records avaialble only to the investigators with the exception of the standard clinical records (lab values, dictations, operative notes, etc).

Standard therapies for metastatic prostate disease include radiotherapy, ADT or observation. Such treatment may or may not be applicable for patients enrolled in this study. Regardless, patients will be expected to forgo standard treatment until there is evidence of clinical, biochemical (PSA >50 ng/ml) or radiographic disease progression.

6.13 Costs

Every patient will be provided a copy of the Insurance and Research Participant Financial Responsibility Information Sheet. This document outlines the financial responsibilities for each study test and procedure based on a standard of care versus research analysis.

6.14 Compensation

Patients will not be financially compensated if they join the study.

6.15 Withdrawal from Study

In accordance with the Declaration of Helsinki, patients have the right to withdraw from the study at any time for any reason, without prejudice to their medical care. The investigator also has the right to withdraw patients from the study for any of the following reasons:

- Subject withdraws consent for follow-up.
- Subject is lost to follow-up.
- Study is terminated for any reason.
- Occurrence of AEs, if discontinuation of study medication is considered necessary by the Investigator and/or patient.
- Lack of patient compliance.
- Protocol violation.
- Progressive disease as defined in section 9.5.5.

7. INVESTIGATIONAL AGENT/DEVICE/PROCEDURE INFORMATION

7.1 Investigational Agent – Radium-223

This information is loaded into the IND application section of eIRB. Additional radiopharmaceutical information not listed below is downloaded on the FDA IND application in the supporting documents section of eIRB and available there for review.

7.2 Investigational Agent - ^{18}F-DCFPyL (“PyL”)

This information is loaded into the IND application section of eIRB. Additional radiopharmaceutical information not listed below is downloaded on the FDA IND application in the supporting documents section of eIRB and available there for review.

8. STUDY CALENDARS

Study Calendars

In order to minimize the need for research-only in-person visits, telemedicine visits may be substituted for in person clinical trial visits or portions of clinical trial visits where determined to be appropriate and where determined by the investigator not to increase the participants risks. Prior to initiating telemedicine for study visits the

study team will explain to the participant, what a telemedicine visit entails and confirm that the study participant is in agreement and able to proceed with this method. Telemedicine acknowledgement will be obtained in accordance with the Guidance for Use of Telemedicine in Research. In the event telemedicine is not deemed feasible, the study visit will proceed as an in-person visit. Telemedicine visits will be conducted using HIPAA compliant method approved by the Health System and within licensing restrictions.

STUDY CALENDAR: SBRT ARM ONLY

	Pre-StudyV	Day 1V	Day 61	Day 91 (+/- 7 days)	Day 181 (+/- 7 days)	Day 361 (+/- 7 days)	Follow-up Q6 Month[T] (+/- 7 days)
ICF	X	Randomization to SBRT arm					
Demographics	X						
Medical History	X	X[F, Q]		X[Q]	X[Q]	X[Q]	X[W]
Concomitant Medications	X	X[F, Q]		X[Q]	X[Q]	X[Q]	X[W]
Physical Exam	X	X[F, Q]		X[Q]	X[Q]	X[Q]	X[W]
Performance Status	X	X[F]		X	X	X	X[W]
Vital Signs	X	X					
Height	X						
Weight	X	X					
CBC w/ Diff[A]	X[C]	X		X	X	X	X[W]
LDH	X[C]	X		X	X	X	X[W]
Serum Chemistry[B]	X[C]	X		X	X	X	X[W]
Testosterone	X[C]				X	X	X[W]
PSA	X[D]			X	X	X	X[W]
Bone Scan	X[E]				X	X	X[W]
CT/MRI of Involved Site[I]	X[E]				X	X	X[W]
PSMA-PET/CT	X[R]	X[R]				X[S]	
CAPP-seq[J]		X[H]		X[H]	X[H]	X[H]	
EPIC HD-CTC[K]		X[H]			X[H]		
Immuno-SEQ[L]		X[H]		X[H]			
Immunophenotyping[M]		X[H]		X[H]	X[H]	X[H]	
Invitae[N]		X[G]					
Rectal Swab[O]		X		X			
AE Evaluation[U]		X	X	X	X	X	X
QoL Brief Pain Inventory		X	X	X	X	X	X
SBRT/SABR[P]		X					

A.	CBC w/ Diff includes: White Blood Cell Count, Red Blood Cell Count, Hemoglobin, Hematocrit, Mean Corpuscular Volume, Mean Corpus HgB, Mean Corpus HgB Conc, RBC Distribution Width, Platelet Count, Neutrophils %, Lymphocyte %, Monocyte %, Eosinophil %, Basophil %, Absolute Neutrophil Count, Absolute Lymphocyte Count, Absolute Monocyte Count, Absolute Eosinophil Count, Absolute Basophil Count, Immature Granulocyte %, and Absolute Immature Granulocyte Count.
B.	Serum Chemistry (CMP) includes Sodium, Potassium, Chloride, Carbon Dioxide (bicarbonate), Urea Nitrogen, Serum Creatinine, Est GFR Afr-Am(MDRD Eqn), Est GFR NonAfrAm(MDRD Eqn), Glucose, Calcium, Total Protein, Albumin, Total Bilirubin, Alkaline Phosphatase, Aspartate Amino Trans (AST), Alanine Amino Trans (ALT), Anion Gap, BUN/Creatinine Ratio, and AST/ALT ratio.
C.	CBC w/ Diff, LDH, Serum Chemistry, and Testosterone labs must be obtained within 6 months prior to enrollment.
D.	PSA lab must be obtained within 1 month prior to enrollment.
E.	Pre-study Bone Scan and CT/MRI need to be obtained within 3 months prior to enrollment. PET-CT scan is an alternative for study entry imaging to CT/MRI and/or Bone Scan
F.	Medical history review, concomitant medication review, physical exam, performance status assessment, adverse event evaluation, and the brief pain inventory assessment should be performed **once**, during either Day 1 or sometime during a SBRT visit (e.g. OTV).
G.	Invitae test should be performed at any time during the study or after follow up for all enrolled patients.
H.	Correlative study blood specimens should be drawn after standard of care labs, from the same line, to prevent epithelial cells from contaminating blood specimens.
I.	CT/MRI or Axumin/Choline/DCFPyL PETs should be used to measure tumor dimensions at baseline and to measure tumor dimensions and evaluate radiographic response on day 181 and on day 361. Imaging used at baseline can be further used at Day 180 and Day 360, respectively.
J.	Instructions for CAPP-Seq Sample Collection & Processing can be found in Appendix III of the protocol.
K.	Instructions for EPIC-HD CTC Sample Collection & Processing can be found in Appendix IV of the protocol.
L.	Instructions for ImmunoSeq Sample Collection & Processing can be found in Appendix V of the protocol.
M.	Instructions for Immunophenotyping Sample Collection & Processing can be found in Appendix VIII of the protocol. Only applicable for JHU/JHSOM. Not applicable for outside sites.
N.	Instructions for Invitae Saliva Kit Sample Collection can be found in Appendix VII of the protocol.
O.	Instructions for Rectal Swab Collection for Microbiome Analysis can be found in Appendix VI of the protocol.
P.	Instructions for SBRT/SABR treatment planning can be found in section 6.3 of the protocol.
Q.	After randomization, only interval concomitant medications, interval medical history, and limited physical exam information needs to be collected. This only needs to occur once, either on Day 1 visit or during SABR treatment.
R.	Per SOC, patient's will have a PSMA PET/CT prior to enrollment/randomization. Patients who are still enrolled in the trial will have a PSMA PET/CT 360 Days from initial PSMA PET/CT (see S).
S.	ONLY for sites participating in PSMA PET-CT: If applicable, patient's will have a PSMA PET/CT 12 Months from the second completed PSMA PET (~approximately 13/14 months from randomization)
T.	Additional follow-up visits are for 1 year. Follow up after 12 months will be conducted Q6 months, as SOC (months 18 and 24).
U.	All AEs will be collected using CTCAE version 4.0. All skeletal related events will need to be collected throughout the trial and during the follow-up period.
V.	If Pre-study and Day 1 occurs within 2 weeks of eachother, the following labs do not need to be repeated for Day 1: PSA, LDH, Testosterone, CBC w/Diff, and CMP. If PSA is completed within 2 weeks from Day 1, regardless of Footnote D, then it may be used. If PSA has been drawn greater than 2 weeks from Day 1, then it must be repeated. Research Blood for Day 1 MUST be completed PRIOR to SABR and/or Xofigo (Radium-223) injection.
W.	During 18 and 24 month follow-up: limited physical exam, ECOG (performance status) changes to conmedications, changes to medical history, Adverse Events, BPI (QoL), and PSA are required for documentation (Epic Note). Any additional labs or imaging are at the discretion of the treating physician.

STUDY CALENDAR: SBRT + Xofigo ARM

	Pre-StudyY	Day 1 Y	Day 15	Day 29	Day 43	Day 57	Day 61	Day 71	Day 85	Day 91 (+/- 7 days)	Day 99	Day 113	Day 127	Day 141	Day 155	Day 181 (+/- 7 days)	Day 361 (+/- 7 days)	Follow-up Q6 Month[W] (+/- 7 days)
ICF	X	Randomization to SBRT + Xofigo arm																
Demographics	X																	
Medical History	X			X[F, T]						X[T]						X[T]	X[T]	X[Z]
Concomitant Medications	X			X[F, T]						X[T]						X[T]	X[T]	X[Z]
Physical Exam	X			X[F, T]						X[T]						X[T]	X[T]	X[Z]
Performance Status	X			X[F]						X						X	X	X[Z]
Vital Signs	X		X		X		X			X		X		X				
Height	X																X	
Weight	X		X		X		X			X		X		X				
CBC w/ Diff[A]	X[C]		X[I and Y]		X[I]		X[I]			X[I]		X[I]		X[I]		X	X	X[Z]
LDH	X[C]		X[Y]							X						X	X	X[Z]
Serum Chemistry[B]	X[C]		X[Y]							X						X	X	X[Z]
Testosterone	X[C]		X[Y]							X						X	X	X[Z]
PSA	X[D]		X[Y]							X						X	X	X[Z]
Bone Scan	X[E]															X	X	X[Z]
CT/MRI/Axumin or Choline PET of Involved Site[K]	X[E]															X	X	X[Z]
PSMA-PET/CT	X[U]		X[U]														X[V]	
CAPP-seq[L]			X[H]							X[H]						X[H]	X[H]	
EPIC HD-CTC[M]			X[H]													X[H]		
Immuno-SEQ[N]			X[H]							X[H]								
Immunophenotyping[O]			X[H]							X[H]						X[H]	X[H]	
Invitae Test[P]										X[G]								
Rectal Swab[Q]			X							X								
AE Evaluation[X]			X		X[F]					X						X	X	X
QoL Brief Pain Inventory			X		X[F]					X						X	X	X
SBRT/SABR[R]					X													
Xofigo[S]			X[J]		X[J]		X[J]			X[J]		X[J]		X[J]				

