REASURE

Radium-223: Evaluation of Activity and SUrrogate REsponse

PROTOCOL

Version: 7.0
Dated: 06 August 2021

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Co-Sponsors:	The Institute of Cancer Research/The Royal Marsden NHS Foundation Trust
Funder:	Bayer HealthCare Pharmaceuticals Inc.
Coordinating Trials Unit:	ICR Clinical Trials and Statistics Unit (ICR-CTSU) The Institute of Cancer Research

EudraCT Number:	2013-004055-20
REC Reference Number:	14/LO/1385
ICR-CTSU Protocol Number:	ICR-CTSU/2013/10040
ISRCTN:	ISRCTN17805587

The REASURE trial is part of the National Institute for Health Research Clinical Research Network Trial Portfolio

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The Trial Management Group (TMG) will be constituted from members of the Protocol Development Group and Principal Investigators from a subset of participating sites. A copy of the current membership of the TMG can be obtained from the REASURE Trial Manager at ICR-CTSU.

Protocol Authorised by:

Name & Role	Signature	Date
Dr Chris Parker (Chief Investigator)		09/08/2021

This protocol describes the REASURE trial and provides information about procedures for entering participants into this trial. The protocol should not be used as a guide for the treatment of patients outside of this trial.

Every care was taken in the preparation of this protocol, but corrections or amendments may be necessary. Protocol amendments will be circulated to participating sites as they occur, but sites entering patients for the first time are advised to contact ICR-CTSU to confirm they have the most recent version.

CONTENTS

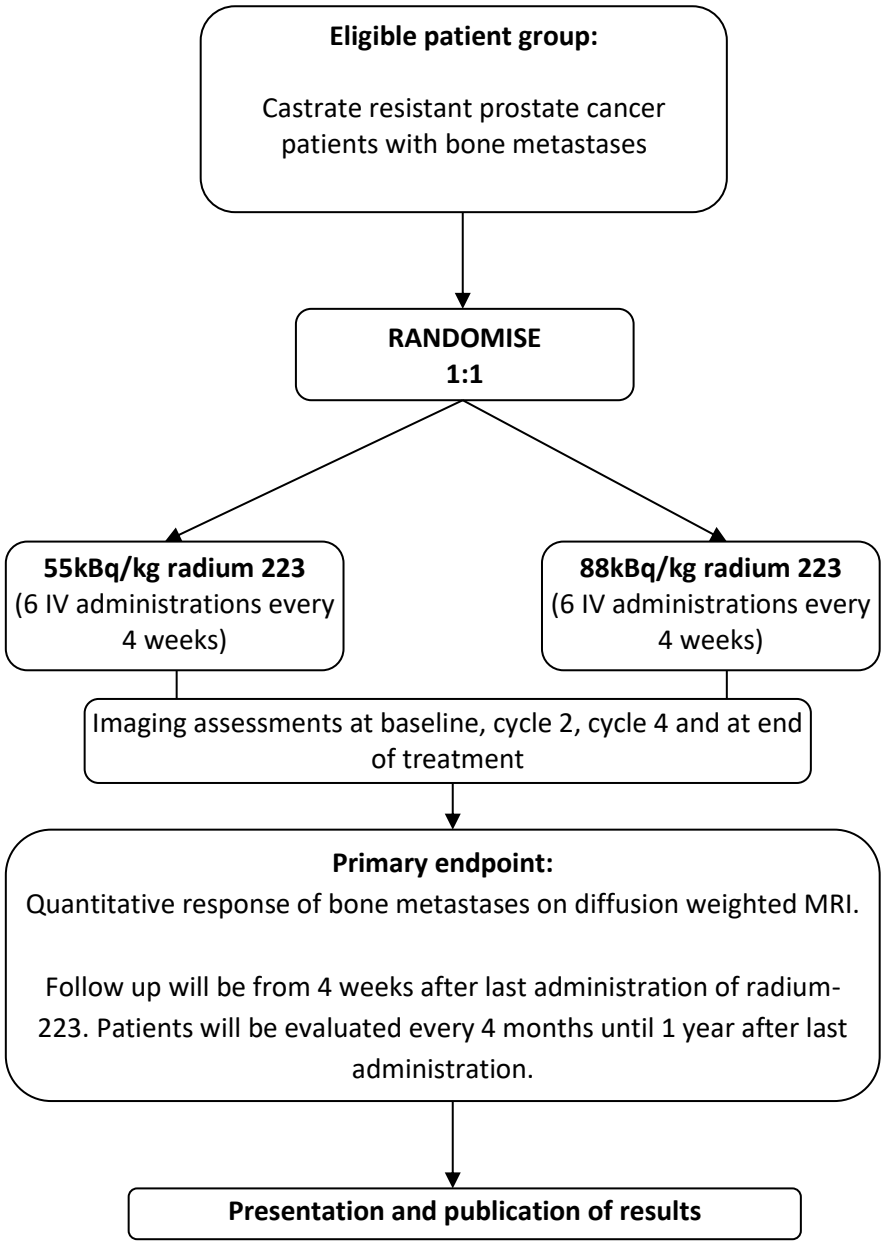
REASURE TRIAL SUMMARY

PROTOCOL TITLE	A phase II open-label study of biomarkers to assess response in patients with metastatic castration resistant prostate cancer treated with radium-223.
STUDY OBJECTIVES	• Evaluate treatment response by whole body diffusion-weighted MRI. • Evaluate treatment response by 18F fluoride and 18F choline PET/CT. • Evaluate treatment response by circulating biomarkers including serum and urinary markers of bone turnover: alkaline phosphatase (ALP), n-telopeptide, PSA, CTCs, plasma tumour DNA. • Explore anti-tumour activity and tumour cell DNA damage by evaluating measures of proliferation, apoptosis, DNA damage and DNA repair utilising Ki67, caspase cleaved cytokeratin, γH2AX and RAD51 foci formation in bone marrow. • Explore relationship between radium-223 dose and response in terms of functional imaging and circulating biomarkers. • Evaluate treatment safety with respect to radium-233 dose. • To explore the incidence of fractures occurring during and after radium-223 treatment. • Explore the relationship between response in terms of functional imaging and overall survival
STUDY DESIGN	Phase II randomised multicentre trial.
TRIAL POPULATION	Castration resistant prostate cancer patients with bone metastases.
RECRUITMENT TARGET	38
TREATMENT REGIMEN	Radium-223 55kBq/kg (standard dose) or 88kBq/kg, given as slow bolus intravenous injection, 6 times, at intervals of 4 weeks. The treatment is given on an outpatient basis under supervision of a nuclear medicine specialist.
PRIMARY ENDPOINT	The proportion of patients showing bone metastases response on diffusion weighted MRI (DW-MRI).
SECONDARY ENDPOINTS	• Quantitative and qualitative assessment of treatment response using DW-MRI • Quantitative and qualitative assessment of treatment response using PET imaging • Quantitative response in circulating/urinary biomarkers (ALP, n-telopeptide, PSA, CTCs) • Time to PSA progression • Time to total-ALP progression • Time to first new bone fracture
EXPLORATORY ENDPOINTS	• Bone marrow samples: evidence and degree of anti-tumour activity and tumour cell DNA damage using measures of proliferation, apoptosis, DNA damage and DNA repair including Ki67, caspase cleaved cytokeratin, γH2AX and RAD51 foci formation in bone marrow

	• Molecular characterisation of CRPC cells, associating disease subtype with anti-tumour activity • Quantitative and qualitative assessment of treatment response depending on dose cohort (55kBq/kg or 88 kBq/kg radium-223) and individual administered dose • Changes in PSA • Changes in total ALP • Quantitative and qualitative assessment of treatment response using DW-MRI according to baseline ALP level. • Correlation between treatment response as measured by regional bone plasma clearance (K_i) and conventional SUV parameters • Association of mean density of lesions on baseline CT scan with response determined by WB DW-MRI • Association of overall survival with response according to DW-MRI and PET-CT
FOLLOW UP	From 4 weeks after last administration of radium-223. Patients will be evaluated every 4 months until 1 year after last administration. Patients will continue to be followed up for overall survival beyond 1 year post-treatment.

TRIAL SCHEMA

Figure 1: Diagram to show eligible patient population, treatment interventions and follow up.



1. INTRODUCTION

1.1. Background

Prostate cancer is the most common cancer in men worldwide and is one of the leading causes of cancer-related morbidity and death. Bone is the commonest site of metastases in castration resistant prostate cancer (CRPC). It is a major problem which impacts on survival and quality of life. Untreated patients face severe morbidity, including pain, fracture, spinal cord compression and the haematological consequences of bone marrow involvement. If treated successfully, these symptoms can be palliated and patients may also gain a survival benefit.

Initial treatment is with androgen deprivation, with the aim of reducing testosterone and its metabolite dihydro-testosterone. This is achieved surgically by bilateral orchidectomy (castration), or medically using LHRH-receptor agonists. Androgen-receptor antagonists (e.g. bicalutamide) are frequently used concomitantly with LHRH treatment to achieve combined androgen blockade (CAB). Patients however ultimately stop responding to this hormone therapy after variable periods of time. Those undergoing CAB at the time of progression may still have short-term response to anti-androgen withdrawal. Patients however continue on LHRH treatment.

Until recently there had been limited subsequent treatment options with proven survival benefit for these patients. Docetaxel with prednisone is currently standard first-line therapy in the US and Europe with a reported 2.4 month median survival benefit versus prednisone and mitoxantrone chemotherapy [1]. In 2010, cabazitaxel, another taxane agent, gained FDA approval following the results of the TROPIC study [2]. 755 patients pre-treated with docetaxel were randomly assigned to either mitoxantrone with prednisolone or cabazitaxel with prednisolone. Those receiving cabazitaxel had a median survival benefit of approximately 3 months.

Abiraterone acetate also has had recent approval for use. It demonstrated survival benefit in CRPC following chemotherapy in a phase III study (14.8 months vs. 10.9 months; hazard ratio, 0.65; 95% confidence interval, 0.54 to 0.77; P<0.001) [3]. Results of MDV3100, a novel androgen receptor antagonist, in phase III studies show significant median survival benefit following chemotherapy of 4.8 months (hazard ratio 0.63; confidence interval, 0.53 to 0.75; P<0.001) [4-6].

There are 2 classes of agents that have been in development to specifically target bone metastases in prostate cancer; those that affect bone resorption and radiopharmaceuticals. Zoledronic acid is a bisphosphonate that has been shown to delay skeletal related events by inhibiting osteoclast mediated bone resorption [7]. However these results may not apply to other bisphosphonates. Use of pamidronate was not positive in the same setting [8]. Denosumab is a human monoclonal antibody against RANKL which has proved more effective than zolendronic acid for skeletal related event (SRE) prevention [9]. Radiopharmaceuticals such as strontium-89 and samarium-153 EDTMP have been developed predominantly for palliation of pain. Radium-223 is a novel alpha emitting radiopharmaceutical. It has bone targeting properties similar to that of other earth alkaline elements like strontium, but radium-223 has demonstrated improvement in survival in patients with CRPC [10, 11]. Radium-223 dichloride was approved by the FDA for the treatment of patients with metastatic castration resistant prostate cancer with bone metastases in May 2013.

The radiation characteristics of alpha-particle emitting nucleotides have several advantages over beta-emitting nucleotides. Radium-223, with a physical half-life of 11.4 days, emits high linear energy transfer (LET) alpha radiation, with a range limited to less than 100 micrometers, and so can be used to generate much more localised radiation zones, reducing exposure of surrounding normal tissue compared to beta emitters. Biodistribution studies have also shown that radium-223 is selectively concentrated in bone compared to soft tissues, and that radium-223 and its progeny are retained in the bone matrix. Due to increased bone metabolism in skeletal metastases, preferential uptake is observed in these lesions compared to normal bone [12, 13].
Radium-223 was brought into clinical development in August 2001. A phase I clinical study in patients with skeletal metastases from breast and prostate cancer was conducted to evaluate whether the product could be administered safely at therapeutically relevant doses. A total of 31 patients were enrolled. 25 patients received a single intravenous injection in the dose escalating part of the study, with 5 patients at each dose level at 46,

93, 163, 213 and 250 kBq/kg b.w. radium-223. The patients were dosed as planned and followed for a period of 8 weeks. Modest and reversible haematological toxicity was observed, which was more pronounced in the higher dose groups. Dose limiting haematotoxicity was not observed. No additional myelosuppression was seen on multiple dosing. No clinically significant trends in serum chemistry parameters were observed during the 8 week period. Transient diarrhoea (Common Terminology Criteria for Adverse Events (CTCAE) grade 1-2) which responded well to medication was reported by some patients in all dose groups and was the most frequent adverse event. Nausea, vomiting and fatigue were reported more frequently by patients in the higher dose groups.

A phase II randomised double blind study, conducted to evaluate the efficacy of 25kBq/kg, 50kBq/kg and 80kBq/kg radium-223 given at 6 week intervals in those with CRPC and bone metastases, demonstrated a dose dependent effect which was well tolerated even at the highest dose level [14].

