Clinical Study Protocol

A phase II clinical trial of radium-223 activity in patients with metastatic castration-resistant prostate cancer (mCRPC) with asymptomaticprogression while on abiraterone acetate or enzalutamide besides AR-V7 mutational status

Protocol number: MedOPP098

Study drug(s):	Xofigo®
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SPONSOR'S SIGNATURE PAGE

Study title:A phase II clinical trial of radium-223 activity in patients with metastatic castration-resistant prostate cancer (mCRPC) with asymptomatic progression while on abiraterone acetate or enzalutamide besides AR-V7 mutational status.

Study code: MedOPP098

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Sponsor	Signature	Signature date (DD-Mmm-YYYY)
Scientific Global Coordinator	Signature	Signature date (DD-Mmm-YYYY)

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Detailed in *Appendix 4.*

Declaration of Investigators

Protocol Title: A phase II clinical trial of radium-223 activity in patients with metastatic castration-resistant prostate cancer (mCRPC) with asymptomaticprogression while on abiraterone acetate or enzalutamide besides AR-V7 mutational status.

Release date: 4, 30th January 2020
Protocol number: MedOPP098
I have received, reviewed and understood the following:

a) Protocol: **A phase II clinical trial of radium-223 activity in patients with metastatic castration-resistant prostate cancer (mCRPC) with asymptomatic progression while on abiraterone acetate or enzalutamide besides AR-V7 mutational status**

b) Summary of Product Characteristics containing clinical and non-clinical data on investigational product that are relevant to the study of the product on human subjects.

I have been adequately informed as to the development to date of the investigational product. I will acknowledge receipt of the updated Summary of Product Characteristics. I have read this study protocol and agree that it contains all the information required to conduct the study. I agree to conduct this study as set out in this protocol.

I understand that any change made by the investigator(s) not previously agreed to by the Sponsor may constitute a protocol non-compliance, including all of the ancillary studies or procedures performed on study patients (other than those procedures needed to ensure the patients' well-being). I am aware that I can only deviate from or apply changes to the protocol without prior approval or the favorable opinion of the EC and/or before Sponsor approval to avoid immediate risk to the trial patients. Should this occur, I agree to inform the Sponsor as to the deviation or changes in writing and their reasons, as soon as possible.

The study will be conducted in accordance with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki and its amendments, the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines (ICH E6 GCP) and applicable laws and regulations.

I agree to receiving, as described in this protocol and in the ICH E6 guidelines forGCP, written informed consent from the patient or their legally authorized representative or the witnessed verbal informed consent from all patients who have been asked to participate in this study, before performing any study procedure.

I will ensure that the study drug(s) provided by the Sponsor will only be used as described in this protocol.

I am aware of the requirements for the correct reporting of serious adverse events, and I commit to document and to report such events as required by the Sponsor and in accordance with Health Authority Regulatory requirements.

I agree to provide the Sponsor or Sponsor's representative, upon request, with evidence of current laboratory accreditation, the name and address of the laboratory, and a list of normal values and ranges.

I agree to the use of study results for national and international registration, publication, and information for medical and pharmaceutical professionals.

I agree to keep all source documents and case report forms as specified in the relevant sections of this protocol.

I agree to submit all the forms required by the health authoritiesand my updated curriculum vitae and those of the sub-investigators and all of the members of my team (if applicable) before the start of this study, which may be submitted, in turn, to the Health Authorities.

I am aware that the Sponsor or their representative may perform audits and that the Health Authorities may carry out inspections as part of the conduct of this study. I will permit monitoring, audit and inspection and provide direct access to source data, documents and reports for these purposes.

In light of the foregoing, I hereby consent to the inclusion by the Sponsor of my contact details and professional profile in their electronic database to be used for internal purposes and for submission to Health Authorities worldwide.

Name: _____

Signature: _____ Date: _____

Protocol synopsis

Investigational drug:	Xofigo®
Protocol number:	MedOPP098
Eudract Number:	2016-001888-36
Protocol Title:	A phase II clinical trial of radium-223 activity in patients with metastatic castration-resistant prostate cancer (mCRPC) with asymptomatic progression while on abiraterone acetate or enzalutamide besides androgen receptor (AR)-V7 mutational status.
Study disease:	Patients with mCRPC with asymptomatic progression while on abiraterone acetate or enzalutamide.The progression on enzalutamide or abiraterone acetate will be defined as patients who confirm radiographic progression and/or prostate-specific antigen (PSA) progression with rapid PSA doubling time (less than three months).
Patients:	Patients ≥ 18 years of age diagnosed with mCRPC with bone metastases and asymptomaticprogression after at least 24 weeks of treatment with abiraterone acetate or enzalutamide.
Number of patients:	52 patients
Screening criteria:	**Inclusion criteria** • Subject is an adult ≥ 18 years at the time of informed consent and has signed informed consent before any trial related activities and according to local guidelines. • Subject has histologically confirmed adenocarcinoma of the prostate without neuroendocrine differentiation or small cell features. • Subject has bone metastases due to the prostate cancer and absence of visceral metastases. • Subject has a serum testosterone of ≤ 1.7 nmol/L (or ≤ 50 ng/dL) at screening. • Subject must have received a minimum of 24 weeks of treatment with abiraterone acetate or enzalutamide within its approved label indication and has discontinued use at least four weeks prior to start of study drug at day 1. • Prior use of docetaxel is allowed in castration-naïve patients (maximum of six cycles). • Subject receives and will continue to receive ongoing androgen deprivation with luteinizing releasing hormone (LHRH) analogue therapy throughout the course of the study or has had a bilateral orchiectomy. • Subject is asymptomaticfrom prostate cancer, defined as patients with the score on brief pain inventory (short form) (BPI-SF) Question #3 must be zero or one and no use of opiate analgesics for prostate cancer-related pain currently or anytime within two weeks prior to screening.

- Subject has an Eastern Cooperative Oncology Group (ECOG) performance status of 0–1 at screening.
- Subject receiving bisphosphonate or other approved bone-targeting therapy must have been on stable doses for at least four weeks prior to start of study drug at day 1.
- Subject has a life expectancy of more than or equal to 12 months.
- Subject agrees not to participate in another interventional study while on study drug.
- Subject and his female partner who is of childbearing potential must use two acceptable methods of birth control (one of which must include a condom as a barrier method of contraception) starting at screening and continuing throughout the study period and for six months after final study drug administration.

Exclusion criteria

Any patient meeting **ANY** of the following criteria will be excluded from the study:

- Subject has received any anti-neoplastic therapy (including ketokonazol and chemotherapy) following abiraterone acetate or enzalutamide discontinuation and prior to start of study drug at day 1.
- Subject has known or suspected brain metastases or active leptomeningeal disease.
- Subject has concurrent disease or any clinically significant abnormality following the investigator's review of the physical examination and safety laboratory tests at screening, which in the judgment of the investigator would interfere with the subject's participation in this study or evaluation of study results.
- Subject has a history of another invasive cancer within three years prior to screening, with the exceptions of non-melanoma skin cancers or a non-infiltrating muscle bladder cancer that have a remote probability of recurrence in the opinion of the investigator in consultation with the medical scientist.
- Subject had major surgery within one month prior to screening.
- Subject has received investigational therapy within 28 days or 5 half lives, whichever is longer, prior to start of study drug at day 1.
- Subject has absolute neutrophil count < 1,500/µL, platelet count < 100,000/µL, and hemoglobin < 6.25 mmol/L (or < 10 g/dL) at screening (Note: Subjects must not have received any growth factors or blood transfusions within seven days of the hematologic laboratory values obtained at screening).
- Subject has total bilirubin > 1.5 times the upper limit of normal (ULN) at screening, except for subjects with documented Gilbert's syndrome.
- Subject has creatinine > 2.5 mg/dL at screening.
- Subject has albumin ≤ 30 g/L (or ≤ 3.0 g/dL) at screening.

Study objectives	**Primary objective**: To assess the efficacy of radium-223 in asymptomaticpatients with mCRPC who have progressed while on abiraterone acetate or enzalutamide treatment. **Secondary objectives:** • Safety profile. • To determine the association between AR-V7 status (positive vs. negative) and progression-free survival (PFS). • To establish the relationship between circulating tumor cells (CTCs) numberwith radium-223 efficacy.
Type of study:	Multicenter, single-arm, open-label, non-controlled phase IIa clinical trial.
Study Treatment:	Once the informed consent has been signed and the study screening criteria have been confirmed, the patient will be treated with radium-223 at a dose of 55 kBq (after 2015 NIST implementation) per kilogram body weight, given at four-week intervals for sixintravenous injections.Adjustment of dose level is not permitted. Patients will receive study treatment according to the protocol and will be prematurely discontinued in the following situations: disease progression, occurrence of unacceptable side effects, death, or withdrawal of consent, whichever occurs first. Patients who complete the study treatment during its active treatment phase will enter a follow-up period, with radiological tumor assessment every three months (±7 working days) until disease progression and safety evaluation to follow-up of symptomatic skeletal related events until reach 2 years from the last dose of study treatment. If patient discontinued treatment for any reason other than progression, tumor reevaluation will be included in the assessment. After disease progression, a safety follow-up visit will be performed within three following months (including survival status and post-study anticancer therapy evaluation) and 2 years after last dose of study drug. Safety evaluation after progression will include follow-up of symptomatic skeletal related events and overall survival.

Safety and efficacy assessments	**Efficacy assessments**: Assessments (CT scan and bone scan) will be performed from the time of enrollment (baseline assessments) and every 12 weeks (± 7 working days) from the time of the first administered dose. Radiographic disease progression will be assessed using The Prostate Cancer Clinical Trials Working Group (PCWG2) modified criteria for bone disease and the Response Evaluation Criteria in Solid Tumors (RECIST criteria) version 1.1 for soft-tissue disease. Radiographic disease progression in bone will require persistence of new lesions on a confirmatory bone scan performed at least six weeks later. It is highly recommended to perform additional CT scans and bone scans if clinical progression disease is suspected. During the active phase of the study treatment, each assessment will be performed in accordance with the schedule (Appendix 1: Schedule of study assessments and procedures), regardless of any delay in administration, to prevent bias in the efficacy assessment. If any of the required assessments are not performed, the disease status at that point in time will not be known. **Safety assessments:** The occurrence and maximum grade of side effects observed throughout the study will be listed and tabulated according to type and dose level. Any adverse events (AEs) that the investigator reports as unrelated to the drug will also be reported. In this study, side effects will be assessed according to the US National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. **Other assessments:** To perform the molecular analyses described in this protocol (Appendix 3: Instructions for blood sample collection. Detection of CTCs), patients will be asked to give their consent to the collection of the following biological material: - Blood samples: These will be collected at the start of the study, at the end of treatmentand when documenting disease progression for the purpose of CTC.
Endpoints	**Primary endpoint:** The primary endpoint of this study is to assess the efficacy of radium-223 in terms of radiological rPFS. **Secondary endpoints:** **Safety** AEs will be evaluated using the NCI-CTCAE version 4.0. Grade 3 or 4 AEs and serious adverse events (SAEs) will be assessed to determine the safety and tolerability of the various combinations of drugs.

	Efficacy • Radiographic progression-free survival (rPFS) depending on AR-V7 status. • Overall survival (OS). • Time to first symptomatic skeletal event (SSE). • Time to PSA progresionaccording to the ALSYMPCA study criteria. • Determination percentage of PSA progresion. • Alkaline phosphatase level response (AF), normalization of alkaline phosphatase level, according to the ALSYMPCA study criteria. **Molecular aspects** • Assessment of AR-V7 mutation evolution during the study treatment. • Determination changes in CTCs number during the study treatment.
Study calendar	Start Recruitment (FPI): Nov-2016 End of Recruitment (LPI): Oct-2018 Follow-up period for efficacy: Nov-2019 Follow-up extension for safety: Apr-2021 Final efficacy report: Sept-2020 Final safety report: Aug-2021

List of abbreviations

Abbreviation	Definition
AE	Adverse Event
AESI	Adverse Event of Special Interest
AF	Alkaline phosphatase
ALT	Alanine transaminase
AR	Androgen Receptor
AR-FL	Full-Length-Androgen Receptor
AR-V7	Androgen Receptor Splice Variant-7
AST	Aspartate transaminase
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
CK	Cytokeratin
CRF	Case Report Form
CRO	Clinical Research Organization
CRPC	Castration-Resistant Prostate Cancer
CT	Computerized Axial Tomography
CTC	Circulating Tumor Cell
CTCAE	Common Terminology Criteria for Adverse Events
DK	DecayCorrection Factor
DNA	Deoxyribonucleic Acid
EC	Ethics Committee
ECOG	Eastern Cooperative Oncology Group
EMA	European Medicine Agency
EoS	End of Study
ESA	Erythropoietin Stimulating Agents
FDA	US Food and Drug Administration
FL	Full-Length
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transferase
GMP	Good Manufacturing Practice
HD	High Definition
HR	Hazard Ratio
HRQOL	Health-Related Quality Of Life
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
IMP	Investigational Medicine Product
IV	Intravenous
LHRH	Luteinizing Releasing Hormone
LPLV	LastPatient Last Visit
mCRPC	Metastatic Castration-Resistant Prostate Cancer
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic Resonance Imaging
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
Non-IMP	Non-Investigational Medicinal Product
OR	Odds Ratio

Abbreviation	Definition
OS	Overall Survival
PCWG	Prostate Cancer Clinical Trials Working Group
PFS	Progression-Free Survival
PSA	Prostate-Specific Antigen
RECIST	Response Evaluation Criteria In Solid Tumors
RNA	Ribonucleic Acid
RR	Relative Risk
RT-PCR	Reverse-Transcription Polymerase-Chain-Reaction
SAE	Serious Adverse Event
SmPC	Summary of Product Characteristics
SOC	System Organ Class
SRE	Skeletal-Related Event
TTP	Time to Progression
ULN	Upper Limit of Normal
UPN	Unique Patient Number
WBC	White Blood Cell

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1. STUDY BACKGROUND AND RATIONALE

1.1. *State-of-the-art treatment of metastatic castration-resistant prostate cancer (mCRPC)*

Prostate canceristhe most frequent urogenital malignancy, the most common solid neoplasm, and the second most common cause of cancer death in men in Europe [1]. In 2015, an estimated 222,800 new cases of prostate cancer will be diagnosed in the United States, and around 27,000 patients died from prostate cancer .

