STUDY TITLE: A single-arm open label biomarker study of standard-of-care Radium-223 chloride for metastatic castration-resistant prostate cancer

Test drug(s):	Radium Ra 223 dichloride (Radium Ra 223 dichloride, BAY 88-8223)		
Study purpose:	Exploratory response/biomarker evaluation		
Clinical study phase:	II		
Version no.:		Date:	30 July 2019
Amendment no.:	Amendment: PET/MRI Clarification	Date:	N/A
Bayer Study no.:	**ONC-2013-119**		
Institution Study no.:	**14-375**		

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The study will be conducted in compliance with the protocol, ICH-GCP and any applicable regulatory requirements.

"The values in this protocol have been revised as per the United States (US) National Institute of Standards and Technology (NIST)standardization update (agreed on 17 MAR 2015)"

Synopsis

[Title]	A single-arm open label biomarker study of standard-of-care Radium-223 chloride for metastatic castration-resistant prostate cancer
Clinical study phase	II
Study objective(s)	The primary objective of this study is to evaluate the quantitative effect of Ra-223 on ^{99m}Tc-MDP bone scan in men with castration-resistant prostate cancer metastatic to bone. Secondary objectives include the description of mean percent change in bone scan lesion area by 18-month survival status as well as the evaluation of the effects of Ra-223 by measures categorized as follows: (A) Imaging biomarkers (B) Standard and novel circulating tumor cell assays (C) Circulating biomarkers of the tumor microenvironment (D) Patient reported pain and quality of life

Name of active ingredient **Dose(s)** **Route of administration**	Radium Ra 223 dichloride 50 kBq/kg body weight (55kBq/kg after NIST update) Intravenous
Indication	Standard-of-care use for castration-resistant prostate cancer metastatic to bone
Diagnosis and main criteria for inclusion	This study will prospectively enroll men with castration-resistant prostate cancer (CRPC) metastatic to bone.
Study design	Exhaustive inclusion/exclusion criteria are detailed in the Section 5 of the protocol. Schema: 1. Screeningassessments include ^{99m}Tc MDP bone scan, & blood testing 2. Enrollment 3. Baseline assessment includes Fluciclovine PET/MRI 4. Ra-223, administered monthly for a total of six anticipated doses with concurrent protocol-specified correlative imaging and blood testing 5. Conclusion of study treatment with sixth dose of Ra-223 6. Follow-up and testing as detailed in experimental protocol Standard androgen deprivation therapy (ADT) will be continued throughout study participation. Men will be treated for 6 months on protocol in the absence of progression or intolerable side effects. Men will be evaluated according to the table provided in protocol Section 7.1.1. At the end of 6 months of treatment, Ra-223 will be discontinued. Subsequent therapies will be at the discretion of the treating oncologist.
Type of control	This is a single-arm exploratory biomarker study.
Number of subjects	22
Study Endpoints	Please see Protocol Section 7.13 & 7.14
Plan for statistical analysis	Bone scan response is defined as a ≥ 30% decrease in quantitative bone scan lesion area (BSA) from baseline to the end of month 2 of Ra-223 treatment (i.e. after 2 of 6 possible Ra-223 doses). In a one-stage design, 22 subjects will be enrolled to evaluate the primary endpoint of bone scan response. Assuming 90% of subjects with adequate data, the evaluable sample size is 20 subjects. With 20 subjects, given target power of 90% and 1-sided 5% alpha, a null hypothesis of ≤ 10% versus an alternative hypothesis of ≥ 35% in the proportion of patients achieving response can be evaluated. If 5 or more responses are observed in 20 subjects, this method of response assessment will be considered promising.

Amendment Rationale

This amendment is to clarify that the baseline Fluciclovine PET/MRI should only be conducted after a subject is successfully registered to the trial, but prior to starting treatment on T1D1.

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List of abbreviations

AE	Adverse Event
ALP	Alkaline Phosphatase
ALSYMPCA	Alpharadin in Symptomatic Prostate Cancer
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
BPI-SF	Brief Pain Index (Short Form)
BSoC	Best Standard of Care
CBC	Complete Blood Count
CRO	Clinical Research Organization
CRPC	Castration Resistant Prostate Cancer
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events; version 4.03
DK	Decay Correction Factor
EBRT	External Beam Radiation Therapy
ECOG	Eastern Co-operative Oncology Group
eCRF	Electronic Case Report Form
EU	European Union
GCP	Good Clinical Practice
GCL	Global Clinical Leader
GMP	Good Manufacturing Practice
HRPC	Hormone Resistant Prostate Cancer
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IDMC	Independent data monitoring committee
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IV	Intravenous
IxRS	Interactive Voice/Web Response System (IVR/IVRS)
kBq	Kilobecquerel; SI Unit of Radioactivity
kg	Kilogram
LHRH	Luteinizing-Hormone-Releasing Hormone; also known as Gonadotropin-Releaseing Hormone (GnRH)
mCi	Millicuries
MedDRA	Medical Dictionary for Regulatory Activities
mL	Milliliter
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
NIST	National Institute od Standards and Technology
NYHA	New York Heart Association
OS	Overall Survival
PS	Performance Status
PSA	Prostate Specific Antigen
QoL	Quality of life
SAE	Serious Adverse event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Software
SRE	Skeletal-related Events
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment-emergent Adverse Event
ULN	Upper Limit of Normal
WHO-DD	World Health Organization – Drug Dictionary

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Definitions of terms

Radium Ra 223 dichloride:
A targeted alpha particle-emitting pharmaceutical (a radiopharmaceutical drug), is a ready-to-use solution for intravenous injection containing the drug substance radium dichloride. The active moiety is the alpha particle emitting nuclide Ra-223, present as a divalent cation ($^{223}Ra^{2+}$).

Dose:
Doses are given as kilobecquerel (kBq) per kilogram body weight, with the corresponding dose given in millicurie (mCi) per kilogram in parenthesis. The term "dose" is used to describe the quantity of radioactivity from Radium Ra 223 dichloride administered.

1. Introduction

1.1 Background

Background: Skeletal Morbidity and Ra-223 Activity in CRPC
Bone metastases are present in 80-90% of men with advanced prostate cancer and account for much of the considerable disease-related morbidity and mortality. Bone-targeted therapies have therefore long been an important potential component of prostate cancer treatment. Until recently, bone-targeted therapies had been shown to produce clinical benefits (e.g. prevention of skeletal-related events with osteoclast inhibition[1, 2] or palliation of bone pain with beta-emitting radiopharmaceuticals[3]) but not to improve overall survival. The systemically-delivered alpha-emitting radiopharmaceutical Ra-223 has recently been shown to significantly improve survival as well as prevent skeletal-related events among men with CRPC predominantly affecting bone.[4-6] These findings led to its 2013 FDA approval.

Ra-223 Efficacy: Need for Biomarkers
The significant survival advantage demonstrated in phase III study of Ra-223 establishes it as an active agent within an expanding group of systemic therapies that improve survival in mCRPC. Biomarkers of its activity, however, are lacking. Ra-223 treatment has not been shown to dependably improve serum PSA, a biomarker that has correlated with drug activity for androgen receptor (AR) targeted therapies (GnRH agonists, androgen biosynthesis inhibitors such as abiraterone[7, 8], antiandrogens such as enzalutamide[9], and taxane chemotherapy[10, 11]) but not for non-AR targeted therapies (immunotherapy with sipuleucel-T[12], radiopharmaceuticals such as Ra-223, and tyrosine kinase inhibitors[13-15]). In addition, Ra-223 has not been shown to produce measurable improvements in CT or bone scan evidence of disease. Biomarkers of Ra-223 activity are needed. Useful biomarkers would be expected to provide any or all of the following: (1) better clarify Ra-223 mechanism of action, (2) improve disease monitoring during Ra-223 therapy, and (3) inform the use of Ra-223 in sequence or in combination with other agents that are active in CRPC.

Quantitative Planar ^{99m}Tc MDP Bone Scan

Bone scan with Technetium-99m methylene diphosphonate (^{99m}Tc MDP) is a widely-used and accepted imaging modality in both clinical practice and research related to prostate cancer.[16] It is an indirect measure of disease activity as its uptake reflects tracer deposition by osteoblasts[17, 18], a cell type that is highly active in the presence of prostate cancer bone metastases. One historical limitation of bone scans is that until recently they have not been interpreted in a quantitative way. Guidelines for their use in clinical trials (e.g. Prostate Cancer Clinical Trials Working Group 2 (PCWG2)) have recommended only their examination for the presence or absence of 2 or more new lesions.[16] More recently, computer-aided quantitative methods for bone scan assessment have been described.[19, 20] Computer-aided quantitative assessments allow for a more rigorous serial assessment of scan evidence of bone metastases without the cost or availability barriers that are presented by alternative imaging strategies such as sodium fluoride positron emission tomography (PET). We hypothesize that serial computer-aided quantitative assessment of ^{99m}Tc MDP bone scans will reveal Ra-223-induced improvements in skeletal disease burden.

Pain and Quality of Life with Metastatic CRPC

Bone metastases are the major cause of morbidity and mortality in men with metastatic prostate cancer. Beta-emitting radiopharmaceuticals that seek bone (Samarium-153 EDTMP, and Strontium-89) are approved to treat bone pain in men with symptomatic bone metastases.[3] These beta-emitting radiopharmaceuticals have a limited role in the management of metastatic prostate cancer, however, because of hematologic toxicity and lack of documented benefit on cancer outcomes (survival or disease progression). The effects of the alpha-emitting Ra-223 on pain and quality of life are unknown because that information was not collected in the pivotal study. Ra-223 may palliate bone pain similar to previously available beta-emitting radiopharmaceuticals.[3] Alternatively, the more limited depth of penetration of Ra-223 (<100 micrometers versus several millimeters for beta emitters) may limit the effect of Ra-223 on pain and quality of life. We hypothesize that the known benefits of Ra-223 in this population will be accompanied by prospectively-observed improvements in patient-reported pain and quality of life.

Molecular Imaging in Advanced Prostate Cancer

Functional imaging of neoplasms using PET/CT has become an integral part of evaluation of a diverse group of malignancies. ^{18}F-fluoro-deoxyglucose (^{18}F -FDG) is the most widely used PET tracer in oncology but has not been useful in prostate cancer due to inadequate sensitivity as well as intense PET signal in the bladder due to renal elimination. Anti-1-amino-3-fluorine 18-fluorocyclobutane-1-carboxylic acid (FACBC; now known as fluciclovine) PET is an alternative PET tracer that is an L-leucine analog and has demonstrated promising sensitivity and specificity for the detection of prostate cancer.[21-23] Fluciclovine was approved by the FDA in 2016. Since fluciclovine uptake depends on the tumor cells themselves and not on physiological response of adjacent bone or lymph tissue, detection based on fluciclovine PET imaging is effective for soft tissue, lymphatic, and osseous metastases. We hypothesize that quantitative assessment of bone tumor volume as assessed by fluciclovine PET will reveal a decrease after 8 weeks of Ra-223 treatment.

Further, we expect that this study will establish fluciclovine PET as an imaging biomarker of Ra-223 activity for potential use in future trials and in clinical practice.