Radium-223 continued its clinical development with a placebo-controlled randomised phase II trial (BC1-02). Sixty-four CRPC patients with painful bone metastases were randomised to receive either radium-223 or placebo (saline), after palliative external radiotherapy, in a blinded design. The patients received multiple dosing of either 50 kBq/kg b.w. radium-223 or saline 4 times at 4 week intervals. All patients were followed for 1 year without breaking the blinding code. There were no treatment discontinuations due to haematotoxicity and no evidence of cumulative toxicity with repeated treatment. The results showed a significant improvement in serum biomarkers for bone turnover, i.e. ALP (the study primary efficacy endpoint) and delayed time to PSA progression with improved median overall survival at 2 years (65.3 weeks for radium-223 versus 46.3 weeks for placebo, p=0.017 based on an ITT population and adjusting for baseline covariates) [15].

Subsequent phase III results have confirmed the overall survival benefit. The ALSYMPCA trial (NCT00699751) recruited 922 patients who were randomised 2:1 to 50kBq/kg radium-223 or placebo. The trial was stopped in 2011 when the pre-planned interim analysis showed significant improvement in survival; patients in the radium-223 group had longer median survival than those in the placebo group (14.0 months versus 11.2 months; hazard ratio, 0.70; 95% confidence interval, 0.55 to 0.88; p = 0.002). There was also significant prolonged time to first SRE (13.6 months versus 8.4 months; hazard ratio 0.610; 95% confidence interval 0.46 to 0.81; P = 0.00046) [10, 11, 16].

1.2. Known Risks and Benefits of Radium-223

Established benefits of treatment with radium-223 include:

- Prolongation of overall survival
- Delay of skeletal related events
- Improvement in quality of life
- Anti-tumour efficacy as measured by PSA
- Palliation of bone pain

The results from phase III trials of radium-223 have also demonstrated a favourable safety profile. Expected side effects from treatment (all grades) include:

- Gastrointestinal: constipation (18%); transient diarrhoea (25%); nausea (36%) and vomiting (18%).
- Potential temporary increase in bone pain a few days after injection (50%).
- Transient reduction in neutrophil count. Mild to moderate reversible myelosuppression may occur. Haematologic toxicity consisted of neutropenia in 5% of patients and thrombocytopenia in 12%.

Grade 3-4 toxicities reported in the ALSYMPCA trial are listed below. With the exception of bone pain, grade 3-4 toxicities were uncommon. All reported grade 5 toxicities occurred in ≤1% of patients. Only 1 grade 5 adverse event was considered to be possibly related to the study drug (thrombocytopenia, reported in 1 patient receiving radium-223) [16].

Event	Radium-223 N-=600		Placebo N=301	
	Grade 3	Grade 4	Grade 3	Grade 4
Haematologic				
Anaemia	65 (11%)	11 (2%)	37 (12%)	2 (1%)
Neutropenia	9 (2%)	4 (1%)	2 (1%)	0
Thrombocytopenia	20 (3%)	18 (3%)	5 (2%)	1 (<1%)
Non-haematologic				
Bone pain	120 (20%)	5 (1%)	74 (25%)	3 (1%)
Diarrhoea	9 (2%)	0	5 (2%)	0
Nausea	10 (2%)	0	5 (2%)	0
Vomiting	10 (2%)	0	7 (2%)	0
Constipation	6 (1%)	0	4 (1%)	0

Radium-223 may in the longer term induce other primary cancers and bone marrow changes however, from the data to date (over 2 years), no long term toxicity has been reported.

In November 2017, it was reported that an increased incidence of deaths and fractures had been identified in a randomised clinical trial of radium-223 in combination with abiraterone acetate and prednisolone/prednisone to treat mCRPC (ERA223, NCT02043678 [17]). In March 2018, Bayer HealthCare Pharmaceuticals Inc (Bayer), as the marketing authorisation holder for radium-223, advised that patients who had received radium-223 should be followed up to assess bone fractures beyond the treatment period and for a minimum of 1 year, but preferentially for 3 years.

As for all radiopharmaceutical agents, written instructions concerning safety precautions will be given to the patients before administration and to the hospital staff before handling and subsequent disposal of radioactive products. Haematological parameters should be closely followed as a safety measure.

1.3. Assessment of Response

1.3.1. Diffusion-weighted MRI

Diffusion-weighted MR imaging (DW-MRI) is a relatively new imaging technique that has shown substantial promise for the detection of metastatic bone disease in patients with prostate cancer, and also for the assessment of treatment response.

Tissue characterisation with DW-MRI is possible based on differences in the mobility of water between tissues. Cellular tumour tissues show greater barrier to water motion, and this impeded water mobility can be quantified by measuring the apparent diffusion coefficient (ADC; unit $\mu m^2/s$) of tissues.

DW-MRI measurements are relatively quick to perform, and do not require the administration of any exogenous contrast medium. A typical whole body (skull base to mid-thigh) DW-MRI study will take approximately 45 minutes and can be used to map sites of metastatic bone disease in the body. An initial pilot study in a group of patients receiving radium-223 treatment showed that the technique can depict sites of metastatic bone disease as well as 18NaF-PET imaging (pilot unpublished data).

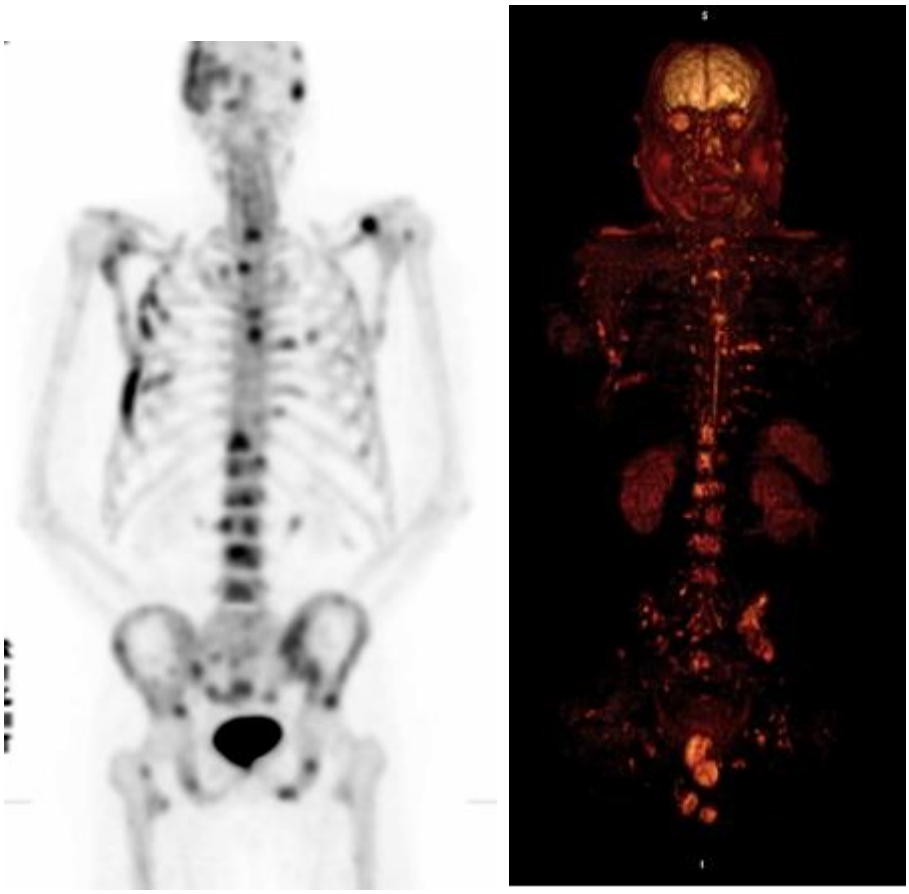


Figure 2: (Left) 18NaF-PET image showing areas of increased tracer activity at metastatic sites involving multiple ribs, spine, pelvis and both scapula. (Right) Colourised maximum projection diffusion-weighted MR image showing good anatomical correspondence of the sites of metastatic bone disease. False negative areas include the skull vault and base, and the anterior ribs. However, normal soft tissue structures such as the brain, kidneys, spleen and testes are also visible on DWI whereas renal excretion is seen on 18NaF-PET.

Following successful therapy, the ADC of metastatic bone lesions significantly increases as early as 1 month after the initiation of treatment. Radiotherapy has been shown to result in significant cell kill resulting in a rise in ADC values. This has also been observed in our study population (Figure B). Total body DW-MRI thus has the potential to be an important response biomarker in patients with metastatic bone disease from prostate cancer.

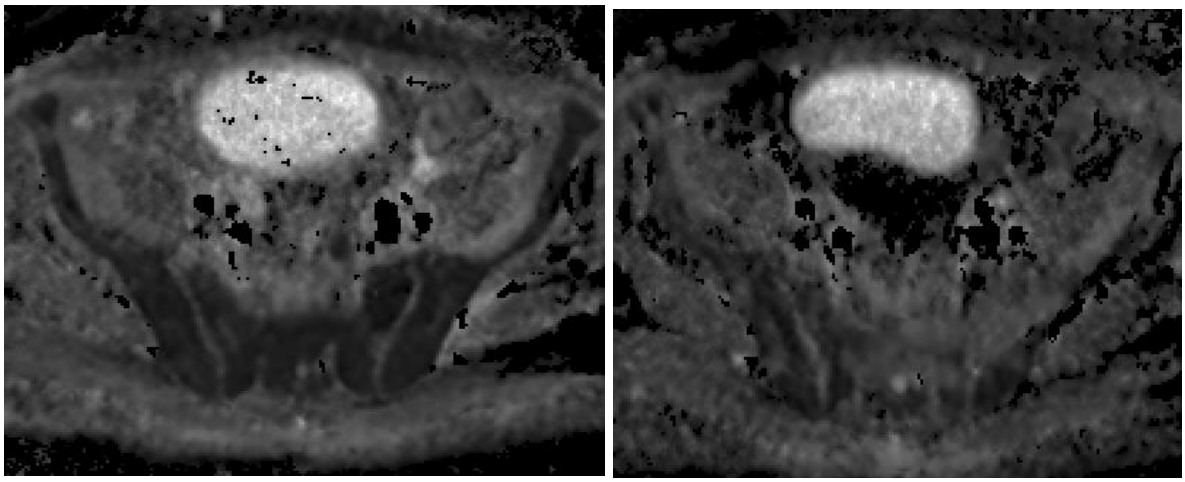


Figure 3: ADC maps of a patient before and at 8 weeks after treatment. Note initial relatively low ADC of diseased marow, which becomes ”brighter” with higher ADC values after novel targeted therapy.

1.3.2. PET/CT: 18F Fluoride and 18F Choline

Bone scan with 99mTc-methylene diphosphonate (MDP) has limited sensitivity to measure treatment effect, with only 52% of responders showing scintigraphic improvement and 62% of non-responders showing scintigraphic deterioration at 6-8 months [18]. In an attempt to increase sensitivity of response measurement, some authors have described various semi quantitative means of following bone metastases with bone scintigraphy [19-22]. However, others have found no advantage over visual interpretation [23] and so quantitative methods have not gained acceptance into routine practice.

Although 18-FDG is the most commonly used tracer in oncological PET/CT assessment, its use in prostate cancer has demonstrated low sensitivity and specificity. In the metastatic setting, sensitivity and specificity is 50% when the PSA exceeds 4ng/ml and 26% and 33% respectively when the PSA is lower than this [24, 25]. 18-FDG can be effective therapy monitoring method but only in patients who are initially positive for FDG uptake. Other tracers have been developed to overcome this.

18F-sodium fluoride is a bone-seeking radiopharmaceutical which accumulates in sites of increased bone formation reflecting sites of increased osteoblastic activity occurring with metastases. It has been shown to have greater diagnostic accuracy as compared to 99mTc-MDP planar or single photon emission computed tomography (SPECT) in prostate and other cancers [26-30]. 18F-fluoride was first described as a bone imaging agent nearly 40 years ago. It is only with recent improvements in PET imaging equipment and image quality that there has been renewed interest in this tracer [31]. PET also offers the inherent advantage of superior quantitative accuracy over planar or SPECT scintigraphy. The study team postulate that semi quantitative 18F-fluoride PET might allow accurate and timely measurement of treatment response in bone metastases from prostate cancer. A pilot study of 5 patients supports 18F-fluoride PET as a potential biomarker for monitoring treatment response in bone metastases following treatment with radium-223 [32]. Further larger prospective studies are required to confirm these observations and to evaluate this method with different tumour and treatment types.

Phosphatidyl choline is an essential component of cell membranes. Malignant tumours have a high turnover of cellular membranes representing their increased proliferation rate. Prostate cancer has high expression of choline transporters and choline kinase which is essential for the transformation of choline into phosphatidyl choline. This forms the basis of 18F choline use in prostate cancer assessment [33]. 18F choline has been extensively evaluated in all stages of prostate cancer [33-35] but there are only small proof of principle studies evaluating therapy response; all showing reductions in choline uptake with successful therapy.

1.3.3. Circulating Biomarkers

Historically the serum biomarker associated with prostate cancer is PSA [36]. Short term changes in PSA, especially during the first 12 weeks of treatment, may not be indicative of treatment response [37]. Other markers may better represent potential response including intact circulating tumour cells (CTCs) and CTC fragments or exosomes [38].