When there is a suspicion that prostatecancer is present based on the digital rectal exam, prostate-specific antigen (PSA) level, and radiological findings, a prostate biopsy must be performed cover with transrectal ultrasound guidance, and a minimum of eight cores must be obtained. The pathology report should include a description of the cells, the cancer grading (Gleason score), and the final pathologist's diagnosis.

Clinically localised prostate cancer should be categorised as low-, intermediate-, or high-risk using a classification that incorporatesGleason score, PSA level, and clinical stage. There is no consensus regarding optimum management of localised disease. Options include watchful waiting, active surveillance, open, laparoscopic or robotic-assisted radical prostatectomy, external beam radiotherapy, and brachytherapy[2].

After radical local therapy, about 30% of patients will develop advanced disease. Androgen-suppressing strategies have become the mainstay treatment of advanced prostate cancer. Androgen suppression using bilateral orchiectomy or an luteinizing releasing hormone(LHRH) agonist/antagonist should be first-line treatment. Furthermore, complete or maximal androgen blockade with the addition of an antiandrogen has been associated with a small five-year survival advantage, of less than 5% [3]. However, more recent results from the CHAARTED study showed an increase in overall survival (OS) in patients with hormone-sensitive prostate cancer treated with continuous androgen deprivation therapy in association with six cycles of docetaxel-based chemotherapy compared with androgen deprivation therapy alone (median, 57.6 vs. 44 months; hazard ratio (HR), 0.61; 95% confidence interval [CI], 0.47 to 0.80; P<0.001)[4]This difference was even higher among patients with high-volume disease(median, 49.2 vs. 39.2 months; HR, 0.60; 95% CI, 0.45 to 0.81; P<0.001). These results contrast with those of the GETUG-AFU 15 trial, performed in a similar population, in which no OS differences were found [5].

Castration-resistant prostate cancer (CRPC) is defined as cancer progression in the setting of “castrate levels” of serum androgens (generally ≤50 ng/dL). However, a number of patients with castrate-resistant disease will continue to respond to secondary hormonal manipulations such as other antiandrogens, estrogen agonists, corticosteroids, and steroidogenesis inhibitors.

Enzalutamide (MDV3100) attacks multiple nodes in the androgen receptor (AR) signaling pathway. It is a competitive inhibitor of AR ligand binding, with greater affinity for the AR and superior potency than other antiandrogens. Additionally, enzalutamide prevents nuclear translocation of the AR, and induces a conformational change that inhibits the receptor-complex

binding to androgen-response elements of deoxyribonucleic acid (DNA), thereby preventing activation of target genes necessary for prostate cancer growth.

Enzalutamide was initially approved for the treatment of patients with mCRPC who have previously received docetaxel. The approval was based on the results of the AFFIRM study, a phase III randomized placebo-controlled multicenter trial that enrolled 1,199 patients with mCRPC who had received prior docetaxel[6]. Patients were randomly assigned, in a 2:1 ratio, to receive enzalutamide 160 mg orally once daily (N = 800) or placebo (N = 399). The primary efficacyendpoint was OS. At the pre-specified interim analysis after 520 events, a statistically significant improvement in OS was observed in enzalutamide-treated patients compared with patients in the placebo group (median, 18.4 vs. 13.6 months; HR, 0.63; 95% CI, 0.53 to 0.75; P<0.0001).Enzalutamide is generally very well tolerated. There were very few toxicities that were more common in the enzalutamide arm, and these included fatigue, diarrhea, musculoskeletal pain, headache, hypertension, and hot flashes.

Subsequently, enzalutamide was also approved for the treatment of patients with mCRPC who are asymptomatic or mildly symptomatic after failure of androgen deprivation therapy in whom chemotherapy is not yet clinically indicated. This indication was based on the results of the PREVAIL study, a phase III randomized placebo-controlled multicenter trial that enrolled 1,717 patients with mCRPC who had not received prior docetaxel[7]. Patients were randomly assigned, in a 1:1 ratio, to receive enzalutamide 160 mg orally once daily (N = 872) or placebo (N = 845). Coprimary endpoints were radiographic progression-free survival (PFS) and OS. At the planned interim analysis after 440 events, a statistically significant improvement in OS was observed in enzalutamide-treated patients compared with patients in the placebo group (median, 32.4 vs. 30.2 months; HR, 0.71; 95% CI, 0.60 to 0.84; P<0.001).

Abiraterone acetate, a prodrug of abiraterone, is a selective inhibitor of androgen biosynthesis that potently blocks cytochrome P450 c17 (CYP17), an essential enzyme in testosterone synthesis, thereby inhibiting androgen synthesis by the adrenal glands and testes and within the prostate tumor.

Like enzalutamide, abiraterone acetate was initially approved in combination with prednisone to treat patients with mCRPC who have received prior docetaxel. The approval was based on the results of the COU-AA-301 study, a phase III randomized placebo-controlled multicenter trial that enrolled 1,195 patients with mCRPC who had received prior docetaxel [8]. Patients were randomly assigned, in a 2:1 ratio, to receive abiraterone acetate 1000 mg orally once dailyplus prednisone 5 mg orally twice daily (N = 797) or placebo plus prednisone (N = 398). The primary efficacyendpoint was OS. At the pre-specified interim analysis after 552 events, a statistically significant improvement in OS was observed in abiraterone-treated patients compared with patients in the placebo group (median, 14.8 vs. 10.9 months; HR0.66; 95% CI, 0.55 to 0.78; P<0.001). Although the most common adverseeffect (AE)was fatigue,AEs associated with elevated mineralocorticoid levels due to CYP17 blockade (fluid retention and edema, hypokalemia, and hypertension), as well as cardiac disorders and liver-function test

abnormalities, were also more common in the abiraterone-treated patients (55% vs. 43%, P<0.001).

Subsequently, abiraterone acetate was also approved for the treatment patients with mCRPC who are asymptomatic or mildly symptomatic after failure of androgen deprivation therapy in whom chemotherapy is not yet clinically indicated. This indication was based on the results of the COU-AA-302 study, a phase III randomized placebo-controlled multicenter trial that enrolled 1,088 patients with mCRPC who had not received prior docetaxel[9]. Patients were randomly assigned, in a 1:1 ratio, to receive abiraterone acetate 1000 mg orally once dailyplus prednisone 5 mg orally twice daily (N = 546) or placebo plus prednisone (N = 542). Coprimary endpoints were radiographic PFSand OS. At the planned interim analysis after 333 events, a statistically significant improvement in OS was observed in abiraterone-treated patients compared with patients in the placebo group (median, not reached vs. 27.2 months; HR, 0.75; 95% CI, 0.61 to 0.93; P=0.01).

Therefore, several agents have become available for the treatment of patients with mCRPC. Front-line therapeutic options for mCRPC include docetaxel, sipuleucel-T[10], abiraterone acetate, enzalutamide, and radium-223. Post-docetaxel options include cabazitaxel[11], abiraterone acetate, enzalutamide, and radium-223. However, it is unclear what the optimal sequencing of these therapies should be in order to maximize clinical outcomes and survival. The results of a preliminary study have suggested that the sequence of abiraterone acetate followed by enzalutamide may produce clinically superior outcomes in patients with mCRPC compared with enzalutamide followed by abiraterone acetate[12]. To address this issue, a prospective phase II clinical trial is currently under way (NCT02125357). Investigators will randomize patients with mCRPC to receive either first-line abiraterone acetate or enzalutamide, and patients will subsequently cross over to the alternative agent at the time of PSA progression.

1.2. *The role of radium-223 in patients with mCRPC*

Radium-223 (molecular formula $^{223}RaCl_2$)is an alpha-emitting radioisotope that targets areas of bone with hight turnover asmetastasis and is excreted by the small intestine. When compared with beta-emitters, radium-223 delivers a high quantity of energy per track length with short tissue penetration.

After injection radium-223 is rapidly cleared from the blood and distributed to bone and intestine. Radium-223 has a physical half-life of 11.43 days and approximately 63% of the administered radioactivity is excreted from the body within seven days, primarily via the fecal route. Dosimetry data suggest that bone, bone marrow, and the intestinal wall have the highest absorbed radiation doses [13].

Thesafetyand tolerability of radium-223 was initially evaluated in a single injection dose ranging study in 25subjects diagnosed with prostate (N = 15) or breast cancer (N = 10)[14]. Five subjects were included at each of the doses: 46, 93, 163, 213, or 250 kBqper kilogram body weightand

followed for eight weeks. In this study, radium-223 presented a dose-proportional increase in exposure with mild and reversible myelosuppression. Preliminaryevidence of efficacywas observed with reduction in serum alkaline phosphatase (AF) levels and improved pain control across the dose levels.Radium-223 was subsequently evaluated in a double-blind, placebo-controlled, multicenter randomized phase II study.Patients diagnosed with mCRPCand bone pain were randomly assigned, in a 1:1 ratio, to receiveexternal beam radiotherapy and radium-223 50 kBq per kilogram body weight (N = 33), given at four-week intervals for four injections, or external beam radiotherapy and placebo (N = 31)[15].External beam radiation therapy was administered at the most painful site in each subject. The primary efficacyendpoints were the activity of radium-223 treatment with respect to the reduction in bone-specific AF levels and time to occurrence of studydefined skeletal events (SSEs). There were no differences in the time to first SSE (median, 14 vs. 11 weeks; 95% CI,9 to 30; P=0.257), although there was a statisticallysignificant reduction in the median of all serum markers forsubjects who received radium-223.

Radium-223 (Xofigo®) is indicated for the treatment of patients with mCRPC with symptomatic bone metastases and no known visceral metastatic disease.In the pivotalphase IIIclinical trial (the ALSYMPCA study), a total of 921 subjectswith mCRPC with bone metastases were randomly assigned, in a 2:1 ratio, to receive six injections of radium-223 (at a dose of 50 kBq per kilogram of body weight intravenously) or matching placebo; one injection was administered every four weeks[16]. Prior to randomization, 58% and 57% of patients had received docetaxel in the radium-223 and placebo arms, respectively. The median duration of treatment was 20 weeks (six cycles) for radium-223 and 18 weeks (five cycles) for placebo. Radium-223, as compared with placebo, significantly improved OS (median, 14.0 vs. 11.2 months; HR, 0.70; 95% CI, 0.55 to 0.88; P=0.002). The updated analysis involving 921 patients confirmed the radium-223 survival benefit (median, 14.9 vs. 11.3 months; HR, 0.70; 95% CI, 0.58 to 0.83; P<0.001). Assessments of all main secondary efficacy endpoints also showed a benefit of radium-233 as compared with placebo. The most common AEs (≥ 10%) in patients receiving radium-223 were nausea (2% grade 3-4), diarrhea (2% grade 3-4), vomiting (2% grade 3-4), and peripheral edema (2% grade 3-4). The most common hematologic laboratory abnormalities in radium-223-treated patients (≥ 10%) were anemia (6% grade 3-4), lymphocytopenia (20% grade 3-4), leukopenia (3% grade 3-4), thrombocytopenia (3% grade 3-4), and neutropenia (2% grade 3-4). Treatment discontinuations due to AEs occurred in 17% of patients who received radium-223 and 21% of patients who received placebo.