A.	CBC w/ Diff includes: White Blood Cell Count, Red Blood Cell Count, Hemoglobin, Hematocrit, Mean Corpuscular Volume, Mean Corpus HgB, Mean Corpus HgB Conc, RBC Distribution Width, Platelet Count, Neutrophils %, Lymphocyte %, Monocyte %, Eosinophil %, Basophil %, Absolute Neutrophil Count, Absolute Lymphocyte Count, Absolute Monocyte Count, Absolute Eosinphil Count, Absolute Basophil Count, Immature Granulocyte %, and Absolute Immature Granulocyte Count.
B.	Serum Chemistry (CMP) includes Sodium, Potassium, Chloride, Carbon Dioxide (bicarbonate), Urea Nitrogen, Serum Creatinine, Est GFR Afr-Am(MDRD Eqn), Est GFR NonAfrAm(MDRD Eqn), Glucose, Calcium, Total Protein, Albumin, Total Bilirubin, Alkaline Phosphatase, Aspartate Amino Trans (AST), Alanine Amino Trans (ALT), Anion Gap, BUN/Creatinine Ratio, and AST/ALT ratio.
C.	CBC w/ Diff, LDH, Serum Chemistry, and Testosterone labs must be obtained within 6 months prior to enrollment.
D.	PSA lab must be obtained within 1 month prior to enrollment.
E.	Pre-study Bone Scan and CT/MRI need to be obtained within 3 months prior to enrollment. PET-CT scan is an alternative for study entry imaging to CT/MRI and/or Bone Scan
F.	Medical history review, concomitant medication review, physical exam, performance status assessment, adverse event evaluation, and the brief pain inventory assessment should be performed only **once,** either during Day 1 or during the SBRT visit (e.g. OTV).
G.	Invitae test should be performed at any time during the study or after follow up for all enrolled patients.
H.	Correlative study blood specimens should be drawn after standard of care labs, from the same line, to prevent epithelial cells from contaminating blood specimens.
I.	CBC w/ Diff must be performed and analyzed prior to each Xofigo infusion.
J.	For patients to receive Xofigo infusion #1, the following criteria must be met: • ANC ≥ 1.5 * 10^3 / L • Platelets ≥ 100 * 10^3 / L • Hemoglobin ≥ 10 g / dL For patients to receive Xofigo infusions #2-6, the following criteria must be met: • ANC ≥ 1* 10^3 / L • Platelets ≥ 50 * 10^3 / L If a patient fails to meet the criteria, the criteria should be re-checked every 7-10 days. If the patient does not recover to the required values within 6-8 weeks, further Xofigo treatments should be discontinued.
K.	CT/MRI or Axumin/Choline PETs should be used to measure tumor dimensions at baseline and to measure tumor dimensions and evaluate radiographic response on day 181 and on day 361. Imaging used at baseline can be further used at Day 180 and Day 360, respectively.
L.	Instructions for CAPP-Seq Sample Collection & Processing can be found in Appendix III of the protocol.
M.	Instructions for EPIC-HD CTC Sample Collection & Processing can be found in Appendix IV of the protocol.
N.	Instructions for ImmunoSeq Sample Collection & Processing can be found in Appendix V of the protocol.
O.	Instructions for Immunophenotyping Sample Collection & Processing can be found in Appendix VIII of the protocol. Only applicable to JHU/JHSOM. Outside sites will not be performing Immunophenotyping
P.	Instructions for Invitae Saliva Kit Sample Collection can be found in Appendix VII of the protocol.
Q.	Instructions for Rectal Swab Collection for Microbiome Analysis can be found in Appendix VI of the protocol.
R.	Instructions for SBRT/SABR treatment planning can be found in section 6.3 of the protocol.
S.	Instructions and information for Xofigo (Radium-223) can be found in section 6.3 of the protocol.
T.	After randomization, only interval concomitant medications, interval medical history, and limited physical exam information needs to be collected only once, either during Day 1 visit or during SABR.
U.	Per SOC, patient's will have a PSMA PET/CT prior to enrollment/randomization. Patients who are still enrolled in the trial will have a PSMA PET/CT 360 Days from initial PSMA PET/CT (see V).
V.	ONLY for sites participating in PSMA PET-CT - If applicable, patient's will have a PSMA PET/CT 12 Months from the first completed PSMA PET (~approximately 13/14 months from randomization)
W.	Additional follow-up visits are for 1 year. Follow up after 12 months will be conducted Q6 months, as SOC (months 18 and 24)
X	All AEs will be collected using CTCAE version 4.0. All skeletal related events will need to be collected throughout the trial and during the follow-up period.
Y.	If Pre-study and Day 1 occurs within 2 weeks of eachother, the following labs do not need to be repeated for Day 1: PSA, LDH, Testosterone, CBC w/Diff, and CMP. If PSA is completed within 2 weeks from Day 1, regardless of Footnote D, then it may be used. If PSA has been drawn greater than 2 weeks from Day 1, then it must be repeated. Research Blood for Day 1 MUST be completed PRIOR to SABR and/or Xofigo (Radium-223) injection.
Z	During 18 and 24 month follow-up: limited physical exam, ECOG (performance status) changes to conmedications, changes to medical history, Adverse Events, BPI (QoL), and PSA are required for documentation (Epic Note). Any additional labs or imaging are at the discretion of the treating physician.

9. MEASUREMENT OF EFFECT

9.1 Antitumor Effect – Metastatic Tumors

For the purposes of this study, patients should be re-evaluated for radiographic response 6- and 12-months after randomization. Trial radiologists evaluating for treatment responses will be blinded to the treatment group and treatment specifics (lesions treated with SABR).

Response and progression will be evaluated in three ways:

1) Using Response Evaluation Criteria in Solid Tumors (RECIST 1.1) Committee definition. Changes in only the largest diameter (uni-dimensional measurement) of the tumor lesions are used in the RECIST criteria.
2) Lesions by bone scan will be evaluated as positive, negative or no change. MRI of the bone lesion can be used to clarify equivocal lesions.
3) Serial PSA changes.

9.2 Definitions

Evaluable Population: will consist of all patients who have received SABR.

Safety Population: Will consist of all subjects who were enrolled and have undergone at least one fraction of SABR. This will be used to assess the clinical safety and tolerability of the study.

Evaluable for Objective Response: Only those patients who have measurable disease present at baseline, have completed all fractions of SABR, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below.

9.3 Disease Parameters

Measurable Disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥10 mm with diagnostic techniques (CT, or MRI). All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters). Serial PSA measurements will also be analyzed.

Non-Measurable Disease: All other lesions (or sites of disease), including small lesions (longest diameter <10 mm with diagnostic techniques), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

Target Lesions: Target lesions in this study will be considered oligometastatic sites up to a maximum of 3 lesions per patient. They should be recorded and measured at baseline. Target lesions should be equal to or larger than 10 mm in the smallest cross-sectional diameter on CT or MRI and/or any lesion that shows

increase uptake on bone scans. A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response. For bone scans we will also use a simple evaluation system composed of positive, negative or no change to lesions.

Non-Target Lesions: N/A

9.4 Methods for Evaluation of Measurable Disease

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

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

Conventional CT, PET/CT, and MRI. These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

9.5 Response Criteria

9.5.1 Evaluation of Target Lesions/PSA Response

Complete Response (CR):	Disappearance of all target lesions and PSA < pre SBRT PSA
Partial Response (PR):	At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD. Or a third of the lesions are negative or no change by bone scan and PSA ≤ pre-SBRT PSA.
Progressive Disease (PD):	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of ≥1 new lesion(s). Or ≥1 new lesion(s) appear by bone scan. Or PSA ≥25% increase in PSA from nadir or > 50 ng/ml.
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as

reference the smallest sum LD since the treatment started. Or PSA ≥ pre-SBRT PSA, but not ≥25% increase in PSA from nadir and <50 ng/ml.

9.5.2 Evaluation of Non-Target Lesions

N/A

9.5.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). Best overall response will be based on the overall response of the target lesions.

9.5.4 Duration of Response

Response will be defined as evidence of CR, PR, or stable disease. The duration of response will be measured from the start of treatment until the criteria for progression are met.

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

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

9.5.5 Clinical Response Parameters

Progression is a composite endpoint defined from the Prostate Cancer Working Group 2 (PCWG2) criteria for metastatic castrate resistant prostate cancer (mCRPC) (75) and our previous trials in a population of men with biochemical failure without metastases (72-74). Progression will be defined as either: 1) a ≥25% increase in PSA from nadir (and by ≥2 ng/mL), requiring confirmation ≥4 weeks later (PCWG2 criteria); and/or, 2) clinical/radiographic-progression defined as symptomatic progression (worsening disease-related symptoms or new cancer-related complications), or radiologic progression (on CT scan: ≥20% enlargement in sum diameter of soft-tissue target lesions [RECIST 1.1 criteria]; on bone scan: ≥1 new bone lesions), initiation of ADT or death due to any cause, whichever occurs first. Death is considered a severe adverse event here.

Progression Free Survival (PFS) is defined as the time from starting treatment to the time of progression as defined above. Subjects who do not progress will be censored at the time of the last contact.

ADT Free Survival (ADT-FS) is defined as the time from starting treatment to the time of initiation of palliative ADT. ADT will typically be initiated on tumor progression and/or development of new metastases. Subjects who do not start ADT will be censored at the time of the last contact.

Time to Progression (TTP) is defined as the time from starting treatment to the time of first documented tumor progression or new lesions by CT and/or bone scan or initiation of ADT. Subjects who do not progress will be censored at the time of the last contact. In addition, death from any cause will also be censored.

Time to New Metastasis (TNM) is defined as the time from starting treatment to the time of a new documented tumor metastasis by CT and/or bone scan. Subjects who do not progress will be censored at the time of the last contact.

Overall Survival (OS) is defined as the time from starting treatment until death due to any cause. For subjects who do not die, time to death will be censored at the time of last contact.

Locoregional Control (LRC) is defined as the time from starting treatment until local and/or regional relapse is documented

9.5.6 Response Review

All responses will be reviewed by the study co-investigator radiologists.