The FDA has approved a CTC system (CellSearch©) as a prognostic indicator for patients with metastatic breast, colon and prostate cancer. A relationship between post-therapy CTC counts and overall survival has been demonstrated in those with CRPC. It was also found to be better at predicting overall survival than PSA algorithms [39].

1.4. Study Rationale

The evaluation of bone predominant disease in patients with metastatic prostate cancer remains challenging. As yet, there is no reliable method to assess and quantify treatment response. Therefore bone metastases are often regarded as non-measurable disease by standard RECIST (v1.1)/PCWG2 criteria [40]. Criteria exist which use radiographic changes to measure response but these are relatively insensitive, taking a number of months for changes to occur [41]. In addition, the criterion of sclerosis of previously osteolytic metastases is not relevant for metastatic disease that is predominantly sclerotic at baseline (as is common in prostate cancer). Given that assessment of response of bone metastases is insensitive and often non-specific, in practice a combination of clinical, biochemical (e.g PSA and ALP) and radiological measures are currently used. However, more accurate means of monitoring response are required to inform early treatment failure or success.

Radium-223 was the first bone targeted alpha emitter to be tested in phase III studies. It has demonstrated improved survival for this group of patients with a favourable risk/benefit profile. Radium-223 was approved by the FDA in May 2013 for the treatment of CRPC with bone metastases and was granted marketing authorisation in the EU in November 2013. It is currently authorised to be marketed in more than 43 countries. The present study will explore the role of functional imaging and circulating biomarkers to assess response to radium-223 and potential dose-response relationships.

2. TRIAL OBJECTIVES

2.1. Primary Objective

To evaluate treatment response to radium-223 by whole body diffusion-weighted MRI.

2.2. Secondary Objectives

- To evaluate treatment response to radium-223 by 18F fluoride and 18F choline PET/CT.
- To evaluate treatment response by circulating biomarkers including serum and urinary markers of bone turnover: alkaline phosphatase (ALP), n-telopeptide, PSA, CTCs, plasma tumour DNA.
- To evaluate treatment safety with respect to radium-233 dose.

2.3. Exploratory Objectives

- To explore anti-tumour activity and tumour cell DNA damage by evaluating measures of proliferation, apoptosis, DNA damage and DNA repair utilising Ki67, caspase cleaved cytokeratin, γH2AX and RAD51 foci formation in bone marrow.
- To explore the relationship between radium-223 dose and response in terms of functional imaging, tumour molecular characterisation and circulating biomarkers.
- To explore the predictive value of ALP as a predictive biomarker for response to radium-223 using DW-MRI.
- To compare the ability of evaluating treatment response to radium-223 using changes in regional bone plasma clearance (K_i) with conventional SUV parameters.
- To explore the incidence of symptomatic and asymptomatic fractures occurring both during and after radium-223 treatment.
- To explore the relationship between response in terms of functional imaging and overall survival.

3. TRIAL DESIGN

This is a prospective phase II, multi-centre, randomised trial of radium-223 incorporating functional MRI and PET imaging, bone marrow samples and blood and urine collection with the primary objective of identifying potential imaging response markers.

Patients will be randomised to receive radium-223 treatment at 55 kBq/kg or 88 kBq/kg slow bolus intravenous injection 6 times, at intervals of 4 weeks. This will allow exploration of the exposure and response relationship between the 2 different dose cohorts. Follow-up will be from 4 weeks after last administration of radium-223. Patients will be evaluated every 4 months until 1 year after last administration.

Adverse events, including toxicity to treatment will be assessed according to The National Cancer Institute (NCI) Common Terminology Criteria (CTC) Version 4 (http://ctep.cancer.gov/reporting/ctc.html).

Please note (REASURE protocol v5.0 and as subsequently amended): A discrepancy of approximately 10% between the published National Institute of Standards and Technology (NIST) primary standardisation [42] and current measurements was confirmed in 2015 and a revised NIST primary reference standard issued. As a result of the revised NIST primary standardisation, an adaption of the numerical description of patient dose and the description of radioactive concentration of the drug product solution is necessary. The REASURE protocol has been updated to reflect the changes, which will come into effect in the UK in April 2016. After the implementation of the new standard the numerical description of the patient dose will be adjusted from

50 kBq/kg to 55 kBq/kg, and from 80 kBq/kg to 88 kBq/kg; the numerical description of the radioactivity in the vial will be changed from 1,000 kBq/mL to 1,100 kBq/mL. There will be no actual change in the patient dose (amount of radioactivity), the change is only in the dose nominal value when corrected according to the new official radium-223 NIST standard.

4. STUDY ENDPOINTS

4.1. Primary Endpoint

The proportion of patients showing bone metastases response on diffusion weighted MRI (DW-MRI)

4.2. Secondary Endpoints

- Quantitative and qualitative assessment of treatment response to radium-223 using DW-MRI
- Quantitative and qualitative assessment of treatment response to radium-223 using PET imaging
- Quantitative response to radium-223 in circulating/urinary biomarkers (ALP, n-telopeptide, PSA, CTCs)
- Time to PSA progression
- Time to total-ALP progression
- Time to first new bone fracture

4.3. Safety Endpoints

- Incidence and severity of adverse events and laboratory abnormalities
- Incidence of treatment discontinuations due to adverse events
- Incidence of fractures occurring during and after radium-223 treatment

4.4. Exploratory Endpoints

- Bone marrow samples: evidence and degree of anti-tumour activity and tumour cell DNA damage using measures of proliferation, apoptosis, DNA damage and DNA repair including Ki67, caspase cleaved cytokeratin, γH2AX and RAD51 foci formation in bone marrow
- Molecular characterisation of CRPC cells, associating subtype with anti-tumour activity
- Quantitative and qualitative assessment of treatment response depending on dose cohort (55kBq/kg or 88kBq/kg radium-223) and individual administered dose
- Changes in PSA
- Changes in total ALP
- Quantitative and qualitative assessment of treatment response to radium-223 using DW-MRI according to baseline ALP level
- Correlation between treatment response changes as measured by quantitative regional bone plasma clearance (K_i) and conventional semi-quantitative SUV parameters
- Association of mean density of lesions on baseline CT scan with response determined by WB DW-MRI
- Association of overall survival with response according to DW-MRI and PET-CT

5. PATIENT SELECTION & ELIGIBILITY

5.1. Number of Participants

The aim is to recruit 38 participants, 19 to each treatment allocation.

5.2. Source of Participants

Participants will be recruited from participating sites in the UK. Potential participants will be identified in oncology clinics and discussed at multi-disciplinary team (MDT) meetings.

5.3. Inclusion Criteria

1. Histologically or cytologically confirmed adenocarcinoma of the prostate.
2. Known castration resistant disease defined as:
   - Castrate serum testosterone level: ≤ 50 ng/dL (2.0nM) and;
   - Bilateral orchidectomy or on maintenance androgen ablation therapy with LHRH agonist or polyestradiol phosphate throughout the study and;
   - Serum PSA progression defined by PCWG II criteria (i.e. 2 consecutive increases in PSA over a previous reference value, each measurement taken at least 1 week apart)
3. Serum PSA value ≥ 2 ng/mL.
4. Available ALP result from a blood sample taken within previous 8 weeks.
5. Multiple skeletal metastases (≥ 2 hot spots) on bone scintigraphy within previous 12 weeks.
6. Age ≥18 years.
7. ECOG performance status 0-2.
8. Life expectancy ≥ 6 months.
9. No prior chemotherapy for CRPC (adjuvant chemotherapy for hormone naïve disease is permissible).
10. Adequate laboratory requirements:
    a. *Absolute neutrophil count (ANC) $\geq 1.5 \times 10^9$/L*
    b. *Platelet count $\geq 100 \times 10^9$/L*
    c. *Haemoglobin ≥ 10.0 g/dL (100 g/L; 6.2 mmol/L)*
    d. *Total bilirubin level ≤ 1.5 x institutional upper limit of normal (ULN)*
    e. *ASAT and ALAT ≤ 2.5 x ULN*
    f. *Creatinine ≤ 1.5 x ULN*
    g. *Albumin >30 g/L*
11. Willing and able to comply with the protocol, including all assessments, scans, procedures, follow-up visits and examinations.
12. Must be fully informed about the study and has signed the informed consent form.

5.4. Exclusion Criteria

1. Any prior radioisotope therapy.
2. Surgery, radiation, chemotherapy, or other anti-cancer therapy within 4 weeks prior to randomisation into the study with the exception of LHRH agonists.
3. Intention to commence cytotoxic chemotherapy within 6 months.
4. Prior other malignancy within 3 years. Adequately treated basal cell or squamous cell skin or superficial (pTis, pTa, and pT1) bladder cancer are allowed.
5. Treatment with any investigational drug within 30 days prior to randomisation into the study.
6. History of visceral metastasis, or visceral metastases, as assessed by chest/abdominal/pelvic CT within previous 8 weeks.
7. Malignant lymphadenopathy exceeding 1.5 cm in short-axis diameter.
8. Known brain or leptomeningeal involvement.
9. Imminent/established spinal cord compression based on clinical findings/MRI (can be re-screened following appropriate treatment).

10. Blood transfusion or erythropoietin stimulating agents within the 4 weeks prior to randomisation.
11. Faecal incontinence.
12. Unsuitable for MRI (patient refusal or clinical contra-indication).
13. Inadequate organ or bone marrow function.
14. Any other serious illness or medical condition.

5.5. Lifestyle Guidelines

Patients should be surgically sterile or must agree to use effective contraception during the period of therapy and for 6 months following the last administration of study treatment. Effective contraception is defined as double barrier contraception (e.g. condom plus spermicide in combination with a diaphragm, cervical cap or intrauterine device).

6. SCREENING

6.1. Screening Log

All participating sites will be required to keep a log of all participants with mCRPC that are potentially eligible for this study. Information collected on the log will include:

- Date patient identified
- Screening outcome (patient approached/accepted participation/declined participation)
- Reasons for not approaching / declining participation (if available)
- Trial ID (if applicable)

This information may be used by the TMG to monitor recruitment activity. No patient identifiable data will be sent to ICR-CTSU at this stage.

6.2. Procedure for Obtaining Informed Consent

The Principal Investigator (or designated individual) must ensure that each trial patient is fully informed about the nature and objectives of the trial and possible risks associated with participation. Participants should be given the current REC approved REASURE patient information sheet for their consideration. Patients should only be asked to consent to the study after they have had sufficient time to consider the trial, and the opportunity to ask any further questions.

No protocol required assessments should only be conducted until the REASURE consent form has been signed and dated by both the patient and the Investigator, unless they are performed routinely as part of standard patient care.

Patients who consent to REASURE consent to having CTCs, plasma, and urine samples collected for translational research. Patients in REASURE will also be asked to donate bone marrow samples but this will be an optional part of the trial.

Confirmation of the patient's consent and the informed consent process must be documented in the patient's medical notes. A copy of the signed consent form should be provided to the patient and the original retained in the investigator site file, which must be available for verification by ICR-CTSU study staff or for regulatory inspection at any time.

6.3. Participation in other Research

Patients who fulfil the eligibility criteria will be given the opportunity to participate in REASURE even if they have participated in other research or other clinical trials prior to recruitment. Treatment with other investigational therapies must have ceased at least 30 days prior to randomisation.

REASURE patients will not be permitted to participate in any other trials of investigational medicinal products whilst they are being treated within REASURE or for 1 month after the date of last treatment administration.

7. REGISTRATION

Participants must be registered centrally with the trials unit (ICR-CTSU) before protocol required screening assessments commence.

Patients should be registered by telephoning ICR-CTSU on:
020 8643 7150
09.00-17.00 (UK time) Monday to Friday

The following information will be required at registration:

- Name of hospital, consultant and person registering patient
- Confirmation that patient has given written informed consent for trial participation
- Patient's initials, hospital number and date of birth

The caller will be given the patient's unique registration number (Registration ID). Assessments of eligibility to be performed are detailed in section 9.1. Please note that bone and CT scans performed as part of standard clinical practice, prior to registration, should be used if conducted within the specified timeframe.

8. RANDOMISATION

Once eligibility has been confirmed, patients must be randomised centrally by the trials unit (ICR-CTSU) before trial treatment can commence.

Patients should be randomised by telephoning ICR-CTSU on:
020 8643 7150
09.00-17.00 (UK time) Monday to Friday

Randomisation should take place as close to the planned start date of treatment as possible. An eligibility and randomisation checklist must be completed prior to randomisation.

The following information will be requested and/or confirmed at randomisation:

- Name of hospital, consultant and person registering patient
- Confirmation that patient has given written informed consent for trial
- Confirmation that patient is eligible for the trial by completion of the eligibility checklist
- Patient's full name, hospital number, date of birth and NHS/CHI number
- Patient's trial registration number
- Patient's weight (Kg), total ALP (U/L) and current bisphosphonate use (yes/no).

The caller will be given the patient's unique randomisation number (Trial ID) and treatment allocation. ICR-CTSU will send written confirmation to the recruiting site to confirm a patient's entry into the trial.

Due to the nature of radium-223, drug can only be ordered for an individual patient after randomisation. Therefore, the first administration of radium-223 should be performed 10 days (+3 days) after randomisation.