Circulating Tumor Cells

Detection and isolation of CTCs in peripheral blood is an emerging analytically-valid biomarker of prognosis and of response to therapy in men with prostate cancer.[24-27] Enumeration of CTCs at baseline and post-treatment is prognostic of survival, and the shedding of cells into the circulation represents an intrinsic property of the tumor that is distinct from extent of disease.[28, 29] These rare peripherally circulating malignant epithelial cells have been identified by several techniques. We propose to use two distinct assays to study Ra-223 induced effects on CTCs. First, we will use the only FDA-approved and broadly-available assay: separation of the CTCs using immunomagnetic beads conjugated with antibody to EpCAM (Veridex CellSearch assay). This assay has been incorporated in recent prospective randomized controlled phase III trials of novel agents such as abiraterone acetate and enzalutamide. Second, we will use our microfluidic device for CTC isolation[30] to perform real-time characterization of androgen receptor (AR) signaling activity in CTCs (termed the Evans Assay[31]) as well as analysis of markers of DNA damage and repair (e.g. gamma-H2AX). We propose to measure treatment-induced changes in CTC number, proliferative index, AR signaling, and DNA damage/repair concurrent with standard and experimental imaging assessments. We hypothesize that these studies will further define the value of CTCs as a biomarker of Ra-223 activity.

Bone Turnover Markers

Most bone metastases in men with prostate cancer are characterized by excess bone turnover. Number and activity of both osteoblasts and osteoclasts are increased in typical bone metastases from prostate cancer. Specific biochemical markers of both osteoblast and osteoclast activity are markedly elevated in men with metastatic prostate cancer and levels correspond to extent of skeletal involvement.[32, 33] In multivariate analyses of data from a large prospective clinical trial of men with CRPC and bone metastases, elevated levels of serum bone specific alkaline phosphatase (BAP), a specific marker of osteoblast activity, was independently associated with shorter overall survival.[34] Higher levels of BAP and urinary N-telopeptide (NTx), a marker of osteoclast activity, are associated with greater risk of skeletal morbidity in men with metastatic CRPC. Greater declines in urinary NTx following treatment with zoledronic acid are associated with lower risk of skeletal morbidity in men with metastatic CRPC.[35] We hypothesize that Ra-223 treatment will be associated with reductions from baseline in BAP and NTx concurrent with improvement in concurrent assessments by standard and experimental imaging studies.

Biomarkers of Angiogenesis & Inflammation

Biomarkers of inflammation and angiogenesis may substantially improve understanding of Ra-223 activity as well as mechanisms of resistance. In particular, SDF1α is a chemokine that is highly expressed in the bone marrow and has been shown in preclinical studies to play important roles in tumor growth and resistance to therapy. Malignant cells may use SDF1α signaling through its receptors CXCR4 and CXCR7 to promote survival and migration. For example, CXCR4 and CXCR7 expression has been associated with decreased cell apoptosis and increased cell proliferation and migration and new vessel formation, including in prostate

cancer. In addition, the SDF1α/CXCR4 pathway promotes inflammatory bone marrow derived cell (BMDC) recruitment that may facilitate tumor resistance to radiotherapy.[36, 37] BMDCs that infiltrate tumors, also known as the "distant stroma", are often increased in tumors after radiation treatment and have led to the hypothesis that this effect mediates resistance.[38] Irradiation increases SDF1α expression in both cancer cells and stromal cells.[39] We have also seen an increase in circulating inflammatory factors after hormonal therapy for prostate cancer, including increased plasma SDF1α.[40] We hypothesize that BMDC infiltration after radiation treatment in prostate cancer is mediated by SDF1α, and that SDF1α pathway mediates progression after systemically administered radiation for prostate cancer metastatic to bone. In exploratory analyses, we will use multiplexed arrays to prospectively measure SDF1α as well as other inflammatory and angiogenic biomarkers in plasma at key timepoints throughout study-directed therapy.

1.2 Rationale of the study

Radium-223 chloride (Ra-223) is the first bone-seeking radiopharmaceutical to significantly improve survival in men with metastatic castration-resistant prostate cancer (mCRPC). Despite this clearly-demonstrated benefit in randomized phase III study, the mechanism of Ra-223 activity is not fully understood. Further, PSA does not appear to be a reliable biomarker of efficacy for therapies such as Ra-223 that do not target the androgen receptor. Ra-223 may improve survival through direct anti-tumor effects, through effects on the bone microenvironment, or through other mechanisms. We seek to carry out a single arm exploratory study that will incorporate comprehensive biomarker studies to clarify its mechanism of activity and to establish relevant and clinically-useful biomarkers. Further, we will use validated patient reported outcome tools to evaluate the effects of Ra-223 on pain and quality of life in the study population.

1.3 Radium Ra 223 dichloride

1.3.1 Description

Radium Ra 223 dichloride is an alpha particle-emitting radio-pharmaceutical, with a half-life (t1/2)of 11.4 days.It is a sterile clear, colorless, isotonic, solution to be administered intravenously.

1.3.2 NIST

The quantification of radium-223 radioactivity 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 (2). 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 theNIST 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 (2) and results obtained by other nationalmetrology institutes (United Kingdom, Germany, Japan). After completion of the re-assessment, NIST reported their findings (3) 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 approx. + 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.

> 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 (BC1-06 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 dosing section).

1.3.3 Mechanism of Action

The active moiety of Radium Ra 223 dichloride is the alpha particle-emitting isotope radium-223 (as radium Ra 223 dichloride), which mimics calcium and forms complexes with the bone mineral hydroxyapatite at areas of increased bone turnover, such as bone metastases. The high linear energy transfer of alpha emitters (80 keV/micrometer) leads to a high frequency of double-strand DNA breaks in adjacent cells, resulting in an anti-tumor effect on bone metastases. The alpha particle range from radium-223 dichloride is less than 100 micrometers (less than 10 cell diameters) which limits damage to the surrounding normal tissue.

1.3.4 Preclinical

In single and repeated dose toxicity studies in rats, the main findings were reduced body weight (b.w.) gain, hematological changes, reduced serum ALP and microscopic findings in the bone marrow (depletion of hematopoietic cells, fibrosis), spleen (secondary extra-medullary hematopoiesis) and bone (depletion of osteocytes, osteoblasts, osteoclasts, fibro-osseous lesions, disruption/disorganization of the physis/growth line). These findings were related to radiation-induced impairment of hematopoiesis and a reduction of osteogenesis and occurred beginning in the dose range of of 20 kBq/kg (22 kBq/kg after implementation of NIST update) b.w. (0.4 times the clinically recommended dose).

In dogs, dose-limiting myelotoxicity was observed after a single dose of 450 kBq/kg (497 kBq/kg after implementation of NIST update), while single doses of 50 kBq/kg (55 kBq/kg after implementation of NIST update) and 150 kBq/kg (166 kBq/kg after implementation of NIST update) were considered safe and repeated administration of the clinically recommended dose of radium-223 dichloride (50 kBq/kg [55 kBq/kg after implementation of NIST update]) at intervals of 4 weeks for 6 months was well tolerated without dose-limiting toxicities.

Osteosarcomas, a known effect of bone-seeking radionuclides, were observed at clinically relevant doses in rats 7 – 12 months after start of treatment. Osteosarcomas were not observed in dog studies. The presence of neoplastic changes, other than osteosarcomas, was also reported in the longer term (12 to 15 months) rat toxicity studies. Due to its mode of action, and as seen with conventional radiotherapy and other radiotherapeutics, radium-223 dichloride may have the potential to induce secondary malignancies. No case of osteosarcoma has been reported in clinical studies with Radium Ra 223 dichloride. The risk for patients to develop osteosarcomas with exposure to Radium Ra 223 dichloride is unknown at present

Studies on reproductive and developmental toxicity have not been performed. Since Radium Ra 223 dichloride binds to bone, the potential risk for toxic effects in the male gonads in cancer patients with castration-resistant prostate cancer is very low, but cannot be excluded. Studies on the mutagenic and carcinogenic potential of Radium Ra 223 dichloride have not been performed.

No histological changes were observed in organs involved in the excretion of Radium Ra 223 dichloride. No significant effects were seen on vital organ systems, i.e. cardiovascular (dog), respiratory or central nervous systems (rat), after single dose administration of up to 450 (497 kBq/kg after implementation of NIST update) in dogs, and up to 1000 kBq/kg (1100 kBq/kg; after the NIST implementation date) in rats, (9 (dog) to 20 (rat) times the clinically recommended dose.

1.3.5 Clinical Experience Summary

The clinical development of Radium Ra 223 dichloride is summarized below[41-51]. It includes phase I and phase II studies in prostate cancer patients with bone metastases. The results of these completed studies indicated that safety and tolerability of Radium Ra 223 dichloride in CRPC/HRPC patients with bone metastases was well tolerated, and that there was evidence of dose related efficacy against bone markers and other markers of disease. In addition there was an effect on median overall survival in a Phase II (BC1-02) placebo-controlled study. These studies enabled the initiation of the Phase III ALSYMPCA (ALpharadin in SYMptomatic Prostate CAncer) study.

The clinical safety and efficacy of Radium Ra 223 dichloride have been evaluated in a double-blind, randomized, multiple dose, phase III multicenter study (ALSYMPCA) in castration-resistant prostate cancer patients with bone metastases. The primary efficacy endpoint was Overall Survival (OS).

At the cut-off date of the pre-planned interim analysis, a total of 809 patients were randomized 2:1 to receive Radium Ra 223 dichloride 50 kBq (0.0014 mCi)/kg (55kBq (.00149 mCiafter implementation of the NIST update) intravenously every 4 weeks for 6 cycles (N=541) plus best standard of care or matching placebo plus best standard of care (N=268). Best standard of care included e.g. local external beam radiotherapy, corticosteroids, antiandrogens, estrogens, estramustine or ketoconazole.

An updated descriptive analysis of safety and of OS was performed in 921 randomized patients prior to implementing crossover (i.e. offering patients in the placebo group to receive Radium Ra 223 dichloride treatment).

The results of both, interim and updated analysis, revealed that OS was significantly longer in patients treated with Radium Ra 223 dichloride plus best standard of care compared to patients treated with placebo plus best standard of care. For the updated analysis, an increase in median overall survival of 3.6 months was seen with Radium Ra 223 dichloride plus best standard of care compared to placebo plus best standard of care (HR =0.695 (95% CI 0.581/0.832), median OS 14.9 months versus 11.3 months, respectively).

In the ALSYMPCA study, the results of the interim analysis and the updated analysis showed also a significant improvement in all main secondary endpoints in the Radium Ra 223 dichloride arm compared to the placebo arm:

Time to first SRE (defined as time to EBRT, time to first pathological bone fracture, time to spinal cord compression and time to surgical intervention) was statistically significantly longer in the radium-223 chloride group compared to placebo (median number of months=15.6 for radium-223 chloride versus 9.8 months for placebo (HR=0.658, 95 CI 0.522–0.830, p= 0.00037).

Time to total ALP progression (defined as ≥ 25% increase compared to baseline/nadir) was statistically significantly longer in the radium-223 chloride group 7.4 months compared to placebo 3.8 months (HR = 0.167, 95% CI 0.129 – 0.217; p= <0.00001).

Time to PSA progression (defined as a ≥ 25% increase and an increase in absolute value of ≥ 2 ng/mL compared to baseline/nadir) was also significantly prolonged in patients receiving Radium Ra 223 dichloride compared to patients receiving placebo (HR = 0.643, 95% CI 0.539,0.768; p = <0.00001)

A total ALP response (defined as a confirmed ≥ 30% or ≥ 50% reduction compared to baseline) at week 12 was observed in higher proportions of subjects who were treated with radium-223 chloride group (47% and 3% respectively) compared to those in the placebo (3% and <1% respectively) group.

Subgroup survival analysis showed a consistent survival benefit for treatment with Radium Ra 223 dichloride, independent of total alkaline phosphatase (ALP), current use of bisphosphonates, prior use of docetaxel and baseline ECOG status. The results from the phase III ALSYMCA study regarding time to external beam radiation therapy (EBRT) for pain relief and fewer patients reporting bone pain as an adverse event in the Radium Ra 223 dichloride group indicate a positive effect on bone pain.