10. ADVERSE EVENT REPORTING REQUIREMENTS

10.1 General

Adverse event collection and reporting is a routine part of every clinical trial. This study will use the descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v 4.0) that is available at http://ctep.cancer.gov/reporting//ctc.html.

Information on all adverse events, whether reported by the participant, directly observed, or detected by physical examination, laboratory test or other means, will be collected, recorded, followed and reported as described in the following sections.

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. The investigator should notify the IRB and any other applicable regulatory agency of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

10.2 Definitions

10.2.1 Adverse Event (AE)

An adverse event is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

10.2.2 Serious adverse event (SAE)

A serious adverse event is an undesirable sign, symptom, or medical condition which:

- is fatal or life-threatening;
- requires or prolongs inpatient hospitalization;
- results in persistent or significant disability/incapacity;
- constitutes a congenital anomaly or birth defect; or
- jeopardizes the participant and requires medical or surgical intervention to prevent one of the outcomes listed above.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

10.2.3 Expectedness

- Expected: Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered <u>expected</u> when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.
- Unexpected: An adverse event is considered <u>unexpected</u> when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk

10.2.4 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely – The AE is doubtfully related to the study treatment.
- Unrelated - The AE is clearly NOT related to the study treatment.

10.3 Potential Adverse Events

Signs and symptoms of disease progression are not considered AEs. The development of a new cancer should be regarded as an AE. New cancers are those that are not the primary reason for administration of study treatment and have been identified after inclusion of the patient into the clinical study.

Because patients are receiving standard treatments, which are not part of this study, their treating physician will be counseling them on the risk of their treatments, including the risk of surgery, radiation therapy, and/or chemotherapy, whichever is appropriate for the type and the stage of their cancer. The procedures related to the study are phlebotomy, SBRT, and Radium-223 administration.

Phlebotomy can cause pain, bleeding, and rare needle site infection.

Reporting: All patients will be seen during the required protocol and calendar time-points, by their radiation oncologist or study team membe, during the entirety of the trial. Any observations durig the entirety of the study (2 years) will be recorded and should include attention toward the following potential side effects:

- Anal hemorrhage, colitis, constipation, diarrhea, fecal incontinence, hemorrhoids, nausea, proctitis, rectal hemorrhage, vomiting
- Anorexia, anxiety, and/or insomia
- Bladder spasms, hematuria, urinary frequence, irinary incontinence, urinarary retion, urinary tract pain, urinary urgency, erectile dysfunction
- Dermatitis radiation
- Fatigue and/or pain
- Hot flashes
- Bone Pain
- Netrophil count decrease and/or platelet count decrease

Any and ALL additional adverse events should be recorded, even if not listed in the above potential side effects.

10.4 Stereotactic Body Radiation Treatment (SBRT)/Stereotactic Ablative Radiation Treatment (SABR)

It is difficult at this time to predict with confidence the complication rate from the proposed SBRT/SABR; however, it is reasonable to extrapolate from the current experience with SBRT/SABR to the lung, prostate, spine, liver and pancreas. One significant toxicity is radiation pneumonitis, which can be manifested as fever, increased excertional dypsnea, pleuritic chest pain, and peritumoral infiltrate on chest imaging. It generally occurs between 1 to 3 months of completion of radiotherapy. The risk of grade 2-4 radiation pneumonitis is aproximately 10-15% in patients treated with standard fractionated large field radiotherapy and higher in patients treated with combined chemoradiotherapy. It is highly dependent on the volume of the lung treated to high dose and the mean lung dose. At this point, the incidence of RT pneumonitis from stereotactic radiosurgery for small pulmonary tumors is unknown. However, if the treated tumor volume is kept ≤ 65 cc, the risk should be < 10-15% with the proposed dose level.

Other toxicities commonly associated with such treatment includes dysphagia, odynophagia, nausea, vomiting, anorexia, and weight loss. Some of these symptoms can also be due to tumor progression. Clinical and radiographic assessments will be performed as indicated to identify all adverse effects, ascertain their etiology, and provide the most appropriate palliative measures. Complications orther than radiation pneumonitis, if any, will be graded according to the Common Toxicity Criteria, National Cancer Institute, version 4.0.

10.5 Reporting Procedures

10.5.1 General and Multi-Site Reporting

10.5.1.1 General

Adverse events will be recorded at each visit. If an adverse event requiring medical attention occurs between visits, this will be recorded as well. The variables to be recorded for each adverse event include, but are not limited to, onset, resolution, intensity, action taken, outcome, causality rating and whether it constitutes an SAE or not. The intensity of the adverse event should be captured using CTCAE criteria, version 4.0, when possible. Additionally, the collection of all skeletal related events will be collected for 2 years.

All Serious Adverse Events (SAEs) will be reported via the AdEERS (Adverse Event Expedited Reporting System) application accessed via the CTEP web site (https://webapps.ctep.nci.nih.gov/openapps/plsql/gadeers_main$.startup).

In addition, any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the industry collaborator Bayer HealthCare Pharmaceutical Inc. When required, and according to local law and regulations, serious adverse events must be reported to the Ethics Committee and Regulatory Authorities. All serious adverse events should be reported to Bayer HealthCare Pharmaceutical Inc. within 24 hours.

All adverse events will be captured on the appropriate study-specific case report forms (CRFs).

10.5.1.2 Reporting SAEs at Multi-Site/Participating Institutions

A subject's AEs and SAEs will be recorded and reported from the signing of the ICF up to at least 30 days after completing salvage radiotherapy. The investigator must instruct the subject to report AEs and SAEs during this time period.

Subjects with evidence of Xofigo related toxicity at the post-treatment follow-up visit will be followed via telephone contact or weekly visits until the drug- related toxicity has resolved. Additionally, the collection of all skeletal related events will be collected for 2 years. Adverse events should be based on the signs or symptoms detected during the physical examination and on clinical evaluation of the subject. In addition to the information obtained from those sources, the subject should be asked the following nonspecific question: "How have you been feeling since your last visit?" Signs and symptoms should be recorded using standard medical terminology. Any unanticipated risks to the subjects must be reported promptly to the IRB/IEC. In the event of an adverse event the first concern will be for the safety of the subject.

All AEs and SAEs must be recorded on source documents. All AEs and SAEs for subjects who meet inclusion and exclusion criteria will be recorded in the CRFs and submitted to the lead site. The investigator must follow up on all AEs and SAEs until the events have subsided, returned to baseline or, in case of permanent impairment, until the condition stabilizes.

Additional reporting is required for SAEs as follows:

- SAEs that are fatal and possibly protocol related should be reported by phone to the Protocol Chair immediately upon knowledge of the event.

Each participating site is responsible for evaluating and completing any additional reporting to their local IRB according to local IRB guidelines.

All SAEs, regardless of causality to study drug and/or administration device, will be reported promptly to the Coordinating Center (e-mail: crocc@jhmi.edu and nradwan1@jh.edu), within 24 hours of recognition of the event. If this falls on a weekend or holiday, an email notification is acceptable but must be followed by an SAE reporting form on the next business day.

CRO Coordinating Center
The CRO Coordinating Center is the central location for the collection and maintenance of documentation of adverse events and is responsible for submitting adverse event reports to the Protocol Chair promptly. The CROCC will maintain documentation of all adverse event reports for each participating site. Adverse event reports submitted to the CRO Coordinating Center must be signed and dated by the participating site's Principal Investigator. The CRO Coordinating Center will provide appropriate forms to be used by all participating sites for reporting adverse events. Information to be provided must include:

- Subject ID number, and initials
- Date of the event
- Description of the event
- Description of site's response to the event
- Assessment of the subject's condition
- Subject's status on the study (on study, off study, etc.)
- Attribution of event to study drug

Participating Sites
Participating sites are responsible for reporting adverse events to their IRB according to its specific requirements and to the CRO Coordinating Center as follows:
Fatal Events whether anticipated or unanticipated, and whether or not related to the study must be reported to the CRO Coordinating Center within 24 hours of the participating site Principal Investigator's learning of the event.

Serious and Unanticipated Adverse Events as defined above must be reported to the CRO Coordinating Center within 24 hours of the participating site Principal Investigator's learning of the event.

Other Serious Adverse Events which may result in a change to the protocol, informed consent, or risk to subjects as specified in the protocol must be reported within three (3) working days of the participating site Principal Investigator's learning of the event.

Adverse Events which result in no change to protocol, informed consent, or risk to subjects must be reported to the CRO Coordinating Center on a monthly basis.

Adverse event reports are to be emailed (use fax as a back-up) to the CRO Coordinating Center at SKCCC. Follow-up reports are faxed, mailed, or sent electronically to the Coordinating Center as necessary.

The investigator must also report follow-up information about SAEs within the same time frames.

If a non-serious AE becomes serious, this and other relevant follow-up information must also be provided within the same time frames described above.

All SAEs must be collected whether or not they are considered causally related to the investigational product. Investigators and other site personnel are responsible for reporting all casually related SAEs to their IRB and the Protocol Chair.

The principal investigator will notify the appropriate regulatory agencies of any serious adverse event due to any cause during the course of this investigation. These include the Johns Hopkins Cancer Center Data and Safety Monitoring Committee, and the Johns Hopkins Medical Institutional Review Board (JHM-IRB) of The Johns Hopkins Medical Institutions. The required reporting time period is 3 days for fatal events, and 10 days for all other events.

10.5.2 Expedited FDA Reporting Requirements for Unexpected and Related Serious Adverse Events (per 21CFR312.32)

<u>7 Calendar-Day Telephone or Fax IND Safety Report</u>
The Sponsor-Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the Sponsor-Investigator to be possibly related to the use of Radium-223 within 7 calendar-days of first learning of the event. An unexpected adverse event deemed possibly related to the use of an investigational study drug is defined as any adverse drug experience of which the specificity or severity is not consistent with the current investigator brochure, the general investigational plan, or elsewhere in the current application, as amended.

Such reports are to be telephoned or faxed to the FDA within 7 calendar-days of first learning of the event. Each telephone call or fax transmission should be directed to the FDA new drug review division in the Center for Drug Evaluation and Research or in the product review division for the Center for

Biologics Evaluation and Research, whichever department is responsible for the review of the IND.

15 Calendar-Day Written IND Safety Report

The Sponsor-Investigator (Dr. Ana Kiess) is required to notify the FDA, and all participating investigators (as applicable), in a written IND Safety Report, of any serious, unexpected adverse event considered by the Sponsor-Investigator to be possibly related to the use of Radium-223within 15 calendar days of first learning of the event. If applicable, the Sponsor-Investigator must also notify the FDA, and all participating investigators (as applicable), of any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity within 15 calendar-days of first learning of the event.