9. TRIAL ASSESSMENTS

9.1. Screening Assessments

Once a patient has been registered, eligibility for the trial can be assessed. The following screening assessments should be conducted within the specified timeframes prior to randomisation:

9.1.1. Within 12 weeks prior to randomisation

- Technetium bone scan with careful identification of all disease-related hotspots. The Extent of Disease (EOD) grading should be determined (see Appendix A2)

9.1.2. Within 8 weeks prior to randomisation

- Chest/abdominal/pelvic CT scan

9.1.3. Within 14 days prior to randomisation

- Complete medical history including detailed information about patient's prostate cancer history and bone metastases
- Recording of previous treatments of prostate cancer and bone metastases
- Physical examination including height and weight
- Vital signs
- ECOG performance status
- Measurement of testosterone level
- Serum PSA. *Note: whilst PSA value must be ≥2ng/ml at screening, other PSA values showing progression can be obtained earlier*
- Assessment of concomitant medications/treatments
- Biochemistry: sodium; potassium; calcium; alanine aminotransferase (ALAT, ALT); lactate dehydrogenase (LDH); gamma-glutamyltransferase; creatinine; urea; bilirubin (total); albumin; alkaline phosphatase (total ALP); total protein
- Haematology: haematocrit; haemoglobin; platelet counts; red blood cell counts; white blood cell counts; white blood cell differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils)

9.2. On-Study Assessments

Radium-223 should be administered every 4 weeks (-3/+7 days). Every effort should be made to keep the exact treatment interval of 4 weeks and the time between treatments should not be fewer than 25 days. Patients will remain on treatment for up to 6 months (i.e. 6 cycles).

All study assessments should be timed in relation to administration of trial treatment. The date of radium administration will be taken as Day 1 of each cycle. The timings of all on-treatment assessments are detailed below:

- All clinical assessments (with the exception of haematology) and blood and urine collection should be performed on Day 1 (prior to administration of radium) or within 3 days prior to Day 1

- Haematology should be done within 24 hours prior to administration of radium-223. The following laboratory values must be achieved prior to treatment:
  - Haemoglobin level should not be lower than 8.0g/dL within 24 hours before any injection. Between study drug administrations, if haemoglobin levels are below 8.0g/dL, the value needs to recover to ≥8.0g/dL before next study drug administration.
  - Platelet count must be ≥100 x 10^9/L
  - Absolute neutrophil must be ≥1.5 x 10^9/L

Blood transfusion is acceptable between study drug administrations.

- Body weight should be measured prior to each administration of radium-223.
- Imaging (PET/CT and DW-MRI) should be performed within 14 days prior to the first administration of radium-223 at Cycle 1. At Cycle 2 and onwards, all imaging should be performed within ±7 days of Day 1
- 18F-fluoride PET/CT and 18F-choline PET/CT scans should not be performed on the same day; there should be a minimum of 12 hours between the 2 PET/CT scans at each timepoint.
- Bone marrow biopsies should be performed within 7 days prior to next administration of radium-223 and no earlier than 21 days after the previous administration.

Baseline conditions and adverse events, including toxicity to treatment, will be assessed according to The National Cancer Institute (NCI) Common Terminology Criteria (CTC) Version 4 (http://ctep.cancer.gov/reporting/ctc.html).

9.2.1. Cycle 1

The following assessments should be performed at Cycle 1, prior to first administration of radium-223:

- Physical examination including height and weight
- Vital signs including heart rate and blood pressure
- ECOG performance status
- Recording of baseline conditions (symptom assessment) according to NCI CTCAE v4.0. Symptoms should be assessed pre-treatment
- Haematology: haematocrit; haemoglobin; platelet counts; red blood cell counts; white blood cell counts; white blood cell differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils)
- Biochemistry: sodium; potassium; calcium; alanine aminotransferase (ALAT, ALT); lactate dehydrogenase (LDH); gamma-glutamyltransferase; creatinine; urea; bilirubin (total); albumin; alkaline phosphatase (total ALP); total protein
- Serum PSA
- Diffusion weighted MRI*
- 18F-Fluoride PET/CT*
- 18F-Choline PET/CT*
- Assessment of concomitant medications/treatments
- Blood sample collection: circulating tumour cells (2x7.5ml) and plasma tumour DNA (2x10ml)
- Urine sample collection: N-telopeptide analysis
- Bone marrow biopsy

**Note: At Cycle 1, all imaging assessments should be performed within 14 days prior to first treatment with radium-223.*

9.2.1. Cycle 2

The following assessments should be performed at Cycle 2, as per the timings described in section 9.2:

- Physical examination

- Vital signs
- ECOG performance status
- Symptom assessment pre-treatment (NCI CTCAE v4.0)
- Haematology: haematocrit; haemoglobin; platelet counts; red blood cell counts; white blood cell counts; white blood cell differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils)
- Biochemistry: sodium; potassium; calcium; alanine aminotransferase (ALAT, ALT); lactate dehydrogenase (LDH); gamma-glutamyltransferase; creatinine; urea; bilirubin (total); albumin; alkaline phosphatase (total ALP); total protein
- Serum PSA
- Diffusion weighted MRI
- 18F-Fluoride PET/CT
- 18F-Choline PET/CT
- Assessment of concomitant medications/treatments
- Blood sample collection: circulating tumour cells (2x7.5ml) and plasma tumour DNA (2x10ml)
- Urine sample collection: N-telopeptide analysis
- Bone marrow biopsy (to be performed ≤7 days prior to Day 1 Cycle 2 and at least 21 days after previous administration of radium-223)

9.2.2. Cycle 3

The following assessments should be performed at Cycle 3, as per the timings described in section 9.2:

- Physical examination
- Vital signs
- ECOG performance status
- Symptom assessment pre-treatment (NCI CTCAE v4.0)
- Haematology: haematocrit; haemoglobin; platelet counts; red blood cell counts; white blood cell counts; white blood cell differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils)
- Biochemistry: sodium; potassium; calcium; alanine aminotransferase (ALAT, ALT); lactate dehydrogenase (LDH); gamma-glutamyltransferase; creatinine; urea; bilirubin (total); albumin; alkaline phosphatase (total ALP); total protein
- Serum PSA
- Assessment of concomitant medications/treatments
- Blood sample collection: circulating tumour cells (2x7.5ml) and plasma tumour DNA (2x10ml)
- Urine sample collection: N-telopeptide analysis

9.2.3. Cycle 4

The following assessments should be performed at Cycle 4, as per the timings described in section 9.2:

- Physical examination
- Vital signs
- ECOG performance status
- Symptom assessment pre-treatment (NCI CTCAE v4.0)
- Haematology: haematocrit; haemoglobin; platelet counts; red blood cell counts; white blood cell counts; white blood cell differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils)
- Biochemistry: sodium; potassium; calcium; alanine aminotransferase (ALAT, ALT); lactate dehydrogenase (LDH); gamma-glutamyltransferase; creatinine; urea; bilirubin (total); albumin; alkaline phosphatase (total ALP); total protein
- Serum PSA
- Diffusion weighted MRI
- 18F-Fluoride PET/CT

- 18F-Choline PET/CT
- Assessment of concomitant medications/treatments
- Blood sample collection: circulating tumour cells (2x7.5ml) and plasma tumour DNA (2x10ml)
- Urine sample collection: N-telopeptide analysis

9.2.4. Cycle 5

The following assessments should be performed at Cycle 5, as per the timings described in section 9.2:

- Physical examination
- Vital signs
- ECOG performance status
- Symptom assessment pre-treatment (NCI CTCAE v4.0)
- Haematology: haematocrit; haemoglobin; platelet counts; red blood cell counts; white blood cell counts; white blood cell differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils)
- Biochemistry: sodium; potassium; calcium; alanine aminotransferase (ALAT, ALT); lactate dehydrogenase (LDH); gamma-glutamyltransferase; creatinine; urea; bilirubin (total); albumin; alkaline phosphatase (total ALP); total protein
- Serum PSA
- Assessment of concomitant medications/treatments
- Blood sample collection: plasma tumour DNA (2x10ml)
- Urine sample collection: N-telopeptide analysis

9.2.5. Cycle 6

The following assessments should be performed at Cycle 6, as per the timings described in section 9.2:

- Physical examination
- Vital signs
- ECOG performance status
- Symptom assessment pre-treatment (NCI CTCAE v4.0)
- Haematology: haematocrit; haemoglobin; platelet counts; red blood cell counts; white blood cell counts; white blood cell differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils)
- Biochemistry: sodium; potassium; calcium; alanine aminotransferase (ALAT, ALT); lactate dehydrogenase (LDH); gamma-glutamyltransferase; creatinine; urea; bilirubin (total); albumin; alkaline phosphatase (total ALP); total protein
- Serum PSA
- Assessment of concomitant medications/treatments
- Blood sample collection: plasma tumour DNA (2x10ml)
- Urine sample collection: N-telopeptide analysis

9.3. End of Treatment

The following assessments will be performed at the end of treatment visit, 4 weeks (± 7 days) after the last administration of radium-223:

- Physical examination
- Vital signs
- ECOG performance status
- Symptom assessment (NCI CTCAE v4.0)
- Haematology: haematocrit; haemoglobin; platelet counts; red blood cell counts; white blood cell counts; white blood cell differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils)

- Biochemistry: sodium; potassium; calcium; alanine aminotransferase (ALAT, ALT); lactate dehydrogenase (LDH); gamma-glutamyltransferase; creatinine; urea; bilirubin (total); albumin; alkaline phosphatase (total ALP); total protein
- Serum PSA
- Diffusion weighted MRI
- 18F-Fluoride PET/CT
- 18F-Choline PET/CT
- Assessment of concomitant medications/treatments
- Blood sample collection: circulating tumour cells (2x7.5ml) and plasma tumour DNA (2x10ml)
- Urine sample collection: N-telopeptide analysis
- Bone marrow biopsy (to be performed ≤7 days prior to the end of treatment visit)

9.4. Post-treatment Follow-up

Following the end of treatment visit, patients will be followed up in clinic every 4 months until 1 year after last administration of radium-223. Follow-up assessments should be scheduled with a window of ±14days.

The following assessments should be performed at patient follow-up:

- Physical examination, as indicated (at the discretion of the Investigator)
- ECOG performance status
- Recording of adverse reaction/s to radium-223
- Haematology: haematocrit; haemoglobin; platelet counts; red blood cell counts; white blood cell counts; white blood cell differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils)
- Biochemistry: alkaline phosphatase (total ALP)
- Serum PSA
- Recording of any cancer-related treatments (excluding analgesics)

ICR-CTSU will also request details of all subsequent imaging performed during routine care of the patient and will arrange for any relevant imaging data to be transferred for central review to assess incidence of fractures. This will continue beyond 1 year of clinical follow-up. Please also refer to section 19.3.

Patients will be followed up for overall survival beyond the end of the 1 year clinical follow up period.

9.5. Discontinuation from Treatment or Follow-up

Participants may discontinue from trial treatment at any time at their own request, or they may be discontinued at the discretion of the Principal Investigator. Reasons for discontinuation may include:

- Disease progression
- Start of new anti-cancer therapy
- Unacceptable toxicity

Please see section 10.5 for information on concomitant therapies. If a patient terminates treatment prior to having received all 6 injections, he will have the end of treatment visit 4 weeks (± 7 days) after the last injection.

Participants who discontinue treatment should continue to be followed up as per the standard visit schedule. If a patient withdraws consent for further follow-up it should be clarified whether they no longer wish to attend trial specific follow up visits or wish to stop contributing further data to the study. A trial deviation form should be completed for any patient who withdraws consent for information to be sent to the ICR-CTSU or for attending trial follow up visits. In the very rare event that a patient requests that their data is removed from the study entirely, the implications of this should be discussed with the patient first to ensure that this

is their intent and, if confirmed, ICR-CTSU should be notified in writing. If this request is received after results have been published the course of action will be agreed between the Co-sponsors and the independent Trial Steering Committee.