The most common adverse reactions (≥ 10%) in patients receiving Radium Ra 223 dichloride were nausea, diarrhea, vomiting, and peripheral edema (Table 1). Grade 3 and 4 adverse events were reported among 57% of Radium Ra 223 dichloride-treated patients and 63% of placebotreated patients. The most common hematologic laboratory abnormalities in Radium Ra 223 dichloride-treated patients (≥ 10%) were anemia, lymphocytopenia, leukopenia, thrombocytopenia, and neutropenia (Table 2). Treatment discontinuations due to adverse events occurred in 17% of patients who received Radium Ra 223 dichloride and 21% of patients who received placebo.

The most common hematologic laboratory abnormalities leading to discontinuation for Radium Ra 223 dichloride were anemia (2%) and thrombocytopenia (2%).

Table 1 shows adverse reactions occurring in ≥ 2% of patients and for which the incidence for Radium Ra 223 dichloride exceeds the incidence for placebo.

Table 1 Adverse Reactions in the Randomized Trial

System/Organ Class Preferred Term	Radium Ra 223 dichloride (n=600)		Placebo (n=301)	
	Grades 1-4 %	Grades 3-4 %	Grades 1-4 %	Grades 3-4 %
Blood and lymphatic system disorders				
Pancytopenia	2	1	0	0
Gastrointestinal disorders				
Nausea	36	2	35	2
Diarrhea	25	2	15	2
Vomiting	19	2	14	2
General disorders and administration site conditions				
Peripheral edema	13	2	10	1
Renal and urinary disorders				
Renal failure and impairment	3	1	1	1

Laboratory Abnormalities

Hematologic Laboratory Abnormalities	Ra-223 (n=600)		Placebo (n=301)	
	Grades 1-4 %	Grades 3-4 %	Grades 1-4 %	Grades 3-4 %
Anemia	93	6	88	6
Lymphocytopenia	72	20	53	7
Leukopenia	35	3	10	<1
Thrombocytopenia	31	3	22	<1
Neutropenia	18	2	5	<1

Table 2 shows hematologic laboratory abnormalities occurring in > 10% of patients and for which the incidence for Radium Ra 223 dichloride exceeds the incidence for placebo.

Table 2: Hematologic Laboratory Abnormalities

Laboratory values were obtained at baseline and prior to each 4-week cycle.

As an adverse reaction, grade 3-4 thrombocytopenia was reported in 6% of patients on Radium Ra 223 dichloride and in 2% of patients on placebo. Among patients who received Radium Ra 223 dichloride, the laboratory abnormality grade 3-4 thrombocytopenia occurred in 1% of docetaxel naïve patients and in 4% of patients who had received prior docetaxel. Grade 3-4 neutropenia occurred in 1% of docetaxel naïve patients and in 3% of patients who have received prior docetaxel.

Fluid Status

Dehydration occurred in 3% of patients on Radium Ra 223 dichloride and 1% of patients on placebo. Radium Ra 223 dichloride increases adverse reactions such as diarrhea, nausea, and vomiting which may result in dehydration. Monitor patients' oral intake and fluid status carefully and promptly treat patients who display signs or symptoms of dehydration or hypovolemia.

Injection Site Reactions

Erythema, pain, and edema at the injection site were reported in 1% of patients on Radium Ra 223 dichloride.

Secondary malignant neoplasms

No cases of radiation-induced cancer have been reported in reported in clinical trials with radium-223 dichloride in follow-up of up to three years. However, the radiation dose resulting from therapeutic exposure may result in higher incidence of cancer (e.g. sarcomas of the bone, or leukemia), mutations and a potential for development of hereditary defects.

Bone Marrow Suppression

In the randomized trial, 2% of patients on the Radium Ra 223 dichloride arm experienced bone marrow failure or ongoing pancytopenia compared to no patients treated with placebo. There were two deaths due to bone marrow failure and for 7 of 13 patients treated with Radium Ra 223 dichloride, bone marrow failure was ongoing at the time of death. Among the 13 patients who experienced bone marrow failure, 54% required blood transfusions. Four percent (4%) of patients on the Radium Ra 223 dichloride arm and 2% on the placebo arm permanently discontinued therapy due to bone marrow suppression.

In the randomized trial, deaths related to vascular hemorrhage in association with myelosuppression were observed in 1% of Radium Ra 223 dichloride-treated patients compared to 0.3% of patients treated with placebo. The incidence of infection-related deaths (2%), serious infections (10%), and febrile neutropenia (<1%) were similar for patients treated with Radium Ra 223 dichloride and placebo.

Myelosuppression; notably thrombocytopenia, neutropenia, pancytopenia, and leukopenia; has been reported in patients treated with Radium Ra 223 dichloride. In the randomized trial, complete blood counts (CBCs) were obtained every 4 weeks prior to each dose and the nadir CBCs and times of recovery were not well characterized. In a separate single-dose phase 1 study of Radium Ra 223 dichloride, neutrophil and platelet count nadirs occurred 2 to 3 weeks after Radium Ra 223 dichloride administration at doses that were up to 1 to 5 times the recommended dose, and most patients recovered approximately 6 to 8 weeks after administration

Hematologic evaluation of patients must be performed at baseline and prior to every dose of Radium Ra 223 dichloride. Before the first administration of Radium Ra 223 dichloride, 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 Radium Ra 223 dichloride, the ANC should be ≥ 1 x 109/L and the plateletcount ≥ 50 x 109/L. If there is no recovery to these values within 6 to 8 weeks after the last administration of Radium Ra 223 dichloride,despite receiving supportive care, further treatment with Radium Ra 223 dichloride 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 Radium Ra 223 dichloride in patients who experience life-threatening complications despite supportive care for bone marrow failure.

The safety and efficacy of concomitant chemotherapy with Radium Ra 223 dichloride have not been established. Outside of a clinical trial, concomitant use with chemotherapy is not recommended due to the potential for additive myelosuppression. If chemotherapy, other systemic radioisotopes or hemibody external radiotherapy are administered during the treatment period, Radium Ra 223 dichloride should be discontinued.

2. Study objectives

The primary objective of this study is to evaluate the quantitative effect of Ra-223 on ^{99m}Tc-MDP bone scan in men with castration-resistant prostate cancer metastatic to bone. .

Secondary objectives include the description of mean percent change in bone scan lesion area by 18-month survival status as well as the evaluation of the effects of Ra-223 by the following measures:

(A) Imaging biomarkers
I. Concurrent conventional assessments including Computed Tomography scans
II. Baseline assessments including fluciclovine PET (if assigned to PET), ^{99m}Tc MDP bone scan, & blood testing

(B) Standard and novel circulating tumor cell assays
I. CTC number by FDA-approved assay (Veridex CellSearch)
II. CTC translational biomarkers by microfluidic platform
(e.g. enumeration, androgen receptor signaling, proliferative index by Ki67 staining)

(C) Circulating biomarkers of the tumor microenvironment
I. Bone turnover markers (serum bone specific alkaline phosphatase, N-telopeptide)
II. Plasma biomarkers of inflammation and angiogenesis

(D) Patient reported pain and quality of life
I. Determine rate of confirmed pain response at week 12 of study treatment
II. Evaluate change in patient reported quality of life as measured by validated assessment tools

3. Investigator[s] and other study participants

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"The values in this protocol have been revised as per the United States (US) National Institute of Standards and Technology (NIST)standardization update (agreed on 17 MAR 2015)"

4. Study design

This single-arm exploratory biomarker study will prospectively enroll men with castration-resistant prostate cancer (CRPC) metastatic to bone. Exhaustive inclusion/exclusion criteria are detailed in the Section 5 of the protocol. Schema:

1. Screening assessments include ^{99m}TC MDP bone scan & blood testing
2. Enrollment
3. Baseline assessments includes fluciclovine PET (if assigned to PET)
4. Ra-223, administered monthly for a total of six anticipated doses with concurrent protocol-specified correlative imaging and blood testing
5. Conclusion of study treatment with sixth dose of Ra-223
6. Follow-up and testing as detailed in experimental protocol

Standard androgen deprivation therapy (ADT) will be continued throughout study participation. Men will be treated for 6 months on protocol in the absence of progression or intolerable side effects. Men will be evaluated according to the table provided in protocol Section 7.1.1. At the end of 6 months of treatment, Ra-223 will be discontinued. Subsequent therapies will be at the discretion of the treating oncologist.

5. Study population

5.1 Eligibility

5.1.1 Inclusion criteria

- Male age ≥ 18 years.
- Histologically or cytologically confirmed adenocarcinoma of the prostate.
- Life expectancy of at least 6 months.
- ECOG performance status of zero, one, or two.
- Bone-predominant metastatic CRPC: at least two skeletal metastases on bone scan with no lung, liver, and/or brain metastasis (lymph node metastasis is allowed).
- Judged by investigator to have progressive disease sufficient to clinically justify standard-of-care radium-223 treatment.
- Subjects must be able to understand and be willing to sign the written informed consent form.
- All acute toxic effects of any prior treatment have resolved to NCI-CTCAE v4.0 Grade 1 or less at the time of signing the Informed Consent Form (ICF).
- No intention to use cytotoxic chemotherapy within the next 6 months.

- Subjects must agree to use adequate contraception beginning at the signing of the ICF until at least 6 months after the last dose of study drug. The definition of adequate contraception will be based on the judgment of the principal investigator.
- Acceptable hematology and serum biochemistry screening values:
    - White Blood Cell Count (WBC) ≥ 3,000/mm3
    - Absolute Neutrophil Count (ANC) ≥ 1,500/mm3
    - Platelet (PLT) count ≥ 100,000/mm3
    - Hemoglobin (HGB) ≥ 9 g/dl (Please note: it is acceptable from the standpoint of study eligibility to undergo transfusion in order to achieve hemoglobin ≥ 9 g/dl)
    - Total bilirubin level ≤ 1.5 x institutional upper limit of normal (ULN)
    - Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 2.5 x ULN
    - Creatinine ≤ 2 x ULN
    - Albumin > 25 g/L
- Willing and able to comply with the protocol, including follow-up visits and examinations.

5.1.2 Exclusion criteria

- Treatment with cytotoxic chemotherapy within previous 28 days, or failure to recover from AEs due to cytotoxic chemotherapy administered more than 28 days previous (however, ongoing neuropathy is permitted).
- Received any investigational compound within 28 days prior to the first dose of study drug or planned during the treatment period or follow-up.
- Received systemic therapy with radionuclides (e.g., strontium-89, samarium-153, rhenium-186, or rhenium-188, or Radium Ra 223 dichloride) for the treatment of bony metastases.
- Received previous radiotherapy to approximately >25% of bone marrow.
- Other malignancy treated within the last 3 years (except non-melanoma skin cancer or low-grade superficial bladder cancer).
- Visceral metastases as assessed by abdominal or pelvic computed tomography (CT) or other imaging modality.
- Presence of brain metastases.
- Lymphadenopathy exceeding 6 cm in short-axis diameter.
- Any size pelvic lymphadenopathy if it is thought to be a contributor to concurrent hydronephrosis.

- Imminent spinal cord compression based on clinical findings and/or magnetic resonance imaging (MRI). Treatment should be completed for spinal cord compression.
- Any other serious illness or medical condition, such as but not limited to:
  - Any infection ≥ National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.03 Grade 2
  - Cardiac failure New York Heart Association (NYHA) III or IV
  - Crohn's disease or ulcerative colitis
  - Known bone marrow dysplasia
- Fecal incontinence.
- Any condition which, in the investigator's opinion, makes the subject unsuitable for trial participation.

5.1.3 Inclusion of Women and Minorities

Both men of all races and ethnic groups are eligible for this trial. Women are not eligible as women can not develop prostate cancer.