A serious, unexpected adverse event deemed possibly related to the use of an investigational study drug is any adverse drug experience of which the specificity or severity is not consistent with the current investigator brochure, the general investigational plan, or elsewhere in the current application, as amended, and results in any of the following outcomes:

- Death (report first as a 7-day telephone/fax report);
- life-threatening adverse drug experience (report first as a 7-day telephone/fax report);
- inpatient hospitalization or prolongation of existing hospitalization; a persistent or significant disability/incapacity, or a congenital anomaly/birth defect; or is an important medical event that may not result in death, be life-threatening, or require hospitalization but is considered a serious adverse drug experience when, based upon appropriate medical judgment, it may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

All written IND Safety Reports should include an Analysis of Similar Events in accordance with 21CFR312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports and be submitted to the FDA, Bayer Pharmaceuticals, and all participating investigators (as applicable), within 15 calendar-days of first learning of the event. The FDA prefers these reports be documented on a MedWatch 3500A Form, but alternative formats are acceptable (e.g., summary letter). This form is available at http://www.fda.gov/medwatch/report/hcp.htm.

Summary: For studies conducted under an Investigator IND, such as this trial, any event that is both serious and unexpected must be reported to the Food and Drug Administration (FDA) as soon as possible and **no later than 7 days** (for a death or life-threatening event) or **15 days** (for all other SAEs) **after the investigator's or institution's initial receipt of the information.**

BMS will be provided with a simultaneous copy of all adverse events filed with the FDA.

SAEs should be reported on MedWatch Form 3500A or similar form. It MUST include the institutional **AND** [Supporter Name] study ID [per study Agreement]

MedWatch SAE forms should be sent to the FDA at:
MEDWATCH
5600 Fishers Lane
Rockville, MD 20852-9787
Fax: 1-800-FDA-0178 (1-800-332-0178)

Follow-up Reports

All follow-up information concerning IND Safety Reports should be submitted to the FDA as soon as possible.

10.5.3 Institutional Review Board

All adverse events and serious adverse events will be reported to the IRB per current institutional standards. If an adverse event requires modification of the informed consent, these modifications will be provided to the IRB with the report of the adverse event. If an adverse event requires modification to the study protocol, these modifications will be provided to the IRB as soon as is possible.

11. DATA REPORTING AND REGULATORY REQUIREMENTS

The SKCCC Lead Coordinating Center will monitor the conduct and progress of the clinical trial at all participating sites. It will provide administrative, data management, regulatory, and organizational support in the conduct of this multi-center trial. The Coordinating Center will also function as the central location for multi-center trial documents and patient registration. For more information regarding reporting, regulatory requirements and guidelines, see Appendix D.

11.1 Multicenter Guidelines

11.1.1. The Protocol Chair

The Protocol Chair, Ana P. Kiess, is responsible for performing the following tasks:

- Taking responsibility for the overall conduct of the study at all participating institutions and for monitoring the progress of the study.

- Coordinating, developing, submitting, and obtaining approval for the protocol as well as its subsequent amendments.
- Assuring that all participating institutions are using the correct version of the protocol.
- Reviewing and ensuring reporting of Serious Adverse Events (SAEs).
- Reviewing data from all sites

11.1.2. Lead Coordinating Center

The Lead Center SKCCC, Johns Hopkins University is responsible for performing the following tasks:

- Ensuring that IRB approval has been obtained at each participating site prior to the first patient registration at that site.
- Maintaining copies of IRB approvals from each site.
- Implementing central patient registration
- Prepare appropriate data as required for review by the Protocol Chair.
- Establishing procedures for documentation, reporting and submitting of AE's and SAE's to the Protocol Chair and all other applicable parties.
- Facilitating audits by securing selected source documents and research records from participating sites for audit, or by auditing at participating sites.

12. DATA AND SAFETY MONITORING PLAN

12.1 Data Management

The Lead Coordinator, Noura Radwan (nradwan1@jh.edu) will manage the study activities at each of the participating sites. The responsibilities of the Lead Coordinator include project compliance, data collection, data entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of the activities of the protocol team.

De-identified study data may potentially be combined with other data in future studies with the University of Maryland for other research purposes. A DUA and is in process (see Section 36 of the IRB application).

Outside participating sites will share de-identified patient data/information, with our co-investigators/sponsors (Johns Hopkins University) including genetic (somatic and germline) information that has been obtained for patient care and/or research purposes.

Eligible patients will be entered on study centrally at the Sidney Kimmel Comprehensive Cancer Center at the Johns Hopkins University by the RAVENS Lead Study Coordinator. All sites should email the the Lead Study Coordinator

(Nradwan1@jh.edu). The Registration Form, and Eligibility Worksheet will be supplied to each participating site.

At the time of registration:

- Informed Consent Form (signed by the subject)
- Eligibility Checklist
- Source documents related to eligibility and randomization

The Study Coordinator at the participating site will then e-mail the RAVENS Lead Study coordinator (nradwan1@jh.edu) to verify eligibility. To complete the registration process/randomization, the Coordinating Center will:

- Assign a patient study number
- Randomization Patient
- Register the patient on the treatment portion of the study
- E-mail the patient study number to the participating site
- E-mail the research nurse or data manager or coordinator at the participating site

If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Lead Coordinator at the Coordinating Center should be notified of cancellations as soon as possible.

Within 4 weeks after randomization:

- Baseline study case report forms
- Pertinent source documents

Within 18 weeks (4 months) after randomization:

- Day 90 study case report forms
- Xofigo 1, 2, 3 injections case report forms, if applicable
- Pertinent source documents

Within 24 weeks (6 months) after randomization:

- Day 180 study case report forms
- Xofigo 4, 5, 6 injections case report forms, if applicable
- Pertinent source documents

Within 2 weeks after Day 360 :

- On study case report forms
- Pertinent source documents

Within 2 weeks after Month 18 F/U:

- Follow-up case report forms
- Pertinent source documents

12.2 Data Safety

The SKCCC Lead Coordinating Center will monitor the conduct and progress of the clinical trial at all participating sites. It will provide administrative, data management, regulatory, and organizational support in the conduct of this multi-center trial. The Coordinating Center will also function as the central location for multi-center trial documents and patient registration.

The SKCCC Compliance Monitoring Program will provide external monitoring for JHU-affiliated sites in accordance with SKCCC DSMP (Version 6.0, 02/21/2019). The SMC Subcommittee will determine the level of patient safety risk and level/frequency of monitoring.

Data and safety monitoring oversight will be conducted by the SKCCC at Johns Hopkins Safety Monitoring Committee. Per the SKCCC at Johns Hopkins Safety Monitoring plan, the CRO AQ will forward summaries of all monitoring reports to the Safety Monitoring Committee for review. All reportable anticipated and unanticipated protocol events/problems and amendments that are submitted to the IRB will also be reviewed by the Safety Monitoring Committee Chair (or designee) and QA manager.

Interim analysis of toxicity, outcome and ongoing scientific investigations will be performed at least annually by the Sidney Kimmel Comprehensive Cancer Center Data Safety Monitoring Board (SKCCC DSMB). The SKCCC DSMB Recommendation letter will state the timeline for the next required review. The SKCCC DSMB will review aspects of this trial that are outlined in the responsibilities section of the Data and Safety Monitoring Board (DSMB) Guidance. If the committee decides that amendments should be made to this trial, recommendations will be made in writing to the Study Principal Investigator. The study team will submit modifications to the IRB within 60 days of receipt from the DSMB. The Associate Director of Clinical Research, will arbitrate any disagreements between the DSMB and the study Principal Investigator. These changes may include early termination of accrual if deemed appropriate.

12.3 Multicenter Guidelines

Protocol Chair

- The Protocol Chair is responsible for performing the following tasks:
- Coordinating, developing, submitting, and obtaining approval for the protocol
- as well as its subsequent amendments
- Assuring that all participating institutions are using the correct version of the protocol.
- Taking responsibility for the overall conduct of the study at all participating institutions and for monitoring the progress of the study.
- Reviewing and ensuring reporting of Serious Adverse Events (SAE)
- Reviewing data from all sites

De-identified study data may potentially be combined with other data in future studies with the University of Maryland for other research purposes. A DUA is in process with the JH Office of Research Administration (see also Section 36 of the IRB application).

Outside participating sites will share de-identified patient data/information, with our co-investigators/sponsors (Johns Hopkins University) including genetic (somatic and germline) information that has been obtained for patient care and/or research purposes.

CRO Coordinating Center

The CRO Coordinating Center and the Lead Study Coordinator are responsible for performing the following tasks:

- Ensuring that IRB approval has been obtained at each participating site prior to the first patient registration at that site, and maintaining copies of IRB approvals from each site.
- Managing central patient registration.
- Collecting and compiling data from each site.
- Establishing procedures for documentation, reporting, and submitting of AE's and SAE's to the Protocol Chair, and all applicable parties.
- Facilitating audits by securing selected source documents and research records from participating sites for audit, or by auditing at participating sites.

Participating Sites

Participating sites are responsible for performing the following tasks:

- Following the protocol as written, and the guidelines of Good Clinical Practice (GCP).
- Submitting data to the Coordinating Center.
- Registering all patients with the Coordinating Center by submitting patient registration form, and signed informed consent promptly.
- Providing sufficient experienced clinical and administrative staff and adequate facilities and equipment to conduct a collaborative trial according to the protocol.
- Maintaining regulatory binders on site and providing copies of all required documents to the Coordinating Center.
- Collecting and submitting data according to the schedule specified by the protocol

Principal Investigator Responsibilities

The Protocol Chair is responsible for performing the following tasks:

- Coordinating, developing, submitting, and obtaining approval for the protocol as well as its subsequent amendments.
- Assuring that the correct version of the protocol is used.

- Taking responsibility for the overall conduct of the study and for monitoring the progress of the study.
- Reviewing and ensuring reporting of Serious Adverse Events (SAE).
- Reviewing data from all sites.

Data Entry

Data collected during this study will be entered into a secure database. Staff at the SKCCC coordinating center will be responsible for the initial study configuration and setup in the consortium database and for any future changes.

Case report forms

Case report forms will be generated by the coordinating center for the collection of all study data. Investigators will be responsible for ensuring that the CRFs are kept up-to-date.