9.6. Schedule of Assessments

Visit/Assessment [a]	Screening	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	End of Treatment [b]	Follow-up (all visits) [c]
Written informed consent [d]	X								
Eligibility criteria	X								
Demographics	X								
Medical/treatment history [e]	X								
Physical examination [f]	X	X	X	X	X	X	X	X	X [g]
Vital signs [h]	X	X	X	X	X	X	X	X	
ECOG performance status [i]	X	X	X	X	X	X	X	X	X
Bone scan [j]	X								
Chest/abdominal/pelvic CT [k]	X								
Archival tumour sample [l]		X							
Adverse event assessment [m]		X	X	X	X	X	X	X	X [n]
Concomitant treatments [o]	X	X	X	X	X	X	X	X	X [p]
Haematology [q]	X	X	X	X	X	X	X	X	X
Biochemistry	X	X	X	X	X	X	X	X	X
PSA [r]	X	X	X	X	X	X	X	X	X

Serum testosterone [s]	X								
Circulating tumour cells		X	X	X	X			X	
Plasma tumour DNA [t]		X	X	X	X	X	X	X	
Bone marrow biopsy [u]		X	X					X	
Urinalysis: N-telopeptide analysis [v]		X	X	X	X	X	X	X	
18F-fluoride PET/CT [w x]		X[y]	X		X[y]			X	
18F-choline PET/CT [w x]		X	X		X			X	
Diffusion-weighted MRI [w x]		X	X		X			X	
Administration of radium-223 (injection) [z]		X	X	X	X	X	X		

Footnotes:

a. The date of radium-223 administration is always considered to be Day 1 of each cycle. All clinical assessments should be performed before administration of radium-223.
b. The end of treatment visit should be conducted 4 weeks after the last administration of radium-223.
c. Patients should be followed up every 4 months until 1 year after last administration of radium-223.
d. Written informed consent is required before any protocol-specific procedures or assessments are performed.
e. Includes full oncologic history.
f. Physical examination should include measurement of weight during the treatment period. Weight must be measured at each visit prior to administration of radium-223.
g. Physical examination to be performed only as indicated (at the discretion of the Investigator) post-treatment.
h. Vital signs including heart rate and blood pressure.
i. ECOG performance status will be determined prior to treatment at each study visit and at all follow-up assessments.
j. To be performed as part of screening, within 12 weeks prior to randomisation. EOD grading to be determined and recorded.
k. To be performed as part of screening, within 8 weeks prior to randomisation.
l. Archival samples will be collected from all randomised patients, where available.

m. SAEs will be recorded from randomisation until 30 days after the last administration of radium-223. All AEs will be recorded according to CTCAE v4.0 from commencement of treatment until 30 days after last treatment.
n. All adverse reactions (ARs) will be recorded until the end of follow-up.
o. All concomitant treatments will be recorded from randomisation until 28 days after last treatment.
p. All cancer related treatments (excluding analgesics) will be recorded until the end of follow-up.
q. Haematology must be performed within 24 hours prior to administration of radium-223. All values must be within acceptable levels before a patient can commence/continue trial treatment.
r. PSA should be measured at screening, Day 1 (≤3 days) of each treatment cycle and at the end of treatment visit.
s. To be performed as part of screening, within 14 days prior to randomisation.
t. Blood samples should be collected on Day 1 (≤3 days) of each treatment cycle and at the end of treatment visit.
u. Bone marrow biopsies are an optional part of the trial for all patients but should be strongly encouraged (where appropriate). The specified timepoints are preferred but biopsies can be collected at alternative visits.
v. Urine samples should be collected on Day 1 (≤3 days) of each treatment cycle and at the end of treatment visit.
w. Baseline scans should be performed on Day 1 of Cycle 1 (≤14 days). All Cycle 1 imaging should be conducted prior to the first administration of radium-223.
x. Following commencement of trial treatment, scans should be performed at Day 1 (± 7 days) of Cycle 2, Cycle 4 and the end of treatment visit.
y. Venous blood will be collected at Cycle 1 and Cycle 4 18F-Fluoride PET/CTs for patients who have consented to participate in the optional sub-study.
z. Radium should be administered every 4 weeks (-3/+7 days). Every effort should be made to keep an exact treatment interval of 4 weeks. Patients will receive treatment with radium-223 for up to 6 cycles.

10. TRIAL TREATMENT

Radium-223 dichloride solution for injection is the investigational medicinal product within REASURE. Radium-223 dichloride solution for injection is a therapeutic radiopharmaceutical.

A radiation license covering the use of radium-223 dichloride must be in place at each participating REASURE site. A copy of this license should be provided to ICR-CTSU prior to site initiation.

10.1. Dose and Schedule

Radium-223 55 kBq/kg or 88 kBq/kg will be administered as slow bolus intravenous injection 6 times at intervals of 4 weeks. The treatment can be administered on an outpatient basis.

Please note: The dose has been described in previous protocol versions as 50 kBq/kg and 80 kBq/kg, which was based on the original NIST standardisation. Following correction of the systematic 10% error in the radium-223 standardisation, the nominal value of the patient dose becomes 55 kBq/kg and 88 kBq/kg; this is based on the new official radium-223 NIST standard. This change will be implemented in April 2016. There will be no actual change in the administered patient dose (amount of radioactivity).

10.2. Preparation and Administration

Radium-223 dichloride should 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 1 hour prior to use as cold material should not be injected into a patient.

The dosage of radium-223 dichloride administered in REASURE is 55 or 88 kBq/kg b.w. The total activity to be injected will be calculated volumetrically using the patient's body weight on the day of injection (kg), the desired dosage level and the decay correction factor (DK) to correct for physical decay of radium-223 dichloride. A table with decay correction factors according to physical decay of the IMP will be provided with each vial of radium-223 dichloride solution for injection. The total amount (volume to be drawn into the syringe) to be administered to a patient should be calculated as follows:

$$\frac{\text{Body weight (kg)} \times 55 \textbf{ or } 88 \text{ kBq/kg b.w.}}{\text{DK} \times 1100 \text{ kBq/mL}} = \text{volume to be injected (mL)}$$

Filling of the syringe should take place under appropriate safety conditions in the radiopharmacy or nuclear medicine department. Local procedures for the handling of radiopharmaceuticals should always be followed. Personnel should use appropriate protective clothing and equipment (e.g. lab coats, medical gloves, protective glasses) whilst filling the syringe to prevent contamination with the radioactive solution. Sites should adhere to all relevant radiation safety regulations including 'as low as reasonably achievable' (ALARA) principles.

Aseptic technique should be used in the administration of radium-223 dichloride. The filled syringe should be handed over to the individual who will perform the injection. Radium-223 dichloride should be administered as a slow bolus intravenous injection. The IV line should be flushed with sterile normal saline before and after administration of radium-223 dichloride. After administration, the equipment used in connection with the preparation and

administration of the radium-223 dichloride should to be treated as radioactive waste and disposed of in accordance with hospital procedure for the handling of radioactive material.

10.2.1. Dial Setting of Dose Calibration

Radium-223 dichloride can be measured in a normal dose calibrator instrument. Once ICR-CTSU has received a copy of a site's radiation license a vial of radium-223 for technical use can be sent to that site, at their request.

If a calibration sample is requested for dial setting the site will receive a sealed vial containing a radium-223 solution for calibration only. The vial is identical to the vials used for study treatment. The amount of radium-223 in the vial will be stated on the label. Instructions for the dial setting will be enclosed with the dispatch of the calibration sample.

Prior to implementation of the updated NIST standardisation for radium-223, all REASURE sites will be required to add a new dial setting to their dose calibrators (NIST update). The current dial setting should be used until the implementation date.

10.3. Duration of Treatment

REASURE participants will remain on trial treatment for up to 6 months. They will receive 1 administration of radium-223 every 4 weeks during that period, up to a total of 6 treatments.

10.4. Supportive Care

In general, the administration of radioactive treatments involves a potential risk for third parties. This is due to radiation from the patient and possible contamination via spilled urine, faeces or vomit. When radium-223 is injected intravenously into a patient, the risk for external radiation exposure to third parties is extremely low due to the short range of the alpha participles and the very low portion of beta and gamma radiation. For these reasons, the product can be administered on an outpatient basis. The patient and his carers will receive written and oral instructions regarding hygiene precautions after receiving radium-223 to minimise the risk of contamination.

10.5. Concomitant Therapy

All medication considered necessary for the participants' welfare and which is not expected to interfere with the evaluation of the study treatment may be given at the discretion of the Investigator. All concomitant medications must be recorded in the patient's notes, as well as the appropriate pages of the eCRF.

Patients who have not undergone bilateral orchidectomy are to receive LHRH agonists throughout the study.

Patients receiving bisphosphonates prior to randomisation should be maintained on bisphosphonate therapy throughout the treatment period. However, bisphosphonate treatment may be stopped at the discretion of the investigator if clinically indicated. Injection of bisphosphonates should be done at least 2 hours before or after administration of radium-223. Ideally, patients who are not receiving bisphosphonate therapy upon enrolment in REASURE should not begin bisphosphonates whilst on trial treatment. They may, however, if the Investigator believes this to be in the patient's best interests. Commencement or

cessation of concurrent treatment with bisphosphonates will not necessitate premature discontinuation of radium-223.

Blood transfusions are permitted during the study period. These procedures should be recorded in the eCRFs until study termination.

Non-permissible concurrent medications/therapies during the treatment period include:

- Cytotoxic chemotherapy
- Any systemic radioisotope or hemi body external radiotherapy
- New hormonal cancer therapy
- Any other investigational drugs

Any disease progression that requires other specific anti-cancer therapy will be cause for discontinuation from trial treatment. Where possible, alternative treatments should not be commenced until 4 weeks after a patient's last administration of study treatment.

10.6. Dose Modifications

Dose modifications are not permitted within the REASURE trial.

Permitted treatment interruptions for the management of specific toxicities are described in Appendix A3.

10.7. Discontinuation and Subsequent Therapy

Discontinuation of treatment for the management of specific toxicities is described in Appendix A3.

10.8. Compliance

Patients should receive treatment with radium-223 under the supervision of a nuclear medicine specialist. Delegated individual/s at each participating site should check the administration volume and total radioactivity injected. The activity dose and the volume injected should be recorded on an eCRF.

10.9. Patient Cards

Small wallet sized cards will be produced by ICR-CTSU on request by the participating site. Each card will state:

- the name of the participating site
- that the patient is participating in the REASURE trial
- that the patient is taking radium-223
- an emergency contact number

10.10. Supply and Distribution

Radium-223 dichloride solution for injection is provided free of charge by Bayer for REASURE. Local R&D approval, the local ARSAC license(s) and a study agreement must be in place before any drug can be shipped to a site.

Study drug required for administration during the conduct of the trial should be ordered directly via the Interactive Voice/Web Response System (IXRS) at least 10 days prior to each planned administration. Drug should be ordered by participating sites on a per patient/per treatment basis; bulk supplies cannot be requested. When ordering drug, the site will need to provide the patient's trial ID, the dose, the planned treatment date and the patient's weight. Information and training on the use of IXRS will be provided to each study site prior to the start of recruitment at that site.

The product is produced according to Good Manufacturing Practice (GMP). It will be delivered in a glass vial, ready-to-use, with a certified activity. Radium-223 dichloride is shipped in a lead container and Type A radioactive package according to international transportation guidelines for radioactive materials.

Each vial should be used for 1 patient only.

10.11. Formulation, Packaging, Storage Conditions and Labelling

The radiopharmaceutical radium-223 dichloride solution for injection is a ready-to-use, sterile, non-pyrogenic, clear and colourless aqueous solution of radium-223 dichloride ($^{223}RaCl_2$) for intravenous administration. Radium-223 is predominantly an alpha particle emitter with a physical half-life of 11.4 days. The product is isotonic and has a pH of 6.0-8.0. The radioactive concentration at the reference date is 1100kBq/ml (after implementation of the NIST update). The product has a pre-calibration of 14 days. When administered on a day other than the reference day, the volume should be corrected according to the physical decay table supplied with each shipment.

The product is supplied in 10mL single dose glass vials, closed with rubber stoppers and aluminium seals. The amount per vial is 6mL, corresponding to 6.6MBq (after implementation of the NIST update), at reference day.

Radium-223 dichloride solution for injection has a shelf life of 28 days from production day, when stored at ambient temperature. The shelf life has been demonstrated for temperatures from cold storage (2-8°C) up to 40°C. In addition, it has been shown that the product quality is not jeopardized upon freezing. 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. The radioactivity is measured by use of a radioisotope dose calibrator, calibrated for radium-223.

The radioactivity in the vial should be controlled at the hospital upon receipt of the radium-223 and after dispensing. The radioactivity of the syringe should be controlled before and after injection.

The radium-223 used in REASURE will be labelled in accordance with the MHRA approved label and according to GMP requirements. A system of numbering will be used, ensuring that each dose of study drug can be traced back to the respective manufacture of the ingredients. As

for all radiopharmaceutical products the lead container will display the main Investigational Medicinal Product label.

10.12. Pharmacy Responsibilities and Drug accountability

The study treatment must not be used outside the context of the REASURE protocol.

Records must be kept of all deliveries, dispensing and destruction in accordance with the REASURE Radiopharmacy Guidance Notes. These records may be requested by ICR-CTSU during the trial to monitor supply and usage of stock. Account must be given of any discrepancies and certificates of delivery should be signed.

11. PHARMACOVIGILANCE

11.1. Definitions

Adverse Event (AE)
An AE is any untoward medical occurrence in a patient or clinical trial subject administered an investigational medicinal product; the event does not necessarily have a causal relationship with the treatment or usage.

Serious Adverse Event (SAE)
An SAE is any untoward medical occurrence that occurs after the commencement of study treatment and within 30 days of the last administration and:

- results in death,
- is life-threatening
- requires hospitalisation or prolongation of existing inpatients´ hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect

Important adverse events that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, may also be considered serious.

Progression of the indicated disease and death due to progression of the indicated disease are not considered SAEs.

Pregnancy or aid in the conception of a child whilst participating in a trial is not itself considered an SAE but should be followed up for congenital anomalies or birth defects.

Serious Adverse Reaction (SAR)
A serious adverse reaction is an SAE that is suspected as having a causal relationship to the investigational medicinal product, as assessed by the investigator responsible for the care of the patient. A suspected causal relationship is defined as possibly, probably or definitely related (see definitions of causality table).