5.1.4 Excluded therapies and medications, previous and concomitant

- Concurrent anti-cancer therapy (chemotherapy, radiation therapy, surgery, immunotherapy, biologic therapy, or tumor embolization) other than Ra 223 dichloride and standard methods of androgen deprivation therapy (GnRH agonist, GnRH antagonist, or bilateral orchiectomy).
- Prior use of Radium Ra 223 dichloride.
- Concurrent use of another investigational drug or device therapy (i.e., outside of study treatment) during, or within 4 weeks of trial entry (signing of the informed consent form).
- Major surgery within 30 days prior to start of study drug.

5.2 Withdrawal of subjects from study

5.2.1 Registration Procedures

General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the QACT protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Notify the QACT Registrar of registration cancellations as soon as possible

Registration Process for DF/HCC and DF/PCC Institutions

The QACT registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time. In emergency situations when a participant must begin protocol therapy during off-hours or holidays, call the QACT registration line at 617-632-3761 and follow the instructions for registering participants after hours.

The registration procedures are as follows:

- Obtain written informed consent from the participant prior to the performance of any protocol specific procedures or assessments.

- Complete the QACT protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical record and/or research chart. **To be eligible for registration to the protocol, the participant must meet all inclusion and exclusion criterion as described in the protocol and reflected on the eligibility checklist.**

  Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for a treatment protocol. Registration to both treatment and ancillary protocols will not be completed if eligibility requirements are not met for all studies.

- Fax the eligibility checklist(s) and all pages of the consent form(s) to the QACT at 617-632-2295. For Phase I protocols, attach participant dose level assignment confirmation from the sponsor.

- The QACT Registrar will (a) review the eligibility checklist, (b) register the participant on the protocol, and (c) randomize the participant when applicable.

- An email confirmation of the registration and/or randomization will be sent to the Overall PI, study coordinator(s) from the Lead Site, treating investigator and registering person immediately following the registration and/or randomization.

5.2.2 Withdrawal

Subjects **must be withdrawn from the trial** (treatment and procedures) for the following reasons:

- Subject withdraws consent from study treatment and study procedures. A subject must be removed from the trial at his own request or at the request of his legally acceptable representative. At any time during the trial and without giving reasons, a subject may decline to participate further. The subject will not suffer any disadvantage as a result.
- Subject is lost to follow-up.
- Death.

Subjects **may be** withdrawn from the study for the following reasons:

- The subject is non-compliant with study drug, trial procedures, or both; including the use of anti-cancer therapy not prescribed by the study protocol.
- If, in the investigator's opinion, continuation of the trial would be harmful to the subject's well-being.
- The development of a second cancer (with the exception of non-melanoma skin cancer).
- Development of an intercurrent illness or situation which would, in the judgment of the investigator, significantly affect assessments of clinical status and trial endpoints.
- Deterioration of ECOG performance status to 4.
- Use of illicit drugs or other substances that may, in the opinion of the investigator, have a reasonable chance of contributing to toxicity or otherwise skewing trial result.

Any subject removed from the trial will remain under medical supervision until discharge or transfer is medically acceptable.

In all cases, the reason for withdrawal must be recorded in the CRF and in the subject's medical records. Alternative care options will be discussed with the participant.

A QACT Treatment Ended/Off Study Form will be filled out when a participant is removed from protocol therapy. This form can be found on the QACT website or obtained from the QACT registration staff.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Philip J. Saylor M.D. at 617-724-4000.

5.2.3 Duration of Follow-up

Participants will be followed for 18 months after removal from protocol therapy or until death, whichever occurs first. Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.2.4 Screen Failures/Dropouts

A subject who discontinues study participation prematurely for any reason is defined as a "dropout" if the subject has already been registered.

A subject who, for any reason (e.g. failure to satisfy the selection criteria), terminates the study before the time point used for the definition of "dropout" (see above) is regarded a "screening failure".

5.2.5 Replacement

No withdrawn subjects will be replaced.

6. Treatment

6.1 Treatments to be administered

6.1.1 Radium Ra 223 dichloride, 50 kBq/kg body weight, will be administered as a bolus intravenous (IV) injection (up to 1 minute) at intervals of every 4 weeks for up to 6 cycles (55kBq/kg body weight after implementation of the NIST update)

6.2 Treatment assignment

This is an open label, single arm study. A patient number (a unique identification number) will be assigned when a subject is evaluated for inclusion into the study. The patients will then undergo screening procedures and will enter the trial after successful screening.

6.2.1 Radium Ra 223 dichloride

The alpha-pharmaceutical Radium Ra 223 dichloride will be administered per standard-of-care following standard institutional clinical practice. It is a ready-to-use, sterile, non-pyrogenic, clear and colorless aqueous solution of Radium Ra 223 dichloride ($^{223}RaCl_2$) for IV administration. It should not be diluted or mixed with any solutions. Each vial for a single use only.

Radium Ra 223 dichloride is an alpha particle emitter with a physical half-life of 11.4 days. The product is isotonic and has a pH of 6.0-8.0. The radioactive concentration at the reference date is 1000 kBq/mL (1,100 kBq/mL after implementation of NIST update). The product has a pre-calibration of 14 days. When administered on a day other than the reference day, the volume should be corrected according to the physical decay table accompanying each shipment..

Bayer Healthcare LLC will provide Radium Ra 223 dichloride, which will be manufactured by Algeta's contract manufacturer: Institute for Energy Technology, Isotope laboratories, Kjeller, Norway. The product is produced according to Good Manufacturing Practice (GMP). The product will be delivered in a glass vial, ready-to-use with a certified activity. Radium Ra 223 dichloride is shipped in a lead container

and Type A radioactive package according to international transportation guidelines for radioactive materials.

The volume per vial is 6 mL, corresponding to 6 MBq (6.6 MBq after implementation of NIST update) at the reference day. Radium Ra 223 dichloride has a shelf life of 28 days from production day, when stored at ambient temperature. The shelf life has been demonstrated for temperatures from cold storage (2-8°C) up to 40°C. In addition, it has been shown that the product quality is not jeopardized upon freezing.

All study drugs will be labeled according to the requirements of local law and legislation. For all study drugs, a system of numbering in accordance with all requirements of GMP will be used, ensuring that each dose of study drug can be traced back to the respective bulkware of the ingredients.

6.2.1.1 General warning

Radium 223 dichloride should be received, used and administered only by authorized persons in designated clinical settings. The receipt, storage, use, transfer and disposal Radium 223 dichloride are subject to the regulations and/or appropriate licenses of the competent official organization. Radium 223 dichloride should be handled by the user in a manner which satisfies both radiation safety and pharmaceutical quality requirements. Appropriate aseptic precautions should be taken.

6.2.1.2 Instructions for Use

The administration of Radium Ra 223 dichloride is associated with potential risks for other persons (e.g. medical staff, care givers and members of the patient's family) from radiation or contamination from spills of body fluids such as urine, feces, or vomit. Therefore, radiation protection precautions must be taken in accordance with national and local regulations.

<u>For drug handling</u>
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 Ra 223 dichloride, 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.

<u>For patient care</u>
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 Ra 223 dichloride or patient fecal matter or urine should be washed promptly and separately from other clothing.

Radium-223 is primarily an alpha emitter, with a 95.3% fraction of energy emitted as alpha-particles. The fraction emitted as beta-particles is 3.6%, and the fraction emitted as gamma-radiation is 1.1%. The external radiation exposure associated with handling of patient doses is considerably lower in comparison to other radiopharmaceuticals for therapeutic purposes as the administered radioactivity will usually be below 8 MBq (0.216 mCi). 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 Ra 223 dichloride and the detection of contamination with standard instruments.

6.2.2 Dose calibration

Radium Ra 223 dichloride can be measured in a normal dose calibrator instrument. When written approvals for the use of Radium Ra 223 dichloride from the Radiation Protection Agency for the specific center have been received by the sponsor, a vial of Radium Ra 223 dichloride for technical use will be sent to the study center. Different clinical study centers possess dose calibrators from various suppliers; thus, the isotope calibration factor may differ from center to center. Consequently, each center must perform the Radium Ra 223 dichloride dial setting on their relevant dose calibrator(s). For dial setting, the clinical study center will receive a sealed vial 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.

6.2.3 Dosimetry

The absorbed radiation dose calculation was performed based on clinical biodistribution data. Calculations of absorbed doses were performed using OLINDA/EXM (Organ Level INternal Dose Assessment/EXponential Modeling), a software based on the Medical Internal Radiation Dose (MIRD) algorithm, which is widely used for established beta and gamma emitting radionuclides. For radium-223, which is primarily an alpha emitter, additional assumptions were made for the intestine, red marrow and bone/osteogenic cells to provide the best possible absorbed dose calculations for Radium Ra 223 dichloride, considering its observed biodistribution and specific characteristics.

For an administered activity of 3.65 MBq (0.0987 mCi) (50 kBq (0.00135 mCi) (55kBq (.00149 mCi after NIST update) per kg body weight to a 73-kg adult), the calculated absorbed doses to the bone (osteogenic cells) is 4.2050 Gy (420.5 rad) and to the red marrow is 0.5066 Gy (50.66 rad). The calculated absorbed doses to the main excretory organs are 0.0265 Gy (2.65 rad) for the small intestine wall, 0.1180 Gy (11.8 rad) for the upper large intestine wall and 0.1696 Gy (16.96 rad) for the lower large intestine wall.

The calculated absorbed doses to other organs are low, e.g. heart wall (0.0063 Gy, 0.63 rad), lung (0.0003 Gy, 0.03 rad), liver (0.0109 Gy, 1.09 rad), kidneys (0.0117 Gy, 1.17 rad), urinary bladder wall (0.0147 Gy, 1.47 rad), testes (0.0003 Gy, 0.03 rad), and spleen (0.0003 Gy, 0.03 rad).

The hematological adverse drug reactions observed in the clinical studies with Ra-223 are much lower in frequency and severity than what could be expected from the calculated absorbed doses to the red marrow. This may be related to spatial distribution of alpha particle radiation resulting in non-uniform radiation dose to the red marrow.

6.2.4 Dose handling

The Radium Ra 223 dichloride vials must be stored inside their lead container in a secure facility. The study drug should be used within 28 days of production. Radium Ra 223 dichloride is an alpha-pharmaceutical and should be handled by individuals who are qualified by training and experience in the safe handling of radionuclides. One dedicated person and a back-up designee will have responsibility as assigned from the Primary Investigator for handling and storage of Radium Ra 223 dichloride. All administrations of Radium Ra 223 dichloride are based on the certified activity of Radium Ra 223 dichloride at the calibration date.

6.2.5 Dose calculation

The dosage of Radium Ra 223 dichloride is 50 kBq/kg body weight (55kBq/kg after NIST update). The patient dose is calculated based on date of injection, a decay correction (DK) factor specific to number of days from reference date applied to correct for physical decay of radium-223 , and patient weight. A table with DK values according to physical decay of the study medication will be provided with every shipment of Radium Ra 223 dichloride. Radium-223 is an alpha particle emitter with a physical $t^{1/2}$ of 11.4 days. The radioactive concentration at the reference date is 1,000 kBq/mL (1,100 kBq/mL after implementation of NIST update).

The volume to be administered for the current dose is calculated as follows:

$$\frac{\text{Body weight (kg)} \quad \mathbf{X} \text{ dose (50kBQ/kg body weight)}^{a}}{\text{DK factor X 1000kBq (0.027mCi)/mL}^{b}}$$

Data regarding activity should be recorded on the appropriate electronic case report form (eCRF) page.