The schedule for completion and submission of Case Report Forms and **all** supporting documents to the Lead Coordinating Center is as follows:

Form	**Submission Timeline**
Eligibility Checklist	Complete prior to registration
Baseline Assessment Form	Within 14 days of registration
On-Treatment Forms (including drug diary)	Within 10 days of course completion
Adverse Event Report Form	Within 14 days of each visit date
Follow-up Form	Within 14 days of visit date
Off Study Form	Within 14 days of being taken off study for any reason

Data should also be submitted within the Johns Hopkins OneDrive Folder or RedCap.

Source documents

Study personnel will record clinical data in each patient's source documents (ie, the patient's medical record). Source documentation will be made available to support the patient research record. Study monitors will review entries on the CRFs at regular intervals, comparing the content with source documents.

Record retention

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. After study closure, the investigator will maintain all source documents, study-related documents, and the CRFs. Because the length of time required for retaining records depends upon a number of regulatory and legal factors, documents should be stored until the

investigator is notified that the documents may be destroyed. In this study, records are to be retained and securely stored for a minimum of 3 years after the completion of all study activities.

12.4 Biospecimen Management

Specimens will be received by JHU from other participating sites, per protocol calendar, and then stored at JHU. Specimens will be sent to collaborating universities (Stanford University and Mayo Clinic) and vendors for analysis as per protocol appendices and materials transfer agreements.

The rectal swab samples will be used for DNA sequencing of the microbiome. The fundamental DNA sequencing technology is common and available at most institutions, but the bioinformatic pipelines and expertise needed to ensure good data quality and analysis is niche. Dr. Michael Liss (UCSD) and Dr. Rob Knight (UCSD) are experts in this regard to help with end-to-end sample handling, sample preparation, sequencing, and data analysis.

The role of Dr. Liss, Dr. Knight, and their team at UCSD will be to process the swab samples and perform DNA sequencing on the microbiota. The role of the JHU team in this collaboration will be to provide outcome data with no obvious PHI from the RAVENS trial to allow for clinical correlations to be made based on results of the DNA sequencing done at UCSD. No physical materials will be transferred back to Hopkins. The analysis will be performed at UCSD and the results will be shared with the JHU team once completed to facilitate writing of a manuscript. Dr. Liss' team will be leading the manuscript writing.

The JHU Office of Research Administration is initiating a Material Transfer Agreement with UCSD regarding this data analysis.

13. REGULATORY CONSIDERATIONS

13.1 Protocol Review and Amendments

Information regarding study conduct and progress will be reported to the Institutional Review Board (IRB) per the current institutional standards.

Any changes to the protocol will be made in the form of an amendment and must be approved by the IRB prior to implementation.

13.2 Informed Consent

The investigator (or his/her designee) will explain to each subject the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject will be informed that participation in the study is voluntary, that she may withdraw from the study at any time, and that withdrawal of consent will not affect her subsequent

medical treatment or relationship with the treating physician(s) or institution. The informed consent will be given by means of a standard written statement, written in non-technical language, which will be IRB approved. The subject should read and consider the statement before signing and dating it, and will be given a copy of the document. No subject will enter the study or have study-specific procedures done before his/her informed consent has been obtained.

In accordance with the Health Information Portability and Accountability Act (HIPAA), the written informed consent document (or a separate document to be given in conjunction with the consent document) will include a subject authorization to release medical information to the study sponsor and supporting agencies and/or allow these bodies, a regulatory authority, or Institutional Review Board access to subjects' medical information that includes all hospital records relevant to the study, including subjects' medical history.

13.3 Ethics and GCP

This study will be carried out in compliance with the protocol and Good Clinical Practice, as described in:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
2. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
3. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The investigator agrees to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

14. STATISTICAL CONSIDERATIONS

14.1 Endpoints

14.1.1 Primary Objective

To determine progression-free survival in patients with oligometastatic (three metastases or less) hormone sensitive prostate cancer and at least one bone metastasis who have been are treated with SABR *versus* SABR + Radium-223.

14.1.2 Secondary Objectives

- To describe the toxicity of SABR and SABR + Radium-223 delivered for the population enrolled using grading with CTCAE v. 4.0
- To determine local control at 12-months after SABR and SABR + Radium-223 in patients with oligometastatic disease.

- To assess time to locoregional progression, time to distant progression, time to new metastasis and duration of response after randomization to SABR *versus* SABR + Radium-223.
- To assess ADT-free survival (ADT-FS) of this patient population after randomization defined as the time interval between the day of randomization and the initiation of ADT. ADT will typically be initiated on progression and/or development of new metastases.
- To assess quality of life following SABR *versus* SABR + Radium-223 arms. Brief Pain Inventory form which will be filled out by the patient at the treatment response intervals outlined above.

14.2 Sample Size/Accrual Rate/Follow-Up

The primary endpoint will be progression-free survival (PFS). Data from STOMP and ORIOLE on this patient population indicate that ≥50-60% would show progression as defined above within a 12-month period from SABR, and a median PFS around 10 months. (72-74). We hypothesize that the addition of radium-223 will be able to reduce the risk of progression by 50%. A sample size using a 1:1 randomization scheme of 30 patients per arm will provide 80% power to detect an increase of median PFS from 10 months to 20 month (corresponding to hazard ratio 0.5) with type I error = 0.1, using one-sided log-rank test. The calculation assumes 18 months of accrual time with an additional follow-up of 12 months after the last patient is randomized. To account for 5% early drop out, we will randomize a total of 64 patients (32 per arm).

There will be no interim analysis for futility, since the progression endpoint will not have been reached by a meaningful number of patients before full accrual.

Follow-up (Every 3-6 months for 24 months or until death)

Patients will be followed for 2 years after Day One of trial or until death. Patients withdrrawn from the study because of AEs will be followed until the adverse event has either resolved or stabilized. Reasons for premature withdrawl should be determined and noted.

14.3 Stopping Guidelines

This study will monitor site-specific grade 4/5 toxicity in the SABR + Radium-223 arm. If it becomes evident that the proportion of grade 4/5 toxicity at specific sites convincingly exceeds 20%, the study will be halted for a safety consultation. Specifically, we will apply a Bayesian toxicity monitoring rule that suspends the enrollment if the posterior probability of toxicity being larger than 20% threshold is 75% or higher. The monitoring rule uses Beta (0.5, 5.5) as prior distribution. This means that our prior guess of the proportion of toxicity is 8.3%, and there is

90% chance that this proportion is 0.04%-30.6%. The monitoring will start from the first patient, and the decision rule for safety stopping is as follows:

Stop if:

# grade 4/5 toxicity >=	3	4	5	6	7	8	9	10
Out of # patients	3 - 5	6 - 10	11 - 14	15 - 18	19 - 23	24 - 27	28 - 32	33 - 36

The operating characteristics of the stopping rule are shown below and are based on 5000 simulations:

True AE rate	**% simulated trials declaring unsafe**	**Average sample size**
0.10	2.4	31.5
0.2	29.5	26.8
0.25	52.6	22.8
0.3	74.9	18.4
0.35	89.2	14.2

14.4 Analysis of Primary Objective

This is a randomized, Phase II trial of SABR *versus* SABR + Radium-223 in oligometastatic hormone sensitive prostate cancer patients. Minimization approach (77) will be applied to ensure balanced assignment to each treatment arm by stratification factors: 1) Institional; 2)Initial treatment with surgery vs. radiation therapy; 3) Prior hormonal therapy vs. no prior hormonal therapy; and 4) PSADT <6 mos vs. 6-14.9 mos. Baseline PSA level is defined as that measured Day 1 following randomization. Progression is defined as per section 9.6.2.

The primary outcome of interest is PFS, defined as the time from the date of randomization to the date of disease progression or death, whichever happens earlier. For those who are alive and do not have progressive disease, PFS will be censored at the time of the last scan. Kapla-Meier method will be used to summarize PFS and log-rank test will be used to compare PFS between the two arms. The analysis population includes all randomized subjects based on intent-to-treat principle.

14.5 Analysis of Secondary Objectives

- For safety analysis, adverse events will be summarized by type and grade.
- Kaplan-Meier (KM) estimates will be used to summarize ADT-free survival (ADT-FS), time to locoregional progression (TTLP), time to distant progression (TTDP), time to new metastasis (TNM) and duration of response over time. The median PFS, ADT-FS, TTLP, TTDP TNM and duration of response will be reported.
- The efficacy of SABR + Radium-223 in men with oligometastatic disease will also be determined by measuring local control of each lesion at 12-months.

Each metastatic lesion will be considered a target lesion and independently evaluated for response using RECIST 1.1 or bone scan evaluation criteria above. The lesion will be coded as being locally controlled if it is considered stable radiographic disease or if there is evidence of a partial or complete response. Local control assessment will start at three months following randomization and continuous assessment will be pursued during the follow-up period. The proportion of the lesions that have a stable or better response will be estimated using generalized estimating equation.

- Quality of life will be assessed using the Brief Pain Inventory form. An overall score will be calculated pre-treatment and at the time of the 2nd radiologic reassessment. The change in score will be evaluated with a paired t-test.

14.6 Evaluation of Toxicity

All patients who receive at least one fraction of SABR and Radium-223 will be evaluable for toxicity from the time of their first treatment for SABR and Radium-223.

14.7 Correlative Science

Descriptive statistics will be performed to correlate the temporal CTC, ctDNA, immunophenotyping and immune repertoire metric changes with clinical outcomes.

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APPENDIX I: Brief Pain Inventory Form

STUDY ID# _____ HOSPITAL # _____

DO NOT WRITE ABOVE THIS LINE

Brief Pain Inventory (Short Form)

Date: _____/_____/_____ Time: _____

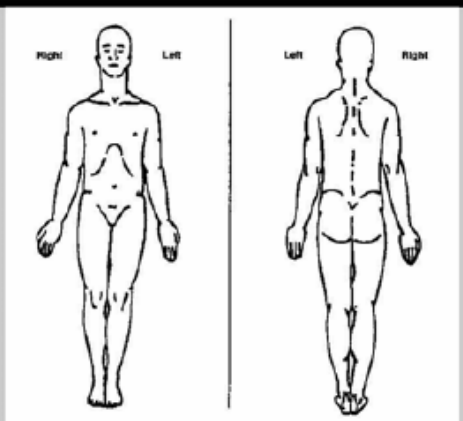
Name: _____ _____ _____

Last First Middle Initial

1. Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than these every-day kinds of pain today?

1. Yes 2. No

2. On the diagram, shade in the areas where you feel pain. Put an X on the area that hurts the most.



3. Please rate your pain by circling the one number that best describes your pain at its worst in the last 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

4. Please rate your pain by circling the one nuimber that best describes your pain at its least in the last 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

5. Please rate your pain by circling the one number that best describes your pain on the average.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

6. Please rate your pain by circling the one number that tells how much pain you have right now.

0	1	2	3	4	5	6	7	8	9	10
No Pain										Pain as bad as you can imagine

7. What treatments or medications are you receiving for your pain?

8. In the last 24 hours, how much relief have pain treatments or medications provided? Please circle the one percentage that most shows how much relief you have received.