Definitions of causality

Relationship	Description
Unrelated	There is no evidence of any causal relationship with the trial drug
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial

	medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatment)
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments)
Probable	There is evidence to suggest a causal relationship, and the influence of other factors is unlikely
Definitely	There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

Suspected Unexpected Serious Adverse Reaction (SUSAR)
A serious adverse reaction, the nature or severity of which is not consistent with the safety information provided in the applicable Investigator's Brochure (IB) and is assessed as unexpected by the Chief Investigator.

11.2. Reporting Adverse Events to ICR-CTSU

Any toxicity, sign or symptom that occurs after commencement of study treatment and within 30 days of the last administration of study treatment, which is not unequivocally due to progression of disease, should be considered an AE. In REASURE, all AEs which are considered by the Principal Investigator or designee to be related to administration of the study drug (adverse reactions [ARs]) will continue to be reported until the end of the follow-up period.

All AEs must be reported on the relevant CRF and submitted to ICR-CTSU.

The severity of AEs should be graded according to the NCI-CTC criteria. For each AE, the highest grade observed since the last visit should be reported.

Whenever one or more toxicity/sign/symptom corresponds to a disease or a well-defined syndrome only the main disease/syndrome should be reported.

11.3. Reporting of Serious Adverse Events to ICR-CTSU

Any SAE that occurs after the commencement of study treatment and up to 30 days following the last dose of study drug must be reported. Any SAEs that occur more than 30 days after the last dose of study drug that, in the opinion of the Principal Investigator, are related to the study drug should be reported to ICR-CTSU if the Principal Investigator becomes aware of them.

All SAEs should be reported to ICR-CTSU within 24 hours of the Principal Investigator (or designated representative) becoming aware of the event, by completing the REASURE SAE form and faxing to:

The ICR-CTSU safety desk
Fax no: **0208 722 4368**
For the attention of the REASURE trial team

As much information as possible, including the Principal Investigator's assessment of causality, must be reported to ICR-CTSU in the first instance. Additional follow up information should be reported as soon as it is available.

All SAE forms must be completed, signed and dated by the Principal Investigator or designated representative.

All reported SAEs and follow up information will be forwarded to Bayer upon receipt at ICR-CTSU.

11.4. Review of Serious Adverse Events

The Chief Investigator (or designated representative) will assess all reported SAEs for causality and expectedness (NB. The Chief Investigator cannot down-grade the Principal Investigator's assessment of causality.)

SAEs assessed as having a causal relationship to study drug and as being unexpected (SUSARs) will undergo expedited reporting to the relevant authorities and all other interested parties by ICR-CTSU (see figure 4 for SAE reporting).

Sites should respond as soon as possible to requests from the Chief Investigator or designated representative (via ICR-CTSU) for further information that may be required for final assessment of an SAE.

11.5. Expedited Reporting of SUSARs

If an SAE is identified as being a SUSAR by the Chief Investigator, and is fatal or life threatening, it will be reported by ICR-CTSU to the MHRA, the main REC and the Co-sponsors within 7 days of being notified of the event.

If an SAE is identified as a SUSAR by the Chief Investigator, and is not fatal or life threatening, it will be reported by ICR-CTSU to the MHRA, the main REC and the Co-sponsors within 15 days of ICR-CTSU being notified of the event.

ICR-CTSU will report any additional relevant information to the MHRA, main REC and the Co-sponsors as soon as possible, or within 8 days of the initial report of a fatal/life threatening SUSAR.

The Principal Investigators at all actively recruiting sites will be informed of any SUSARs occurring within the trial at appropriate intervals. All SAEs will be reported to Bayer within 24 hours of ICR-CTSU becoming aware of the event. Any additional information will be reported as soon as possible.

11.6. Follow up of Serious Adverse Events

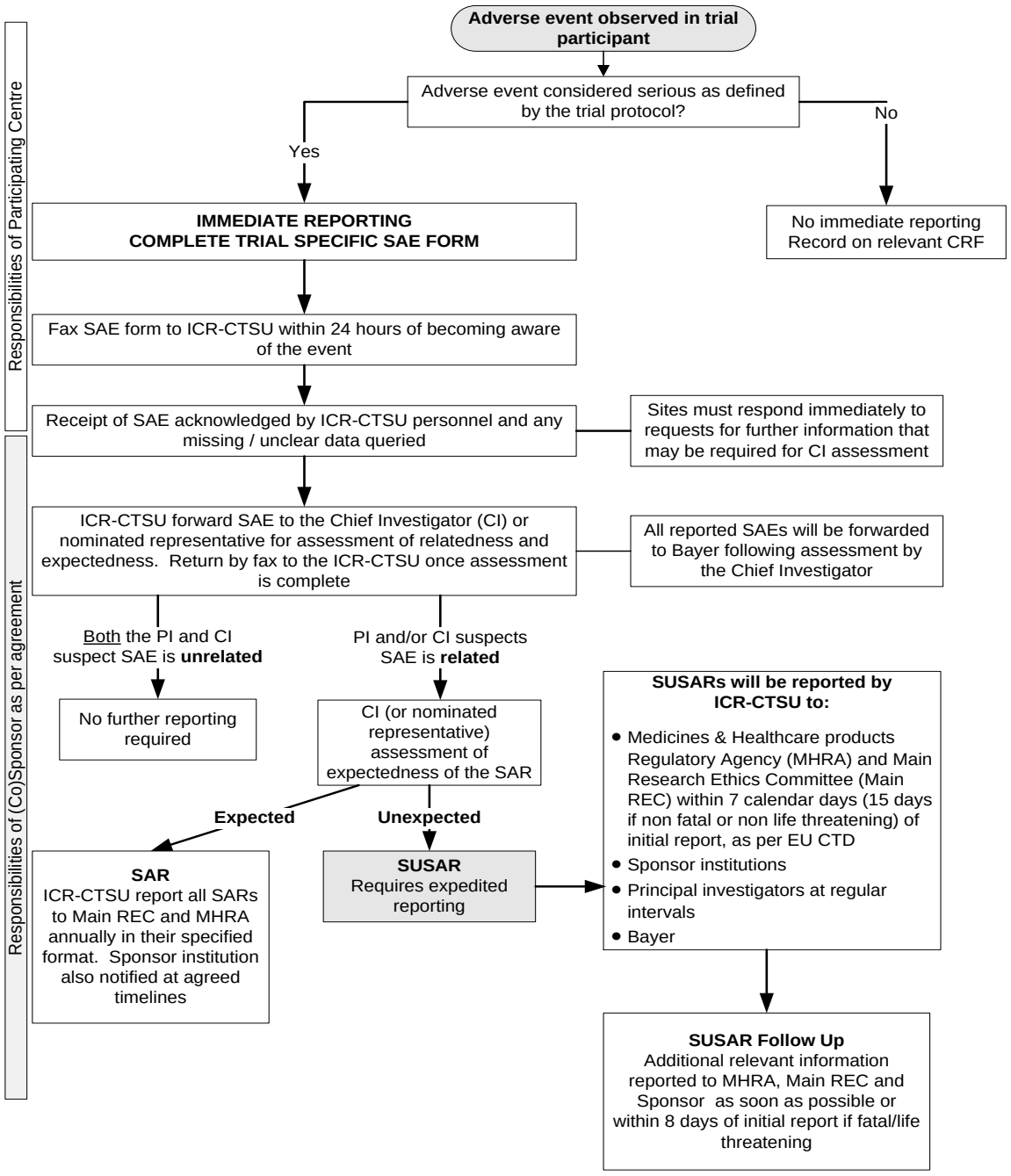
SAEs should be followed up until clinical recovery is complete or until disease has stabilised. SAE outcomes should be reported to ICR-CTSU using the relevant section of the SAE form as soon as the Principal Investigator or designee becomes aware of the outcome.

NB: The partner of any patient who fathers a child whilst on study medication, must be followed up and the outcome reported to ICR-CTSU who will then forward the details to Bayer.

11.7. Annual Reporting of Serious Adverse Reactions

An annual report will be provided to the MHRA and the main REC by ICR-CTSU and copied to the Co-sponsors at the end of the reporting year.

Figure 4: Flow diagram for SAE reporting, and action following report



NB. All SAEs should continue to be followed up as specified above

12. STATISTICAL CONSIDERATIONS

12.1. Statistical Design and Sample Size Justification

The recruitment target is 38 patients.

Power calculations using ADC changes on DW-MRI are challenging due to the lack of data in this setting. The correlation between ADCs and clinical response is unknown and how treatment dose may affect the ADCs is to be explored. In a reproducibility study the coefficient of repeatability of bone disease on DW-MRI was approximately 15% [43]. If 2 x coefficient of repeatability is considered a threshold for significant change, then the ADC increase observed is unlikely to be due to chance. Using a relative increase of 30% or more in ADC to classify as response it is possible to give an impression of the size of difference (post/pre-treatment) that can be detected.

It is hypothesised that 25% of untreated patients demonstrate this level of response due to biological variability or chance. Using a 1 sample comparison of a proportion to this hypothesised value, then with 36 treated patients, α=0.05 (1-sided) and 83% power, the study would be able to detect a proportion of 45% amongst those treated that have demonstrated at least a 30% increase in ADCs post treatment. Response rates of at least 48% could be concluded with 90% power. Radium-223 is a new treatment that prolongs survival and has very favourable toxicity profile; a 5% drop-out rate has been incorporated giving a target sample size of 38 patients.

Patients will be randomised to receive either 55kBq/kg or 88kBq/kg radium-233. As a secondary objective we are interested in exploring whether a dose-response relationship can be detected. With 80% power and α=0.05 (1-sided) 38 patients, 19 per group, would be sufficient to detect a difference in the proportion of patients “responding” (i.e. showing a 30% increase in ADCs post-treatment) between the dose cohorts of 45% (e.g. 35% response rate in 55kBq/kg group vs. 80% in 88kBq/kg group).

All analyses are of exploratory nature and there will be no formal interim analysis. No multiplicity adjustment has been made therefore all results and p-values will be interpreted with caution.

12.2. Treatment Allocation

All trial participants will receive radium-223.

Treatment dose allocation is by minimisation with a random element; balancing factors will be based on patient’s weight, total ALP (U/L) and current bisphosphonate use (yes/no) and specified in detail in the Statistical Analysis Plan.

12.3. Endpoint Definitions

12.3.1. Primary endpoint

The proportion of patients overall showing bone metastases response on diffusion-weighted MRI (DW-MRI).

DW-MRI explores the random motion of water molecules in the body and yields qualitative (disease progression, no change, response) and quantitative information (Apparent Diffusion Coefficients (ADCs)) that can provide an insight into tumour characteristics. This study will

explore the response to radium-223 using the differences in the ADCs pre-treatment and post start of treatment. A 30% increase in global median ADC from pre-treatment to any point after 1st injection and the end of cycle 6 will be used to define response.

12.3.2. Secondary endpoints

Quantitative (ADC) and qualitative (disease progression, stable disease, partial response, complete response) assessment of treatment response using DW-MRI. Further research [44] suggests that up to five target lesions are sufficient to evaluate response. Associations between the percentage change in the per-patient mean of the mean ADC value and the overall clinical, radiological and biochemical definitions of disease response to treatment and total activity of radium-223 administered will be explored. A 30% increase in the mean of the mean ADC values up to the end of cycle 6 will be used to define a response.

Quantitative (SUV_{60}, K_i) and qualitative (disease progression, stable disease, partial response, complete response) assessment of treatment response using PET imaging. The mean (or median if the data are skewed) of the quantitative measurements will be used in the analysis.

Quantitative response in circulating/ urinary biomarkers (ALP, n-telopeptide, PSA, CTCs). PSA response will be defined according to The Prostate Cancer Working Group (PCWG2) criteria. Total-ALP response is defined as ≥30% reduction of the blood level, compared to the baseline value, confirmed by a second total-ALP value approximately 4 or more weeks later. CTC response will be defined either by conversion of CTC counts (CTC count from ≥5 cells/7.5ml blood at baseline to <5 cells/7.5mls confirmed by 2 consecutive readings at least 4 weeks apart) or percentage decline of ≥ 30%.

Time to PSA progression is defined as the time interval between trial entry and first documented PSA progression. PSA progression will be defined according to The Prostate Cancer Working Group criteria (PCWG2).

Time to total-ALP progression is defined as the time interval between trial entry and first documented ALP progression. Total ALP progression is defined in: a) patients with no total-ALP decline from baseline as ≥ 25% increase from the baseline value, at least 12 weeks from baseline; b) in patients with an initial total-ALP decline from baseline as ≥25% increase above the nadir value, which is confirmed by a second value obtained 3 or more weeks later.

Total ALP response is defined as ≥30% reduction of the blood level, compared to the baseline value, confirmed by a second total-ALP value approximately 4 or more weeks later.

12.3.3. Safety endpoints

Adverse events will be graded according to the NCI-CTCAE v4 and will be summarised according to grade, dose cohort and time point. The number and percentage of patients experiencing a grade 3-4 AE will also be calculated at the end of treatment. The safety analysis will include all randomised patients who received at least 1 injection (dose) of radium-223.