"The values in this protocol have been revised as per the United States (US) National Institute of Standards and Technology (NIST)standardization update (agreed on 17 MAR 2015)"

[a]55 kBq/kg after implementation of NIST update
[b]1100 kBq (0.0297 mCi)/mL after implementation of NIST update; NIST: National Institute of Standards and Technology; DK: Decay correction

6.2.6 Dose preparation

Personnel should use appropriate protective clothing and equipment during syringe filling and application to prevent contamination with the radioactive solution (medical gloves / protective glasses). The individual responsible for study drug preparation will draw the correct volume of study drug into a syringe. The size of the syringe should be chosen according to the applied volume to reach the required dosing accuracy. Radium Ra 223 dichloride should not be diluted or mixed with any solutions. Do not store above 40°C (104°F). If the vials have been stored in a refrigerator, they should be left at room temperature for 1 hour prior to use, since cold material should not be injected in a patient. Store Radium Ra 223 dichloride in the original container or equivalent radiation shielding. This preparation is approved for use by persons under license by the Nuclear Regulatory Commission or the relevant regulatory authority of an Agreement State.

6.2.7 Dose administration

Before administration of study drug, the patient must be well hydrated; the patient should be instructed to drink ad libitum. Aseptic technique should be used in the administration of Radium Ra 223 dichloride. The syringe should be handed over to the individual who will perform the injection. The study medication will be administered as a bolus intravenous (IV) injection (up to 1 minute). After administration, the equipment used in connection with the preparation and administration of drug is to be treated as radioactive waste and should be disposed in accordance with local procedure for the handling of radioactive material.

6.2.8 Dose Modification/Delays

Every effort should be made to administer the full dosing regimen of Radium Ra 223 dichloride. Adjustment of dose level is not permitted.

Study visits during the treatment period will occur at protocol-defined intervals (within a window of +/- 7 days).

Dosing delays may be instituted under the following circumstances:

Disease progression: The Investigator should delay cytotoxic chemotherapy, other systemic radioisotope, hemibody external radiotherapy or other investigational drug until the follow-up period. If such treatments have to be given during the treatment period, further study drug administrations must be discontinued. Patients with disease progression may continue treatment at the Investigator's discretion.

Myelosuppression: Treatment-related changes in hematology parameters may occur.

- If a patient experiences CTCAE v4.03 Grade 3 or 4 neutropenia, thrombocytopenia, or anemia the administration of study drug should be delayed until recovery to Grade 2 or better.
- If a patient experiences CTCAE v4.03 Grade 3 or 4 neutropenia, thrombocytopenia, or anemia lasting > 14 days, further study drug administrations must be discontinued.
- Blood transfusion is acceptable. Use of biologic response modifiers, such as G-CSF or GM-CSF, is allowed in the management of acute toxicity.

Gastrointestinal events: Diarrhea should be treated as per local practice. A further dose of study medication should not be given before diarrhea is recovered to CTCAE v4.03 Grade 2 or baseline levels. Nausea or vomiting should be treated as per local practice. A further dose of study medication should not be given before nausea or vomiting is recovered to CTCAE v.4.03 Grade 2 or baseline levels.

Spinal Cord Compression: If the patient experiences spinal cord compression during the treatment period, the patient should be treated for the event, and may receive further study drug administration if adequately recovered.

Surgical Intervention: If surgery is required, the patient should continue with study treatment, if this is considered safe in the treating Investigator's opinion. The surgeon needs to be notified that the patient has been given radioactive drug, and needs to follow the guidelines for radioactive protection.

Non-Pathological Fractures: For traumatic fractures in weight-bearing bones during treatment phase, the study drug administration should be delayed for 2-4 weeks from the time of fracture.

Pathological fractures: Pathological fractures may occur as the result of either progressive disease or increased physical activity associated with significant pain palliation. Pathologic fractures are to be treated in a manner that attempts to maintain the best functional status and quality of life. Study treatment may continue as planned.

Any Other Toxicity: Local practice will apply.

6.3 Drug logistics and accountability

All study drugs will be stored at the investigational site in accordance with Good Clinical Practice (GCP) and Good Manufacturing Practices (GMP) requirements and the instructions given by the clinical supplies department of the Institution and will be inaccessible to unauthorized personnel.

6.3.1 Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the agent(s) (investigational or free of charge) using the NCI Drug Accountability Record or another comparable drug accountability form. (See the CTEP website at http://ctep.cancer.gov/protocolDevelopment

for the "Policy and Guidelines for Accountability and Storage of Investigational Agents" or to obtain a copy of the drug accountability form.)

6.3.2 Destruction and Return

At the end of the study, unused supplies of Ra 223 dichloride and other investigational agents should be destroyed appropriately and according to institutional policies.Destruction will be documented in the Drug Accountability Record Form. The certificate of destruction for Ra 223 dichloride should be sent to Bayer.

6.4 Treatment compliance

Subject compliance with the treatment and protocol includes willingness to comply with all aspects of the protocol, and to have blood collected for all safety evaluations. At the discretion of the principal investigator, a subject may be discontinued from the trial for non-compliance with follow-up visits or study drug.

6.5 Prior and concomitant therapy-

All medication that is considered necessary for the subject's welfare, and which is not expected to interfere with the evaluation of the study treatment, may be given at the discretion of the investigator. All medications (including contrast media) taken within 2 weeks prior to the start of the study and during the study must be recorded in the subject's source documentation and in the CRF (including start/stop dates, dose frequency, route of administration, and indication).

Permitted

- Treatment with non-conventional therapies (e.g., herbs [with the exception of St. John's Wart], acupuncture) and vitamin/mineral supplements is acceptable provided that, in the opinion of the investigator, such treatment will not interfere with the trial endpoints.
- Subjects may receive standard of care for any underlying illness.
- In the event of neutropenia, anemia, or thrombocytopenia, subjects may receive appropriate supportive care (e.g., transfusion, biologic response modifiers such as G-CSF or GM-CSF, prophylactic antibiotics, antifungals and/or antivirals, hematopoietic growth factors).
- Blood transfusions and erythropoietin are allowed.
- If surgery is required during study drug treatment, the surgeon needs to be notified that the patient has been treated with a radioactive product and adequate precautions for radioactive protection should be applied during the surgical procedure. The patient should continue with study treatment if considered safe in the treating Investigator's opinion.

- Concomitant treatments for prostate cancer will be recorded in the CRFs.

7. Procedures and variables

7.1 Schedule of procedures

7.1.1 Tabulated overview

Table 1: Treatment	S	B	T1 D1	T1 D4	T2	T3	T4	T5	T6	F/U 1M	F/U 4M	F/U 18M
History & physical exam	X		X		X	X	X	X	X	X	X	X
^{99m}Tc MDP bone scan	X					X[1]				X		
CT a/p	X					X[1, 2]				X		
Fluciclovine PET/MRI[3]		X				X[1]						
Medication history	X		X		X	X	X	X	X	X	X	X
Chemistry panel	X		X		X	X	X	X	X	X		
CBC with differential	X		X		X	X	X	X	X	X		
PSA			X		X	X	X	X	X	X	X	X
CTCs by CellSearch[4]			X		X	X						
CTCs by microfluidic chip[5]			X		X	X						
BAP & NTx			X	X	X	X	X	X	X	X	X	X
Blood biomarkers[6]			X	X	X	X	X	X	X	X	X	X
Patient reported outcomes[7]			X		X	X	X	X	X	X	X	X
Adverse event reporting			X		X	X	X	X	X	X		

Notations:
Screening studies "S" must be done within 60 days of start of treatment ("T1 D1").
Baseline study "B" must be done after a subject is successfully registered to the trial, but prior to start of treatment ("T1 D1").
Treatments T1 through T6 occur at 4 week intervals ± 7 days.
(1) T3 timepoint scans (bone scan, and CT a/p, and fluciclovine PET/MRI in some) will be performed within 10 days prior to the T3 treatment day.
(2) T3 timepoint CT a/p will be performed only for participants that do not undergo fluciclovine PET/MRI.
(3) fluciclovine PET/MRI will be performed at two timepoints each for 10 of the study participants. They will be chosen by investigator discretion in light of their willingness and medical eligibility to undergo PET/MRI (e.g. pacemaker is a contraindication, claustrophobia is a contraindication). The baseline PET/MRI will only be done after the subject has successfully been registered to the trial, but prior to start of treatment ("T1 D1").
(4) CTCs, circulating tumor cells; 7.5 mL of whole blood will be collected in a special CellSave tubes as described in Appendix G for BWH core lab testing using the commercially available Veridex CellSearch assay; subsequent CTC testing will only be carried out on participants who had ≥ 1 detectable CTC on study T1 D1.
(5) 20 mL of blood will be collected in vacutainer tubes (usually purple top EDTA tubes) for Haber/Maheswaran laboratory analysis of circulating tumor cells as detailed in Appendix B;

subsequent CTC testing will only be carried out on participants who had ≥ 1 detectable CTC on study T1 D1.
(6) Plasma biomarkers; 10 mL of blood will be collected in a purple top (EDTA plasma) tube and processed as detailed in Appendix C for batched analysis of inflammatory and angiogenic biomarkers.
(7) Analgesic use and other patient reported outcomes will be collected as detailed in Appendices D, E, and F.

Abbreviations:
S, screening; B, baseline;
T1 D1, Ra-223 treatment 1 day 1;
F/U 1M, follow-up visit 1 month after final Ra-223 treatment;
F/U 4M, follow-up visit 4 months after final Ra-223 treatment;
F/U 18M, follow-up visit 18 months after final Ra-223 treatment;
CT a/p, CT of the, abdomen, and pelvis;
Chemistry panel, includes liver panel also known as LFTs; CBC, complete blood count;
PSA, serum prostate specific antigen;
PET, positron emission tomography;
CTCs, circulating tumor cells;
BAP, bone-specific alkaline phosphatase; NTx, n-telopeptide.

7.1.2 Timing of assessments

Relevant timing is as detailed above in Section 7.1.1 (notations immediately below table).

7.1.3 Medical history

Medical history findings (i.e. previous diagnoses, diseases or surgeries) meeting all criteria listed below will be collected:

1. Not pertaining to the study indication

- Start before signing of the informed consent
- Considered relevant to the study

Detailed instructions on the differentiation between (i) medical history and (ii) adverse events can be found in Section 7.15.1.1.

7.2 ^{99m}Tc MDP bone scan: Standard clinical bone scan will be carried out per institutional practice.

7.3 CT a/p: Standard clinical CT of the abdomen and pelvis will be carried out per institutional practice.

7.4 Medication history: Medication history, including any changes in medication use since last assessment, will be reviewed.

7.5 Chemistry panel: Standard comprehensive metabolic panel (including sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, total protein, albumin, AST, ALT, total bilirubin, direct bilirubin, and alkaline phosphatase) will be carried out at the MGH clinical laboratory in real time.

7.6 CBC with differential: Standard comprehensive metabolic panel (including white blood cell count, hemoglobin, hematocrit, platelets, and differential) will be carried out at the MGH clinical laboratory in real time.

7.7 PSA: Standard PSA assay will be carried out at the MGH clinical laboratory in real time.

7.8 Fluciclovine PET/MRI: This testing will be carried out only for 10 study participants. They will be chosen by investigator discretion in light of their willingness and medical eligibility to undergo PET/MRI (e.g. pacemaker is a contraindication, claustrophobia is a contraindication). The agent used in this study will be the radiolabled agent (fluciclovine) that will be administered prior to standard whole body PET imaging which includes skull base to thigh. Fluciclovine will be handled by qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment. PET/MRI imaging will be carried out per standard/clinical institutional protocol. Thus far, there is limited experience using fluciclovine PET/MRI; it is investigational.