0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
No Relief										Complete Relief

9. Circle the one number that describes how, during the past 24 hours, pain has interfered with your:

A. General Activity

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

B. Mood

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

C. Walking Ability

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

D. Normal Work (includes both work outside the home and housework)

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

E. Relations with other people

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

F. Sleep

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

G. Enjoyment of life

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

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APPENDIX II: Performance Status Criteria

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX III: CAPP-Seq Sample Collection & Processing

Diehn Lab Blood Processing Protocol (version 122314)

❖ **Materials** (check to make sure you have all of it before you start)

- Filtered P2000 pipette tips
- P-2000 pipette
- 2.0-mL Eppendorf tubes
- Patient's blood - usually 30 mL in 3 "purple top" tubes (EDTA) per time point. Blood should be kept on ice or in refrigerator after drawing and processed as soon as possible to minimize lysis of WBCs and release of cellular genomic DNA into plasma

❖ **Methods**

1. Spin samples in the clinical centrifuge using the settings: 3,500rpm, 10min, 4C
2. While you wait, label tubes
3. After spinning, carefully remove lavender top tubes from centrifuge. Do not disturb the separated plasma and cell-free whole blood
    i. Tip: It helps to put all the tubes into one holder and carefully carry the holder to the hood
4. Using a filtered tip and p-2000 pipet aliquot ~1.8 mL clear plasma (not all the way to the top since tops of tubes tend to pop open upon freezing if filled all the way) into a 2.0 mL Eppendorf tube. Repeat until you have aliquoted the plasma from all the purple-top tubes into 2.0 mL Eppendorf tubes. With the tubes that have only the buffy coat and RBC remaining, mix the buffy coat and cell-free whole blood using a pipette tip and aliquot ~1.8 mL into a 2.0 mL Eppendorf tube. Repeat so you have a second 2.0 mL Eppendorf tube containing the buffy coat and RBC mixed together.
5. Put Eppendorf tubes into -80C freezer.
6. Write down **# of plasma tubes stored, box number, date of blood draw, time of storage** on the top of patients' requisition forms.
7. Enter the blood draw information into REDCap database.

APPENDIX IV: EPIC-HD CTC Sample Collection & Processing

EPIC SCIENCES

EPIC SCIENCES, INC.
9381 Judicial Dr, Suite 200
San Diego, CA 92121

CTC Sample Collection in Streck Cell-Free DNA BCT Tubes

IMPORTANT: The first 5 mL of blood collected from the fresh venipuncture **cannot** be used for the collection into the Streck tubes due to possibility of contaminating epithelial cells during venipuncture. **Please ensure that at least one blood tube of 5 mL or more is collected prior to collection of the CTC sample to avoid adversely affecting the test results.**

Prevention of Backflow:

Since Streck Cell-Free DNA BCT tubes contain chemical additives, it is important to avoid possible backflow from the tube. To guard against backflow, observe the following precautions:

- Keep patient's arm in the downward position during the collection procedure.
- Hold the tube with the stopper uppermost.
- Release tourniquet once the blood starts to flow into the tube, or within 2 minutes of application.
- Tube contents should not touch stopper or the end of the needle during the collection procedure.

Blood Collection Instructions:

**Schedule courier for same-day sample pic-up prior to collection

1. Confirm blood tube is not expired. Expired tubes should not be used for blood collection.
2. Draw whole blood sample into 10 mL Streck Cell-Free DNA BCT tube (*see note regarding prevention of backflow). Fill tube until blood flow stops. NOTE: Epic requires a minimum of 4mL blood per sample, but a full 10 mL tube of blood should be provided when possible.
3. Remove tube from adapter and immediately mix by gentle inversion 8 to 10 times. Tube inversion prevents clotting. Inadequate or delayed mixing may result in inaccurate test results.
4. Label the tube with subject's identification and date and time of blood draw.
5. Keep sample at ***room temperature*** and ***ship on day of collection.***

Sample Shipment Instructions:

Samples are processed the same day as receipt. Priority is given to the samples that had notification the day prior. Epic is able to process samples up to 96 hours after the initial blood draw, but it is preferable to process the blood before the 48 hour mark for optimal CTC retention and data integrity purposes.

EPIC SCIENCES

EPIC SCIENCES, INC.
9381 Judicial Dr, Suite 200
San Diego, CA 92121

The total number of slides created is dependent upon the patient's WBC count and amount of blood provided. Each tube should be labeled with the Study and Subject ID. Each sample sent should be accompanied by a requisition form, filled out by the site, listing the study ID, subject ID, draw date, draw time, and time point (if applicable). To avoid weekend delays, mark Saturday delivery if shipping on Friday.

Samples should be sent overnight in ambient shippers on the day the blood sample is drawn.

Epic Sciences receives samples Monday through Saturday from 8:00 a.m. to 5:00 p.m., excluding U.S. holidays. Incoming sample notifications should be sent during normal business hours on the day of shipment by email to partners@epicsciences.com or by fax at 858-356-5852. For samples that will be processed at LabCorp Belgium, please send the sample notification email to partners.eu@epicsciences.com. For samples that will be processed at LabCorp Singapore, please send the sample notification email to LabCorp.Singapore@epicsciences.com.

This email/fax should include:

1) Study ID
2) Subject ID (and timepoint if applicable)
3) Tracking Number
4) Number of samples being shipped
5) Date and time of each blood sample draw

APPENDIX V: ImmunoSEQ Sample Collection & Processing

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Human Sample Preparation Guidelines

Sample Types Accepted
- Sorted T and B cells
- Peripheral blood mononucleated cells (PBMCs)
- Whole blood
- Tissue (including Fresh Frozen [FF] or formalin-fixed, paraffin-embedded [FFPE] tissue)
- gDNA and cDNA

Assays available:
- T-cell receptor beta (TCRB)
- T-cell receptor alpha/delta (TCRA/D)
- T-cell receptor gamma (TCRG)
- IGH
- IG kappa/lambda (IGK/L)

TCR IMMUNOSEQ ASSAY SAMPLE GUIDELINES

Sample type	Resolution			
	Survey	Deep	Ultra Deep	Max Depth
Sorted T cells	60,000 cells[a] 1 µg DNA 50 µL vol.	200,000 cells 3 µg DNA 125 µL vol.	800,000 cells 12 µg DNA 400 µL vol.	4,000,000 cells 48 µg DNA 700 µL vol.
PBMCs	120,000 cells 2 µg DNA 50 µL vol.	400,000 cells 6 µg DNA 125 µL vol.	1,600,000 cells 24 µg DNA 400 µL vol.	8,000,000 cells 96 µg DNA 700 µL vol.
Whole blood	2 mL blood 4 µg DNA	4 mL blood 12 µg DNA	10 mL blood 48 µg DNA	Contact Technical Support
Lymphoid tissue[b]	25 microns FFPE 10 mg FF tissue 1 µg DNA	3 µg DNA	Contact Technical Support	
Non-lymphoid tissue	25 microns FFPE 10 mg FF tissue 3 µg DNA	9 µg DNA	Not recommended	

[a] 1000 cells is the absolute minimum number of cells accepted.
[b] Deep resolution is recommended for lymphoid tissue samples.

NOTE: In order to sequence only rearranged TCRG receptors originating from gamma/delta T cells in PBMCs and tissue samples, a cell sort must be performed prior to receipt of samples by Adaptive Biotechnologies. Unsorted cells will result in the amplification of rearranged TCRG receptors from both gamma/delta and alpha/beta T cells.

cDNA GUIDELINES

- A minimum of 150 ng of RNA is recommended as starting material for the reverse transcription step
- Adaptive targets 10-fold sequencing coverage of each TCR or BCR template
- Using cDNA as an input source will limit available applications

BCR IMMUNOSEQ ASSAY SAMPLE GUIDELINES

Sample type	Resolution			
	Survey	Deep	Ultra Deep	Max Depth
Sorted B cells	60,000 cells[a] 1 µg DNA 50 µL vol.	200,000 cells 3 µg DNA 125 µL vol.	800,000 cells 12 µg DNA 400 µL vol.	4,000,000 cells 48 µg DNA 700 µL vol.
PBMCs/bone marrow[b]	600,000 cells 4 µg DNA 50 µL vol.	2,000,000 cells 12 µg DNA 125 µL vol.	Contact Technical Support	
Lymphoid tissue	10 mg FF tissue 1 µg DNA	3 µg DNA		
Non-lymphoid tissue	10 mg FF tissue 3 µg DNA	9 µg DNA	Not recommended	

[a] 1000 cells is the absolute minimum number of cells accepted.
[b] B cells represent a small fraction of the total PBMC population; this resolution may not be appropriate for all projects.

DESCRIPTION OF PROFILING RESOLUTIONS: SURVEY VS. DEEP

Resolution	Considerations for choosing resolution
Survey	• Clonal samples • Samples with low numbers of T or B cells (<100,000 estimated T or B cells cells) • Samples derived from most non-lymphoid tissues
Deep	• Studying the peripheral immune repertoire (e.g. whole blood, peripheral blood mononuclear cells [PBMCs], or lymphoid tissue) • Samples requiring greater sensitivity (detection of rare clones) • Experiments assessing a broader range of the repertoire

amplify **discovery**™

RECOMMENDATIONS FOR SAMPLE PREPARATION

Isolating DNA from different sample types

Sorted cells

- Sorting fixed cells into HEPES buffer (PBS with 2% FBS and 0.025M HEPES) can boost the DNA yield from the cell pellets
- When preparing fixed cells for fluorescence-activated cell sorting (FACS), a concentration of 0.5%–2.0% PFA is recommended. Higher concentrations of PFA can fragment the DNA, which will result in reduced PCR amplification efficiency
- Cells should arrive in no more than 200 µL of buffer

Tissue

- A tissue homogenizer with homogenization buffer is recommended for disruption of fresh or frozen tissue samples
- Example kit for DNA extraction:
  - Qiagen DNeasy™ Blood & Tissue Kit (Mini Spin Columns)
  - Qiagen QIAamp™ DNA FFPE Tissue Kit

Blood, PBMCs, or bone marrow

- ACD or EDTA is recommended as anticoagulant for whole blood or bone marrow collection
- Sodium heparin and sodium citrate are compatible with the immunoSEQ Assay. However, excessive amounts of sodium heparin can inhibit PCR
- Roughly 50% of cells frozen in DMSO will lyse during the thawing process. To recover all DNA do not centrifuge the sample after thawing. Instead, extract DNA from the entire thawed sample
- Possible extraction kits:
  - Qiagen DNeasy™ Blood & Tissue Kit (Mini Spin Columns): Bone marrow and <1 mL blood
  - Qiagen QIAamp™ DNA Blood Maxi Kit: 1–10 mL blood

Shipping Samples

- Cells and tissue: 1.5 or 2.0 mL Eppendorf tubes/ cryotubes (snap top or screw cap)
- Whole blood: Vacutainer ACD or EDTA tube, completely filled
- gDNA or cDNA: Uniquely barcoded sample containers will be provided by Adaptive Biotechnologies.