Incidence of fractures occurring during and after radium-223 treatment. The time to first new bone fracture is defined as the time interval between trial entry and the first new bone fracture detected on trial-specific or routine imaging (e.g. CT, MRI, bone scan or X-ray) not

present on baseline imaging. Further analyses will quantify the number and location of bone fractures that have occurred at 1 and 2 years post treatment.

12.3.4. Exploratory endpoints

- Bone marrow samples: evidence and degree of anti-tumour activity and tumour cell DNA damage using measures of proliferation, apoptosis, DNA damage and DNA repair including Ki67, caspase cleaved cytokeratin, γH2AX and RAD51 foci formation in bone marrow.
- Characterisation of CTCs.
- Quantitative and qualitative assessment of treatment response depending on dose cohort (55kBq/kg or 88 kBq/kg radium-223) and individual administered dose.
- Changes in PSA and total ALP.
- Quantitative and qualitative assessment of treatment response using DW-MRI according to baseline ALP level.
- Correlation between treatment response changes as measured by quantitative regional bone plasma clearance (K_i) and conventional semi-quantitative SUV parameters.
- Association of mean density of lesions on baseline CT scan with response determined by WB DW-MRI
- Association of overall survival with response according to DW-MRI and PET-CT

12.4. Analysis Methods

As discussed in section 1.3.1, DW-MRI endpoints are novel and there is no validated definition of response by DW-MRI. All the analyses in the REASURE trial are of an exploratory nature. Sample size calculations were based on a difference in treatment group between the proportion of patients achieving a $\geq$30 increase in global median ADC but a number of alternate definitions of response by ADC are to be explored and investigated for correlation with other response endpoints. Subgroup analyses by allocated dose group will be performed.

Quantitative evaluation of imaging markers: The proportion of patients having achieved a response as defined in section 12.3.1 according to DW-MRI imaging will be calculated along with its 1-sided 95% confidence interval. In addition, best response since first injection will also be reported. Response rates by ADC values and PSA/CTC response pre- and post-treatment will be cross tabulated and a Fisher's exact test will be used to assess their association. Logistic regression models will be used to test for an association between ADC response and PSA/CTC response. The model will be adjusted for weight, total ALP, current bisphosphonate use and other known prognostic factors. As ADC use is still an exploratory field, ROC analysis will be performed to compare the greatest percentage increase in ADC value with the overall clinical, radiological and biochemical definition of disease response to treatment. The correlation between best % increase in ADC and the mean total dose of radium-223 administered per cycle will also be explored. Post-treatment quantitative PET markers of response (SUV60, K_i) will be summarised by their mean or median (whichever is deemed more appropriate) and t-test or the non-parametric Mann–Whitney–Wilcoxon test will be used to compare their mean/median according to ADC response at each timepoint. Linear regression models will be fitted to evaluate the association between the imaging markers and ADC values adjusting for other prognostic variables.

Qualitative evaluation of imaging markers: To compare the performance of other functional imaging techniques as biomarkers of response the agreement between the PET scan assessment and the DW-MRI will be assessed using the Cohen's K statistic. Statistical significance for kappa will not be reported but the interpretation will be in line with Landis and Koch, who characterised values <0 as indicating no agreement, 0–.20 as slight, .21–.40 as fair, .41–.60 as moderate, .61–.80 as substantial, and .81–1 as almost perfect agreement. Response concordance will also be examined between the PET markers of response and PSA/CTCs response.

CTCs: To assess the CTC count conversion from ≥5 cells/7.5ml to < 5/7.5ml only those patients with ≥5/7.5ml CTC rate at baseline will be analysed. The proportion of patients with a CTC conversion to <5/7.5 ml blood at nadir (confirmed by a second consecutive reading obtained at least 4 weeks later) will be presented along an exact 2-sided 95% confidence interval. To evaluate post-treatment falls in CTC the proportion of patients with at least 30% fall in CTC will be reported with a 95% CI.

Changes in PSA and total ALP: Waterfall plots will be used to show the percentage change in PSA and ALP from baseline to 12 weeks (or earlier for those who discontinue therapy), as well as the maximum decline that occurs at any point after treatment.

Time to PSA/ALP progression and time to ALP response will be compared using a log-rank test between responders and non-responders (defined by DW-MRI imaging) and will be summarised using Kaplan-Meier methods.

Any changes of PSA and ALP (not necessarily reaching the response or progression levels) will also be explored as a continuous variable between DW-MRI responders and non-responders. Depending on the distribution of these measures t-tests or the non-parametric Mann–Whitney U test will be employed.

Characterisation of CTCs: DW-MRI response and CTC conversion rate data will be cross-tabulated when all 4 weeks post commencement of treatment data are available. These variables will be correlated with time-to-PSA progression using Kaplan-Meier methods and the log-rank test to evaluate their performance as an early response biomarker.

Adverse Events: To assess the safety and tolerability of radium-223 the extent of exposure to study drug will be summarised and details will be provided. Adverse events will be graded according to the NCI-CTCAE v4 and will be summarised according to grade, dose cohort and time point. The number and percentage of patients experiencing a grade 3/4 AE will also be calculated at the end of treatment. The safety analysis will include all randomised patients that received at least 1 injection (dose) of radium-223.

Time to first new bone fracture. The time to first bone fracture will be summarised using the Kaplan-Meier method. Log-rank tests will compare the time to first bone fracture by randomised dose group and by responders and non-responders (defined by DW-MRI imaging). A Cox model will determine the relationship with mean total dose administered per cycle and response status. The model will be adjusted for weight, total ALP and current bisphosphonate use.

Assessment of treatment response by dose cohort and individual administered dose: To explore whether there is any indication of a dose-response relationship, an analysis (including

interaction tests) will compare the ADC responses by dose cohort and by mean total dose administered per cycle.

Bone marrow samples: Exploratory analyses of profiles associated with resistance and response will include evaluation of the treatment effect using serial bone marrow samples and further characterisation of CTCs. Analyses will also include measures of proliferation, apoptosis, cell-free plasma tumour DNA, DNA damage and DNA repair utilising Ki67, and caspase cleaved cytokeratin, γH2AX and RAD51 foci formation in bone marrow.

Assessment of treatment response by ALP level: Response rates by ADC values and baseline ALP level will be cross tabulated and a Fisher's exact test will be used to assess their association. The use of appropriate cut-points of continuous ALP scores will be decided before examining their association with response. Logistic regression models will be used to test for an association between ADC response and baseline ALP level.

Correlation between treatment response by K_i and SUV: Spearmans rank correlation and linear regression models will be used to describe the correlation between change in quantitative K_i values and SUV parameters between baseline and 12 weeks post commencement of treatment.

Correlation between baseline CT scans and WB DW-MRI response: The mean CT density of individual lesions at baseline will be investigated and association with WB DW-MRI response (increase in ADC) determined using multivariable logistic regression analyses adjusting for max axial diameter at baseline and other relevant characteristics.

Association of overall survival with response according to DW-MRI and PET-CT: Overall survival will be calculated as time from randomisation to date of death, patients alive at time of analysis will be censored at the last time the patient was known to be alive. The association between overall survival and response will be assessed using log rank tests and Cox proportional hazards models and displayed graphically using Kaplan Meier methods.

Further details will be provided in a Statistical Analysis Plan.

12.5. Interim Analyses and Stopping Rules

This is a phase II trial and there will be no formal early stopping rules for efficacy or toxicity. The Independent Data Monitoring Committee (IDMC) will review accumulating safety and efficacy data in full at regular intervals (to be determined by recruitment rates) and will advise if there are any safety signals.

13. TRIAL MANAGEMENT

13.1. Trial Management Group (TMG)

A Trial Management Group (TMG) will be set up and will include the Chief Investigator, ICR-CTSU Scientific Lead, Co-investigators and identified collaborators, the Trial Statistician and Trial Manager. Principal Investigators and key study personnel will be invited to join the TMG as appropriate to ensure representation from a range of sites and professional groups. Where possible, membership will include a lay/consumer representative. The TMG will meet at regular intervals, and at least annually. Notwithstanding the legal obligations of the co-sponsor(s) and Chief Investigator, the TMG have operational responsibility for the conduct of the trial. The Committee's terms of reference, roles and responsibilities will be defined in a charter issued by ICR-CTSU.

13.2. Trial Steering Committee (TSC)

A Trial Steering Committee (TSC) will be set up and will comprise an independent Chairman and at least 2 further independent members with clinical or statistical expertise (at least 1 member must be a statistician). The TSC will meet at regular intervals, and at least annually. The TSC will provide expert independent oversight of the trial on behalf of co-sponsors. The Committee's terms of reference, roles and responsibilities will be defined in charter issued by ICR-CTSU.

13.3. Independent Data Monitoring Committee (IDMC)

An Independent Data Monitoring Committee (IDMC) will be set up to monitor the progress of the trial and will comprise a Chairman and at least 2 further members with clinical or statistical expertise (at least 1 member must be a statistician). Membership of the IDMC will be proposed by the TMG and approved by the TSC.

The IDMC will meet in confidence at regular intervals, and at least annually. A summary of findings and any recommendations will be produced following each meeting. This summary will be submitted to the TMG and TSC, and if required, the main REC and the MHRA.

The IDMC reserve the right to release any data on outcomes or side-effects through the TSC to the TMG (and if appropriate to participants) if it determines at any stage that the combined evidence from this and other studies justifies it.

The Committee's terms of reference, roles and responsibilities will be defined in a charter issued by ICR-CTSU.

14. RESEARCH GOVERNANCE

14.1. Sponsor Responsibilities

The co-sponsors of the REASURE trial are The Institute of Cancer Research (ICR) and The Royal Marsden NHS Foundation Trust (RM), the Chief Investigator's host institution. Sponsor responsibilities, as defined by The Medicines for Human Use (Clinical Trials) Regulations 2004 as amended, are allocated between ICR and RM, as set out in an agreement letter between ICR and RM.

14.2. Bayer Responsibilities

Bayer is responsible on behalf of the Co-sponsors for the manufacture, packaging, labelling and distribution of study treatment to sites in accordance with Good Manufacturing Practice and all applicable local legislation. Responsibilities are defined in an agreement between Bayer and the Co-sponsors.

14.3. Participating Site Responsibilities

Responsibilities delegated to participating sites are defined in an agreement between the Co-sponsors and the individual site.

15. TRIAL ADMINISTRATION & LOGISTICS

15.1. Site Activation

Before activating the trial, participating sites are required to sign an agreement accepting responsibility for all trial activity which takes place within their site.

Sites may commence recruitment once the site agreement has been signed by all required signatories, the required trial documentation is in place (as specified by ICR-CTSU) and a site initiation (visit or teleconference) has taken place. Site initiation visits will be conducted at sites where the Principal Investigator has requested one or where ICR-CTSU deems it is appropriate.

15.2. Investigator Training

Written information about radium-223 and instructions about handling and injection of radioactive material will be provided to relevant study personnel.

15.3. Data Acquisition

Electronic (e) Case Report Forms (CRF) will be used for the collection of trial data. ICR-CTSU will provide guidance to sites to aid the completion of the eCRFs. The Trial Management Group reserves the right to amend or add to the eCRF template as appropriate. Such changes do not constitute a protocol amendment, and revised or additional forms should be used by sites in accordance with the guidelines provided by ICR-CTSU.

15.4. Central Data Monitoring

Once data has been entered on the eCRF by the site personnel, ICR-CTSU will review it for compliance with the protocol, and for inconsistent or missing data. Should any missing data or data anomalies be found, queries will be raised for resolution by the site.

Any systematic inconsistencies identified through central data monitoring may trigger an on-site monitoring visit.

15.5. On-Site Monitoring

If a monitoring visit is required, ICR-CTSU will contact the site to arrange the visit. Once a date has been confirmed, the site should ensure that full patient notes of participants selected for source data verification are available for monitoring.

ICR-CTSU staff conducting on-site monitoring will review essential documentation and carry out source data verification to confirm compliance with the protocol. If any problems are detected during the course of the monitoring visit, ICR-CTSU will work with the Principal Investigator or delegated individual to resolve issues and determine appropriate action.

15.6. Completion of the Study and Definition of Study End Date

The study end date is deemed to be the date of last data capture.

15.7. Archiving

Essential trial documents should be retained according to local policy and for a sufficient period for possible inspection by the regulatory authorities (at least 5 years after the date of last data capture). Documents should be securely stored and access restricted to authorised personnel.

16. PATIENT PROTECTION AND ETHICAL CONSIDERATIONS

16.1. Trial Approvals

This trial has been formally assessed for risk by ICR-CTSU.

ICR-CTSU, on behalf of the Co-sponsors, will ensure that the trial has received ethics approval from a research ethics committee for multi-centre trials, regulatory approval from the MHRA and global R&D approval via the NIHR Coordinated System for gaining NHS Permission. Before recruiting patients, the Principal Investigator at each site is responsible for submitting Site Specific Information and gaining local Research and Development approval of this protocol.

16.2. Trial Conduct

This trial should be conducted according to the approved protocol and its amendments, supplementary guidance and manuals supplied by the Co-sponsors and in accordance with The Medicines for Human Use (Clinical Trials) Regulations 2004 as amended, the Research Governance Framework for Health and Social Care and the principles of GCP.