7.9 CTCs by CellSearch (see Appendix G for detailed instructions):

At study-designated timepoints, 7.5 mL of whole blood will be collected in a special CellSave tube (available from Veridex/Janssen Diagnostics), which is then stored at room temperature until processed. It will be sent to the BWH CTC Lab for analysis. The BWH Circulating Tumor Cell Laboratory provides circulating tumor cell (CTC) services using the Veridex/Janssen Diagnostics CellSearch immunomagnetic platform. The lab is located to a CLIA-approved space within the BWH Cytology Division (Brigham and Women's Hospital, Medical Research Building, 3rd Floor; 75 Francis St., Boston, MA 02115). **Major equipment includes the** CellSearch Autoprep, and CellTracks Analyzer II. The CTC Lab provides clinical enumeration of CTCs for patients with metastatic prostate cancer using the FDA-cleared Veridex/Janssen Diagnostics CellSearch System. It also functions as the BWH CTC Core Lab

(http://www.partners.org/researchcores/CTC/CTC_BWH.html) providing enumeration and isolation of CTC for research purposes. The current director is Alarice Lowe, M.D. (**Phone**: 617-525-8696, **Fax:** 617-739-6192, **Email:** aclowe@partners.org).

7.10 CTCs by microfluidic chip: (see Appendix B for detailed instructions)

Two 10 mL samples of peripheral blood will be collected into vacutainer tubes (Becton-Dickinson) containing the anticoagulant EDTA. Samples will be shipped by U.S. Ground courier to the MGH Charlestown Navy Yard laboratories of Drs. Daniel Haber and Shyamala Maheswaran. Alternatively, a member of the Haber or Maheswaran labs will personally transport the samples from the MGH Cancer Center to the MGH Charlestown Navy Yard laboratories. Samples will be processed through the experimental platform within 6 hours of collection, according to previously established protocols[52,53] with modifications subject to current lab protocol. Briefly, a 5 mL aliquot of blood will be placed into an air-tight conical tube on a rocker assembly, and blood will be pneumatically driven through the microfluidic chip at a protocol-specified flow rate. The microfluidic chip will then be flushed with phosphate-buffered saline at a protocol specified flow rate to remove nonspecifically bound cells. CTCs on the chip will be fixed, permeabilized, and stained with antibodies. Staining for PSA, PSMA, DAPI, Ki-67, and other proteins will be performed. Captured CTCs will then be identified and enumerated using the automated detection system as previously described[52]. RNA and/or DNA will also be isolated from CTCs captured on a separate chip for gene expression analyses. Any remaining isolated RNA or DNA will be stored frozen at -70 to -90 degrees C for batch global profiling analyses. See Appendix B additional processing instructions.

7.11 Bone turnover markers (see Appendix H for detailed instructions):

Sera will be collected from subjects at study-specified timepoints. It will be processed and stored as detailed in Appendix H. Serum N-telopeptide concentrations will be determined at a Massachusetts General Hospital core laboratory using a competitive inhibition enzyme-linked immunosorbent assay (ELISA/EIA) (Osteomark® NTx Serum, Alere Scarborough, Inc., Scarborough,ME). The intra-assay and inter-assay variability are 4.6% and 6.9%, respectively. Quantitative bone-specific alkaline phosphatase (BAP) will be determined at a Massachusetts General Hospital core laboratory using an enzyme immunoassay (MicroVue™ BAP EIA; Quidel Corporation, San Diego, CA).

7.12 Blood biomarkers (see Appendix C for detailed instructions):

Blood samples will be collected at study specific timepoints. For the purposes of this testing, at least 10 mL will be collected in a PURPLE top (EDTA plasma) tube. After processing, separated peripheral blood cells will be immediately

stained using fluorescently labeled antibodies and analyzed using an LSR-II flow cytometer within the Steele Laboratory's facility. Dr. Dan G. Duda, D.M.D., Ph.D. (Associate Professor at MGH/HMS), who has over 12 years of experience in clinical correlative studies, supervises the Steele Lab's Core operation. Plasma analysis will be carried out for a panel of circulating angiogenic and inflammatory molecules. They include vascular endothelial growth factor (VEGF), placental-derived growth factor (PlGF), soluble (s)VEGFR1, basic fibroblast growth factor (bFGF), VEGF-C, VEGF-D and sTie2 using multiplex (7-plex) and IL-1β, IL-6, IL-8 and TNF-α (4-plex) protein array kits from Meso-Scale Discovery (Gaithersburg, MD), and stromal cell-derived factor 1a (SDF1a), hepatocyte growth factor (HGF), soluble (s)c-MET, and s-c-KIT using ELISA from R&D Systems (Minneapolis, MN). Finally, we will evaluate biomarkers of tumor hypoxia, by measuring plasma carbonic anhydrase IX (CAIX) levels as well as biomarkers of osteoclast and osteoblast activity (plasma C-telopeptide and total alkaline phosphatase) using ELISA from R&D Systems. Samples will be run in duplicate.

7.13 Patient reported outcomes: Key patient reported outcome measures will include:

1. Analgesic use as recorded in a medication diary (see Appendix D)
2. Changes in quality of life as measured by a validated assessment tool (EQ-5D-3L; see Appendix E)
3. Changes in pain and narcotic analgesic use as assessed by 4-item questionnaire taken from the MD Anderson Brief Pain Inventory (BPI; see Appendix F)[54]:
   A. Pain at its worst in the last 24 hours (BPI question 3)
   B. Average pain in the last 24 hours (BPI question 5)
   C. Effect of pain on general activity (BPI question 9A)
   D. Effect of pain on sleep (BPI question 9F)

7.14 Efficacy

Primary efficacy variable: The primary objective of this study is to evaluate the efficacy of Ra-223 based on bone scan response measured by quantitative analysis of ^{99m}Tc-MDP bone scan. The bone scan area that we will employ for analysis was developed as a quantitative tool to improve the interpretability and clinical relevance of the bone scan. The BSA is a method of expressing the tumor burden in bone as a percent of the total skeletal mass. For analysis, scan images are intensity normalized, and the lesions are identified and segmented by anatomic region-specific intensity thresholding, and area summed (resulting in a bone scan area measurement for the skeleton and lesions). This calculated ratio of lesion area to bone scan area (calculated from two dimensional planar images) will be used to assess percent change with treatment. Bone scan response is defined as a ≥ 30% decrease in quantitative bone scan lesion area (BSA) from baseline to the end of month 2 of Ra-223 treatment (i.e. after the second of six possible Ra-223 doses).

Secondary variables: Please see statistical methods in protocol Section 8.

7.15 Safety

The sponsor is responsible to comply with the local regulation and legislation for adverse events reporting.

All subjects who receive at least one dose of study treatment will be valid for the safety analysis.

All observations pertinent to the safety of the study treatment will be recorded and included in the final report.

All AEs whether considered drug-related or not, will be reported with a diagnosis, start/stop dates, action taken, whether treatment was discontinued, any corrective measures taken, outcome, and other possible causes. For all events, the relationship to treatment and the intensity of the event will be determined by the investigator.

This trial will use the NCI-CTCAE v4.0 criteria for assessment of toxicity and SAE reporting with regard to toxicity grade.

Safety variables may include but not limited to the following: laboratory changes (complete blood counts, electrolytes, chemistry, and coagulation), changes in vital signs (blood pressure, heart rate, respiratory rate, and temperature) and ECG and, in some instances, changes in chest x-ray images, as produced at the investigator's discretion (e.g., for evaluation for pneumonia).

7.15.1 Adverse events

Investigators should refer to the Safety Information section of the current IB for Ra 223 dichloride, including the DCSI (development core safety information), for the expected side effects of Ra 223 dichloride. As with any agent, there is always the potential for unexpected AEs, including hypersensitivity reactions. The IB will be updated if any new relevant safety data are obtained.

Therapeutic monitoring should be performed following dose selection of Ra 223 dichloride in a manner consistent with the local clinical standard of care. In general, subjects should be closely monitored for side effects of all concomitant medications regardless of the path of drug elimination.

All concomitant medications must be recorded in the subject's source documentation.

Subjects must be carefully monitored for AEs. This monitoring also includes clinical laboratory tests. Adverse events should be assessed in terms of their seriousness, intensity, and relationship to the study drug, or other chemotherapy/treatment.

7.15.1.1 Definitions

Definition of adverse event (AE)

In a clinical study, an AE is any untoward medical occurrence (i.e. any unfavorable and unintended sign [including abnormal laboratory findings], symptom or disease) in a patient or clinical investigation subject after providing written informed consent for participation in the study. Therefore, an AE may or may not be temporally or causally associated with the use of a medicinal (investigational) product.

A surgical procedure that was planned prior to the start of the study by any physician treating the subject should not be recorded as AE (however, the condition for which the surgery is required may be an AE if the condition worsens compared to baseline).

- Conditions that started before signing of informed consent and for which no symptoms or treatment are present until signing of informed consent are recorded as medical history (e.g. seasonal allergy without acute complaints).
- Conditions that started before signing of informed consent and for which symptoms or treatment are present after signing of informed consent, at *unchanged intensity*, are recorded as medical history (e.g. allergic pollinosis).
- Conditions that started or deteriorated after signing of informed consent will be documented as adverse events.

Definition of serious adverse event (SAE)

An SAE is classified as any untoward medical occurrence that, at any dose, meets any of the following criteria (a – f):

a. Results in death.

b. Is life-threatening.

The term 'life-threatening' in the definition refers to an event in which the patient was at risk of death at the time of the event, it does not refer to an event which hypothetically might have caused death if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization.

A hospitalization or prolongation of hospitalization will not be regarded as an SAE if at least one of the following exceptions is met:

- The admission results in a hospital stay of less than 12 hours.
- The admission is pre-planned.
  (i.e. elective or scheduled surgery arranged prior to the start of the study)
- The admission is not associated with an AE.
  (e.g. social hospitalization for purposes of respite care).

However, it should be noted that invasive treatment during any hospitalization may fulfill the criterion of 'medically important' and as such may be reportable as an SAE

dependent on clinical judgment. In addition, where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedence.

d. Results in persistent or significant disability / incapacity.

   Disability means a substantial disruption of a person's ability to conduct normal life's functions.

e. Is a congenital anomaly / birth defect.

f. Is another medically important serious event as judged by the investigator.

7.15.1.2 Classifications for adverse event assessment

All AEs will be assessed and documented by the investigator according to the categories detailed below.

7.15.1.2.1 Seriousness

For each AE, the seriousness must be determined according to the criteria given in Section 7.15.1.1.

7.15.1.2.2 Intensity

The intensity of the AE is classified according to the CTCAEv4.0. Grade refers to the severity (intensity) of the AE:

CTCAEv4 Grade 1: mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention is not indicated.

CTCAEv4 Grade 2: moderate; minimal, local, or noninvasive intervention is indicated; limiting to age-appropriate instrumental activities of daily living (ADL; instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc).

CTCAEv4 Grade 3: Severe or medically significant but not immediately life threatening; hospitalization or prolongation of hospitalization is indicated; disabling; limiting to self care ADL (self care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).

CTCAEv4 Grade 4: life-threatening consequences; urgent intervention is indicated.

CTCAEv4 Grade 5: death due to an AE.

7.15.1.2.3 Causal relationship

The assessment of the causal relationship between an AE and the administration of treatment is a clinical decision based on all available information.

The assessment is based on the question whether there was a "reasonable causal relationship" to the study treatment in question.

Possible answers are "yes" or "no".

An assessment of "no" would include:

1. The existence of a clear alternative explanation, e.g. mechanical bleeding at surgical site.

   or

2. Non-plausibility, e.g. the subject is struck by an automobile when there is no indication that the drug caused disorientation that may have caused the event; cancer developing a few days after the first drug administration.

An assessment of "yes" indicates that there is a reasonable suspicion that the AE is associated with the use of the study treatment.