Quality of input DNA

Once DNA is isolated, quantification using a spectrophotometer or comparable method is highly recommended. For optimal results the absorbance ratios of DNA samples should be:

- A260/280 = 1.8–2.0
- A260/230 = 2.0–2.2

Potential PCR inhibitors

Sample source(s) containing any of the following may inhibit PCR steps used in the immunoSEQ Assay:

- **Heparin, EDTA,** common anticoagulants in blood and bone marrow samples
- **Melanin,** common to skin and melanoma tissue samples
- **B5 Reagent,** commonly used for bone marrow storage
- **Collagen,** can be at high levels in some tissue samples
- **Myoglobin,** common to muscle tissue
- **Bacterial contamination** from all sample sources
- **Phenol, ethanol,** and **other organic contaminants** remaining after DNA extraction

For questions or Technical Support contact:
techsupport@adaptivebiotech.com, or (855) 466-8667

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APPENDIX VI: RECTAL SWAB COLLECTION FOR MICROBIOME ANALYSIS

OPTION 1: Flocked Swabs & Plastic Applicator - Reagents and Supplies Needed:

Regular flocked swab, plastic applicator, sterile in dry tube (Copan, catalog #552C)

Protocol:

1. Remove swab from plastic tube. Hold the swab by the end of the plastic handle and take care not to touch the cotton tip of the swab to any surfaces or with fingers.
2. Insert the swab through the rectal sphincter and approximately 3 cm into the anal canal. Rotate the swab and withdraw.
3. Place the swab back into the plastic tube.

OPTION 2: Peel-Pouch Swabs - Reagents and Supplies Needed:

- Regular flocked swab, plastic applicator, sterile in peel pouch (Copan, catalog #519CS01)
- Sterile 15mL conical tube
- Pair of purple nitrile powder free exam gloves
- Biohazard collection bag

Protocol:

1. Put on purple nitrile gloves.
2. Remove swab from peel pouch. Hold the swab by the end of the plastic applicator and take care not to touch the cotton tip of the swab to any surfaces or with fingers.
3. Insert the swab directly into the rectum and approximately 3 cm into the anal canal. Rotate the swab and withdraw. **If fecal matter is on the swab, it has been done correctly.**
4. Place the swab into the 15mL conical tube and snap off at the red break-off point. Discard gloves as to not get any material on the outside of the tube.
5. Screw cap back onto tube securely and place tube into biohazard bag.

ALL OPTIONS: The swab samples can be kept at 4°C or -20°C prior to transport to the Sfanos lab.

APPENDIX VII: Ordering from Invitae

Ordering from Invitae

INVITAE

PLACE YOUR ORDER

Place your order online for the most efficient processing.

1. Create an account by visiting www.invitae.com/signup.
2. Sign in and then click **Start an order**.
3. Under **Test selection**, browse Invitae's panels to select a pre-curated panel or create a custom test and add it to your order. You can also select a test from your recent or custom orders; order family follow-up testing; or order through a sponsored testing program or clinical trial by choosing the **Partnership programs** tab.
4. Fill out the requested information, including patient information in any order; save your entries at any time to come back to them later.
5. Enter billing information.
6. Submit your order.

If you prefer to open an account by speaking with an Invitae representative, please contact us at 800-436-3037.

COLLECT A SPECIMEN

1. Order Invitae blood or saliva collection kits, which you need for collecting and returning your specimen, at www.invitae.com/request-a-kit.
2. Label the specimen tube with the patient's full name, date of birth, and specimen collection date.
3. If you need additional details on our specimen requirements, please visit www.invitae.com/specimen-requirements.

Continued on back

Ordering from Invitae *(continued)*

PRINT THE REQUISITION FORM

1. Print the requisition form that was created during the online ordering process.
2. If you prefer to place a paper-based order for panel testing, you can also download our paper test requisition from www.invitae.com/order-forms. Please note that exome testing is ordered exclusively online.

SEND THE SPECIMEN AND FORMS TO INVITAE

1. Package the requisition form with your patient's specimen in the provided collection box.
2. You're now ready to call your shipping carrier to schedule a pick-up. Within the US, return shipping is offered at no additional charge and a label is included in the collection kit.
3. We recommend shipping the specimen overnight, on the same day the specimen is collected. We also recommend shipping at the beginning of the week to avoid any transport delays over a weekend.

RESULTS

1. Once Invitae receives the shipment, you will receive the results in:
   - Panel testing: 10–21 calendar days (14 days on average)
   - STAT panel testing: 5–12 calendar days (7 days on average)
   - Exome testing: 20 weeks on average
2. If you ordered online, you can view the status of your order by logging in to your account. Alternatively, if you provided your email address on your paper-based order form, you can create an online account following the steps above to view the status of your order.
3. You will receive a notification email once the test results are ready.

If you have any questions about the ordering process, please contact Client Services at clientservices@invitae.com or 800-436-3037. Local contact information outside the US can be found at www.invitae.com/contact.

HEADQUARTERS | 1400 16th Street, San Francisco, CA 94103 | ONLINE | www.invitae.com | CONTACT | www.invitae.com/contact | p: 800-436-3037 | f: 415-276-4164
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APPENDIX VIII: Immunophenotyping

Peripheral blood will be collected into two heparin tubes (green top tubes) according to the study calendar. Following collection, Elizabeth Thompson from the Powell lab will be notified for collection (see contact information below). Peripheral blood mononuclear cells (PBMCs) will be isolated using standard protocols of Ficoll isolation and cryopreserved in freezing media (FBS, 10%DMSO). Cells will be stored long-term in liquid nitrogen until thawed for batch analysis. Upon thawing, cells will be immunophenotyped using multiparameter flow cytometry to evaluate the immunologic and metabolic phenotype of the following cell subsets: CD4 and CD8 T cells, FOXP3+ Tregs, B cells, NK cells, monocytes, dendritic cells, and myeloid derived suppressor cells (Table 1). Samples will be run on a Cytek Aurora machine, which allows for the simultaneous detection of up to 25 markers. Additionally, the metabolic fitness of the total PBMCs will be evaluated using the mitochondrial stress test on the Seahorse analyzer. Functional assays will be run on PBMCs to determine the relative capacity of peripheral T cells to metabolically respond to T cell receptor stimulation through activation of CD3 and CD28. Additional vials will be stored for RNA-sequencing of relevant populations identified in initial immunophenotyping.

Contact information

Primary point of contact

Elizabeth Thompson

Ethomp58@jhmi.edu

410-814-1261

Secondary point of contact

Jiayu Wen

Jwen1@jhmi.edu

410-502-7444

Table 1. Immuno- and metabolic phenotyping of PBMCs

Marker	Purpose
Live Dead	Dead cell exclusion
H3K27me3	Histone Methylation
CTLA4	Immune phenotype-Co-inhibitory
PD1	Immune phenotype-Co-inhibitory
41BB	Immune phenotype-Co-stimulatory
CCR7	Immune phenotype-Memory
CD45RA	Immune phenotype-Memory
Ki67	Immune phenotype-Proliferation

CD19	Immune subset-B cell
CD15	Immune subset-MDSC
CD33	Immune subset-MDSC
HLA-DR	Immune subset-Monocyte/DCs
CD14	Immune subset-Monocyte/MDSC
CD56	Immune subset-NK cell
CD3	Immune subset-T cell
CD4	Immune subset-T cell
CD8	Immune subset-T cell
FOXP3	Immune subset-Treg
CPT1a	Metabolic phenotype-Fatty acid oxidation
GLUT1	Metabolic phenotype-Glycolysis
Hexokinase II	Metabolic phenotype-Glycolysis
LDHA	Metabolic phenotype-Glycolysis
pS6	Metabolic phenotype-mTOR activity
TOMM20	Metabolic phenotype-OXPHOS
VDAC1	Metabolic phenotype-OXPHOS

APPENDIX IX: RAPID SPECT Imaging Sub-Contract Study

Purpose: To measure radiation exposure to specific metastases by performing SPECT scans at 2-4 hours, 24 hours (+/- 4 hours), and 48 hours (+/- 4 hours) following Xofigo infusions #1 and #6 to calculate the total radiation exposure to the specific metastases.

All patients participating in Hopkins Xofigo/XRT (RAVENS) trial will be eligible for participation in the Rapid SBIR-contract supported CXαRPT (companion) trial.

Patient treatment will not change. A data collection effort for retrospective evaluation of results will be conducted by Hopkins via a companion protocol that patients participating in the parent trial may sign on to. The target population is 20 out of the 30-34 patients that will be enrolled in the SABR + Xofigo arm of the RAVENS trial. The expected accrual is 10 patients in year 1 and 10 patients in year 2.

Patients will be compensated with a $100 gift card for the 3 ^{223}Ra SPECT/CT scans following Xofigo infusion #1, and a $100 gift card for the 3 ^{223}Ra SPECT/CT scans following Xofigo infusion #6. gift cards and scans will be paid for by the research budget from the NIH sub-contract.

Hypothesis: The ^{223}Ra dosimetry calculations performed will demonstrate, retrospectively, that > 30% of the patients would have required an adjustment in the XRT treatment plan, to meet the following criteria:

1. Tumor absorbed dose (to at least 5 lesions) ≥ 80 EQD2 -Gy (BED=96 Gy; α/β=10 Gy),
2. Normal organ doses within tolerance limits.