1.3. *AR mutations as a driver of therapeutic resistance in mCRPC*

The AR is a steroid receptor; a sub-family of receptors characterised as ligand dependent, sequence specific transcription factors, and like other nuclear receptors, the AR has a modular structure. The AR gene is situated at Xq11-12 and consists of 8 exons with exon 1 encoding the N-terminal domain and the entire 5' untranslated region, exons 2 and 3 encoding the DNA binding domain (DBD), and exons 4-8 encoding the "hinge" region and ligand binding domain (LBD).The

androgen induced transcriptional activation of AR is modulated by the interaction of AR with coregulators and by phosphorylation of AR and AR coregulators in response to growth factors[17].

AR and the modulators of AR activity remain important in prostate cancer. Approximately 80-90% of prostate cancers are dependent on androgen at initial diagnosis, and endocrine therapy of prostate cancer is directed toward the reduction of serum androgens and inhibition of AR. However, androgen ablation therapy ultimately fails, and prostate cancer progresses to a hormone refractory state.

AR mutations have been extensively reported in patients with prostate cancer. In early stages of prostate cancer, mutations of the AR are rare, but their frequency is significantly increased in advanced androgen-independent tumors, suggesting that AR mutations play a role in tumor progression[18].

Recently, it has been published the clinical relevance of AR variants in mCRPC through the prospective evaluation of the AR splice variant 7 messenger ribonucleic acid(RNA) (AR-V7) in circulating tumor cells (CTCs) from patients receiving enzalutamide and abiraterone acetate [19]]. A total of 31 enzalutamide-treated patients and 31 abiraterone-treated patients were included, of whom 39% and 19%, respectively, had detectable AR-V7 in CTCs. The overall proportion of patients who had a PSA response while receiving enzalutamide was 32% (95% CI, 17 to 51; 10 of 31 men). However, the PSA response rate among AR-V7–positive patients was 0% (95% CI, 0 to 26; 0 of 12 men), whereas the rate among AR-V7–negative patients was 53% (95% CI, 29 to 76; 10 of 19 men; P=0.004). Regarding abiraterone-treated patients, the overall proportion of patients who had a PSA response while receiving abiraterone acetate was 55% (95% CI, 36 to 73; 17 of 31 men). In the same way, the PSA response rate among AR-V7–positive patients was 0% (95% CI, 0 to 46; 0 of 6 men), whereas the rate among AR-V7–negative patients was 68% (95% CI, 46 to 85; 17 of 25 men; P=0.004). The authors concluded that the detection of AR-V7 in CTCs from patients with mCRPC may be associated with resistance to enzalutamide and abiraterone acetate.

1.4. *Study rationale*

Radium-223 is indicated for the treatment of patients with mCRPC with symptomatic bone metastases and no known visceral metastatic disease. However, very few data have been reported in patients with mCRPC who are asymptomatic or mildly symptomatic. Recently, results from an International Expanded Access Program have also suggested a benefit of radium-223 in asymptomatic patients with mCRPC. In addition, the mechanism of action of radium-223 should not be correlated with the presence/absence of the AR-V7 mutation, although this issue has not yet been evaluated.

The aim of this study is to assess the efficacy of radium-223 in asymptomatic patients with mCRPC,and to establish the association between AR-V7 status and radium-223 activity.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. *Primary objective*

To assess the efficacy of radium-223 in asymptomatic patients with mCRPC who have progressed while on abiraterone acetate or enzalutamide treatment.

Primary endpoint:

To determine the efficacy of radium-223 in terms of radiological rPFS.

2.2. *Secondary objectives*

- Safety profile.
- To determine the association between AR-V7 status (positive vs. negative) and PFS.
- To establish the relationship between CTCs number with radium-223 efficacy.

Secondary endpoints:

Safety

AEs will be evaluated using the Common Terminology Criteria for Adverse Events(CTCAE) of the US National Cancer Institute (NCI) version 4.0[20]. Grade 3 or 4 AEs and serious adverse events (SAEs) will be assessed to determine the safety and tolerability of the various combinations of drugs.

Efficacy

- Radiographic progression-free survival (rPFS) depending on AR-V7 status.
- Overall survival (OS).
- Time to first symptomatic skeletal event (SSE).
- Time to PSA progresionaccording to the ALSYMPCA study criteria.
- Determination percentage of PSA progresion.
- Alkaline phosphatase level response (AF), normalization of alkaline phosphatase level, according to the ALSYMPCA study criteria.

Molecular aspects

- Assessment of AR-V7 mutation evolution during the study treatment.
- Determination changes in CTCs number during the study treatment.

3. STUDY OVERVIEW

3.1. *Type of study*

This is a multicenter, single-arm, open-label, non-controlled phase IIa clinical trial.

3.2. *Study schedule*

The design of this study is divided into three well-defined phases:

- **Screening phase**

During this phase, subject eligibility is determined, including the documentation of baseline characteristics. This phase of the study will begin once the informed consent is signed by the patient and the procedures to be performed are described in section 5.1 of the protocol.

- **Treatment phase:**

When a patient is identified and all the study screening criteria have been met, a baseline blood sample will be collected to perform CTCs the patient will be treated with radium-223 at a dose of 55 kBq (after 2015 NIST implementation) per kilogram body weight, given at four-week intervals for six intravenous (IV) injections.

Patients will receive study treatment according to the protocol and will be prematurely discontinued if one of the following situations arises:

- Disease progression is confirmed radiologically and unequivocally. An event for progression will be determined by The Prostate Cancer Clinical Trials Working Group (PCWG2) modified criteria defined as the time from inclusion to the first event in clinical, radiological, or biochemical progression, or death as[21]:

- Bone metastases: The appearance of ≥ two new lesions, and, for the first reassessment only, a confirmatory scan performed six or more weeks later that shows a minimum of two or more additional new lesions. The date of progression is the date of the first scan showing the changes.
- Soft-tissue lesions: Assesed according to the Response Evaluation Criteria in Solid Tumors (RECIST criteria) version 1.1.[22].
- Symptoms: Consider independently of other outcome measures. Document pain/analgesia at entry and measure repeatedly at three- to four-week intervals. Ignore early changes (≤ 12 weeks) in pain or health-related quality of life (HRQOL) in the absence of compelling evidence of disease progression. Confirm progression of pain or HRQOL endpoints ≥ three weeks later.
- Clinical deterioration: Consider independently of other outcome measures. A deteriorating patient is one who moves from one clinical state to a worse clinical state which increases their individual risk of morbidity, including organ dysfunction, protracted hospital stay, disability, or death.[23].

- AEs which, according to the protocol or in the opinion of the investigator, can cause serious or permanent damage or which rule out further treatment with the study drug.
- Major study protocol non-compliance.
- Patient's withdrawal from the study.

- Dead.
- Study is canceled by the Sponsor.

Patients may continue receiving commercial radium-223 (Xofigo®), if it is found to offer clinical benefits in the opinion of the investigator.

- **Follow-up phase:**

Once the treatment phase is completed, patients will enter a follow-up period with radiological tumor assessment every three months (±7 working days) until disease progression and safety evaluation during 2 years from the last dose of study treatment. If patient discontinued treatment for any reason other than progression, tumor reevaluation will be included in the assessment. After disease progression, a safety follow-up visit will be performed within three following months and 2 years after the last dose of study treatment (including survival status and post-study anticancer therapy evaluation). Safety evaluation after progression will include follow-up of symptomatic skeletal related events and overall survival.

3.3. *General concomitant medication and additional assistance guidelines*

Concomitant treatment and prior medication are defined as non-investigational medicinal product (non-IMP). Concomitant treatment includes any prescribed medication or phytotherapy between the 28 days prior to the administration of the first treatment dose until disease progression. All concomitant treatments will be recorded. After this time, information will only be collected on any anti-cancer drugs and other possible medications for symptomatic skeletal related events taken by the patient until end of study (EoS).

Information on concomitant medication will include start date, end date, brand or generic name, route of administration, dose, and treatment indication.

The following concomitant tratments are permitted during the study:

- Erythropoiesis-stimulating agents (ESA) are permitted (such as Procrit®, Aranesp®, Epogen®) for the supportive treatment of anemia. Blood transfusions are permitted during the study.
- The prophylactic use of granulocyte-colony stimulating factors (G-CSF; GM-CSF) is not allowed during the first treatment cycle, but can be used for cases of neutropenia arising during treatment, in accordance with theNational Comprehensive Cancer Network (NCCN) guidelines[24]. In any case, the investigator should request approval from the medical scientist.
- The use of medication for the treatment of diarrhea, nausea, or vomiting is permitted.
- Approved bone-targeting therapy must have been on stable doses for at least four weeks prior to start of study drug at day 1.

- Any medications deemed necessary to ensure patient safety and well-being may be administered at the discretion of the investigator.

3.4. *Prohibited therapies*

Cytotoxicchemotherapy, othersystemic radioisotopes, radiotherapy, abiraterone acetate, enzalutamide, any antiandrogen, or other investigational drugs shouldnotbe used during the treatment period.Ifsuchtreatments are to be introduced during the treatment period, further studydrug administrations must be discontinued and treatment with the disallowed therapy should not be initiated until at least four weeks after last studydrug administration, if possible.

3.5. *Duration of study treatment (treatment phase)*

The study treatment period is defined as the time elapsed between the first treatment dose and the last dose of treatment administered.

The study treatment should be interrupted if at least one of the reasons listed in section 3.2 is present, and the reason(s) for interruption should be recorded in the patient record and in the corresponding case report form (CRF).

3.6. *Duration of post-treatment follow-up period (follow-up phase)*

The efficacy follow-up period is defined as the time relapsed between the last treatment administration and disease progression, death, withdrawal of consent, or 34 months after the first patient has been included, whichever occurs first. Safety follow-up period is defined as 2 years since the last treatment administration.

3.7. EoS

The EoS is defined as the Last Patient LastVisit (LPLV) at the end of the safety follow-up period (24 months after last dose of treatment). This is the last timepoint for data collection.
The last visit for efficacy evaluation will be take place within three following months after last patient's disease progression, death, withdrawal of consent or 34 months after the first patient has been included, whichever occurs first.
The last visit for safety and overall survival evaluation will be take place 2 years after last dose of study treatment of last patient. We include a 2-years extension after the end of the treatment to evaluate symptomatic skeletal related events. Additionally, analysis of overall survival will be also extended. Contact by telephone is permitted.

4. PATIENT SCREENING

The following eligibility criteria can be used in the screening of patients for whom the protocol treatment is deemed suitable. In order to determine whether this protocol is suitable for a given patient, all medical and non-medical criteria should be taken into consideration.

4.1. *Study population*

Patients with mCRPC with asymptomaticprogression while on abiraterone acetate or enzalutamide. The progression on enzalutamide or abiraterone acetate will be defined as patients who confirm radiographic progression and/or PSA progression with rapid PSA doubling time (less than three months).

The patient's signed informed consent should be obtained before any trial related activities and according to local guidelines.

4.2. *Inclusion criteria*

Patient eligibility will be reviewed and documented by a suitable member of the investigator's study team before the patients are enrolled in the study.

Patients must meet all the following inclusion criteria to be enrolled in the study:

- Subject is an adult ≥ 18 years at the time of informed consent and has signed informed consent before any trial related activities and according to local guidelines.
- Subject has histologically confirmed adenocarcinoma of the prostate without neuroendocrine differentiation or small cell features.
- Subject has bone metastases due to the prostate cancer and absence of visceral metastases.
- Subject has a serum testosterone of ≤ 1.7 nmol/L (or ≤ 50 ng/dL) at screening.
- Subject must have received a minimum of 24 weeks of treatment with abiraterone acetate or enzalutamide within its approved label indication and has discontinued use at least four weeks prior to start of study drug at day 1.
- Prior use of docetaxel is allowed in castration-naïve patients (maximum of six cycles).
- Subject receives and will continue to receive ongoing androgen deprivation with LHRH analogue therapy throughout the course of the study or has had a bilateral orchiectomy.
- Subject is asymptomaticfrom prostate cancer, defined as patients with the score on brief pain inventory (short form) (BPI-SF) Question #3 must zero and no use of opiate analgesics for prostate cancer-related pain currently or anytime within two weeks prior to screening.
- Subject has an Eastern Cooperative Oncology Group (ECOG) performance status of 0–1 at screening.
- Subject receiving bisphosphonate or other approved bone-targeting therapy must have been on stable doses for at least four weeks prior to start of study drug at day 1.
- Subject has a life expectancy of more than or equal to 12 months.
- Subject agrees not to participate in another interventional study while on study drug.
- Subject and his female partner who is of childbearing potential must use two acceptable methods of birth control (one of which must include a condom as a barrier method of contraception) starting at screening and continuing throughout the study period and for six months after final study drug administration.