Factors to be considered in assessing the relationship of the AE to study treatment include:

- The temporal sequence from drug administration: The event should occur after the drug is given. The length of time from drug exposure to event should be evaluated in the clinical context of the event.
- Recovery on drug discontinuation (de-challenge), recurrence on drug re-introduction (re-challenge):
- Subject's response after de-challenge or subjects response after re-challenge should be considered in the view of the usual clinical course of the event in question.
- Underlying, concomitant, intercurrent diseases:
  Each event should be evaluated in the context of the natural history and course of the disease being treated and any other disease the subject may have.
- Concomitant medication or treatment:
  The other drugs the subject is taking or the treatment the subject receives should be examined to determine whether any of them may be suspected to cause the event in question.
- The pharmacology and pharmacokinetics of the study treatment:
  The pharmacokinetic properties (absorption, distribution, metabolism and excretion) of the study treatment, coupled with the individual subject's pharmacodynamics should be considered.

Causal relationship to protocol-required procedure(s)

The assessment of a possible causal relationship between the AE and protocol-required procedure(s) is based on the question whether there was a "reasonable causal relationship" to protocol-required procedure(s).

Possible answers are "yes" or "no".

7.15.1.2.4 Action taken with study treatment

Any action on study treatment to resolve the AE is to be documented using the categories listed below.

- Drug withdrawn
- Drug interrupted
- Dose not changed
- Dose increased
- Not applicable
- Unknown

7.15.1.2.5 Other specific treatment(s) of adverse events

- None
- Remedial drug therapy
- Other

7.15.1.2.6 Outcome

The outcome of the AE is to be documented as follows:

- Recovered/resolved
- Recovering/resolving
- Recovered/resolved with sequelae
- Not recovered/not resolved
- Fatal
- Unknown

7.15.1.3 Assessments and documentation of adverse events

7.15.1.4 Reporting of serious adverse events

The definition of serious adverse events (SAEs) is given in Section 7.15.1.1.

Each serious adverse event must be followed up until resolution or stabilization, by submission of updated reports to the designated person. An isolated laboratory abnormality that is assigned grade 4, according to CTC definition, is not reportable as an SAE; unless the investigator assesses that the event meets standard ICH criteria for an SAE. CTC grade 4 baseline laboratory abnormalities that are part of the disease profile should not be reported as an SAE, specifically when they are allowed or not excluded by the protocol inclusion/exclusion criteria.

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 within 24 hours. In the event of such an event, the investigator should refer to the Pharmacovigilance section of the contract for reporting procedures.

Requirements for Reporting of Serious Adverse Events:
All SAEs must be reported to Bayer within 24 hours of the Principal Investigator's awareness and must include the following minimum information:
1. The name and contact information of the reporter
2. The name of the study drug(s)
3. A description of the reported SAE
4. A patient identified by one or more of the following:
a. Patient initials
b. Patient number
c. Knowledge that a patient who experienced the adverse event exists
d. Age
e. Sex
5. An investigator assessment of study drug causality. For studies with combination therapy, a separate causality assessment should be provided for each study drug.

Additional data which would aid the review and causality assessment of the case include but are not limited to:
The date of onset
The severity
The time from administration of study drug(s) to start of the event
The duration and outcome of the event
Any possible etiology for the event
The final diagnosis or syndrome, if known

The Investigator may report serious adverse drug reactions (SADRs) using either:

An ADEERS form (Adverse Event Expedited Reporting System) available at
http://ctep.cancer.gov/reporting/adeers.html

OR

A MedWatch form available at http://www.fda.gov/medwatch/

All reports shall be sent electronically to:

Electronic Mailbox: DrugSafety.GPV.US@bayer.com

Facsimile: (973) 709-2185

Address: Global Pharmacovigilance - USA
Mail only: Bayer HealthCare
P.O. Box 1000

"The values in this protocol have been revised as per the United States (US) National Institute of Standards and Technology (NIST)standardization update (agreed on 17 MAR 2015)"

Montville, NJ 07045-1000

Address: 340 Changebridge Road
FDX or UPS only Pine Brook, NJ 07058

Reports for all Bayer products can also be phoned in via our Clinical Communications Dept:
Phone: 1-888-842-2937

7.15.1.5 Expected adverse events

For this study, the applicable reference document is the most current version of the investigator's brochure (IB) / summary of product characteristics.

The expectedness of AEs will be determined by Bayer according to the applicable reference document and according to all local regulations.

7.15.2 Pregnancies

The investigator must report to Bayer any pregnancy occurring in a study subject's partner, during the subject's participation in this study. The report should be submitted within the same timelines as an SAE, although a pregnancy per se is not considered an SAE. For the pregnancy of a study subject's partner, all efforts should be made to obtain information on course and outcome, subject to the partner's consent. For all reports, the forms provided are to be used.

7.15.3 Further safety

Progressive disease

If progressive disease leads to signs and symptoms that meet the criteria for an SAE (i.e., hospitalization, disability, death, or important medical event), the signs and symptoms should be reported as an SAE and not the underlying progressive disease.

Death

If any subject dies during the trial or within 30 days of the end-of-treatment visit, the investigator will inform Bayer and record the cause of death in detail (using the SAE Form) within 24 hours.

7.16 Other procedures and variables

7.17 Appropriateness of procedures / measurements

The assessments described in the previous sections are widely used and generally recognized as reliable, accurate, and relevant for determining the safety and efficacy of therapies in this disease.

8. Statistical methods and determination of sample size

Primary Aim

Bone scan response is defined as a ≥ 30% decrease in quantitative bone scan lesion area (BSA) from baseline to the end of month 2 of Ra-223 treatment (i.e. after 2 of 6 possible Ra-223 doses). In a one-stage design, 22 subjects will be enrolled to evaluate the primary endpoint of bone scan response. Assuming 90% of subjects with adequate data, the evaluable sample size is 20 subjects. With 20 subjects, given target power of 90% and 1-sided 5% alpha, a null hypothesis of ≤ 10% versus an alternative hypothesis of ≥ 35% in the proportion of patients achieving response can be evaluated. If 5 or more responses are observed in 20 subjects, this method of response assessment will be considered promising.

Operating characteristics

	True but unknown rate of Bone Scan Response Rate					
	10%	15%	20%	25%	30%	35%
Probability of ≥ 5 responses in 20 pts	0.04	0.17	0.37	0.59	0.76	0.88

Secondary Aims

A key secondary objective is to evaluate whether quantitative analysis of ^{99m}Tc-MDP bone scan after 2 months of therapy can identify patients who will achieve long term clinical benefit. Towards this end, all subjects will be treated and followed for at least 18 months. Survival status at 18 months will be ascertained (i.e. dichotomized as survivor or non-survivor at 18 months). A two-sample t-test will be used to determine the difference in mean percent change in BSA between survivor and non-survivor groups. Reported median survival for the study cohort of the ALSYMPCA trial was 14.9 months. Assuming events follow an exponential distribution this parallels an 18-month survival rate of 43%. The detectable difference will depend on the variability in change in BSA as measured by standard deviation (SD). There is 80% power to detect an effect of 1.332 SD given a 2-sided 0.05 alpha and 20 evaluable patients divided as 9 survivors and 11 non-survivors.

Bone scans are scheduled for the beginning of month 3/end of month 2 (T3), and one month post month 6 (FU1M). Descriptive statistics on absolute and percent change in BSA from baseline to these study timepoints will be provided for the overall study cohort and within survivor groups. By the end of month 2, bone scan response is expected in 35% of subjects. Patients will be cross-classified by bone scan response status at 2 months and survival status at 18 months. Agreement will be quantified using Cohen's Kappa; however, the sample size is too small to produce a realistic confidence interval. Kappa is assessed as follows: <0.00, poor; 0.00-0.20, slight; 0.21-0.40, fair; 0.41-0.60, moderate; 0.61-0.80, substantial; 0.81-1.00, almost perfect; and 1.00, perfect. Survival time defined as the time from registration to death, or if censored to date last known alive will be calculated. Correlation between change in BSA after 2 months and survival time will be assessed graphically with scatter plots and using Pearson correlation coefficient. Survival distributions will also be estimated using the Kaplan-Meier method in a landmark analysis at 2 months. Survival time is defined as the time from response classification (at 2 months) to death, or if censored to date last known alive. Cox proportional hazards (PH) regression will be used to estimate the bone scan response hazard ratio along with 95% confidence limits.

This study also affords insights into several other potential biomarkers including measures related to circulating tumor cells (number, proliferative index, AR signaling, markers of DNA damage/repair), bone turnover (BAP, NTx), inflammation and angiogenesis. Fluciclovine PET imaging will occur in 10 subjects at baseline and the end of month 2 of treatment. All other measures will occur at baseline, during treatment, and post treatment as detailed in the required data table. Descriptive statistics will be provided for each measure and timepoint along with absolute and percentage change from baseline. Association with overall survival and response will be analyzed as exploratory similar to the methods above for bone scan.

Accrual Targets

Accrual Targets								
Ethnic Category	**Sex/Gender**							
	Females			**Males**			**Total**	
Hispanic or Latino	0		+	1		=	1	
Not Hispanic or Latino	0		+	21		=	21	
Ethnic Category: Total of all subjects	0	(A1)	+	22	(B1)	=	22	(C1)
Racial Category								
American Indian or Alaskan Native	0		+	0		=	0	
Asian	0		+	1		=	1	
Black or African American	0		+	1		=	1	
Native Hawaiian or other Pacific Islander	0		+	0		=	0	
White	0		+	20		=	20	
Racial Category: Total of all subjects	0	(A2)	+	22	(B2)	=	22	(C2)
	(A1 = A2)			(B1 = B2)			(C1 = C2)	

Timeline

We anticipate an average of 2 subjects to be accrued monthly (approximate accrual time: 12 months). The primary endpoint (assessment of change in total area of bone metastases by ^{99m}Tc MDP bone scan) is assessed 2 months after the start of treatment; the primary analysis will take place 14 months after study activation. With an anticipated 12 month enrollment period and per-subject 18 months of study-directed treatment and follow-up, the last enrolled patient will conclude follow-up 30 months after activation.

Notable anticipated dates and analyses are as follows:

T: 0m	Study opens for accrual at MGH
T: 12m	Study accrual completed
T: 14m	Primary analysis
T: 30m	Completion of all study follow-up

9. Data handling and quality assurance

9.1 Data recording

It is the expectation that all data has source documentation available at the site. The site must implement processes to ensure this happens.

The QACT will collect, manage, and perform quality checks on the data for this study.

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the QACT according to the schedule set by the QACT.

9.2 Monitoring

The DF/HCC Data and Safety Monitoring Board (DSMB) will review and monitor study progress, toxicity, safety and other data from this study. The board is chaired by a medical oncologist from outside of DF/HCC and has external and internal representation. Information that raises any questions about participant safety or protocol performance will be addressed by the Overall PI, statistician and study team. Should any major concerns arise, the DSMB will offer recommendations regarding whether or not to suspend the study.

The DSMB will meet twice a year to review accrual, toxicity, response and reporting information. Information to be provided to the DSMB may include: participant accrual; treatment regimen information; adverse events and serious adverse events reported by category; summary of any deaths on study; audit results; and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

9.3 Audit and inspection

Inspections by regulatory health authority representatives i.e. FDA and IEC(s)/IRB(s) are possible. The investigator should notify Bayer immediately of any such inspection.

9.4 Archiving

Essential documents shall be archived safely and securely in such a way that ensures that they are readily available upon authorities' request.

Patient (hospital) files will be archived according to local regulations and in accordance with the maximum period of time permitted by the hospital, institution or private practice.

10. Premature termination of the study

- If risk-benefit ratio becomes unacceptable owing to, for example,
  - Safety findings from this study (e.g. SAEs)
  - Results of any interim analysis
  - Results of parallel clinical studies
  - Results of parallel animal studies
    (on e.g. toxicity, teratogenicity, carcinogenicity or reproduction toxicity).
- If the study conduct (e.g. recruitment rate; drop-out rate; data quality; protocol compliance) does not suggest a proper completion of the trial within a reasonable time frame.

The investigator has the right to close his/her center at any time.