16.3. Informed Consent

Patients should be asked to sign the current ethics approved REASURE consent form at trial registration after receiving both verbal and written information about the trial, having been given sufficient time to consider this information. All consent forms must be countersigned by the Principal Investigator or a designated individual. A signature log of delegated responsibilities, listing the designated individuals and the circumstances under which they may countersign consent forms, must be maintained at the participating site. This log, together with original copies of all signed patient consent forms, should be retained in the Site Investigator File and must be available for inspection. The current ethics approved REASURE patient information sheet should be provided in addition to any standard patient information sheets that are provided by the site and used in routine practice.

16.4. Patient Confidentiality

Patients will be asked to consent to their full name being collected at trial entry in addition to their date of birth, hospital number, postcode and NHS number or equivalent to allow linkage with routinely collected NHS data and ensure accuracy in handling biological samples.

Each investigator should keep a separate log of all participants' Trial IDs, names, addresses and hospital numbers. The investigator must retain trial documents (e.g. participants' written consent forms) in strict confidence. The investigator must ensure the participants' confidentiality is maintained at all times.

Representatives of ICR-CTSU and the regulatory authorities will require access to participants' hospital notes for quality assurance purposes. ICR-CTSU will maintain the confidentiality of participants at all times and will not reproduce or disclose any information by which participants could be identified.

16.5. Data Protection Act (DPA)

ICR-CTSU will comply with all applicable data protection laws.

16.6. Insurance and Liability

Indemnity to meet the potential legal liability of investigators participating in this trial is provided by the usual NHS indemnity arrangements.

17. FINANCIAL MATTERS

REASURE is investigator designed and led and meets the criteria for R&D support as outlined in the Statement of Partnership on Non-Commercial R&D in the NHS in England.

The trial is part of the NIHR CRN Cancer portfolio. NIHR resources should therefore be made available for the trial to cover UK specific research costs.

ICR has received an Investigator Initiated Study (IIS) grant from Bayer for the central coordination of the trial. If further funding is received from any other source this will be made apparent in the patient information sheet and to the main REC, but will not require a protocol amendment.

18. PUBLICATION POLICY

The main trial results will be published in a peer-reviewed journal, on behalf of all collaborators. The manuscript will be prepared by a writing group, consisting of members of the TMG. Participating clinicians may be selected to join the writing group on the basis of intellectual and time input. All participating clinicians will be acknowledged in the publication.

Any presentations and publications relating to the trial must be authorised by the TMG. Authorship of any secondary publications e.g. those relating to sub-studies, will reflect the intellectual and time input into these studies.

No investigator may present or attempt to publish data relating to the REASURE trial without prior permission from the TMG.

19. IMAGING

Details of all imaging assessments are given in the accompanying REASURE Imaging Manual, supplied by ICR-CTSU, which should be used as the primary source of information for planning and assessment of all scans within REASURE.

19.1. Diffusion-weighted MRI (DW-MRI)

Whole body DW-MRI will be performed on a 1.5T MR system using surface imaging coils from the base of skull to the mid-thigh area.

The DWI images will be processed to generate 'at a glance' maximum intensity projection (MIP) images. Whole body ADC maps will also be generated. All images will be reviewed by central expert radiologist/s in the following way:

1) Qualitative evaluation:

All acquired images, as well as the MIP and ADC maps will be visually assessed and scored as either: i) disease progression; ii) stable disease; iii) partial response; iv) complete response.

2) Quantitative evaluation:

- Total disease assessment. Global disease in the body will be estimated using a semi-automatic segmentation technique and global median ADC value will also be obtained. A 30% increase in the global median ADC value before and after treatment will be used to define treatment response. However, ROC analysis will also be performed to compare the percentage increase in ADC value with the overall clinical, radiological and biochemical definition of disease response to treatment.
- Target lesions. The mean ADC value of up to 5 target lesions in bone measuring 1.0 cm in diameter or larger will be measured and the mean taken. In the absence of pre-defined gold standard to determine DWI bone response a 30% or more change in ADC value will be used to define response versus non-response per patient and per lesion.

The central expert DWI reviewers will be blind to each patient's dose allocation.

19.2. PET/CT (18F-Fluoride and 18F-Choline)

The protocol specified PET scans should not be conducted on the same day. A minimum gap of 12 hours should be maintained between the 18F-fluoride PET/CT and the 18F-choline PET/CT.

18F-fluoride PET/CT static imaging: Half body 18F-fluoride PET imaging will be performed 1 hour after the patient receives an injection of 250 MBq (+/- 10%) F-18 fluoride.

18F-choline PET/CT static imaging: Half body (skull base to thighs) 18F-choline PET imaging will be performed at 1 hour following injection of 300 MBq (+/- 10%) of F-18 choline.

PET/CT image analysis will be performed centrally by expert readers.

QA/QC of the PET scans will be undertaken by the NCRI PET Core Lab which is part of the NCRI PET Research Network and provides national accreditation for PET research trial studies.

19.2.1. Optional sub-study: 18F-Fluoride PET/CT quantitative assessment of treatment response using regional bone plasma clearance (K_i)

Patients at selected participating REASURE centres will be invited to take part in an optional sub-study to assess the feasibility of using a new validated quantitative method of acquiring and analysing 18F-Fluoride PET/CT scans to estimate regional bone clearance (K_i). This method enables regional K_i to be estimated from static scans acquired at multiple sites in the skeleton following a single injection of tracer [45].

This part of the study is optional for patients. If a patient has consented, following the injection of 18F-Fluoride a plasma sample will be collected prior to the start of the scan and again at the end of the scan. This will be performed at the baseline 18F-Fluoride PET/CT and at Cycle 4. Plasma samples collected should be assayed using an appropriate gamma counter in order to accurately assay radioactivity concentration.

It is not expected that this sub-study will run at all participating sites. Patients who do not wish to participate in this optional sub-study will not be precluded from joining the main trial.

19.3. Post-treatment standard of care imaging

At the request of ICR-CTSU, all available post-radium-223 imaging will be transferred by participating sites for the attention of a radiologist (based at the Royal Marsden Hospital) who will review the imaging for occurrence of fractures. Participating REASURE centres will also be asked to provide a minimal dataset of associated data including, but not limited to, patient fractures reported locally and whether these were symptomatic or imaging findings only. Data and images will be collected beyond the 1 year post-trial treatment clinical follow up period.

20. TRANSLATIONAL STUDY

All REASURE trial samples should be collected, processed, stored and shipped as detailed in the REASURE Investigator Laboratory Manual. Please also refer to section 9.6 (Schedule of Assessments) for timing of sample collections.

20.1. Archival tumour sample

An archival tumour sample should be obtained, wherever possible, for each patient. However, unavailability of archival material will not preclude a patient from taking part in REASURE. Samples will be processed and analysed for comparison to circulating and bone marrow tumour cells.

20.2. Blood samples: circulating tumour cells and plasma DNA analysis

Collection of blood samples to be used in biomarker research is an essential part of REASURE so these will be mandatory for all participants.

Two 7.5ml blood samples should be collected for CTC analysis (characterisation and enumeration) prior to study drug administration at Cycle 1, 2, 3 and 4 and at the end of treatment visit.

In addition, REASURE patients should be asked to provide 20ml of blood at the start of each cycle (prior to administration of study treatment) and at the end of treatment visit. These samples will be used for evaluation of plasma tumour DNA.

20.3. Bone marrow biopsies

Where patients consent to bone marrow biopsies, samples should be collected: prior to first treatment and/or at the start of Cycle 2 and/or at the end of treatment visit. However, whilst efforts should be made to keep to these timepoints, biopsies can be performed and samples collected at any visit. Specific consent should be sought from patients for bone marrow samples and it should be made clear that this procedure is optional and not mandated by the trial. However, where patients are willing to donate bone marrow samples, this should be supported by study site staff and strongly encouraged. A decision not to undergo biopsy for research purposes will not preclude patients from participating in the main trial.

Trained medical staff should perform bone marrow biopsies after appropriate patient education. Entonox (laughing gas) should be administered during the biopsy to make the procedure more tolerable for the patient.

Analysis of these samples may include measures of proliferation, apoptosis, DNA damage and DNA repair utilizing Ki67, and caspase cleaved cytokeratin, γH2AX and RAD51 foci formation in bone marrow.

20.4. Serum and urinary markers of bone turnover

Urinary samples (second morning void) should be collected for measurement of N-telopeptide of type I collage (NTX) and stored for subsequent batch analysis. These are mandatory for all trial participants. Urine samples will be transferred to the University of Sheffield for analysis.

Serum samples should be collected for measurement of alkaline phosphatase, as part of the routine biochemistry evaluation.

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A1. ECOG PERFORMANCE STATUS

Activity Performance Description	**Score**
Fully active, able to carry out all normal activity without restriction	0
Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, for example, light housework, office work.	1
Ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours.	2
Capable of only limited self-care; confined to bed or chair more than 50% of waking hours.	3
Completely disabled; cannot carry out any self-care; totally confined to bed or chair.	4

A2. EXTENT OF DISEASE (EOD) GRADING SYSTEM

Bone scans will be used to document that a patient has skeletal metastases prior to inclusion in REASURE. Baseline bone scans will be performed using Technetium-99m scintigraphy a maximum of 12 weeks prior to randomisation.

The number of metastatic deposits identified on the bone scans will be graded locally according to the Extent of Disease (EOD) grading system.

Description	EOD Grade
Normal; abnormal because of benign bone disease	0
Fewer than 6 metastatic sites	1
6-20 metastatic sites	2
More than 20 lesions but not a superscan*	3
Superscan*	4

*A superscan is defined as diffuse, intense, skeletal uptake of the tracer with absent renal and background activity.

A3. MANAGEMENT OF SPECIFIC TOXICITIES

Administration of study drug should be delayed until recovery of toxicities to the levels described below. However, treatment should not be delayed by more than 2 weeks. In the case of a treatment delay greater than 2 weeks, treatment should be discontinued.

A3.1 Myelosuppression

Changes in haematology parameters may occur after injection of radium-223.

Grade 3-4 neutropenia: Study drug administration should be delayed until recovery to ≤grade 2 (minimum 1.0 x 10^9/L).

Grade 3-4 thrombocytopenia: Study drug administration should be delayed until recovery to ≤grade 2 (minimum 50 x 10^9/L).

Grade 3-4 neutropenia or thrombocytopenia lasting more than 14 days: Study drug should be discontinued.

Grade 3-4 haemoglobin: The value must recover to ≤grade 2 (minimum 8.0 g/dL) before next study drug administration. Blood transfusion is acceptable between study drug administrations.

A3.2 Gastrointestinal events

Diarrhoea: No prophylactic treatment for diarrhoea is recommended. Anti-diarrheals can be used as required. Study drug administration should be delayed until recovery to grade 2 or baseline.

Nausea/vomiting: No prophylactic treatments for nausea or vomiting are recommended but anti-emetic drugs can be used as required. Study drug administration should be delayed until recovery to grade 2 or baseline.

A3.3 Spinal cord compression

If a patient experiences spinal cord compression during the treatment period, they should be treated for the event and may continue to receive study drug if the event is adequately treated within the treatment window.

A3.4 Non-pathological fractures

For traumatic fracture in weight bearing bones during treatment phase, study drug administration should be delayed 2-4 weeks from fracture.

A3.5 Any other toxicity

If a patient experiences any grade 4 non-haematological toxicity lasting longer than 7 days despite adequate treatment, trial treatment should be discontinued.

A4. GLOSSARY

ADC	Apparent Diffusion Coefficient
AE	Adverse Event
ALARA	As Low As Reasonably Achievable
ALP	Alkaline Phosphatase
AL(A)T	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AR	Adverse Reaction
AS(A)T	Aspartate Aminotransferase
b.w	Body Weight
CAB	Combined Androgen Blockade
CI	Chief Investigator
CRF	Case Report Form
CPT	Cell Preservation Tube
CRPC	Castration Resistant Prostate Cancer
CT	Computerised Tomography
CTC	Circulating Tumour Cell
CTSU	Clinical Trials and Statistics Unit
DW-MRI	Diffusion-Weighted Magnetic Resonance Imaging
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
EOD	Extent of Disease
FDA	(US) Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HR	Hazard Ratio
IB	Investigator's Brochure
ICR	The Institute Of Cancer Research
IDMC	Independent Data Monitoring Committee
IMP	Investigational Medicinal Product
ITT	Intention To Treat
IXRS	Interactive Voice/Web Response System
mCRPC	Metastatic Castration Resistant Prostate Cancer
MDT	Multi-disciplinary team
MHRA	Medicines and Healthcare products Regulatory Agency
MIP	Maximum Intensity Projection
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NIHR	National Institute for Health Research
NIST	National Institute of Standards and Technology
PCWG	Prostate Cancer Working Group
PET	Positron Emission Tomography
PI	Principal Investigator
PIS	Patient Information Sheet
PSA	Prostate-Specific Antigen
R&D	Research and Development
REC	Research Ethics Committee
RM	The Royal Marsden NHS Foundation Trust
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SmPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction

TMG	Trial Management Group
TSC	Trial Steering Committee
ULN	Upper Limit of Normal