4.3. *Exclusion criteria*

Any patient meeting **ANY** of the following criteria will be excluded from the study:

- Subject has received any anti-neoplastic therapy (including ketokonazol and chemotherapy) following abiraterone acetate or enzalutamide discontinuation and prior to start of study drug at day 1.
- Subject has known or suspected brain metastases or active leptomeningeal disease.
- Subject has concurrent disease or any clinically significant abnormality following the investigator's review of the physical examination and safety laboratory tests at screening, which in the judgment of the investigator would interfere with the subject's participation in this study or evaluation of study results.
- Subject has a history of another invasive cancer within three years prior to screening, with the exceptions of non-melanoma skin cancers or a non-infiltrating muscle bladder cancer that have a remote probability of recurrence in the opinion of the investigator in consultation with the medical scientist.
- Subject had major surgery within one month prior to screening.
- Subject has received investigational therapy within 28 days or 5 half lives, whichever is longer, prior to start of study drug at day 1.
- Subject has absolute neutrophil count < 1,500/μL, platelet count < 100,000/μL, and hemoglobin < 6.25 mmol/L (or < 10 g/dL) at screening (Note: Subjects must not have received any growth factors or blood transfusions within seven days of the hematologic laboratory values obtained at screening).
- Subject has total bilirubin > 1.5 times the upper limit of normal (ULN) at screening, except for subjects with documented Gilbert's syndrome.
- Subject has creatinine > 2.5 mg/dL at screening.
- Subject has albumin ≤ 30 g/L (or ≤ 3.0 g/dL) at screening.

5. STUDY ASSESSMENTS AND PROCEDURES

5.1. *Screening phase: Patient enrollment procedures*

Study-specific assessments performed during the screening phase: From Day 0 (patient allocation) to day 28 (to confirm remaining screening criteria during days 1-28 after day 0 of the screening phase).

Written informed consent from the patient must be signed before performing any study procedure (before screening assessment).By giving their consent, patients will be informed as to the nature of the study drug and will receive pertinent information regarding the study objectives, possible benefits, and potential AEs. They will also receive information on the follow-up procedures and

possible risks they will be exposed to. This document also informs patients about how biological samples will be obtained and collected and its legal implications. After receiving the document, the patient will read it (or receive information verbally before witnesses) and will sign the previously approved informed consent. The patient will receive a signed copy of the informed consent. The patient can withdraw their consent and discontinue the study, this will not affect any future medical treatment.

At inclusion:

1. The Sponsor will request the patient's demographic and clinical data related to screening criteria.
2. Each patient will be given a Unique Patient Number (UPN) for this study, provided by the Sponsor. All data will be recorded in the appropriate CRF using this identification number. This number will be provided to the central laboratory to ensure traceability of study samples.

Confirmation of patient's eligibility for study participation will be recorded in the CRF. The investigator is responsible for safeguarding patient information (e.g., age, name, address, telephone number, social security number, and study identification number), ensuring access to this information by Health Authorities if necessary. These records will remain confidential for the period of time stipulated by current legislation.

The following will be obtained during the screening phase:

- Anamnesis, medical history, and assessment of symptoms.
- Physical examination and measurement of height, weight, vital signs, and performance status.
- Basic blood test that includes hematological test [hemoglobin, hematocrit, red blood cell count, platelet count, white blood cell count (WBC) with differential count (neutrophils, lymphocytes, monocytes, eosinophils and basophils)], coagulation, chemistry with renal function analysis (serum creatinine, creatinine clearance according to the Cockroft-Gault formula), liver function [aspartate transaminase (AST), alanine transaminase (ALT), Alkaline phosphatase (AF), gamma-glutamyl transferase (GGT), total and direct bilirubin], glucose, sodium, potassium, calcium, chloride, magnesium, uric acid, total protein, albumin, and lactate dehydrogenase.
- PSA and testosterone determination.
- BPI-SF.
- HRQoL questionnaire (FACT-P, Version 04).
- Blood sample for translational study, which is then sent to central laboratory.
- Assessment of concomitant medications (as it is specified in section 3.3).
- Radiological tumor assessments [torax, abdomen, and pelviscomputerized axial tomography(CT) scan or magnetic resonance imaging (MRI), and bone scan] prior to start first cycle.

Results of standard of tests or examinations available performed as part as clinical practice prior to obtaining informed consent and within 28 days prior to treatment start may be used; such tests do not need to be repeated for screening.

5.2. *Treatment phase: Assessments*

All screening assessments must be completed and reviewed to confirm that patients meet all eligibility criteria before treatment starts (day 1). The investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable.

Visits are organized in programmed cycles of 28 days (if there are no delays in treatment owing to the occurrence of a side effect). Adjustment of dose level is not permitted. Dose delays are permitted, as described in section 6.11. All visits must occur within ± 7 working days from the scheduled date, unless otherwise noted in the schedule of assessments. All assessments will be performed on the day of the specific visit unless a time window is specified. Assessments scheduled on the day of study treatment administration should be performed prior to study treatment administration unless otherwise indicated. If a mandatory procedure described in the protocol falls on a bank holiday and/or weekend, this procedure should be performed on the day before or after the holiday (i.e. within a period of ± 3 working days).

Local laboratory tests scheduled for day 1(before treatment) of all cycles must be performed within 72 hours prior to confirm to the patient if treatment can be followed up. The results of local blood tests should be reviewed, and this review recorded before administration of the study treatment.

Specific study assessments during treatment phase:

During basic safety visits: Day 1of each cycle (every four weeks):

- Assessment of symptoms.
- Physical examination and measurement of weight, vital signs, and ECOG performance status.
- Basic blood test that includes blood count, coagulation, chemistry with renal function analysis (serum creatinine, creatinine clearance according to the Cockroft-Gault formula), liver function (AST, ALT, AF, GGT, total and direct bilirubin), glucose, sodium, potassium, calcium, chloride, magnesium, uric acid, total protein, albumin,and lactate dehydrogenase.
- PSA determination.
- HRQoL questionnaire (FACT-P, Version 04).
- Assessment of concomitant medications.
- Assessment of AEs.
- Survival.

During full visits: Day 1 of each cycle every three cycles (every 12 weeks):

- Assessment of symptoms.
- Physical examination and measurement of weight, vital signs, and ECOG performance status.
- Basic blood test that includes blood count, coagulation, chemistry with renal function analysis (serum creatinine, creatinine clearance according to the Cockroft-Gault formula), liver function (AST, ALT, AF, GGT, total and direct bilirubin), glucose, sodium, potassium, calcium, chloride, magnesium, uric acid, total protein, albumin,and lactate dehydrogenase.
- PSA determination.
- HRQoL questionnaire(FACT-P, Version 04).
- Assessment of concomitant medications.
- Assessment of AEs.
- Tumor assessment: torax, abdomen, and pelvisCT scan or MRI, and bone scan prior to start of cycle every three cycles, every 12 weeks, until progression disease.
- Survival.

Blood assessment schedule for translational study:

- Blood draws prior to the start of study treatment, at the end of treatment and at the time to documented progression disease.

Biological sampling:

The first blood samples should be taken before starting study treatment (see appendix3 for more details on blood sampling procedures). The following samples should be taken at the end of treatment and at the time of disease progression.

At the time of site activation, the Sponsor will provide streck cell-free DNA BCT tubes as well as labels for the storage and transport of samples. No other tubes or labels should be used for this study. If more tubes and labels are required, these should be requested from the Sponsor in writing at least three weeks before they are required (see appendix 3).

Anamnesis and demographic data:

Anamnesis includes clinically significant diseases, surgical interventions, history of cancer (including prior antineoplastic treatments and procedures), history of smoking, alcoholism, drug addiction, as well as any medications (e.g., prescribed drugs, over-the-counter drugs, medicinal plants, homeopathic remedies, or food supplements) used by the patient in the 28 days prior to screening visit. Demographic data will include age, sex, and self-reported race/ethnicity.

Vital signs:

These will include the measurement of height (only during screening), weight, respiratory rate, heart rate, blood pressure, and body temperature. Abnormal or significant changes in vital signs from baseline should be recorded as AEs, if appropriate.

Physical Examination:

A complete physical examination should include an examination of head, eyes, ears, nose, and throat as well as cardiovascular, dermatological, musculoskeletal, respiratory, digestive, genitourinary, and neurological systems. Changes to abnormalities identified during the baseline period should be recorded at all subsequent physical examinations. New or worsening abnormalities should be recorded as AEs, if applicable.

Physical exams should also include, as part of tumor assessment, evaluation of presence and degree of increase of lymph nodes, hepatomegaly, and splenomegaly. Limited physical exams will focus on symptoms.

Response and tumor assessments:

Tumor response will be assessed for all patients, unless they withdraw from the study for any reason not attributable to disease progression confirmed radiologically or clinically and who have not received an acceptable complete assessment of the disease. The non-measurable disease should be documented at screening and be re-assessed at every tumor assessment thereafter.
The initial assessment of tumor response will be performed at the end of cycle three. Subsequently, an efficacy follow-up will be carried out for all patients every 12 weeks ±7 working days during the study until disease progression, the patient withdraws their consent, or death, whichever comes first. In this study, response and disease progression will be assessed using the PCWG2 guidelines for bone disease and the RECIST criteria version 1.1 for soft-tissue disease. Radiographic disease progression in bone will require persistence of new lesions on a confirmatory bone scan performed at least six weeks later. Soft-tissue progression will not require a confirmatory scan for purposes of analysis (Appendix 2: Response Evaluation Criteria in Solid Tumors Guidelines (RECIST criteria) version 1.1).
A torax, abdomen, and pelvis CT scan or MRI, and a bone scan must be performed at screening (no more than 28 days before the first trial treatment). Tumor assessments should include an assessment of all known and/or suspected sites of the disease wherever possible. Patients should have a series of selected lesions that can be assessed at each tumor assessment.The same radiographic procedure employed at screening should be used throughout the study (e.g., the use of the same contrast protocol for CT scans).

Laboratory assessments:

Laboratory tests will be performed in accordance to local standard treatment and clinical indications before treatment administration. These values should include: blood count, coagulation, chemistry with renal function analysis (serum creatinine, creatinine clearance according to the Cockroft-Gault formula), liver function (AST, ALT, AF, GGT, direct and total

bilirubin), glucose, sodium, potassium, calcium, chloride, magnesium, uric acid, total protein, albumin, lactate dehydrogenase, PSA, and testosterone.

ECOG performance status:

Performance status will be determined using the ECOG performance status scale (see table below). Wherever possible, the patient's performance status should always be assessed by the same personnel throughout the study.

Table 1. ECOG performance status scale

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

(http://www.ecog.org/general/perf_stat.html)

AEs and toxicity to treatment:

Safety and tolerability of all patients will be closely monitored throughout study treatment and the follow-up period using the NCI-CTCAE version 4.0[25]. Patients will be assessed in order to detect any side effects before administering new study treatment during each treatment visit. Treatment will only be administered if clinical evaluation and local laboratory test results are acceptable.

5.3. *Follow-up phase*

Patients who complete the study treatment during its active treatment phase will enter a follow-up period with radiological tumor assessment every three months (±7 working days) until documented disease progression and safety evaluation during 2 years from the last dose of study treatment. If patient discontinued treatment for any other reason than progression, tumor reevaluation will be included in the assessment.

- Assessment schedule after progression or at the end of treatment for any other reason: After progression or at the end of treatment for any other reason the following assessments will be performed within a period of 28 days (if end of treatment is not confirmed in some of the scheduled full visits):
  - Assessment of symptoms.

- Physical examination and measurement of weight, vital signs, and ECOG performance status.
- Basic blood test that includes blood count, coagulation, chemistry with renal function analysis (serum creatinine, creatinine clearance according to the Cockroft-Gault formula), liver function (AST, ALT, AF, GGT, direct and total bilirubin), glucose, sodium, potassium, calcium, chloride, magnesium, uric acid, total protein, albumin, and lactate dehydrogenase.
- PSA determination.
- HRQoL questionnaire(FACT-P, Version 04).
- Assessment of concomitant medications.
- Assessment of AEs.
- Tumor assessment: torax, abdomen, and pelvisCT scan or MRI, and bone scan.
- Blood sample for translational study, which is then sent to central laboratory.
- Survival.

After disease progression, a safety follow-up visit will be performed within three following months and 2 years after last dose of study treatment (including survival status and post-study anticancer therapy evaluation). Safety evaluation after progression will include follow-up of symptomatic skeletal related events and overall survival. Contact by telephone is permitted.

In addition, any events ≥ grade 2 will be followed until improvement to baseline levels is observed, complete recovery or resolution to grade 1, consent is withdrawn by the patient, patient death, or until follow-up is no longer possible.

5.4. *Assessment schedule*

The summary of study assessments will be included in Appendix 1: Schedule of study assessments and procedures.

5.5. *Discontinuation of patient, study, or site participation*

Treatment discontinuation: Patients have the right to withdraw from the study at any time and for any reason, without being required to state their reasons for doing so. The investigator has the right to discontinue a patient from study treatment or from the study for any medical condition that the investigator determines may jeopardize the patient's safety if he continues in the study, for reasons of non-compliance (e.g., missed doses, visits), or if the investigator determines it is in the best interest of the patient.