For any of the above closures, the following applies:

- Closures should occur only after consultation between involved parties.
- All affected institutions (e.g. IEC(s)/IRB(s); competent authority(ies); study center; head of study center) must be informed as applicable according to local law.
- In case of a partial study closure, ongoing subjects, including those in post study follow-up, must be taken care of in an ethical manner.

Details for individual subject's withdrawal can be found in Section 5.2.1.

11. Ethical and legal aspects

11.1 Ethical and legal conduct of the study

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the investigator abide by Good Clinical Practice (GCP) guidelines and under the guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in keeping with applicable local law(s) and regulation(s).

Documented approval from appropriate IEC(s)/IRBs will be obtained for all participating centers before start of the study, according to GCP, local laws, regulations and organizations. When necessary, an extension, amendment or renewal of the EC/IRB approval must be obtained and also forwarded to Bayer.

Strict adherence to all specifications laid down in this protocol is required for all aspects of study conduct; the investigator may not modify or alter the procedures described in this protocol.

Modifications to the study protocol will not be implemented by the investigator without discussion and agreement by Bayer. However, the investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to the trial subjects without prior IEC/IRB/Bayer approval/favorable opinion. As soon as possible, the

implemented deviation or change, the reasons for it and if appropriate the proposed protocol amendment should be submitted to the IEC/IRB/head of medical institution. Any deviations from the protocol must be explained and documented by the investigator.

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and properly documented.

11.2 Subject information and consent

Each subject / legal representative or proxy consenter will have ample time and opportunity to ask questions and will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision.

Only if the subject / legal representative or proxy consenter voluntarily agrees to sign the informed consent form and has done so, may he/she enter the study. Additionally, the investigator and other information provider (if any) will personally sign and date the form. The subject / legal representative or proxy consenter will receive a copy of the signed and dated form.

The signed informed consent statement is to remain in the investigator site file or, if locally required, in the patient's note/file of the medical institution.

In the event that informed consent is obtained on the date that baseline study procedures are performed, the study record or subject's clinical record must clearly show that informed consent was obtained prior to these procedures.

1. If the patient is not capable of providing a signature, a verbal statement of consent can also be given in the presence of an impartial witness (independent of Bayer and the investigator). This is to be documented by a signature from the informing physician as well as by a signature from the witness.

The informed consent form and any other written information provided to subjects / legal representatives or proxy consenters will be revised whenever important new information becomes available that may be relevant to the subject's consent, or there is an amendment to the protocol that necessitates a change to the content of the subject information and / or the written informed consent form. The investigator will inform the subject / legal representative or proxy consenter of changes in a timely manner and will ask the subject to confirm his/her participation in the study by signing the revised informed consent form. Any revised written informed consent form and written information must receive the IEC/IRB`s approval / favorable opinion in advance of use.

11.3 Publication policy

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

Bayer recognizes the right of the investigator to publish results upon completion of the study. However, the investigator must send a draft manuscript of the publication or abstract to Bayer at least thirty days in advance of submission in order to obtain approval prior to submission of the final version for publication or congress presentation. This will be reviewed promptly and approval will not be withheld unreasonably. In case of a difference of opinion between Bayer and the investigator(s), the contents of the publication will be discussed in order to find a solution which satisfies both parties. All relevant aspects regarding data reporting and publication will be part of the contract between Bayer and the investigator/institution

The Principal Investigator will ensure that the information regarding the study be publicly available on the internet at www.clinicaltrials.gov.

11.3.1 Publications and NIST

Bayer recommends inclusion of the new NIST standard in abstracts/ publications submitted OR pending publication January 2016 onwards from IIRs and other non-Bayer supported abstracts/publications for consistency. Investigators may choose to add a footnote to the publication or within the body of the publication include the new NIST standard.

11.4 Confidentiality

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

Should direct access to medical records require a waiver or authorization separate from the subject's statement of informed consent, it is the responsibility of the Investigator to obtain such permission in writing from the appropriate individual.

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Appendix A: 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 B: Standard Operating Procedure for CTC Capture

Materials: Three (3) EpCAM functionalized CTC capture devices; 20 mL of patient's blood; 20 mL of PBS, 20 mL of buffer (PBS with 1% bovine serum albumin and 0.01% sodium azide), anti-cytokeratin PE (CAM 5.2, BD Biosciences); CD45 FITC (BD Pharmingen); 4',6-diamidino-2-phenylindole (DAPI, BD Biosciences); anti-PSA antibody; anti-PSMA antibody; Ki67 antibody; PicoPure RNA isolation kit (Arcturus, Sunnyvale, CA); TransPlex Whole Transcriptome Amplification Kit (Rubicon Genomics, Ann Arbor, MI), Fluidigm Biomark system kit.

Transport of samples: Samples for CTC analyses will be transported from the MGH Cancer Center in Boston to the MGH Charlestown Navy Yard laboratories of Drs. Daniel Haber and Shyamala Maheswaran. After collection of blood samples, the two vacutainer tubes will be de-identified and labels with the subject's study identification number will be affixed. Transport will be performed by U.S. Ground Services, or alternatively, by personal transport via a member of the Haber/Maheswaran laboratories. It is expected that the elapsed time between blood draw and assay will be under six hours.

Protocol: Protocol specifics for the samples are subject to ongoing modifications. CTC capture, enumeration, and other analysis will be carried out per current Haber/Maheswaran lab protocol and will be described in detail in concert with all data reporting.

Appendix C: Standard Operating Procedure for Blood Biomarkers at the CLIA certified Clinical Correlative Studies Core within the Steele Laboratory

Blood will be collected and processed at study specific timepoints as described above.
Dr. Dan G. Duda, D.M.D., Ph.D. (Associate Professor at MGH/HMS), who has over 13 years of experience in clinical correlative studies, supervises the Steele Lab's Core operation.

1. Collect at least 10 mL of blood in a purple top (EDTA plasma) tube.
2. Maintain sample on wet ice and transport within 1-2 hours of collection to the Steele Laboratory facility at MGH main campus (Cox 7).
3. Separate plasma by centrifugation, then aliquot and store at –80ºC until later batched analysis.
4. Separated peripheral blood cells (remaining from step <3> above) will be immediately stained using fluorescently labeled antibodies and analyzed using an LSR-II flow cytometer within the Steele Lab's facility at MGH main campus as previously described.[55]
5. Plasma analysis (samples stored in step <3> above) will later be carried out for a panel of circulating angiogenic and inflammatory molecules when all samples have been collected at the Steele Lab facility in Charlestown Navy Yard. They include protein array kits from Meso-Scale Discovery (Gaithersburg, MD): Human ProInflammatory Panel 1 V-PLEX™ Plus (#K15049G-2) – IFN-γ, IL-1β, IL-10, IL-12 p70, IL-13, IL-2, IL-4, IL-6, IL-8, TNF-α; and Human Angiogenesis Panel 1 V-PLEX Kit (#K15190D-2) – bFGF, Flt-1, PlGF, Tie-2, VEGF, VEGF-C, VEGF-D, and single-analyte ELISA kits from R&D Systems (Minneapolis, MN): for stromal cell-derived factor 1a (SDF1α), hepatocyte growth factor (HGF), soluble (s)c-MET, and s-c-KIT. Finally, we will evaluate biomarkers of tumor hypoxia, by measuring plasma carbonic anhydrase IX (CAIX) levels as well as biomarkers of osteoclast and osteoblast activity (plasma C-telopeptide and total alkaline phosphatase) using single-analyte ELISA kits from R&D Systems. Samples will be run in duplicate.

Appendix D: Opioid/Narcotic Pain Medication Diary

Participant name: _____

Visit date: _____

Subject ID: _____

Visit identifier: _____

Instructions to study participant: Please complete this diary on the date indicated above as "Visit date." Please enter the number of units you have taken of each pain mediation over the prior 24 hour period. If you did not take any listed pain medication, record "NONE" in the "# Units Taken" box below.

Medication Name (write in name)	**Route of Administration** (examples: oral, patch, other)	**Strength per Unit** (example: 10 mg tablets)	**# Units Taken**

Appendix E: EuroQol (EQ)-5D-5L

Under each heading, please check the ONE box that best describes your health TODAY

MOBILITY

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

SELF-CARE

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

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

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

PAIN / DISCOMFORT

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

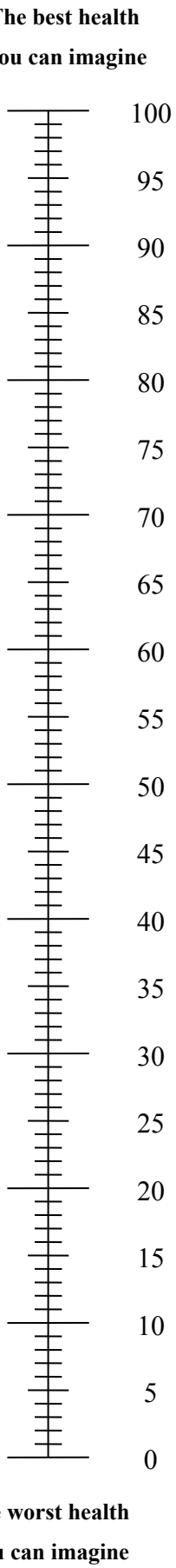
ANXIETY / DEPRESSION

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

"The values in this protocol have been revised as per the United States (US) National Institute of Standards and Technology (NIST)standardization update (agreed on 17 MAR 2015)"

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



Appendix F: Brief Pain Inventory

Participant name: _____

Visit date: _____

Subject ID: _____

Visit identifier: _____

Instructions to study participant: Please complete this diary on the date indicated above as "Visit date."

A. 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

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

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

C. Please circle the one number that describes how, during the past 24 hours, pain has interfered with your general activity.

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

D. Please circle the one number that describes how, during the past 24 hours, pain has interfered with your sleep.

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

Appendix G: Sample Processing for Veridex CellSearch CTC Analyses

Specimen Collection, Handling, Storage:

- Venipuncture or venous port collection only.
- Blood samples must be obtained in CellSave Preservative Tubes. Proprietary preservative stabilizes CTCs for up to 96 hours at room temperature.
- If multiple tube types are to be drawn, draw the CellSave Tubes last.
- Draw 1 CellSave tube of blood (approximately 7.5 mL).

Supplies: CellSave Preservative Tubes

Procedure:

1	Fill tube completely – until blood flow stops.
2	Immediately invert gently 8 times.
3	Check for clots.
4	Label tubes with two patient identifiers.
4	Send as soon as possible to Brigham and Women's Hospital, Cytology Division, Circulating Tumor Cell Lab, Medical Research Building room 315.

Procedure notes:

- Invert the tube immediately after drawing to avoid clotting. Clotted samples cannot be submitted to laboratory.
- Do not rock, vortex or shake samples.
- Store samples at room temperature. Do not refrigerate.

Reference: Veridex, LLC, CellSearch System Training Guide. Page 1-6, section 1-4.

Appendix H: Sample Processing for Bone Turnover Markers

Collection of Specimens: Blood draws will take place at three timepoints (baseline, 3-months, and 6-months). Each blood draw will be for 10 mL.

Handling of Specimens: Each sample will be processed for storage as serum as follows:

1. Label as many 1 mL cryovials as necessary for the serum collected (label to include study number, participant identifier, collection time & date, study timepoint, and "serum."

2. Allow red top tube to clot for 30 minutes at room temperature.

3. Spin in a standard clinical centrifuge at ~2500 RPM for 10 minutes at 4 degrees C (or at room temperature if done within 2 hours of blood draw).

4. Aliquot 0.5 mL serum into as many cryovials as are necessary for the sample collected (as labeled under item (1)).

5. Place cryovials into biohazard bag and immediately freeze at -70 to -90 degrees C.
Storage of Specimens: Cryovials will be frozen and stored at -70 to -90 degrees C.