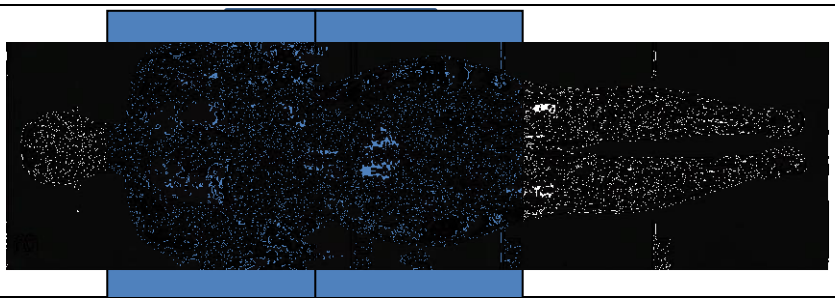
Data/Workflow:

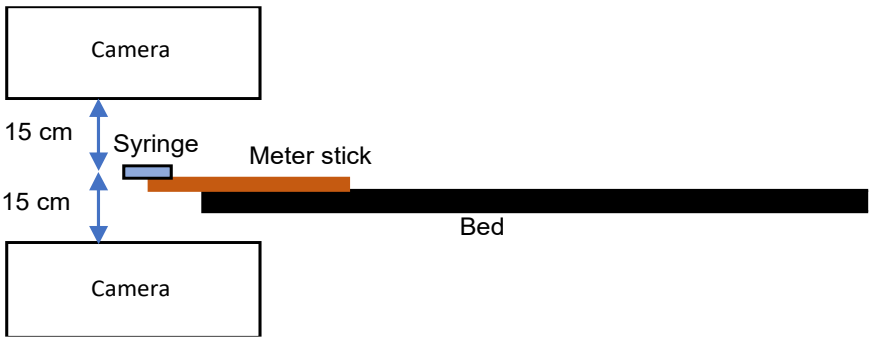
- For patients participating in the RAVENS protocol, Hopkins will collect ^{223}Ra SPECT/CT at 2-4 hours, 24 hours (+/- 4 hours), 48 hours (+/- 4 hours) after first and last Xofigo infusion.
- Rapid receives anonymized SPECT projection data, performs quantitative recon, 3D-RD dosimetry, and provides combined RPT-XRT (CXRPT) dose map to Hopkins.
- Hopkins compares XRT dose map w/ and w/o αRPT dosimetry and determines if a change would have been merited.
- Rapid receives Hopkins assessments.

Standard Operating Procedure

Quantitative Imaging and Dosimetry of Radium-223
V2.2; July 8, 2019

Characteristics of Ra-223	Radium-223 is primarily an alpha emitter (95.3% abundance). The fractions emitted as beta and gamma particles are 3.6% and 1.1%. The gamma component allows for the in-vivo imaging.	
Purpose of imaging procedure	Exploratory – to evaluate variability in Ra-223 uptake across patients and how the proposed combination agents impact this, also how tumor burden impacts distribution and dosimetry.	
Dosage level[1]	As per Xofigo package insert	
Radiation dose to main organs[1] (Gy/MBq)	Adrenals	0.00012
	Brain	0.00010
	Breasts	0.00005
	Gallbladder wall	0.00023
	Lower large intestine wall	0.04645
	Small intestine wall	0.00726
	Stomach wall	0.00014
	Upper large intestine wall	0.03232
	Heart wall	0.00173
	Kidneys	0.00320
	Liver	0.00298
	Lungs	0.00007
	Muscle	0.00012
	Ovaries	0.00049
	Pancreas	0.00011
	Red marrow	0.13879
	Osteogenic cells	1.15206
	Skin	0.00007
	Spleen	0.00009
	Testes	0.00008
	Thymus	0.00006
	Thyroid	0.00007
	Urinary bladder wall	0.00403
	Uterus	0.00026
	Whole body	0.02311
Patient preparation	Nothing beyond what is already specified in the protocol	
SPECT imaging length	~30 mins per FOV	
FOVs taken	Chest & Abdomen, if there are relevant lesions outside these two fields of view then additional views could be collected as needed. After first field is collected, to extent possible, patient should remain on couch for subsequent FOV collection.	

	
Imaging Time points (post-injection)	2-4 hours, 24 hours ± 4 hours, 48 hours ± 4 hours
SPECT Acquisition parameters for SPECT/CT	Collimator: MEGP Matrix: 128 x 128 Time / view: 30 seconds/view # of projection views: 120 Angle range: 360 Energy Window Setting (KeV): 71.4 96.6 (30% at 84); 138.5 168.5 (20% at 154); 249.7 290.2 (15% at 270)
CT acquisition parameters for SPECT/CT	The CT portion of the first SPECT/CT scan for each patient should be in high res (i.e., diagnostic mode); attenuation mode (low-dose) may be used for subsequent scans. Standard vendor settings for high resolution/diagnostic vs low-dose or attenuation acquisition may be applied.
Quality control required	Standard camera quality control (QC) as specified by the vendor should be up to date on all cameras that will be used for this study. Typical QCs include uniformity, linearity/spatial resolution, and center of rotation alignment. Dose calibrator QCs should also be routinely performed and documented (accuracy, consistency, linearity, and geometry).

Additional requirement	Sensitivity needs to be measured prior to or immediately after the patient scan. Suspend a calibration source (with known amount of Ra-223 – 2MBq) between the two camera heads (by taping it to a yard stick and supporting the stick on the bed). The source should be such that there are no attenuating materials between, including the bed or meter stick, between the source and collimator. Position the source so it is 15 ± 0.5 cm from the face of each collimator and collect a 5 min dual head single view scan (256x256 matrix, same collimator/energy windows as above). 
Recommended phantom calibration scan	There is a recommended phantom calibration scan with either a NEMA PET IEC Body Phantom or an ACR Phantom to be collected for each camera that may be used for Ra-223 patient imaging. This scan is collected once prior to camera's use for Ra-223 patient imaging. The body of the phantom should be filled with water at room temperature removing as much of the air as possible. All spheres must be removed except the largest one. The activity and measurement time of the syringe prepared earlier should be recorded. All the activity (~2 MBq) contained in the syringe should be injected into the largest sphere (a long needle might be needed). Both, activity and measurement time after the injection (with needle on) must be recorded. The rest of the large sphere can be completely filled with water, using a new syringe and needle. The image technician must make sure that the largest sphere is in place before putting the lid on the phantom and hand-tighten the screws. The phantom should be placed flat-side down on the bed (pad can be removed, if necessary) and in the middle of the camera field of view. SPECT and CT images should be acquired using pre-defined patient imaging protocol(s) and parameters used should be recorded.
Reporting requirements	None, a table of results will be provided for each patient to the institution or to the PI of the protocol with the expectation that this will lead to a publication.
Interpretation for dosimetry (or any other) purpose	N/A; Results will be analyzed and used for publication to evaluate patient to patient variability and impact of tumor burden. Study will also provide accurate SPECT-imaging based dosimetry.

Step by step imaging procedure	Routine SPECT/CT imaging procedure in clinical practices should be used with the parameters specified above.
Data to send	SPECT projections, attenuation map, and all CT slices (in DICOM format).
Image quantification procedure	SPECT projections will be reconstructed using previously published iterative quantitative reconstruction methods established by Rapid[2-5]. Volume of interest (VOIs) will be drawn around the major organs and lesions of interest at each time points. Total counts in the VOIs at each time point will be calculated and converted to activity by measured sensitivity. The time integrated activity coefficients (TIACs), mathematically equivalent to the number of decays per unit injected activity, will be calculated from single or double exponential fits for the main source organs and the lesions. The TIACs in the red marrow (TIACRM) will be derived from contours drawn around lumbar vertebrae L3-L5, the TIACRM will be divided by the marrow mass in this region to give TIACRM concentration. Total TIACRM will be obtained as the product of this concentration and the estimated patient RM mass.
Dosimetry analysis procedure	The absorbed dose values for target organs will be derived by using whole organ S-value based method and MC-calculation using images and 3D-RD approach (RBE =5 for alphas). Importantly, Rapid follows SNMMI MIRD Committee recommendations as published in its series of pamphlets and will update calculation accordingly if necessary. Mean absorbed dose (mAD) and mean (mBED) will be obtained using the software package 3D-RD[6-10]. The methodology used in 3D-RD is briefly summarized below: 1. Using the Electron Gamma Shower (EGS) Monte Carlo (MC) software, one million events will be run for the beta- and photon components of the isotope decay spectra, for each time point. 2. The energy deposition distribution from the contributing components from MC will be weighted for probability and unit activity then converted to absorbed dose rate for each VOI (spectra probability distributions obtained from LBL/Lund web site[11]). 3. The dose rates for the each VOI will be fitted using the simulation Analysis and Modeling II (SAAM II) software package (The Epsilon Group, Charlottesville VA), and the area under the curve calculated as the absorbed dose mAD. 4. The mBED will be calculated using the linear-quadratic (LQ) BED methodology described in reference[12].
References	1. Xofigo (radium Ra 223 dichloride) Prescribing Information. https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/203971lbl.pdf 2. Frey EC, Tsui BMW. A new method for modeling the spatially-variant, object shape dependent scatter response function in SPECT. *IEEE Nuclear Science Symp Conference Record.* 1996:1082-1086.

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- approval signatures page. This will be have to be signed off at each imaging site.

Responsibility for:

- Data transfer, receiving / accepting images at Rapid:
  - Bin He (bhe@rapiddosimetry.com)
- Assurance of de-identification of patient data:
  - TRIAD
- Storing images (and for how long), and interpreting these images
  - Images and data related to dosimetry will be transferred back to TRIAD

APPENDIX X: XOFIGO PATIENT INFORMATION SHEET

Johns Hopkins Hospital
Department of Radiation Oncology and Molecular Radiation Sciences

Patient Information – Radium-223 (Xofigo) Therapy

- Prior to all injections of Radium-223 it is important that you obtain required labs.
- Report any signs of bleeding or infection.
- Shut the lid and double flush the toilet after each urination/stool for the next 7 days (for 7 days after each and every treatment).
- If urine/feces/blood soils your clothing, you should wash the clothing separately and promptly.
- If you wear an undergarment (Depends, pad, etc.), you should double bag it and discard it in the regular trash.
- Caregivers should use gloves and gowns when handling bodily fluids and wash hands well.
- You may return to work or school the day of treatment.
- You may have sexual contact immediately but use a condom during the 6 months of treatment and 6 months after treatments are completed. Female partners with reproductive potential should use effective birth control.
- There are no restrictions regarding contact with other people after receiving Radium-223.

Any concerns regarding Radium-223 (Xofigo) may be addressed by calling 410-955-6980 during normal business hours (Monday-Friday, 8AM – 4:30PM).

For concerns after hours, please call 410-955-4331 and ask for the Radiation Oncology doctor on-call.

_____ _____

Patient Signature Date Provider Signature

APPENDIX XI: BAYER PATIENT RELEASE CARD

Please print and fill out the card for all patient's randomized to the SABR + XOFIGO Arm.