Patients should also be withdrawn from the study treatment if they experience disease progression as defined by the RECIST criteria version 1.1. Further details regarding study discontinuation due to side effects are described in section 6.11.

Patients who withdraw from study treatment prematurely will be followed up in accordance to section 5.3 except for those patients who withdraw their consent and who do not wish to undergo a follow-up. The main reason for discontinuation should be recorded in the appropriate section in the CRF.

Study discontinuation:

Patients will be withdrawn from the study (e.g., from any subsequent study procedure) for any of the following reasons:

- Withdrawal of consent.
- Lost to follow-up.

If patient is lost to follow-up, site personnel must do their utmost to re-establish contact with the patient and determine the reason for their withdrawal. All measures taken to undertake follow-up should be recorded.

When a patient withdraws before the end of the study, the reason for withdrawal should be recorded in the CRF and in the original document. Patients who withdraw from the study will not be replaced.

In addition, the Sponsor reserves the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- The incidence or seriousness of AEs in this or other studies indicates a potential health risk to patients.
- Patient enrollment is unsatisfactory.
- Data recording is inaccurate or incomplete.

6. STUDY DRUG INFORMATION

6.1. *Study treatment*

Once enrolled in the study, the patient will receive study treatment.

Patients will be treated with radium-223 at a dose of 55 kBq (1.49 microcurie) per kilogram body weight, given at four-week intervals for six IV injections.

6.2. *Drug supply*

The latest version of study document Summary of Product Characteristics (SmPC) for radium-223 (Xofigo®) includes updated information on this drug.
The Sponsor will provide radium-223, which will be manufactured by the contract manufacturer: Institute for Energy Technology, Isotope laboratories, Kjeller, Norway. The products will be produced according to Good Manufacturing Practice (GMP), and will be delivered in a glass vial,

ready-to-use, with a certified activity. Radium-223 will be shipped in a lead container and type A radioactive package according to international transportation guidelines for radioactive materials.
The alpha-pharmaceutical radium-223 is a ready-to-use, sterile, non-pyrogenic, clear, and color less aqueous solution of $^{223}RaCl_2$ for IV administration.The product isisotonic and has a pH of 6.0-8.0 with a pre-calibration of 14days.
Single-use vials contain 6 mL solution at concentration of 1100 kBq/mL (27 microcurie/mL) at the reference date with a total radioactivity of 6.6 MBq the reference date (after 2015 NIST implementation). When administered on a day other than the reference day, the volume should be corrected according to the physical decay table.
Radium-223 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 qualityis not jeopardized upon freezing. All study drugs will be labeled according to the requirements of local law and legislation. Label text will be approved according to the Sponsor's agreed procedures.
For all study drugs, a system of numbering in accordance with all requirements of GMP wil be used, ensuring that each dose of study drug can be traced back to the respective bulkware of the ingredients. Lists linking all numbering levels will be maintained by the Sponsor. A complete record of batch number and expiry dates for all study treatments as well as the labels will be maintained in the Sponsor study file.
Once the study has ended, patients may continue receivingcommercial radium-223, if it is found to offer clinical benefits in the opinion of the investigator

6.3. *Dosage and administration*

Written information about radium-223 and instruction about handling and injection of radioactive material will be provided to study personnel.
In general, the administration of radioactive drugs involves a potential risk for third parties, due to radiation from thepatient and due to possible contamination by spilling urine or feces. When radium-223has been injected intravenously into apatient, the risk for external radiation exposure to third parties is extremelylow, due to the short range of the alpha particlesand the low portion of beta and gamma radiation. For these reasons the product can be administered on an out-patient basis. To minimize the risk of contamination, the patient and his caregivers will receive instructions regarding hygiene precautions to abide by after receiving the radioactive drugaccording to the investigational site radiation protection guidelines.

6.4. *Dose calibration*

Radium-223 can be measured in a normal dose calibrator instrument. When written approvals for the use of radium-223 from the Radiation Protection Agencyfor the specific center have been received bythe Sponsor, a vial of radium-223 for technical use will be sent to the studycenter.Different clinical study centers possess dose calibrators from various suppliers; thus,

the isotope calibration factor maydiffer from center to center. Consequently, each center must perform the radium-223 dial setting on their relevant dose calibrator(s). For dial setting, the clinical studycenter will receive a sealed vial containing a radium-223 solution for calibration only. The vial is identical to the vials used for studytreatment. The amount of radium-223 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.

6.5. *Dose handling*

Ordering of study drug will be done using Drug Order Form, the site will send this request to MedSIR before each planned administration and MedSIR will manage the study drug shipment with Bayer. The radium-223 vials must be stored inside their lead container in a secure facility. The study drug should be used within 28 days of production.

Radium-223 is an alpha-emitting pharmaceutical and should be received, used and administered only by authorized persons in designated clinical settings. The receipt, storage, use, transfer, and disposal aresubject to the regulations and/or appropriate licenses of the competent official organization. Radiopharmaceuticals should be prepared bythe user in a manner which satisfies both radiation safety and pharmaceutical qualityrequirements. Appropriate aseptic precautions should be taken.

Control measurements of both the radium-223 vial (before and after dispensing) and syringes (before and after administration) are performed as part of the clinical trial documentation.All administrations of radium-223 arebased on the certified activityof radium-223 at the calibration date.

6.6. *Dose calculation*

The dosage of radium-223 is 55 kBq per kilogram body weight. The total activity to be injected will be calculated volumetrically using the patient's bodyweight on the dayof injection, the 55 kBq per kilogrambodyweight dosage level, and the decaycorrection factor (DK) to correct for physicaldecayof radium-223 (after NIST implementation). A table with DKs according to physicaldecayof the study medication will be provided with each vial of radium-223. The total amount (volume to be drawn into the syringe) to be administered to apatient should be calculated as follows:

$$\frac{\text{Body weight (kilograms)} \times 55\ \text{kBqper kilogram body weight}}{\text{DK} \times 1100\ \text{kBq/ml}} = \text{Volume to be injected (mL).}$$

6.7. *Dose preparation*

Personnel should use appropriate protective clothing and equipment during syringe filling and application to prevent contamination with the radioactive solution (lab coats, medical gloves/protective glasses). Sites should adhere to all relevant radiation safetyregulations as prescribed by local authorities administering their site radiation license, including ALARA principles.

Filling of the syringe should take place in a safetybench or similar cabinet in the Radiopharmacy/Nuclear Medicine department. The individual responsible for studydrug 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-223should not be diluted or mixed with any solutions. If the vials have been stored in a refrigerator, they should be left at room temperature for one hour prior to use, since cold material should not be injected in a patient.

6.8. *Dose administration*

Before administration of study drug, the patient must be well hydrated and should be instructed to drink ad libitum.Aseptic technique should be used in the administration of radium-223. The syringe should be handed over to the individual who will perform the injection. The study medication will be administered as a slow bolus IV injection over one 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 hospital procedure for the handling of radioactive material. Information about radium-223 and instruction about handling and injection of radioactive material will be given to study personnel.

6.9. *Medication errors and overdose*

Medication errors in this study may arise when the drug is administered at the wrong time or when the wrong dose strength is taken. Patient medication errors should be recorded in the relevant section in the CRF. In the event of an error in the administration of the medication, the Sponsor should be informed immediately.

Medication errors must be reported irrespective of the presence of an associated AE/SAE, including:

- Medication errors involving patient exposure to the IMP.
- Any possible medication errors or use of the medication not defined in the protocol which implicate the participating patient or not.

Regardless of whether the medication error is accompanied by an AE or not, in the judgment of the investigator, the medication error should be recorded in the AE page.

6.10. *Treatment compliance*

Patients will receive treatment under the supervision of a physician licensed in the administration of radioisotopes. Personnel will check the administration volume and total radioactivityinjected. The activitydose (total and per bodykilogram) will be recorded in the source data and the appropriate CRF.

6.11. *Dose adjustments*

Everyeffort will be made to administer the full dosing regimenofradium-223. Adjustment of dose level is not permitted.Studyvisits during the treatment period should occur at four weeks intervals (within a window ± 7 working days).
Dosing delays may be instituted under the following situations:

- Disease progression:

In this patient population, disease progression is expected. Bone pain is a prominent symptom; other symptoms mayinclude fatigue, nausea, anorexia, depression, constipation (also secondaryto opioids), bowel and bladder symptoms, lymphedema, neurological, and hematological complication as well as metastases to other organs, such as liver and lung.The investigator should delay cytotoxic chemotherapy, other systemic radioisotope, 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 apatient experiences grade 3 or 4 neutropenia, thrombocytopenia, or anemia lasting ≤ 14 days, the administration of studydrug should be delayeduntil recoveryto grade 2 or better. A delay of up to 4 weeks is permited.

- If a patient experiences grade 3 or 4 neutropenia, thrombocytopenia, or anemia lasting >14 days, further study drug administrations must be discontinued.

- Blood transfusion is acceptable between studydrug administrations. Use of biologic response modifiers, such as G-CSF or GM-CSF, is allowed in the management of acute toxicity.

- Gastrointestinal events:

- Diarrhea. Diarrhea should be treated as per local practice. A further dose of study medication should not be given before diarrhea is recovered to no higher than CTCAE grade2.

- Nausea/Vomiting. Nausea or vomiting should be treated as per local practice. A further dose of study medication should not be given before nauseaor vomiting is recovered to no higher than CTCAE grade 2.

- Surgical intervention:

  If surgery is required, the patient should continue with studytreatment, if this is considered safe in the treating investigator's opinion. The surgeon needs to be notified that thepatient 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 two to four weeks from the time of fracture.

- Any other toxicity:
  Local practice will apply. A dose delay of more than four weeks (maximum eight weeks between two injections) will lead to study drug discontinuation.

6.12. *Possible drug interactions*

No formal clinical drug interaction studies have been performed with radium-223. Any medications deemed necessary to ensure patient safety and well-being may be administered at the discretion of the investigator with the exceptions indicated in section 3.4.

7. SAFETY DEFINITIONS AND REPORTING REQUIREMENTS

Safety assessments will consist of monitoring and recording AEs, adverse events of special interest (AESI), and SAES as defined in the protocol; measurement of blood count and chemistry values specified in the protocol; and the measurement of vital signs and other tests defined in the protocol which are deemed important to the safety assessment of the study drug(s).

The Sponsor or its designee is responsible for reporting SAEs to the competent authorities, other applicable regulatory authorities, and participating investigators, in accordance with International Conference on Harmonization (ICH) guidelines, European Clinical Trials Regulation (Regulation (EU) No. 536/2014), and/or local regulatory requirements.

The Sponsor or designee is responsible for reporting unexpected fatal or potentially life-threatening events related to the use of the study drug to the Health Authorities and relevant authorities, by fax within seven calendar days of learning of its occurrence. The Sponsor or designee will report any other significant expeditable SAEs related to the use of the study medication to the corresponding competent authorities (in accordance with local guidelines), in the form of a written safety report within 15 calendar days.

7.1. *Definitions of AEs*

An AE is defined as an incident that is harmful to the health of the patient who has been treated with an IMP as part of a clinical trial, even though there may not be a causal relationship between the two. Therefore, an AE can be any unfavorable and/or unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP, regardless of whether it is considered to be related to the investigational product or not. Abnormal test results should only be reported as AEs if they meet any of the following criteria, are associated with concomitant symptoms, and cannot be defined in general diagnostic terms. This includes abnormal symptoms and test results, or symptoms that call for additional diagnostic testing, pharmacologic intervention or surgery, a change in study drug dose or withdrawal from the study, those requiring additional concomitant drug treatment, or those that are considered an AE by the investigator or Sponsor.

The causal relationship between an AE and the IMP will be defined as follows:

Unrelated: The time relationship between the AE and the administration of the IMP makes a causal relationship between the two highly unlikely, or the patient's clinical status or the study procedures/conditions sufficiently explain the cause of the AE.

Related: The time relationship between the AE and the administration of the IMP makes a causal relationship between the two very likely, and the patient's clinical status or the study procedures/conditions do not sufficiently explain the cause of the AE.

The investigator will assess each AE to determine whether or not there exists a reasonable possibility that the AE is related to radium-223.

Toxicities will be reported using the descriptions and grade scales provided by the NCI-CTCAE version 4.0. A copy of the NCI-CTCAE criteria version 4.0 can be downloaded from the CTEP website (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-4_QuickReference_5x7.pdf).

When reported, the severity of an adverse event will be classified as one of the following:

- Mild - easily tolerated AE: It does not interfere with daily activities. CTCAE grade 1.
- Moderate: It causes some disruption of daily activities, may require intervention or treatment. CTCAE grade 2
- Severe: Considerable disruption of daily activities, need for hospitalization and/or surgical intervention or treatment. CTCAE grade 3 or 4.
- Fatal: Death. CTCAE grade 5
- Not applicable: Abnormalities in laboratory tests that are clinically significant and asymptomatic, or abnormal assessments not covered by the CTCAE scale, even though they are considered an AE.

A mild, moderate, or severe AE may or may not be serious. These terms are used for describing the severity of a specific event. Nevertheless, the medical significance of a severe event may be considered relatively minor (such as a severe headache) and may not be necessarily serious. For example, nausea lasting several hours may be considered severe, but may not be clinically serious. A fever of 39 °C which is not considered severe, may become serious if it results in a one-day hospital stay. Seriousness, rather than severity, serves as a guide for determining whether the event should be reported to authorities.

Adverse reactions to medicines: Any harmful and unexpected responses to an IMP (i.e. when there is a at least reasonable possibility that there is a causal relationship between the IMP and the AE) that are related to any dose should be considered an adverse reaction to the medicine. In the case of marketed drugs, an adverse reaction to medicine is defined as a drug response that is harmful and unexpected and is produced at the dose that is normally administered to human subjects for prophylaxis, diagnosis or treatment of diseases, or to alter physiological functions.

Anunexpected adverse reaction to a medicine is defined as an adverse reaction whose nature or severity is not consistent with the information on the product.
SAEs: By definition, a SAE is defined as any event that:

- Results in death.
- Is life-threatening.
- Requires hospitalization or prolongs existing hospitalization.
- Results in persistent or significant disability/incapacity (incapacity is defined as a significant disruption in a person's ability to perform normal life activities).
- Constitutes a congenital anomaly/birth defect.

Definition of life-threatening: An AE islife-threatening if the patient was at immediate risk of death due to the event; i.e. not including a reaction that could have caused death if it had been more serious. For example, a drug-induced hepatitis that resolved without signs of liver failure will not be considered life-threatening, even though drug-induced hepatitis can be fatal.

Definition of hospitalization: AEs that require hospitalization are considered serious. In general, hospitalization is considered as such when the patient has stayed (usually overnight) at a hospital or emergency room for observation or treatment that could not have been performed in a doctor's office or outpatient site. If there is some doubt as to whether the hospital stay can be considered "hospitalization" or whether it was necessary or not, the AE should be considered serious.

Hospitalization for scheduled surgery or standard clinical procedures which are not the result of an AE will not be considered an AE. If an incident arises during the procedure that is considered harmful, this should be reported as an AE and should be designated "serious" or "not serious" as per the standard criteria.

Definition of an event that is clinically or medically significant: Significant medical events that do not have a fatal outcome, regardless of whether they are life-threatening or require hospitalization, may be considered SAEs when, according to the appropriate medical opinion they put the patient's life at risk and may require medical or surgical intervention to prevent any of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm which requires intensive treatment in an emergency room or at home; blood dyscrasias; convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.Clinically/medically significant events must be reported as SAEs.

For this clinical study and, according to this protocol, SAEs and hospitalizations related solely and unequivocally to the progression of the established tumor disease will not be treated as SAEs with regards to reporting obligations.

If the investigator becomes aware of a SAE at any time after the end of administration of study treatment and believes that it is possibly related to the study treatment (serious adverse reactions), he/she should notify the Sponsor.

7.2. *Reporting of AEs*

AEs will be collected starting from the first procedure required by the study up to three months after patient's disease progression. Safety evaluation after progression will include follow-up of symptomatic skeletal related events until reach 2 years from the last dose of study treatment.

All study patients who experience AEs will be closely monitored during this time. All AEs should be recorded in the CRF.

All signs, symptoms, and results of procedures with abnormal diagnoses that are clearly related should be grouped together and reported as a single diagnosis or disorder wherever possible. Any other event that does not fulfill this definition will also be reported separately.

Reporting of SAES and reporting period

Reporting requirements will comply with all applicable local regulations regarding safety reporting.

All SAEs and AESIs defined in the protocol will be reported to the Sponsor within a period of 24 hours from the time the investigator or any other member of the site staff becomes aware of the event, as follows:

- Regardless of the study drug received by the patient and whether or not the investigator considers this event to be related or not to the study drug, all SAEs and AESIs (as defined in this protocol), will be reported to MedSIR immediately, no later than 24 hours after any member of the study site staff becomes aware of the event.
- All information regarding the SAE and AESI should be obtained and recorded in full using the SAE form and sent to MedSIR.
- Follow-up information, copies of test results, event outcome and the opinion of the investigator as to the relationship between the IMP and the SAE and AESI, accompanied by other applicable documentation when it is requested, will be sent along with the SAE form, if available on the day the event is reported or as soon as possible if it is not.
- The original SAE reporting form of the Sponsor should be kept on file with the documentation from the CRF at the study site(s).

All SAE forms will be sent by the investigator or investigator staff to the Sponsorby e-mail and fax as indicated below:
E-mail: farmacovigilancia@experior.es

Fax: + 34 96 145 21 91

Follow-up of the SAEs and AESIs will continue until they resolve, a stable outcome is reached, patient is lost to follow-up, or the patient dies.

MedSIR will be responsible for ensuring that events are reported to the European Medicine Agency (EMA), competent authorities, within the stipulated period, as needed.

AESIs

The investigator must report any AESIs to the Sponsor immediately (i.e. within 24 hours of learning of its occurrence) regardless of their seriousness (see section 7.2). In this study, AESIs include:

- An agent is considered infectious when it is suspected that an infectious agent has been transmitted via medication, any organism, virus or infectious particle (e.g.,the prion protein that causes transmission of transmissible spongiform encephalopathies), pathogenic or not. Transmission of an infectious agent may be suspected due to clinical symptoms or laboratory results which indicate the presence of infection in an individual exposed to a medicine. This term will only be applied when drug contamination is suspected and not to any infections supported by mechanism of drug action, e.g. immunosuppression.

- Potential drug-induced liver damage which meets the analytic criteria of Hy's Law. The following laboratory abnormalities are indicative of possible Hy's Law cases and should be reported as AESIs:
    - Any increase in AST and/or ALT> 3 times the ULN.
    - Simultaneous increase in total bilirubin > 2 times ULN (or clinical jaundice if total bilirubin measurements are not available), except for patients who have been diagnosed with Gilbert's syndrome. In the case of patients with Gilbert's syndrome, an increase in direct bilirubin > 2 times ULN should be used.

Non-compliance

Definition: Non-compliance with applicable laws or institutional policies that govern research on human subjects to follow-up on ethics committee (EC) decisions. Non-compliance may occur due to ignorance or a deliberate decision to ignore the law, institutional policies, or EC decisions.

Non-compliance should be reported to MedSIR, the principal investigator of the study site and, if necessary, to the EC immediately, within 24 hours.

Serious non-compliance

Definition: Non-compliance that materially increases the risk of serious harm to patients or other persons, or which materially compromises patient rights or well-being.

Serious non-compliance should be reported to MedSIR, the principal investigator of the study site and, if necessary, to the EC immediately, within 24 hours.

Expedited reporting to Health Authorities

To determine the reporting requirements in cases of a single AE, the Sponsor will assess the expectedness of these events using the radium-223 SmPC.

The Sponsor will compare the severity of each event and its cumulative frequency as reported in the study with the severity and frequency reported in the pertinent source document.

Reporting requirements are also based on the investigator's assessment of causality and seriousness, which may be updated by the Sponsor as needed.

7.3. *Safety reporting flow chart*

Non-serious AE reporting responsibilities:

Investigational site (completion of CRF)	⇨	Data management department of CRO and MedSIR (review, evaluation, and processing of safety data): Quarterly lists of non-serious AEs	⇨	CRO/MedSIR will provide quarterly lists of non-serious AEs for Bayer

AESI and SAE reporting responsibilities (as defined in the protocol):

Investigational site (SAE report, 24 hours)	⇨	CRO/MedSIR (review, evaluation, and processing of safety information): Reporting to CIOMS	⇨	To Bayer (copy): • SUSARs & AESIs requiring expedited reporting within 15 days • Other SAEs, other AESIs & pregnancy reports within 30 days
			↘	Expedited safety reports &Periodic Safety Line Listings (PSLLs) according to regulatory reporting obligations (including timelines)

Expedited safety reports &Periodic Safety e

Radium-223 treatment DSUR [DSUR form: Non-commercial Sponsor (MedSIR)]

CRO/ MedSIR (review, evaluation, and processing of safety information): Drafting DSUR): Drafting DSUR	⇨	Bayer (copy): ASAP
	↘	Health authorities, EC in accordance with regulatory reporting obligations

n, and processing of safety information):
DraftDrafting DSUR

Note:Bayer will send MedSIR an executive summary of Bayern's DSUR, at the request of MedSIR. MedSIR may cross reference Bayern's executive summary of DSUR.

Bayer (review, evaluation, and processing of safety data from sources other than those used in this clinical trial): Latest version of IB and IB updates, medical alert letters, and any new data that could modify or complement known data on radium-223, especially any new AEs and data related to radium-223 tolerance that may indicate a risk to patients:	⇨	MedSIR: ASAP

Managing safety crisis and safety-related requests from Health Authorities or for publications:

In the event of a safety crisis, e.g. cases where safety issues will potentially impact indication(s) or the conduct of the study that may give rise to changes in labeling or regulatory actions that limit or restrict the way in which radium-223 is used or in cases where the media is involved, the party (MedSIR) where crisis originated will contact the other party as soon as possible. Bayer will have final say and control over aspects related to the management of the safety crisis regarding.
MedSIR will not reply to any request related to the safety of radium-223 that it receives from the Health Authorities, the media or other sources (publications). Instead, it will direct requests to Bayern in accordance with and communicating the deadlines regarding necessary replies.

8. STATISTICAL CONSIDERATIONS AND STATISTICAL ANALYSIS PLAN

8.1. *Determination of sample size*

A total of 52 patients will be enrolled into this trial. We expect to include 13 AR-V7-positive and 39 AR-V7-negative patients.

8.2. *Justification of sample size*

The sample size is determined on the basis of the primary endpoint. The primary endpoint is defined as PFS according tothe PCWG2 criteria. We will screen the AR-V7 mutation retrospectively, after the patient was included. We expect the number of AR-V7-positive patients will be 25% of total sample. Therefore, sample size has been estimated on AR-V7-positivecohort to ensure a minimum of 80% power in two cohort analysis. We hypothesized equally efficacy of radium-223 in AR-V7-negative and AR-V7-positive cohorts.

Previous studies in advanced refractory prostate cancer patientstreated with docetaxel plus estramustine against mitoxantrone plus prednisone showed a median PFS of 6.3 and 3.2 months, respectively[26]. According to these results, excluding a PFS ≤ 3 months while targeting an improvement of the PFS ≥ 6.3 months would be a conservative approach to evaluation of the radium-223 effect inboth cohorts. The analysis will be performed with one arm one-sided Log-Rank test. We program a 23 months of accrual period and 11 months of follow-up period (maximum follow-up of 34 months). We assume an uniform accrual of 2.26 patients per month (both cohorts). A sample size of 52 patients will be needed to attain 80% power at nominal level of one-sided alpha of 0.05. Therefore, we expect to include a total sample of 52 patients, 39 of them will be AR-V7-negative and 13 AR-V7-positive patients.

<u>Safety follow-up extension of the study.</u>

The maximum follow-up of the study for primary and secondary efficacy analysis will be 34 months. However we will include a 2-years extension after the end of the treatment (April 2019) to evaluate bone lesions. Additionally, analysis of overall survival will be also extended.

8.3. *Statistical plan*

Unless otherwise mentioned all tests will be two-sided.

8.3.1. Primary efficacy endpoint

The primary efficacy endpoint is the median PFS achieved with radium-223 treatment. The PFS will be evaluated at the first 34 months period of the study. The PFS will not be evaluated at the 2-years extension of the study for safety. PFS is a composite endpoint defined as the time from the start of the radium-223 treatment to disease progression in bone or soft-tissue, symptoms, or death, according to the modifiedPCWG2 criteria. Objective radiographic disease progression is defined as the presence of at least one of thefollowing conditions:

- Bone lesion progression (appearance of ≥ two new bone lesions compared to baseline).
- Soft-tissue lesion progression according to the RECIST criteria version 1.1.
- Presence of symptomatic skeletal events.

Patients with no progression will be censored at the date of their last evaluation. Censoring rules are specified in Table 2.

Table 2. Censoring rules for PFS

Situation	Date of progression or censoring	Outcome
Progression documented between scheduled visits	Earliest of: • Date of radiological assessment showing new lesion (if progression is based on new lesion); or • Date of last radiological assessment of measured lesions (if progression is based on increase in sum of measured lesions); or • Bone lesion progression (appearance of ≥ two new bone lesions compared to baseline); or • Presence of symptomatic skeletal events (pathological fractures, spinal cord compression, radiation or surgery to bone)	Progressed
Death before first progression disease assessment	Date of death	Progressed
Death between adequate assessment visits	Date of death	Progressed
No progression	Date of last radiological assessment of measured lesions	Censored
Treatment discontinuation for undocumented progression	Date of last radiological assessment of measured lesions	Censored
Treatment discontinuation for toxicity or other reason	Date of last radiological assessment of measured lesions	Censored
Death or progression after more than one missed visit	Date of last radiological assessment of measured lesions	Censored

8.3.2. Secondary endpoints

All efficacy endpoints will be described in both study cohorts (AR-V7-negative and positive) and combining both cohorts.
All efficacy endpoints will be evaluated at the first 34 months period of the study. Additionally, OS will be evaluated at the 2-years safety extension after the end of the treatment. The other efficacy endpoints will not be evaluated at the 2-years safety extension.

-OS defined as the time from inclusion until death from any cause or the last date the patient was known to be alive.
Patients who are lost to follow-up and the patients who are alive at the date of data cut-off are censored at the date the patient was last known alive. Censoring rules are specified in **Table 3.**
- Time to first SSE defined as the time from treatment initiation untilSSE (pathological fractures, vertebral or non-vertebral, spinal cord compression, radiation or surgery to bone).For all other events, the start date of the event/medication/therapy was used as the time of the event. If an event has not occurred at the time of the analysis or the patient has been lost to follow-up, the time-to-event variables will be censored at the last disease assessment date.
- Alkaline phosphatase level response, normalization of alkaline phosphatase level(progression defined as FAelevation ≥ 25% after 12 weeks).
- The time from the first study drug administration to when PSA progresion was observed, defined as: 1) In subjects with no PSA decline from baseline; a greater than or equal to 25% increase from baseline value and an increase in absolute value of greater than or equal to 2 ng/mL, at least 12 weeks from baseline; 2) In subjects with initial PSA decline from baseline; the time from start of treatment to first PSA increase that is greater than or equal to 25% increase and at least 2 ng/mL above the nadir value, which was confirmed by a second value obtained three or more weeks later.

- Determination percentage of PSA progression(defined as PSA elevation ≥ 25% and ≥ 2 ng/mL after 12 weeks).

-Radiographic progression-free survival (rPFS) depending on AR-V7 status

Predictive biomarkers

- Assessment of AR-V7 mutation prior to the start of study treatment and at time to documented progression disease or treatment end (see Appendix 3).

-Determination changes in CTCs number prior to the start of study treatment and at time to documented progression disease or treatment end (see Appendix 3).

Table 3. Censoring rules for OS

Situation	End date	Status
Death during study follow-up	Date of death	Death
The patient is alive at last follow-up	Date of last follow-up	Censored

8.3.3. Analysis sets

We propose to test efficacy and safety of experimental treatment using the full analysis set. We include in the analysis all patients that accomplished selection criteria and receive one drug exposure.

8.3.4. Analysis of baseline and demographic variables

The demographics and baseline characteristics including disease history and prior therapy are summarized using descriptive statistics.

8.3.5. Efficacy analysis

Tumor response, bone lesion, SSE evaluation, biochemistry determinations,and predictive biomarkers are obtained at investigators' sites. There is not central and independent review of evaluations. Methodology for tumor assessment will be according tothe RECIST version 1.1 and the PCWG2 criteria.

8.3.6. Decision rules and adjustment of alpha for primary endpoint

The study will be declared positive if the median PFS of radium-223 in total sample is statistically significantly better compared with null hypothesis ($p<0.05$).

8.4. *Efficacy results*

Primary endpoint

We will analyze PFS in both cohorts with one arm Log-Rank test. This time endpoint will be described with Kaplan-Meier method. We will calculate number and proportion of events and median survival time, with corresponding 95% CI.

Secondary endpoints

We will describe the number and percentage of patients with AR-V7 mutation and CTCs at the start and at the end of the study. The evolution of these measures in the study will be analyzed with Mc Nemar's test and paired odds ratio (OR) with corresponding 95% CI.

OS, time to first symptomatic SSE, rPFS and alkaline phosphatase level response (AF) will be described with Kaplan-Meier method. Number and proportion of events and median survival time, with corresponding 95%CI, will be calculated.

Subgroup analysis

The AR-V7-negative and positive cohorts at baseline will be compared for all analysis. For timed endpoints (PFS, OS,time to first symptomatic SSE, TTP-AF, and TTP-PSA), we will use the Kaplan-Meier method and Log-Rank test. For binary outcomes we will use chi-squared or fisher´s exact tests.

Levels of CTCsat the start and at the end of the study will be correlated with timed and binary endploints. For timed and binary endpoints we will use cox analysis and logistica regression, respectively.

We also analyse PFS and OS with multivariable Cox proportional hazards models for adjusting for AR-V7 status, CTCs levels and relevant baseline covariates, including Gleason score, PSA level, number of prior hormonal treatments, the presence or absence of visceral metastases, the Eastern Cooperative Oncology Group (ECOG) score, prior use of abiraterone acetate or enzalutamide, and the level of full-length (FL) androgen receptor. All tests were two-sided, and P values of 0.05 or less were considered to indicate statistical significance. We will examine the residuals to assess model assumptions and only first-order interactions will be evaluated. We will calculate relative risk (RR) and HR with corresponding 95% CI to compare dichotomous and time to event variables, respectively. Up-to-date versions of R will be used to conduct analyses.

8.5. *Safety results*

All safety date will be evaluated at the first 34 months period of the study. Additionally, symptomatic skeletal related events will be evaluated at the 2-years safety extension after the end of the treatment.

Analysis of safety-related data will be considered at three levels:

- First, the degree of exposure (dose, duration, number of patients) will be assessed to determine the degree to which study safety can be assessed.
- Second, clinically relevant tests, concomitant medications, and reported AEs will be described. For AEs, severity, expectedness, causality, relationship, body system, action taken, and outcome will be reported.
- Third, SAEs, deaths, and study discontinuations for each study group will be described and assessed.

8.6. *Missing data management*

The analysis of the primary and secondary timed endpoints (progression and death) will be based on a Log-Rank or Cox regression tests and, therefore, not affected by patient withdrawals (as they will be censored) provided that dropping out is unrelated to prognosis. Patients with missing information in other outcomes, such as clinical benefit rate or overall response, will be considered as no responders. Furthermore, we will report reasons for withdrawal for each randomization group and compare the reasons qualitatively.

8.7. *Scientific committee review*

A Steering Committee (SC) has been established for this study. Initially, it is composed of the investigators, the study medical scientist, the Scientific Global Coordinator and two physicians expert in radium-223 management.

The SC will meet on demand to review, discuss and evaluate all of the gathered safety data. In case of any arising safety concern, these meetings can also be called at any time at request of a participating investigator. At these meetings, MedSIR and the participating investigators must reach a consensus on safety data. MedSIR will prepare minutes from these meetings and circulate them to each investigator for comment prior to finalization.

The study site Investigators and MedSIR will review patient data at least every four months. Each study site Investigator will monitor patients data for serious toxicities on an ongoing basis.

9. ETHICAL CONSIDERATIONS

9.1. *Regulatory and ethical compliance*

The study will be conducted and reported in accordance with the protocol, the International Conference on Harmonization (ICH) guidelines, the ethical principles laid down in the Declaration of Helsinki, European Clinical Trials Regulation (Regulation (EU) No. 536/2014), and any applicable regulatory requirement.

9.2. *EC*

The conduct of the study must be authorized by a duly constituted EC. Authorization is required for study protocol, protocol amendments, informed consent forms, patient information sheets, and promotional materials.

Wherever necessary, the investigator and/or Sponsor should contact the EC to ensure that accurate and timely information is being provided at every phase of the study.

The principal investigator and/or Sponsor are responsible for providing, when required, written summaries of the study status to the EC annually or more frequently, in accordance with the requirements, policies and procedures set out by the EC. The investigators are also responsible for immediately informing the EC of any protocol amendment.

In addition to reporting the AEs defined in the protocol to the Sponsor, the investigators must immediately reportany unexpected issues that entail a risk to patients to their respective ECs. Some ECs may request immediate reporting of all SAEs, while others may only require reporting of events if they are serious, related to the study treatment or unexpected. The Sponsor may provide the investigators with written safety reports or other safety-related reports. Safety reports should be made available to the EC for review and processing in accordance with the regulatory requirements and policies and procedures established by the EC and kept on file at the study site.

9.3. *Informed consent*

Before any protocol-related activity, the signed informed consent should be obtained from each patient. As part of this procedure, the study site investigator or his/her representative should explain verbally and in writing, the nature, duration and purpose of the study, as well as the action of the drugs so that the patient is aware of the possible risks, discomfort and adverse effects that may occur. Study patients will be informed of their right to withdraw from the study at any time and without any reason. The patient will receive all the information required by local regulations and ICH guidelines.

Consent forms must be signed and dated by the patient or their authorized legal representative before starting their participation in the study. Each patient’s medical history or medical records should include an entry detailing the process of obtaining informed consent and that it was obtained in writing before the patient’s participation in the study.

One copy of the signed consent form should be provided to the patient or the patient's authorized legal representative.

Signed and dated consent forms should be kept in each patient's study file. They should be made available to study monitors who may request them for review at any time.

Consent forms should be reviewed whenever changes have been made to the procedures described in the informed consent or when new information becomes available that may affect the patient's willingness to participate.

In case of review or update of consent forms, each patient's medical historyshould include an entry detailing the process of obtaining informed consent and that it was obtained in writing using the updated/reviewed consent form in order to continue their participation in the study. The final informed consent form approved by the EC should be provided to the Sponsor for regulatory purposes.

9.4. *Data protection*

The Sponsor will ensure confidentiality of the patient's medical information, in accordance with the applicable laws and regulations.

The study Sponsor, as data controller according to the European Directive on the protection of individuals with regard to the processing of personal data and on the free movement of such data confirms herewith compliance with Regulation (EU) 2016/679 of the European Parliament and of the Council, of 27 April 2016, on the protection of natural persons with regard to the processing of personal data and on the free movement of such data in all stages of data management.

The data generated by this study will be made available to the representatives of national and local Health Authorities, Sponsor monitors, Sponsor representatives, and partners, as well as the EC of each study site for review, as needed.

10. ORIGINAL DOCUMENTS, STUDY MONITORING AND QUALITY ASSURANCE

10.1. *Source data record*

Source data refers to all the information found in source files and certified copies of source files containing clinical findings, observations, or other activities that are part of the clinical study and which are necessary for study reconstruction and assessment. Source data is contained in source documents (comprising source documents and certified copies).

Source documents are original documents, data, and records (e.g., hospital records, medical histories and office records, laboratory notes, memoranda, patients' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, patients files, and records kept at

the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical study).

The Sponsor's quality assurance group may assist in determining whether electronic records generated using computerized medical record systems used at investigational sites can be considered source documentation for the purposes of this protocol.

If the site computerized medical record system has not been adequately validated for the purposes of clinical research (instead of general clinical practice), all paper source documentation should be kept on file to ensure that important protocol data entered on the CRFs can be verified.

At a minimum, source documentation must be available to substantiate patient identification, eligibility, and participation; proper informed consent procedures; dates of visits; adherence to protocol procedures; adequate reporting and follow-up of AEs; administration of concomitant medication; study drug receipt/dispensing/return records; study drug administration information; and date of completion and reason.

Data recorded on the CRF will be verified by checking the CRF entries against source documents (i.e., all original documents, laboratory reports, medical records) in order to ensure data completeness and accuracy as required by study protocol. The investigator and/or site staff must make CRFs and source documents of patients enrolled in this study available for inspection by Sponsor or its representative at the time of each monitoring visit.

The source documents must also be available for inspection, verification, and copying, as required by regulations, officials of the regulatory Health Authorities [e.g., US Food and Drug Administration(FDA), EMA, and others], and/or ECs. The study investigator and site staff must comply with applicable privacy, data protection, and medical confidentiality laws for use and disclosure of information related to the study and enrolled patients.

The patient must also allow access to the patient's medical record. Each patient should be informed of this requirement prior to study start.

10.2. *Study monitoring and source data verification*

Sponsor or its representative will monitor study progress as frequently as necessary to ensure:

- Protection of study patients' rights and well-being.
- Study data are accurate, complete, and verifiable from the source documents.
- Conduct of the trial is in compliance with the approved protocol/amendment, good clinical practices (GCP), and applicable regulatory requirements.

Study staff contact details are included in a document located on the investigator site file

Data recorded on the CRF will be verified by checking the CRF entries against source documents (i.e., all original documents, laboratory reports, medical records, patients' diaries) in order to ensure data completeness and accuracy as required by study protocol. The investigator and/or

site staff must make CRFs and source documents of patients enrolled in this study available for inspection by the Sponsor or its representative at the time of each monitoring visit.

10.3. *Record retention*

Investigators should retain all study records in a secure place with restricted access as per applicable regulatory requirements. The investigator must consult a Sponsor representative before disposal of any study records and must notify the Sponsor (of any change in the location, disposition, or custody of the study files.

Essential documents must be retained by the investigator for at least two years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or at least two years have elapsed since the formal discontinuation of clinical development of the investigational product. "Essential documents" are defined as those documents which individually and collectively allow for the assessment of the conduct of the study and the quality of the resulting data. These documents must be retained for a longer period if required by the applicable regulatory requirements or as a result of an agreement with the Sponsor. It is the Sponsor's responsibility to notify the investigator or site when these documents are no longer needed(ICH E6 Guidelines, 4.9.5).

The study site investigator should not dispose of records belonging to this study without first (1) obtaining written approval from the Sponsor (2) offering the Sponsor an opportunity to collect these records. The study site investigator will be responsible for retaining suitable and accurate electronic or paper copies of source documentation containing observations and data collected from this study.

10.4. *Data quality assurance*

During the study or upon its completion, the quality assurance auditor(s) designated by Sponsor or the competent authorities may wish to perform an audit of the sites. Investigators are expected to cooperate in these audits, offering their assistance, and providing auditors with the documentation they require (including source documents).

The Sponsor's representatives are responsible for contacting and visiting the investigator in order to inspect the facilities and, upon request, to inspect clinical trial records (e.g., CRF and other pertinent data), provided that patient confidentiality is respected.

The investigator agrees to cooperate with the monitor to ensure that all issues detected duringthese monitoring visits are resolved, including delays in completing CRFs.

In accordance with the ICH E6 Guideline for GCP and the Sponsor's audit plans, the Sponsor's Department of Quality Assurance or its representatives may perform an audit of this study. An inspection of site facilities (e.g., pharmacy, drug storage areas, laboratories) and review of study-

related records will be carried out in order to assess the conduct of the study and compliance with the protocol, the ICH guidelines for GCP (ICH E6), and relevant regulatory requirements.

11. DATA MANAGEMENT

11.1. *Data entry and processing*

In this study, the investigator and/or site staff will regularly enter all data into the CRFs.

The investigator must review all data included in the CRF to ensure its accuracy.

Reconciliation of the data will be performed by the designated CRO. At the end of the study, any protocol non-compliances will be identified and recorded as part of the clinical database. Once all of these tasks are complete and the accuracy and completeness of the database has been verified, it will be locked and the data will become available for data statistical analysis.

11.2. *Data clarification*

As part of the conduct of the trial, Sponsor may have questions about the data entered by the site, referred to as queries. The monitors and the Sponsor are the only parties that can generate a query.

11.3. *Data coding procedures*

Coding of AEs, medical history, and prior and concomitant medications will be performed using standard dictionaries described in the data management plan.

12. STUDY MANAGEMENT

12.1. *Study discontinuation*

MedSIR reserves the right to discontinue the study at any time for safety or administrative reasons. If the study is discontinued and/or the site is closed for any reason, all of the investigational study drugs can be destroyed locally at the site according local law regarding radioactive products) . All of the actions needed to assess or maintain study patient safety will continue as required, despite study discontinuation.

12.2. *Protocol amendments*

Any change or addition to the protocol requires a written protocol amendment or administrative letter that must be approved by Sponsor, the global scientific coordinator, the study site investigator, and the EC/Health Authorities when applicable. This requirement should not, under any circumstances, prevent any immediate action taken by the study site investigator or Sponsor to ensure the safety of all the patients enrolled in the study. If, in the opinion of the study site investigator, an immediate protocol amendment is required and it is applied for safety reasons,

this process should be reported to Sponsor as soon as possible (within 24hours) and the EC will be informed, as needed.

12.3. *Publication policy and protection of trade secrets*

All of the data generated by this study should be considered highly confidential and should not be disclosed to persons who are not directly related to the study without prior written permission from the global scientific coordinator and Sponsor. Nevertheless, full access is granted to authorized officials of Health Authorities, the global scientific coordinator or study site investigator, and Sponsor staff (or their representatives) for record inspection and copying. All of the investigational products, patient body fluids and/or other collected materials will only be used in accordance with this protocol, unless otherwise agreed in writing between the global scientific coordinator or study site investigator and Sponsor.

The Sponsor will ensure that, as far as possible, the study results are published in the form of scientific/clinical articles in prestigious scientific peer-reviewed journals. These documents will be prepared with the full participation of the principal investigators and in accordance with applicable guidelines for good publication practice and with international recommendations, such as those from the International Committee of Medical Journal Editors (ICMJE) and all elements of the Consort Statement (2010).

The Sponsor will be notified of any attempt to publish data obtained during the study and permission to do so must first be obtained from the Sponsor before publication.

12.4. *Insurance*

The Sponsor contracts an insurance policy to cover the responsibilities of the investigator and other parties participating in the study, according to the applicable Spanish legislation.

- Insurance company: CHUBB Insurance Company of Europe SE. c/ José Abascal, nº56, 5a planta, 28003 Madrid (Spain).

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Appendix 1: Schedule of study assessments and procedures

Study Period	**Screening**	**Treatment period**		**Follow-up period**		
Day	**-28 to -1**	**Each cycle* (every 28 days)**	**End of treatment ******	**Every 3 months (Efficacy FUP)[12]**	**At time of DP****	**EoS (Safety FUP) [13]**
Informed Consent[1]	X					
Baseline signs/symptoms	X	X	X	X	X	
Check of inclusion/exclusion criteria	X					
Brief pain inventory (short form) (BPI-SF)	X					
Medical History[2]	X					X
Physical Examination and ECOG status[3,4]	X	X	X	X	X	

Study Period	**Screening**	**Treatment period**		**Follow-up period**		
Day	**-28 to -1**	**Each cycle* (every 28 days)**	**End of treatment ******	**Every 3 months (Efficacy FUP)[12]**	**At time of DP****	**EoS (Safety FUP) [13]**
Concomitant Medication Reporting[5]	X	X	X	X	X	
Health-related quality of life (HRQoL) questionnaire[6]	X	X	X	X	X	
AE reporting[7]	X	X	X	X	X	X
Radiological Tumor Assessments[8]	X	every 3 cycles	X	X	X	
Standard Laboratory Procedures						
Hematology[9,^]	X	X	X	X	X	
Biochemistry[10, ^]	X	X	X	X	X	
PSA determination[^]	X	X	X	X	X	

Study Period	**Screening**	**Treatment period**		**Follow-up period**		
Day	**-28 to -1**	**Each cycle* (every 28 days)**	**End of treatment ******	**Every 3 months (Efficacy FUP)[12]**	**At time of DP****	**EoS (Safety FUP) [13]**
Testosterone determination^	X					
Treatment Administration						
Radium-223 at dose of 55 kBq/Kg one IV injection		D1 (for a total of six IV injections)				
Research Central Laboratory Procedures						
Blood sample collection (split variant AR-V7 determination)[11]	X		X		X	
Blood sample collection (CTC count)[11]	X		X		X	

* All visits must occur within ± 7 working days from the scheduled date.

** If disease progression coincides with the end of treatment visit, the evaluations planned for both visits will coincide, they will only be performed once (including blood sample for central laboratory).

^ Local laboratory tests scheduled for day 1 of all cycles must be performed within 72 hours prior toconfirm to the patient if treatment can be followed up.

1. Signed written informed consent obtained prior to any trial-specific procedure.
2. Complete medical history and demographics (including age, gender, and ethnic origin) and all medications taken the last 28 days prior to enrollment will be collected.
3. Physical examination and vital signs (including respiratory rate, blood pressure measurements, heart rate, and body temperature) and weight measurement will be performed prior to enrollment.
4. ECOG performance status will be assessed before trial drug adminstration and before the administration of each cycle.
5. Relevant concomitant medication will be recorded at screening and on an ongoing basis.
6. FACT-P, Version 04
7. All AEs occurring during the trial and until the treatment discontinuation visit (end of study visit) have to be recorded with grading according to NCI-CTCAE version 4.0.
8. Baseline assessments of the torax, abdomen, and pelvis (preferably CT or MRI in case of contrast allergy) must be performed no more than 28 days before the first trial treatment. Baseline bone scan must be performed no more than 28 days before the first trial treatment. Post-baseline assessments have to be performed every 12 weeks ± 7 working days after the first dose using the same imaging method and where possible obtained at the same institution for an individual patient as used during screening until progression disease. Radiographic bone progression will require the persistence of new lesions on a confirmatory scan at least six weeks later. In those patients who receive 6 treatment cycles, the radiological tumour assessment corresponding to week 24 will be performed for the EOT visit.
9. Hematological test will be performed as per local standard of care and clinical indication before treatment administration. These values should be included: hemoglobin, hematocrit, red blood cell count, platelet count, and white blood cell count with differential count (neutrophils, lymphocytes, monocytes, eosinophils, and basophils).
10. Biochemistry test will be performed as per local standard of care and clinical indication before first treatment administration and first day of follow-up. These values should be included: coagulation, chemistry with renal function analysis (serum creatinine, creatinine clearance according to the Cockroft-Gault formula), liver function (AST, ALT, ALP, direct and total bilirubin), glucose, sodium, potassium, calcium, chloride, magnesium, uric acid, total protein, albumin, and lactate dehydrogenase.
11. Blood sample for central laboratory prior to the start of study treatment, at the end of treatment and at time to documented progression disease (if the patient ends treatment due to progressive disease, only one blood sample will be sent at this point).
12. After end of treatment (if no progressive disease documented), efficacy follow-up visits will be performed every 3 months including radiological tumour assessments until disease progression.
13. After disease progression, a safety follow-up visit will be performed within three following months and 24 months after last dose of treatment to evaluate symptomatic skeletal related events, overall survival and post-study anticancer therapy.

Appendix 2: Response Evaluation Criteria in Solid Tumors Guidelines (RECIST criteria) (version 1.1)

Eisenhauer, E.A., et al., *New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1).* Eur J Cancer, 2009. 45(2): p. 228-47.

Appendix 3: Instructions for blood sample collection. Detection of CTCs

CTC analysis will be conducted using high definition system to identify CTCs(HD-CTCs). It is distinct in that it does not rely on any single protein enrichment strategies. Instead, all nucleated cells are retained and immunofluorescently stained with monoclonal antibodies targeting cytokeratin (CK), an intermediate filament found exclusively in epithelial cells, a pan leukocyte specific antibody targeting CD45, and a nuclear stain, DAPI. All nucleated blood cells are imaged in multiple fluorescent channels to produce high quality and high resolution digital images that retain fine cytologic details of nuclear contour and cytoplasmic distribution. This enrichment-free strategy results in high sensitivity and high specificity, while adding high definition cytomorphology to enable detailed morphologic characterization of a CTC population known to be heterogeneous. A key advantage of this approach is that multiple analysis parameters can be pursued to identify and characterize specific populations of interest.

Blood samples (10 mL) will be collected using standard streck cell-free DNA BCT by venipuncture. The first 5 ml of blood collected from the fresh venipuncture cannot be used for the collection into de Streck tubes due to possibility of contaminating epithelial cells during venipuncture. These samples will be stored at room temperature and ship on day of collection in shipper at ambient temperature (date and time of draw are required) to Covance Central Laboratory Services (Switzerland) and they will be received and processed within 24 hours.

<u>Blood sample processing for HD-CTC detection</u>

Blood specimens will be rocked for five minutes before a WBC will be measured using the Hemocue white blood cell system (HemoCue, Sweden). Based upon the WBC count, a volume of blood will be subjected to erythrocyte lysis (ammonium chloride solution). After centrifugation, nucleated cells will be re-suspended in PBS and attached as a monolayer on custom made glass slides. The glass slides will be the same size as standard microscopy slides but have a proprietary coating that allows maximal retention of live cells.

Each slide can hold approximately three million nucleated cells, thus the number of cells plated per slide depend on the patients WBC. For HD-CTC detection, four slides will be used as a test. The remaining slides created for each patient will be stored at −80°C for future experiments.

Four slides will be thawed from each patient, then cells will be fixed with 2% paraformaldehyde, permeabilized with cold methanol, and non-specific binding sites will be blocked with goat serum. Slides will be subsequently incubated with monoclonal anti-pan CK antibody (Sigma) and CD45-Alexa 647 (Serotec) for 40 minutes at 37°C. After PBS washes, slides will be incubated with Alexa Fluor 555 goat anti-mouse antibody (Invitrogen) for 20 minutes at 37°C. Cells will be counterstained with DAPI for 10 minutes and mounted with an aqueous mounting media.

Detection of AR-V7 in CTCs

mRNA expression analyses will be performed using the ProstateCancerDetect kit with multiplexed reverse-transcription polymerase-chain-reaction (qRT-PCR) primers to detect the presence of CTCs, and custom primers designed to detect the full-length-AR (AR-FL) and AR AR-V7. The relative AR-V7 transcript abundance will be determined by calculating the ratio of AR-V7 to AR-FL.

Details will be provided in the study laboratory manual. All samples will be labelled with patient study number, date and time of collection.

Appendix 4: Principal investigators